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Mineral content of propolis tinctures in relation to the extraction time and the ethanol content of the extraction solvent

Áron SOÓS*[a] – Éva BÓDI[a] – Szilvia VÁRALLYAY[a] – Szabolcs MOLNÁR[b] – Béla KOVÁCS[a]

[a]Institute of Food Science, University of Debrecen, 138 Böszörményi str, H-4032 Debrecen, Hungary

[b]Food and Wine Research Centre, Eszterházy Károly University, 6 Leányka str, H-3300 Eger, Hungary

***corresponding author: soos.aron@agr.unideb.hu (Á. Soós)**

tel.: +36 52 508444/88170

Abstract

Propolis tincture is an extracted form of raw propolis with an extraction solvent, which can be used against several diseases and makes it a possible nutraceutical. Raw propolis is often the objective of geographical identification by its element content. It is harder to identify tinctures, because different production processes may influence the mineral content. Therefore the effect of the extraction time (1 hour, 1 day, 1 week and 1 month) and the ethanol content (0, 50, 80 and 100% (v/v)) was checked and recorded based on the element content of the tinctures. The 33 elements were classified into 3 groups by their behaviour. Most of the elements dissolved better in water than in ethanol-content solvents. Essential elements were extracted well also in up to 80% (v/v) ethanol content solvents. The concentration of most of the elements did not change greatly after 1 week. Geographical identification can be applied to the tinctures by their element content.

Keywords

propolis, tincture, element content, extraction time, extraction solvent

1. Introduction

Propolis or bee glue is a product of the *Apis mellifera* L. bees, which is collected from buds, leaves and bark, which then exudes mixing with their enzymes and beewax. Propolis is used in the beehive to cover the walls, to block the holes, to repair combs and to embalm any alien species which invade the hive, so they cannot escape (Pierini, Granero, Di Nezio, Centurión, Zon, & Fernández, 2013). It has several beneficial effects well known in traditional medicine and has been used for thousands of years. Antioxidant (Choi, Noh, Cho, Suh, Kim, & Kim, 2006), anti-inflammatory (Wang et al., 2014), antibacterial (Chen, Ye, Ting, & Yu, 2018), antiviral (Takeda, Nagamatsu, & Okumura, 2018), antifungal effects (Freires et al., 2016) and hepatoprotective (Banskota et al., 2001) as well as antitumoral (Carvalho et al., 2011) qualities are demonstrated. The main constituents are 50-60% of resin and balm and 30-40% of wax. Other components, such as pollens, vitamins and minerals (Barth, Freitas, Alex da Silva de, Matsuda, & Almeida-Muradian, 2013) are present in about 5% concentration. The chemical composition can vary based on the botanical origin of the propolis (Aliboni, 2014). Analytical techniques or chemical parameters that can be used in geographical or botanical identification are the following: thin layer chromatography and image analysis (Sârbu & Moţ, 2011), reflectance spectroscopy (Moţ, Soponar, & Sârbu, 2010), gas chromatography-mass spectrometry (Isidorov, Szczepaniak, & Bakier, 2014), physicochemical characteristics (Park, Alencar, & Aguiar, 2002), however the macro- and microelement content can also be used for this purpose. Chinese (Gong, Luo, Gong, Gao, & Xie, 2012) and Argentinean (Cantarelli, Camiña, Pettenati, Marchevsky, & Pellerano, 2011) raw propolis was identified by their element content, but other researchers have also analysed the mineral content of raw propolis (Bonvehí & Bermejo, 2013; González-Martín et al., 2015; Golubkina, Sheshnitsan, Kapitalchuk, & Erdenotsogt, 2016).

Propolis is used as a component of pastes, nasal sprays, lozenges, nutritional supplements or as an additional component in honey (Osés, Pascual-Maté, Fernández-Muiño, López-Díaz, & Sancho, 2016). However in some countries, e.g. in Hungary, it is known as a tincture which is a product of an extraction method, most commonly used with solvents containing ethanol. Tinctures are a

possible nutraceutical used in apitherapy, which can be part of a regular diet (Sadhana, Lohidasan, & Mahadik, 2017; Galeotti, Maccari, Fachini, & Volpi, 2018). Nutraceutical identifies a food or part of a food, which can be of vegetal or animal origin, has a potential pharmaceutical activity and should have proven health beneficial effect as a requirement (Santini & Novellino, 2018; Daliu, Santini, & Novellino, 2019). The process can be described as one of fractionation, which is a classification of an analyte or a group of analytes from a certain sample according to physical (e.g. size, solubility) or chemical (e.g. bonding, reactivity) properties (Szpunar & Lobinski, 2003). The tinctures can be prepared at home but the methods vary with different extraction times and different ethanol content. The recommended amount of ethanol used, however, should be at least 60% (v/v). The extraction can last for 30 days, but aqueous extract can also be applied (Nagai, Inoue, Inoue, & Suzuki, 2003).

The organic content of the tinctures has been extensively studied and analysed using different extraction methods (Kalogeropoulos, Konteles, Troullidou, Mourtzinos, & Karathanos, 2009; Tylkowski, Trusheva, Bankova, Giamberini, Peev, & Nikolova, 2010; De Zordi et al., 2014; Galeotti, Maccari, Fachini, & Volpi, 2018), however the mineral content has not been so thoroughly studied (Cvek, Medić-Šarić, Vitali, Vedrina-Dragojević, Šmit, & Tomić, 2008). While the raw propolis can be identified by its geographical origin based on element analysis, with tinctures it is a more complex problem, because they can be affected by different factors.

The aim of this study is to evaluate two factors in the extraction method of propolis tinctures, namely the ethanol content of the extraction solvent and the time of the extraction process in relation to the element content of the tinctures.

2. Materials and methods

2.1. Reagents and tools

High purity deionized water (18.2 MΩ cm) was used from MilliQ system (Bedford, MA, USA). Analytical grade nitric acid (65% (w/w)) and hydrogen-peroxide (30% (w/w)) came from Scharlab S.L. (Sentmenat, Spain). Calibration stock solution was made from 1000 mg L^{-1} monoelement stock

solutions from Scharlab S.L. (Sentmenat, Spain), except rare earth elements (REEs), which were made from 100 mg L^{-1} multielement stock solution of REEs from Teknolab A/S (Drøbak, Norway). Rhodium (Fluka, Buchs, Switzerland) was used as an internal standard in 40 µg L^{-1} concentration. High purity anhydrous ethanol (≥99.8% (v/v)) was used when the extraction solvents were being from VWR International (Fontenay-sous-Bois, France). The ≥99.8% (v/v) ethanol is referred to as 100% (v/v) below. All the new plastic tools were cleaned and soaked in 2% HNO_3 for 3 days, then soaked in MilliQ water for 1 day, and finally rinsed with distilled water and dried prior to use.

