Chapter

Summer Gifts from the Hive: Botanical Origin, Antioxidant Capacity, and Mineral Content of Hungarian Honeys

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Abstract

Although Hungary is one of the biggest honey producers in the EU, there is little information on diagnostic traits, nutritional value, and potential health benefits of the honeys produced in this Central European country. The aim of this study was to perform a complex analysis of eight Hungarian summer honeys, focusing on melissopalynology, antioxidant measurements with three different assays, and the macro- and microelement profile. Light-colored honey types included a multifloral honey and unifloral phacelia, milkweed, and linden honeys; dark-colored honeys were represented by unifloral goldenrod, sunflower, and chestnut honeys and a dark multifloral honey. Pollen analysis and sensory traits confirmed the botanical origin of each unifloral honey, while the dominance of *Tilia*- and Lamiaceae-pollen was observed in the light- and dark-colored multifloral honeys, respectively. The total reducing capacity (TRC) assay and the microelement content clearly separated the light- and dark-colored honeys. The oxygen radical absorbance capacity (ORAC) assay highlighted the strong antioxidant activity of linden honey, comparable to that of dark-colored honeys. Multivariate statistical analysis revealed correlations between antioxidant assays, color, and mineral content of honeys. The results contribute to establishing unique character sets for each honey type, aiding proper identification and quality control of these natural products.

Keywords: *Phacelia*, *Asclepias*, *Tilia*, *Solidago*, *Helianthus*, *Castanea*, melissopalynology, antioxidant activity, macroelement, microelement, correlation analysis, principal component analysis

1. Introduction

Honey, as a complex food, offers several nutritional and health benefits, due to its favorable composition and high levels of antibacterial and antioxidant activity. In addition to sugars which constitute the largest part of honey, polyphenolic substances, vitamins, and minerals are also essential for the beneficial effects.

The quality of honey is largely determined by the floral source, which can be a single or multiple plant species [1, 2]. The correct identification of honey types is based on their physicochemical properties, pollen composition, and marker compounds [3]. However, it is difficult to find reliable chemical markers, since the chemical composition of honey depends on several factors, like geographical origin, season of harvest, and storage conditions. Although melissopalynology is considered as one of the most effective tools in checking the identity of honey [4], and detailed pollen analyses are available for different honey types from various countries [5–7], it is acknowledged that determining the pollen quality and quantity in honey samples requires considerable time and botanical expertise [8]. Thus, the need arises to establish a set of quality traits that are suitable to unequivocally identify and characterize each honey type.

One of the most intensively researched health benefits of honey is its antioxidant capacity, attributed to high levels of plant-derived compounds, like flavonoids and carotenoids that are efficient against reactive oxygen species [3, 9]. The antioxidant activity of honey has been evaluated recently in several countries, e.g. Turkey [10], Poland [11–13], Greece [14], Serbia [15], Romania [16], and Hungary [17, 18]. Total antioxidant capacity (TAC) of honeys can be determined with several assays. The most frequently used methods are based on single electron transfer (SET), such as total reducing capacity (TRC) also known as Folin-Ciocalteu assay, Trolox Equivalent Antioxidant Capacity (TEAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, and the Ferric Reducing Antioxidant Power (FRAP). In honey research, the hydrogen atom transfer (HAT)-based assays like the oxygen radical absorbance capacity (ORAC) assay are used less commonly [19]. Maurya et al. [20] and more recently Martinello and Mutinelli [21] provided a comprehensive overview of the antioxidant parameters in different honey types, involving several multifloral honeys from different floral and geographical origin. The close relationship between the color and antioxidant activity of honey has been confirmed by several research groups [13, 22–24]. Other factors that can have an impact on the antioxidant capacity of honey include environmental and climatic conditions, harvesting treatment, and storage [25]. Floral origin of honey was found to play a decisive role in its biological activities, regarding both antioxidant and antibacterial properties [4, 15]. In turn, Dżugan et al. [26] reported that antioxidant capacity can be a useful indicator of honey's botanical origin.

Although minerals are only minor constituents of honey, they contribute significantly to its quality [27]. In addition, minerals can be used for honey identification [28], since the composition of elements has strong botanical specificity and may reflect the mineral content of the soil, too [29–31]. The mineral composition of honeys has been studied in various countries: Altun et al. [32] analyzed Turkish honeys, Sager et al. [33] analyzed Austrian honeys, Conti et al. [34] analyzed Italian honeys, and Czipa et al. [35] and Sajtos et al. [36] analyzed Hungarian honeys. Solayman et al.'s [27] review compared the mineral content of honeys from different countries, revealing that the mineral composition of honey can vary within wide ranges, influenced by both botanical and geographical origin throughout the world. The mineral content of honey was found to correlate with its color: dark honeys contain higher amounts of certain minerals when compared to pale-colored ones.

Less attention has been paid to the possible relationships between mineral content of honeys and their antioxidant activity. Such studies included Hungarian, Indian, Italian, and Turkish honeys, establishing some correlations between the composition of macro- and microelements and the antioxidant capacity of respective honeys [37–39]. Principal component analysis (PCA) allowed differentiation of honeys based on factors like mineral, physicochemical, and enzymatic analysis, antioxidants and physicochemical

properties, metal content and contamination (antibiotic and pesticides residues), and browning index and antioxidant activity [16, 37, 40, 41], respectively.

Based on the observation that the color of honey can be an indicator of antioxidant capacity and mineral content, eight Hungarian honey types from light- to dark-colored repertoire were selected for the purposes of the present study. The honey types chosen for this study included representatives of cultivated plants with high nectar production, to relatively rare specialty honeys, which are produced by few beekeepers in Hungary and worldwide. The comprehensive analysis included melissopalynology, antioxidant measurements with three different assays, and the macro- and micro-element profile. An additional goal was to reveal correlations among the analyzed parameters. Although Hungary is one of the biggest honey producers in the EU, little is known about the diagnostic traits, nutritional value, and potential health benefits of various Hungarian uni- and multifloral honeys. The results of the current study are intended to facilitate comprehensive characterization of eight Hungarian summer honeys, aiding their proper identification and quality control.

2. Botanical sources, pollen analysis, sensory characteristics, and color of honey samples

2.1 Plant sources of honey samples

The botanical sources of the eight honey samples (**Figure 1**) included cultivated plants like phacelia and sunflower, natural flora elements like linden and sweet chestnut, as well as invasive plants like milkweed and goldenrod.

