Thesis of doctoral (PhD) dissertation

ELEMENT ANALYSIS AND GEOGRAPHICAL IDENTIFICATION OF RAW AND EXTRACTED PROPOLIS SAMPLES

Written by:
Soós Áron
PhD candidate

Supervisor:
Prof. Dr. Kovács Béla
Professor

UNIVERSITY OF DEBRECEN

Kerpely Kálmán Doctoral School

Debrecen
2020

1. INTRODUCTION AND GOALS OF THE RESEARCH

Propolis, as well as their products are one of the parts of apitherapy, which have an effect to maintain or improve the human health by its antioxidant, anti-inflammatory and antibacterial properties (Anjum et al., 2018). The positive effect of the propolis connects to e.g. its flavonoid content. More than 300 compounds were identified by analyzing the organic content of propolis (De Groot et al., 2014). However, the mineral content of propolis has not been so thoroughly studied, especially in the case of propolis products such as tinctures. The mineral content of raw propolis or any kind of food also can be often useful to identify the geographical origin of them (Cantarelli et al., 2011; Gong et al., 2012). Moreover, the geographical origin can have an impact to the organic content as well as the biological properties of the propolis (Bankova et al., 2000). Therefore my research was chiefly focusing on the analysis of the mineral content of propolis and propolis products. The questions should be answered are the following:

- What are the possibilities to speed up the sample preparation? Moreover is it possible to use the applied sample preparation and instrumental analytical method for the analysis of the mineral content of raw and extracted propolis?
- What is the mineral concentration of Hungarian raw propolis, and how they match with the element content of foreign propolis?
- Is it possible to apply the mineral content of raw propolis for the geographical identification of them, as well as what are the elements which are useful in this situation?
- How affect the extraction parameters the mineral content of the tincture, such as the ethanol content and the volume of the extraction solvent compared to raw propolis, as well as the extraction time?
- What is the relationship between the flavonoid content and mineral composition of tinctures?
- What is the transfer coefficient of the elements between the raw propolis and the tinctures?
- Is it possible to determine the mineral content of the original raw propolis based on the element content of its tincture?
- Is it also possible to apply the mineral content of propolis tincture for the geographical identification?

In addition, I wanted to make a database which contains the mineral composition of Hungarian raw propolis.

2. MATERIALS AND METHODS

Totally 252 Hungarian **raw propolis were analyzed,** which settlement and region of the collection was known. Propolis were collected in autumn of 2014 by the beekeepers from standing apiaries, and the collection was controlled by the National Beekeepers' Association of Hungary. I have got the homogenized samples from Eszterházy Károly University, Food and Wine Research Centre. Samples were homogenized in a mortar after freezing them with liquid nitrogen. I have stored the propolis in scintillation vials till chemical digestion or extraction. I have prepared the samples for element analysis by microwave digestion in University of Debrecen, Agricultural, Food and Environmental Management, Institute of Food Science. While there were a small amount of samples, moreover I wanted to increase the sample throughput, therefore I have used vessel inside vessel method.

Approximately 0.1000±0.0100 g raw propolis was deposited into quartz tubes. After this, 2 mL concentrated HNO_3 was added and the samples were left overnight. On the following day 0.6 mL (w/w) H_2O_2 was added and the quartz tubes were sealed with Teflon tape. Up to three closed tubes were placed in polytetrafluoroethylene (PTFE, Teflon) vessels containing 10 mL MilliQ water around the quartz vessels. The water was necessary because it helps for the temperature control, slows down the reaction and compensates for the inner pressure inside the quartz tubes. Closed PTFE vessels were placed into a microwave digester (Milestone Start D, Milestone Srl, Sorisole, Italy). The digestion steps were the following: heating up to 180°C in 15 min, then being kept at a constant heat at 180°C for 20 min and finally a ventilation step was done for 60 min. The samples were quantitatively washed into 15 mL centrifuge tubes with Pasteur pipette and filled up to 9.5-10.5 mL with MilliQ water. Because the scale of the centrifuges tube do not fit the accuracy required therefore I have calculated the exact volume by the mass of the digested sample filled up with water multiplied by its density. Density of digested samples considered as 1.07 g cm^{-3}.

I have done the analysis with a Thermo Scientific iCAP 6300 Dual view inductively coupled plasma optical emission spectrometer (ICP-OES) and a Thermo Scientific X-Series II inductively coupled plasma mass spectrometer (ICP-MS) (Bremen, Germany) in University of Debrecen, Agricultural, Food and Environmental Management, Institute of Food Science. Samples were diluted by five times before ICP-MS measurement because of the acid content decrease, moreover Rh was added as an internal standard in 40 $\mu g\ L^{-1}$ final concentration. There was no dilution and internal standardization in the case of ICP-OES measurement.

For the analysis of extraction from raw propolis to tinctures (extract) I have created a mixed raw propolis sample from the collected samples. This sample was created from at least

30 individual raw propolis by homogenizing them. Almost all the counties were represented in the mixed sample at equal ratio. These combined samples formed an average composition of raw propolis from across the country. About 0.5000 ± 0.0100 g of homogenized raw propolis was measured and put into 15 mL plastic centrifuge tubes. I have checked the effect of the composition of extraction solvent (0, 50, 80 and 100% (v/v) by their ethanol content in MilliQ deionized water), the volume of the extraction solvent (2.5 mL, 5.0 mL and 10 mL), moreover the extraction time (1 hour, 1 day, 1 week and 1 month). The extraction took place at room temperature (23 ± 2°C) in triplicate. The tubes with the raw propolis and the extraction solvents were intensively mixed by Vortex at the beginning of the extraction process. Samples, which were extracted for 1 day, 1 week and 1 month, were mixed twice in every working day. After the extraction period the samples were mixed again and were centrifuged. The supernatant was filtered into centrifuge tubes.

I failed to measure the element content of tinctures by direct introduction them into the plasma without digestion, therefore I had to use a destructive sample preparation. Since ethanol content of tinctures and nitric acid can enter into an intensive reaction and can be dangerous, therefore solvents were evaporated before digestion. After having been shaken, 2 mL of the tinctures were pipetted into quartz vessels, except when 2.5 mL extractant was used, because that time 1 mL liquid were measured. Tinctures were dried in an oven at 40°C to their constant weight. A gentle temperature was used to avoid quit the volatile elements and have a negative mistake. To compare, not only tinctures containing ethanol, but water as an extraction solvent were also evaporated from the samples. The following steps were the same as in the case of raw propolis digestion.

Analysis of transfer coefficient was done using 27 raw propolis, which were randomly chosen from collected samples. The chosen samples were not necessarily matching with those were used for the mixed propolis. The samples were extracted with a chosen extraction condition, namely with 5 mL of 80% (v/v) ethanol content solvent for a week. The sample preparation and element analysis procedure were the same as mentioned before. Transfer coefficient (TC) was calculated by the following equation:

$$\mathrm{TC}\ (\%) = \frac{\text{mineral content in tincture }(\mathrm{mg\ L^{-1}})}{\text{mineral content in raw propolis }(\mathrm{mg\ kg^{-1}})} * \text{dilution factor }(\mathrm{L\ kg^{-1}})*100$$

In order to **improve limit of detections** I have paid attention to improve the purity in the laboratory, and also use high purity chemicals for element analysis. I tried to make the cleanest conditions as possible. The surfaces were cleaned regularly to avoid anthropogenic contaminants from the environment. Moreover, the samples were covered by paper to avoid external contamination. Filter papers were changed regularly. Water used for preparing solutions was coming from a MilliQ system (Millipore Corp., Bedford, MA, USA). The second highest amount of chemical was nitric acid. It was purified by Milestone subPUR sub-boiling distillation system (Milestone Srl, Sorisole, Italy). All the new plastic tools were cleaned and soaked in 2% (w/w) HNO_3 for at least 3 days, then soaked in MilliQ water for at least 1 day, after that rinsed with distilled water, finally dried on clean filter paper. Cleaned plastic tools were hold in plastic bags prior to use. I have used new plastic tools for every sample to avoid cross-contamination. Quartz tubes after usage were washed several times with distilled water, then scrubbed with cotton buds, finally washed again several times with distilled water. Tubes were left for drying.

Polyphenol content of tinctures prepared in different extraction conditions was analyzed by Folin-Ciocalteu method (Singleton et al., 1999) with minor modification. Absorbance was measured by Thermo Electron Corporation Nicolet Evolution 300 spectrophotometer at 760 nm. Results were expressed in gallic acid equivalent (mg GAE L^{-1}). **Flavonoid content of tinctures** prepared in different extraction conditions was analyzed by the method of Zhishen et al. (1999) with minor modification. Absorbance was measured by the aforementioned spectrophotometer at 510 nm. Results were expressed in catechin equivalent (mg CE L^{-1}).

The used software are the following: ICP-OES and ICP-MS were controlled by iTEVA 2.8.0.97 and Thermo PlasmaLab 2.5.10.319 software. IBM SPSS 22.0 was used for one-way analysis of variance (ANOVA) and Tukey-test, Pearson correlation, principal component analysis and linear discriminant analysis. The graphs and calculation of results were made by Microsoft Excel 2013.

3. RESULTS AND DISCUSSION

3.1. Evaluation of sample preparation and instrumental analysis

Detection limit of sample preparation and instrumental analysis is a very important point in trace element analysis. To achieve this, as low amount of reagent should be used as possible. Therefore I have added 2 mL HNO_3 + 0.6 mL H_2O_2 for 0.1000 g sample. The limit of detections (LOD) related to the solid material are the following for ICP-MS: Lu, Tm, Ho, Tb, Eu, Er, Yb, Dy, Gd, Pr, U, Cs and Sm LODs are between 0.0957-0.686 µg kg^{-1}, Nd, La, Ce, Co, Cd, V and Mo LODs are between 1.51-14.8 µg kg^{-1}, while the LODs for Ni, Cu, Mn and Cr are between 39.0-55.5 µg kg^{-1}. The limit of detections for ICP-OES are the followings: Sr, Ba and B LODs are between 0.0811-0.639 mg kg^{-1}, Zn, Fe, Mg, P, S, Al, Na and K LODs are between 1.28-6.91 mg kg^{-1}, while the highest LOD has the Ca with 56.7 mg kg^{-1} concentration. Totally 35 element concentrations, except La could be measured from all the raw propolis samples, because of the low limit of detections. I have noticed that the element concentrations were under the LOD of some elements in case of tinctures despite the lower dilution factors. However it was also adequate for most of the elements depending on the extraction process of the tinctures. The measurement of lanthanides were mainly the exception. Moreover it can be concluded that there is no cross-contamination between the quartz tubes placed inside the same Teflon vessel. Therefore independent samples can be prepared in the same vessel.

