Scientific Papers. Series B, Horticulture. Vol. LXVIII, No. 1, 2024
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EVALUATION OF SOME ESSENTIAL ELEMENTS IN WALNUT KERNEL

Maria RADA[1], Simion ALDA[2], Ariana VELCIOV[2], Daniela SCEDEI[2], Iasmina Mădălina ANGHEL[3], Alina DOBREI[2], Eleonora NISTOR[2], Marcel DANCI[2]

[1]"Victor Babes" University of Medicine and Pharmacy Timisoara, 2 Piata Eftimie Murgu, 300041 Timisoara, Romania
[2]University of Life Sciences "King Mihai I" from Timisoara, 119 Calea Aradului, 300645, Timisoara, Romania
[3]Politehnica University Timisoara, 1 Mihai Viteazul Blvd, 300222, Timisoara, Romania

Corresponding author email: marcel_research@yahoo.com

Abstract

The paper presents the results in determining essential element content in walnut kernel marketed in agri-food markets in Timisoara (Romania) and in estimating the supply of minerals of this food drug. Results of mineral elements analysis through flame atomic absorption spectrometry show that walnut kernel analyzed contains increased amounts (mg/100g) dry matter of K (338-457), Mg (163-270) and Ca (62.60-119) and significant amounts of Mn (2.56-10.40), Fe (l.19-5.58), Zn (1.23-3.58), Na (0.75-6.45), Cu (0.58-3.14), Cr (0.08-0.60), unevenly distributed according to the origin of the kernel and the nature of the analyzed element. The results obtained when evaluating the mineral intake, show that a consumption of 25 g of walnut kernel contributes differently to ensuring the daily mineral needs: is not of interest from the point of view of the contribution of Na, Ca and K, but can cover significant percentages of the required Zn, Fe and Mg and high Cr, Cu and Mn. Given the results of our experiment, we can say that the analyzed walnut kernel can be considered as a good additional source, especially of Cr, Mn, Cu, Mg and less as the source of Zn and Fe.

***Key words**: walnut kernel, essential element, mineral intake.*

INTRODUCTION

Walnut, the fruit of the Juglas tree directed L., is the most valuable of all hard-shelled fruits (Ros, 2010). Walnuts contain a lot of nutrients, on average: 60-70% lipids (especially mono and polyunsaturated fats), 12-24% protein, 12-18% carbohydrates, 6.7% dietary fiber and 1.7-2.0% minerals (Simsek, 2016; Cosmulescu, 2009; Leahu et al., 2013). Walnut kernel, the edible part of walnut, is one of the most appreciated fruits and it is an excellent source of essential nutrients such as: essential minerals, carbohydrates, phenolic compounds, vitamins and omega-3 and omega-6 polyunsaturated fatty acids etc. (Cindrić et al., 2018; Şen and Karadeniz, 2015; Trandafir et al., 2016); Yilmaz and Akça, 2017; Al-Snafi, 2018; Momchilova et al., 2016). Walnut kernel are a rich source of α-linolenic acid, phytosterols, nonsodium minerals, γ-tocopherol, melatonin and polyphenols (Ros, 2018). Walnut kernels also contain important vitamin structures such as riboflavin, niacin, thiamine, pantothenic acid, vitamin B_6 and folates (Şen and Karadeniz, 2015).

Regarding the composition of minerals, walnut is considered a good source of minerals. Potassium (K), phosporous (P), magnesium (Mg), sulphur (S), copper (Cu), zinc (Zn), mangane Mn, iron (Fe), selenium (Se), chromium (Cr), etc. (Simsek, 2016; Cindrić et al., 2018; Trandafir et al., 2016, Yilmaz et Akça, 2017; Chatrabnous et al., 2018; Polat et al., 2015; Ozyigit et al., 2018; Rada et al., 2018; Cosmulescu et al., 2010; Zhai et al., 2014), essential for the normal functioning of the human body. Walnuts are low in Na, but rich in K, Mg, and Ca. These three minerals are involved in many aspects of cellular metabolism and other biological processes, including insulin sensitivity, blood pressure regulation and vascular reactivity (Ros, 2018). Microelements, such as Fe, Cu, Zn, Mn, and Se are essential components of biological structures and have an important effect on and play a key role in a variety of the processes necessary for life throughout mediate vital