The flowers of phacelia (*Phacelia tanacetifolia* Benth., Hydrophyllaceae) provide an excellent nectar source for honeybees, and their strong odor attracts large numbers of bees throughout the summer. The flowers cluster in scorpioid cymes, the long stamens protruding from the corolla tube (**Figure 1a**). Phacelia is frequently used outside its native North American range as a cover crop and bee plant, being among the best honey plants worldwide, performing well particularly in temperate climates [42, 43].

Figure 1.
Botanical sources of the honey samples. (a) phacelia—Phacelia tanacetifolia, *(b) milkweed*—Asclepias syriaca, *(c) linden*—Tilia platyphyllos, *(d) goldenrod*—Solidago gigantea, *(e) sunflower*—Helianthus annuus, *(f) chestnut*—Castanea sativa *(photos: Á. Farkas, E. Zajácz).*

Common milkweed (*Asclepias syriaca* L., Apocynaceae) is native to North America. Having been introduced to Europe as early as the seventeenth century, today in Hungary it is considered an invasive weed species, widespread on sandy soils of the Great Hungarian Plain. The plants provide a good nectar flow, and the sweet-scented, white to purple flowers are very attractive for honeybees (**Figure 1b**), even though they get occasionally trapped in a blossom and their motion may be hindered by pollen masses (pollinia) sticking to the bee's body [44].

Representatives of the linden genus (*Tilia* spp., Malvaceae) that are common in Hungary include small-leaved linden (*T. cordata* Mill.), large-leaved linden (*T. platyphyllos* Scop.), and silver linden (*T. tomentosa* Moench). The flowers of these deciduous trees (**Figure 1c**) can provide good honey flow in favorably hot and humid weather [44].

Goldenrod species (*Solidago* spp., Asteraceae) of North American origin, today widespread in Europe, include giant goldenrod (*S. gigantea* Ait.) and Canadian goldenrod (*S. canadensis* L.). Similar to milkweed, they are treated as invasive species in Hungary, which, however, are valued as medicinal and bee plants. Together with European goldenrod (*S. virgaurea* L.), the yellow flowers of *Solidago* species (**Figure 1d**) are good nectar and pollen sources in late summer and early fall, providing ample food for overwintering bees.

Sunflower (*Helianthus annuus* L., Asteraceae) is the second most important honeybee plant in Hungary, following black locust (*Robinia pseudoacacia* L.). The plant is cultivated on large areas, being exploited primarily as an oil plant. Blooming from June through July, the disk florets of the capitulum (**Figure 1e**) provide both pollen and nectar for the bees [44].

Sweet chestnut (*Castanea sativa* Mill., Fagaceae), native to Southern Europe and Asia Minor, is a deciduous tree with edible fruits. The flowers serve as abundant pollen source, and the staminate flowers (**Figure 1f**) provide nectar, as well. Although single flowers yield low volumes of nectar, the total nectar production of a tree can be significant, due to the large number of flowers in an inflorescence [44].

2.2 Pollen profile and sensory traits of honey samples

The botanical origin of honey samples was determined with a combination of microscopic pollen analysis and spectrophotometric color determination (**Table 1** and **Figure 2**), following the methodology of Polish and Spanish research groups [22, 45]. Furthermore, to confirm the floral sources of honey samples, the sensory traits odor and consistency were evaluated. In case of multifloral honeys, a more detailed melissopalynological analysis was carried out to reveal the pollen spectrum of the samples and to determine their floral origin (**Table 2**).

Linden (*Tilia*) honey belongs to honey types with under-represented specific pollen. In the comprehensive study of Oddo et al. [46], mean linden pollen percentage was 23%, with extreme values 1–56%, while Kuś et al. [22] found only 22–26% *Tilia* pollen in linden honeys. The 46% *Tilia* pollen in our linden honey sample support the unifloral origin of this honey. Similar to our characterization, light amber color was reported for certain Polish linden honeys, although most of them were described as extra white [22]. In accordance with our linden honeys, Polish linden honey was characterized by solid, fine granulated consistency. The 47% *Helianthus* pollen in our sunflower honeys fits nicely within the range of 12–92% *Helianthus* pollen in European sunflower honeys [46], similar to Turkish sunflower honey samples, which contained *Helianthus* pollen as their dominant pollen type (45–70%), proving their

Nr.	Honey type plant name	Dominant pollen (%)	Sensory characteristics (color, odor, and consistency)	ABS_{450} (mAU)
1	Phacelia *Phacelia tanacetifolia*	*Phacelia tanacetifolia* (74.1%)	Light beige, moderately intense odor, fine granulated, semisolid	247 ± 11^{a}
2	Milkweed *Asclepias syriaca*	Brassicaceae (45.3%)	Light yellowish amber, moderately intense flower-like odor, liquid, viscous	248 ± 12^{a}
3	Linden *Tilia* spp.	*Tilia* spp. (45.9%)	Light amber, strong odor, fine granulated, semisolid	285 ± 8^{b}
4	Multifloral-*Tilia* (MF-*Tilia*)	See **Table 2**	Light amber, intense odor, semisolid, fine granulated	306 ± 8^{b}
5	Goldenrod *Solidago gigantea*	*Solidago gigantea* (45.3%)	Amber, moderately intense odor, semisolid, fine granulated	531 ± 15^{c}
6	Multifloral-Lamiaceae (MF—Lamiaceae)	See **Table 2**	Brownish amber, intense malt odor, semisolid, fine granulated	606 ± 18^{d}
7	Sunflower *Helianthus annuus*	*Helianthus annuus* (47.4%)	Bright yellow, moderately intense odor, coarsely granulated, solid	719 ± 5^{e}
8	Chestnut *Castanea sativa*	*Castanea sativa* (90.8%)	Dark amber with reddish tone, strong odor, liquid, viscous	920 ± 10^{f}

Each code number in the first column represents three biological replicates (n = 3) of honey types. Means in the same column with different superscripted letters are significantly different according to Student's t-test ($p < 0.05$).

Table 1.
Identification and sensory characteristics of analyzed honey samples.