Accuracy was measured by at least 22 spike recoveries during the sample preparation and analysis of raw propolis. The average recoveries of the elements were between 87.4-109.9%. The highest and lowest spike recoveries in the case of tinctures were 83.4 and 116.2%, respectively, while the average of all the elements was 99.1%. Based on this, there is no effect of the evaporation of samples at 40°C to the accuracy of the sample preparation method. To sum up, the accuracy of the method is proper based on the spike recoveries. I have analyzed a plant sample in every analysis from an international plant-analytical exchange (Wageningen Evaluating Program for Analytical Laboratories). The sample was a rice straw (*Oryza sativa*) or a kind of silvergrass (*Miscanthus sp*.). Moreover I have measured also the element content of BCR 189 wholemeal flour certified reference material. We can say that the results are in good agreement with the theoretical concentrations with a few exceptions. Based on this, the accuracy of the method is suitable.

I have measured the **repeatability** of the method in all the 252 raw propolis samples by analyzing 3 replicates. It was found that the relative standard deviation (RSD) of the macro and microelements measured by ICP-OES, except Ba was lower. The average of the RSDs were between 5.3 and 9.2%, while the RSD of Ba was 12.3%. The repeatability was a bit worse in the case of microelements measured by ICP-MS between 7.5 and 12.0%, while in the case of lanthanides and U the RSDs were between 10.9 and 14.4%.

One of the aims of the used sample preparation method was to **improve the limited sample throughput of the microwave digester**, which was achieved by this method. The sample throughput was increased up to three times, because the number of the quartz tubes which can be placed into the Teflon vessel is three. Based on the requirements of green chemistry smaller volume of nitric acid (2 mL) and hydrogen peroxide (0.6 mL) was used for each sample, compared to the normal microwave digestion. Moreover, the amount of the sample which is necessary is also smaller. That is good, because in many cases a limited quantity of propolis are available for measurement.

3.2. Evaluation of the element composition of raw propolis

The descriptive statistics of the element content of 252 raw propolis sample is presented in *Table 1*. The analyzed samples presented in the database are representing the characteristics of Hungarian propolis. I have documented firstly the results of 36 elements measured in Hungarian raw propolis. The measured elements except one element were over the limit of detection in all the samples. This database is unique worldwide, because there is no publication in the literature which consists of such a number of samples, moreover presents 36 element concentrations of the propolis of the given country in a representative way. I have found that element concentrations are present in a wide range by evaluating the statistical data. The difference is huge by comparing the lowest and the highest concentrations of each elements. The smallest difference is 9.0-fold, while all the other element concentrations are higher that 10-fold. This means that the element concentrations are not in the same order of magnitude except Mg. The ratio of the maximum and minimum concentration is up to 25 in the case of Al, B, Ca, K, Mg, Mn, Na, P, S, Cs, Ce, Pr, Nd, Sm, Gd, Tb, Dy, Ho, Er, Tm and Yb elements. Besides that, the concentration difference is within two order of magnitude, namely the maximum and minimum concentration is up to 100-fold in the case of Cu, Fe, Sr, Co, Mo, Lu and U elements. The difference of minimum and maximum concentration is higher than 100-fold in the case of Ba, Zn, V, Cr, Ni, Cd and Eu elements. The Zn and Ba should be highlighted, because the difference between the minimum and maximum concentration is 522-fold and 1638-fold, respectively.

Table 1.: Descriptive statistics of the measured Hungarian raw propolis samples (n=252)

elements	median	average	SD	minimum	maximum	max / min*	average / median*	skewness*	kurtosis *
K (mg kg^{-1})	792	900	414	289	3440	11.9	1.14	2.299	9.468
Ca (mg kg^{-1})	493	590	346	211	2600	12.3	1.20	2.413	8.243
S (mg kg^{-1})	234	256	118	90.4	1010	11.2	1.09	1.897	7.064
P (mg kg^{-1})	213	235	96	60.5	606	10.0	1.11	0.813	0.420
Fe (mg kg^{-1})	171	213	160	36.8	1450	39.3	1.25	3.215	16.74
Mg (mg kg^{-1})	156	171	77	51.8	465	9.0	1.09	1.279	1.963
Al (mg kg^{-1})	116	140	90	39.7	938	23.6	1.20	3.585	25.06
Zn (mg kg^{-1})	54.7	144	331	5.34	2790	522	2.63	5.209	30.62
Na (mg kg^{-1})	32.8	39.8	25.5	9.93	158	15.9	1.21	2.440	7.104
Ba (mg kg^{-1})	8.59	25.5	79.6	0.690	1130	1638	2.97	11.10	149.0
B (mg kg^{-1})	5.41	5.78	2.12	2.00	20.3	10.1	1.07	1.739	7.987
Mn (mg kg^{-1})	4.79	5.72	3.47	0.887	21.1	23.8	1.19	1.961	4.916
Sr (mg kg^{-1})	1.72	2.26	1.86	0.520	20.2	38.9	1.31	4.580	35.81
Cu (mg kg^{-1})	1.47	2.08	2.32	0.573	26.9	47.0	1.41	6.561	59.86
Cr (mg kg^{-1})	0.502	1.01	2.52	0.0909	38.4	422	2.01	13.13	193.4
Ni (mg kg^{-1})	0.283	0.573	2.227	0.0903	28.8	319	2.02	11.27	131.0
V (mg kg^{-1})	0.204	0.276	0.396	0.0578	5.81	101	1.35	11.29	153.9
Ce (mg kg^{-1})	0.182	0.229	0.157	0.0592	1.01	17.1	1.26	2.096	6.337
Co (mg kg^{-1})	0.103	0.163	0.197	0.0182	1.30	71.2	1.59	3.580	15.11
La (μg kg^{-1})	96.9	n.c.	n.c.	<1.53	544	n.c.	n.c.	1.940	6.169
Nd (μg kg^{-1})	77.1	97.1	69.1	25.5	476	18.6	1.26	2.446	8.608
Mo (μg kg^{-1})	76.7	91.7	80.5	17.0	889	52.2	1.20	6.747	60.17
Cd (μg kg^{-1})	33.3	64.1	131.8	5.99	1480	247	1.93	7.433	66.49
Pr (μg kg^{-1})	20.2	25.9	18.6	6.71	121	18.1	1.28	2.405	8.217
Gd (μg kg^{-1})	15.5	19.1	13.4	4.74	95.1	20.1	1.23	2.357	7.993
Sm (μg kg^{-1})	15.1	19.1	13.6	5.18	97.9	18.9	1.27	2.505	9.174
Cs (μg kg^{-1})	14.3	16.6	10.5	4.86	89.1	18.3	1.16	2.735	12.64
Dy (μg kg^{-1})	9.35	11.9	8.4	3.17	66.9	21.1	1.27	2.555	10.25
U (μg kg^{-1})	6.01	8.17	8.71	1.73	113	65.0	1.36	7.468	82.76
Eu (μg kg^{-1})	5.19	8.99	18.68	1.14	270	237	1.73	11.82	165.9
Er (μg kg^{-1})	5.11	6.32	4.35	1.65	35.2	21.4	1.24	2.453	9.809
Yb (μg kg^{-1})	3.91	5.01	3.59	1.20	29.5	24.6	1.28	2.617	10.96
Tb (μg kg^{-1})	2.02	2.53	1.70	0.641	12.9	20.1	1.25	2.229	7.789
Ho (μg kg^{-1})	1.78	2.22	1.57	0.562	12.7	22.5	1.24	2.518	10.19
Tm (μg kg^{-1})	0.643	0.796	0.570	0.191	4.33	22.7	1.24	2.427	8.833
Lu (μg kg^{-1})	0.542	0.697	0.506	0.106	3.99	37.8	1.29	2.497	9.656

*no unit; n.c.: not calculable

I have checked the distribution of the element concentrations within the samples by statistical tests among others. I have found positive values for the skewness and kurtosis in all the cases (*Table 1.*). The lowest skewness was found in the case of P (0.813), while it was between 1 and 2 in the case of Mg, Mn, S and La. The skewness was between 2 and 3 in most of the elements. However, Ba, Ni, V and Eu elements had a skewness around 11, while Cr element had above 13. Kurtosis of P and Mg element was below 2, but other elements had extremely high kurtosis values. Kurtosis is above 100 in the case of Ba, Ni, V, Eu and Cr elements. Therefore, we can say that most of the element concentrations in the propolis samples have a non-normal distribution, but have a positive skew and a positive kurtosis.

The order of the element concentrations was best described by the median (*Table 1.*), which is the following: K>Ca>S>P>Fe>Mg>Al>Zn>Na>Ba>B>Mn>Sr>Cu>Cr>Ni>V>Ce>Co>La>Nd>Mo>Cd>Pr>Gd>Sm>Cs>Dy>U>Eu>Er>Yb>Tb>Ho>Tm>Lu.

It should be highlighted by **comparing my results of Hungarian propolis with results of raw propolis originating from other countries** that the results are in the same range in most cases. The results of South Spanish (Bonvehí and Bermejo, 2013) and Chinese (Gong et al., 2012) propolis are often higher, such as the concentration of K, Ca, Fe or Mg. This may be due to the different botanical origin. I have found, that the concentration of Ca is usually higher than the concentration of K in South Spanish, Chinese and Croatian (Cvek et al., 2008) samples, in contrast with most of the Hungarian propolis. The microelement concentrations in Hungarian and Argentinian raw propolis are in good agreement, but concentrations of measured lanthanides are 2 to 3 orders of magnitude lower in the article of Cantarelli et al. (2011).

I have checked the **possibility of geographical identification** in case of 252 Hungarian raw propolis. The settlement of the collection was known, and the geographical identification was done by separating the different regions. The independent variables should have a normal distribution for doing a statistical analysis of the data. Because I have confirmed that this requirement has not been fulfilled, therefore I have used the decimal logarithm of the concentrations of measured elements. Concentrations presented in a logarithm scale are closer to the normal distribution, because the skewness and kurtosis are in the ±1 range for most of the elements. Those elements, which had higher skewness or kurtosis than 1 were excluded from chemometric analysis. Therefore Cd, Cr, Cu, Eu, Mo, Ni, U, V and Zn were not used for evaluation. La was also not evaluated, because its concentration was under the limit of detection in some of the samples.

