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COMPARISON OF BEEKEEPERS' AND ANALYTICAL DETERMINATIONS OF HONEY ORIGIN

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ABSTRACT

Honey is a natural sweetener evaluated in accordance with Council Directive 2001/110/EC. 336 honeys from Czech hobby beekeepers were evaluated in this work. The honeys were classified by the beekeepers using questionnaires, and all samples were subjected to laboratory analysis using physico-chemical and melissopalynological methods. The honey samples were categorized by the beekeepers into blossom honeys (n=272), honeydew honeys (n=32), and blended honeys (n=32). Statistically significant differences between beekeepers' and analytical determinations of honey origin were confirmed. For blossom honey, incorrect classification was due to hight electric conductivity (39%), hight moisture (29%), low F+G (14%), and high sucrose content (0,4%). For honeydew honey, incorrect classification was mainly due to low electric conductivity (100%). For blended honey due to high electric conductivity (3.2%) and high acidity (3.2%). Our results show that although the beekeeper has a great deal of information at his disposal for the proper classification of honey, the determination of a wide range of honey contents and properties is always crucial. The cumulative assessment of blossom honeys also showed that there are more monofloral honeys in the country than beekeepers themselves identify. The 6.8% and 23.0% of blossom honey was in compliance with the definition for monofloral honeys for upper and lower limit according to Czech and German regulation.

Keywords: melissopalynology; monofloral honey; beekeepers practice

INTRODUCTION

Currently, the consumer can choose of a whole range of kinds and types of honey, which are defined in the Czech Republic in Decree No. 76/2003 Coll. **(Decree, 2003)** which incorporates European Directive No. 110/2001 **(Directive, 2001)**. The Directive describe honeys depending on their origin into blossom, honeydew and their combination, i.e. blended honeys. Blossom honeys can be classified according to the botanical taxa from which they come, then we talk about monofloral honeys. Based on honey origin the directive classifies honeydew honey from abroad, however, in the Czech Republic honeydew honey are not divided according to the type of aphids from which it comes. Considering the climatic and botanical conditions in the Czech Republic, it is said that it is not easy to produce monofloral honey. There is no honey in the Czech Republic that is protected by any of the international quality marks, nor by a national mark that protect the region of origin of the honey. Meanwhile, in the European Union (EU) there are 8 honeys with the quality mark of protected geographical indication and 30 with the mark of protected designation of origin. At the national level, regional delimitation is provided by the national standard "Český med" (translated as "the Czech Honey") that defines stricter physical and chemical conditions for traditional Czech honey and mainly narrows the origin of honey down to the territory of the Czech Republic **(Kamler *et al.*, 1999)**. From the point of view of Regulation No. 1169/2011, the designation by the name of the country is also binding and such labelling of the food must not be misleading, so the honey must come from the given country if it bears its name **(Regulation, 2011)**. The amount and type of pollen present in the honey is important for determining its botanical origin. But the dominant taxon is not necessarily the defining taxon – i.e. the specific taxon – of the monofloral honey. The reason is the different pollen-producing capacity of botanical taxa, as well as the ability of pollen to get on the body of the bees and then into honey. Pollen-producing capacity of the main taxa important for bees is summarized in a Polish study **(Demianowicz, 1964)** which is still used today. During a nectar collection period, bees utilize all available botanical taxa within a reachable range. Therefore, honey with a specific taxon content of more than 45% is generally considered to be monofloral honey. However, this rule is not accurate for taxa that are characterized by high (*Brassica sp.*) or low (*Robinia pseudoacacia*) pollen-producing capacity **(Anklam, 1998)**. Therefore, the basic assumption is to examine a minimum amount of pollen grains in honey in order to eliminate sampling error by systematic examination as described in an European study (**Ohe *et al.*, 2004**). Identifying botanical taxa microscopically is difficult, although many botanical taxa are identified to genus level based on the morphological structure of the pollen grain. For this reason, taxa are classified according to their frequency of occurrence as predominant pollen, secondary pollen, important minor pollen, minor pollen, and present pollen (**Louveaux et al. 1970**). Generally, the first two groups determine the origin of the honey. Important minor pollen can determine the origin of honey in some exceptions, such as Robinina pseudoacacia or Citrus spThe expected abundance of the main taxon may vary according to the data of different authors **(Beckh & Camps, 2009; El-Labban, 2020; Persano Oddo *et al.*, 1995; Persano Oddo & Piro, 2004)**, as well as according to national standards. For the Czech Republic, a national standard has not yet been established, however, values taken from the German specification are mainly used **(Beckh & Camps, 2009)** and a range of specific pollen taxa included in the Czech National Methodology for Pollen Analysis is also available **(Pospiech *et al.*, 2021)**. . In view of the above, it is important not only to identify pollen grains, but also to interpret the results correctly. In addition to the pollen representation of specific taxa, monofloral honeys must also be in accordance with physico-chemical parameters and sensory characteristics. From the physico-chemical characteristics, the most important for determining the origin of honey is the electric conductivity (el. cond.) and the sum of fructose and glucose (F+G), which divides honeys into blossom and honeydew honeys. The ratio of fructose and glucose (F/G) is also an important parameter especially for determining its botanical origin. Of the sensory parameters, colour, taste and aroma are important. In addition to the verbal description, the colour is also expressed analytically in the PFUND unit. The mentioned physico-chemical and sensory parameters for some monofloral honeys are described in the German specification, recommendations of the International Honey Commission and in professional literature **(Beckh & Camps, 2009; Persano Oddo *et al.*, 2004; Persano Oddo & Piro, 2004; Piana *et al.*, 2004)**.

