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Microwave-assisted sample preparation of Hungarian raw propolis in quartz vessels and element analysis by ICP-OES and ICP-MS for geographical identification

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ABSTRACT

One of the aims of this study was to improve the sample throughput of a microwave-assisted closed vessel digestion system by using small quartz vials in polytetrafluoroethylene (PTFE) vessels for the sample preparation of raw propolis samples in small amounts. The digested samples were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) analyzing 36 elements. Limit of detection was low enough to measure all the elements, with the exception of La, in all raw propolis samples. There were no cross-contamination between the quartz vials, therefore independent samples can be prepared in the same PTFE vessel. Accuracy of the method was checked by spike recoveries and by analyzing BCR 189 wholemeal flour and two plant samples from a collaborative study. The means of RSDs were 5.3%–14.4% in the case of measured elements. The sample throughput was increased by three times using quartz vials in PFTE vessels besides matching with the requirements of green chemistry.

Another goal was the characterization of the element content and thereby geographical identification of Hungarian raw propolis. In total, 252 samples were analyzed and their statistical characteristics were described. We cannot find globally such results of propolis element content, which is representing one country and with such a number of elements and samples. All the elements have positive skew and positive kurtosis. Concentration range is above two orders of magnitude in the case of Ba, Zn, V, Cr, Ni, Cd and Eu elements. The decimal logarithm of element concentrations was used for geographical identification of raw propolis samples originating from seven regions of Hungary by linear discriminant analysis (LDA). Grouping of the samples of the Northern Great Plain was the most effective with 96.3% and 77.8% based on the original method and the cross validation, respectively. The same indicators for all the groups are 76.6% and 61.5%.

1. Introduction

Propolis, also called “bee glue”, 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 [1]. The main constituents are 50–60% of resin and balm and 30–40% of wax. Other components, such as pollens, vitamins and minerals [2] are typically below 5%. More than 300 compounds were identified by analyzing the organic content of propolis [3], such as alcohols, aliphatic acids, aromatic acids, esters, flavonoids, anthraquinones, ketones, sugars, terpenes [4]. The biologically positive effect of the propolis connects to e.g. its flavonoid content. However, the mineral content of raw propolis has not been so thoroughly studied, despite several articles published in this topic [5–11]. Raw propolis is the base of propolis tincture, a possible nutraceutical [12], therefore the concentration of essential and potentially toxic elements are often analyzed [5, 13]. However, it should be highlighted that the physiological effect of propolis to the human body are more complex from the point of essential and toxic minerals. Propolis is usually consumed in processed form, like tincture or capsule, therefore the effect of preparation circumstances is not negligible. For example, extraction conditions dramatically influence the essential and potentially toxic element content of the propolis tincture [5,14]. The mineral content of raw propolis or any kind of food also can be often useful to identify the geographical origin of them [10,

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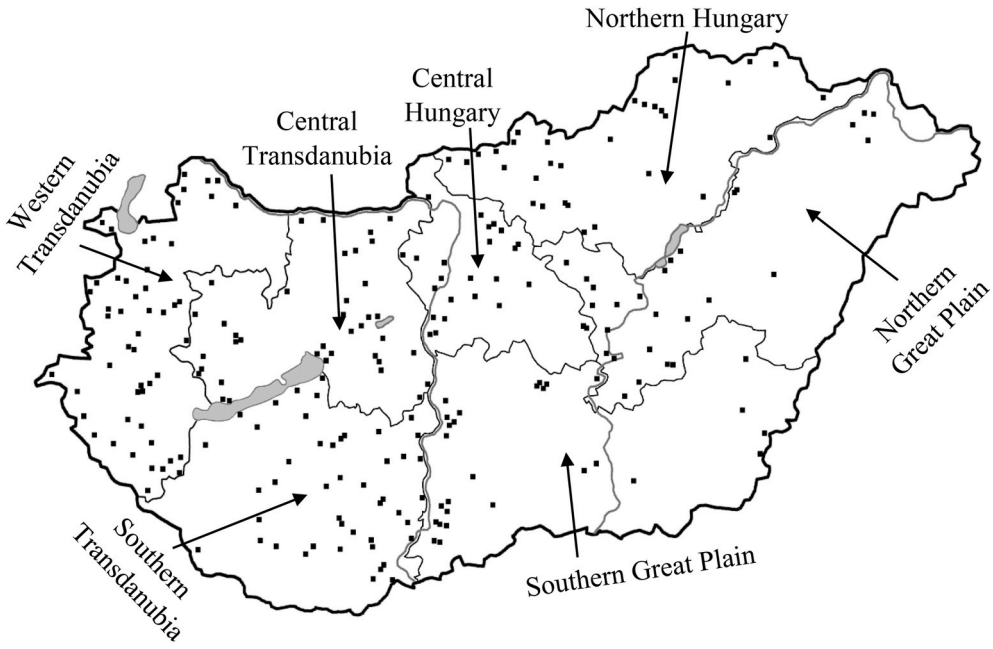

Fig. 1. The sampling locales of Hungarian raw propolis samples.

11,15–17]. This can be beneficial, because the latter can have an impact on the organic content as well as on the biological properties of the propolis [18–20].

Inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are often used techniques in element analysis. A fundamental part of the analysis is the sample preparation before measurement. Usually a microwave-assisted digestion in closed polytetrafluoroethylene (PTFE) vessels is applied before analysis to digest the organic content of the biological samples and produce a liquid material [5,9,10,17]. However the lower limit of acid volume is often established, thus dilution is high in the case of samples in small amounts. A high dilution factor is often a challenge in the case of analysis of low concentration elements.

By using a smaller vessel the aforementioned problem can be improved. Single Reaction Chamber technology allows digestion of samples in small amounts in digestion vials [21,22]. A sample also can be digested in mini PTFE vessels by microwave-assisted digestion [23] or quartz tubes placed inside a holder within a household microwave oven [24]. For the aforementioned techniques one may need to purchase a new instrument or a special equipment to perform this kind of digestion. This vessel-inside-vessel technique allows to digest the sample inside the primary PTFE carrier by being encapsulating it in a smaller closed container made from quartz or Teflon. This means the sample throughput can be increased, besides using smaller amounts of reagents, all the while lowering the investment costs. Decreasing the amount of reagents and waste is an important part of green chemistry [25].

In this study we digested 0.1 g of raw propolis samples together with 2.0 mL HNO_3 and 0.6 mL H_2O_2 in quartz vials placed inside the primary PTFE vessel. This kind of sample preparation method meets with the requirements of green chemistry because of low amount of reagents. Digested samples were analyzed by ICP-OES and ICP-MS. Performance characteristics of the method were also checked. We have measured the element content of 252 Hungarian raw propolis samples and created a database. This kind of database made from Hungarian propolis has not existed so far. Moreover, we cannot find globally such results of propolis element content, which is representing one country and with such a number of elements. Element concentrations were used for geographical identification of raw propolis from different regions of Hungary.

2. Materials and methods

2.1. Chemicals

Deionized water was produced from a MilliQ system (MilliQ, Millipore Corp., Bedford, MA, USA) with 18.2 MΩ cm^{-1} conductivity during the analytical process. Calibration standards were prepared from 1000 mg L^{-1} monoelement standards (Scharlau, Barcelona, Spain) except for rare earth elements, where a multielement standard solution was used with 100 mg L^{-1} concentration (Teknolab A/S, Drøbak, Norway). Rhodium was used as an internal standard diluted from 1000 mg L^{-1} monoelement standard (Fluka, Buchs, Switzerland) for ICP-MS analysis. Nitric acid (65% w/w) and hydrogen peroxide (30% w/w) from Scharlab S.L. Sentmenat, Spain was applied for the digestion. Nitric acid was distilled with a Milestone subPUR sub-boiling distillation system (Milestone Srl, Sorisole, Italy) for further purification.