Principal component analysis (PCA) may be an appropriate method for finding the connection between the independent variables. It creates ≤n number of principal components from n number of independent variables, which are uncorrelated with each other. I have left three principal components (PC1, PC2, PC3) by the eigenvalue of principal components. The created principal componenst were representing the 85.3% of the total variance. The result of the principal component analysis is presented on a three-dimensional scatter plot in *Figure 1*. It shows the individual samples with different colors according to their geographical origin by region. The results show no correlation with geographical origin. The points originating from the same region do not form separated groups on the scatter plot, but they are mixed with other regions. Therefore I have rejected the use of principal component analysis.

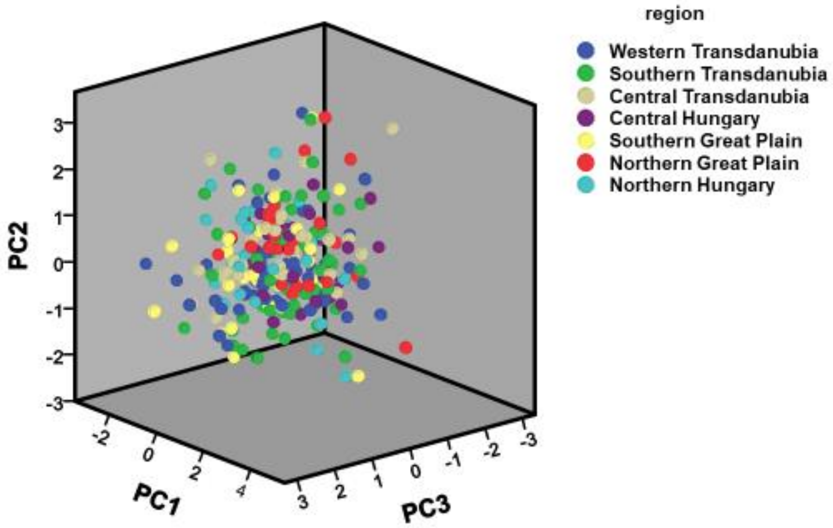

Figure 1: Evaluation of the logarithm element concentrations of raw propolis samples by principal component analysis (PCA) for checking the geographical origin

I have used linear discriminant analysis (LDA) instead of PCA for the discrimination of the geographical regions. This method is also a dimension reduction procedure like PCA, however minimizes the difference between the same group of samples, while maximizes the difference between the different group of samples. I got the impact of the single variables for the grouping after running the LDA analysis. The impact is expressed by the Wilks' Lambda and the significance level. I found that the impact of Ba and Co is very low, therefore I removed them from the grouping model. Henceforward I used Al, B, Ca, Fe, K, Mg, Mn, Na, P, S, Sr, Cs, Ce, Pr, Nd, Sm, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu elements in the grouping method. It can be seen based on the loading plot (*Figure 2.*) that Northern Great Plain propolis samples are separated the most efficiently from other regions' propolis samples. They overlap only partially with the others, especially propolis from the Northern Hungarian region. In contrast, propolis from other regions could not be completely separated from each other. Samples from Southern Great Plain region are almost totally overlap with Central Hungarian and Central Transdanubian propolis samples, respectively. Western Transdanubian propolis samples are a bit further from them on the loading plot, but Southern Transdanubian and Northern Hungarian samples are also partially overlap with each other.

Numerical results of the classification show us that grouping of the samples of Northern Great Plain was the most effective based on the Original method. The 96.3% of the samples goes into the correct category (Predicted Group Membership), that is the grouping was successful. The classification of the other groups is not so effective. The second highest match is in the case of Central Hungarian samples with 80.0%. Discrimination of propolis from other regions were between 64.7 and 78.1%. Cross validation can be used for verification of the classification result. The classification efficiency of Northern Great Plain samples decreased to 74.1% in the cross validation, which is also quite high. The correct matches in other regions are lower between 44.7 and 72.0%. To sum up, 75.4% of the samples were correctly classified by the original classification method, while it is decreased to 60.3% by the cross validation.

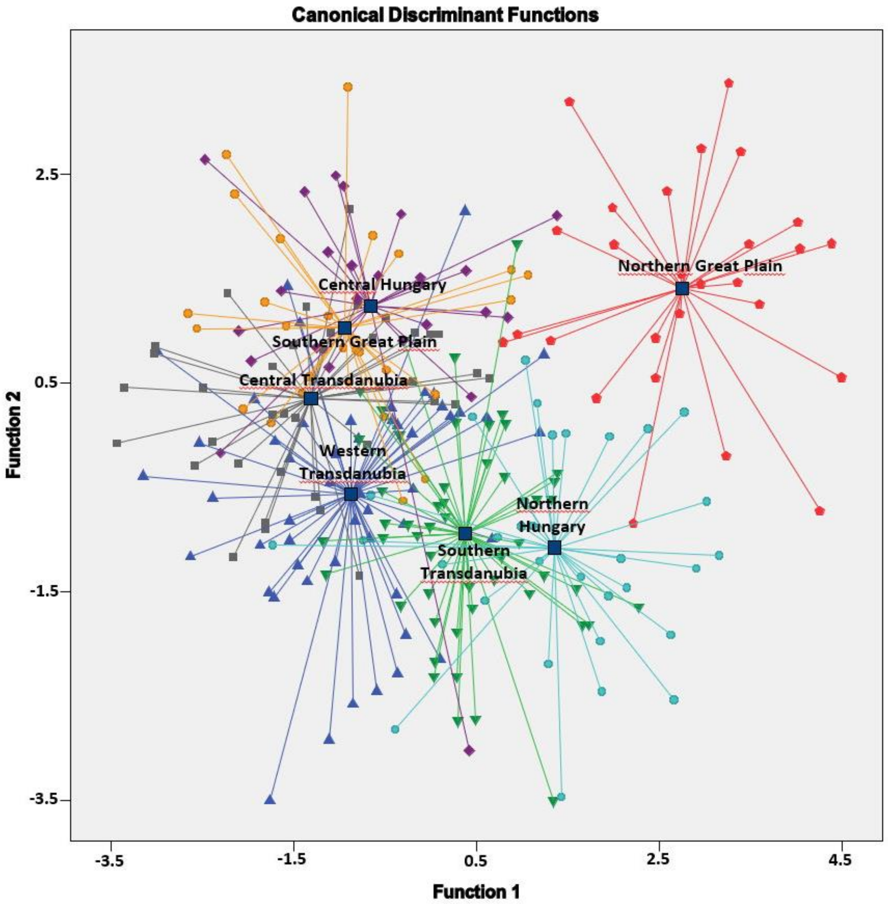

Figure 2. Loading plot for the discrimination of raw propolis originating from different regions with linear discriminant analysis

3.3. Effect of the tincture preparation method to the composition of propolis tinctures

I would like to measure the effect of the preparation method of propolis tinctures for the element content of the tinctures. I have measured the mineral content of the tinctures in relation to the extraction time (1 hour, 1 day, 1 week and 1 month) and the ethanol content (0, 50, 80 and 100% (v/v)). The measured elements can be classified into three main groups (i; ii; iii) according to their behavior during the extraction process, depending on the extraction time and the composition of the extraction solvent (Soós et al., 2019). I have established, that elements of **group (i)** dissolved considerably better in aqueous extraction solvents than in solutions containing ethanol. In many cases, this has resulted that the concentrations fell under the LOD in tinctures extracted by 100 or even 80% (v/v) ethanol content solvents. However, it happened that tinctures prepared with 50 or 0% (v/v) ethanol content extraction solvents did not contain the given element in measurable concentration. I have classified Ba, Ca, Sr, Cd, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu elements into this group. Ca, Sr and Ba are alkaline earth metals, which can explain why their behaviour was similar. However Mg was also an alkaline earth metal is not included in the (i) group. Other elements, except Cd are belonging to lanthanides, which may also be related to their similar behaviour. Elements in **group (ii)**, namely B, K, Mg, Na, P, S, Zn, Mn, Co and Cs dissolved the most in aqueous extraction solvents after 1 month, but the concentrations were decreased by the increasing ethanol content in the extraction solvent. However the reduction was not as high as in the aforementioned first group. It happened, that there was no significant difference between tinctures extracted with 0 and 50% (v/v) ethanol content extraction solvent after 1 month. Therefore, the extraction with 0 and 50% (v/v) ethanol content extraction solvent is favorable for increasing the concentration of these elements, however extraction with 80, but mainly with 100% (v/v) ethanol gives lower element concentration in the tincture. Elements belonging to this group, except Cs are essential for the human body. Elements classified under the **group (iii)** had one common property, namely that they were extracted the worst in water, but results did not improve proportionally by the increasing ethanol content in the solvent. This group contains the Cu, Fe, V, Cr, Mo and U elements. The solubility of elements was the highest in 80% (v/v) ethanol in most of the cases. However there was no significant difference between the mineral content of tinctures prepared with 50, 80 and 100% (v/v) ethanol content solvent depending on the extraction time in some of the cases. It did not fully meet the criteria for the mentioned groups, but Al and Ni was closer to the properties of group (i) and group (ii), respectively. Examples are shown for each of the three main groups in *Figure 3.*