2.2. Samples

Tinctures were made from mixed raw propolis samples collected in 2014 from Hungarian beekeepers from all over the country. Almost all the counties were represented in the mixed sample, collected from at least 30 individual raw propolis producers in equal quantities. These combined samples formed an average composition of raw propolis from across the country. About 0.5±0.01 g of homogenized raw propolis was measured and put into plastic centrifuge tubes, then 5 ml of extraction solvent was added. The extraction solvents were 0, 50, 80 and 100% (v/v) by their ethanol content in MilliQ deionized water. The extraction time was 1 hour, 1 day, 1 week and 1 month, respectively. 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 Biosan Vortex V-1 at the beginning of the extraction process, then twice every working day. After the extraction period the samples were mixed again and centrifuged at 1600×g for 10 min at 21±2 °C. The supernatant was filtered with Filtrak 388 filter paper and kept at room temperature in a dark place in closed centrifuge tubes until digestion.

2.3. Sample preparation

The sample preparation was completed by microwave digestion. Since ethanol 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 and dried in an oven at 45°C to their constant weight. To compare, not only tinctures containing ethanol,

but water as an extraction solvent were also evaporated from the samples. In the case of the raw propolis, 0.1 g was deposited into quartz tubes but was not dried in an oven. All the other steps in the process stayed the same. After this, 2 ml HNO_3 was added and the samples were left overnight. On the following day 0.6 ml H_2O_2 was added and the quartz tubes were sealed with Teflon tape. Up to three closed tubes were placed in polytetrafluoroethylene (PTFE) vessels containing 10 ml MilliQ water around the quartz vessels. The water was necessary because it slows down the reaction and compensates for the inner pressure inside the quartz tubes. Closed PTFE vessels were placed into a Milestone Start D digester (Milestone Srl, Sorisole, Italy). The digestion steps were the following: heating up to 180°C in 15 mins, then being kept at a constant heat at 180°C for 20 mins and finally cooling down to room temperature. The samples were filled with up to 10±0.5 mL with MilliQ water in centrifuge tubes. We did not use a volumetric flask in order to avoid cross contamination and reach the lowest limit of detection. 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.

2.4. Apparatus

Element analysis was carried out with Thermo Scientific iCAP 6300 Dual view inductively coupled plasma optical emission spectrometry (ICP-OES) and Thermo Scientific X-Series II inductively coupled plasma mass spectrometry (ICP-MS). The main parameters are listed in *Table 1*. Other parameters of ICP-MS were optimized prior to analysis to maximize ^{59}Co, ^{115}In and ^{238}U signals and minimize Ba^{2+}/Ba^{+} and CeO^{+}/Ce^{+} ratios. Before analysis by ICP-MS, a fivefold dilution was applied with distilled water to reduce the acid content of the samples. The ICP-OES and ICP-MS were controlled by iTEVA 2.8.0.97 and Thermo PlasmaLab 2.5.10.319 software.

2.5. Limit of detection, accuracy and precision

Three blanks were made separately for each of the 0, 50, 80 and 100% (v/v) extraction solvents, respectively. The blanks were prepared and digested in the same way as the samples. The limit of detections was calculated by taking into account the standard deviation of the blanks multiplied by

3 and multiplied by the dilution factor, separately for the different extraction solvents. The mean of the separately determined LODs was between 0.00117 and 1.30 µg L^{-1} in the case of ICP-MS and between 0.0014 and 0.961 mg L^{-1} in the case of ICP-OES. To check the accuracy, another propolis tincture was prepared with 80% (v/v) ethanol content and was spiked with the analytes. The sample of the original and that of the spiked propolis were digested in the same way as the other samples. The lowest spike recovery was B with 83.4% and the highest was the Sr with 117.9%. The average recoveries of the 33 elements were 100.5±6.9%. The precision of the measurements were demonstrated by the relative standard deviation (RSD) of the replicates in different propolis tinctures. The average RSD was between 2.59-27.4% in the case of B and Er. The RSD was quite low in the case of higher concentration elements, however it was quite high if the concentration was small in the tinctures, e.g. Sr, V or lanthanides. The mean of the limit of detections, the average, minimum and maximum values of RSD and the spike recoveries of the propolis tinctures are shown in *Table 2.*

2.6. Statistical analysis and calculation of the transfer coefficient

The graphs were made by using Microsoft Excel 2013. SPSS 22.0 for one-way ANOVA and Tukey-test with a $p<0.05$ significance level was used. The transfer coefficient (TC) was calculated by the concentration of propolis tinctures divided by their concentration in raw propolis, multiplied by 10 with the dilution factor in tinctures compared to raw propolis, then multiplied by 100.

3. Results and discussion

3.1. Behaviour of elements in different extraction processes

The measured elements can be classified into three main groups (i; ii; iii) according to their behaviour during the extraction process, depending on the extraction time and the composition of the extraction solvent.

(i) Most of the elements dissolved considerably better in aqueous extraction solvents than in solutions containing ethanol. This means that the concentration of these elements decreased by 50-90% in 50% (v/v) solutions compared to 0% (v/v) extracts after 1 month. Further decreases of at

least 80% were observed in 80% (v/v) tinctures against 50% (v/v), if they could be calculated at all, that is, if the concentration was above the limit of detection (LOD). The concentrations depending on the extraction time and the ethanol content of the extraction solvent in the first group of elements are represented for instance by Ba, Ca, Sr, Cd and La as shown in *Figure 1*.

Only Sr and Cd were above the limit of detection in every extraction process out of the elements belonging to the first group. In most cases element concentrations were reduced below the LOD by the increasing ethanol content in the extraction solvent. The concentrations fell under the LOD in all the 100% (v/v) tinctures in the case of Ca. The same changes occurred in all the 80 and 100% (v/v) extracts in the case of Ba, La, Ce, Pr, Eu, Gd, Dy, Ho, Er, Yb and in all the 50, 80 and 100% (v/v) solutions in the case of Nd. Some elements were detectable only in some solutions, such as the Sm and Tm in aqueous extracts after 1 week and 1 month, and Tb could be detected in all the aqueous and in the 50% (v/v) extracts after 1 week and 1 month. Lu was below the limit of detection in all the cases but all the other lanthanides fell into this group, so Lu most probably has the same behaviour.

It follows from the above that Ba Ca, Sr, Cd, La, Ce Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu belong to the first group. Ca, Sr and Ba are alkaline earth metals, which can explain why their behaviour was similar, however Mg was not included in the first group. Additionally, lanthanides also belonged to the first group.

(ii) There were elements which 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. In the case of Na, K and P there was no significant difference between the aqueous and the 50% (v/v) extracts after 1 month. There was a significant increasing trend in aqueous extracts depending on the time for all the elements of the group. On the other hand, this trend was not typical in all the other extraction solvents. Low, but detectable increases were found in the case of Mg, P, S and Co, in 100% (v/v) tinctures. The concentration of B increased up to 1 week but decreased after 1 month. No significant

differences were found in the case of Na in relation to the extraction time in 100% (v/v) tinctures. Low, but observable decreases were found, however, in the case of K and Mn, while the Zn content was also significantly reduced.