2.2. Samples

Raw propolis samples were collected in autumn of 2014 by the beekeepers from Hungarian fixed-location apiaries, and the collection was controlled by the National Beekeepers' Association of Hungary. The evaluation of the effect of collection period was not the goal of this article. In total, 252 samples were collected from all over the country. The regions were Western Transdanubia (51 samples), Central Transdanubia (38 samples), Southern Transdanubia (51 samples), Central Hungary (25 samples), Northern Hungary (32 samples), the Northern Great Plain (27 samples) and Southern Great Plain (28 samples), respectively. The sampling locales are shown in Fig. 1. Southern and Northern Great Plain are plain regions, Central Hungary, Southern and Western Transdanubia are hilly, while Northern Hungary and Central Transdanubia are mountainous parts of Hungary.

The climate in Hungary is dominantly continental with oceanic and Mediterranean effects. There is generally warm/moderately warm and dry in Southern and Northern Great Plain, moderately warm/moderately cool/cool and dry/moderately dry in Northern and Central Hungary and Central Transdanubia, moderately warm and moderately wet/moderately dry in Southern Transdanubia, while moderately cool and moderately wet/moderately dry in Western Transdanubia [26]. Resin of propolis is mainly collected from *Poplar* sp. in temperate zone

Table 1
Characteristics of the calibration and the additional concentrations in spike.

Analyzed element (unit)	Calibration range	Nr. of calibration points	Sensitivity [a]cps/unit	R^2	Median concentration in digested propolis	Additional concentration 1 (n = 17)	Additional concentration 2 (n = 5)	Additional concentration 3 (n = 1)
K (mg L^{-1})	0.5–100	8 + 1	37700	0.99997	7.92	10	50	–
Ca (mg L^{-1})	0.25–50	8 + 1	1220	1.00000	4.93	5	25	–
P (mg L^{-1})	0.25–50	8 + 1	142	1.00000	2.13	5	25	–
Mg (mg L^{-1})	0.125–25	8 + 1	382	1.00000	1.56	5	25	–
Na (mg L^{-1})	0.125–25	8 + 1	714	0.99996	0.328	1	5	–
S (mg L^{-1})	0.1–20	8 + 1	81.7	0.99997	2.34	7	35	–
Fe (mg L^{-1})	0.05–10	8 + 1	2130	1.00000	1.71	–	–	10
Al (mg L^{-1})	0.025–5	8 + 1	2960	0.99999	1.16	–	–	10
Zn (mg L^{-1})	0.025–5	8 + 1	3030	0.99999	0.547	–	–	10
B (mg L^{-1})	0.01–2	8 + 1	941	1.00000	0.0541	0.1	0.5	–
Ba (mg L^{-1})	0.005–1	8 + 1	614000	0.99999	0.0859	–	–	1
Sr (mg L^{-1})	0.005–1	8 + 1	733000	0.99962	0.0172	0.03	0.15	–
Mn (μg L^{-1})	0.2–200	10 + 3	10900	0.99993	9.58	20	100	–
Cu (μg L^{-1})	0.2–200	10 + 3	1960	0.99992	2.94	2	10	–
V (μg L^{-1})	0.1–100	10 + 3	5950	0.99991	0.408	1	5	–
Cr (μg L^{-1})	0.1–100	10 + 3	6130	0.99994	1.00	1	5	–
Co (μg L^{-1})	0.1–100	10 + 3	8310	0.99993	0.206	1	5	–
Ni (μg L^{-1})	0.1–100	10 + 3	1660	0.99995	0.566	1	5	–
Mo (μg L^{-1})	0.1–100	10 + 3	2060	0.99987	0.153	2	10	–
Cs (μg L^{-1})	0.1–100	10 + 3	15000	0.99987	0.0286	1	5	–
Cd (μg L^{-1})	0.04–40	10 + 3	1750	0.99987	0.0666	1	5	–
La (μg L^{-1})	0.1–20	8 + 3	12500	0.99963	0.194	1	5	–
Ce (μg L^{-1})	0.1–20	8 + 3	13800	0.99971	0.364	1	5	–
Pr (μg L^{-1})	0.1–20	8 + 3	24700	0.99972	0.0404	1	5	–
Nd (μg L^{-1})	0.1–20	8 + 3	5280	0.99973	0.154	1	5	–
Sm (μg L^{-1})	0.1–20	8 + 3	5290	0.99984	0.0302	1	5	–
Eu (μg L^{-1})	0.1–20	8 + 3	21400	0.99969	0.0104	1	5	–
Gd (μg L^{-1})	0.1–20	8 + 3	6750	0.99986	0.0310	1	5	–
Tb (μg L^{-1})	0.1–20	8 + 3	42600	0.99979	0.00404	1	5	–
Dy (μg L^{-1})	0.1–20	8 + 3	11500	0.99978	0.0187	1	5	–
Ho (μg L^{-1})	0.1–20	8 + 3	47900	0.99972	0.00356	1	5	–
Er (μg L^{-1})	0.1–20	8 + 3	16300	0.99954	0.0102	1	5	–
Tm (μg L^{-1})	0.1–20	8 + 3	52800	0.99965	0.00129	1	5	–
Yb (μg L^{-1})	0.1–20	8 + 3	12300	0.99983	0.00782	1	5	–
Lu (μg L^{-1})	0.1–20	8 + 3	58000	0.99966	0.00108	1	5	–
U (μg L^{-1})	0.1–20	8 + 3	48500	0.99952	0.0120	1	5	–

[a] Cps: counts per second, unit of sensitivity is corresponding with the unit in the first column (cps/mg L^{-1}or cps/μg L^{-1}).

[27]. About 2.7% of Hungarian forests are white poplar (*Populus alba*), which is a possible source of propolis, present mostly in Mid-Danube-Tisza Plain (Southern Great Plain) [28]. Although different *Poplar* sp. are present all over the country, but not do not necessarily form a continuous forest. However, bees take up to 0.5 km distance to find the appropriate tree for the collection of resin [29].

To the best of our knowledge, certified reference material for element composition of propolis does not exist, therefore plant reference material with high carbohydrate (generally cellulose or starch) content is usually used as a quality control [9–11,30]. In this study rice straw (*Oryza sativa*) and silver grass (*Miscanthus* sp.) plant samples from a collaborative study (Wageningen Evaluating Program for Analytical Laboratories) as well as BCR 189 wholemeal flour certified reference material were analyzed as a quality control.

2.3. Sample preparation

Before homogenization raw propolis samples were frozen with liquid nitrogen then were ground in a mortar. They were stored in plastic scintillation vials. Approximately 0.1 g raw propolis were weighted into quartz tubes (80 mm × 16 mm, total volume is approx. 11 mL), then 2.0 mL nitric acid were pipetted and were left overnight. The next day 0.6 mL H_2O_2 were added and the quartz tubes were sealed with household Teflon tape and were closed during the whole digestion process. Maximum three closed quartz tubes were placed into PTFE vessels (total volume 100 mL, maximum volume 50 mL) of the microwave digestion system. Ten mL distilled water was added between the quartz and the PTFE container, because it slows down the reaction and compensates the inner pressure inside the quartz tubes. The temperature controller needle was dipped into water between the PTFE and quartz vessel. Therefore, the temperature of samples mixed with the acid were not directly measured. Then PTFE receptacles were closed smoothly and were placed into the SK-10 high pressure segmented rotor then into the microwave digestion system (Milestone Start D, Milestone Srl, Sorisole, Italy). The samples were heated up to 180 °C in 15 min, were kept at a constant temperature at 180 °C for 20 min, finally cooled down to room temperature at about 60 min. Digestion program was adapted from Milestone cookbook used for similar biological materials in the case of digestion in PTFE vessels. After the quartz tubes were opened, digested samples were washed into 15 mL centrifuge tubes and filled up to 9.5–10.5 mL with MilliQ water. A volumetric flask was not used in order to avoid cross-contamination and reaching the lowest limit of detections. The accurate volume and the dilution factor were calculated in relation to the mass of the digested sample multiplied by the density of the solution. Sample preparation was done in triplicate. Digestion of propolis samples results a homogenous liquid without any visual particles inside, therefore samples were not filtered. Rice straw and silver grass samples were also not filtered after digestion, despite they contained solid particles. Supernatant was separated from insoluble matters after deposition in a short time.