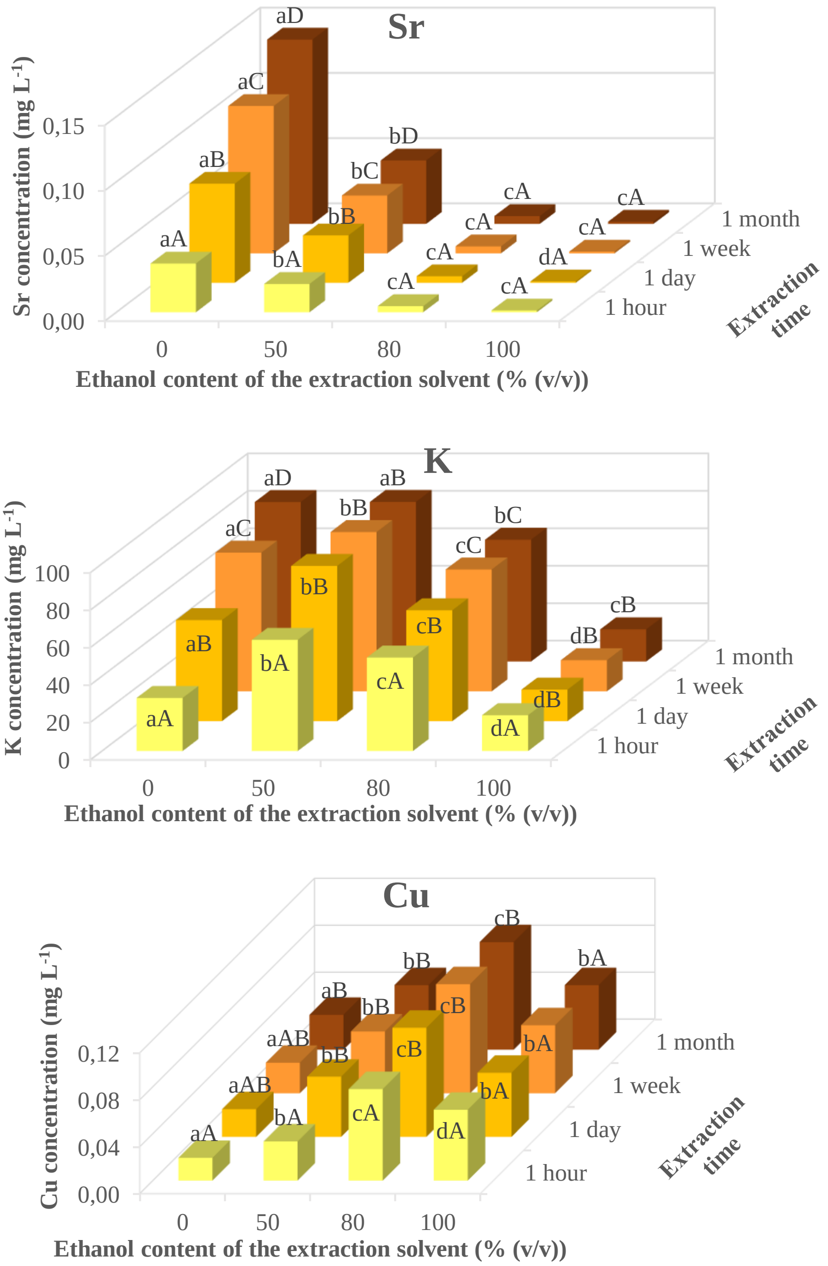

Figure 3. Sr, K and Cu concentrations of the propolis tinctures depending on the ethanol content of the extraction solvent and the extraction time. Different small letters mean the significant differences ($p<0.05$) between the effect of the different extraction solvents. Capitals mean significant differences ($p<0.05$) between the effect of the different extraction times.

I have found that most of the element concentrations in group (i) and (ii) had increased in the aqueous tinctures **in relation to the extraction time**. It was found that the element concentrations had increased after 1 month extraction compared to 1 hour between 2.78 and 5.40 times in the (i) group, while in the (ii) group they had increased between 2.27 and 4.79 times. This means that there was a remarkably high difference in mineral concentration of tinctures at the shortest and the longest extraction time. On the other hand, the concentration growth between 1 week and 1 month extraction was between 1.01 and 1.28 times for the former group and between 1.10 and 1.28 times for the latter.

The increase or decrease of the low concentration elements are not significant in most of the cases, especially when ethanol content solvent was used. The concentration of the remaining elements in the first group, except lanthanides had changed between 1.28 and 2.57 times in addition to 0.90 and 1.73 times between 1 hour and 1 month, as well as between 1 week and 1 month. The change in the tincture element composition generated by a higher ethanol content solvent could not be calculated, because elements were either below the LOD or, in the case of Ca, Sr and Cd there was no significant difference in relation to the extraction time. Concentration of elements in the (ii) group, except Zn, had increased in the ethanol content extraction solvents in relation to the extraction time. It was increased by 1.41-2.78 and 1.00-1.11 times in 50% (v/v) ethanol content solution, changing to 1.09-1.58 and 0.97-1.03 times in 80% (v/v) tinctures, while they changed by 0.75-1.52 and 0.96-1.47 times in 100% (v/v) tinctures between 1 hour and 1 month, as well as between 1 week and 1 month. It was noted, however, that the concentrations did not increase significantly after 1 week or even 1 day in 50 and 80% (v/v) ethanol content tinctures. It can be concluded that the extraction solvent dissolved almost all the accessible elements from raw propolis within this time period. Thus, no increase or decrease of these elements was expected in the 50 and 80% (v/v) ethanol content tinctures after 1 week. It was also observed that the concentrations changed in a remarkably different way in 80 and 100% (v/v) ethanol content tinctures in relation to the extraction time in the (iii) group of elements. There was a small decrease, then the concentration stagnated after 1 day in both 80 and 100% (v/v) ethanol content tinctures by the extraction time in case of Cu and Cr. On the other hand, in the case of Fe, V, Mo and U, the decrease was the highest in 1 week compared to 1 hour extraction time. The change (reduction), however, was not significant between 1 week and 1 month extraction period in the aforementioned cases, except for one element (Fe in 80 and 100% (v/v) tinctures).

Previous articles highlight that flavonoids can make complexes with metals, such as with Fe^{2+} and Cu^{+} ions, respectively (Havsteen, 2002). While the mineral content and the flavonoids may be related to each other in propolis, therefore **I have measured the flavonoid content also** (Soós et al., 2018), which is presented in *Figure 4*.

The flavonoid content was the lowest in aqueous solutions (≤679 mgCE L^{-1}), but it is increased by the increasing extraction time. Higher concentrations were found in 50% (v/v) ethanol content solutions, in which I have found significant increment in most of the cases in relation to the extraction time (2390-4200 mgCE L^{-1}).

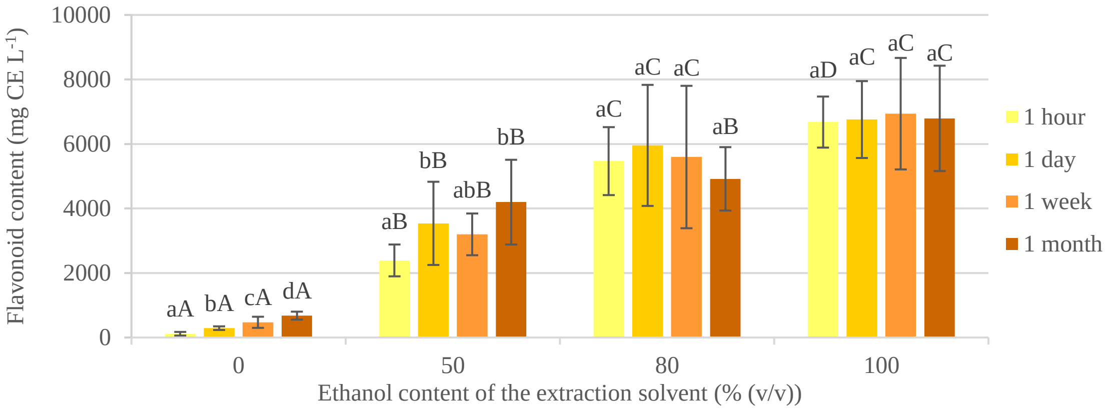

Figure 4. Flavonoid content of the propolis tinctures depending on the ethanol content of the extraction solvent and the extraction time. Different capitals mean the significant differences ($p<0.05$) between the effect of the different extraction solvents. Small letters mean significant differences ($p<0.05$) between the effect of the different extraction times. Error bar represents the uncertainty of the measurement.

The highest flavonoid content was found in 100% (v/v) ethanol content tinctures, but difference is often not significant compared to 80% (v/v) ethanol content solution, moreover I have found no increment in all of the cases in relation to the extraction time. The flavonoid contents were between 4920 and 6940 mg CE L^{-1} in 80 and 100 % (v/v) ethanol content tinctures.

By evaluating the **correlation relationship between minerals and flavonoid content**, I have found similar tendency just in aqueous extracts between flavonoids and group (i) and (ii) elements. Macroelement contents of these groups and flavonoid concentrations were increased in relation to the extraction time in aqueous solutions, moreover their molar concentrations are also near to each other. However this can be explained by the concentration equalization of the elements and the flavonoids between the raw propolis and the extraction solvent depending on the time. As a result, group (i) and (ii) elements chiefly are not connected to flavonoids, but may connected to other molecules or present in ionic form in raw propolis. There is a stronger correlation with elements in group (iii) and flavonoids, but there was no element, which has a significant correlation with flavonoid concentration in all circumstances, namely the extraction time span and the extraction solvent, but Fe, Cu, U and Cr elements were the closest to that. On the other hand, the molar concentrations of the aforementioned elements were negligible compared to the flavonoid content. So, if they made complex with flavonoids in the propolis, the concentration of complex would be very small according to the total flavonoid content.

I have evaluated the **transfer coefficient** (TC) in randomly chosen 27 raw propolis and in the mixed propolis sample. I have extracted them similarly to the most often used method in propolis tincture preparation, namely with 80% (v/v) ethanol content extraction solvent for 1 week extraction time span. Transfer coefficients are shown in *Figure 5*.