The aim of this study was the comparison of the botanical origin of honey by beekeeper´s declaration and on the basis of physico-chemical parameters and melissopalynological analysis of honey. All the honey samples were from the Czech Republic and collected by hobby beekeepers.

MATERIAL AND METHODS

As part of this work, 336 honey samples from hobby beekeepers from the Czech Republic were evaluated. The honey was taken in the form of mature honeycombs and individually extracted under controlled conditions to prevent any cross-contamination between the processed samples.. It was mainly the first and second collection periods, in some localities the third collection was also included. The honeys were collected and analysed in 2019 to 2021. The honey came from hobby beekeepers, who voluntarily participated in this study. Data on the botanical origin of honey came from a questionnaire survey where each beekeeper was asked the questions below:

1. Is this blossom or honeydew honey?
2. If blossom honey, is it a polyfloral or monofloral honey? If monofloral honey, what is the dominant botanical taxon?
3. Unclassified honey samples were ranked as blended honey.

To confirm the questionnaire survey, the honeys were further analysed in order to confirm the beekeepers´statements. The limit for the analytical method used was taken from the European Directive No. 110/2001 (**(Directive, 2001)**. For monofloral honey upper and lower limit was used. These limits are taken from **(Beckh & Camps, 2009; Pospiech *et al.*, 2021)**
The following analyses were performed.

Determination of water content – the tempered and homogenized sample of honey was applied to the optical prism of a digital Abbe refractometer RM 40 (Mettler-Toledo, CH) with the whole tempered to 20 °C. The refractometer was calibrated using the refractometric index of distilled water before use. Each sample was measured in duplicate. The method was performed according to the recommendations of the International Honey Committee (IHC) **(Bogdanov, 2009)**. This parameter was only used for quality determination and was not used for determination of honey origin.

Determination of electrical conductivity (el.cond.) – the honey sample was weighed to represent 20g of honey dry matter. The sample was added to 100 ml of distilled water at a temperature of 20 °C. The el. cond. was determined in the conductivity cell of the conductometer Multi 9310 IDS (WTW GmbH, GER. The electrode used was IDS Tetra Con 925 (WTW GmbH, GER). The method was performed according to the approved IHC procedure **(Bogdanov, 2009)**.

Determination of free acidity was provided by alkalimetric titration to a final pH of 8.3. An automatic titrator T5 (Mettler-Toledo, CHE) and an electrode DGi115-SC (Mettler-Toledo, CHE) The method was performed according to the approved IHC procedure **(Bogdanov, 2009)**.