After digestion, quartz tubes were washed with deionized water several times, then scrubbed with cotton buds, finally being washed again several times with deionized water. These tubes were left to dry. 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 deionized water. Cleaned and dried plastic tools were held in

plastic bags prior to use. We have used new plastic tools for every sample to avoid cross-contamination.

2.4. Apparatus

Digested samples were measured by inductively coupled plasma optical emission spectrometry (ICP-OES; Thermo Scientific iCAP 6300 Dual view) and inductively coupled plasma mass spectrometry (ICP-MS, Thermo Scientific X-Series II). ICP-OES parameters were the following: forward power 1350 W, plasma gas flow rate 12.0 L min^{-1}, nebulizer gas flow rate 1.0 L min^{-1}, auxiliary gas flow rate 1.0 L min^{-1}, sample uptake speed 50 rpm with white/orange Tygon tubing, plasma view axial, integration time 10-10 s at low and high wavelength ranges. A concentric nebulizer was used with a cyclonic spray chamber. No internal standard correction was applied for ICP-OES analysis. The analyzed elements (and their wavelengths in nm) were Al (394.401), B (208.959), Ba (455.403), Ca (315.887), Fe (238.204), K (766.490), Mg (279.079), Na (818.326), P (213.618), S (182.034), Sr (407.771) and Zn (213.856). The measurement was controlled by iTEVA 2.8.0.97 software. ICP-MS parameters were as follows: forward power 1400 W, plasma gas flow rate 14.0 L min^{-1}, nebulizer gas flow rate 0.86 L min^{-1}, auxiliary gas flow rate 0.88 L min^{-1}, sample uptake speed 20 rpm with yellow/orange Tygon tubing, pole bias −16.0 V, hexapole bias −10.0 V, dwell time 100 ms, sweep 9, main run 3. H_2–He (in 7:93%) collision cell gas was applied with 6.0 mL min^{-1} flow rate. A concentric nebulizer was used with a Peltier cooled conical spray chamber (2 °C). Other parameters were optimized daily to maximize ^{59}Co, ^{115}In and ^{238}U signals and minimize Ba^{2+}/Ba^{+} and CeO^{+}/Ce^{+} ratios from tune solution. Digested samples were diluted five times for decreasing the acid content of the solution. Rh was used as an internal standard in 40 μg L^{-1}. Analyzed isotopes were ^{51}V, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{95}Mo, ^{111}Cd, ^{133}Cs, ^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{153}Eu, ^{157}Gd, ^{159}Tb, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{172}Yb, ^{175}Lu and ^{238}U. Thermo PlasmaLab 2.5.10.319 software was applied for the measurement.

2.5. Statistical analysis

The graphs were made by using Microsoft Excel 2013. IBM SPSS 25.0 was used for principal component analysis (PCA), linear discriminant analysis (LDA), Kolmogorov-Smirnov and Shapiro-Wilk tests, moreover for testing skewness and kurtosis.

3. Results and discussion

3.1. Performance characteristics of the analytical method

3.1.1. Calibration range, linearity and sensitivity

The calibration ranges, coefficient of determination (R^2) and the sensitivities of the calibration curves are present in Table 1. Calibration ranges were adjusted to the expectable concentration in digested propolis. Those cases when analyte concentration was out of calibration range, samples were diluted five-times or as necessary with 3 M HNO_3. Eight calibration points were used in ICP-OES analysis as well as for lanthanides and U, while ten calibration points were applied for the remaining elements measured by ICP-MS. In addition, one and three blanks were measured in the calibration of ICP-OES and ICP-MS, respectively. Coefficient of determination of the calibration curves were higher than 0.99990 for all elements measured by ICP-OES, with the exception of Sr, while R^2 were exceeded 0.99950 for all elements measured by ICP-MS.

3.1.2. Limit of detection (LOD) and cross-contamination

Limit of detections (LODs) can be increased by limiting the volume of reagents during the sample preparation. Firstly, we have checked 1.0 mL HNO_3 and 0.3 mL H_2O_2 reagent volumes, however the digestion was not acceptable, because solid particles remained from the raw propolis samples. Therefore 2.0 mL HNO_3 and 0.6 mL H_2O_2 was necessary for the digestion using approximately a 0.1 g sample, which after sample preparation results in a homogenous liquid. Although quartz tubes were cleaned by just scrubbing and washing several times with deionized water between sample preparation periods, there was no submersion into acid or any different cleaning step, and the dilution factors were quite high (500-fold in the case of ICP-MS, while 100-fold in the case of ICP-OES), LODs were low enough for analyzing all the elements from raw propolis samples except one. Limit of detections were determined by the followings. Two blanks were digested in every digestion process, and 8 to 10 digested blanks were analyzed in an analysis period. The measured concentrations of blanks were averaged and it was deducted from the concentration of samples measured in the analysis period. The threefold of standard deviation was determined and multiplied by the dilution factor (500-fold in the case of ICP-MS, while 100-fold in the case of ICP-OES). These values were averaged from all analysis periods and were given as LODs.

Table 2
Limit of detection (LOD) in raw propolis by the applied method.

Analyzed element (unit)	Limit of detection (LOD)
Lu (μg kg^{-1})	0.0957
Tm (μg kg^{-1})	0.123
Ho (μg kg^{-1})	0.127
Tb (μg kg^{-1})	0.154
Eu (μg kg^{-1})	0.196
Er (μg kg^{-1})	0.223
Yb (μg kg^{-1})	0.229
Dy (μg kg^{-1})	0.370
Gd (μg kg^{-1})	0.421
Pr (μg kg^{-1})	0.478
U (μg kg^{-1})	0.529
Cs (μg kg^{-1})	0.601
Sm (μg kg^{-1})	0.686
Nd (μg kg^{-1})	1.51
La (μg kg^{-1})	1.53
Ce (μg kg^{-1})	3.17
Co (μg kg^{-1})	4.29
Cd (μg kg^{-1})	4.22
V (μg kg^{-1})	12.4
Mo (μg kg^{-1})	14.8
Ni (μg kg^{-1})	39.0
Cu (μg kg^{-1})	40.9
Mn (μg kg^{-1})	53.5
Cr (μg kg^{-1})	55.5
Sr (mg kg^{-1})	0.0811
Ba (mg kg^{-1})	0.258
B (mg kg^{-1})	0.640
Zn (mg kg^{-1})	1.29
Fe (mg kg^{-1})	2.10
Mg (mg kg^{-1})	2.94
P (mg kg^{-1})	3.00
S (mg kg^{-1})	4.47
Al (mg kg^{-1})	4.94
Na (mg kg^{-1})	5.31
K (mg kg^{-1})	6.91
Ca (mg kg^{-1})	56.7

Limit of detections were shown in Table 2. The limit of detections in relation to ICP-MS are the following: Lu, Tm, Ho, Tb, Eu, Er, Yb, Dy, Gd, Pr, U, Cs and Sm LODs are between 0.0957 and 0.686 μg kg^{-1}, Nd, La, Ce, Co, Cd, V and Mo LODs are between 1.51 and 14.8 μg kg^{-1}, while the LODs for Ni, Cu, Mn and Cr are between 39.0 and 55.5 μg kg^{-1}. The limit of detections in relation to ICP-OES are: LODs of Sr, Ba and B are between 0.0811 and 0.639 mg kg^{-1}, LODs of Zn, Fe, Mg, P, S, Al, Na and K are between 1.28 and 6.91 mg kg^{-1}, while Ca has the highest LOD with 56.7 mg kg^{-1} concentration. In all 35 element concentrations, with the exception of La, could be measured from all the raw propolis samples.

