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QUALITATIVE PARAMETERS OF MONOFLORAL HONEYS FROM SLOVAKIA AND AUSTRIA AND THE ROLE OF WATER ACTIVITY

Kristína Kukurová[1], Zuzana Ciesarová[1], Viera Jelemenská[1], Martina Orolínová[2], Zerina Duhović[3], Janka Kubincová[1], Zuzana Dubová[1], Jana Horváthová[1], Blanka Tobolková[1], Michael Murkovic[3], Barbara Siegmund[4]*

Address(es): Ing. Kristína Kukurová, PhD.
[1]National Agricultural and Food Centre, Food Research Institute, Priemyselná 4, 824 75 Bratislava, Slovak Republic.
[2]Slovak Technical University, Faculty of Chemical and Food Technology, Radlinského 9, 812 37 Bratislava, Slovak Republic.
[3]Graz University of Technology, Faculty of Technical Chemistry, Chemical and Process Engineering, Biotechnology, Institute, Petersgasse 10-12/II, 8010 Graz, Austria.
[4]Graz University of Technology, Faculty of Technical Chemistry, Chemical and Process Engineering, Biotechnology, Institute of Analytical Chemistry and Food Chemistry, Stremayrgasse 9 / II + III, 8010 Graz, Austria.

*Corresponding author: kristina.kukurova@nppc.sk

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ABSTRACT

The quality evaluation and physicochemical parameters assessment of Slovak and Austrian monofloral honeys were performed. In total 65 local monofloral honeys denoted by beekeepers as acacia (n = 20), rapeseed (n = 10), linden (n = 10), honeydew (n = 6), forest (n = 12) and chestnut (n = 7) were collected. The parameters such as moisture content, water activity, electrical conductivity, pH, free acidity, and 5-hydroxymethylfurfural (HMF) content were assessed for physicochemical characterization and comparison of all monofloral honeys.
Electrical conductivity showed to be the most significant indicator for honeydew origin, that together with pH and a_w led to 81.54 % correct classification of samples according to honey type using multivariate statistical analysis. In context to undesirable fermentation and spoilage, a_w value below 0.600 showed to be an important control parameter that could be recommended to control especially for creamed rapeseed honeys.

Keywords: honey, water content, water activity, electrical conductivity, free acidity, pH, HMF, botanical origin

INTRODUCTION

Honey is a precious traditional natural bee product with health-promoting effects recognized worldwide. The composition of honey is specified by Council Directive 2001/110/EC with the aim to guarantee quality as well as to protect consumers from adulterating practices. Honey is one of the most frequently adulterated commodities due to the increasing price of honey on the market and its hard recognition resulting from the huge variability in composition according to the type and origin of honey. From a qualitative point of view, honey must not have any foreign tastes or odors, have begun to ferment, be heated to such an extent that its natural enzymes are inactivated, or contain a substance that endangers human health (**Official Journal of the European Communities, 2002**).
Honey quality is evaluated through physicochemical analysis of its constituents including water content, water-insoluble content, electrical conductivity, free acidity, and sugars profile composed predominantly by fructose and glucose with a limited content of sucrose. Further legislative parameters such as 5-hydroxymethylfurfural (HMF) content and diastase activity are used as indicators of honey quality and good practices of processing and storage. However, additional quality standards reflecting biological activity of honey (**Majtán *et al.*, 2021**) and new methods for assessing quality and botanical origin of honeys are discussed by researchers (**Puscion-Jakubik *et al.*, 2020**). In data processing methods of multivariate statistical analysis that are applied for classification of honeys according to their authenticity, botanical, and geographical origin, help to untangle all interactions among individual components and understand their combined effects on the whole object (**Kukurová *et al.*, 2008**).
Slovak and Austrian honey samples in the study were provided by small beekeepers. In both countries, local monofloral honeys were collected according to the current production year. Selected legislative and additional qualitative parameters such as water content, water activity, pH, acidity, electrical conductivity and HMF content were evaluated, and processed using statistical methods to discriminate honey samples according to type and origin.

