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Complex analysis of Hungarian nectar sources, multi- and unifloral honeys from different botanical and geographical origin

Theses of Ph.D. Dissertation

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1. Introduction

Nectar is a sweet, viscous secretion of the nectaries (nectar glands) of plants (Amtmann, 2009). Nectar volume and composition is thought to be influenced by several ecological parameters, such as microclimatic conditions (air temperature, relative air humidity, evapotranspiration, amount of solar radiation, wind speed) and soil features like moisture level, temperature and aeration e.g. (Fahn, 1949; Dafni et al., 1988; Zimmerman, 1988; Petanidou et al., 2000; Mačuković-Jocić et al., 2004; Silva et al., 2004; Mačuković-Jocić et al., 2005; Mačuković-Jocić, 2006; Pacini & Nepi, 2007; Mačuković-Jocić et al., 2008; Lu et al., 2015).

Wild garlic or ramson (*Allium ursinum* L.) is a bulbous perennial herbaceous monocot, widely distributed in mesic, deciduous woodlands of Europe (Tutin, 1942), as well as in certain regions of Asia (Tutin, 1942; Stearn, 1947) and Africa (Schmid, 1975). The species has been used traditionally both for food and medical purposes, such as lowering blood pressure and cholesterol level (Reuter, 1995; Sobolewska et al., 2015). Besides, unifloral honey can be obtained from the nectar produced by the flowers. Thus, it may be interesting, which of the above-mentioned climatic and soil properties and to what extent affect the nectar production of flowers. In Hungary, previous research has already clarified how habitat and microclimatic conditions of wild garlic influence the quantity and quality of nectar (Farkas et al., 2012). However, the effect of different soil parameters on *A. ursinum* nectar production was not revealed.

Honey is a complex food, which has played an important role in human nutrition and medicine since ancient times. It mainly contains various carbohydrates and to a lesser extent, other components such as polyphenols, minerals, vitamins, organic acids and enzymes (Bertoncelj et al., 2007). Due to its constituents, honey has antifungal, antiviral, antibacterial and antioxidant effects (Alvarez-Suarez et al., 2010a,b,c).

The antioxidant capacity of honeys is a relatively widely analyzed topic (Aljadi & Kamaruddin, 2004; Beretta et al., 2005; Bertoncelj et al., 2007; Chua et al., 2013; Gorjanović et al., 2013; Isla et al., 2013; Silva et al., 2013). However, while the mineral content of honeys is well researched internationally (Rashed és Soltan, 2004; Terrab et al., 2005; Conti et al., 2007; Pisani et al., 2008; Almeida-Silva et al., 2011; Vanhanen et al., 2011; Rajs et al., 2017; Sager, 2017), it is a barely studied parameter in the case of Hungarian honeys (Amtmann, 2009; Czipa és mtsai., 2015; Sajtos és mtsai., 2019).

2. Objectives

2.1. Study of nectar traits of wild garlic (*Allium ursinum* L.)

Among the various ecological factors influencing nectar yield, soil properties may be as important as microclimatic characteristics. In order to exclude the effect of microclimatic conditions during the blooming period and to focus on the influence of soil properties, *Allium* plants were kept in different types of soil, but under the same microclimatic conditions. We addressed the following questions: (i) which nectar traits of *A. ursinum* are affected by sampling site/soil properties; (ii) whether the effect of soil features is the same in each year; (iii) which soil properties have the greatest influence on the number of nectar producing flowers, and the volume and sugar content of nectar in individual flowers. Our hypotheses were the following: (i) between-site differences in soil characteristics affect the number of wild garlic flowers producing nectar, the amount of nectar produced and the sugar content; (ii) the effect of soil factors can be modified by annually changing climatic factors.

2.2. Identification of Hungarian honeys on the basis of pollen spectrum, examination of their bioactivity, analysis of their minerals and toxic elements

Main quality parameters of honey depend primarily on the botanical origin which can be determined by the composition and amount of pollen in the honeys. The first objective of our work was to identify the purchased honey samples by accurately mapping their pollen spectrum. By detecting the source of nectar, it can be clearly demonstrated whether the honey can be considered as a unifloral or multifloral honey. Based on this, we proceeded and selected the group that truly contained unifloral honeys; samples of honey purchased as unifloral honey but actually proving to be multifloral honeys; and multifloral honeys, whose various pollen content also provided important information.