The cross-contamination between the quartz vessels was also checked. A quartz vessel with a raw propolis sample and another two quartz tubes with just the reagents were placed into the same PTFE vessel. The element concentrations were under the LOD in the

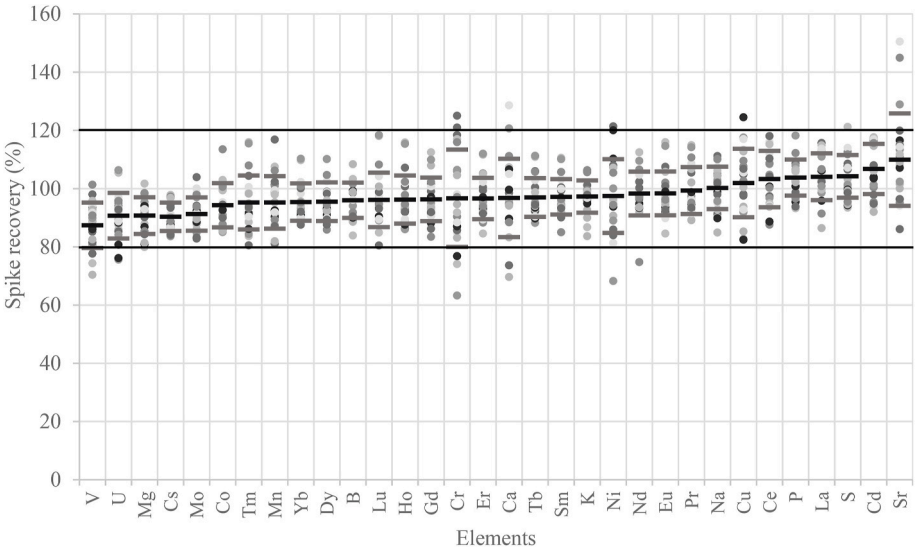

Fig. 2. Spike recoveries in raw propolis samples (n = 22).

Table 3
Reported and measured values of plant samples (rice straw and silver grass) from a collaborative study.

Element (unit)	Rice straw (*Oryza sativa*)			Silver grass (*Miscanthus* sp.)		
	Reported value (median ± MAD)	Measured value (mean ± standard deviation)	Similarity (%)	Reported value (median ± MAD)	Measured value (mean ± standard deviation)	Similarity (%)
Al[a] (mg kg^{-1})	143 ± 34	110 ± 8	77.1	256.5 ± 166	253 ± 3	98.8
B (mg kg^{-1})	11.0 ± 4.0	12.6 ± 0.7	114.6	3.583 ± 0.623	3.09 ± 0.52	87.6
Ba (mg kg^{-1})	23.6 ± 3.2	23.8 ± 1.0	100.8	18.37 ± 1.50	18.3 ± 0.2	94.9
Ca (g kg^{-1})	3.25 ± 0.150	2.98 ± 0.24	91.8	3.100 ± 0.210	2.74 ± 0.04	89.6
Cd (μg kg^{-1})	10.0 ± 3.0	12.6 ± 1.8	126.2	115.0 ± 8.7	129 ± 3	108.8
Co (μg kg^{-1})	208 ± 23	194 ± 11	93.1	83.33 ± 11.27	72.3 ± 2.5	100.7
Cr (μg kg^{-1})	6630 ± 1840	9800 ± 700	147.8	1168 ± 116	830 ± 118	83.5
Cs (μg kg^{-1})	48.6 ± 8.3	52.0 ± 2.8	106.9	56.75 ± 15.35	33.4 ± 1.4	58.9
Cu (mg kg^{-1})	3.00 ± 0.24	2.98 ± 0.14	99.4	4.240 ± 0.625	4.07 ± 0.20	99.8
Fe (mg kg^{-1})	172 ± 23	170 ± 16	98.9	350.0 ± 39.0	326 ± 1	91.7
K (g kg^{-1})	15.7 ± 1.03	14.1 ± 0.6	89.8	3.585 ± 0.260	3.46 ± 0.20	95.8
Mg (g kg^{-1})	1.18 ± 0.063	1.13 ± 0.05	95.8	0.8400 ± 0.0410	0.804 ± 0.040	96.4
Mn (mg kg^{-1})	336 ± 35	340 ± 25	101.2	68.48 ± 4.95	65.6 ± 1.6	94.4
Mo (μg kg^{-1})	2000 ± 290	2220 ± 150	111.1	1201 ± 129	1070 ± 30	92.5
Na (mg kg^{-1})	88.0 ± 16.28	97.7 ± 5.0	111.0	92.00 ± 10.92	92.3 ± 0.9	109.2
Ni (μg kg^{-1})	5440 ± 1240	6160 ± 370	113.1	440.3 ± 60.7	359 ± 9	86.6
P (g kg^{-1})	0.776 ± 0.044	0.830 ± 0.035	107.0	0.7720 ± 0.042	0.775 ± 0.012	101.2
S (g kg^{-1})	0.810 ± 0.058	0.907 ± 0.041	111.9	0.7845 ± 0.054	0.821 ± 0.027	107.6
Sr (mg kg^{-1})	12.3 ± 1.00	12.2 ± 0.7	99.0	6.950 ± 0.300	7.30 ± 0.09	100.7
V (μg kg^{-1})	300 ± 9.0	308 ± 25	102.7	982 ± 110.0	783 ± 45	93.2
Zn (mg kg^{-1})	17.3 ± 2.32	17.6 ± 0.9	101.8	28.27 ± 2.17	27.3 ± 0.5	97.4

[a] Acid soluble total element content.

aforementioned two tubes, so it can be concluded that there is no cross-contamination between the quartz tubes placed into the same PTFE container. Therefore, independent samples can be prepared in the same digestion vessel.

3.1.3. Accuracy

The accuracy of the method was checked by spiking of 22 samples during the measurement process. Two different additional concentrations were used ("additional concentration 1 and 2"), which are present in Table 1. At least one spiking was checked in an analyzed sample set. Spiked propolis samples were chosen randomly from prepared samples. The means of spike recoveries were between 87.4 and 109.9% in the case of V, Cr, Mn, Co, Ni, Cu, Mo, Cd, Cs, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, U (ICP-MS), B, Ca, K, Mg, Na, P, S and Sr (ICP-OES), respectively. The spike recoveries of individual spiked samples are presented with points, the means are shown with black rectangles, while the standard deviations are marked with grey rectangles in Fig. 2. The spiking of Al, Ba, Fe and Zn was done separately in one another sample with "additional concentration 3" which is presented in Table 1. Recoveries were 86.6%, 119.6%, 100.0% and 101.5%, respectively.

Accuracy was also checked by analyzing two plant samples from Wageningen plant exchange program, moreover BCR 189 wholemeal flour was also prepared. The results are shown in Table 3 and Table 4. Results are in good agreement with the reported values except in the case of the next elements. We have measured notable higher concentration in the case of Cd and Cr content of rice straw, while in the case of Al in rice straw and Cs in silver grass lower concentrations were measured than the expected. Other results match 80–120% with the reported values. Besides, results in BCR 189 wholemeal flour match well with the reported values. Based on the spike recoveries and the analysis of plant samples we have evaluated the accuracy of the method to be acceptable.

Table 4
Certified, indicative and analyzed values in BCR 189 Wholemeal Flour certified reference material.