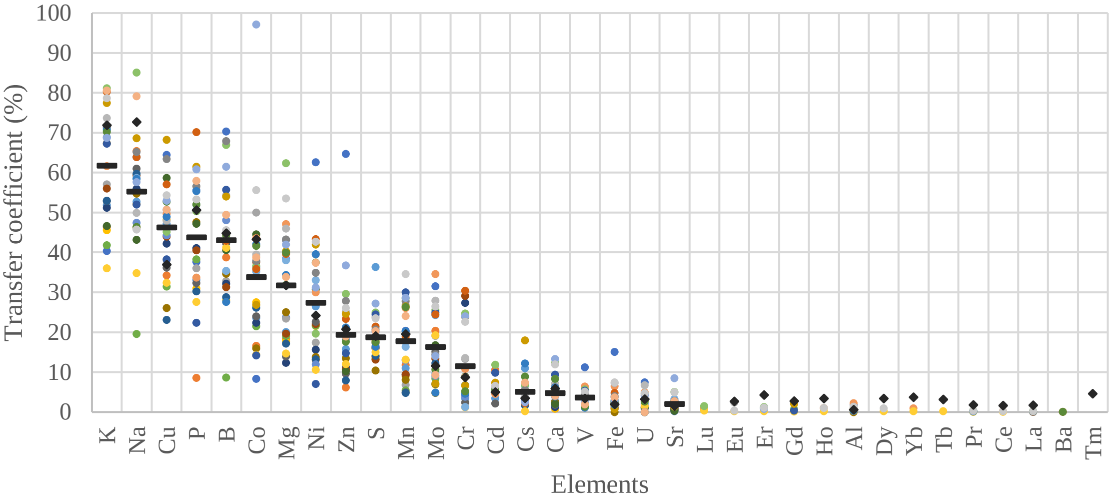

Figure 5. Transfer coefficient of the analysed elements from raw propolis to their tinctures extracted with 80% (v/v) ethanol content extractant for 1 week (n=27

It can be seen from the results, that in elements of group (i) which is created based on the behavior related to extraction time and the ethanol content of the extraction solvent, that are dissolved considerably better in aqueous extraction solvents, transfer coefficient was obviously low in the tinctures prepared with 80% (v/v) ethanol. TC of Ca and Sr was just 4.81±3.60% and 2.02±1.87%, respectively. Cd was under the limit of detection in more than half of the tinctures, therefore I could not calculate nor the average neither the median, but the highest value was just 11.8%. Ba was over the LOD just in two samples from 27, but the transfer coefficient was neither reach 0.1% in these cases. The situation was similar in the case of rare earth elements, because the TC did not reach 2.35% in none of the cases. TC was about the same in the mixed propolis sample in the case of Cd, Ca and Sr, moreover Ba was below the limit of detection, but rare earth elements were extracted from raw propolis with higher efficiency, that is 1.63-4.28%. Lu was not measurable from tincture of mixed sample, in contrast with some of the individual 27 propolis, however Tm was measureable from the mixed one, in contrast with the others. Nevertheless, transfer coefficient of lanthanides was below 5%, which is negligible.

Group (ii) elements, which are dissolved the best in aqueous solvents, but dissolved well in 80% (v/v) ethanol content solutions also, transfer coefficient was quite high in the analyzed conditions. In ascending order 17.8%, 18.8%, 19.4%, 31.8%, 33.8%, 43.1%, 43.8%, 55.3% and 61.8% of the Mn, S, Zn, Mg, Co, B, P, Na and K content on average is transferred from raw propolis into their tincture. Transfer coefficient is lower for Cs with 5.13%, which is not essential for the human body in contrast to the previous ones. It can be stated, that the elements of group (ii) were present in all the tinctures in measurable concentration. Although the highest transfer coefficient had K with 61.8±13.7% on average, anyway it has not the highest TC in all the samples, because Na and Co overtook it in one-one sample. Previous element had 85.1%, the latter one had 97.1% topmost transfer coefficient, respectively, while the highest TC of K was just 81.2%. I have found a bit higher transfer coefficient in mixed propolis compared to the average results of 27 samples, but they are not differing in their typical range. The highest difference had Na compared to the results of 27 samples, in which the transfer coefficient is 55.3% on average, while 72.7% of the mixed sample is extracted with 80% (v/v) ethanol. By this way, Na had the highest transfer coefficient from the measured elements in mixed propolis.

Nevertheless, elements of group (iii) were usually dissolved with the highest efficiency not in aqueous, but in 80 or 100% (v/v) ethanol content solvents, in most cases, however, TC was relatively low in tinctures containing 80% (v/v) ethanol after 1 week extraction. Cu had the highest transfer coefficient from group (iii) with 46.32±11.36% on average. V, Cr and Mo was below with 3.63±2.10%, 11.54±9.40% and 16.38±8.61%, respectively. Other elements belong to this group, namely Fe and U were below the LOD in one and six tinctures, respectively, therefore I could not calculate the average, just the median of TC. The median of the aforementioned elements was 2.53% and 2.59%, respectively. The TC from the mixed propolis is slightly lower or similar for the elements mentioned above. The transfer coefficient of Cu and Mo was just 37.0% and 11.6%, respectively, while in the case of other element difference is negligible, so their TC can be considered similar to the average or median of the 27 samples.

Among the elements not included in the mentioned groups, the transfer efficiency of Al, like that of group (i), was extremely low at 2.25% or less in any sample, moreover in a tincture was below the limit of detection, as well as Ni TC was 27.4% on average, similar to group (ii).

Based on the element concentrations measured in the tinctures, it was found that K remains at first place in relation to the mean (55.6±19.5 mg L^{-1}) and the median (50.6 mg L^{-1}), respectively. It is followed by P, Mg and S elements with 12.4±7.0, 5.30±2.10 and 5.00±2.37 mg L^{-1} concentration on average. Ca, Na and Zn were followed by the median and average also. In the following elements, at least one tincture was present at a concentration below the limit of detection, so in this case the mean and the standard deviation could not be calculated. In addition, a non-normal distribution was also observed, like in raw propolis samples, which may influence the order of the elements. So, in this case it was also advisable to sort the items by the median instead of the average.

In terms of median concentration, only Fe and B are present in the order of 0.1-1 mg L^{-1}. In addition, the median of Mn, Cu, Al, Ni, Cr, Co, Sr and Mo was in the range of 1-100 µg L^{-1}. Concentrations of lanthanides were below the limit of detection in at least half of the samples. Therefore, I could not calculate the median, but nor the highest concentration did not reach 60 ng L^{-1}. Based on the median of element concentrations measured in the 27 tinctures, the elements can be sorted in the following order:

K>P>Mg>S>Ca>Na>Zn>Fe>B>Mn>Cu>Al>Ni>Cr>Co>Sr>Mo>V>Cs>U.

I calculated how accurately the **element composition of raw propolis could be determined** based on the transfer coefficient on average and the mineral composition of the tincture. In this case I did not take into account the effect of the different preparation methods, as each tincture was prepared with the same preparation method (5 mL, 80% (v/v) ethanol content extractant, 1 week extraction time). Since I calculated the estimated element composition of raw propolis based on the average transfer coefficient of the 27 propolis samples and the element composition of the same tinctures, the mean of the estimated match rate in each case is 100% compared to their actual concentration. The level of the error was described the best by the difference from 100% (namely by the standard deviation). Most accurately, that is with the smallest error, the concentration of K can be predicted, where the standard deviation is 22.2%. For Na and Cu elements, the standard deviation of the match level would be ±23.5 and ±24.5%, respectively, while the concentration of S, P and B in the raw propolis can be predicted with a 25-40% standard deviation. The standard deviation of Mg, Ni, Mn, Co, Mo and V elements would be relatively high with 40 to 60%. The level of mismatch is higher in terms of the other elements.

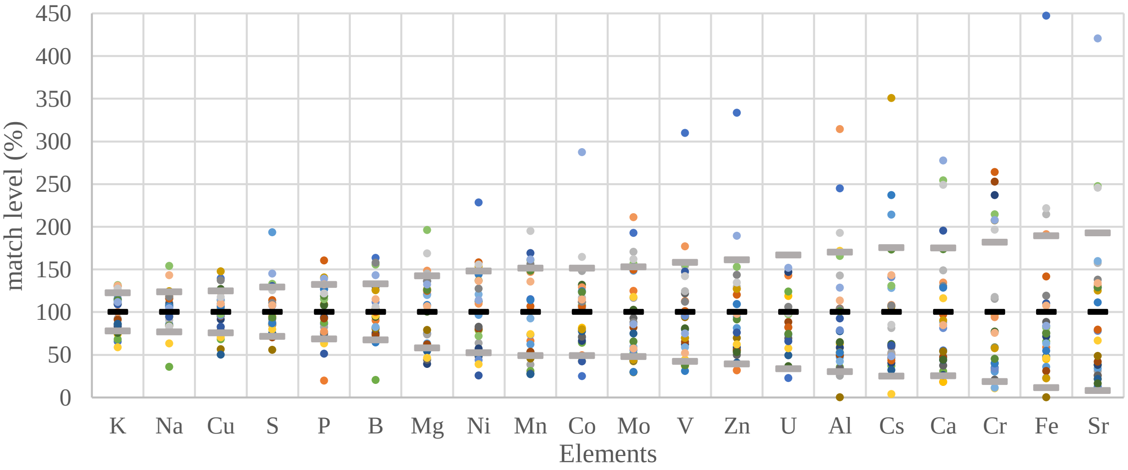

Figure 6. Estimation of raw propolis element composition based on the element composition of their tincture (5 mL, 80% (v/v) ethanol content extractant, 1 week extraction time)

4. NEW SCIENTIFIC RESULTS

1. I have determined some analytical characteristics of the applied method used for sample preparation of small amount of sample. The sample preparation was done by microwave assisted digestion, while digested samples were measured by ICP-OES and ICP-MS techniques. I have proved that it is appropriate for analyzing 35 element concentrations in raw propolis.

2. I have created a database which contains information about 36 element composition of 252 Hungarian raw propolis samples. The analyzed samples presented in the database are representing the characteristics of Hungarian propolis. This kind of database has not existed so far from Hungarian propolis. Moreover, we cannot find globally such a results of element contents of propolis, which is representing one country and consists of such number of elements.

3. It was proved, that the geographical identification of raw propolis is partially efficient to discriminate samples originating from the seven Hungarian regions based on their element composition. Linear discriminant analysis was identified the samples with 75.4% efficiency, while in the case of the cross validation efficiency was decreased up to 60.3%.

4. It was proved that the analyzed elements were classified into three main groups by their behavior by analyzing the element content of tinctures prepared with different ethanol content extraction solvents (0, 50, 80 and 100% (v/v)) and different extraction times (1 hour, 1 day, 1 week and 1 month). Elements in group (i) dissolved considerably better in aqueous solvent, elements in group (ii) dissolved mostly in aqueous extraction, but extracted well also in 50% (v/v) ethanol content solutions, while elements classified into group (iii) were extracted mostly in 80% (v/v) ethanol content solvents.

5. By extracting the tinctures for different extraction times (1 hour, 1 day, 1 week and 1 month) using ethanol content extractant (50, 80 and 100% (v/v)), it was found, that after 1 week extraction period usually there is no significant change in measured element concentrations. The recommended extraction time, which is a few weeks applied by most producers, results in a constant element concentration in tinctures in terms of extraction time.