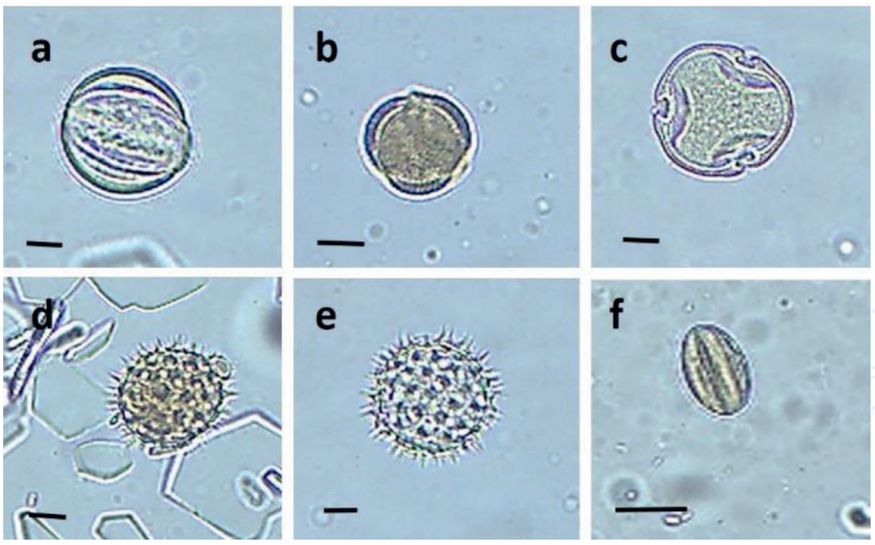

Figure 2.
Honey samples—observed pollen types (a) phacelia—Phacelia tanacetifolia*, (b) milkweed—Brassicaceae pollen, (c) linden*—Tilia *ssp., (d) goldenrod*—Solidago gigantea*, (e) sunflower*—Helianthus annuus*, (f) chestnut*—Castanea sativa*. Scale bar = 10 μm (photo: K. J. Csetneki).*

unifloral origin [47]. Turkish sunflower honeys were characterized by similar color, texture, and crystallization traits as our Hungarian samples, being bright yellow, fragrant, creamy, crystallizing quickly. From the eight honey samples in this study, phacelia and chestnut honeys belong to honeys with over-represented pollen, containing more than 60% *Phacelia* and at least 90% *Castanea* pollen, respectively [46, 48].

Pollen type relative frequency (%)	MF-*Tilia*	MF-Lamiaceae
Brassicaceae	—	15.6
Tilia	21.5	1.6
Solidago	—	6.8
Lamiaceae	—	30.8
Robinia	12.1	1.6
Rosaceae	10.1	4.0
Asteraceae	10.7	5.6
Caryophyllaceae	4.7	—
Poaceae	1.3	—
Apiaceae	3.4	—
Fagopyrum	—	3.6
Trifolium	—	1.2
Fabaceae	—	1.2
Others	29.5	23.2

Dominant pollen >45%, secondary pollen 16–45%, important minor pollen 3–15%, minor pollen <3% of the pollen grains counted.

Table 2.
Pollen spectrum of the studied multifloral honeys.

The 74% *Phacelia* and 91% *Castanea* pollen content in our phacelia and chestnut honey samples, respectively, met these requirements. In case of milkweed honey, we cannot rely on melissopalynology to determine if the nectar was collected from *Asclepias* flowers, since the honeybees are not able to collect the large-size pollinia. However, sensory traits like odor, taste, and color can aid identification. The color of milkweed honeys can range from yellowish green [44] to somewhat darker amber color [49]. The color of our milkweed honey sample was in the darker range, between the light beige phacelia and the light amber linden honeys [18]. According to our microscopic analysis, *Brassica* pollen was the most abundant in this sample; however, 45% relative frequency of *Brassica* pollen is far from the values required in unifloral rape honeys (minimum 60% [50], and in some regions above 80%) [17]).

The unifloral origin of our goldenrod honey samples was supported by the dominance of *Solidago* pollen, as well as sensory characteristics. The amber color of our samples was darker than the extra light amber color of Polish (138–205 mAU) [51, 52] and Croatian goldenrod honeys (287 mAU) [53]. This might be explained by a difference in their floral origin: the botanical source of goldenrod honey in Poland and Croatia can be the native European species, *Solidago virgaurea*, while our samples with darker color (535 mAU) originated from the North American invasive species, *S. gigantea*. Similarly, dark, rich amber color characterized goldenrod honey from the US, the native region of *S. gigantea* [54].

The light and dark-colored multifloral honeys (MF-*Tilia* and MF-Lamiaceae) in our study showed remarkable differences in their pollen spectrum, as expected. The most abundant pollen type of the lighter colored multifloral honey was *Tilia*, while the darker colored one was dominated by Lamiaceae pollen (**Table 2**). Multifloral honeys show great variability, with diverse features from all aspects, among others pollen composition, antioxidant parameters, or mineral content [20, 21, 34].

Color represents an important characteristic of honey, referring to its botanical origin and also its composition [55]. Based on the color, the studied honeys were divided into light- and dark-colored groups, exhibiting color intensity from 200 to 300 mAU (phacelia, milkweed, linden, and MF-*Tilia* honeys) and from 500 to 1000 mAU (goldenrod, MF-Lamiaceae, sunflower, and chestnut honeys), respectively. Color intensity distinguished the dark honeys from each other, while in the light-colored group, phacelia and milkweed honeys and also linden and MF-*Tilia* honeys did not differ significantly in their absorbance. The reported color intensities were similar to the Romanian honeys, which were in the range of 210 mAU (acacia honey) to 1228 mAU (forest honey) [56]. Net absorbances of Slovenian and Croatian linden honeys were four times higher than that of chestnut honey [57, 58]; however their color values were lower than those of the same honey types in our study. Beretta et al. [59] reported lower color parameter for chestnut honeys (610 mAU), Cimpoiu et al. [56] measured lower color intensity for sunflower honey (512–556 mAU) compared to our results. Based on the Pfund scale, according to which honey can be water white, extra white, white, extra light amber, light amber, amber, and dark amber [60], the color of sunflower honey samples was extra light amber [16].

3. Total antioxidant capacities of honeys

In order to provide the most reliable results, the combination of nonenzymatic antioxidant assays is required. In the current study, three different TAC methods were used to determine the antioxidant behavior of the honey samples. Light-colored honeys clearly separated from dark-colored honeys based on the results of TRC, whereas ORAC assay did not distinguish these two groups from each other (**Table 3**).