biochemical reactions (Al-Fartusie and Mohssan, 2017).
The special quality and richness of bioactive nutrients and phytochemicals that are part of the walnut kernel also confers numerous therapeutic qualities: reduce risk of developing cancer, cardiovascular risk decreases, help to reduce body weight, improve endocrine parameters in polycystic ovary syndrome, help to control diabetes, improve thinking ability etc. (Şen and Karadeniz, 2015; Ros, 2018; Fatima et al., 2018; Kalkişim et al., 2014)**.**
The fact that in the walnut kernel, the edible part of the walnut fruit (*Juglans regia L.*), are concentrating increased amounts of essential mineral elements is supported by numerous studies in the field published in the last ten years.
In the analysis of walnut kernels from some walnut cultivars in Kirşehir province (Turkey), Özcan et al. determined important concentrations of essential mineral elements: 11.99-15.11 mg/kg B, 2462-2758 mg/kg Ca, 1.695-3.323 mg/kg Cr, 5.676-5.944 mg/kg Cu, 17.88-21.82 mg/kg Fe, 5380-3478 mg/kg K, 4163-5488mg/kg Mg, 17.59-22.20 mg/kg, Mn, 1.671 mg/kg Mo, 617-833 mg/kg Na, 1.651-1.915 mg/kg Ni, 2226-2604 mg/kg P and 0.623-17.981 mg/kg - Zn (Özcan et al., 2010).
Turkish walnuts were also investigated for contamination with heavy metals. Kalkişim et al., determined the levels of essential and non-essential elements in walnut powder (*Juglans regia* L.) collected near and far from the Gumushane highway area. The average concentrations of trace elements Al, Cd, Cr, Cu, Fe, Ni, Mn, Zn, Ba, Na and Sr in the walnut samples collected near the motorway area were (μg/g): 10.39, 0.055, 0.503, 15.82, 32.54, 1.16, 19.96, 21.48, 3.90, 116.12, respectively, 3.22 (Kalkişim et al., 2014). Macro-elements: Ca, K, and Mg were determined in much higher concentrations: 0.72, 3.40, respectively, 1.15 mg/g (Kalkişim et al., 2014). Comparing the values obtained with the average data obtained for the control samples, the study authors observed that the levels of Al, Cd, Cr, Cu, Ca, K, Na and Sr are higher in the walnut samples collected near the highway area. Kafaoğlu et al. reported increased amounts of macroelements and significant amounts of trace elements: Mg (1034.96 mg/kg), Ca (793 mg/kg), B (15.5 mg/kg), Co (29.11 μg/kg), Cu (11.73 mg/kg) Fe (22.08 mg/kg), Mn (24.23 mg/kg), Ni (718.66 μg/kg) and Zn (23.25 mg/kg) (Kafaoğlu et al. 2016). The fact that in the composition of the walnut kernel from different genotypes and varieties of walnut (*Juglans regia* L.) from Turkey are concentrated increased amounts of K, Mg and Ca as well as significant amounts of Na, Fe, Mn, Zn, Cu is to be demonstrated and the works elaborated by Polat et al. (2015), Simsek (2016) and Yilmaz et al. (2017). The authors of these studies showed that the walnut kernel samples have the following mineral profile (mg/100 g): K (408.37-569.48), Ca (94.79-267.85, Mg (241-426), Cu (0.72-1.43), Zn (1.93-3.47) (Polak et al., 2015); K (534.3-778.6), P (346.0-584.8), Ca (100.9-233.9) and Mg (117.8-181.4), Na (8.67–19.29), Fe (3.13–5.37), Mn (2.02-4.50), Zn (1.44-3.63) and Cu (0.77-2.44) (Simsek, 2016); P (230.65-344.40), K (163.92-308.86), Na (7.94-22.53), Cu (2.25-364), Fe (2.21-4.32), Zn (1.97-5.48), Mn (0.91-439) (Yilmaz and Akça, 2017).
The Persian walnut kernel was also analyzed by Aryapak and Ziarati, which when evaluating the traditional walnut kernel in Teheran and Karaj (Iran) determined high concentrations (mg/100 g dry weight) of: Na (58.42-66.54), K (546.46-617.36), Ca (235.19-276.46), Mg (580.11-692.49); Fe (444.02-559.52), Cu (281.20-322.73), Se (0.003) and Zn (133.92-142.0) (Aryapak and Ziarati, 2014). These nuts, are distinguished by high contents of Fe, Cu, Zn, and Na.
Important data regarding the mineral profile are provided by Ebrhimi et al. which in a study on the importance of Persian walnuts (*Juglans egia* L.) in human health, shows that the Persian walnut kernel is a food rich in essential minerals (mg/100 g): 358, 414, 372.69, and 340 K; 362 P; 153, 316, 140, and 115 Mg; 125, 61, 120, and 72 Ca; 3.8, 4.6, and 3.2 Fe; 4.4, 6.4, 7.4, and 11.5 Na; 3.8, 2.12, and 2.2 Cu (Ebrhimi et al., 2018).
Interest in knowing the concentrations of mineral elements in walnut kernels from cultivated walnut species or from various domestic markets have also been reported in papers published by researchers in China. Analyzing the mineral concentration of walnut kernel from 17 types of walnut (*Juglans regia*