Determination of diastase – diastase activity (DN) was determined by Phadebas method using a commercially available kit. The method was carried out according to the instructions supplied with the kit **(Bogdanov, 2009; Phadebas, 2018)**.

Determination of saccharides content – a sample of honey weighing 2.5 g was dissolved in 12.5 ml of a 25% aqueous methanol solution and made up to 50 ml in a volumetric flask. Before analysis, samples were filtered with a 0.45 µm membrane filter. The content of mono- and di-saccharides was determined by the HPLC method (high performance liquid chromatography) with RI detection (detection based on changes in the refractive index). The flow rate of the mobile phase was 1.2 ml/min, the temperature of the detector was 35°C, the temperature of the column 35°C, the volume of the dosing loop 10 µl. The method is based on the approved IHC methodology **(Bogdanov, 2009)**.

Melissopalynological analysis – before analysis, the samples were homogenized and tempered at a temperature of 40°C until complete dissolution. 5 g of the sample was weighed into 20 ml of tempered water. The quantitative method of filtering honey according to **(von der Ohe *et al.*, 2004)**. Briefly, a filter of 3µm, Ø 25mm (Millipore, Merc, USA) was applied, filtration was performed by Eisco™ Glass Filtration Assembly (Fisherscientific, USA). After drying, the filter was mounted using solacryl on a 76 x 26 mm glass slide. The samples were analysed under the Eclipse Ci-L microscope (Nikon, JPN) with motorized stage of Proscan III (Prior, USA). Images were captured by the DFK 23U274 camera (Imaging Source, GER). The pollens were classified by an expert from super resolution pictures.

Statistical analysis – nonparametric McNemar's test (Contingency table test) was used for comparison, significance level was alpha 0.05. McNemar's test compared 2 values with binary responses for randomized complete blocks. Xlstat 2022.4.1.1370 software was applied. Results which are not in agreement between beekeepers declaration and analytical results (inconsistent results) are interpreted as relative value calculated from inconsistent results, not from all evaluated samples.

RESULTS AND DISCUSSION

According to legislative requirements, honey can be divided into blossom honey and honeydew honey. The division reflects the origin of honey, i.e. the source from which a significant part of the nectar comes. Blossom honey is honey produced from the nectar of plants. Honeydew honey comes mainly from the excretions of aphids (*Hemiptera sp.*) sucking on plant tissue. Honey can also be further labelled by its origin as monofloral and polyfloral **(Decree, 2003; Regulation, 2001)**. The third category defined by national legislation **(Decree, 2003)** is blended honey that represents honey containing an undefined ratio of nectar and honeydew. It could also be said that it is a transition between blossom and honeydew honey. Such honey has different characteristics and, given the diversity, it is not easy to be defined, therefore both, the national legislation as well as the professional literature, do not define a specific parameter that would be typical for this type of honey. European Directive **(Regulation, 2001)** considers "blends of honeydew honey with blossom honey" to be honeydew honey and it must meet its minimum requirements. To identify the origin of honey, the beekeeper applies his knowledge of nectar-producing plants in the vicinity of the hives, but commercially defined physico-chemical parameters and melissopalynological analysis are used to determine/confirm the origin of honey nectar. A comparison of blossom honey origin indicated by beekeepers and results based on physico-chemical parameters and melissopalynological analysis is presented in **Table 1**.

Blossom Honey

McNemar's test confirmed a statistically significant difference ($p < 0.05$) between the honey samples determined by the beekeeper and the laboratory analysis. The result clearly confirmed that sensory evaluation, knowledge of the location, observing the flight of bees, as is commonly done by beekeepers, does not allow a clear identification of the origin of honey. Relatively speaking, 20% of blossom honeys were not correctly identified by beekeepers.