Results showed that in the shortest extraction time almost all the elements were found to be significantly higher in concentration in the 50 or 80% (v/v) tinctures as opposed to the aqueous extracts, while they were reduced in 100% (v/v) solvents. Mn was an exception, because its concentration was considerably decreased by the increasing ethanol content after 1 hour, which means that Mn dissolved the best in water after 1 hour.

Consequently, the second group contains B, K, Mg, Na, P, S, Zn, Mn and Co, which are essential elements for the human body. *Figure 2* shows, for instance, the K, Na, P, Zn and Mn concentrations from the second group of elements in the tinctures and their relation to the extraction time and the ethanol content of the extraction solvent.

(iii) Elements classified under the third group had one common property, namely that they did not extract well in water, but results improved in solvents containing ethanol, although they had differing other properties. This group contains the elements Cu, Fe, V, Cr, Mo and U, their concentrations are shown in *Figure 3*, in relation to the extraction time and the ethanol content of the extraction solvent.

Even though they were not dissolved by water in the highest amounts, their concentrations were not raised by the increased ethanol content in every extraction solvent. The highest Cu content was found in the 80% (v/v) solvents after every extraction time. Cr showed the same behaviour as Cu, except that the concentration increased in the aqueous extracts. Mo was below the limit of detection in all extractions of the 0% (v/v) solvents, however it had the highest concentration in 80% (v/v) tinctures, but the difference was not significant compared to 50 and 100% (v/v) tinctures, after 1 month. An increasing trend was observed in the case of V when the ethanol content was also raised, however it was not significant in all cases.

3.2. Element content change in relation to extraction time

It is noteworthy that there was an increasing tendency in most of the element contents in aqueous extracts, except in the case of some elements in the third group. It was observed that two elements, namely Fe and Mo, had no significant differences. Two other elements (Cu and V) had small but statistically significant growth. Two additional elements (Cr and U) showed significant increases in aqueous extracts at the time of extraction.

It was found that the elements in the first group had increased after 1 month compared to 1 hour between 2.78 and 5.40 times, while in the second group they had increased between 2.27 and 4.79 times. This means that there was a remarkably high difference between the shortest and the longest extraction time. On the other hand, the difference between 1 week and 1 month was between 1.01 and 1.28 times for the former group and 1.10 and 1.28 times for the latter. The average increase was 1.23 and 1.18 times, in the latter cases. The highest increase was found for Ba, which was between 5.40 and 1.47 times for the 1 hour and 1 month time spans and also after 1 week and 1 month. It can be seen that the concentration did not increase to its maximum after 1 week, and it would have raised the extractable amount of Ba. However the changes to Ca were close to the average increase by 3.66 and 1.19 times between 1 hour and 1 month as well as 1 week and 1 month. Thus, the extraction process was nearly completed after 1 week in the aqueous solvent, because the increase was just 19% after 1 week.

The percentile change can be misleading, especially for solvents containing ethanol, because of the increase and decrease of the small concentration elements, which is often not significant. For example the change in the concentration of some lanthanides, namely Eu, Gd, Ho and Yb was not significant in 50% (v/v) tinctures regarding the extraction time. In addition, the change in Nd, Sm, Tb, Tm and Lu could not be calculated, because the elements in at least one treatment remained below the limit of detection or did not show a tendency to change. The remaining elements in the first group 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 tinctures generated by

a higher ethanol 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.

Elements in the second group, except Zn, had increased by 1.41-2.78 and 1.00-1.11 times in 50% (v/v) 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 day or 1 week in 50 and 80% (v/v) 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) tinctures after 1 week. The concentration of Zn fell in 100% (v/v) tinctures, but did not show any change in 80% (v/v) tinctures and only a 1.13 fold increase was observed in 50% (v/v) extract after 1 week.

It was also observed that the concentrations changed in a remarkably different way in 80 and 100% (v/v) tinctures in relation to the extraction time in the third group of elements. There was a small increase, then the concentration stagnated after 1 day in both 80 and 100% (v/v) 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 0.26-0.62 fold after 1 month compared to 1 hour, while the decrease was reduced to 0.60-1.05 times between 1 week and 1 month. The reduction, however, was not significant between 1 week and 1 month in the aforementioned cases, except for one element (Fe in 80 and 100% (v/v) tinctures).

3.3. A possible explanation of the increase and decrease of the element content in the tinctures in relation to extraction time

So far, studies have not investigated the element content of the propolis tinctures in relation to their extraction time spans, therefore we compared them to other matrixes, chiefly tea infusions. Pytlakowska, Kita, Janoska, Połowniak & Kozik (2012) made herbal tea infusions prepared in 10 and 30 minutes, respectively. There were different trends observed for the analysed elements in

some herbal infusions. The content of Cu increased in some cases, however in most herbal tea infusions it was reduced. The Fe content increased when infused with chamomile, while it decreased in peppermint and nettle, depending on the production time. The concentration of Zn grew in the case of St. John's wort, but reduced in the case of melissa, sage, and chamomile. Al increased in St. John's wort, but different trends were observed for peppermint. In general, concentrations increased in the infusion of linden, while the element contents decreased in the infusion of peppermint (Pytlakowska, Kita, Janoska, Połowniak, & Kozik, 2012). Other researchers also found similar decreases in the element content of herbal tea infusions in relation to the extraction time (Özcan, 2005). On the other hand the mineral content of white hawthorn infusions increased depending on the time span (Juranović Cindrić, Zeiner, Mihajlov Konanov, & Stingeder, 2015).

The element content can also decrease during or after the fermentation process of wines. Some elements can be precipitated from the wine, mostly potassium bitartrate or a smaller quantity of calcium tartrate (Lasanta & Gómez, 2012). There was a decrease in Na, K and Ca content, however an increase in P, Cu and Mg concentrations has been found in apple juices treated by ultrasonic methods (Abid et al., 2014).

The changes in the element content can be explained by describing two different processes. Firstly, the longer the contact of the raw propolis with the extraction solvent, the higher the concentration of the components will be in the solvent from the raw material (Paul, Laurila, Vuorinen, & Divinski, 2014). Secondly, it was noted that the propolis is not a real but a colloid solution. It was observed that in some filtered solvents under longer storage there was appeared a small amount of precipitation at the bottom of the tinctures, although this precipitation dissolved again in the solvent when shaken. The decrease may be accounted for by the fact that the ethanol could not keep the elements solved during the longer extraction time. The same process may result in the decrease of element content in different kinds of tea. In addition, elements were capable of making complexes with organic components and had a bad solubility in aqueous extracts at the

given pH (Pytlakowska, Kita, Janoska, Połowniak, & Kozik, 2012). Before digestion, the tube was shaken, so any remaining precipitation dissolved again and digestion was made from a homogenous solvent, thus precipitation could not affect the accuracy of results after the filtration.