MATERIAL AND METHODS

Honey samples collection

A total of 65 local monofloral honeys denoted by beekeepers as acacia (*Robinia pseudoacacia)* (n = 20), rapeseed (*Brassica napus)* (n = 10), linden (*Tilia platyphyllos)* (n = 10), chestnut (*Castanea sativa)* (n = 7) and mixed floral honeydew (n = 6) and forest (n = 12) honeys were collected in the production year of 2021. The samples from Slovakia represented acacia, creamed rapeseed, linden, and honeydew honeys. The samples from Austria were of chestnut and forest origin. The honeys were stored in the dark at room temperature.

Determination of water content and water activity

The water content of honey was determined directly using an optical refractometer with automatic temperature compensation (ATC) and a digital honey refractometer PAL22-S (Atago, Japan) after previous liquefying the crystallized samples in a water bath set at 50 °C to allow all sugar crystals to dissolve and cooling down for 24 h before analysis. Water activity was analyzed in liquid or crystallized honeys directly transferred into plastic sample containers using a_w meter LabMaster (Novasina, Switzerland).

Determination of free acidity, pH and electrical conductivity

Free acidity, pH and electrical conductivity were measured with a 9310 IDS multimeter (WTW, Germany). The electrical conductivity of a 20% (w/v) honey solution in demineralized water at 20 °C was measured using an electrical conductivity cell, a platinized double immersion electrode TetraCon 925. For pH and free acidity determination, 10 g of honey was dissolved in 75 mL of carbon dioxide-free water in a 250 mL beaker, stirred with a magnetic stirrer, the pH electrode SenTix 940 was immersed in the solution and the first pH value was recorded followed by titration with 0.1M NaOH to pH 8.30 according to harmonized methods of the International Honey Commission IHC (**Bogdanov, 2009**, **Bogdanov *et al.*, 1997**).

Determination of 5-hydroxymethylfurfural (HMF)

HMF content in honey was determined by a reverse-phase HPLC with UV detection at 280 nm and a C18 column according to the IHC methods (**Bogdanov, 2009; Bogdanov *et al.*, 1997; Kukurová et al., 2006; Kukurová et al., 2008**) with some modifications in chromatographic conditions. A HPLC Series 1200 (Agilent Technologies, USA), a Zorbax C18 SB column (250 x 4.6 mm, 5 µm) tempered to 30 °C and a mobile phase consisted of water (A), methanol (B) and acetonitrile (C) with a gradient elution at a flow rate 0.8 mL/min were used for separation. 1 g of honey 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 syringe nylon filter (0.45 µm), samples were transferred to 2mL 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.

Statistical analysis

Data were reported as mean and standard deviation of at least two or three replications. Statistical evaluation of experimental data was performed using statistical package Unistat 6.0 (United Kingdom). Correlations among analyzed parameters were calculated by Pearson's correlation coefficients. Multiple comparison of qualitative characteristics between individual honey's types was assessed by ANOVA Tukey´s HSD (Honesty significant difference) test. The level of significance was accepted at $P < 0.05$. Canonical discriminant analysis (CDA) was used to interpret and visualize differences between compared honeys and to define the most appropriate characteristics for honeys differentiation.

RESULTS AND DISCUSSION

In Europe more than 100 botanical species are known to produce monofloral honeys of which 15 honey types are the most important in terms of abundance of production or commercial relevance (**Persano Oddo & Piro, 2004**). In presented collaborative study, 6 botanical types of honeys from Slovakia and Austria produced in 2021 have been collected including acacia, rapeseed, linden, chestnut, honeydew, and forest honeys for evaluation of their quality based on selected physico-chemical parameters and legislative requirements.