Nr.	Honey types	TRC (mg GAE kg^{-1})	DPPH (IC_{50} mg mL^{-1})	ORAC (μmol TE g^{-1})
1	Phacelia	91.67 ± 19.03^{a}	55.78 ± 1.95^{a}	13.79 ± 0.58^{a}
2	Milkweed	144.72 ± 17.17^{ab}	37.61 ± 0.41^{b}	22.67 ± 0.97^{b}
3	Linden	119.14 ± 13.80^{b}	35.86 ± 0.62^{c}	71.68 ± 5.43^{c}
4	MF-*Tilia*	195.44 ± 9.87^{c}	37.16 ± 1.57^{bc}	63.00 ± 4.43^{c}
5	Goldenrod	255.27 ± 22.44^{d}	33.65 ± 2.20^{c}	19.50 ± 1.69^{a}
6	MF-Lamiaceae	475.71 ± 40.63^{e}	28.52 ± 0.81^{d}	32.41 ± 2.41^{d}
7	Sunflower	230.25 ± 8.35^{d}	26.62 ± 0.49^{e}	34.32 ± 3.57^{e}
8	Chestnut	232.82 ± 24.97^{d}	17.37 ± 0.57^{f}	75.20 ± 4.71^{c}
	Total			
Light-colored honeys (nr. 1–4)		137.74 ± 44.15^{a}	41.60 ± 9.48^{a}	43.42 ± 29.33^{a}
Dark-colored honeys (nr. 5–8)		298.51 ± 118.67^{b}	26.54 ± 6.80^{b}	40.16 ± 24.51^{a}

TRC—total reducing capacity; DPPH—antiradical power; ORAC—oxygen radical absorbance capacity; data are means ± standard deviations of three independent determinations (n = 3). Means in the same column with different superscripted letters (a-f) are significantly different according to Student's t-test ($p < 0.05$).

Table 3.
Total antioxidant capacities of selected honey samples.

Phacelia honey displayed the lowest antioxidant activity with each assay, while the highest values were measured in MF-Lamiaceae and chestnut honeys with the SET-based methods such as TRC and DPPH, respectively. Remarkably, the light-colored linden and MF-*Tilia* honeys showed as high antioxidant capacity as the dark-colored chestnut honey, according to the HAT-based ORAC assay.

In addition, TRC distinguished the light-colored members from each other, except milkweed honey. In the dark group, only MF-Lamiaceae separated from the others, using this SET-based method. A high variation of TRC was reported for various honeys from different parts of the world. Chestnut honey was reported to have high antioxidant potential in several studies [24, 38, 39]. Our results on this honey type are in accordance with values reported for Italian [59] and Slovenian [57] chestnut honeys, but in case of linden honey the values of the above research groups were slightly lower than ours. Flanjak et al. [58] measured somewhat lower values for these two types of honeys from Croatia, while Kus et al. [22] obtained higher TRC parameters for linden honey (192.5 ± 17.8 mg GAE kg^{-1}) compared to our results. Furthermore, Gül and Pehlivan [10] measured even higher reactivities for linden and chestnut honeys, respectively (268.81 mg GAE 100 g^{-1} and 327.60 mg GAE 100 g^{-1}). TRC values of sunflower honey in this study were in line with those reported by Pauliuc et al. [16]. Sari and Ayyildiz [47] measured TRC of 50 sunflower honeys in Turkey, with results in a broad range (6.9–23.2 mg GAE 100 g^{-1}). Our samples approached the upper limit reported by them. Similar to linden and chestnut honeys, much higher values were calculated by Gül and Pehlivan [10] for Turkish sunflower honeys (77.64 ± 0.86 mg GAE 100 g^{-1}). The TRC values of our goldenrod honey, which originated from *S. gigantea*, were higher than those of Polish goldenrod honeys derived from *S. virgaurea* (147–199 mg GAE kg^{-1} [51], and 210.3 mg GAE kg^{-1} [52]), but lower than those measured in Croatian goldenrod honey, also originating from *S. virgaurea* (492 mg GAE kg^{-1}) [53]. Goldenrod honey is unique in the sense that its color does not necessarily correlate with its antioxidant capacity, which might reach the level measured in dark-colored honeydew honey [26]. The TRC of multifloral honeys can range between 32 and 147 mg GAE kg^{-1} [20]. Our multifloral honeys were characterized by higher TRC values than multifloral honey from Serbia (87 mg GAE kg^{-1}) [15]. TRC values of our MF-Lamiaceae honey were in the same range as those of Polish multifloral honeys [26, 61], but were much lower compared to the values (325–937 mg GAE kg^{-1}) measured in 18 multifloral honeys from Burkina Faso (Africa) [62].

The results of the DPPH assay, which was used to determine the free radical-scavenging activity of the honey samples, showed parallel tendency with the darkness of honeys. In this assay, the lower is the IC_{50} value, the higher is the antioxidant activity. In this study, the highest radical scavenging activity was identified for chestnut honey and the lowest for phacelia honey. In case of dark honeys, the IC_{50} values varied significantly ($p < 0.05$) depending on the botanical origin of the honey samples, while the values of the light-colored honeys were only partially different from each other. The assay has been frequently used to characterize the antioxidant activity of honeys, e.g. Polish honeys [11, 12, 22], Czech honeys [63], Romanian honeys [16], Indian honeys [37], or Lithuanian honeys [64]. The activity of linden honey in this study was in the upper range of values measured by Bertoncelj (28.8 ± 5.4 mg ml^{-1}) and in the lower range of values measured by Flanjak (42.77 ± 10.32 mg ml^{-1}) [57, 58]. The activity of our chestnut honey was lower compared to data obtained by the above-mentioned researchers. The multifloral honeys from Greece exhibited a broad range of DPPH activity from 7.5 to 109.0 mg ml^{-1} [14]. The IC_{50} values of the DPPH analysis were significantly lower for the dark-colored honeys in this study, which means

that their antiradical power was significantly higher than that of the lighter honeys, except the goldenrod honey. Its DPPH value was close to those of linden and MF-*Tilia* honeys. Slovenian and Turkish linden (*Tilia*) honeys showed similar DPPH values as our above-mentioned honeys. Beretta et al. [59] reported lower antioxidant values in unifloral dandelion and acacia honeys, while their multifloral honey was much more active (IC_{50} = 5.32 ± 0.2 mg mL^{-1}) compared to our honeys in this study.