3.4. Translocation from raw propolis to tinctures

The translocation level was demonstrated by the transfer coefficient (TC). The TC was calculated for 80% (v/v) tinctures which represented a tincture containing the average amount of ethanol, and, for the aqueous extracts (0% (v/v), which revealed the highest element content produced from raw propolis for most of the measured elements. The element concentration in raw propolis and tinctures of 0 and 80% (v/v) extraction solvents after 1 week together with the TCs are shown in *Table 3*. It was observed that TCs varied significantly in the three main groups of elements. High TC was found in the case of the second group of elements in 0% (v/v) extracts, where its value was between 30.9 and 81.9%, while in 80% (v/v) tinctures it was between 19.1 and 71.9%. Of the elements from the second group, K had the highest and S had the lowest TC in both of these cases. The first group of elements had very different TCs. The TC was between 31.8-61.6% and 1.50-5.93 in the case of Ca, Sr and Co in 0 and 80% (v/v) tinctures. On the other hand the TC of the Ba and lanthanides remained below 5% in aqueous extracts, and was not calculable in 80% (v/v) tinctures. The third group of elements had low TCs between not calculable and 10.3% and between 1.97-37.0% in 0 and 80% (v/v) tinctures. Of the third group of elements, Cu had the highest TC in both processes. Cvek, Medić-Šarić, Vitali, Vedrina-Dragojević, Šmit & Tomić (2008) also made tinctures with 80% (v/v) ethanol, but with a shorter extraction time and more intensive mixing. They also found high TC in the case of K and B, while Mn and Zn were also in the same range, however Mg and Na were found to be lower than in our study. Ca Sr and Ba were notably higher in content, but Fe and Cu had results similar to those in our study.

4. Conclusion

This study has demonstrated that the element content of the propolis tinctures is considerably affected by the composition of the extraction solvent, and the extraction time has a great effect in

most cases. Most of the analysed elements classified into the first and second group dissolved better in aqueous solutions than in solvents containing ethanol. However, the elements essential to the human body with few exceptions were extracted well also in solvents containing 50 or 80% (v/v) of ethanol.

The increase or decrease was statistically significant and noticeable after 1 month compared to 1 hour, but was not significant or perceptible compared to 1 week. It can be concluded, that the concentration of most of the elements did not change greatly after 1 week. The recommended extraction time for most of the methods, which is a few weeks applied by most producers, results in a constant element concentration in tinctures. However the differences in extraction solvents lead to statistically significant differences in the element content of the tincture, which can be diminished if the composition of the extraction solvent is known. Geographical identification can be applied to propolis tinctures, if the ethanol concentration is known, and the same holds true for the geographical identification of raw propolis according to its element content.

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430

	ICP-OES		ICP-MS
RF power	1350 W	RF power	1400 W
Plasma gas flow rate	12.0 L min^{-1}	Plasma gas flow rate	14.0 L min^{-1}
Auxiliary gas flow rate	1.0 L min^{-1}	Auxiliary gas flow rate	0.86 L min^{-1}
Nebuliser gas flow rate	1.0 L min^{-1}	Nebuliser gas flow rate	0.88 L min^{-1}
Sample uptake	50 rpm	Sample uptake	20 rpm
Plasma view	axial	Dwell time	100 ms
Integration time low WL range	10 s	Sweep	9
high WL range	10 s	Main run	3
Repeats	2	Pole bias	-16.0
		Hexapole bias	-10.0
		CCT gas	H_2-He in 7:93
		CCT gas flow rate	6 mL min^{-1}
Nebulizer	concentric	Nebulizer	concentric
Spray chamber	cyclonic	Spray chamber	conical (2°C)
Elements and wavelengths (nm)	B (208.959), Ba (455.403),	Analysed masses	^{51}V, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{65}Cu,

	Ca (315.887), Fe (238.204), K (766.490), Mg (279.079), Na (818.326), P (213.618), S (182.034), Sr (407.771), Zn (213.856)		^{95}Mo, ^{111}Cd, ^{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, ^{238}U
Internal standard	-	Internal standard	^{103}Rh

Table 1: Main parameters of inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS)

Element (unit)	Limit of detection	RSD average (min-max) (%)	Original propolis tincture (n=3)	Spiked propolis tincture (n=3)	Spike concentration	Spike recovery (%)
B (mg L^{-1})	0.0209	2.59 (0.83-12.1)	0.146±0.002	0.578±0.013	0.519	83.4
Ba (mg L^{-1})	0.0034	6.84 (2.08-14.3)	<LOD	0.0480±0.0008	0.0519	92.1
Ca (mg L^{-1})	0.961	4.97 (1.67-11.4)	2.02±0.07	27.3±0.5	25.9	97.7
Fe (mg L^{-1})	0.0153	7.01 (1.31-20.9)	0.258±0.008	0.786±0.022	0.519	100.2
K (mg L^{-1})	0.101	2.32 (0.18-8.62)	37.0±0.2	89.6±0.7	51.9	102.2
Mg (mg L^{-1})	0.0239	2.94 (1.07-9.17)	3.80±0.03	28.0±0.4	25.9	93.0
Na (mg L^{-1})	0.121	4.23 (1.15-10.5)	1.64±0.02	6.87±0.09	5.19	100.9
P (mg L^{-1})	0.0629	3.06 (1.45-10.3)	8.10±0.06	35.7±0.4	25.9	106.6
S (mg L^{-1})	0.0999	2.94 (0.16-9.37)	3.48±0.03	43.1±0.8	35.3	111.9
Sr (mg L^{-1})	0.0014	14.9 (1.18-43.3)	<LOD	0.184±0.002	0.156	117.9
Zn (mg L^{-1})	0.0302	4.68 (0.42-11.6)	2.19±0.08	2.37±0.07	0.259	96.8
V (µg L^{-1})	0.0157	18.9 (5.85-38.1)	0.929±0.217	26.7±0.0	25.9	98.8
Cr (µg L^{-1})	0.750	11.5 (1.27-24.6)	8.12±1.36	37.1±0.1	25.9	111.7
Mn (µg L^{-1})	0.490	4.51 (0.94-14.1)	97.0±1.2	635±1	519	103.7
Co (µg L^{-1})	0.083	8.00 (1.15-16.5)	2.84±0.16	29.4±0.0	26.5	99.7
Ni (µg L^{-1})	1.30	12.6 (2.41-32.3)	8.58±0.31	36.1±0.0	25.9	106.1
Cu (µg kg^{-1})	0.0734	4.44 (1.06-9.73)	46.8±1.0	98.6±0.0	51.9	101.1
Mo (µg L^{-1})	0.211	12.9 (1.46-33.7)	0.500±0.598	51.3±0.0	51.9	100.3
Cd (µg L^{-1})	0.090	14.1 (1.26-33.7)	0.265±0.118	25.7±0.0	25.9	97.5
La (µg L^{-1})	0.0258	11.1 (1.25-18.2)	<LOD	26.1±0.0	25.9	100.1
Ce (µg L^{-1})	0.0153	9.90 (4.18-17.9)	<LOD	28.2±0.0	25.9	108.3