Water content and water activity

A water content below 20% represents a general criterion for honey ripeness and maturity, which is considered sufficient to ensure good shelf life and microbial stability during long-term storage (**Council Directive 2001/110/EC**). In the countries of middle European climate, a lower moisture with a maximum water content of 18% should be naturally attainable. For this reason, presented lower limit of 18% for honeys with geographical origin label in the Slovak Republic is recommended. In the Slovak Republic, beekeepers who want to use a trademark of a geographical origin must meet a stricter requirement for maximum water content of 18%, together with some other parameters (**ZN SZV 1/2006**).
A water content in liquid honeys is measured by refractometry and this parameter is often self-checked by small producers themselves using an affordable optical refractometer available in specialized stores for beekeepers. For this reason, measurements of water content in all honey samples were performed by two instruments, an optical refractometer with ATC and a laboratory digital honey refractometer PAL22-S (Atago, Japan). Both instruments provided satisfactory and comparable results for practical usage with a standard deviation up to 5%.
All Austrian honey samples were within a legislative limit below 20% as well as below a stricter geographical regulation, with values in the range from 13.7% to 16.1% for forest honeys, and 14.9% to 17.1% for chestnut honeys. Among Slovak honeys, one sample of linden honey did not meet a legislative requirement with 21.4% water content, and several others had water content higher than 18% (2 linden honeys, 2 honeydew honeys and 1 acacia honey). Regarding the honey type, it can be summarized that water content was in the range from 14.1% to 18.8% for acacia, 14.6% to 21.3% for linden, 15.8% to 18.4% for honeydew, and 16.3% to 17.8% for rapeseed honeys. Similar observations of monofloral honeys have been reported by the IHC working group with exceeded water content detected in some heather honeys (**Persano Oddo & Piro, 2004**). The results of water content in honey samples under study are summarized in Fig. 1A, which shows the classification according to the type of honey. It was evident that the lowest water content was found in Austrian forest honeys in comparison to other types of honey. In general, water content is not a very decisive parameter for specification of botanical origin.

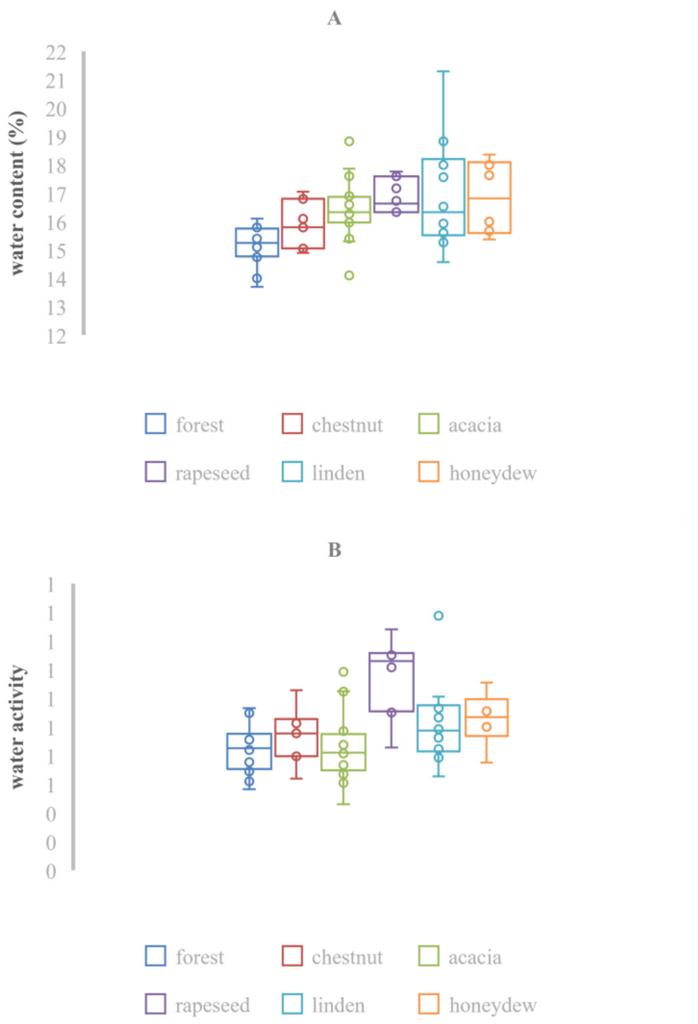

Figure 1 Comparison of water content (A) and water activity (B) of monofloral honeys