The properties of honey, to lesser extent, are influenced by other environmental factors, e.g. geographical origin and the year of harvest. We focused on this in our study on honey samples of rape and wild garlic (identified as early spring flower honey based on pollen composition) from two years of harvest and several locations. Our objective in this case was to study the possible influence of the two external factors mentioned above on the antioxidant parameters, macro- and microelements and possible toxic substances of the examined honeys.

Furthermore, by examining the color, bioactivity, multielement content of Hungarian unifloral and multifloral honeys, we aimed to compile a set of properties based on which the given honey types can be well identified. The result of our work is to fill a gap because there is

no analysis in the literature on Hungarian honeys with such a viewpoint and depth. Furthermore, we also have little data on the relationship between botanical origin, antioxidant capacity and element content. Accordingly, the assortment of selected honeys ranged from honeys harvested from abundant nectar-producing plants to relatively rare, special honeys produced by only a few apiaries both in Hungary and worldwide. An additional benefit of our complex analysis, which we have formulated as the ultimate goal, is to compare the character sets examined with respect to their efficiency to accurately identify and differentiate honey types.

3. Materials and methods

3.1. Study of nectar traits of wild garlic (*Allium ursinum* L.)

Wild garlic specimens were collected in two different years (2013 and 2015) from different natural sites (fourteen and eight sampling sites, respectively). Sampling sites included mesic deciduous forests (oak-hornbeam, beech and ravine forests) and alluvial forests (hardwood gallery forest with oak-ash-elm), according to Kevey (2008) and Borhidi et al. (2012). Each plant, together with their original soil, was potted separately. Afterward plants were kept under the same climatic conditions in a growth room.

Prior to nectar sampling, *A. ursinum* flower buds just prior to anthesis were covered with a mosquito net to exclude flower-visiting insects for 24 h before the measurement. Nectar was collected with the microcapillary method from the base of the ovary. The solute concentration (as %w/w) was measured using a handheld sugar refractometer (ATAGO N-50E).

In order to analyze the effect of soil parameters on nectar production, complete soil analysis protocol was performed on three replicate samples per habitat. Soil analysis was carried out according to the Hungarian Standards at the Accredited Soil Laboratory (104/2015/LAB/NÉBIH) of Kaposvár University, Hungary. The investigated soil parameters included upper limit of plasticity according to Arany (PA), pH(KCl), pH(H₂O), calcium carbonate (CaCO₃), organic matter (humus), water soluble salts (salinity), nitrogen (N) content [nitrite (NO₂) + nitrate (NO₃)], iron, potassium oxide (K₂O), magnesium (Mg), manganese (Mn), phosphorus (P), zinc (Zn), sulphate (SO₄) and copper (Cu).

Each nectar producing parameter of *A. ursinum* was analyzed with linear mixed models using lm4-package (Bates et al., 2014). Models hypothesis testing was performed with Chi-square tests. For pair-wise comparisons, Tukey post-hoc tests were conducted in both cases with multcomp package (Fijen et al., 2020) to compare the differences among all experimental set-ups.

3.2. Identification of Hungarian honeys on the basis of pollen spectrum, examination of their bioactivity, analysis of their minerals and toxic elements

Honey samples were purchased from the producers. They were stored at room temperature (20-21°C) in the dark for a maximum of 3 weeks.

Qualitative microscopic analysis of the principal pollen types was carried out using the method of Von Der Ohe et al. (2004), with slight modifications. The percentage frequency of the characteristic pollen taxon was counted in each sample. Maurizio's (1975) specification was considered as an initial point, according to which a honey can be considered as a unifloral honey if it contained at least 45% of the taxon during the pollen analysis, while the representation classes introduced by Von Der Ohe et al. (2004) were taken into account, as well. Pollen spectrum analysis of honeys was also performed, in which pollen types were classified into one of four frequency groups: predominant/very common (dominant) pollen types (>45% of all pollen grains counted), secondary/common pollen types (16%-45%), rare pollen types (3%-15%), and individual pollen types (<3%).

The measurement of color intensity of honey samples was carried out using the method of Beretta et al. (2005). The net absorbance was defined as the difference between absorbance at 450 and 720 nm, measured using a Shimadzu UV-1800 spectrophotometer, and the results were expressed as milli-absorbance unit (mAU).