Pr (µg L^{-1})	0.00674	20.9 (6.99-45.3)	<LOD	26.7±0.0	25.9	102.4
Nd (µg L^{-1})	0.0463	17.4 (7.69-30.4)	<LOD	26.0±0.0	25.9	99.7
Sm (µg L^{-1})	0.0185	24.3 (16.7-31.8)	<LOD	24.6±0.0	25.9	94.3
Eu (µg L^{-1})	0.00186	14.7 (0.87-27.9)	<LOD	25.6±0.0	25.9	98.3
Gd (µg L^{-1})	0.00435	18.8 (9.88-29.8)	<LOD	23.0±0.0	25.9	88.4
Tb (µg L^{-1})	0.00234	22.4 (6.69-55.8)	<LOD	28.3±0.0	25.9	108.6
Dy (µg L^{-1})	0.00699	15.1 (6.10-26.4)	<LOD	24.8±0.0	25.9	95.0
Ho (µg L^{-1})	0.00117	22.1 (5.82-40.1)	<LOD	28.2±0.0	25.9	108.2
Er (µg L^{-1})	0.00221	27.4 (9.45-44.9)	<LOD	25.2±0.0	25.9	96.7
Tm (µg L^{-1})	0.00205	11.5 (5.91-17.2)	<LOD	25.8±0.0	25.9	99.1
Yb (µg L^{-1})	0.00429	18.4 (4.88-34.6)	<LOD	26.8±0.0	25.9	102.9
Lu (µg L^{-1})	0.00177	-	<LOD	26.5±0.0	25.9	101.5
U (µg L^{-1})	0.00517	22.1 (4.07-51.8)	0.0227	24.4±0.0	25.9	93.7

Table 2. Limit of detection (LOD), relative standard deviation (RSD) of the replicates, concentration in the original and spiked samples, moreover spike recoveries of analysed elements in propolis tincture. *<LOD: under the limit of detection

Element	Unit	Raw propolis (n=3)	Unit	Propolis tincture, 0% (v/v) (n=3)	TC (%)	Propolis tincture, 80% (v/v) (n=3)	TC (%)
B	mg kg^{-1}	5.92±0.51	mg L^{-1}	0.360±0.003	60.8	0.266±0.023	44.9
Ba	mg kg^{-1}	54.1±9.0	mg L^{-1}	0.073±0.002	1.35	<LOD	n.c.
Ca	mg kg^{-1}	707±84	mg L^{-1}	43.6±1.1	61.6	4.19±0.27	5.93
Cu	mg kg^{-1}	2.52±0.33	mg L^{-1}	0.026±0.001	10.3	0.093±0.002	37.0
Fe	mg kg^{-1}	390±70	mg L^{-1}	0.058±0.008	0.15	0.770±0.042	1.97
K	mg kg^{-1}	905±48	mg L^{-1}	74.1±0.8	81.9	65.1±2.1	71.9
Mg	mg kg^{-1}	217±18	mg L^{-1}	12.8±0.5	58.8	6.90±0.12	31.8
Mn	mg kg^{-1}	8.47±0.33	mg L^{-1}	0.477±0.012	56.3	0.166±0.005	19.6
Na	mg kg^{-1}	39.2±7.4	mg L^{-1}	2.69±0.15	68.6	2.85±0.04	72.7
P	mg kg^{-1}	283±18	mg L^{-1}	17.5±0.4	61.8	14.3±0.3	50.6
S	mg kg^{-1}	328±41	mg L^{-1}	10.1±0.6	30.9	6.25±0.01	19.1
Sr	mg kg^{-1}	3.56±0.50	mg L^{-1}	0.113±0.003	31.8	0.005±0.001	1.50
Zn	mg kg^{-1}	194±16	mg L^{-1}	11.3±0.8	58.3	4.03±0.03	20.8
V	µg kg^{-1}	385±54	µg L^{-1}	0.199±0.054	0.52	1.33±0.27	3.44

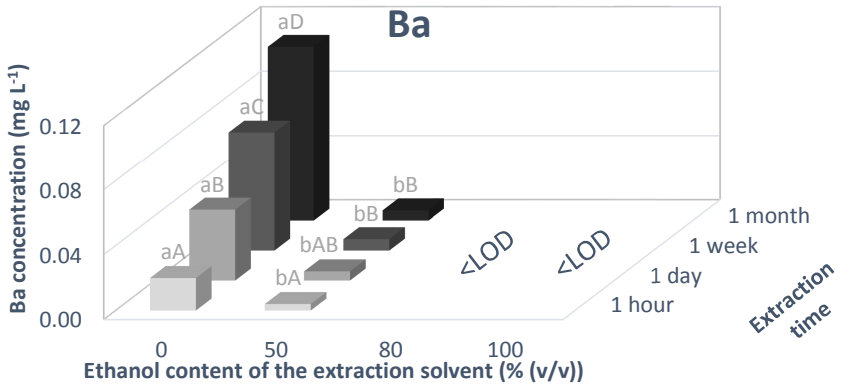
Cr	µg kg^{-1}	1940±210	µg L^{-1}	6.63±0.62	3.42	16.9±2.1	8.74
Co	µg kg^{-1}	238±17	µg L^{-1}	12.9±1.3	54.3	10.3±0.4	43.3
Mo	µg kg^{-1}	111±13	µg L^{-1}	<LOD	n.c.	1.28±0.04	11.6
Cd	µg kg^{-1}	103±8	µg L^{-1}	4.91±0.57	47.6	0.520±0.124	5.5
La	µg kg^{-1}	137±25	ng L^{-1}	235±35	1.71	<LOD	n.c.
Ce	µg kg^{-1}	265±53	ng L^{-1}	430±34	1.63	<LOD	n.c.
Pr	µg kg^{-1}	33.0±5.5	ng L^{-1}	61.2±4.3	1.85	<LOD	n.c.
Nd	µg kg^{-1}	118±19	ng L^{-1}	221±17	1.87	<LOD	n.c.
Sm	µg kg^{-1}	23.2±3.4	ng L^{-1}	54.7±17.4	2.36	<LOD	n.c.
Eu	µg kg^{-1}	10.4±0.9	ng L^{-1}	27.8±2.5	2.68	<LOD	n.c.
Gd	µg kg^{-1}	21.8±2.8	ng L^{-1}	60.1±7.8	2.76	<LOD	n.c.
Tb	µg kg^{-1}	3.03±0.32	ng L^{-1}	9.56±2.05	3.15	<LOD	n.c.
Dy	µg kg^{-1}	14.5±1.8	ng L^{-1}	49.3±3.0	3.39	<LOD	n.c.
Ho	µg kg^{-1}	2.85±0.35	ng L^{-1}	9.70±2.72	3.41	<LOD	n.c.
Er	µg kg^{-1}	7.44±0.52	ng L^{-1}	31.9±4.8	4.28	<LOD	n.c.
Tm	µg kg^{-1}	1.01±0.05	ng L^{-1}	4.67±0.28	4.64	<LOD	n.c.