Crystallization of honey is a natural phenomenon during storage and shelf life, that is mainly caused by the precipitation of glucose, which is converted to glucose monohydrate. Honey with large crystal grains and coarse texture that causes greater friction on the palate is not easily accepted by consumers. Honey crystallization is often confused with honey adulterated with sugar (**Ji *et al.*, 2023**).
A special production method of induced crystallization was performed to improve sensory and physical properties of all Slovak rapeseed honeys to obtain a smooth spreadable consistency and a typical lighter, butter-like color. This type of honey known as creamed honey, whipped honey, spun honey, churned honey, or honey fondant is very preferable by consumers in many countries (**Karasu *et al.*, 2015**).
A critical aspect that has been observed in relation to the processing of honey by induced crystallization is the higher risk of undesirable fermentation that some consumers have noticed during storage. For this reason, beekeepers recommend adapting the storage conditions of creamed honeys and keep products at a controlled temperature below 15 °C. The presented study was focused on evaluating the existing water content limit and possibly proposing some better parameters with a higher informative value for the evaluation of the storage of this type of honey.
Despite low water content (Fig. 1A), creamed rapeseed honeys were characterized by high water activity (Fig. 1B), with a moderate correlation between these two parameters (r = 0.636). The average a_w value of creamed rapeseed honeys was significantly higher compared to other honey types (Fig. 1B). Water activity of crystallized honeys is often higher than that of liquid honeys with the same water content (**Gletier *et al.*, 2006**). Crystallization of honey decreases the glucose concentration in the liquid phase and thus increases the water activity, which sometimes can allow naturally occurring yeasts cells to multiply, causing fermentation of the honey (**Zamora & Chirife, 2006**). Stirring and the presence of impurities such as air bubbles or pollen particles in honey lead to inhomogeneity within the phase, reducing the surface energy barrier, with crystals nucleus forming, followed by the growth of more crystals attached, thus accelerating the crystallization of honey (**Ji *et al.*, 2023**). In our study, some samples of creamed rapeseed honey exceeded a_w value above 0.600, which is considered as a maximum value typical for honey (**Zamora & Chirife, 2006)** which may not be sufficient to inhibit the growth of osmophilic yeasts.

As shown in Fig. 1A, although the water content in all rapeseed honeys was below 18% and within even stricter limits, this type of honey was susceptible to undesirable fermentation during storage at room temperature, which was manifested by the lid cracking when opening, or by visible foaming and acidic odor (interestingly, without exceeding the limit for free acidity).

In comparison to rapeseed honeys, one sample of linden honey had also a higher a_w value (0.628), however this sample was characterized also by exceeded water content of 21.4%. In general, a_w values between 0.530 and 0.630 are safe for food in terms of microbial spoilage. The problem in honey is fructose. The hydrogen bonding between water molecules and fructose results in low-energy H-bonds, which means that water retained around fructose molecules to hydrate them is mobile enough to be available for microorganism growth. The crystallization of glucose releases water, but the most important phenomenon is the change in glucose/fructose ratio in favor of fructose (**Gletier *et al*., 2006**).

It is recognized in the honey industry that the water content of honey is a key factor involved in fermentation spoilage. The results presented indicated the relevance of adding the a_w value in the evaluation of honey quality, especially for creamed and crystallized honeys.

Free acidity and pH

Acidity and water content are indicators of microbial stability of honey. In general, free acidity is limited to 50 milliequivalents/kg (**Council Directive 2001/110/EC**) determined by titration. The negative logarithm of hydrogen ion concentration is the pH, while the free acidity is the sum of all free acids present in honey. Among the most common organic acids are citric and gluconic acids, as well as succinic, malic, butyric, lactic, formic, acetic, and pyroglutamic acids, which are responsible for its taste (**Puscion-Jakubik *et al*., 2020**). Although nectar is a source of organic acids (citric, malic, oxalic), most of the acid is formed through the enzymatic activity of bees, mainly gluconic acid, which is formed from glucose by the enzyme glucose oxidase which is active in unripe or diluted honey. High acidity is usually associated with dark-colored honey.