Compared to other assays used for characterizing the antioxidant capacity of honeys, ORAC is thought to be the most biologically relevant method, which is based on hydrogen atom transfer [21]; thus, it may evaluate different groups of antioxidants than the SET methods. This assay distinguished the linden and MF-*Tilia* honeys from the light-colored group with similarly high ORAC values as those of the dark-colored chestnut honey. Similar exceptional cases were reported by Bogdanov [65], who measured particularly high antioxidant power in the relatively lighter arbutus and sourwood honeys, similar to dark honeys. Goldenrod honey in the present study provided similarly low ORAC value as acacia honey in a previous Hungarian study, while the antioxidant activity of our multifloral honey was significantly higher compared to the dark-colored fennel and the amber-colored sunflower honeys [18]. The ORAC value of our milkweed honey was comparable to that of strawberry tree honey, while the values measured in our other honey samples were significantly higher than those of the dark African or buckwheat honeys [59].

4. Multi-element analysis of honeys

The macro- and microelement contents measured in our honey samples are shown in **Tables 4** and **5** and **Figures 3** and **4**. The samples in our study were harvested in the Western part of Hungary, the mineral content of which was compared with honey samples originating from South and East Hungary [35, 36, 66].

4.1 Macroelements

Expectedly, K was found to be the most abundant mineral in all studied honeys. Linden honey had significantly higher K content than the other light-colored honeys, similarly high as the darker MF-Lamiaceae honey. The mean K level in linden honey was comparable to that reported for this honey type in Croatia [67]. Relatively high amounts of K and Ca were measured also in linden and chestnut honey samples from other parts of Hungary [35, 36]. Previous results are in accordance with our observation regarding the K content of honeys, increasing in the order: phacelia < sunflower < linden < chestnut. MF-*Tilia* and MF-Lamiaceae honeys had similar K content as sunflower and linden honeys, respectively, in the previous Hungarian studies. Significantly lower K content was measured in our milkweed and goldenrod honey samples, the values being similar to those of acacia, phacelia, and rape honeys from the Eastern part of Hungary. Comparing Ca to P and S to Mg contents within the same honey provided differences ($p < 0.05$) among honey types as follows: linden, MF-*Tilia*, goldenrod, sunflower, and chestnut honeys had higher Ca content than P, while the pale phacelia, milkweed, and the dark MF-Lamiaceae honeys showed reverse relation. The macroelement values obtained for the above-mentioned honey types were variable among the previously studied Hungarian honeys [35, 66]; however, the relation between their average amount of Ca and P was in agreement with each other. Similarly, the ratio of S and Mg was different in various honey types.

Nr.	Honey types	K (mg kg^{-1})	Ca (mg kg^{-1})	P (mg kg^{-1})	S (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})
1.	Phacelia	145.62 ± 4.23^{a}	19.88 ± 3.11^{a}	33.68 ± 2.46^{a}	13.04 ± 0.88 ^{a}	6.04 ± 0.33^{a}	6.38 ± 1.38 ^{a}
2.	Milkweed	340.28 ± 11.12^{b}	19.06 ± 2.67^{a}	39.19 ± 3.56^{a}	15.98 ± 1.88 ^{ab}	11.78 ± 0.10^{b}	3.56 ± 3.09 ^{a}
3.	Linden	1278.08 ± 18.97^{c}	67.85 ± 8.01^{b}	41.52 ± 4.46^{a}	15.89 ± 4.46 ^{b}	16.51 ± 0.30^{c}	9.29 ± 1.03 ^{b}
4.	MF-*Tilia*	845.88 ± 35.67^{d}	53.71 ± 11.05^{b}	37.73 ± 2.69^{a}	14.22 ± 0.94^{ab}	15.74 ± 1.30^{c}	37.02 ± 8.49^{c}
5.	Goldenrod	342.73 ± 12.29^{b}	75.79 ± 10.44^{b}	40.74 ± 3.85^{a}	16.67 ± 1.15^{b}	24.30 ± 0.11^{d}	8.69 ± 0.36^{b}
6.	MF-Lamiaceae	1264.73 ± 70.79^{c}	73.78 ± 12.22^{b}	127.04 ± 4.20^{b}	52.31 ± 0.67^{c}	34.54 ± 2.07^{e}	8.80 ± 1.43^{b}
7.	Sunflower	758.95 ± 18.69^{e}	126.37 ± 14.93^{c}	76.25 ± 8.22^{c}	26.53 ± 8.22^{e}	33.26 ± 1.28^{e}	13.23 ± 1.70^{e}
8.	Chestnut	1815.79 ± 20.69^{f}	153.01 ± 12.60^{c}	79.04 ± 5.41^{c}	35.55 ± 5.41^{e}	45.38 ± 17.32^{e}	20.94 ± 0.80^{f}

Data are means ± standard deviations of three independent measurements (n = 3). Means in the same column with different superscripted letters (a-f) are significantly different according to Student's t-test ($p < 0.05$).

Table 4.
Macroelement content of the studied honey samples.

Nr.	Honey types	B (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
1.	Phacelia	4.10 ± 0.52 ^{a}	<0.10	<0.05	<0.10	1.17 ± 0.25^{a}
2.	Milkweed	3.79 ± 0.63^{a}	0.13 ± 0.01^{a}	0.73 ± 0.00^{a}	0.12 ± 0.03^{a}	0.44 ± 0.00^{b}
3.	Linden	2.70 ± 0.09^{b}	0.12 ± 0.02^{a}	<0.05	1.01 ± 0.03^{b}	0.15 ± 0.08^{c}
2.	MF-*Tilia*	2.41 ± 0.52^{b}	0.13 ± 0.01^{a}	0.62 ± 0.08^{a}	0.62 ± 0.05^{c}	0.63 ± 0.00^{d}
3.	Goldenrod	4.90 ± 1.02^{ac}	0.13 ± 0.01^{a}	1.80 ± 0.60^{b}	0.16 ± 0.01^{a}	2.15 ± 0.20^{e}
4.	MF-Lamiaceae	6.49 ± 0.43^{c}	0.77 ± 0.03^{b}	1.53 ± 0.29^{b}	0.77 ± 0.01^{d}	3.32 ± 0.04^{f}
5.	Sunflower	4.90 ± 0.61^{a}	0.23 ± 0.02^{c}	0.75 ± 0.09^{a}	0.45 ± 0.01^{e}	4.87 ± 0.15^{g}
6.	Chestnut	4.51 ± 0.44^{a}	0.34 ± 0.20^{ac}	1.16 ± 0.85^{ab}	8.45 ± 2.81^{f}	3.88 ± 1.23^{fg}

Data are means ± standard deviations of three independent measurements (n = 3). Means in the same column with different superscripted letters (a-g) are significantly different according to Student's t-test (p < 0.05).