L.) from China, Zhai et al. determined increased concentrations (mg/100 g) of Mg (401.47-749.27) and Ca (253.90-504.07), as well as significant amounts (mg/100 g) of Fe (4.44-10.45), Mn (2.73-17.12), Zn (6.84-13.32) and Cu (1.37-5.21) (Zhai et al., 2014). Proceeding to determine some elements that have essentiality or toxicity for the body from the walnut kernel marketed in Chinese markets, Ni et al. and Yin et al., found that this food contains different amounts (mg/kg) of essential and potentially toxic elements: 0.24-0.35 (Cr); 34.4-57.9 (Mn); 34.3-44.5 (Fe); 0.32-0.67 (Mo); 9.30-24.7 (Cu); 14.8-27.7 (Zn); 0.035-0.072 (Se); 8.43-14.2 (Sr), 0.99-2.22 (Al); 0.0032-0.013 (As), 0.012- 0.022 (Cd); 0.0051-0.012 (Pb) (Ni et al., 2016), respectively: 10 μg/g, Mn; 0.02 μg/g, Co; 8.8 μg/g, Cu; 20 μg/g, Zn; 0.01 μg/g, Li; 0.12 μg/g, As; 2.9 μg/g, Ru; 6.4 μg/g, Sr; 0.45 μg/g Ba; 0.11 μg/g Mo; 0.02 μg/g Cd (Yin et al., 2015). Finally, the authors of these studies consider that the analyzed walnut kernel samples show appreciable concentrations of essential elements and a normal consumption of walnut kernels does not present a risk of contamination with toxic metals.

Arpadjan et al. determined the distribution of essential and toxic elements in 12 types of nuts grown in two locations in Bulgaria: an area contaminated with lead metallurgy and an area without pollution. Average values obtained (mg/kg): 0.013-0.045, Cd; 0.05-0.38, Co; 0.032-0.076, Cr; 15.4-20, Cu; 28-33, Fe; 31-36, Mn; 0.64-1.62, Ni; 0.08-0.20, Pb; 0.006-0.01, Sb; 0.034-0.047, Se; 28-36, Zn - in the polluted area and 0.08-0.12, Co; 0.030-0.038, Cr; 15.3-17.4, Cu; 23-38, Fe; 28-35, Mn; 0.40-0.96, Ni; 0.034-0.047, Se; 28-34, Zn; 0.005-0.009, Cd; <0.01-0.04 Pb; <2, Sb - in the unpolluted area, shows a general tendency to increase the Cd, Co, Cr, Ni and Pb content in the contaminated area (Arpadjan et al. 2013). Under these conditions, the average intakes of Co, Cr, Cu, Fe, Mn, Ni, Se and Zn corresponding to a consumption of 50 g of walnut kernels in the polluted area were below the toxicological reference values. Also, the maximum exposure of nuts to Cd, Pb and Sb represented only 10.5%, 4.7% and 0.1% of the maximum tolerable weekly intakes. Therefore, walnuts can be grown even in a polluted area, without losing their nutritional qualities. Analyzing the distribution of Cd, Cu, Fe, Mn, Pb, Zn in six varieties of walnuts (*Juglans regia* L.) grown in Bulgaria, Sv. Momchilova et al. (2016) found that the concentration (mg/kg) of the analyzed elements: 0.0235 - 0.0298 (Cd); 15.7-17.2 (Cu); 24.2-32.8 (Fe); 32.0-39.5 (Mn); 0.095-120 (Pb) and 26.8-34.2 (Zn), is conditioned by the walnut variety (Momchilova et al., 2016).

