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Research Article

Cu, Fe, Mn AND Zn CONTENTS IN PROPOLIS SAMPLES FROM MALATYA, TÜRKİYE AND HEALTH RISK ASSESSMENT

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ABSTRACT

In this study, copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) contents of propolis samples obtained from five different districts of Malatya, Türkiye were investigated. Element contents were determined using flame atomic absorption spectrometry (FAAS). The concentration ranges of propolis samples taken from five different districts of Malatya province varied between 1.24 ± 0.06 mg/kg and 6.28 ± 0.02 mg/kg for Cu, 206.28 ± 21.50 mg/kg and 663.08 ± 55.24 mg/kg for Fe, 15.40 ± 0.17 mg/kg and 27.11 ± 1.83 for Mn, 39.36 ± 1.82 mg/kg and 52.57 ± 2.13 mg/kg for Zn. The results obtained for each district and element were evaluated in detail considering the estimated daily intake (EDI), recommended dietary allowance (RDA), provisional maximum tolerable daily intake (PMTDI) of these elements for adults. Moreover, hazard coefficient (HQ) was calculated for risk assessment and it was determined that HQ values for the studied elements in bee propolis from all districts were below 1.

INTRODUCTION

Elements that play an important role in maintaining vital functions in the human body (Ca, Mg, K, Cu, Zn, Fe, etc.) are involved in many functions in the body, such as physiological functions, metabolic mechanisms, and enzyme functions (Stacewicz-Sapuntzakis, Bowen, Hussain, Damayanti-Wood & Farnsworth, 2001; Mezzaroba, Alfieri, Simao & Reiche, 2019; Vatansever, Ozyigit & Filiz, 2017). For these functions to be maintained in the body, it is very important to take nutritious foods rich in mineral elements into the body through nutrition. However, taking too little or too many of these elements into the body can negatively affect body functions. In recent years, bee products (such as propolis, royal jelly and pollen) have begun to be consumed by humans due to their high nutritional value. Propolis, a natural mixture of these products, is a wax resin and is collected by honeybees from shoots, plant resin, leaves, and flowers. Propolis has antioxidant, anti-inflammatory, antitumor, antibacterial, antifungal and antiviral properties (Zulhendri et al., 2021; Aboulghazi et al., 2024; Chan, Cheung & Sze, 2013; Kalogeropoulos, Konteles, Troullidou, Mourtzinos & Karathano, 2009; Nainu et al., 2021; Tumbarski et al., 2023; Keskin & Cetin, 2020; Silva, Rodrigues, Feas & Estevinho, 2012;

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Wozniak et al., 2020). It has been reported that propolis is rich in many bioactive components such as flavonoids, phenolic acids, vitamins, essential minerals and oils (Di Capua, Bejarano, Adami & Reverchon, 2018; Jansen-Alves et al., 2018; Yesiltas et al., 2014). These bioactive components vary depending on the vegetation, geographical and climatic characteristics of the region where bees live and feed, and the chemical composition of each propolis species found in different regions of the world is different (Di Capua, Bejarano, Adami & Reverchon, 2018; Ozdal et al., 2019; Yen et al., 2017). Today, because of human activities (such as urbanization, mining, industrialization and agricultural activities), the damage caused by chemical pollutants to the environment is increasing (Tutun et al., 2022). In addition to essential trace elements that have a toxic effect when taken excessively, it is very important to monitor toxic elements. The accumulation of toxic and essential trace elements in food products and the consumption of these foods by humans can pose a risk to human health (Tutun et al., 2022; Mititelu et al., 2022).

The contents of essential trace elements such as Cu, Fe, Mn and Zn in propolis samples collected from five different districts of Malatya/Türkiye were determined by FAAS (flame atomic absorption spectrometry), and the obtained elemental results were evaluated statistically. Considering the elemental contents, the daily contribution of the studied elements to human nutrition and the risk factors for health were evaluated in detail.