6. By analyzing propolis samples of Hungary, I have proved that the elements have extremely variable transfer coefficient from raw propolis to tincture, extracted with 80% (v/v) ethanol for one week extraction time. The transfer coefficient on average is in the following order: K>Na>Cu>P>B>Co>Mg>Ni>Mn>Zn>S>Mo>Cr>Ca>Cs>V>Fe>Sr>U>Al. The transfer coefficient of Cd, Ba and lanthanides could not be determined in at least half of the samples.

7. While identifying the geographical origin of propolis tinctures, I have come to the conclusion that this cannot be done solely on the basis of the element composition. Although extraction results in a stable composition after 1 week, the element composition is drastically determined by the concentration and volume of ethanol used in the extraction. Even when extracted under the same conditions, the element composition of the original raw propolis can be estimated with a high level of error.

5. SCIENTIFIC RESULTS FOR PRACTICAL APPLICATION

1. I have described some analytical characteristics of the sample preparation and measurement method which had favorable analytical characteristics for the analysis of propolis or tinctures. However, this sample preparation may also be used to determine the element composition of other small amount of samples of plant or animal origin. It can be used e.g. for clinical samples which often have the problem of limited amount of sample available.

2. Based on the element results, I proved that the origin of raw propolis can be partially identified by the element composition in case of the Hungarian samples. This is relevant because its geographical origin may influence the physiological effects of propolis. In addition, element analysis may provide a possible way of identifying the origin.

3. I proved that a notable part of the essential elements (B, K, Mg, Na, P, S, Zn, Mn and Co) from raw propolis is transferring the most effectively into the aqueous and 50% (v/v) ethanol extraction media, but using 80% (v/v) ethanol content extractant the effectiveness of extraction is also remarkable. However, some toxic elements, such as Cd or Al, have low transfer coefficient under these conditions. As tincture are generally prepared using 80% (v/v) ethanol, it is beneficial to human health in terms of the element composition of the tincture.

6. REFERENCES

Anjum, S.I. – Ullah, A. – Khan, K.A. – Attaullah, M. – Khan, H. – Ali, H. – Bashir, M.A. – Tahir, M. – Ansari, M.J. – Ghramh, H.A. – Adgaba, N. – Dash, C.K.: 2018. Composition and functional properties of propolis (bee glue): a review. Saudi Journal of Biological Sciences. In press.

Bankova, V. – De Castro, S. – Marcucci, M.: 2000. Propolis: recent advances in chemistry and plant origin. Apidologie. 31. (1): 3-15.

Bonvehí, J.S. – Bermejo, F.O.: 2013. Element content of propolis collected from different areas of South Spain. Environmental Monitoring and Assessment. 185. 6035-6047.

Cantarelli, M.Á, Camiña, J.M. – Pettenati, E.M. – Marchevsky, E.J. – Pellerano, R.G.: 2011. Trace mineral content of Argentinean raw propolis by neutron activation analysis (NAA): Assessment of geographical provenance by chemometrics. LWT – Food Science and Technology. 44. 256-260.

Cvek, J. – Medić-Šarić, M. – Vitali, D. – Vedrina-Dragojević, I. – Šmit, Z. – Tomić, S.: 2008. The content of essential and toxic elements in Croatian propolis samples and their tinctures. Journal of Apicultural Research. 47. (1): 35-45.

De Groot, A.C. – Popova, M.P. – Bankova, V.S.: 2014. An update on the constituents of poplar-type propolis. Wapserveen, The Netherlands: acdegroot publishing. 11.

Gong, S. – Luo, L. – Gong, W. – Gao, Y. – Xie, M.: 2012. Multivariate analyses of element concentrations revealed the groupings of propolis from different regions in China. Food Chemistry. 134. 583-588.

Havsteen, B.H.: 2002. The biochemistry and medical significance of the flavonoids. Pharmacology and Therapeutics. 96, (2-3): 67-202.

Singleton, V.L. – Orthofer, R. – Lamuela-Raventós, R.M.: 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology. 299. 152-178.

Soós Á. – Tóth N. – Bódi É. – Várallyay Sz. – Molnár Sz. – Kovács B.: 2018. The effect of the preparation method on the physical and chemical characteristics of propolis tinctures. Acta Agraria Debreceniensis. 74. 163-168.

Soós Á. –Bódi É. – Várallyay Sz. – Molnár Sz. – Kovács B.: 2019. Mineral content of propolis tinctures in relation to the extraction time and the ethanol content of the extraction solvent. LWT – Food Science and Technology. 111. 719-726

Zhishen, J. – Mengcheng, T. – Jianming, W.: 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 64. (4): 555-559.

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/29/2020.PL
Subject: PhD Publikációs Lista

Candidate: Áron Soós
Neptun ID: DBIVSW
Doctoral School: Kálmán Kerpely Doctoral School
MTMT ID: 10055249

List of publications related to the dissertation

Hungarian scientific articles in Hungarian journals (4)

1. **Soós, Á.**, Reichenbach, R., Várallyay, S., Bódi, É., Kovács, B.: Pest, Zala és Bács-Kiskun megyékből származó propolisz minták elemtartalmi összetétele. *Agrártud. Közl. 72*, 149-153, 2017. ISSN: 1587-1282.
2. **Soós, Á.**, Várallyay, S., Kovács, B.: Hazai kékfrankos borok toxikus elem koncentrációja. *Agrártud. Közl. 68*, 87-90, 2016. ISSN: 1587-1282.
3. **Soós, Á.**, Andrási, D., Kovács, B.: Folyékony élelmiszerminták destruktív minta-előkészítést nem igénylő nyomelemtartalom-meghatározási lehetőségei induktív csatolású plazma tömegspektrométerrel. *Élelmvizsg. Közl. 60* (2), 178-189, 2014. ISSN: 0422-9576.
4. **Soós, Á.**, Andrási, D., Kovács, B.: Belső sztenderdek alkalmazhatósága élelmiszerek induktív csatolású plazma tömegspektrometriás mikroelem-tartalmának vizsgálatában. *Élelmvizsg. Közl. 59* (3), 106-119, 2013. ISSN: 0422-9576.

Foreign language scientific articles in Hungarian journals (1)

5. **Soós, Á.**, Tóth, N., Bódi, É., Várallyay, S., Molnár, S., Kovács, B.: The effect of the preparation method on the physical and chemical characteristics of propolis tinctures. *Agrártud. Közl. 74*, 163-168, 2018. ISSN: 1587-1282. DOI: http://dx.doi.org/https://doi.org/10.34101/actaagrar/74/1683

Foreign language scientific articles in international journals (1)

6. **Soós, Á.**, Bódi, É., Várallyay, S., Molnár, S., Kovács, B.: Mineral content of propolis tinctures in relation to the extraction time and the ethanol content of the extraction solvent. *LWT-Food Sci. Technol. 111*, 719-726, 2019. ISSN: 0023-6438. DOI: http://dx.doi.org/10.1016/j.lwt.2019.05.090 IF: 3.714 (2018)

Hungarian conference proceedings (1)

7. **Soós, Á.**, Reichenbach, R., Várallyay, S., Bódi, É., Molnár, S., Kovács, B.: Kis mennyiségű propolisz minták előkészítése és mérése ICP-OES és ICP-MS technikákkal.
   In: Magyar Spektrokémia vándorgyűlés és XIII. Környezetvédelmi Analitikai és Technológiai Konferencia. Debrecen, 2017. 08. 23-25 : Program és előadás összefoglalók, [Debreceni Egyetem], [Debrecen], 113-116, 2017.

Foreign language conference proceedings (1)

8. **Soós, Á.**, Andrási, D., Kovács, B.: Evaluation of internal standards and calibration methods in measurement of micro-elements in wines with direct analysis by ICP-MS.
   In: 28th ISMAS Symposium cum Workshop on Mass Spectrometry. Ed.: S. K. Aggarwal, P. G. Jaison, Arnab Sarkar, Prudent Arts & FAb Private Limited, Mumbai, 208-211, 2014. ISBN: 9788190444262

Hungarian abstracts (3)

9. **Soós, Á.**, Andrási, D., Kovács, B.: Mátrixillesztés és belső standared vizsgálata borok direkt mintabevitelű mikroelem-tartalom meghatározására, induktív csatolású plazma tömegspektrométerrel.
   In: XXXVI. Kémiai Előadói Napok: Program és előadás-összefoglalók. Szerk.: Endrődi Balázs, Laufer Noémi, JATEPress - Szegedi Egyetemi Kiadó, Szeged, 58-59, 2013. ISBN: 9789633151457
10. **Soós, Á.**, Andrási, D., Kovács, B.: Mikroelemtartalom-meghatározási módszer kidolgozása borra, direkt mintabevitel esetén, multielemes ICP-MS mérésnél. Utánközlés párhuzamos közlés,
   In: XI. Környezetvédelmi Analitikai és Technológiai Konferencia. Program és előadás-összefoglalók. Szerk.: Princz Péter, Magyar Kémikusok Egyesülete, Hajdúszoboszló, 57, 2013. ISBN: 9789639970403
11. **Soós, Á.**, Andrási, D., Kovács, B.: Mikroelemtartalom-meghatározási módszer kidolgozása borra, direkt mintabevitel esetén, multielemes ICP-MS mérésnél.
   In: A Magyar Szabadgyök-Kutató Társaság VII. Konferenciája, Magyar Szabadgyök-Kutató Társaság, Debrecen, 35, 2013.

Foreign language abstracts (5)

12. **Soós, Á.**, Bódi, É., Várallyay, S., Molnár, S., Kovács, B.: The Element Content of Propolis Tinctures by the Volume of the Extraction Solvent.
   In: FSD 2018 3rd Food Structure & Design Conference : Abstract Book. Ed.: Zoltán Győri, Debreceni Egyetem, Debrecen, 41, 2018. ISBN: 9789634900245

13. **Soós, Á.**, Bódi, É., Várallyay, S., Szegő, R., Molnár, S., Kovács, B.: Transfer Coefficient of Minerals from Raw Propolis to Their Tinctures.
    In: Scientific researches in food production, University of Debrecen - Proceedings of abstracts. Ed.: Béla Kovács, Nikolett Czipa, Ferenc Peles, Éva Bacskainé Bódi, Andrea Kántor, Flóra Mária Szabóné Petróczki, Loránd Alexa, Debreceni Egyetem, Mezőgazdaság-, Élelmiszertudományi és Környezetgazdálkodási Kar, Debrecen, 27, 2018. ISBN: 9789634900412

14. **Soós, Á.**, Bodnár, S., Czipa, N., Kovács, B.: Evaluation of Sample Preparation Techniques for the Measurement of Honey by ICP-MS.
    In: Meeting of Young Researchers from V4 Countries : Book of abstracts. Ed.: Monika Wesoowska, University of Rzeszow, Rzeszow, 77, 2016.