Element (unit)	Certified value ± uncertainty	Indicative value	Measured value (mean ± standard deviation)	Similarity (%)
Ca (g kg^{-1})	–	0.52	0.503 ± 0.016	96.7
Cd (μg kg^{-1})	71.3 ± 3	–	67.5 ± 0.9	94.7
Cr (μg kg^{-1})	–	57–73	47.4 ± 8.4	–
Cu (mg kg^{-1})	6.4 ± 0.2	–	5.85 ± 0.09	91.4
Fe (mg kg^{-1})	68.3 ± 1.9	–	63.7 ± 1.3	93.3
K (g kg^{-1})	–	6.3	5.45 ± 0.01	86.5
Mn (mg kg^{-1})	63.3 ± 1.6	–	56.3 ± 0.7	88.9
Na (g kg^{-1})	–	0.04	0.0357 ± 0.0003	89.3
Ni (mg kg^{-1})	–	0.38	0.303 ± 0.029	79.7
P (g kg^{-1})	–	5.3	5.35 ± 0.02	100.9
Zn (mg kg^{-1})	56.6 ± 1.7	–	57.1 ± 0.2	100.9

3.1.4. Repeatability

Repeatability was presented in Fig. 3 based on three replicates of 252 raw propolis samples. We have found that macro and microelements measured by ICP-OES have <10% relative standard deviation (RSD) except for Ba. The mean of RSD was 5.3%–9.2%, while Ba has 12.3%. Microelements measured by ICP-MS have 7.5–12.3% RSD, while lanthanides and U have between 10.9% and 14.4%.

3.1.5. Uncertainty of measurement

The uncertainty of the measurement was estimated by considering the uncertainty of the balance (u_b), the stock standard solutions (u_{std}) and the repeatability (u_{rep}). The uncertainties of the separate factors were calculated by the $\frac{RSD}{\sqrt{3}}$ equation, while the combined uncertainty (u_{comb}) was calculated as follows: $u_{comb} = \sqrt{u_b^2 + u_{std}^2 + u_{rep}^2}$. The expanded uncertainty (U) was calculated by multiplying the u_{comb} with 2 as a coverage factor (k = 2). It was found that the uncertainty of the balance and the standard stock solution is negligible (≤0.5%) of the u_{comb}, and the expanded uncertainty is mostly depending on the uncertainty of the repeatability. As an example, the expanded uncertainty of potassium, strontium, ytterbium and lutetium are 6.1%, 9.7%, 13.5% and 16.6%, respectively.

3.1.6. Stability

While analysis periods lasted between 8 and 10 h, stability should be checked. The stability of the analysis was checked by examining the 10% calibration point of at least 25 samples as quality control (QC) points.

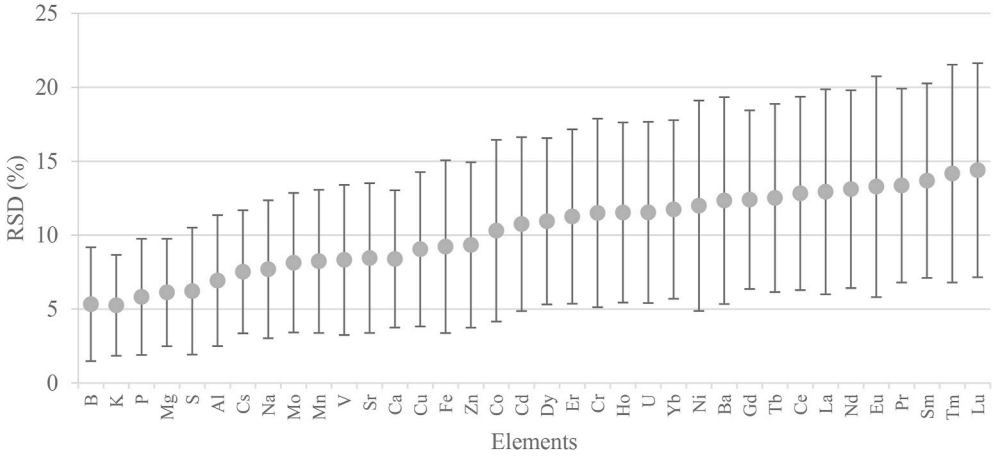

Fig. 3. Relative standard deviation (RSD) of analyzed element concentrations in three replicates of raw propolis samples (n = 252).

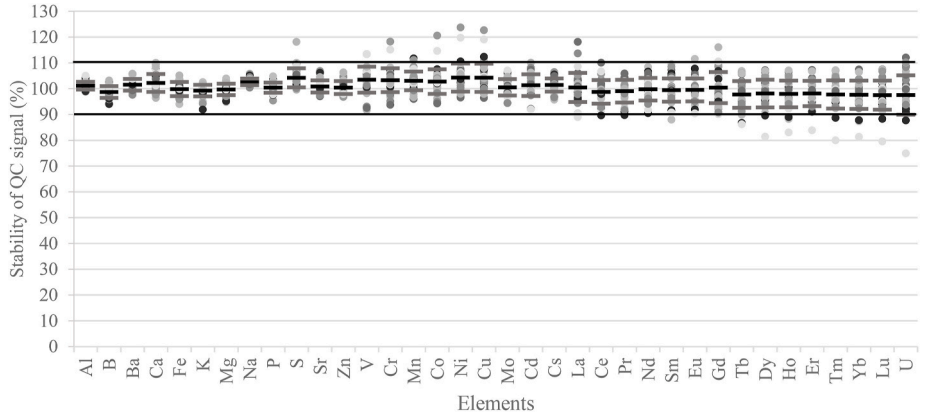

Fig. 4. Stability of the signal of quality control (QC) solution (n = 34) during the analyses.

Table 5
Descriptive statistics of the measured Hungarian raw propolis samples (n = 252).

elements (unit)	median	mean	SD	minimum	maximum	max/min[a]	mean/median[a]	skewness[a]	kurtosis[a]
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.[b]	n.c.[b]	<LOD[c]	544	n.c.[b]	n.c.[b]	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

[a] No unit
[b] Not calculable
[c] Under the limit of detection

The measured 34 QC points were presented in Fig. 4. Graph points show the individual measurements, black rectangles represent the means, while grey rectangles demonstrate standard deviations of quality control solution. It was observed that most of the results are in the 90–110% interval, therefore drift during measurement is negligible. Rhodium was used as an internal standard for compensating drift in the case of ICP-MS analysis. Thereby QC recovery is in the ±10% range. At the same time standard deviation is bigger in the case of elements measured by ICP-MS compared to elements measured by ICP-OES. Long-term stability of ICP-OES signal is known by long time ago [31]. In a single case a considerable drift was observed for the isotopes heavier than ^{157}Gd (^{159}Tb–^{238}U). The measurement of sample set should have been repeated, however other measurements have provided reliable information from element content. The means of QC measurements are between 98.7% and 104.2% for all the elements.

3.1.7. Sample throughput and green chemistry

One of the aims of the applied digestion method was to improve the limited sample throughput of the microwave digestion system, which was successful. While the samples with the nitric acid were left overnight, just one series of sample could be prepared daily, because we possessed only one series of quartz tubes. However, soaking in nitric acid or another cleaning process of the tubes is not necessary, time can be reduced to washing them with distilled water. Samples could be weighted after tubes were dried, then samples could be digested the next day. While the maximum number of the quartz tubes placed into one PTFE vessel was three, the sample throughput was increased by three.

Based on manufacturers' instructions at least 8 mL liquid (e.g. 7 mL HNO_3 and 1 mL H_2O_2) should be had inside the PTFE vessels of the Milestone Start D microwave digestion system for appropriate temperature control. However with the used technique the necessary amount of nitric acid (2.0 mL) and hydrogen peroxide (0.6 mL) is lower for one sample. Moreover, the necessary amount of sample is also lower. This is favorable in those situations when the amount of the sample is limited. The Vessel-inside-vessel method may be appropriate for homogeneous biological samples in small amounts.