Cindrić et al. also concluded that walnut is an important element which when analyzing the core of some mineral elements from walnut kernel products from France and Canada determined high quantities (mean values in mg/kg) of Ca (1062), K (2772), Mg (1426) and significant amounts of Na (42.5), Mn (31.7), Fe (27.4), Zn (24.0) and Cu (8.38); microelements with toxic properties: As, Cd, Pb, were determined in concentrations (mean values, mg/kg) below the maximum allowed limit: 0.027, 0.0039, respectively 0.331 (Cindrić et al., 2018).

Proceeding to evaluate the bioaccessibility of some essential macro and microelements: Ca, Mg, Fe and Zn from walnut (*Juglans regia* L.), Suliburska and Krejpcio showed that the walnut kernel from the local market from Poznan (Polska) contains increased amounts of Mg (140.1 mg/100 g) and Ca (73.1 mg/100 g) and notable amounts of Fe (2.1 mg /100 g) and Zn (1.8 mg/100 g) (Suliburska and Krejpcio, 2014).

When analyzing some mineral nutrients and heavy metals from walnut kernel samples collected from four areas of natural walnut-fruit forests in Osh Region (Kyrgyzstan), Ozyigit et al. determined high amounts (mg/100 g) of K (311.6-397.1), Ca (138.5-152.1), Mg (158.6-220.1), as well as important quantities of: Mn (3.045-4.562), Fe (3.356-4.892), Zn (2.320-3.463), Cu (1.251-2.067) and Na (0.878-1.700) (Ozyigit et al., 2018). The experimental results obtained allowed the evaluation of the mineral intake in the daily diet, for a certain consumption of walnut kernels. Under the conditions described by the study authors, a daily consumption of 100 g of walnut kernel covers a large part of the recommended mineral requirement: Ca (15%), Cu (173%), Fe (52.25%), K (7.6%), Mg (45.40%), Mn (135.7%), Na (0.09%), Zn (26.8%) for adults and Ca (15%), Cu (173%), Fe (23.22%), K

(7.6%), Mg (59.5%), Mn (211.1%), Na (0.09%), Zn (36.9%) for premenopausal women).

Concerns for knowing the mineral profile of the core are also recorded in the works of researchers in Romania, which show that the Romanian walnut kernel is a rich source of essential mineral elements, and the consumption of walnut kernels contributes to a well-balanced diet. Analyzing the mineral composition of some walnut kernels obtained from nine local walnut varieties, Cosmulescu et al. determined identified high contents (mg/100 g) of essential elements, unevenly distributed, depending on the variety of walnut and the analyzed element: 0.134-2.387, Na; 357.1-499.6, K; 37.0-90.84, Ca; 189.2-234.2, Mg; 3,815-5,927, Fe; 3,134-18.37, Mn; 1.41-3.223, Cu; 0.002-0.005, Se; 0.102-0.525, Al; 0.255-0.692, Cr; 0.160-0.537, Sr .; 0.001, V; 0.356-2.607, Rb; 1,948-3,613, Zn (Cosmulescu et al., 2009). In another study, Cosmulescu et al., determined the distribution of Na, K, Ca, Mg, Fe, Mn, Cu, Se, Al, Cr, Zn, Sr and Rb, from walnut kernels from three varieties of Romanian walnut. The concentration of these elements (mg/100 g) in the analyzed walnut kernel varies in relatively wide limits between: 387.25-444.35, K; 264.7-272.3, Mg; 62.78-72.91, Ca; 10.45-18.06, Mn; 5.44-5.90, Fe; 3.19-4.10, Zn; 2.93-3.47, Cu; 0.17-0.33, Al; 0.59-0.84, Cr; 0.39-0.55, Sr.; 0.13-0.56, Rb. (Cosmulescu et al., 2010). Analyzing some nutritional and functional components of the walnut kernel and pellicle across twelve genotypes of *Juglans regia* L. (six with a red pellicle and six with a light yellow pellicle), Trandafir et al. reported that walnuts are a good dietary source of total phenolics with high antioxidant potential, minerals (Ca, Mg and K) and essential elements (Fe, Mn, Cu and Zn), most of which are concentrated in the walnut pellicle (Trandafir et al., 2016). The concentration of essential elements (mean values/dry matter) analyzed presents the following values: 74.82 mg/ 100 g, Ca; 228.45 mg/100 g, Mg; 354.11 mg/100 g, K; 0.62 mg/100 g, Na; 3.89 mg/100g, Fe; 11.16, Mn; 2.14, mg / 100 g, Cu; 0.50 mg/100 g, Cr; 2.89 mg/100 g, Zn - in walnut kernel with red pellicle and 60.82 mg/100 g, Ca; 202.95 mg/100 g, Mg; 764.99 mg/100 g, K; 0.97 mg/100 g, Na; 3.50 mg/100 g, Fe; 6.83 mg 100 g, Mn; 2.03 mg/100 g, Cu; 0.68 mg/100 g, Cr; 2.96 mg/100 g, Zn - in walnut kernel with a light yellow pellicle (Trandafir et al., 2016). More recent data on the concentration of some essential microelements in the Romanian walnut kernel are presented by Rada et al. (Rada et al., 2018). The study authors reveal that the analyzed walnut kernel contains significant amounts (mg/kg) of Mn (18.59-51.65), Fe (20.5-29.1), Zn (16.06-26.15, Cu (9.64-10.07) and reduced amounts of Cr (0.78-1.08). These values allowed the evaluation of the mineral intake of the walnut kernel analyzed in the diet of people aged between 19 and 50 years. The authors of the study show that a daily consumption of 20 grams of walnut kernels covers a certain percentage of the daily mineral requirement: Cr (51.43%), Mn (34.15%), Cu (22.31%), Fe (6.13%), Zn (3.58%) - for man and Cr (72.00%), Mn (43.63%), Cu (22.31), Zn (4.93%), Fe (2.72%), - for women.