15. **Soós, Á.**: Elemental analysis of wines by ICP-MS.
    In: International Conference for Students : Students in Bucovina Abstracts, Faculty of Food Engineering, Stefan cel Mare University of Suceava, Suceava, Románia, 156, 2015, (ISSN 2068-7648)

16. Andrási, D., **Soós, Á.**, Fekete, I., Kovács, B.: Method development for direct wine analysis by inductively coupled plasma mass spectrometry.
    In: EuroFoodChem : Book of abstracts. Ed.: Hamit Köksel, Hacettepe University, Food Engineering Department, Isztambul, 315, 2013. ISBN: 9786056393501

List of other publications

Hungarian scientific articles in Hungarian journals (7)

17. Várallyay, S., Veres, S., Bódi, É., **Soós, Á.**, Kovács, B.: Arzénterhelés hatásvizsgálata a napraforgó fejlődésének korai stádiumában.
    Növénytermelés. 67 (3), 95-114, 2018. ISSN: 0546-8191.

18. Várallyay, S., Veres, S., Bódi, É., **Soós, Á.**, Kovács, B.: Eltérő arzénformák hatása a fejlődés korai stádiumában lévő napraforgó növény (Helianthus annuus L.) szárazanyag produktumára, valamint foszfor-, kén- és mikroelem-koncentrációjára.
    Növénytermelés. 67 (2), 57-73, 2018. ISSN: 0546-8191.

19. Várallyay, S., Balláné Kovács, A., **Soós, Á.**, Kovács, B.: Arzénnal szennyezett talajon termesztett zöldborsó élelmiszer- és takarmánybiztonsági megítélése.
    Élelmvizsg. Közl. 63 (3), 1661-1670, 2017. ISSN: 0422-9576.

20. Papp-Topa, E. A., Nyeste, E., **Soós, Á.**, Bódi, É., Várallyay, S., Czipa, N., Kovács, B.: Egy rektori elképzelés továbbgondolása: egy induktív csatolású plazma optikai emissziós spektrométer optimálása.
    Növénytermelés. 66 (3), 75-94, 2017. ISSN: 0546-8191.

21. Várallyay, S., Balláné Kovács, A., **Soós, Á.**, Kovács, B.: Élelmiszeripari felhasználásra szánt zöldborsó ásványi anyag összetételének vizsgálata növekvő koncentrációjú arzénkezelések függvényében.
Agrártud. Közl. 72, 203-208, 2017. ISSN: 1587-1282.

22. Várallyay, S., Balláné Kovács, A., **Soós, Á.**, Kovács, B.: Arzénkezelés hatása zöldborsó (Pisum sativum L.) arzén- és foszforkoncentrációjára, valamint szárazanyag-produktumára.
Agrokém. Talajt. 65 (2), 275-295, 2016. ISSN: 0002-1873.
DOI: http://dx.doi.org/10.1556/0088.2016.65.2.7

23. Várallyay, S., Szilva, D., **Soós, Á.**, Kovács, B.: Talaj- és növényminta arzéntartalmának meghatározására irányuló minta-előkészítési módszerek összehasonlító vizsgálata.
Agrártud. Közl. 69, 167-170, 2016. ISSN: 1587-1282.

Foreign language scientific articles in international journals (7)

24. Both, E. B., Stonehouse, G. C., Lima, L. W., Fakra, S. C., Aguirre, B., Wangeline, A. L., Xiang, J., Yin, H., Jókai, Z., **Soós, Á.**, Dernovics, M., Pilon-Smits, E. A. H.: Selenium tolerance, accumulation, localization and speciation in a Cardamine hyperaccumulator and a non-hyperaccumulator.
Sci. Total Environ. 703, 1-12, 2020. ISSN: 0048-9697.
DOI: http://dx.doi.org/10.1016/j.scitotenv.2019.135041
IF: 5.589 (2018)

25. Kolláth, I. S., Molnár, Á. P., **Soós, Á.**, Fekete, E., Sándor, E., Kovács, B., Kubicek, C. P., Karaffa, L.: Manganese Deficiency Is Required for High Itaconic Acid Production From D-Xylose in Aspergillus terreus.
Front. Microbiol. 10, 1-10, 2019. EISSN: 1664-302X.
DOI: http://dx.doi.org/10.3389/fmicb.2019.01589
IF: 4.259 (2018)

26. Sumanszki, C., Kiss, E., Simon, E., Galgóczi, E., **Soós, Á.**, Patócs, A., Kovács, B., Nagy, E. V., Reismann, P.: The Association of Therapy Adherence and Thyroid Function in Adult Patients with Phenylketonuria.
Ann. Nutr. Metab. 75 (1), 16-23, 2019. ISSN: 0250-6807.
DOI: http://dx.doi.org/10.1159/000501301
IF: 3.051 (2018)

27. Molnár, Á. P., Németh, Z., Kolláth, I. S., Fekete, E., Flipphi, M., Ág, N., **Soós, Á.**, Kovács, B., Sándor, E., Kubicek, C. P., Karaffa, L.: High oxygen tension increases itaconic acid accumulation, glucose consumption, and the expression and activity of alternative oxidase in Aspergillus terreus.
Appl. Microbiol. Biotechnol. 102 (20), 8799-8808, 2018. ISSN: 0175-7598.
DOI: http://dx.doi.org/10.1007/s00253-018-9325-6
IF: 3.67

28. Várallyay, S., Balláné Kovács, A., **Soós, Á.**, Kovács, B.: Effect of different arsenic-treatments on the dry weight of vegetative plant parts of green pea in the different phenophase of the plant development.
    Annals of Academy of Romanian Scientists Series on Agriculture, Silviculture and Veterinary Medicine Sciences. 6 (1), 144-151, 2017. ISSN: 2069-1149.

29. Bódi, É., Várallyay, S., **Soós, Á.**, Kovács, B.: Effect of molybdenum tratments on growth and molybdenum uptake of green pea.
    Annals of Academy of Romanian Scientists Series on Agriculture, Silviculture and Veterinary Medicine Sciences. 6 (1), 130-135, 2017. ISSN: 2069-1149.

30. Garousi, F., Domokos-Szabolcsy, É., Jánószky, M., Balláné Kovács, A., Veres, S., **Soós, Á.**, Kovács, B.: Selenoamino acid-enriched green pea as a value-added plant protein source for humans and livestock.
    Plant Food Hum. Nutr. 72 (1), 168-175, 2017. ISSN: 0921-9668.
    DOI: http://dx.doi.org/10.1007/s11130-017-0606-5
    IF: 2.465

Hungarian conference proceedings (5)

31. Posta, J., Nagy, D., Csámer, Á., **Soós, Á.**, Nyeste, E., Kovács, B.: A kabai meteorit elemanalitikai vizsgálata.
    In: Átfogó kutatások a kabai meteoriton : a kabai meteorit hullásának 160. évében rendezett nemzetközi konferencia előadásai : 2017. november 8. Református Kollégium, Debrecen. Szerk.: Nagy Mihály, Rózsa Péter, McIntosh Richard William, Debreceni Egyetemi Kiadó, Debrecen, 159-168, 2018, (Acta geographica ac geologica et meteorologica Debrecina. Acta Geoscientia Debrecina, ISSN 1788-4497 ; 1. különszám) ISBN: 9789633180532

32. Papp-Topa, E. A., Nyeste, E., **Soós, Á.**, Kovács, B.: Thermo iCAP 6300 ICP-OE spektrométer optimálása.
    In: 60. Magyar Spektrokémiai Vándorgyűlés és XIII. Környezetvédelmi Analitikai és Technológiai Konferencia : Program és előadás összefoglalók / Mihucz Viktor Gábor, Debreceni Egyetem, Debrecen, 53-57, 2017. ISBN: 9789639970779

33. Novák, A., **Soós, Á.**, Nagy, É., Kovács, B., Czipa, N.: Gyógynövény kivonatos méhkészítmények mikroelem tartalmának vizsgálata.
    In: XXXVI. Óvári Tudományos Nap - Hagyomány és innováció az agrár- és élelmiszergazdaságban: Tudományos Nap Összefoglalók. Szerk.: Bali Papp Ágnes, Szalka Éva, Széchenyi István Egyetem Mezőgazdaság- és Élelmiszertudományi Kar, Mosonmagyaróvár, 125-133, 2016. ISBN: 9786155391798

34. Novák, A., **Soós, Á.**, Kovács, B., Czipa, N.: Mézek és gyógynövény kivonatos méhkészítmények makroelemtartalmának vizsgálata.
    In: Őshonos- és tájfajták, ökotermékek, egészséges táplálkozás, vidékfejlesztés : a XXI. század mezőgazdasági stratégiái : tudományos konferencia, Nyíregyháza. Szerk.: Tóth Csilla, Nyíregyházi Egyetem Műszaki és Agrártudományi Intézet, Nyíregyháza, 279-285, 2016. ISBN: 9786155545696
35. Várallyay, S., Veres, S., **Soós, Á.**, Bódi, É., Kovács, B.: Arzén kezelés hatása kukorica növény tápanyagfelvételére rhizoboxos nevelési körülmények között.
    In: Georgikon Napok konferenciakötet : 2015-ös előadások, Pannon Egyetem, [Keszthely], 458-464, 2015. ISBN: 9789639639829

Foreign language conference proceedings (2)

36. Diósi, G., **Soós, Á.**, Sipos, P.: Mineral element composition of grain.
    In: Integration of science in food chain : Book of proceedings. Ed.: Tekla Engelhardt, István Dalmadi, László Baranyai, Csilla Mohácsi-Farkas, Faculty of Food Science, Corvinus University, Budapest, 61-64, 2015. ISBN: 9789635036035
37. Kovács, B., Bódi, É., **Soós, Á.**, Andrási, D.: Application of ICP-MS in various fields of research.
    In: 28th Indian Society for Mass Spectrometry Symposium cum Workshop on Mass Spectrometry. Ed.: Suresh K. Aggarwal, P. G. Jaison, Arnab Sarkar, Prudent Arts & FAb Private Limited, Mumbai, 69-80, 2014. ISBN: 9788190444262

Hungarian abstracts (6)

38. Posta, J., Nagy, D., **Soós, Á.**, Kovács, B.: A kabai meteorit elemanalitikai vizsgálata.
    In: Átfogó kutatások a kabai meteoriton, Debreceni Református Kollégium, Debrecen, 15, 2017.
39. Várallyay, S., Balláné Kovács, A., **Soós, Á.**, Kovács, B.: Arzénkezelés hatása a zöldborsó kén, magnézium, valamint vas felvételére.
    In: Magyar Spektrokémia vándorgyűlés és XIII. Környezetvédelmi Analitikai és Technológiai Konferencia. Debrecen, 2017. 08. 23-25 : Program és előadás összefoglalók, [Debreceni Egyetem], [Debrecen], 150-153, 2017.
40. Bódi, É., Várallyay, S., **Soós, Á.**, Kovács, B.: Molibdén kezelés hatása a zöldborsó makroelem felvételére.
    In: Magyar Spektrokémia vándorgyűlés és XIII. Környezetvédelmi Analitikai és Technológiai Konferencia. Debrecen, 2017. 08. 23-25 : Program és előadás összefoglalók, [Debreceni Egyetem], [Debrecen], 147-149, 2017.