3.2. Evaluation of the element composition of raw propolis

The descriptive statistics of the element content of 252 raw propolis samples is presented in Table 5., namely the median, mean, standard deviation (SD), minimum, maximum, moreover some calculated values, that is, ratio of maximum and minimum, as well as ratio of median and mean. The difference is huge by comparing the lowest and the highest concentrations of the same element in the whole sample set. The smallest difference is ninefold, while all the other element concentration differences are higher than tenfold. This means that the element concentrations except Mg are not in the same order of magnitude. The ratio of the maximum and minimum concentrations 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 the above, the concentration difference is within two orders of magnitude, namely the maximum and minimum concentration ratio in the case of Cu, Fe, Sr, Co, Mo, Lu and U elements which is up to 100. The difference of minimum and maximum concentrations in the case of Ba, Zn, V, Cr, Ni, Cd and Eu elements is higher than hundredfold. Zn and Ba should be highlighted, because the difference

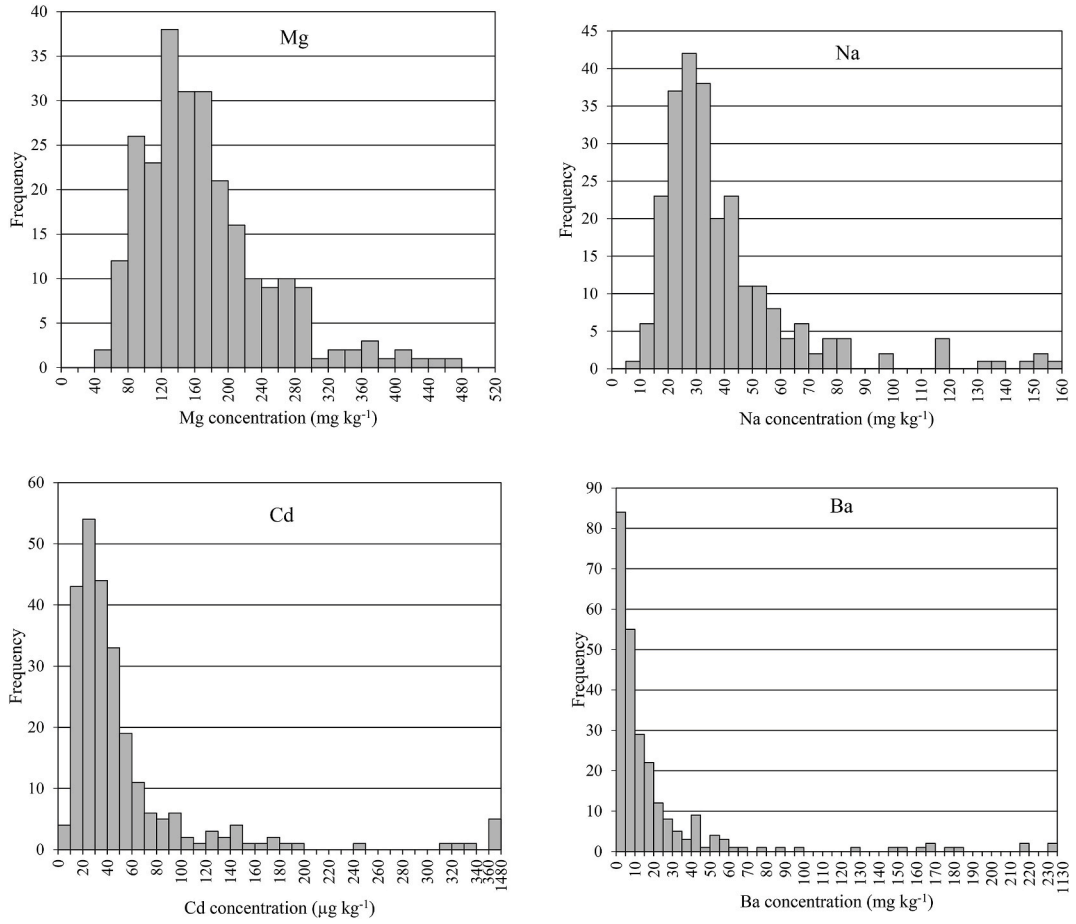

Fig. 5. Histogram of Mg, Na, Cd and Ba concentrations in raw propolis samples (n = 252).

between the minimum and maximum concentrations is 522-fold and 1638-fold, respectively. Concentration of La was below the LOD in some of the samples, therefore some statistics may not be calculated, but has similar characteristics to other rare earth elements.

By comparing the mean and median it is seen that mean is always higher, resulting in a positive skewness. Elements with a small difference of minimum and maximum concentrations also have a low ratio of median and mean (<1.3). However, when difference between minimum and maximum concentrations was more than 100, this infers a higher ratio of mean/median (1.73–2.97). This can be explained by outliers in the sample set. Distributions of element concentrations are presented by a histogram of Mg, Na, Cd and Ba (Fig. 5.).

The histogram of Mg represents nearly normal distribution, which could also be seen from the low value of mean/median as well as maximum/minimum. The histogram of Na shows that the number of the interval is not equal on the left and on the right side from the most frequent category (25–30 mg kg^{-1}). There are 4 bins on the left side and 18 bins on the right side with equal intervals. The highest concentration sample belongs to the 155–160 mg kg^{-1} bin. The histogram of Cd shows that most of the bins are on the right side of the most frequent bin. Mean and median of Cd concentrations are 64.1 µg kg^{-1} and 33.3 µg kg^{-1}, respectively, while ratio of the aforementioned parameters is 1.93. Positive skewness could also be seen from maximum/minimum ratio as 247. We have used a different interval than before on the right side of the scale (350–1480 µg kg^{-1}) for better visibility, because some samples contained much higher concentrations than others. The histogram of Ba has extreme positive skewness. The most frequent bin is on the left side of the scale, that is, 0–5 mg kg^{-1} and 84 samples from 252 are in this interval. Median and mean were 8.59 mg kg^{-1} and 25.5 mg kg^{-1}, respectively, however Ba concentrations above 200 mg kg^{-1} were also measured in 4 samples. Aforementioned results present that primarily Ba, but Cd and Na show a positive skewness.

We have checked the distribution of the element concentrations in the sample set by statistical tests. We have found positive values for the skewness and kurtosis in all the cases (Table 5.). The lowest skewness was found in the case of P (0.813), while values were 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 elements 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. Kolmogorov-Smirnov and Shapiro-Wilk tests also showed that significance value is below 0.05. This means that all of the element distribution is non-normal in the sample set, neither Mg nor P, but have a positive skew and a positive kurtosis.