From the above values it can be stated that the walnut kernel contains increased quantities of essential mineral elements, distributed differently depending on a series of factors: variety, soil and climatic conditions and culture, nature of the element, etc. To evaluate the nutritional intake of walnut, it is very important to determine the concentrations of these essential elements. The purpose of this study is to determine the concentrations of some essential elements: Na, K, Ca, Mg, Fe, Mn, Zn, Cu and Cr from walnut kernel samples sold in agri-food markets in Timisoara (Romania) and evaluating the mineral intake of this precious food.

MATERIALS AND METHODS

Samples

To carry out this study, five samples of walnut kernels were taken from agri-food markets in Timisoara (Romania). Until the time of analysis, these walnut kernel samples, from the 2018 harvest, were kept cold (about 4-5ºC) in plastic bags.

Instrumentation

- Nabertherm thermoregulatory calcination furnace model 6/11, used to burn walnut kernels;

- Varian 280 FS atomic flame absorption spectrophotometer, used to measure the absorbances of mineral elements in standard solutions and in the solution of problems obtained after mineralization.

Chemicals and Reagents

- Merck nitric acid, 65% (ρ = 1.39 g/cm^3), required for the preparation of the 0.5 N nitric acid solution;
- Multi - element standard solution (1 g/L) Merck - Germany, used in the preparation of working standards for Na, K, Ca, Mg, Fe. Mn, Zn, Cu and Cr, in concentrations covering the concentration range of the elements present in the analyzed samples;
- Double-distilled water.

Method

The determination of the concentrations of the mineral elements in the walnut kernel samples was performed by the atomic absorption method in the air-acetylene flame according to the procedure described by Rada et al. and Yerlikaya et al. (Rada et al., 2018; Yerlikaya et al., 2012).

Basically, 1±0.0002 g of walnut kernel, previously ground and homogenized were calcined at 550°C in two batches of 4 hours each. To prevent loss of the sample during calcination, before calcination the sample was treated with 2 mL 65% HNO_3, after which it was evaporated on an electric hob to dryness. After cooling, the ash obtained was treated with 20 ml of 0.5 N HNO_3 solution, after which it was evaporated (on an electric hob) until almost sec. This operation was repeated twice, after which it was brought quantitatively with small portions of 0.5 N nitric acid and distilled water at 50 ml. The determination of the concentrations of the elements in the clear solution brought to the level of 50 ml was performed with the help of the Varian 280 FS Spectrometer, in the air-acetylene flame. Working parameters of the atomic absorption spectrometer: wavelength, air flow and acetylene, etc. were recommended by the device supplier. Simultaneously with the measurement of the analyzed samples and in the same working conditions, the calibration solutions were measured. All the mineral composition analyses were performed in triplicate.

Statistical analysis

The average values of the concentrations of the mineral elements were calculated using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA), and then they were statistically tested in order to find significant differences by the T-Test: Two-Sample Assuming Unequal Variances was used for p = 0.05.