Table 1 Comparison of beekeepers' and analytical determinations of blossom honey

		Results of analysis		
		Blossom	Non-Blossom	Total
Beekeepers declaration	Blossom	205 (61.0%)*	67 (19.9%)	272 (81.0%)
	Non-Blossom	27 (8.0%)	37 (11.0%)	64 (19.0%)
	Total	232 (69.0%)	104 (31.0%)	336 (100)

*relative expression of the frequencies

Inconsistency with analytical values was most often (39% of inconsistent results) due to the high el. cond. of honey, which is a maximum of 0.8 mS/cm for blossom honey **(Regulation, 2001)**. Other reasons were the low F+G content (14%), which is legislatively limited to a minimum content of 60% for blossom honeys and high water content of 29% honey, which is legislatively limited to a maximum of 20% **(Decree, 2003; Regulation, 2001)**. The physico-chemical parameters of blossom and non-blossom honey are shown in **table 2**.

Table 2 Physico-chemical parameters of blossom honey

Physico-chemical parameters	Blossom	Non- Blossom
Water content (%)	17.7±1.5	18.0±2.7
Electrical conductivity (mS.cm-1)	0.4±0.2	0.8±0.4
Free acidity (meq/kg)	19.6±7.7	28.5±10.2
Diastase (DN)	25.6±8.5	24.2±6.9
F+G (%)	73.0±4.9	65.5±7.9

DN – diastase number

In 16% of honeys, more than two parameters were inconsistent with the analytical values. In one case only a high sucrose content was recorded (9.5 g/100g). The botanical profile of this honey (45% lime, 0% acacia) did not correspond to *Robinia pseudoacacia* honey and cannot be considered *Robinia pseudoacacia* honey from the legislative point of view, where an exception of 10 g/100g of sucrose content is allowed **(Regulation, 2001)**.
For blossom honey, legislative allow the labelling as floral or vegetable origin in case that honey comes mainly from the indicated source with condition that sensory, physico-chemical and microscopic characteristics are in accordance with botanic source. For blossom honey, the beekeepers were also asked to determine its botanical origin in the case of the assumption of monofloral honey. The agreement with the beekeepers' statements is shown in **Table 3.** Determining monofloral honeys is not easy, and therefore, both in the literature and in national recommendations or standards, a certain range of achievement values is allowed for them. In particular, this applies to the range in the pollen content, but the range can also be for some physico-chemical parameters. Therefore, the agreement between the determination of honey by the beekeeper and the analytical values is expressed separately for the upper limit and lower limit (**Table 3.**).

Table 3 Comparison of beekeepers' and analytical determinations of monofloral honey

		Result of analysis					
		Upper Limit			Lower Limit		
		Agreement	Disagreement	Total	Agreement	Disagreement	Total
Beekeepers declaration	Agreement	0	7	7	2	5	7
	Disagreement	18	311	329	61	268	329
	Total	**18**	**318**	**336**	**63**	**273**	**336**

Upper and lower limits of pollen content and physico-chemical parameters **(Beckh & Camps, 2009; Pospiech *et al.*, 2021)**

McNemar's test confirmed a statistically significant difference between the monofloral honeys determined by the beekeeper and the laboratory analysis ($p < 0.05$) for the lower as well as upper limit. The calculated value of the test was ($p = 0.045$) for the upper limit and ($p < 0.0001$) for the lower limit. But it should also be mentioned that there can be differences even between laboratory methods, especially with regard to non-harmonized melissopalynological analysis. In Europe, different laboratories may achieve different results on pollen content. For example, in a Spanish study, interlaboratory differences reached 5-54% for *Brassica sp*., 8.7-31% for *Coriandrum sativum*, 0-17% for *Castanea sativa* and 75.7-99% for *Eucalyptus sp*. **(Escriche *et al*., 2023)**. In order to minimize errors, several studies have been developed that deal with the issue of the melissopalynological method **(Bogdanov, 2009; Jones & Bryant, 1992, 2001; Louveaux *et al.*, 1970; Low *et al.*, 1989)**. One recommendation is to count at least 300 pollen grains, or better 500-1000 pollen grains per sample **(Silici & Gökceoglu, 2007; Stawiarz & Wróblewska, 2010; Terrab *et al.*, 2004)**. In order to verify the method, it is then advisable to at least meet the repeatability and reproducibility of the results, as defined in the IHC recommendation **(Ohe *et al.*, 2004)**. Or it can also be use mathematical models that evaluate all possible combinations of fields of view of the examined honey sample **(Pospiech *et al.*, 2021)**.