MATERIAL AND METHOD

Reagents and Standards

Standard solutions of Cu, Fe, Mn, Zn (1000 mg/L), HNO_3 and H_2O_2 were purchased from Merck (Merck & Co., Inc., Whitehouse Station, NJ, USA). All chemicals and solvents used in all experimental studies were analytically pure. Ultrapure water (Milli-Q, Millipore 18.2 $\mu\Omega$ cm^{-1} resistivity) was used in all experiments. Standard solutions of the elements were prepared by diluting the stock solutions of the studied elements. Standard solutions prepared at different concentrations were used to obtain the calibration graph of each element. All glassware was cleaned with a 10% HNO_3 solution before use and then rinsed with deionized water.

Instrumentation

Cu, Fe, Mn and Zn analyses were performed using Perkin Elmer AAnalyst 800 FAAS (Perkin Elmer, Inc., Shelton, CT, USA). The system is equipped with a hollow cathode lamp, air-acetylene flame, and a single slot-burner head. The operation conditions used for FAAS are given in Table 1.

Table 1. Instrumentation operation condition for FAAS*

Element	Cu	Fe	Mn	Zn
Wavelength (nm)	324.8	248.3	279.5	213.9
Slit Wight (nm)	0.7	0.2	0.2	0.7

*Flow of air: 17.0 L min^{-1}, Flow of acetylene: 2.0 L min^{-1}.

Sampling and Sample Preparation

Propolis samples were supplied from different honey producers from five different districts (Arapgir, Arguvan, Hekimhan, Kale, Puturge) of Malatya province in the Eastern Anatolia Region of Türkiye in 2022. These samples were cleaned, ground into small pieces, and stored at +4 °C until analysis time. The propolis samples obtained from Arapgir, Arguvan, Hekimhan, Kale and Puturge districts were called *Propolis I, Propolis II, Propolis III*, *Propolis IV* and *Propolis V*, respectively.

About 0.5 g of propolis samples obtained from different districts of Malatya were weighed in 3 parallel samples for each district. They were digested with 2 mL of a mixture of concentrated HNO_3 and H_2O_2 prepared in a one-to-one ratio on a hot plate. The samples were evaporated until almost dry, and 2 mL of a mixture of the same concentration was added to the samples again and evaporated until dry. After the samples were cooled, the final volume was taken with 15 mL of 1.0 M HNO_3 and filtered with Whatman filter paper. Cu, Fe, Mn and Zn analyses were performed with FAAS in clear solutions. Blank samples were prepared in the same way as the samples. The results obtained for different districts are the average of three values, and the results were given as the average value ± standard deviation on a dry weight basis. The accuracy of the method was tested with the standard reference material NIST-1547 peach leaves and all the procedures applied to the samples were applied to the standard reference material. Experimental results were statistically evaluated using one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests. The differences were considered statistically significant when $p < 0.05$.

Estimation of Daily Intake (EDI) and Hazard Quotient

In human nutrition, adequate intake of Cu, Fe, Mn and Zn elements varies according to age and gender. In this study, the intake values of these elements over the age of 19 were evaluated considering females and males (Institute of Medicine, 2006). The calculation of estimated daily intakes (EDI, mg/kg bw/day) is expressed as element concentration (mg/kg) and meal size (kg/day) divided by adult body weights (kg) (Tutun et al., 2022; Mititelu et al., 2022).

Hazard quotient (HQ) was calculated to evaluate the non-carcinogenic risks for Cu, Fe, Mn, and Zn with propolis consumption, and hazard index (HI), which is the sum of the hazard quotients, was calculated to assess the potential risk of elements in food. Systemic effects may occur when the risk of HQ is above 1, which is an indicator higher than the reference dose of HQ (US-EPA, 2007). The calculation of HQ is expressed in the equation below (Tutun et al., 2022; Mititelu et al., 2022):

$$HQ = C \times EF \times ED \times IR/RfD \times BW \times AT$$

C: Element content in propolis, mg/kg

MS or IR: Meal size or Ingestion rate, 0.00315 kg/day propolis for adults (Tutun et al., 2022)

BW: Body weight, 70 kg for adults (US-EPA, 2000)

EF: Exposure frequency, 52 days/year for people who eat propolis one time a week

ED: Exposure duration, 70 years for adults

AT: Averaging time, ED × 365 days/year

RfD: Oral reference dose, 0.04 mg/kg bw/day for Cu, 0.7 mg/kg bw/day for Fe, 0.14 mg/kg bw/day for Mn, 0.3 mg/kg bw/day for Zn (US-EPA, 2007).