RESULTS AND DISCUSSIONS

The results obtained in determining the essential mineral elements from the walnut kernel samples studied are presented in Table 1.

Table 1. The concentration of some essential element in walnut kernel

Specification	Mineral element, mg/100 g*								
	Na	K	Ca	Mg	Mn	Fe	Zn	Cu	Cr
Source 1	4.32± 0.16^{a}	401± 15.89^{a}	112± 10.03^{a}	226± 14.17^{a}	3.93± 0.36^{a}	2.45± 0.23^{a}	1.97± 0.22^{a}	1.02± 0.21^{a}	0.19± 0.09^{a}
Source 2	1.22± 0.10^{b}	412± 15.90^{b}	80.1± 8.67^{b}	270± 15.58^{b}	9.52± 0.51^{b}	5.58± 0.39b	3.49± 0.27^{b}	3.14± 0.26^{b}	0.49± 0.14^{b}
Source 3	0.75± 0.08^{b}	376± 14.20^{c}	75.2± 7.68^{b}	221± 8.27^{a}	10.4± 08.4^{b}	3.71± 0.31^{c}	1.48± 0.20^{a}	2.10± 0.20^{c}	0.60± 0.15^{b}
Source 4	6.45± 0.47^{a}	338± 10.20^{d}	62.6± 6.16^{b}	163± 10.98^{c}	2.56± 0.24^{a}	1.19± 0.21^{a}	1.23± 0.31^{a}	0.58± 0.11^{a}	0.16± 0.05^{a}
Source 5	1.93± 0.14^{b}	457± 18.55^{b}	119± 6.68^{a}	176± 10.03^{c}	3.05± 0.38^{a}	4.22± 0.26^{e}	3.58± 0.28^{b}	1.86± 0.18^{a}	0.08± 0.02^{a}
Mean value	***2.93***	***397***	***89.8***	***211***	***5.89***	***3.43***	***2.35***	***1.74***	***0.30***

*a-e: Values in the same column not sharing a common superscript differ significantly (P< 0.05)

As can be seen from this table, the walnut kernel contains increased amounts of K, Mg, Ca as well as significant amounts of Na, Fe, Mn, Zn, Cu and Cr unevenly distributed, depending on the origin of the walnut kernel and the nature of the element analyzed. This non-uniformity, even within each analyzed element, can be explained by the origin of the analyzed walnut kernel. The best represented among the analyzed elements are the macro elements (average values): K (397 mg/100 g), Mg (211 mg/100 g) and Ca (89.8 mg/100 g), which represent a mass weight of 97.59% of the total analyzed elements. The other ingredients were determined in much smaller amounts, as follows: 2.93 mg/100 g Na, 5.89 mg/100 g Mn, 3.43 mg/100 g Fe, 2.35 mg/ 100 g Zn, 1.74 mg/100 g Cu and 0.30 mg/100 g Cr, but high enough given the quantities needed for the normal functioning of the human body.

Considering the average concentrations of the analyzed mineral elements, it is stated that their distribution in the walnut kernel samples studied (mg/100 g) shows the following decreasing trend (in mg/100 g): K (397) > Mg (211) > Ca (89.8) > Mn (5.89) > Fe (3.43) > Zn (2.35) > Cu (1.74) > Cr (0.30).

Regarding the total concentrations of the mineral elements determined in the walnut kernel samples from the five sources: 752.88 mg/100 g - from S1, 785.54 mg/100 g - from S2, 691.24 mg/100 g - from S3, 575.77 mg/ 100 g - from S4 and 766.72 mg/100 g - from S5, the statistical analysis performed for $p = 0.05$ ensures the existence of significant differences in favor of the S2 source, closely followed by S5 and S1. Sources S3 and S4 are significantly different from those mentioned above. As such, source S2 is the richest source of minerals, and source S4 is the poorest source of minerals.