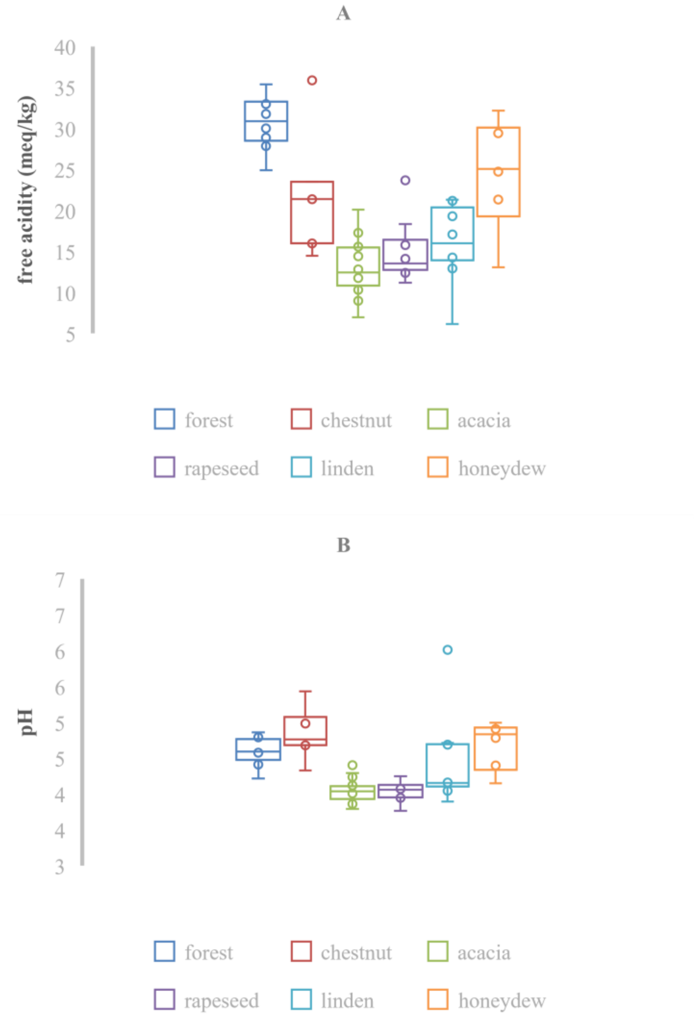

Figure 2 Comparison of free acidity (A) and pH (B) of monofloral honeys

The honey type has significant effect on acidity (**Akgün *et al*., 2021**). In our study, significantly higher free acidity was characteristic for honeydew, forest, linden, and chestnut honeys, compared to nectar acacia and rapeseed honeys (Fig. 2A). Free acidity of all honey samples varied within the legislative limit, including samples where some fermentation processes were already perceptible by the sensory senses. The results summarized in Fig. 2 pointed out differences between honey type for both parameters, free acidity and pH value, with a weak correlation between them (r = 0.263).

The highest free acidity was determined in chestnut and honeydew honeys followed by forest and linden honeys with honeydew content that is documented in Fig. 3A. Rapeseed and acacia honeys had the lowest acidity and the lowest pH value. However, in total, a correlation between pH value and free acidity was weak (r = 0.263) due to the influence of other honey constituents such as mineral compounds on pH value of honey. All honey samples met qualitative requirement for maximum free acidity and good microbial stability.

Electrical conductivity and HMF content

Mineral compounds, amino acids, and organic acids, present in honey, form ionic particles in aqueous honey solutions, which consequently affects the conduction of electrical current and the measurable parameter termed electrical conductivity (**Puscion-Jakubik *et al*., 2020**). The honey type was found to be a significant factor affecting the conductivity value (**Akgün *et al*., 2021).** Electrical conductivity not less than 800 µS/cm represents a legislative discriminating parameter for the declaration of honeydew and chestnut origin (**Council Directive 2001/110/EC**). In our study, the results of conductivity measurements are summarized in Fig. 3A. All Austrian chestnut honeys met the legislative requirements with values in the range from 903 µS/cm up to 1053 µS/cm. Based on the values of electrical conductivity also almost all Austrian forest honeys were categorized to honeydew honeys.