An important fact resulting from this assessment is that there is potentially a larger amount of monofloral honey in the Czech Republic than beekeepers estimate for blossom honeys. In relative terms, 6.8% of blossom honeys with an upper limit and 23.0% of blossom honeys with a lower limit of specific taxa pollen content and compliance with physico-chemical parameters could be considered as monofloral. Their representation is shown in **Table 4** including the specific taxon for monofloral honey.

Table 4 Monofloral honey classified according to upper and lower limit of pollen content

	Total	
Limit	Upper Limit	Lower Limit
Acacia honey	0	1
Clover honey	0	6
Lime honey	4	13
Mustard honey	1	1
Honey from fruit trees	2	2
Dandelion honey	0	1
Buckwheat honey	0	1
Rapeseed honey	10	34
Sunflower honey	0	1
Goldenrod honey	1	1
Total	18	61

Of the analysed blossom honeys from the Czech Republic, rapeseed honey was most often confirmed. Another monofloral honey with a greater occurrence was lime honey. About half of that amount was clover honey, which is not often described but is common in countries with more grassland, such as Ireland **(Downey *et al.*, 2005)**. Clover honey may increase in the future in the Czech Republic with regard to the recognition of clover incarnate (*Trifolium incarnatum*) among registered agricultural varieties since 2018 **(Mezlík, 2019)**. The reason for growing clover incarnate, in combination with honey-producing blue tansy (*Phacelia tanacetifolia*) and non-honey-producing annual ryegrass (*Lolium multiflorum*), is that they represent a combination with the lowest erosion factor, and on the contrary, they enrich the soil with atmospheric nitrogen and thus improve the yields of the later crops **(Kincl *et al.*, 2022)**.

Monofloral rapeseed honey was confirmed in 34 cases. While the upper limit, i.e. 80% or more rapeseed pollen, would correspond to 10 honey samples, the lower limit, i.e. more than 60%. In addition to the pollen profile, this honey must also meet physico-chemical parameters. Specifically, it is a el. cond. lower than 0.25 mS/cm and F/G ratio lower than 1.05 **(Beckh & Camps, 2009; Persano Oddo & Piro, 2004)**. The rapeseed honey samples in this study had an average el. cond. of 0.21 mS/cm and the F/G ratio was 1.00.

Acacia honey has been confirmed in one case. This honey was declared by the beekeeper as floral, but the beekeeper did not provide a botanical definition. In addition to the minimum pollen content (10%), acacia honey must have an F/G ratio greater than 1.5 and a el. cond. less than 0.20 mS/cm. For this honey, the el. cond. was 0.20 mS/cm and the F/G ratio was 1.5. Both values were borderline. Of the analysed honey samples, three more met the requirement for the minimum amount of acacia pollen grains. However, these honeys did not meet the physico-chemical parameters for acacia honey.

Lime honey is a typical monofloral honey for the Czech Republic. This honey is characterized by a strong aroma and is popular for many consumers. From the point of view of lime honey production, however, compared to other monofloral honeys, there is a difference in the source of sweet secretions. Lime tree is not only a nectar-producing tree, but is also a good source of honeydew. A higher acidity of 23.5 meq/kg, or a pH of 4.4, is therefore typical for these honeys. With regard to honeydew, this honey is also characterized by a higher el. cond., with an average of around 0.63 mS/cm **(Persano Oddo & Piro, 2004)**. According to the German standard, the minimum el. cond. is 0.20 mS/cm, and an F/G ratio of 1.0 and above **(Beckh & Camps, 2009)**. Interestingly, these values are not in accordance with the already mentioned German standard, which is more liberal in this case. All analysed honeys in this study with a lime tree pollen content above 10% also met the physico-chemical parameters. They can therefore be considered monofloral honeys, if the sensory properties are also suitable. For lime honey in this study, the average el. cond. was 0.65 mS/cm and F/G ratio was 1.2, acidity was 26.98 meq/kg. The pollen content of the honeys in this study was above 20% in only four honeys, which confirms the lower pollen-producing capacity of lime trees.

