Determination of trace and major elements in commercial propolis samples by ICP-OES after microwave digestion

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Propolis is a resinous product collected by bees from specific plant sources.There are more than 300 compounds in the structure of propolis. The main ones are phenolic compounds (flavonoids and phenolic acids), benzoic acid and its derivatives, cinnamic alcohol, cinnamic acid and its derivatives, monoterpenes, diterpenes, triterpenes and sesquiterpenes and their alcohol and benzaldehyde derivatives, other phenolic acids and derivatives, alcohols, sugars, ketones, heteroaromatic compounds, aliphatic hydrocarbons, minerals, steroid hydrocarbons and amino acids. Studies have reported that propolis has anti-inflammatory, antihepatotoxic, anticancer, antiviral and antibacterial activities. The concentrations of Na, Mg, K, Ca, P, Fe, Cu, B, Mn, Zn and Al were determined in commercial propolis drop products by ICP-OES. For microwave digestion method, 1 mL of propolis samples in reaction vessels directly, added to each flasks 9 mL concentrated HNO_3. The method was validated according to the parameters of accuracy, precision, linearity, recovery, LOD and LOQ. The recovery percentages were obtained to be between 69.67 to 104.05% in propolis samples.

Keywords: Propolis; Microwave digestion; ICP-OES, Food supplement, Spectroscopy

INTRODUCTION

Bee products such as honey, pollen, royal jelly and propolis have been used since the early ages of mankind for nutrition, health protection and treatment of diseases. Propolis is a sticky substance collected by honey bees from bark, leaves and plant secretions [1]. Propolis, which contains many phenolic compounds such as phenolic acids, esters and flavonoids, is also rich in biological and pharmacological properties. These compounds in the chemical structure of propolis give propolis many biological activities such as antibacteriyel, antiviral, antifungal, antitumor, anti-inflammatory and antiulcer. Propolis, which has many different effects, was first discovered and widely used by the Greeks as a natural antibiotic due to these properties [2]. In addition to this, it is nowadays used versatile in the food sector, apitherapy centers, dermatological and cosmetic applications as well as medicine. In recent years, especially during the coronavirus (covid-19) pandemic, interest in propolis, which is a unique mineral, has increased considerably since it is known to strengthen the immune system and increase body resistance.

Raw propolis contains many different structures and has a mixed composition. Propolis contains resin, vegetable balsam, wax, essential and aromatic oils, pollen, other organic compounds and minerals [3]. Other components consist of other compounds such as vitamins, minerals and elements [4-5]. Deficiency can cause health problems and excessive amounts can have toxic effects [6-8]. Determination of elemental amounts is important for human survival and ecological cycle. Therefore, it is important to make chemical measurements of the elements in dietary supplements.

Macro and micro (trace) elements play a role in many important functions of the organism. Regulation of biological functions of vitamins, enzymes and hormones, oxygen transport, elimination of free radicals, and their use as building and support materials are examples of these functions [6]. In our country and in the world, there are studies on the quantitative analysis of some phenolic compounds and elements in propolis, but there is no study on the quantitative determination of commercial propolis elemental content by ICP-OES. ICP-OES is one of the most frequently used techniques in metal determinations due to its wider working range compared to AAS (Atomic absorption spectroscopy), its ability to determine many elements at the same time (even if their concentrations are very low and different), and its more sensitive results in the determination of refractory elements [9].

In this study, we investigated the multielement contents in commercial propolis drop products by ICP-OES. For elemental analysis, samples were solubilized by microwave and then analyzed by ICP-OES.

EXPERIMENTAL

Instrumentation

ICP-OES (Spectro/ Spectroblue) and MarsXpress (CEM) were used for measurements and microwave digestion. Rf power was 1.4 kW, nebulizer gas flow

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was 1.0 L min^{-1}, plasma-Ar flow was 12 L min^{-1} and auxiliary gas flow was 1.0 L min^{-1}.

Reagents and standards

Nitric acid (65%) and Hydrogen peroxide (35%) were supplied from Sigma-Aldrich, Steinheim, Germany. and Nitric acid and Hydrogen peroxide were used for digestion procedure. Concentration ranges of standard solutions prepared by dilution from 1000 µg/mL stock solution 1.25 - 25 µg/mL for Na, 5-100 µg/mL for Mg, 15-300 µg/mL for Ca, 15-300 µg/mL for K, 25-1000 ng/mL for P, Fe, Cu and B, respectively. Standard stock solutions were supplied from the Merc k (Darmstadt, Germany). The stock solution and working standards were diluted in HNO_3.

Preparation of samples

Commercial propolis samples were purchased in Turkey. The brand names of the propolis drop samples were labeled to A, B and C. A, B and C brands were used water, ethyl alcohol and glycol-water mixture as solvent, respectively.