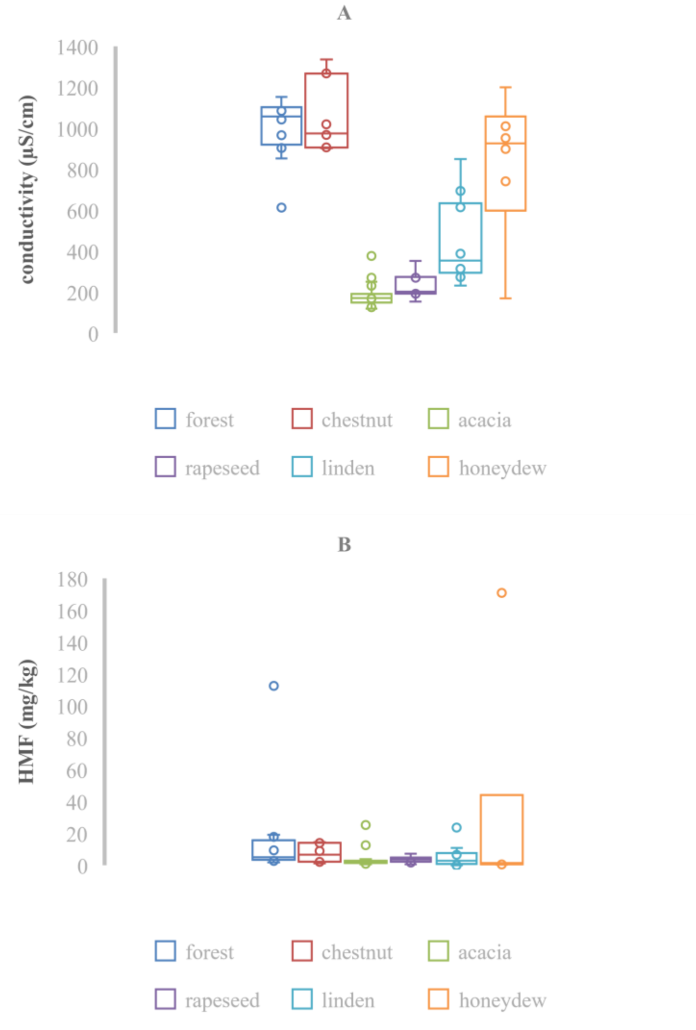

Figure 3 Comparison of electrical conductivity (A) and HMF (B) content of monofloral honeys

Only one sample of forest honey had a conductivity below 800 µS/cm (612 µS/cm) typical for honey from nectar. The EU legislative regulation does not have any specific requirements for forest honey in terms of honeydew and nectar contents as well as for a conductivity value. The designation of the type of honey as forest refers to the origin, where the source for honey collection is both nectar and honeydew in specific proportions depending on location. On the other hand, in most samples of Slovak honeydew honeys, the values of electrical conductivity were out of the limit. It should be noted that the specification of honey type was provided directly by the beekeepers according to their considerations. One sample of honeydew honey had a slightly lower value of electrical conductivity (740 µS/cm), which indicated an insufficient honeydew proportion and a misclassification. However, the second honeydew sample, with a very representative dark brown color, was characterized by an extremely low conductivity value of 169 µS/cm, which instantly led to the suspicion of adulteration practices, which was also supported by the very high HMF content (170.7 mg/kg). Based on these data, it was possible to assume that this honeydew honey is adulterated with nectar honey by coloring with caramel or other sugar syrup and this sample was excluded from the authentic honey sample set of this study.

HMF is a chemical compound form by sugar degradation, the Maillard reaction or caramelization, and its concentration increases with heat treatment and exposure (**Kukurová *et al*., 2006; Akgün *et al*., 2021**). It was found that one sample of Slovak honeydew honey and one sample of Austrian forest honey significantly exceeded the legislative limit of 40 mg/kg HMF content reaching values of 170.70 mg/kg and 112.50 mg/kg, respectively. One sample of Slovak linden honey was at the limit (40.0 ± 0.5 mg/kg) with an increase in the HMF content in the first year of storage to 43 mg/kg. The results of HMF determination in honeys are summarized in Fig. 3B with a statistical evaluation in Tab. 1.
It can be summarized that lower HMF content was characteristic for acacia, rapeseed, and linden honeys in comparison with forest, chestnut, and honeydew honey.