RESULTS AND DISCUSSIONS

Limit of detection (LOD) and limit of quantification (LOQ) values were determined for Cu, Fe, Mn and Zn. For this purpose, calculations were made with a formula defined as 3×SD/b for LOD and 10×SD/b for LOQ, where SD is the standard deviation of the blank and b is the slope of the analytical curve. Repeatability was calculated as the relative standard deviation (RSD%) and was found to be less than 10% for the studied elements. LOD and LOQ values for Cu, Fe, Mn and Zn elements are presented in Table 2. The essential trace element (Cu, Fe, Mn, Zn) contents of propolis samples collected from five districts including Arapgir (*Propolis I*), Arguvan (*Propolis II*), Hekimhan (*Propolis III*), Kale (*Propolis IV*), Puturge (*Propolis V*) of Malatya, Türkiye were determined, and these results were evaluated considering EDI, RDA, PMTDI, HQ values calculated for each element. As seen in Table 2, the concentration ranges of propolis samples taken from five different districts of Malatya province varied between 1.24 ± 0.06 mg/kg and 6.28 ± 0.02 mg/kg for Cu, 206.28 ± 21.50 mg/kg and 663.08 ± 55.24 mg/kg for Fe, 15.40 ± 0.17 mg/kg and 27.11 ± 1.83 for Mn, 39.36 ± 1.82 mg/kg and 52.57 ± 2.13 mg/kg for Zn. When the element contents of each district are evaluated separately, the highest Fe and Zn contents were determined in *Propolis V,* and the results were

found to be 663.08 ± 55.24 mg/kg and 52.57 ± 2.13 mg/kg. The highest Cu and Mn contents were determined in *Propolis III* and *Propolis IV*, and the results were found to be 6.28 ± 0.02 mg/kg and 27.11 ± 1.83 mg/kg. When the lowest essential element contents were evaluated, it was found that the lowest Mn (15.40 ± 0.17 mg/kg) and Zn (39.36 ± 1.82 mg/kg) contents were determined in *Propolis III*. Similarly, when Cu and Fe contents were evaluated, the lowest results were observed in *Propolis IV*, and the results were found to be 1.24 ± 0.06 mg/kg for Cu and 206.28 ± 21.50 mg/kg for Fe. The results obtained for the studied element were also evaluated statistically. When the statistical results of the Cu contents of propolis samples were evaluated, significant differences were found between both propolis samples except *Propolis II* and *Propolis V* ($p < 0.05$). According to the statistical results, the Fe contents of propolis samples showed significant differences between both propolis samples except *Propolis I* and *Propolis III*, *Propolis I* and *Propolis IV*, *Propolis III* and *Propolis IV* ($p < 0.05$). The Mn contents of propolis samples were evaluated statistically, and no significant difference was found between both propolis samples except *Propolis I* and *Propolis IV*, *Propolis II* and *Propolis IV*, *Propolis III* and *Propolis IV*, *Propolis IV* and *Propolis V* ($p < 0.05$). When the statistical results of the Zn contents of propolis samples were evaluated, no significant differences were found between both propolis samples except *Propolis III* and *Propolis IV*, *Propolis III* and *Propolis V* ($p < 0.05$). The accuracy of the method was verified using NIST-1547 peach leaves standard reference material. The recoveries of the elements were found to be 95% for Cu, Mn and 98% for Fe, Zn.

Table 2. The element contents of propolis belonging to five different districts of Malatya, Türkiye (n=3).

Propolis samples	Cu, mg/kg	Fe, mg/kg	Mn, mg/kg	Zn, mg/kg
Propolis I	4.98 ± 0.26^{a}	313.41 ± 1.76^{a}	19.49 ± 1.72^{a}	46.88 ± 2.87^{a}
Propolis II	3.25 ± 0.34^{b}	452.50 ± 26.16^{b}	16.15 ± 1.45^{a}	44.89 ± 3.38^{a}
Propolis III	6.28 ± 0.02^{c}	259.54 ± 10.93^{a}	15.40 ± 0.17^{a}	39.36 ± 1.82^{a}
Propolis IV	1.24 ± 0.06^{d}	206.28 ± 21.50^{a}	27.11 ± 1.83^{b}	$50.24 \pm 0.06^{a,b}$
Propolis V	2.67 ± 0.03^{b}	663.08 ± 55.24^{c}	19.63 ± 1.52^{a}	$52.57 \pm 2.13^{a,b}$

Average values of different letters were significantly different from each other ($p < 0.05$).
LOD: 0.03 mg/kg for Cu and Fe, 0.04 mg/kg for Mn, 0.02 mg/kg for Zn; LOQ: 0.09 mg/kg for Cu, 0.10 mg/kg for Fe, 0.11 mg/kg for Mn, 0.06 mg/kg for Zn.