Among the antioxidant capacity measuring methods, three methods were based on single electron transfer (SET: TRC, TEAC, DPPH) and one was based on hydrogen atom transfer (HAT: ORAC). Total reducing capacity (TRC) was determined by Folin-Ciocalteu method according to Singleton et al. (1999), with minor modifications, and results were expressed as mg gallic acid equivalent (GAE) kg⁻¹ honey. Measurement based on DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging was performed according to the method developed by Beretta et al. (2005) and Bertonecelj et al. (2007), adapted to a plate reader. Trolox standards were used for calibration. The radical scavenging activity was expressed as IC₅₀, which means the concentration of the honey sample (mg ml⁻¹) needed to scavenge 50% of DPPH. The Trolox equivalent antioxidant capacity (TEAC) method is based on the spectrophotometric measurement of quantity of ABTS cation radical (ABTS^{•+}). For calibration Trolox was used. The results were expressed as μmol TE 100 g⁻¹ honey (Re et al., 1999). The oxygen radical absorbance capacity (ORAC) assay was based on the procedure previously described by Patay et al. (2016) and Kőszegi et al. (2017). The antioxidant capacity values were expressed as Trolox equivalent (TE) g⁻¹ honey.

The determination of macro- and microelement content was done using an inductively coupled plasma atomic emission spectroscopy (ICP-AES) instrument in the Research Institute of Viticulture and Enology of the University of Pécs. The instrument was calibrated using inorganic reference standards for the 20 different elements.

Statistical analyses were carried out using Excel® (Microsoft Corp., Redmond, WA, USA) and the paleontological statistics software package (PAST) version 3.11 (Hammer et al., 2001), at 5% significance level ($p < 0.05$), after normality checking with the Shapiro-Wilk test. Data were expressed as means \pm standard deviations (SD). For the correlation matrix, the 5% ($p < 0.05$), 1% ($p < 0.01$) and 0.1% ($p < 0.001$) significance levels were used. Pairwise comparisons were performed with Student's t-test. Interactions between the measured parameters were investigated with Pearson's Rank Correlation. To describe relatedness among honey types, we performed a centered and standardized principal component analysis (PCA) with all measured parameters using the ggfortify 0.4.8. package (Horikoshi et al., 2016) in R, version 3.5.3. Distances among object points (honey types) were calculated with Euclidean distances.

4. Results and discussion

4.1. Study of nectar traits of wild garlic (*Allium ursinum* L.)

The ratio of *A. ursinum* flowers producing nectar ranged from 0.0 to 82.8%, and from 3.9 to 55.7% in the first and second year of the study, respectively. We found that both sampling years and sites, and even their interaction influenced this parameter significantly. In addition, the ratio of nectar producing flowers was influenced stronger by the site ($df=7$; $\chi^2=69.043$; $p < 0.001^{***}$) than by the year ($df=1$; $\chi^2=15.477$; $p < 0.001^{***}$). Differences between sites could be attributed mainly to the fact that at some habitats a large proportion of flowers did not produce any nectar at all. When analyzing the relationship of nectar volume in individual flowers to the years of measurement, a highly significant relationship was found ($df=1$; $\chi^2=83.641$; $p < 0.001^{***}$), while a minor yet significant difference was evident with respect to the sites ($df=7$; $\chi^2=14.233$; $p < 0.05^*$) and the interaction ($df=7$; $\chi^2=18.325$; $p < 0.05^*$). There was a highly significant difference between the two years regarding the sugar content of nectar of individual flowers ($df=1$; $\chi^2=57.867$; $p < 0.001^{***}$), and a lower but statistically relevant difference was also found among the sites ($df=7$; $\chi^2=23.776$; $p < 0.01^{**}$). In addition, the interaction 'Year x Site' was highly significant ($df=7$; $\chi^2=41.428$; $p < 0.001^{***}$). Mean sugar concentration of nectar produced by *A. ursinum* plants was $34.09 \pm 3.92\%$, ranging from 10.0 to

45.0% and from 25.0 to 47.0 in individual flowers, in first and second year, respectively. Mean sugar concentrations of nectar in individuals were significantly different in the two years for most of the sites ($df=1$; $\chi^2=9.946$; $p<0.01^{**}$), except for Site 10 and Site 12. No significant difference was evident with respect to the sites ($df=7$; $\chi^2=6.106$; $p=0.527$), but the 'Year x Site' interaction was highly significant ($df=7$; $\chi^2=24.176$; $p<0.001^{***}$). Our study revealed that total nectar volume of individual *A. ursinum* plants did not differ significantly between study sites or years; while nectar sugar concentrations of individual flowers differed significantly between sites and years.