Table 1 Descriptive statistics of HMF content in monofloral honeys

Honey type	Acacia	Rapeseed	Linden	Honeydew	Forest	Chestnut
Valid Cases	20	10	10	6	12	7
Mean	3.7	3.8	5.4	29.5	16.0	7.5
Median	2.0	3.7	2.9	1.5	5.2	6.8
Variance	32.3	3.5	52.6	4788.6	956.2	28.9
Standard Deviation	5.7	1.9	7.3	69.2	30.9	5.4
Standard Error	1.3	0.6	2.3	28.3	8.9	2.0
Coefficient of Variation	1.5	0.5	1.3	2.3	1.9	0.7
Minimum	0.8	0.7	0.0	0.5	1.9	1.3
Maximum	25.4	7.3	23.8	170.7	112.5	14.6
Range	24.6	6.6	23.8	170.2	110.6	13.3

Classification of honeys according to the type

The results of statistical analysis revealed insignificant differences in HMF between individual honey types. However, HMF contributed significantly to the discrimination of honey types with 84.54 % of correctly classified honey samples (Fig. 4). Besides HMF, parameters of electrical conductivity and a_w also played dominant role in honeys discrimination. It can be assumed that these parameters are the most relevant for the specification of botanical origin and honey type.

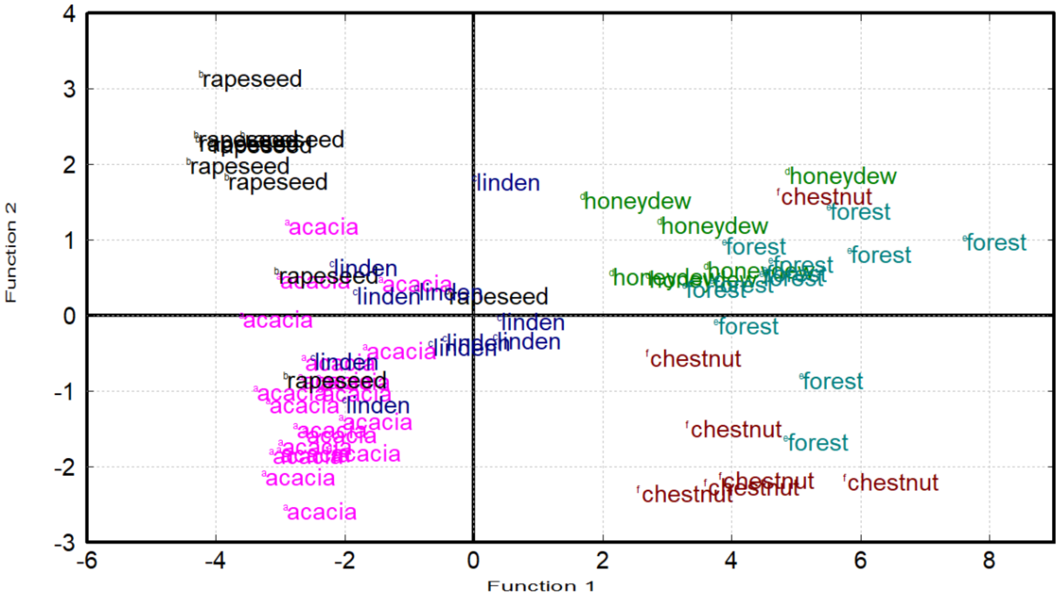

Figure 4 Classification of honey samples according to honey type by canonical discriminant analysis (CDA).

Although the discrimination of honey samples according to the geographical origin reached more than 93% correctness, this discrimination is not relevant due to the diversity of local honey types collected in both countries. In 2021, the Slovak samples consisted of acacia, rapeseed, and linden honeys with a limited number of honeydew honeys, while the Austrian samples from Graz region consisted of chestnut and forest honey types. Nevertheless, the parameters of water activity and electrical conductivity were the most important for the classification of honeys according to the country.

CONCLUSION

The quality of 65 monofloral honeys from Slovak and Austrian beekeepers were evaluated based on selected physico-chemical analysis, including water content, water activity, electrical conductivity, pH, free acidity and HMF. Water content was exceeded only in some honeys. However, it was observed that water activity could be a better parameter for evaluation of honey shelf-life stability, especially for creamed rapeseed honeys. Another problematic qualitative parameter showed to be HMF, which was exceeded significantly in one Slovak honeydew honey and one Austrian forest honey, indicating excessive heat treatment during processing. The Slovak honeydew honey sample had also very low electrical conductivity, pointing on low quality and/or on probable fraud practices. Statistical analysis identified parameters of electrical conductivity, a_w and HMF as the most important for honeys discrimination.

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