Table 5.
Microelement content of honey samples.

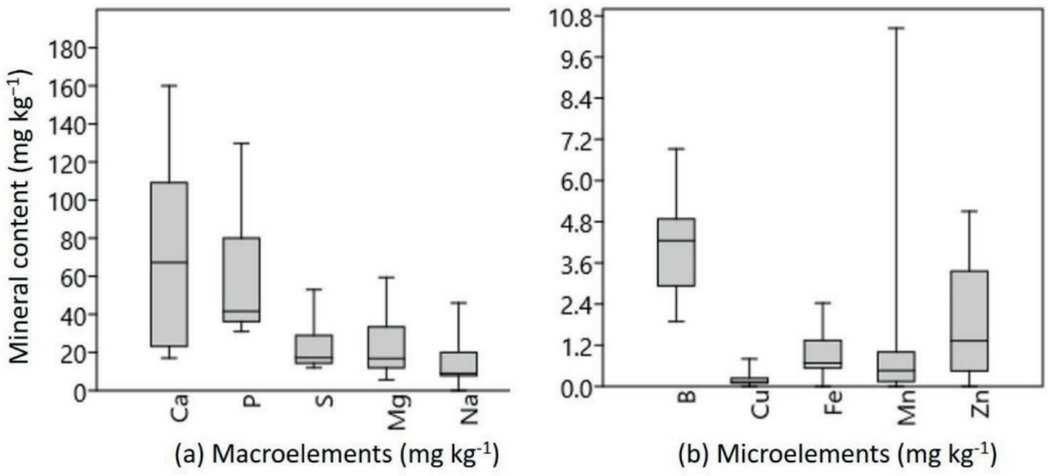

Figure 3.
The range of the measured elements in our honeys. (a) Macroelement content (Ca, P, S, Mg, Na, K not shown due to values differing by one order of magnitude) and (b) microelement content (B, Cu, Fe, Mn, and Zn).

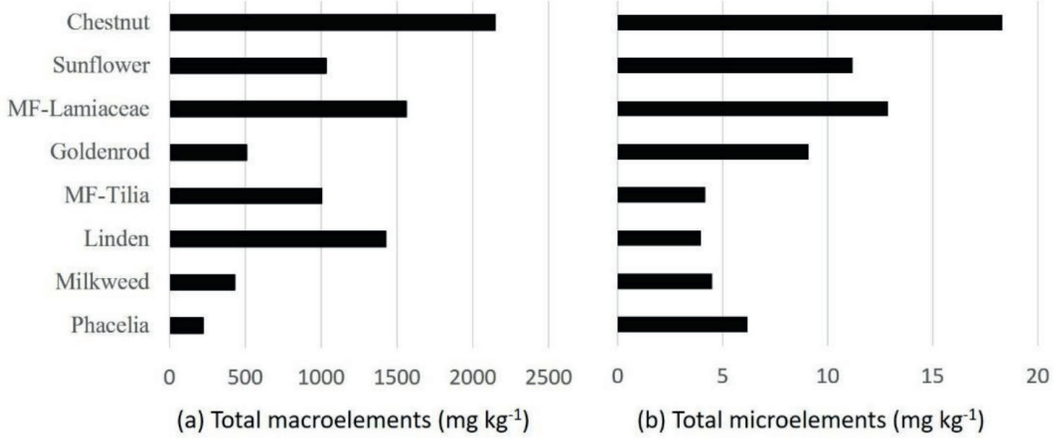

Figure 4.
Reported average concentrations of (a) macroelements (K, Ca, P, S, Mg, and Na) and (b) microelements (B, Cu, Fe, Mn, and Zn) in the studied honeys.

S content was higher than Mg in the light-colored phacelia, milkweed, and MF-Lamiaceae honeys, while in the goldenrod honey Mg exceeded the S. Linden, sunflower, and chestnut honeys contained similar amounts from both elements. Similar to our results, S content of phacelia was significantly higher than its Mg content in an earlier Hungarian study [35]. The range of Mg content was similar to that reported for honeys from Bulgaria, France, Italy, and Poland [27]. Among the macroelements, the amount of Na was the lowest, except in MF-*Tilia* honey, where Na proved to be the third main macroelement together with P, after K and Ca. Nayik et al. [37] measured higher amount of Na than P content in Indian acacia honey. High Na level characterized also the avocado honey, in which Na was the second most abundant mineral [68]. The Na content of honeys can show differences between continents: European (Bulgarian, Italian, Polish, and Spanish) honeys were characterized by lower Na levels (7.2–152 mg kg^{-1}), compared to those measured in Asian (Indian and Malaysian) honeys (83–732 mg kg^{-1}) [27]. The range of macrominerals (**Figure 3a**) clearly shows the decreasing amount of the measured elements after K (Ca > P > S > Mg > Na). Compared to our results, the macromineral content of Italian multifloral honeys decreased in different order (K > S > Ca > P > Na > Mg) [34].

Regarding the total macroelement content (**Figure 4a**), the dark-colored chestnut honey was found to be particularly rich in minerals, while the light-colored phacelia honey was found to be poor in elements with average values of 2150 mg kg^{-1} and 222 mg kg^{-1}, respectively. The total macroelement content did not differentiate the dark- and light-colored group from each other. The light milkweed and the twice darker goldenrod honeys represented similarly low values (430 mg kg^{-1} and 500 mg kg^{-1}, respectively), while the macroelement content of the light-colored linden and MF-*Tilia* honeys was similarly high as that of the dark-colored MF-Lamiaceae and sunflower honeys (around 1500 mg kg^{-1} and 1000 mg kg^{-1}, respectively).

4.2 Microelements

The amounts of microelements determined in our honey samples are summarized in **Table 5** and **Figure 3b** and **4b**. The majority of the measured microelements was present in each honey, whereas Cu, Fe, and Mn were under detection limit in phacelia honey and Fe in linden honey. The range of Zn and B was the broadest among the selected microelements, while the Cu content was under 1 mg kg^{-1} in all of the samples (**Figure 3b** and **Table 5**). The dark-colored group contained significantly higher amount of microelements than the light-colored group (**Figure 4b**), but there were differences in the ranking of each element. Chestnut honey was the richest and linden honey the poorest in microelements, with average amounts of 18.3 mg kg^{-1} and 3.9 mg kg^{-1}, respectively. Regarding the dark-colored honeys, MF-Lamiaceae was the richest in B and Cu, goldenrod in Fe, and chestnut in Mn, while high Zn content characterized the sunflower honey.