Potassium is the main cation in intracellular fluid with important role in acid-base balance, regulation of osmotic pressure, conduction of nerve impulses, muscle contraction particularly the cardiac muscle, cell membrane function and Na^+/K^+ - ATPase (Cosmulescu et al., 2010; Soetan et al., 2013). This macro element is the best represented of all the analyzed elements, the concentration limits being between 338-457 mg/100g. Statistically, the highest potassium concentrations were determined in walnut kernels from sources S5 and S2, values without statistically significant differences. Lower values were identified in the sample from source S1, respectively from sources S3 and S4 (these two statistically significant differences). These values are comparable to those reported in literature: 347.8757-547.6201 mg/100 g (Özcan et al., 2010), 349, 358, 372.69, 414 mg/ 100 g (Ebrhimi et al., 2017), 387.23-444.35 mg/100 g (Cosmulescu et al., 2009; Cosmulescu et al., 2010), 354.11 mg/100 g - kernel with red pellicle (Trandafir et al., 2016), but lower than these: 408.37-569.4 mg/100 g (Polat et al., 2015), 534.3-778.6 mg/100 g (Simek, 2016), 546.46-617.36 mg/100 g (Aryapak and Ziarati, 2014), 746.99 mg/100 g - kernel with yellow pellicle (Ros, 2018). The higher values were reported by Yilmaz and Akça, 2017 (63.92-308.86 mg/100 g), Cindrić et al., 2014 (200.6-322.1 mg/100 g).

Magnesium, as an essential macroelement is a remarkable cofactor, necessary for ATP synthesis, glycolysis, oxidative phosphorylation, protein synthesis, muscle contraction, neuromuscular conduction, bone structure, blood sugar metabolism, membrane stabilization, and DNA transcription (Ozyigit et al., 2018). In addition, it is involved in immune system processes (Ozyigit et al., 2018). This element was determined in lower concentrations than potassium but higher than calcium and much higher than the other elements. The highest concentration of Mg was identified in walnut kernel from source S2, followed by walnut kernel from sources S1 and S3 (no statistically significant differences) and S4 and S5 (no statistically significant differences). The limits of Mg concentrations determined in the analyzed walnut kernel (163-270 mg/100 g), are comparable to those obtained by Ozyigit et al., 2018 (158.6-220.1 mg/100 g), Cosmulescu et al., 2009; Cosmulescu et al. 2010 (189.2-234.2, and 264.7-272.3 mg/100 g), Trandafir et al., 2016 (202.95, 228.45 mg/100 g). Magnesium concentrations in the analyzed walnut kernel samples show lower values than those obtained by Özcan et al.(2010): 416.36-548.81 mg/100g, Polat et al. (2015): 241-426 mg/100 g, Aryapak et al. (2014): 580.11-692.49 mg/100 g, Zhai et al. (2014): 401.47-749.27 mg/100 g and higher than those obtained by Kalkışım et al. (2014): 115 mg/100 g, Kafaloglu (2016): 103,496 mg/100 g, Simsek (2016): 117.8-181.4 mg/ 100 g, Ebrhimi et al. (2017): 115 and 140 mg/100 g.

Calcium, an essential macro element, is an important constituent of bones and teeth, helps regulate nerve and muscle function and is important for blood clotting, blood pressure regulation, immune system health (Soetan et al., 2010). The walnut kernel analyzed contains high amounts of Ca, but lower than K and Mg and less than the rest of the elements analyzed. The concentration limits determined experimentally, between 62.6-119 mg/100 g, are comparable to the values reported: 79.3 mg/100 g (Kafaloglu, 2017), 72 mg/100 g (Kalkışım et al., 2014), 86.6–143.5 mg/100 g (Cindrić et al., 2018), 73.1 mg/100 g (Suliburska and Krejpcio, 2014), 37.0-90.84 and 62.78-72.91 mg/100 g (Cosmulescu et al., 2009; Cosmulescu et al., 2010), 74.82 mg/ 100 g - kernel with yellow pellicle (Trandafir et al., 2016), but smaller than those determined by 246.2315-275.7883 mg/100g (Özcan et al. 2010), Polat: 194.79-267.85 mg/100 g (Polat, 2015), Simsek: 100.9-233.9 mg/100 g (Simsek, 2016), 235.19-276.46 mg/100 g (Aryapak et al., 2014), 253.90-504.07 mg/100 g (Zhai, 2014), 138.5-152.1 mg/100 g (Ozyigit et al. 2018).

The highest concentrations of calcium, with no statistically significant differences, were determined in samples from S1 and S5 sources. Lower calcium concentrations with no statistically significant differences were identified in samples from S2, S3 and S4 sources.