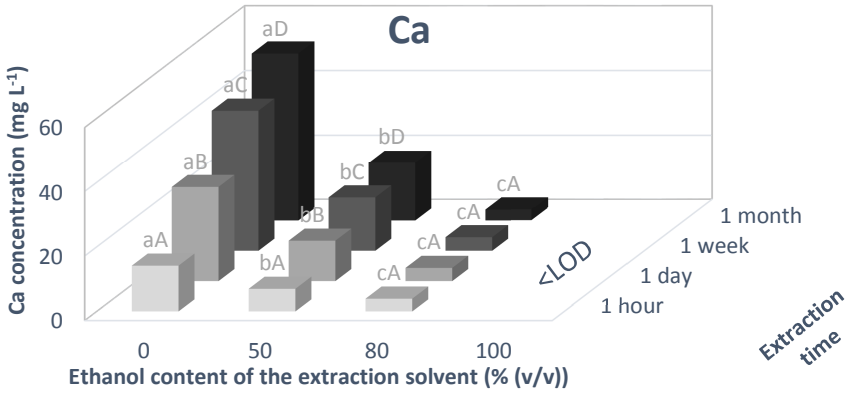
Yb	µg kg^{-1}	5.95±0.37	ng L^{-1}	22.4±3.0	3.77	<LOD	n.c.
Lu	µg kg^{-1}	0.841±0.122	ng L^{-1}	<LOD	n.c.	<LOD	n.c.
U	µg kg^{-1}	12.4±1.3	ng L^{-1}	15.8±1.6	1.28	40.4±3.8	3.26

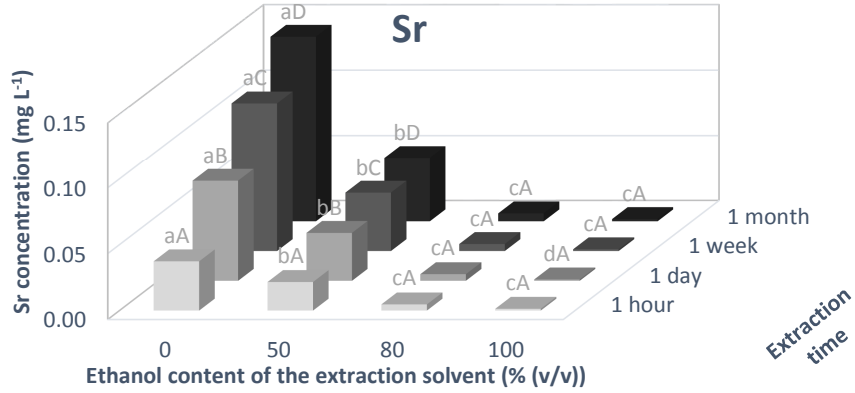
Table 3. The element content and the transfer coefficient (TC) of the raw propolis to the 0 as well as 80% (v/v) propolis tinctures after 1 week

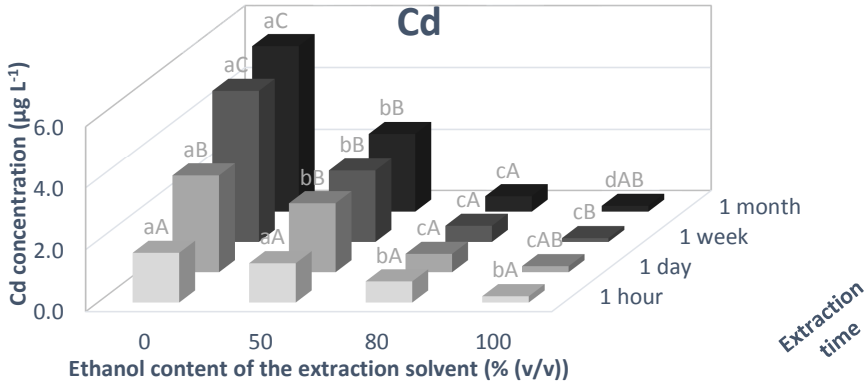
*<LOD: under the limit of detection

**n.c.: not calculable


Ba
Ba concentration (mg L-1)
0.00
0.04
0.08
0.12
aA
aB
aC
aD
bA
bAB
bB
bB
<LOD
<LOD
1 hour
1 day
1 week
1 month
Extraction time
0
50
80
100
Ethanol content of the extraction solvent (% (v/v))


Ca
Ca concentration (mg L-1)
0
20
40
60
aA
aB
aC
aD
bA
bB
bC
bD
cA
cA
cA
cA
<LOD
1 hour
1 day
1 week
1 month
Extraction time
0
50
80
100
Ethanol content of the extraction solvent (% (v/v))


Sr
Sr concentration (mg L-1)
0.00
0.05
0.10
0.15
aA
aB
aC
aD
bA
bB
bC
bD
cA
cA
cA
cA
cA
cA
dA
1 hour
1 day
1 week
1 month
Extraction time
0
50
80
100
Ethanol content of the extraction solvent (% (v/v))


Cd
Cd concentration (µg L-1)
0.0
2.0
4.0
6.0
aA
aB
aC
aC
aA
bB
bB
bB
bA
cA
cA
cA
bA
cAB
cB
dAB
1 hour
1 day
1 week
1 month
Extraction time
0
50
80
100
Ethanol content of the extraction solvent (% (v/v))