The situation is complicated with clover honey. There is no clearly defined pollen content for this monofloral honey, or the German trade standard states 60-70%. Compared to it, other literary sources report differences according to the type of clover, for example white clover (*Trifolioum repens*) 5-78% and red clover (*T. pratense*) 18%, and, in contrast, a Turkish study reports a range of 10-72% **(Dogan, 2008; Downey *et al.*, 2005)**. There are also differences in the physico-chemical parameters, where the German standard states el. cond. less than 0.40 mS/cm and F/G less than 1.25 **(Beckh & Camps, 2009)**, and literature reports el. cond. in the range 0.16-1.09 mS/cm, F/G in the range 1.1-1.5 **(Dogan, 2008)**. In the case of honeys from the Czech Republic, the average pollen content was 49%, the average el. cond. was 0.61mS/cm, F/G 1.16. The lower content of pollen in honey is most likely caused by different types of clover, when the German standard specifies them together for all, which does not appear to be an optimal limiting factor with respect to the literature.

Sunflower, buckwheat, and dandelion honeys were also occasionally recorded. For these honeys, there is a specification from a German standard, and a European descriptive study **(Beckh & Camps, 2009; Persano Oddo & Piro, 2004)**. For sunflower honey, a minimum pollen content of 30%, el. cond. 0.2 -0.4 mS/cm, F/G 1.2 is allowed. In the European descriptive study, however, a large variability in pollen content is allowed, ranging between 20-90% **(Persano Oddo & Piro, 2004)**. The recorded honey had a pollen content of 33%, a el. cond. of 0.22 mS/cm and an F/G of 1.0. Buckwheat honey is characterized by its specific sensory properties and is unacceptable to some consumers **(Kortesniemi *et al.*, 2017)**. The pollen content should be over 30%, el. cond. up to 0.3 mS/cm. The F/G ratio is not defined in the German standard**(Beckh & Camps, 2009)**, in the Serbian study, F/G in six samples was 1.4 **(Nešović *et al.*, 2020)**. The honey in this study had a pollen content of 49%, a el. cond. of 0.4 mS/cm, and an F/G of 1.1. With regard to its sensory properties, it is classified among monofloral honeys, the different el. cond. can be justified by the admixture of other taxa and honeydew. Fast crystallization and light colour are typical for dandelion honey. Dandelion honey has a minimum dandelion pollen content of 15% **(Beckh & Camps, 2009; Persano Oddo & Piro, 2004)**. Physico-chemical parameters for dandelion honey differ in the literature, similarly to lime honey, according to the German standard, the minimum el. cond. is greater than 0.40 mS/cm, the F/G ratio is less than 1.05. The European descriptive study reports an average el. cond. of 0.50 mS/cm and F/G ratio of 0.99 **(Beckh & Camps, 2009; Persano Oddo & Piro, 2004)**. The honey confirmed in this study did not meet the parameter of the F/G ratio, which was 1.07. However, we consider the honey to be monofloral due to ambiguous data in the literature and also due to the fact that differences in individual physico-chemical parameters have been confirmed between individual states for species honeys **(Juan-Borrás *et al.*, 2014)**.

Monofloral honeys from the flowers of fruit trees, goldenrod or mustard are less commonly described in the Czech Republic. For these types of honey, there is no officially or pan-European defined characteristic. When determining, we therefore apply the general assumption that more than 45% of the honey (nectar) comes primarily from this taxon **(Ohe *et al.*, 2004)**. Honey from fruit trees with regard to planting in the Czech Republic was expected, but the occurrence is relatively small and is due to the fact that nectar and pollen from early spring plants are used more for the development of the bee colony than for the creation of honey reserves.