Based on the microelements, phacelia was distinguishable from other light-colored honeys due to its significantly higher B and Zn content; and chestnut honey stood out from dark-colored honeys due to its extremely high Mn content. Among the studied microelements, B content showed the highest amount in all honey types, except chestnut honey. However, Amtmann et al. [66] measured lower B content in sunflower, chestnut, and even phacelia honey (0.98 mg kg^{-1}), compared to our results, while that of linden honey was similar in the two studies. In contrast, Sajtos et al. [36] measured higher B content in the above-mentioned honeys, compared to our and to

Amtmann et al.'s [66] results. Linden and MF-*Tilia* honeys contained similar amount of this microelement. Similar to our results, Cu content of phacelia was under detection limit in the study of Czipa et al. [35]. They reported similar average amount of Cu in sunflower, while higher Cu content in linden honey (0.320 ± 0.073 mg kg^{-1}). Low Cu content was measured in milkweed, linden, MF-*Tilia*, and goldenrod honeys in this study, while MF-Lamiaceae showed the highest Cu content. The microelement Fe was missing or present only in low amounts in the light-colored honeys and the dark sunflower honey. The highest Fe content was measured in goldenrod and MF-Lamiaceae honeys, but much higher upper limit of Fe content had been detected in honeys from France (86.76 mg kg^{-1}) and Malaysia (233 mg kg^{-1}) [27]. Regarding microelements, chestnut honey is exceptional due to its particularly high Mn content, in accordance with the results of Sajtos et al. [36], who measured even higher level of Mn in this honey type (11.9 mg kg^{-1}). The high Mn content of chestnut honey was confirmed by several authors, being similarly high as that of fir honey, but slightly lower than that of oak honey [29, 38]. Light-colored honeys contained significantly lower Zn content than the dark-colored ones. However, the light acacia and rape honeys from the eastern part of Hungary had significantly higher Zn content, similar to our darker goldenrod and MF-Lamiaceae honeys [35]. The highest Zn level was found in our sunflower honey samples, supporting the observations of Sajtos et al. [36], who compared the Zn content of sunflower honey to that of linden, phacelia, and chestnut honeys. Regarding the linden honey in Bogdanov et al.'s study [29], it showed similar Cu and Fe content, and higher Zn content than chestnut honey, while in our study linden honey contained lower amount of each microelement than chestnut honey. Compared to our results, higher Cu and Fe levels have been reported for linden and chestnut honeys in Croatia, while the Zn content of chestnut honey was higher in our samples [67].

The results of multi-element analysis in Egyptian and Italian honeys proved that mineral content can serve also as a marker of geographical origin [34, 69, 70]. However, the minor components in honeys cannot be considered as a reliable biomonitor of environmental pollution [34]. Mohammed et al. [31] showed evidence that minerals in some honeys were not in strict relation with the soil mineral content. Bogdanov et al. [25] and Nayik et al. [37] concluded that the mineral composition in honey has been primarily attributed to the botanical origin rather than geographical and environmental exposition of nectar sources.

5. Correlation analysis

The data matrix of color (absorbance), antioxidant values, and mineral contents was analyzed by Pearson's correlation (**Table 6**). The following significant relationships were found. Correlation was obtained between color and the SET-based antioxidant capacities (TRC and DPPH), as well as between these methods. As expected, color and TRC did not show correlation with ORAC. Most of the minerals correlated with color, except Na. TRC and DPPH were in strict correlation with the macrominerals P, S, and Mg and the microminerals Cu and Mn. TRC was the strongest predicting factor regarding B and Fe, while DPPH was related to K, Ca, and Mn content. ORAC showed strict correlation with the K content of honeys. Ca, Na, B, and Mn also correlated with this assay.

Similar to our results, many studies reported that darker honeys had higher antioxidant capacities, while lighter honeys were characterized by relatively low

Variable	Color	TRC	DPPH	ORAC
TRC	0.5388**			
DPPH	−0.8429***	−0.5429**		
ORAC	0.2596	−0.0583	−0.5091*	
K	0.6191**	0.3845	−0.7529***	0.8176***
Ca	0.9199***	0.3372	−0.8373***	0.4906*
P	0.6572***	0.8834***	−0.6082**	0.0798
S	0.6611***	0.8673***	−0.6113**	0.1078
Mg	0.9287***	0.6273***	−0.8742***	0.3506
Na	0.1546	0.0280	−0.3005	0.6241**
B	0.5404**	0.7541***	−0.3309	−0.4405*
Cu	0.5152**	0.9021***	−0.5415**	0.1266
Fe	0.4714*	0.6496***	−0.2368	−0.3112
Mn	0.6913***	0.1063	−0.6322***	0.5895**
Zn	0.8847***	0.5437**	−0.646***	−0.0104

TRC, total reducing capacity; DPPH, antiradical power; ORAC, oxygen radical absorbance capacity.
**Significant at $p < 0.05$.*
***Significant at $p < 0.01$.*
****Significant at $p < 0.001$.*

Table 6.
Correlation matrix (Pearson's correlation coefficients) of color, antioxidant, and mineral parameters in Hungarian honeys.

antioxidant values [15, 24, 51, 52, 71]. The DPPH assay showed the strongest correlation with the color of honey samples, in accordance with the results of Dżugan et al. [11], Flanjak et al. [58], and Gorjanovich et al. [72]. The used SET-based methods gave positive linear correlation in this study, consistent with the results of other authors [15, 22, 24, 37]. However, ORAC as an exception showed correlation only with DPPH, in contrast to the results reported by Beretta et al., Gorjanovich et al., and Bodó et al. [18, 59, 72]. Similarly, ORAC and TRC, known also as total polyphenolic content (TPC), were found to correlate with each other [13]. However, TPC does not always correlate with antioxidant activity [73]. Our study highlights the fact that in a comprehensive study on antioxidant activity of honeys, it is essential to include HAT-based methods as well, besides the SET-based assays.