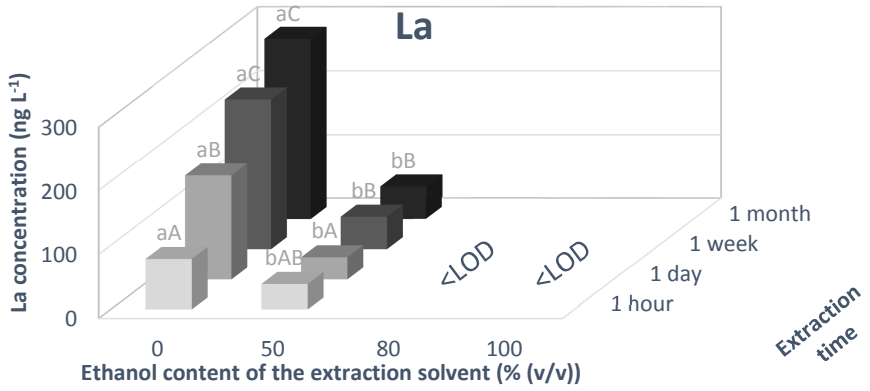
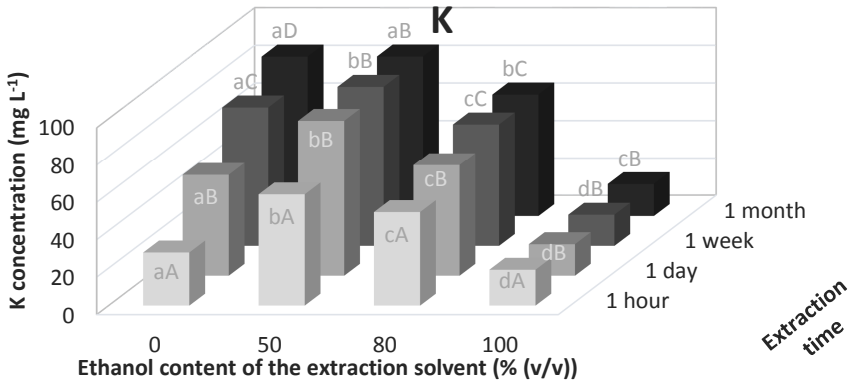
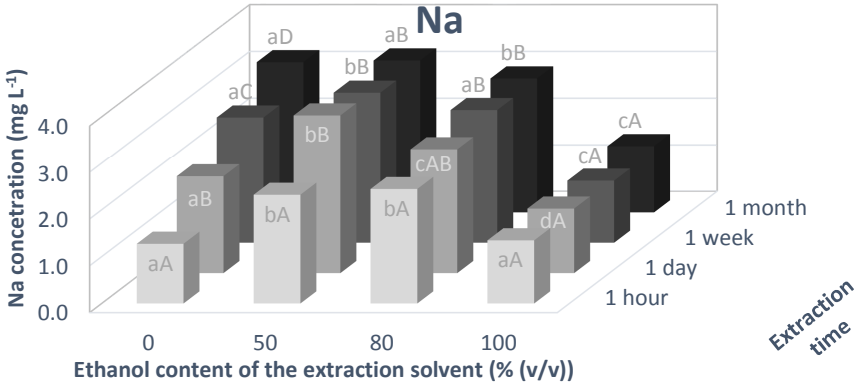
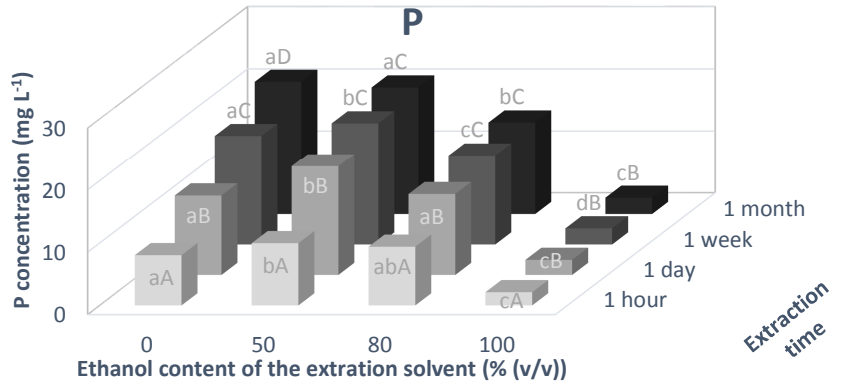
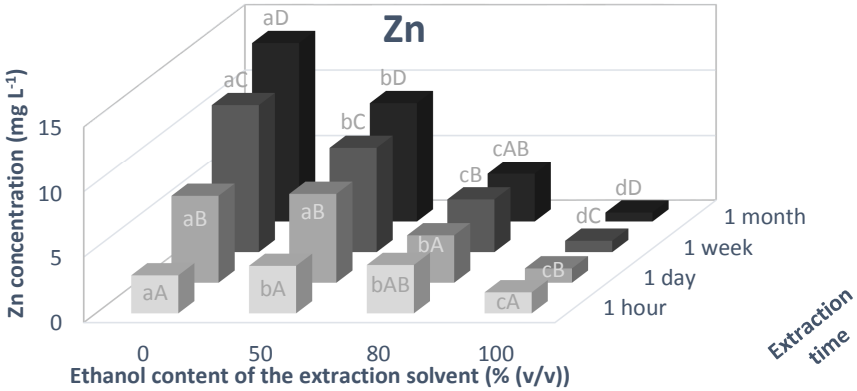


Figure 1: The Ba, Ca, Sr, Cd and La concentrations of the propolis tinctures (n=3) depending on the ethanol content of the extraction solvent and the extraction time. Different small letters mean the significant differences ($p<0.05$) between the effect of the different extraction solvents. Capitals mean significant differences ($p<0.05$) between the effect of the different extraction times. *<LOD: under the limit of detection.


K
K concentration (mg L-1)
100
80
60
40
20
0
aD
aB
aC
bB
bC
cC
cB
dB
aA
aB
bA
bB
cA
cB
dA
dB
1 month
1 week
1 day
1 hour
Extraction time
0
50
80
100
Ethanol content of the extraction solvent (% (v/v))


Na
Na concetration (mg L-1)
4.0
3.0
2.0
1.0
0.0
aD
aB
aC
bB
bB
aB
cA
cA
aB
bA
cAB
bA
dA
aA
aA
1 month
1 week
1 day
1 hour
Extraction time
0
50
80
100
Ethanol content of the extraction solvent (% (v/v))


P
P concentration (mg L-1)
30
20
10
0
aD
aC
aC
bC
bC
cC
cB
dB
aA
aB
bA
bB
abA
aB
cA
cB
1 month
1 week
1 day
1 hour
Extraction time
0
50
80
100
Ethanol content of the extration solvent (% (v/v))


Zn
Zn concentration (mg L-1)
15
10
5
0
aD
aC
bD
bC
cAB
cB
dD
dC
aA
aB
bA
aB
bAB
bA
cA
cB
1 month
1 week
1 day
1 hour
Extraction time
0
50
80
100
Ethanol content of the extraction solvent (% (v/v))