However, in some regions and countries, the migration of bee colonies is used in a targeted manner in order to increase the yield of fruit trees **(Cunningham *et al.*, 2016)**. It is then easier to obtain monofloral honey from fruit trees from these bee colonies. Goldenrod honey is characterized by a relatively high pollen content, ranging from 40 to 84%. This honey is typically light to watery white or amber. There are certain differences between the regions where this honey comes from **(Czigle *et al.*, 2022)**. A lower amount of goldenrod pollen in monofloral honey is admitted by a Croatian study (28%), this study also reports the el. cond. of this honey as 0.39 mS/cm, the F/G ratio is 1.3 according to another study **(Zielińska *et al.*, 2014)**. The goldenrod honey in this study had a pollen content of the main taxon in the amount of 64%, a el. cond. of 0.45 mS/cm, and an F/G of 1.3. Honey from mustard is also not ordinary honey and only a general rule can be applied to it, which is to meet the requirements for blossom honey and a minimum mustard pollen content of 45%. In this study, the pollen content reached 69%, el. cond. 0.27 mS/cm, F/G 1.0, F+G 72.79 g/100g.

Honeydew Honey

As in blossom honey, a comparison of the determinations of the origin by the beekeepers and analytical methods in honeydew honey samples confirmed a statistically significant difference by McNemar's test ($p < 0.05$). The result of the analytical determination and determination by the beekeepers is shown in **Table 5**.

Table 5 Comparison of beekeepers' and analytical determinations of honeydew honey

		Result of analysis		
Beekeepers declaration		Honeydew	Non-Honeydew	Total
	Honeydew	16 (4.8%)*	16 (4.8%)	32 (9.5)
	Non-Honeydew	39 (11.6%)	265 (78.9%)	304 (90.5)
	Total	55 (16.4)	281 (83.6)	336 (100)

*relative expression of the frequencies

Determination by beekeepers was incorrect in 50% of honey samples, as confirmed analytically. The reason for non-compliance with the legislative limit was always low el. cond. (100% non-compliant results) **(Directive, 2001)**. In the case of the analytical methods, it was also a question of low el. cond. (99.7%), which is due to the fact that el. cond. is the decisive criterion for determining honey origin. Two honey samples (0.3%) did not meet the requirement for the maximum value of total acidity, which was above 50 meq/kg. The legislative parameter for the analytical determination of honeydew honey is also F+G, which is set at a minimum content of 45g/100g **(Directive, 2001)**. The Physico-chemical parameters of honeydew and non-honeydew honey are shown in **table 6**.

Table 6 Physico-chemical parameters of honeydew honey

Physico-chemical parameters	Honeydew	Non-Honeydew
Water content (%)	17.0±1.2	17.6±1.3
Electrical conductivity (mS/cm)	1.1±0.2	0.5±0.1
Free acidity (meq/kg)	32.2±7.3	27.8±7.5
Diastase (DN)	25.5±6.6	29.6±9.9
F+G (%)	61.3±2.8	70.1±5.0

DN – diastase number

The lower permitted value of the sum of F+G in honeydew honeys than in blossom honeys is due to the different carbohydrate composition. Sucking insects, which make up a significant part of the sugar solutions used by bees for the production of honeydew honey, produce in addition to glucose and fructose other carbohydrates that subsequently become part of the honey. Honeydew honey can therefore contain 16 other carbohydrates in addition to glucose and fructose. The most represented include disaccharides (but not sucrose), trisaccharides and a certain percentage of tetrasaccharides. At the same time, it is not possible to say unequivocally which carbohydrates could be used to differentiate honeydew honeys, because they differ depending on two basic conditions. The first condition is the botanical species **(Pita-Calvo & Vázquez, 2018)**, on which the insect sucks, and the second condition is the species of the insect itself **(Shaaban *et al.*, 2020)**.

Blended Honey

Blended honey forms a transition between blossom and honeydew honey, both in terms of origin and variable analytical values. This group is the most difficult to characterize, and beekeepers rank here honeys for which they are not able estimate the source of the honey based on their practice. McNemar's test confirmed a statistically significant difference ($p < 0.05$) between the honey samples determined by the beekeeper and the laboratory analysis also for blended honey. The comparison results are presented in **Table 7**.