All of the studied macro- and microelements were in good correlation with color, except Na. The contribution of minerals to the color of honey has already been established [74]. Perna et al. [39] proved that color intensity is positively correlated with metal content in honey; furthermore, with an increase in metal content, there is an increase in antioxidant capacities of honeys.

The above-described correlations could be clearly interpreted in the light of principal component analysis (PCA). Three groups of parameters—antioxidant assays, macroelements, and microelements—were selected to establish their identification power in terms of honey types. The results are presented in the biplots of **Figure 5**.

The first principal component, PC1, included most of the information with 95.3% for the antioxidant results, 99.4% for the macroelements, and 65.9% for the microelements of the total variance, while the second principal component, PC2, explained

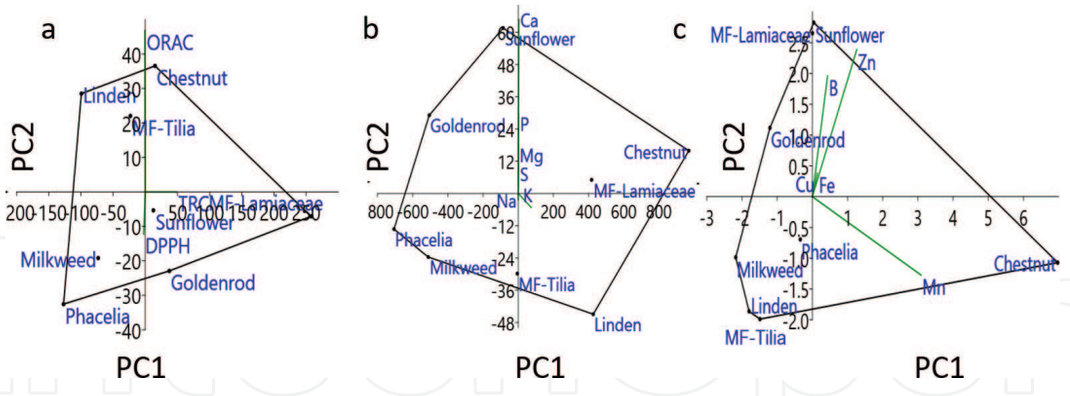

Figure 5.
Principal component analysis (PCA) of datasets consisting of (a) antioxidant parameters, (b) macroelements, and (c) microelements analyzed from the honey samples.

4.43%, 0.37%, and 28.7%, respectively. The light-colored phacelia and milkweed, furthermore the linden and MF-*Tilia* honeys, were located close to each other in each biplot. Most of the analyzed parameters of phacelia and milkweed honeys showed low variability, locating them always on the negative PC1 and PC2 quarter. The relation between linden and MF-*Tilia* honey types suggested the influence of their linden (*Tilia*) plant origin.

Regarding antioxidant variables, MF-Lamiaceae, sunflower, and goldenrod honeys were located on the negative PC2, while ORAC activity clustered linden, MF-*Tilia*, and chestnut on the positive PC2 coordinate. The analysis of macroelements revealed a different picture, separating the honeys from each other according to their color. The light-colored honeys represented negative, the dark-colored ones positive PC2 value. K played a key role in clustering of linden, MF-Lamiaceae, and chestnut honeys on the positive PC1 coordinate. The third group, the microelements, separated clearly the Mn-rich chestnut honey. Similarly to the macroelement analysis, the biplot separated the light- and dark-colored honeys from each other.

Several attempts have been made to identify and differentiate honey types from each other by means of PCA. For example, PCA of three Indian honeys based on antioxidant properties and minerals was able to separate different honey types, but analyzing the same honeys based on their sugar content was not so powerful [37]. In a Turkish study, antioxidant capacity, mineral contents, and vitamin B_2 values were useful in distinguishing honeys from various botanical origin [38]. Romanian honey types could be separated with PCA based on mineral content, color, antibiotic, and pesticide residues [40], while the analysis of physicochemical parameters was only partly able to identify various honey types [16]. PCA working with nine variables (browning index, color parameters, and antioxidant values) successfully distinguished light- and dark-colored Polish honeys [41].

However, in this study, PCA was applied not only to identify honey types but also to interpret the relationships of the studied parameters, namely the antioxidant activities, macroelement contents, and botanical origin of honeys.

6. Future prospective

The complex approach applied in this study can be used in further investigations aimed at identification of various honey types and establishing their nutritional

values and biological activities. Since the issue of honey adulteration poses a great challenge internationally [75, 76], it is essential to recognize the unique traits of each honey type, which can aid proper identification of these beehive products. As there is a growing interest in applying natural agents, such as the products of the beehive, for health maintenance [21, 77–79], the differences in antioxidant power, antibacterial property, and mineral content should be revealed for each honey type by further studies.

7. Conclusions

Our complex analysis of four light- and four dark-colored Hungarian summer honeys revealed the characteristic traits and unique values of each honey type. Melissopalynological analysis combined with the sensory traits color, odor and consistency, and spectrophotometric color determination was found to be suitable for confirming the botanical origin of honey samples. Our study supported the general view that light-colored honeys possess lower antioxidant activity compared to darker colored ones, except for linden honey, which acted as strong antioxidant despite its light color. The color of honey can be a good predictor of its mineral content. The PCA based on macro- and microelements composition, successfully separated the light- and dark-colored honeys, the latter typically containing more of both macro- and microelements. The honeys with a dominance of *Tilia* pollen were exceptions, with macroelement contents comparable to those of dark-colored honeys. The SET-based antioxidant assays TRC and DPPH were found to correlate with the macrominerals P, S, and Mg and also with the microminerals Cu and Mn. Chestnut honey can be a good source of Mn, whereas goldenrod honey and multifloral honey with Lamiaceae pollen dominance can supply Fe. The unique character sets based on the above analyses can aid the botanical authentication of each honey type and reveal their individual nutritional values and health benefits.

Acknowledgements

This research was supported by a grant from the National Research, Development and Innovation Office NKFIH K 132044; by Bolyai Research Scholarship of the Hungarian Academy of Sciences BO/00701/19/4; by the ÚNKP-21-5 New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund.

Thanks

The authors are grateful to Dr. Rita K. Csepregi and Dr. Tamás Kőszegi for help with the antioxidant assays and to Márta Lauferné Kajdi and Sarolta Gausz for performing the ICP-AES measurements of macro- and microelements.

DOI: http://dx.doi.org/10.5772/intechopen.108175

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