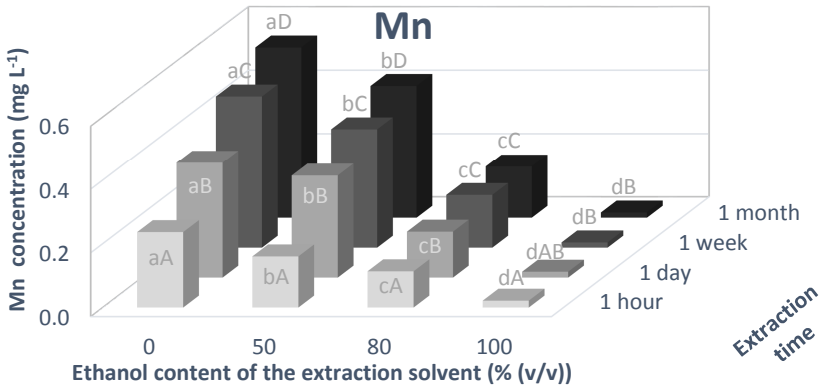
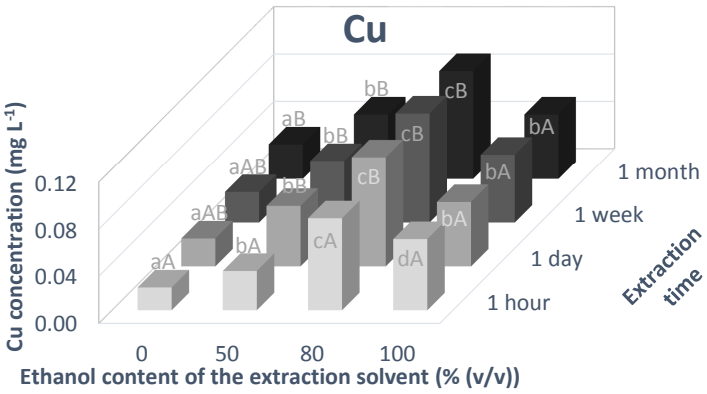
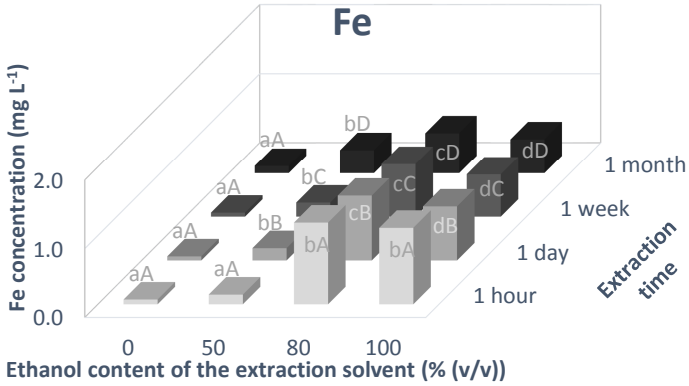
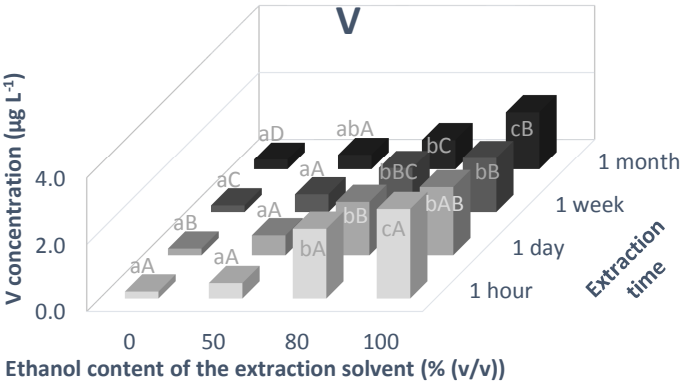
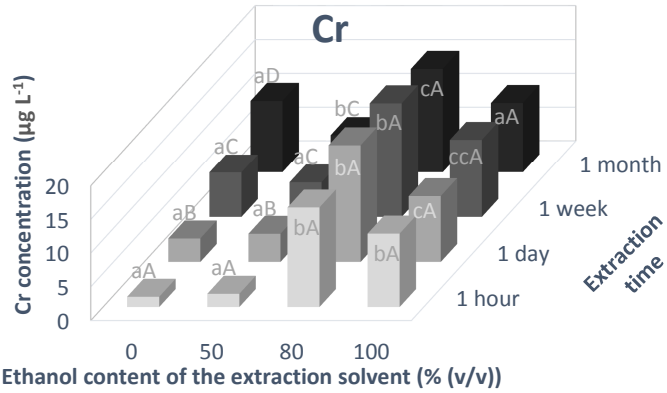


Figure 2: The K, Na, P, Zn and Mn concentrations of the propolis tinctures (n=3) depending on the ethanol content of the extraction solvent and the extraction time. Different small letters mean the significant differences ($p<0.05$) between the effect of the different ethanol content of the extraction solvents. Capitals mean significant differences ($p<0.05$) between the effect of the different extraction times.


Cu
Cu concentration (mg L-1)
0.12
0.08
0.04
0.00
aA
aAB
aAB
aB
bA
bA
bB
bB
cA
cB
cB
cB
dA
bA
bA
cB
1 month
1 week
1 day
1 hour
Extraction time
0
50
80
100
Ethanol content of the extraction solvent (% (v/v))


Fe
Fe concentration (mg L-1)
2.0
1.0
0.0
aA
aA
aA
aA
bB
bC
bA
bA
bD
cB
cC
cD
dB
dC
dD
1 month
1 week
1 day
1 hour
Extraction time
0
50
80
100
Ethanol content of the extraction solvent (% (v/v))


V
V concentration (µg L-1)
4.0
2.0
0.0
aA
aA
aB
aA
aC
aD
bA
bB
bA
bBC
abA
bC
cA
bAB
bB
cB
1 month
1 week
1 day
1 hour
Extraction time
0
50
80
100
Ethanol content of the extraction solvent (% (v/v))


Cr
Cr concentration (µg L-1)
20
15
10
5
0
aA
aA
aB
aB
aC
aC
aD
bA
bA
bA
bC
cA
cA
ccA
aA
1 month
1 week
1 day
1 hour
Extraction time
0
50
80
100
Ethanol content of the extraction solvent (% (v/v))

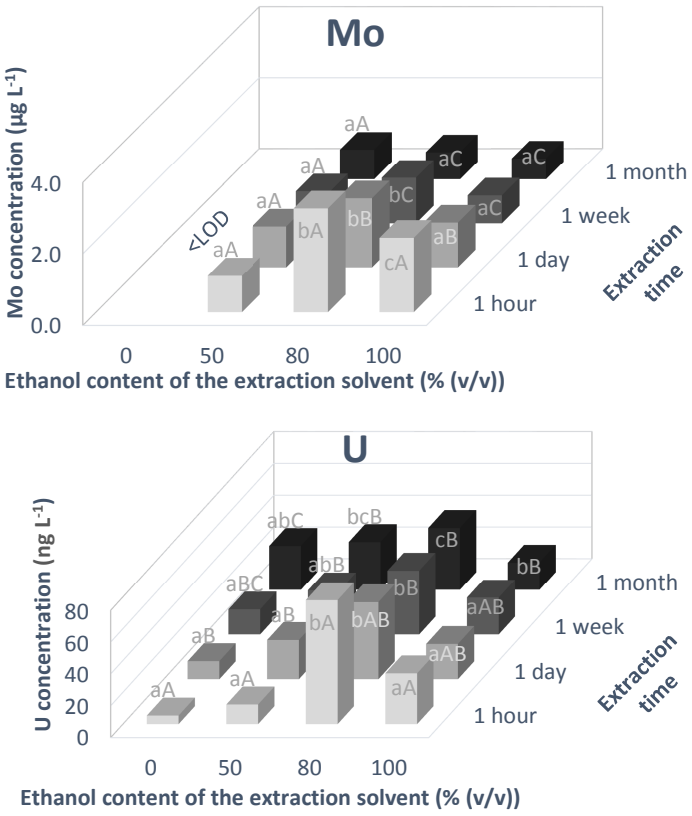


Figure 3: The Cu, Fe, V, Cr, Mo and U concentrations of the propolis tinctures (n=3) depending on the ethanol content of the extraction solvent and the extraction time. Different small letters mean the significant differences ($p<0.05$) between the effect of the different ethanol content of the extraction solvents. Capitals mean significant differences ($p<0.05$) between the effect of the different extraction times. *<LOD: under the limit of detection

- Extraction process of raw propolis highly affects the element content of tinctures
- Most of the elements were dissolved better in aqueous solutions from raw propolis
- Essential elements with some exceptions were extracted well in up to 80% ethanol
- A one week extraction time was enough to extract most of the elements