Estimated daily intakes (EDI) values for females and males over 19 years of age were evaluated separately for each element. When examined in Table 3, EDI in propolis samples varied between 0.0001 and 0.0003 mg/kg bw/day for Cu, 0.0093 and 0.0298 mg/kg bw/day for Fe, 0.0007 and 0.0012 mg/kg bw/day for Mn, and 0.0018 and 0.0024 mg/kg bw/day for Zn. Recommended daily allowance (RDA) values in propolis samples were evaluated considering the recommended values for females and males (Table 3). The RDA values for Cu varied

between 0.006% and 0.031% in females and males, the RDA values for Fe varied between 0.052% and 0.373% in females and 0.116% and 0.373% in males, the RDA values for Mn varied between 0.038% and 0.068% in females and 0.030% and 0.053% in males, the RDA values for Zn varied between 0.022% and 0.030% in females and 0.016% and 0.022% in males (Table 3). PMTDI values were calculated considering the element contents for propolis samples in Table 3, and the results are presented as %. PMTDI values varied between 0.01% and 0.03% for Cu, 0.93% and 2.98% for Fe, 0.07% and 0.12% for Mn, and 0.18% and 0.24% for Zn. Additionally, HQ values were calculated for health risk assessment; the highest HQ values were found for *Propolis III* (0.0011) for Cu, *Propolis V* (0.0061) for Fe, *Propolis IV* (0.0012) for Mn and *Propolis IV and V* (0.0011) for Zn. The lowest HQ values were found for *Propolis IV* (0.0002) for Cu, *Propolis IV* (0.0019) for Fe, *Propolis II and III* (0.0007) for Mn and *Propolis III* (0.0008) for Zn. HQ values below 1 indicate that there is no health risk.

Table 3. EDI, RDA, PMTDI, and HQ values calculated considering the studied contents of propolis samples of Malatya, Türkiye.

		Propolis samples				
Element		*Propolis I*	*Propolis II*	*Propolis III*	*Propolis IV*	*Propolis V*
Cu	EDI (mg/kg bw/day)	0.0002	0.0001	0.0003	0.0001	0.0001
	RDA (%)	0.025(F,M)	0.016(F,M)	0.031(F,M)	0.006(F,M)	0.013(F,M)
	PMTDI (%)	0.02	0.02	0.03	0.01	0.01
	HQ	0.0008	0.0005	0.0011	0.0002	0.0004
		Propolis I	*Propolis II*	*Propolis III*	*Propolis IV*	*Propolis V*
Fe	EDI (mg/kg bw/day)	0.0141	0.0204	0.0117	0.0093	0.0298
	RDA (%)	0.078-0.176(F) 0.176(M)	0.113-0.255(F) 0.255(M)	0.065-0.146(F) 0.146(M)	0.052-0.116(F) 0.116(M)	0.166-0.373(F) 0.373(M)
	PMTDI (%)	1.41	2.04	1.17	0.93	2.98
	HQ	0.0029	0.0041	0.0024	0.0019	0.0061
		Propolis I	*Propolis II*	*Propolis III*	*Propolis IV*	*Propolis V*
Mn	EDI (mg/kg bw/day)	0.0009	0.0007	0.0007	0.0012	0.0009
	RDA (%)	0.049(F) 0.038(M)	0.040(F) 0.032(M)	0.038(F) 0.030(M)	0.068(F) 0.053(M)	0.049(F) 0.038(M)
	PMTDI (%)	0.09	0.07	0.07	0.12	0.09
	HQ	0.0009	0.0007	0.0007	0.0012	0.0009
		Propolis I	*Propolis II*	*Propolis III*	*Propolis IV*	*Propolis V*
Zn	EDI (mg/kg bw/day)	0.0021	0.0020	0.0018	0.0023	0.0024
	RDA (%)	0.026(F) 0.019(M)	0.025(F) 0.018(M)	0.022(F) 0.016(M)	0.028(F) 0.021(M)	0.030(F) 0.022(M)
	PMTDI (%)	0.21	0.20	0.18	0.23	0.24
	HQ	0.0010	0.0010	0.0008	0.0011	0.0011