Among the soil parameters, the relationship with the number of nectar producing *A. ursinum* flowers was minor yet significant regarding humus content ($df=1$; $\chi^2=4.283$; $p<0.05^*$, $r^2=0.303$), while highly significant with respect to iron ($df=1$; $\chi^2=12.376$; $p<0.001^{***}$; $r^2=0.371$) and sulphate ($df=1$; $\chi^2=11.662$; $p<0.001^{***}$; $r^2=0.402$). In our study, we found that the phosphate content of soil had nearly significant positive effect on the number of flowers producing nectar ($df=1$; $\chi^2=3.741$; $p=0.089$) and the concentration of nectar sugar ($df=1$; $\chi^2=2.900$; $p=0.099$). A statistically relevant negative correlation was also found between ratio of nectar producing flowers and sulphate ($df=1$; $\chi^2=6.428$; $p<0.05^*$; $r^2=0.352$), as well as between ratio of nectar producing flowers and humus ($df=1$; $\chi^2=7.063$; $p<0.01^{**}$). If soil parameters were related to corresponding total nectar volumes (of individuals), significant relationship was found regarding humus ($df=1$; $\chi^2=6.332$; $p<0.01^{**}$; $r^2=0.362$) and iron ($df=1$; $\chi^2=6.752$; $p<0.01^{**}$; $r^2=0.298$), and lower but relevant correlation was detected with magnesium ($df=1$; $\chi^2=6.162$; $p<0.05^*$; $r^2=0.247$) and sulphate ($df=1$; $\chi^2=5.152$; $p<0.05^*$; $r^2=0.310$). All observed correlations were negative, except for magnesium, which showed positive correlation. Mean nectar volume showed minor yet significant correlation merely with pH(KCl) ($df=1$; $\chi^2=5.271$; $p<0.05^*$; $r^2=0.185$), whereas mean nectar sugar concentration was related to neither of the soil parameters. In case of the other soil factors analyzed (PA; soluble salts; Ca, Cu, K, N, Na, Zn content), no statistically significant results were obtained.

4.2. Identification of Hungarian honeys on the basis of pollen spectrum, examination of their bioactivity, analysis of their minerals and toxic elements

During the melissopalynological examination of the honeys, we determined that rape honeys can be classified as unifloral honeys with a dominant pollen ratio of above 80%, while samples purchased as ramson honey can be considered as early spring multifloral honeys.

In addition, during our investigations we could identify 10 types of unifloral honey (acacia, amorphia, phacelia, linden, sunflower, chestnut, fennel, meadow sage, milkweed, goldenrod) and two other multiflower honeys (MF-*Tilia* and MF-Lamiaceae).

Examination of color of rape and early spring multifloral honeys revealed that the latter has a significantly higher color intensity. All of the antioxidant capacity assays also showed higher activity in multifloral honeys than in rape honeys. The positive correlation between these two parameters is supported by several studies (Bertoncelj et al., 2007; Alvarez-Suarez et al., 2010; Gorjanović et al., 2013). The results of our first study showed that year of harvest and geographical origin did not significantly affect the total antioxidant capacity (TAC) of honeys, whereas floral origin and color has a decisive character.

In case of both types of honey, the most abundant macronutrient was potassium, followed by calcium, phosphorus, sulfur, magnesium and sodium. Regarding macronutrients, we found no relevant difference between the years of harvest, while the geographical origin had an effect on the macronutrient profile of rape honey samples. Among the examined micronutrients, boron, iron and manganese were detected in the rape honey samples, while copper and zinc were detected in the multifloral honey samples in addition to the above-mentioned elements. Out of these, only manganese content showed significant difference between honey types, in favor of multifloral honeys. Depending on the year of harvest or geographical origin, the amount of the above-mentioned microelements did not differ.

In our second study, we performed complex analyses of 8 different types of unifloral honeys. Based on the color intensity results, we were able to divide these into two groups, light-colored and dark-colored honeys. The color intensity of light-colored honeys (acacia, amorphia, phacelia and linden) ranged from 136 to 285 mAU, while that of dark-colored honeys (sunflower, chestnut, fennel, meadow sage) ranged from 719 to 1459 mAU.

To determine the antioxidant behavior of Hungarian unifloral honeys, we used three different TAC assays (TRC, DPPH, ORAC). We have established that the light-colored honeys showed significantly lower antioxidant activity and their values ranged on a wider scale than in case of dark-colored honeys. Using the colors, we were able to distinguish all honeys from each other, while the TRC, DPPH and ORAC values of amorphia and phacelia honeys did not differ

significantly. However, linden honey had an outstanding value, its ORAC activity being significantly higher than that of light-colored honeys and even similar to that of dark-colored chestnut honey. In our research, we obtained the highest TAC values for meadow sage honey. The observation that light-colored honeys have lower antioxidant activity than dark honeys has also been confirmed in our research, with the exception of linden honey.