Microwave digestion procedure

1 mL of commercial propolis drop samples and 9 mL of $HNO_3$was added in digestion vessels. For microwave digestion, commercial propolis samples were kept at 200°C for 45 minutes. After cooling processes, 3 mL of digested samples were took and were diluted to 20.0 mL with ultrapure water. Analyzes were repeated 3 times.

Recovery

Percentage recovery values were calculated with the standard addition method. Percent recovery value was calculated using the formula Cs-C_t/Ck x100, where Cs is element concentration of the spiked sample, Ct is element concentration of the blank target sample, Ck is known spike concentration.

RESULTS AND DISCUSSION

The elemental contents of commercial propolis drop samples dissolved in three different solvents were investigated. Microwave digestion procedure was used because solubilization using a closed system microwave oven for solubilization of samples process is fully realized and evaporation losses are eliminated. Na, Ca, Mg, K, Al, B, Cu, Fe, Mn, P, and Zn were investigated in propolis samples. Analytical parameters are presented in Table 1.

LOD and LOQ were analysed according to 3xσ and 10xσ. %RSD values were below 5 %. All experiments were performed in triplicate and the results expressed as mean ± SD. The concentrations (µg/mL) of Na, Ca, Mg, K, Al, B, Cu, Fe, Mn, P, and Zn in commercial propolis samples are given in Table 2.

Table 1. Values of LOD and LOQ

Element	LOD, ng/mL	LOQ, ng/mL
Na	159.1	530
Ca	19.3	64
Mg	28.7	96
K	129.2	431
Al	0,4838	1,613
B	2,949	9,830
Cu	0,0619	0,206
Fe	0,6769	2,253
Mn	0,6791	2,264
P	7,937	26,457
Zn	0,6433	2,144

Table 2. Element concentrations of commercial propolis samples

	A , µg/mL	**B , µg/mL**	**C, µg/mL**
Na	68.40±1.77	57.28±3.34	3144.02±35.76
Ca	391.15±2.70	9.28±0.08	5.53±0.20
Mg	40.40±1.54	10.08±0.05	2.40±0.07
K	670.03±4.67	209.92±4.45	131.0±2.22
Al	24.11±0.77	16.15±0.10	1.19±0.03
B	5.51±0.12	0.70±0.03	0.00±0.00
Cu	0.79±0.04	0.00±0.00	0.00±0.00
Fe	19.45±0.33	48.14±0.18	4.36±0.19
Mn	4.71±0.05	0.76±0.02	0.14±0.01
P	127.57±1.30	29.59±0.37	2.40±0.25
Zn	8.86±0.16	2.09±0.01	0.12±0.01

The order of the element levels after application of microwave digestion procedure was determined to be C>A>B for Na, A>B>C for Ca, A>B>C for Mg, A>B>C for K, A>B>C for Al, A>B>C for B, A>B=C for Cu, B>A>C for Fe, A>B>C for Mn, A>B>C for P and Zn. The highest mean concentrations were obtained for A, and the lowest concentration was obtained for C in commercial propolis samples. Na was found as the highest element concentration (3144.02 µg/mL) in C sample. K was observed as the second highest element concentration (670.03 µg/mL) in A sample. Ca was found as the third highest element concentration in A sample. Cu and B were found as the lowest element concentration (0 µg/mL) in C sample. Zn was observed as the second lowest element concentration (0.12 µg/mL) in C sample. Zn was found as the third lowest element concentration (0.12 µg/mL) in C sample. The highest element concentration (24.11 µg/mL) of Al was found in A sample. All Al concentrations are acceptable levels by Turkish Food Codex. Recovery results of commercial propolis samples are given in Table 3. Currently, in analytical procedures, recovery percentages in the range from 69.67 to 101.94%.

Table 3. Recovery results of commercial propolis samples

Element	A,%	B,%	C,%
Na	95.6	97.7	100.9
Ca	87.75	89.44	89.31
Mg	102.3	104.05	94.06
K	69.67	97.39	92.61
Al	91.61	91.41	88.24
B	101.94	101.84	102.2
Cu	101.22	101.92	97.52
Fe	94.15	98.6	90.99
Mn	96.05	95.23	89.65
P	96.36	100.1	98.8
Zn	98.78	97.52	91.44

Korn and et al. found Zn, Fe, Mn and Cu concentrations in natural propolis samples by ICP-OES. Zn, Mn Fe and Cu concentrations were higher than our results for all propolis samples [10]. Ferreira et al. analyzed the elemental content of geopropolis after microwave solubilization method by ICP-OES. Zn, Mg, K, Mn and Cu concentrations were higher than our results for all samples and Na concentrations were lower than our C samples and Fe values were lower than our A and B samples. [11]. Tosic and et al. were investigated macro and microelements in Serbian propolis samples by ICP-OES. Cu, B, Mn and Fe values were higher than our results for all samples [12]. Matuszewska and et al were analyzed bee pollen, propolis, and royaljelly collected in west-central Poland by ICP-OES. Cu , P and Fe were higher than our results for all samples [13].Liben and et al. were investigated elemental contents in honey and propolis samples in Ethiopia. Total phenolic and flavonoid contents were analysed in propolis but element contents of propolis weren't investigated [14].