41. Várallyay, S., Veres, S., **Soós, Á.**, Bódi, É., Kovács, B.: Arzén kezelés hatása kukorica növény tápanyagfelvételére rhizoboxos nevelési körülmények között.
In: LVII. Georgikon Napok = 57th Georgikon Scientific Conference : Kivonat-kötet : Programfüzet, valamint az elhangzó és poszter előadások rövid kivonatainak gyűjteménye : [agrárgazdaság a növekedéskor után] : [2015. október 1-2., Keszthely]. Szerk.: Nagy Zita Barabara, Pannon Egyetem Georgikon Kar, Keszthely, 123, 2015. ISBN: 9789639639812

42. Várallyay, S., Andrási, D., **Soós, Á.**, Kovács, B.: Mintaelőkészítési módszerek összehasonlítása tejminta elemtartalom vizsgálatára.
In: XI. Környezetvédelmi Analitikai és Technológiai Konferencia : Program és Előadás Összefoglalók, KAT13, Hajdúszoboszló, 93, 2013.

43. **Soós, Á.**: Mikroelemtartalom-mérési módszer kidolgozása borra, direkt mintabevitel esetén, multielemes ICP-MS mérésnél.
In: Kari Tudományos Diákköri Konferencia. Kiadta: a Debreceni Egyetem Mezőgazdaság-Élelmiszertudományi és Környezetgazdálkodási Kar Tudományos Diákköri Tanács, Debreceni Egyetem Mezőgazdaság- Élelmiszertudományi És Környezetgazdálkodási Kar Tudományos Diákköri Tanács, Debrecen, 42, 2012.

Foreign language abstracts (16)

44. Sumanszki, C., Kiss, E., Simon, E., Galgóczi, E., **Soós, Á.**, Patócs, A., Kovács, B., Nagy, E. V., Reismann, P.: Iodine and selenium status in adult PKU.
Endocrine Abstracts. 63, P781, 2019. ISSN: 1470-3947.
DOI: http://dx.doi.org/10.1530/endoabs.63.P781

45. Karaffa, L., Molnár, Á. P., Kolláth, I. S., Kovács, B., **Soós, Á.**, Kubicek, C. P., Fekete, E.: Aspergillus terreus itaconic acid fermentation technology reflects the physiological requirements of overflow metabolism.
In: 11th International Mycological Congress: Mycological Discoveries for a Better World : Abstract Book, Ana G. Méndez University, San Juan, Puerto Rico, 355-356, 2018.

46. Bódi, É., Várallyay, S., **Soós, Á.**, Kovács, B.: Changes of Microelement Content Of Green Pea and Maize by Molybdenum Enrichment.
In: FSD 2018 3rd Food Structure & Design Conference : Abstract Book. Ed.: Zoltán Győri, Debreceni Egyetem, Debrecen, 46-47, 2018. ISBN: 9789634900245

47. Kolláth, I. S., Fekete, E., Sándor, E., **Soós, Á.**, Kovács, B., Karaffa, L.: Effect of copper (II) ions on itaconic acid prodoction by Aspergilius terreus.
In: VII. Interdiszciplináris Doktorandusz Konferencia 2018 absztraktkötet. Szerk.: Bódog Ferenc, Pécsi Tudományegyetem Doktorandusz Önkormányzat, Pécs, 74, 2018. ISBN: 9789634292104

48. Bódi, É., Balláné Kovács, A., Várallyay, S., **Soós, Á.**, Kovács, B.: Growth of Green Pea by Effect of Molybdenum Treatments at Different Developmental Stages.
In: Scientific researches in food production, University of Debrecen - Proceedings of abstracts. Ed.: Béla Kovács, Nikolett Czipa, Ferenc Peles, Éva Bacskainé Bódi, Andrea Kántor, Flóra Mária Szabóné Petróczki, Loránd Alexa, Debreceni Egyetem, Mezőgazdaság-, Élelmiszertudományi és Környezetgazdálkodási Kar, Debrecen, 17, 2018. ISBN: 9789634900412

49. Kovács, B., Nagy, K., Lévai, L., Várallyay, S., **Soós, Á.**, Papp-Topa, E. A., Bódi, É.: The Effect of Selenium Treatment on Maize and Sunflower Plants in Nutrient Solution and Soil-Plant System.
In: FSD 2018 3rd Food Structure & Design Conference : Abstract Book. Ed.: Zoltán Győri, Debreceni Egyetem, Debrecen, 38-39, 2018. ISBN: 9789634900245

50. Várallyay, S., Veres, S., Bódi, É., **Soós, Á.**, Papp-Topa, E. A., Kovács, B.: Effect of Arsenic on the Growing Parameters of Sunflower Seedlings.
In: Scientific researches in food production, FBFS, SUA in Nitra - Proceedings of abstracts. Ed.: Miroslava Kačániová, Slovak University of Agriculture in Nitra, Nitra, 40, 2017. ISBN: 9788055217369

51. Várallyay, S., Veres, S., Bódi, É., **Soós, Á.**, Papp-Topa, E. A., Kovács, B.: Effect of Inorganic Arsenic forms on the Uptake of Boron and Calcium by Sunflower Seedlings.
In: Scientific researches in food production, FBFS, SUA in Nitra - Proceedings of abstracts. Ed.: Miroslava Kačániová, Slovak University of Agriculture in Nitra, Nitra, 41, 2017. ISBN: 9788055217369

52. Bódi, É., Várallyay, S., **Soós, Á.**, Papp-Topa, E. A., Kovács, B.: Examination of the Molybdenum Uptake of the Green Pea at Different Developmental Stages.
In: Scientific researches in food production, FBFS, SUA in Nitra - Proceedings of abstracts. Ed.: Miroslava Kačániová, Slovak University of Agriculture in Nitra, Nitra, 19, 2017. ISBN: 9788055217369

53. Papp-Topa, E. A., Nyeste, E., **Soós, Á.**, Bódi, É., Várallyay, S., Kiss, D., Kovács, B.: Optimization of Inductively Coupled Plasma Optical Emission Spectrometer.
In: Scientific researches in food production, FBFS, SUA in Nitra - Proceedings of abstracts. Ed.: Miroslava Kačániová, Slovak University of Agriculture in Nitra, Nitra, 12, 2017. ISBN: 9788055217369

54. Kolláth, I. S., Molnár, Á. P., Fekete, E., Sándor, E., **Soós, Á.**, Kovács, B., Kubicek, C. P., Karaffa, L.: Production of itaconic acid from D-xylose by Aspergillus terreus. Utánközlés párhuzamos közlés,
In: 2nd Symposium on Plant Biomass Conversation by Fungi, Westerdijk Fungal Biodiversity Institute, Utrecht, Hollandia, 25, 2017.

55. Kolláth, I. S., Molnár, Á. P., Fekete, E., Sándor, E., **Soós, Á.**, Kovács, B., Kubicek, C. P., Karaffa, L.: Production of itaconic acid from D-xylose by Aspergillus terreus.
    In: Abstracts of the 5th Central European Forum for Microbiology. Ed.: K. Márialigeti, O. Dobay, Akadémiai Kiadó, Budapest, 136, 2017, (Acta Microbiologica et Immunologica Hungarica, ISSN 1217-8950 ; 64. (Suppl))

56. Várallyay, S., Veres, S., **Soós, Á.**, Bódi, É., Kovács, B.: Effect of different arsenic treatments on the uptake of Ca, K, S, P and Mg by maize seedlings.
    In: 1st Meeting of Young Researchers from V4 Countries. Book of Abstract. Ed.: Monika Wesołowska, University of Rzeszow, Rzeszow, 90, 2016.

57. Bódi, É., Várallyay, S., **Soós, Á.**, Kovács, B.: Effect of molybdenum treatment on the element content of maize and green pea in a long-term field experiment.
    In: 1st Meeting of Young Researchers from V4 Countries : Book of Abstract. Ed.: Monika Wesołowska, University of Rzeszow, Rzeszow, 17, 2016.

58. Novák, A., **Soós, Á.**, Kovács, B., Czipa, N.: Mézek és gyógynövény kivonatos méhkészítmények makroelem tartalmának vizsgálata = Analysis of makroelement content in honeys and herdhoneys.
    In: Őshonos- és tájfajták - Ökotermékek - Egészséges Táplálkozás - Vidékfejlesztés: A XXI. század mezőgazdasági stratégiái Konferencia. Szerk.: Irinyiné Oláh Katalin, Kosztyuné Krajnyák Edit, Tóth Csilla, Lajtos István, Nyíregyházi Egyetem, Nyíregyháza, 62, 2016. ISBN: 9786155545696

59. Kovács, B., Bódi, É., Garousi, F., Várallyay, S., **Soós, Á.**, Vágó, X., Andrási, D.: Problems and solutions in the application of ICP-MS for analysis of trace elements in various samples.
    World Academy of Science, Engineering and Technology. International Journal of Nutrition and Food Engineering. 2 (6), 1, 2015. ISSN: 2010-376X.

Total IF of journals (all publications): 22,748
Total IF of journals (publications related to the dissertation): 3,714

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

13 February, 2020