It should be highlighted by comparing our 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 potassium content of Hungarian propolis are typically lower than the K concentration of Southern Spanish [9], similar to Croatian [5], Chinese [10], Serbian [7], Moldovan and Mongolian [6] samples, but higher than Turkish [8] or Russian [6] propolis. Calcium content is higher in Southern Spanish, Chinese and Croatian propolis, similar to Moldovan, Mongolian and Serbian propolis, but lower in Russian or Turkish samples, compared to Hungarian. We have found, that the concentrations of Ca are usually higher than the concentrations of K in Southern Spanish, Chinese and Croatian samples, in contrast with most of the Hungarian propolis. Phosphorus content is similar to another researches [6,7,9], but sulfur is higher in Southern Spanish samples. Fe content is similar to Serbian and Moldovan samples but a bit lower than the results of

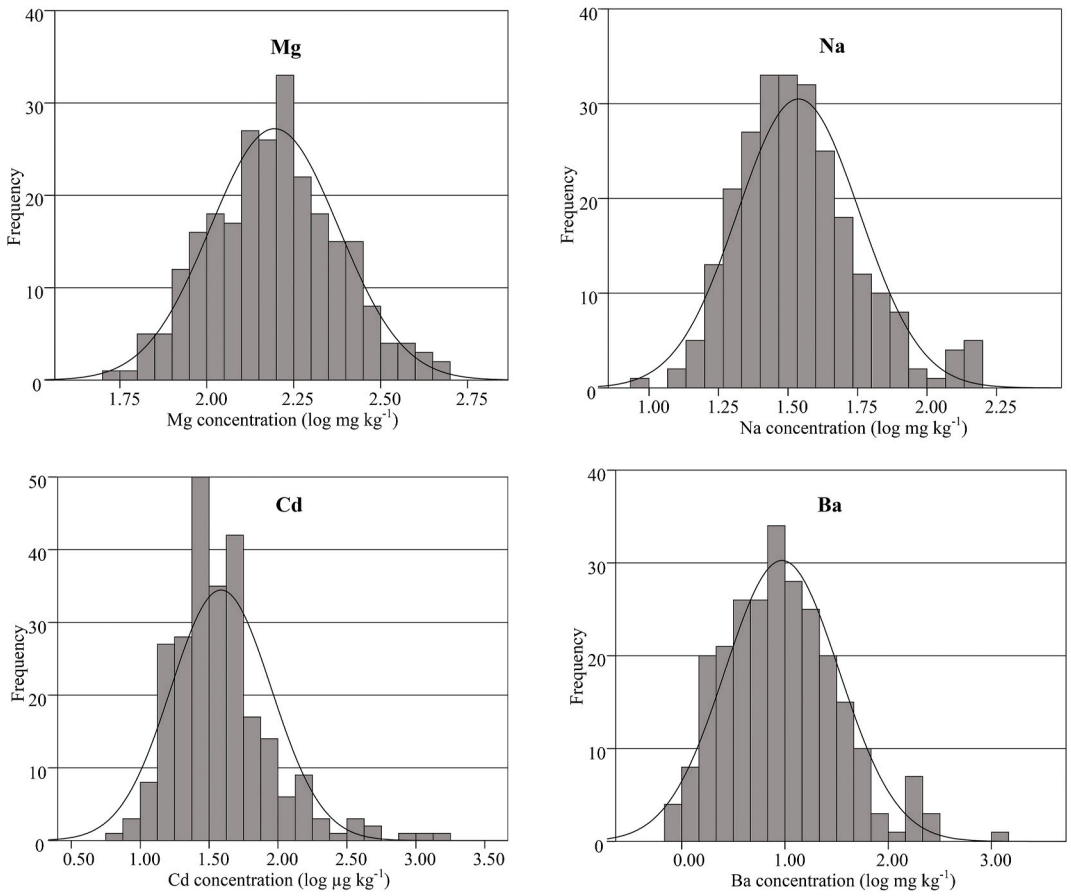

Fig. 6. Histogram of Mg, Na, Cd and Ba logarithm concentrations in raw propolis samples (n = 252).

Southern Spanish, Chinese, Argentinian [32] and Croatian propolis. Magnesium content of our samples is near to the Serbian, Croatian, Moldovan, Mongolian and Turkish propolis, but lower than the Southern Spanish and Chinese propolis. Southern Spanish, Chinese and Croatian samples contained much higher concentration of Al, Serbians propolis was similar to ours, but Moldovan and Russian propolis contained less Al. Zn content was present with a few outliers in our sample batch, which is in agreement with other researches. Croatian samples should be underlined, because of high difference between the lowest and the highest Zn content. Croatian and Southern Spanish propolis have typically higher in Zn, whereas the zinc content of Serbian, Argentinian, Turkish and Chinese samples are like ours. Sodium is notably higher in Chinese, and Turkish samples, also higher in Southern Spanish, Serbian and Croatian propolis, but similar to the results of Golubkina et al. [6]. Mean result of Ba content is similar to Croatian propolis, however the concentration range is much narrower in the publication of Cvek et al. [5]. Copper and boron content of our samples are similar to or a little lower than the concentration of these elements in propolis from foreign countries, but Sr is typically higher in those. Manganese content is similar to the results of Golubkina et al. [6] and Tosic et al. [7], but lower compared to Southern Spanish, Chinese and Croatian propolis. V, Cr, Co, Ni and Cd content of our samples are in the same order of magnitude with the aforementioned articles; however, Mo, Cs and U content was not mentioned. The microelement concentrations in Hungarian and Argentinian raw propolis are in good agreement, but concentrations of measured lanthanides are 2–3 orders of magnitude lower in the article of Cantarelli et al. [32].

3.3. Geographical identification of raw propolis

We have checked the possibility of geographical identification in the case of 252 Hungarian raw propolis samples. 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 we have confirmed that this requirement has not been fulfilled, we have used the decimal logarithm of the measured element concentrations. The histogram of the logarithm concentrations of Mg, Na, Cd and Ba are shown in Fig. 6. The histograms are follow better the Gaussian curve normal distribution.

Concentrations presented in a logarithm scale are closer to 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. Cd, Cr, Cu and Zn elements are often used as environmental indicators, because the human activity may affect their concentrations [33,34]. 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 $\leq n$ number of principal components from n number of independent variables, which are uncorrelated with each other. We have left three principal components (PC1, PC2, PC3) by the eigenvalue of principal components. The created principal components represented 85.8% of the total variance. The result of the principal component analysis is presented on a three-dimensional scatter plot in Fig. 7. It shows the individual samples with different colors and marks according to their geographical origin by region. The results show no correlation with geographical origin. The samples originating from the same region do not form separated groups on the scatter plot, but they are mixed with other regions. Therefore we have rejected the use of principal

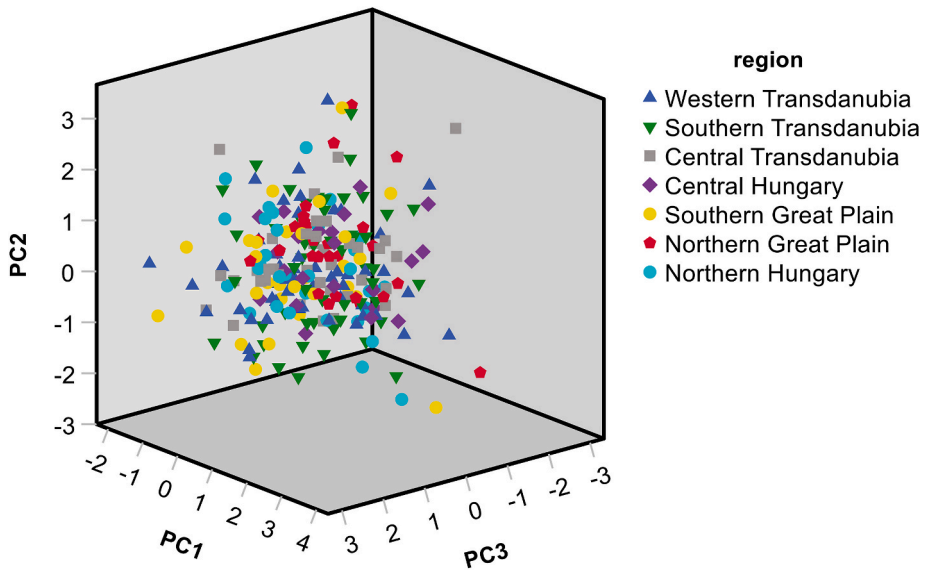

Fig. 7. Evaluation of the logarithm element concentrations of raw propolis samples by principal component analysis (PCA) for checking the geographical origin.