Table 7 Comparison of beekeepers' and analytical determinations of blended honey

		Result of analysis		
Beekeepers declaration		Blended	Non-Blended	Total
	Blended	30 (8.9%)*	2 (0.6%)	32 (9.5%)
	Non-Blended	288 (85.7%)	16 (4.8%)	304 (90.5%)
	Total	318 (94.6%)	18 (5.4)	336 (100%)

*relative expression of the frequencies

Honey incorrectly categorized by the beekeeper had high el. cond. (20.9 g/100ml) in one case and high acidity (62.8 meq/kg) in the other case, so the beekeeper incorrectly classified 6.3% of the honey in the blended honey category. From the legislative point of view, these honey samples cannot be considered as honey, but they could be used as baker's honey, where up to 25 g/100 ml of water and total acidity above 50 meq/kg are allowed. **(Directive, 2001)**. The physico-chemical parameters of blended and non-blended honey are shown in **table 8**.

Table 8 Physico-chemical parameters of blended honey

Physico-chemical parameters	Blended	Non-Blended
Water content (g/100ml)	16.8±1.5	19.7±1.2
Electrical conductivity (mS.cm-1)	0.8±0.4	0.7±0.3
Free acidity (meq/kg)	29.3±9.7	41.9±21.0
Diastase (DN)	27.7±9.7	29.9±9.8
F+G (g/100ml)	67.1±7.5	70.8±4.8

DN – diastase number

An interesting fact about the blended honeys was that six of these honeys could be considered monofloral honeys. Rapeseed was the dominant taxon in four honeys, in varying pollen content (60-91%), and two honey samples contained a high amount of clover pollen reaching 45% and 33%. Honey samples with a rapeseed pollen content of more than 60% cannot be considered blended. Rapeseed is a nectar- and pollen-producing crop. The high pollen content therefore also indicates a large amount of rapeseed nectar in this honey. The situation is different for honey with the presence of clover pollen. Clover pollen is also an accompanying taxon for honeydew honeys, and for these honeys, the judging criterion would be the el. cond. of the honey. In our case, the el. cond. of honey with a pollen content of 33%, 0.84 mS/cm, which points to honeydew honey. Honey with a higher clover pollen content of 45% had a el. cond. of 0.66 mS/cm, which on the other hand points to blossom honey. These results confirmed that both honeydew and clover nectar contributed to the honey production in this sample. This finding is not surprising, as pollen is the main source of protein for bees and therefore, if it is available in the area, bees fly to pollen-producing plants even in the case of abundant honeydew. Primarily, pollen is stored by bees in special honeycomb cells, not in honeycomb cells with honey. However, pollen often gets into the honey, from the surface of the bees' bodies and also from the bees that process and thicken the nectar. The amount of pollen in honey can also be influenced by technological manipulation in the hive, when pollen from the pollen cells is then also stored in the honey **(da Fernandez & Ortiz, 1994)**. Other taxa detected in this honey were lime tree 10% and forget-me-not 35%. Also, these taxa point to a collection from forest areas.

CONCLUSION

Statistically significant differences were confirmed between beekeepers' and analytical determinations of honey origin for all, blossom, honeydew as well as blended honeys. Overall, for blossom honey, honey dew honey and blended honey the results were inconsistent, reaching 24.6% 50%, 6.3%, respectively. Our results show that analytical methods should be used for correct determination of honey origin. For most of the honeys, the beekeepers' classification was different from the measured electrical conductivity, whose legal limit is one of the criteria for determining the origin of the honey. For blossom honey, incorrect classification was given by el. cond. (39%), F+G (14%), moisture (29%), and by high sucrose content in one case. For honeydew honey, incorrect classification was mainly by low el. cond. (100%). For blended honey, beekeepers did not classify two samples correctly, where high el. cond. and total acidity was detected. Our results also show that there is more monofloral honey in Czech Republic than it was determined by the beekeepers. 6.8% or 23.0% of blossom honey was in compliance with the definition for monofloral honey for upper or lower limit respectively. In this study, the beekeepers supposed 2.6% of monofloral honeys but most of them were actually classified incorrectly. The discrepancy in the declaration of monofloral honeys shows that the classification of the origin of honeys is still problematic. Therefore, new methods or a better characterised distinction for monofloral honeys are needed. On the other hand, further research is needed to identify the environmental, agricultural and behavioural conditions that can lead to the production of monofloral honeys.

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