Adequate intakes (AIs) for >19 years of age: for F and M 0.9 mg/day for Cu, for 8-18 mg/day F and 8 mg/day M for Fe, for 1.8 mg/day F and 2.3 mg/day M for Mn, for 8 mg/day F and 11 mg/day M for Zn (Institute of Medicine, 2006). Tolerable upper intake levels (UL) for adults: for 10 mg/day for Cu, 45 mg/day for Fe, 11 mg/day for Mn, and 40 mg/day for Zn (Institute of Medicine, 2001). Provisional maximum tolerable daily intakes (PMTDIs): 0.5

mg/kg bw/day for Cu, 0.8 mg/kg bw/day for Fe, 0.36 mg/kg bw/day for Mn and 1.0 mg/kg bw/day for Zn (FAO/WHO, 2007).

Mutlu et al. (2023) examined the element contents of propolis samples obtained from different parts of Türkiye, including Malatya province. They determined the average element contents for the provinces including Malatya province as the Eastern Anatolia Region, and the propolis contents of this region were found to be 627 ± 122 mg/kg for Fe, 9.43 ± 3.75 mg/kg for Mn, and 5.46 ± 3.84 mg/kg for Zn (Mutlu, Ozer-Atakoglu, Erbas & Yalcin, 2023). Tutun et al. (2022) investigated the element contents of bee pollen and propolis in Türkiye and evaluated their effects on health risk. In propolis samples obtained from different provinces, Cu, Fe, Mn and Zn contents were determined as 2.76 ± 1.46 mg/kg on average in the range of 0.61 to 6.08 mg/kg, 390 ± 181 mg/kg on average in the range of 69 to 658 mg/kg, 10.6 ± 5.88 mg/kg on average in the range of 1.61 to 28.0 mg/kg and 36.3 ± 18 mg/kg on average in the range of 7.98 to 102 mg/kg, respectively, and the results were reported to have no health risk (Tutun et al., 2022). Arslan et al. (2021) investigated the chemical and biological properties of propolis obtained from *Apismellifera caucasica* from Ardahan and Erzurum provinces of Türkiye. The element contents in propolis samples for Ardahan province was found as 2.45 ± 0.16 mg/kg for Cu, 428.51 ± 77.75 mg/kg for Fe, 5.297 ± 0.71 mg/kg for Mn, 30.05 ± 7.30 mg/kg for Zn and the element contents in propolis samples for Erzurum province 2.01 ± 0.79 mg/kg for Cu, 507.62 ± 287.13 mg/kg for Fe, 7.47 ± 3.27 mg/kg for Mn, 41.77 ± 19.65 mg/kg for Zn (Arslan et al., 2021). The elemental contents of propolis samples from different provinces in Türkiye were found to be compatible with the propolis samples from Malatya province. However, the element contents of propolis samples may differ depending on the region due to the environmental conditions in which bees live. Risk assessment can be carried out with element analyses in different provinces and regions to monitor environmental health and contamination levels of pollutants in bee products (Tutun et al., 2022).

CONCLUSION

The elements Cu, Fe, Mn and Zn were determined in propolis samples obtained from five different districts of Malatya, Türkiye. The Cu, Fe, Mn and Zn contents of propolis samples were determined by FAAS, and the obtained results were also evaluated statistically. In addition, the element contents were calculated by considering adult females and males as the estimated daily intake (EDI), recommended dietary allowance (RDA) and provisional maximum tolerable daily intakes (PMTDIs). It was found that there was no risk according to the tolerable limits allowed in the intake of the estimated elements studied in the propolis

samples. In addition, HQ values were calculated for each element and propolis sample for health risk assessment. Since the HQ value was below 1 for all propolis samples and elements, no health risk was found.

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