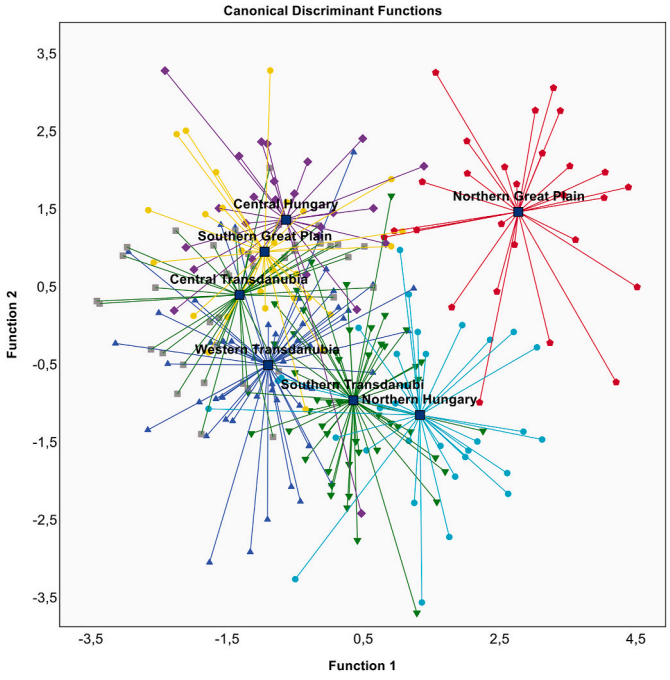

Fig. 8. Loading plot for the discrimination of raw propolis originating from different regions of Hungary with linear discriminant analysis (LDA).

component analysis.

We 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 groups of samples, while maximizes the difference between the different groups of samples. The impact was shown of the single variables for the grouping after running the LDA analysis. The impact is expressed by the Wilks' Lambda and the significance level. The impact of Ba and Co was very low, therefore they were removed from the grouping model. Henceforward 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 were used in the grouping method. It can be seen based on the loading plot (Fig. 8.) that propolis samples from the Northern Great Plain 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 the 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 partially overlap with each other.

Numerical results of the classification (Table 6.) show that grouping of the samples of the Northern Great Plain was the most effective based on the Original method. The 96.3% of the samples go 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 Southern Transdanubia samples with 80.4%. Discrimination of propolis from other regions was between 68.4 and 78.6%. Cross-validation can be used for verification of the classification result. The classification efficiency of Northern Great Plain samples decreased to 77.8% in the cross-validation, which is also quite high. The correct matches in other regions were between 44.7 and 72.0%. To sum it up, 76.6% of the samples were correctly classified by the original classification method, while it decreased to 61.5% by cross-validation.

Climatic and geographical conditions are similar in Northern and Southern Great Plain, however propolis from the latter region cannot be separated well from propolis originated from Central Hungarian and Central Transdanubian regions, even though most of the white poplar population is present in Southern Great Plain. Southern Transdanubia and Northern Hungary are relatively far from each other, however propolis originated from those regions are close to each other in LDA loading plot. The discrimination results can be explained by small differences in botanical and climatic conditions between the Hungarian regions investigated. Additionally, honeybees collect resin of different botanical origin if available [29]. Cantarelli et al. [32] and Gong et al. [10] also used multielement analysis for geographical identification of Argentinian and Chinese propolis and accomplished efficient separation. However regions used in aforementioned articles are larger in size than Hungary, thus may infer stronger botanical and climatic differences.

The applied separation model may be more effective by supplementing flavonoid composition of propolis samples [35]. Geographical identification by multielement analysis can also be improved by isotope distribution of light (e.g. δD, $\delta^{13}C$) [36] or heavy (e.g. $^{87}Sr/^{86}Sr$) [37] elements. Color [38], image analysis of thin layer chromatography [39]

Table 6
Classification results of raw propolis from different regions of Hungary by LDA Classification Results.[a,c]

		region	Predicted Group Membership							Total
			Western Transdanubia	Southern Transdanubia	Central Transdanubia	Central Hungary	Southern Great Plain	Northern Great Plain	Northern Hungary	
Original	Count	Western Transdanubia	**35**	3	7	2	2	1	1	51
		Southern Transdanubia	3	**41**	0	3	3	0	1	51
		Central Transdanubia	1	3	**26**	3	4	0	1	38
		Central Hungary	0	0	4	**19**	0	0	2	25
		Southern Great Plain	2	1	2	1	**22**	0	0	28
		Northern Great Plain	0	0	0	0	0	**26**	1	27
		Northern Hungary	0	3	3	1	0	1	**24**	32
	%	Western Transdanubia	**68.6**	5.9	13.7	3.9	3.9	2.0	2.0	100.0
		Southern Transdanubia	5.9	**80.4**	0.0	5.9	5.9	0.0	2.0	100.0
		Central Transdanubia	2.6	7.9	**68.4**	7.9	10.5	0.0	2.6	100.0
		Central Hungary	0.0	0.0	16.0	**76.0**	0.0	0.0	8.0	100.0
		Southern Great Plain	7.1	3.6	7.1	3.6	**78.6**	0.0	0.0	100.0
		Northern Great Plain	0.0	0.0	0.0	0.0	0.0	**96.3**	3.7	100.0
		Northern Hungary	0.0	9.4	9.4	3.1	0.0	3.1	**75.0**	100.0
Cross-validated[b]	Count	Western Transdanubia	**26**	3	11	3	2	2	4	51
		Southern Transdanubia	6	**35**	0	3	5	0	2	51
		Central Transdanubia	6	3	**17**	4	6	1	1	38
		Central Hungary	1	0	4	**18**	0	0	2	25
		Southern Great Plain	4	1	3	2	**17**	1	0	28
		Northern Great Plain	2	0	0	1	1	**21**	2	27
		Northern Hungary	2	3	3	1	0	2	**21**	32
	%	Western Transdanubia	**51.0**	5.9	21.6	5.9	3.9	3.9	7.8	100.0
		Southern Transdanubia	11.8	**68.6**	0.0	5.9	9.8	0.0	3.9	100.0
		Central Transdanubia	15.8	7.9	**44.7**	10.5	15.8	2.6	2.6	100.0
		Central Hungary	4.0	0.0	16.0	**72.0**	0.0	0.0	8.0	100.0
		Southern Great Plain	14.3	3.6	10.7	7.1	**60.7**	3.6	0.0	100.0
		Northern Great Plain	7.4	0.0	0.0	3.7	3.7	**77.8**	7.4	100.0
		Northern Hungary	6.3	9.4	9.4	3.1	0.0	6.3	**65.6**	100.0

[a] 76.6% of original grouped cases correctly classified.
[b] Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.
[c] 61.5% of cross-validated grouped cases correctly classified.

or reflectance spectroscopy [40] maybe also be a way to improve efficiency of separation.

4. Conclusion

Digestion in quartz tubes by microwave-assisted digestion and measurement by ICP-OES and ICP-MS have proven a powerful method for element analysis of raw propolis. Applying quartz tubes the sample throughput of the sample preparation method can be approved compared to the conventional microwave-assisted digestion system. Performance characteristics of the method enable us to measure up to 36 elements from raw propolis samples. We have created a database which contains information about the 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 made from Hungarian propolis has not existed so far. Moreover, we cannot find globally such results of propolis element content, which is representing one country and with such a number of elements. 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 identified the samples with 76.6% efficiency, while in the case of cross-validation the efficiency was 61.5%.

Credit author statement

Áron Soós; Conceptualization; Methodology; Validation; Formal analysis; Investigation; Writing – original draft; Writing – review & editing; Funding acquisition, Éva Bódi: Investigation, Szilvia Várallyay; Investigation, Szabolcs Molnár; Conceptualization; Resources; Béla Kovács; Resources; Funding acquisition

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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