When we consider the results of the analysis of commercial propolis drop samples in general, it was observed that all the elements (Ca, Mg, K, Al, B, Cu, Mn, P and Zn) except Fe and Na were more abundant in A samples. The elemental concentrations found in studies on natural propolis samples in the literature are very similar to the analysis results of propolis samples with water as solvent in our study. The results obtained as a result of the study show that water is a better solvent for commercial propolis samples, as it was found that the elemental contents of commercial propolis drop samples using water as a solvent are closer to the elemental contents of natural propolis samples in the literature. Calcium is an essential element for enzyme activation and bone development. Sodium plays an important role in the functioning of muscle and nerve cells in our body. Potassium is involved in the functioning of enzymes, cell division and growth, and the regulation of blood pressure. Potassium deficiency can cause heart problems. High doses of copper damage the liver and kidneys, while deficiency causes fatigue and anemia. Iron is found in red blood cells called hemoglobin, which is responsible for blood production and transports oxygen from the blood to the tissues. The most important functions of manganese in our body are to contribute to the formation of carbohydrate and fat metabolism and to regulate blood sugar. Zinc is an important trace element involved in protein and DNA synthesis. Daily intake doses for calcium, magnesium, sodium and potassium are 1000-1200 ppm; 320-420 ppm; 1300-1500 ppm; 2000-2300 ppm, respectively [15].

CONCLUSION

The concentrations of sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), phosphorus (P), iron (Fe), copper (Cu), boron (B), manganese (Mn), zinc (Zn) and aluminum (Al) in commercial propolis samples were investigated. This the first study in propolis samples using microwave digestion by ICP-OES. Ca, Mg and K values were below RDA levels so the findings indicate all propolis samples are not good source of essential elements but Na were above RDA in propolis drop samples dissolved in ethyl alcohol. Fe and Mn values in all propolis samples were above RfD and TDI values but Cu and Zn values in propolis samples dissolved in water were above for RfD and TDI values.

REFERENCES

1. S. Castaldo F. Capasso. *Fitoterapia*,**73**(1), S1 (2002).
2. A. Papachristoforou, E. Koutouvela, I. Mourtzinos, *Chem. & Diversity,* **16**(7), e1900146 (2019).
3. V.S. Bankova, S.L. De Castro, M.C. Marcucci. *Apidologie,* **31,** 3 (2000).
4. R. Christov, B. Trusheva, M. Popova, V.S. Bankova, M. Bertrand, *Natural Product Research* **20**(6), 531 (2006).
5. W. Yang, Z. Wu, Z.Y. Huang, X. Miao. *J. Food Sci. Technol.,* **54**(11), 3375 (2017).
6. Parsons PJ, Barbosa F. Atomic spectrometry and trends in clinical laboratory medicine. *Spectrochim. Acta Part B, Atomic Spect.,* **62**(9), 992v(2007).
7. Y. Zheng, X.K. Li, Y. Wang, I. Cai.. *Hemoglobin,* **32**(1–2), 135 (2008).
8. M.L. Carvalho, T. Magalhães, M. Becker, A. von Bohlen. *Spectrochim. Acta Part B Atomic Spect.,* **62**(9),1004 (2007).
9. C. Douvris, T. Vaughan, D. Bussan, G. Bartzas, R. Thomas, Review of the global application landscape. *Science of The Total Environ.*, **905**, 167242 (2023).
10. M,G.A. Korn, M.A.P. Guida, J.T.P. Barbosa, E.A. Torres, A.P. Fernandes, J.C.C. Santos, K.G.F. Dantas, J.A. Nóbrega, *Food Anal. Methods,* **6**, 872 (2013).
11. R.C. Ferreira, F.S. Dias, C.A. Tannus, F.B. Santana, D.C.B.M. Santos, F.S. Dias, M.S. Castro, H.N. Brandão, A.F.S. Júnior, L.C.R.C. Silva, F.A. Chinalia, Essential and Potentially Toxic Elements from. *Biol. Trace Element Research,* **199**,3527 (2021).
12. S. Tosic, G. Stojanovic, S. Mitic, A. Pavlovic, S. Alagic . *J. Apıc. Sci*., **61**(1),5 (2017).
13. E. Matuszewska, A. Klupczynska, K. Maciołek, Z.J. Kokot, J. Matysiak, *Molecules,* **26**, 2415 (2021).
14. T. Liben M, Atlabachew A. Abebe, *Cogent Food & Agri.,***4**, 1475925 (2018).
15. A. Karasakal, *Biological Trace Element Research*, **197** (2), 683 (2020).