With regard to macronutrients (K, Ca, P, S, Mg, Na), dark-colored meadow sage honey was particularly rich in minerals (on average 3497 mg kg⁻¹), while light-colored phacelia honey was found to be poor (on average 222 mg kg⁻¹). In the group of light-colored honeys, with the exception of linden, the total macroelement content was below 300 mg kg⁻¹, while in the other types of honey this value was above 1000 mg kg⁻¹. As we expected, potassium was the most abundant mineral in all examined unifloral honeys. Linden honey had a significantly higher potassium content, and therefore a higher total macronutrient content (1429 mg kg⁻¹) than other light honeys and even dark-colored sunflower honey (1034 mg kg⁻¹). Calcium-phosphorus and sulfur-magnesium contents were compared in the same honey samples. A significant difference ($p < 0.05$) was found between the honey types in the following order: lime, sunflower and chestnut honeys had higher calcium content than phosphorus, while light-colored acacia, amorpha, phacelia, fennel and meadow sage honeys showed an inverse relation. Sulfur and magnesium also showed different quantitative relationships in the honey types. The sulfur content was higher than magnesium for light-colored acacia, amorpha and phacelia honey, whereas this ratio was reversed for dark-colored fennel and meadow sage honey.

Among the microelements (boron, copper, iron, manganese, zinc), all were present in dark honeys, while copper, iron and manganese were below detection limits in some light-colored honeys. Consequently, the group of dark-colored honeys was significantly richer in micronutrients than light-colored honeys. Chestnut honey was the richest, while amorpha honey was the poorest in terms of micronutrient content, with average amounts of 17.3 mg kg⁻¹ and 3.0 mg kg⁻¹, respectively. Regarding microelements, phacelia was separable from other light-colored honeys due to its significantly higher boron and zinc content, and chestnut honey was unique among dark-colored honeys with its extremely high manganese content. Among the investigated micronutrients, boron appeared in the highest amount in all honey types, except for chestnut honey.

Among trace elements, nine were tested: aluminum, arsenic, cadmium, cobalt, chromium, molybdenum, nickel, lead and vanadium. One out of the three samples of linden, sunflower and chestnut honeys contained Al (1.07, 1.04 and 1.76 mg kg⁻¹, respectively). Chestnut, fennel and meadow sage honeys displayed quantifiable amounts of cadmium (0.12 ± 0.028 , 0.22 ± 0.01

and $0.40 \pm 0.005 \text{ mg kg}^{-1}$, respectively), while nickel could be detected in only one of the chestnut honey samples (0.15 mg kg^{-1}). The other elements were below the detection limits ($<0.1 \text{ mg kg}^{-1}$), indicating that the nectar sources of our honey samples were not contaminated or only to a very small extent.

The data matrix of color, antioxidant values and multielement contents of Hungarian unifloral honeys was analyzed by Pearson's correlation and PCA methods to obtain more information. Similarly to previous research (Beretta et al., 2005; Sowa et al., 2014), a high linear correlation was obtained between color and total antioxidant capacity (TAC) values and among TAC methods. All of the studied macro- and microelements were in good correlation with color, except manganese ($r=0.257$; $p>0.05$). Our study confirmed the possible existence of a link between the following antioxidant assays and macronutrients: TRC with magnesium, phosphorus, and sulfur content; DPPH with calcium content; and ORAC with potassium content of honeys. The microelements, boron, copper and iron significantly correlated with the antioxidant capacities.

Our third research included two types of unifloral honeys, milkweed honey (declared as 'Hungarikum') and goldenrod honey. Both are honeys from invasive plant species introduced to Hungary. Lighter-colored milkweed honey was considered as unifloral honey based on its sensory characteristics, as in case of this special honey type pollen analysis cannot prove its botanical origin, because honeybees cannot collect pollinia from this plant. The pollen spectrum of the darker-colored goldenrod honey, as expected, showed *Solidago* pollen as the dominant pollen type. Together with its sensory characteristics, its unifloral origin was clearly verifiable. For these two types of honey, we looked for multifloral honeys, which were studied earlier, that could be considered similar in terms of color intensity. The most abundant type of pollen in the lighter multifloral honey was linden (*Tilia*) pollen, while in the darker honey it was Lamiaceae pollen.

In case of milkweed, goldenrod and two multifloral (MF-*Tilia* and MF-Lamiaceae) honeys, four different TAC methods were applied for determining their bioactivity. The results of single electron transfer methods (TRC, TEAC and DPPH) showed a parallel tendency with color of honeys. TRC showed a difference among the milkweed, goldenrod and MF-Lamiaceae honeys but MF-*Tilia* did not differ significantly from the unifloral honeys. The light and dark-colored honeys can be clearly separated based on their TEAC result. The DPPH assay distinguished the honeys to the lowest degree. The method based on hydrogen atom transfer, the ORAC, separated uni- and multifloral honeys. The highest antioxidant capacity based on electron transfer was measured for dark-colored MF-Lamiaceae, while the lowest for milkweed

honey. In terms of ORAC results, MF-*Tilia* showed the highest values, whereas the goldenrod sample showed the lowest.

Based on the total macroelement content, unifloral honeys significantly differed from multifloral honeys and the two multifloral honeys from each other. As expected from our previous studies, the most common macronutrient also in these honey samples was potassium, but other macronutrients followed each other in different decreasing quantitative order. Calcium was the second most common element, with the exception of milkweed honey, where it was phosphorus.

The total microelement content of dark-colored honeys was significantly higher than that of light-colored honeys. All honey samples contained boron, while iron, manganese and zinc were under the detection limit in some of the light honey samples. The multifloral honeys were distinguishable from the unifloral ones due to their significantly higher manganese content, while iron and zinc content significantly separated the light and dark colored honeys from each other.

In the study of milkweed, goldenrod and the two multifloral honeys, we also looked for the parameters that can be used to clearly distinguish the four types of honey. By using principal component analysis (PCA), we are able to differentiate these types of honeys from each other based on both antioxidant activity and color, as well as macro- and microelement content.

5. Conclusion

5.1. Study of nectar traits of wild garlic (*Allium ursinum* L.)

Examining the relationship between the nectar parameters of *Allium ursinum* and the year-site variables, it was revealed that the number of nectar producing flowers, as well as the nectar volume and sugar content in individual flowers were influenced by both year and site.

The humus-, iron and sulfate content of soil showed negative correlation with the number of nectar producing flowers. The total nectar volume correlated negatively with humus and iron content, but it was influenced positively by magnesium content of soil.

Changing microclimatic conditions can alter utilization of nutrients and in turn the nectar producing capacity of plants even in the same habitat. Our results suggest that the flowers of *A. ursinum* can provide maximal nectar yields when the soil conditions of the habitat are optimal for nectar production, which does not necessarily mean that conditions are optimal for the plant on the whole.

Wild garlic habitats with greater diversity, such as ravine forests, alder gallery forests and transitory oak-hornbeam forests, can be expected to provide nectar for honeybees more evenly and steadily compared to monodominant wild garlic associations in oak-hornbeam and beech forests. In addition, the yearly climatic differences may have a substantial impact on the nectar producing capacity of plants.

The findings of our study can be useful for beekeepers and producers of ramson honey. Besides species related nectar traits, edaphic and microclimatic characters should be taken into consideration when selecting plant associations with *A. ursinum* where beehives should be placed.

5.2. Identification of Hungarian honeys on the basis of pollen spectrum, examination of their bioactivity, analysis of their minerals and toxic elements

During the complex evaluation of honeys, we revealed that melissopalynological analysis is a good tool for determining the types of honey, taking into consideration also the sensory and physicochemical characteristics. However, pollen analysis is unavoidable in multifloral honeys, while unifloral honeys are generally well identifiable by physicochemical characteristics.

We have confirmed earlier reports in literature in so far as the antioxidant effect of honey is mainly influenced by its botanical origin.

We identified that there is a correlation not only between the botanical origin, color and antioxidant properties of honeys, but also their mineral content.

We have proved that the methods based on hydrogen atom transfer (HAT) can provide further useful information when applied in addition to single electron transfer (SET) assays. Furthermore, the present research has shown a strong correlation between the ORAC antioxidant method and the most important macronutrient, potassium.

Although unifloral honeys have a higher market value than multifloral honeys, the latter have great potential due to their diverse botanical origin, which can result unique quality characteristics.

We can conclude that the revealed markers gave the possibility to assign a characteristic fingerprint to a given honey, which refers to its botanical origin, quality and identity.

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