Sodium is a macro element that helps regulate the osmotic balance of all solutions in the body and adjusts the volume of intra/intercellular solutions; along with K, establishes the electrochemical potential (membrane potential) (Ozyigit et al., 2018). This macro element that helps regulate blood volume, thus affecting blood pressure was determined in very low concentrations compared to the first three macro elements, the concentration limits being between 0.75-6.45 mg/100 g core. The highest Na concentrations, with no statistically significant differences, were determined in sources S4 and S1. The rest of the samples show lower Na concentrations, but without statistically significant differences. Experimentally determined Na concentrations are comparable to those reported: 4.4 and 6.0 mg/100 g (Ebrhimi et al., 2017), 1.87-19.43 mg/100 g (Cindrić, 2018), 0.878-1.70 mg/100 g (Ozyigit et al. 2018), 0.134-2.387 and 0.23-1.38 mg/100 g (Cosmulescu et al., 2009; Cosmulescu et al., 2010), 0.97 mg/100 g - kernel with yellow pellicle (Trandafir et al., 2016) and lower compared to those reported: 61.7713-833.433 mg/100 g (Özcan et al., 2010), 11,612 mg/100 g (Kalkışım et al., 2014), 8.67-19.29 mg/100 g (Simsek, 2016), 7.94-22.53 mg/100 g (Yilmaz and Akça, 2017), 58.42-66.54 mg/100 g (Aryapak and Ziarati, 2014).

Manganese is an essential microelement that helps the body to form connective tissue, bones, blood-clotting factors, and sex hormones. It also plays a role in fat and carbohydrate metabolism, calcium absorption, and blood sugar regulation (Al-Fartusie and Mohssan, 2017; Ozyigit et al., 2018). Manganese is the best represented among the analyzed microelements, its concentration presenting values between 2.56-10.04 mg/100g, values comparable to those reported: 2.02-4.05 mg/100 g (Simsek, 2016), 0.91-4.39 mg/100 g (Yilmaz and Akça, 2017), 3.44-5.79 mg/100 g (Ni et al., 2015), 2.8-3.6 mg/100 g (Arpadjan et al., 2013), 1.37-4.6 mg/100 g (Cindrić, 2018), 3.045-4.462 mg/100 g (Ozyigit et al., 2018), 3.134-18.37 mg/100 g (Cosmulescu et al., 2009), 1.859-5.165 mg/100 g (Rada et al., 2018).

The highest concentrations of Mn were determined in walnut kernels from sources S3 and S2 (no statistically significant differences). At lower concentrations, but without statistically significant differences was determined in the walnut kernel from sources S1, S5 and S4.

Iron is an essential component of myoglobin, a protein that provides oxygen to muscles and is necessary for the growth, development, normal functioning of cells and the synthesis of hormones and connective tissue (Al-Fartusie and Mohssan, 2017). The concentrations of this essential microelement in the analyzed walnut kernel show values between l.19-5.58 mg/ 100 g. Statistically, the highest concentration in Fe was determined in the walnut kernel from source S2. Lower concentrations, statistically significantly different, were identified in samples from sources S5, S3, S1 and S4.

These values are found in the concentration limits obtained by: 3.254 mg/100 g (Kalkışım et al., 214), 1.02-3.98 mg/100 g (Polat, 2015), 2.21-4.32 mg/100 g (Yilmaz and Akça, 2017), 3.2, 3.8, 4.6 mg/100 g (Ebrhimi et al., 2017), 3.43-4.45 mg/100 g (Ni et al., 2016), 2.3-3.8 mg/100 g (Aryapak and Zirati, 2014), 3.356-4.892 mg/100 g (Ozyigit et al., 2018), 3.815-5.927 mg/100 g (Cosmulescu et al., 2009), 3.50 mg/100 g - kernel with yellow pellicle (Trandafir et al., 2016), 2.05-2.91 mg/100 g (Rada et al., 2018).

Zinc is an essential microelement that functions as a cofactor in almost 300 enzymes involved in the metabolism of proteins, carbohydrates, lipids, and energy metabolism [20]. Zinc also plays an important role in growth and cell division (where it is required for protein and DNA synthesis), in insulin activity, in the metabolism of the reproductive organs, and in liver function (Al-Fartusie and Mohssan, 2017). The Zn concentration in the analysed walnut kernel samples shows values between 1.23-3.58 mg/100 g, values that are found in the range of values obtained by 1.93-3.47 mg/100 g (Polat, 2015), 1.44-3.63 mg/100 g (Simsek, 2016), 1.97-5.48 mg/100 g (Yilmaz and Akça, 2017), 2.12, 2.2 and 3.8 mg/100 g (Ebrhimi et al., 2017), 1.48–2.77 mg/100 g (Ni et al., 2016), 2.98 and 2.96 mg/100 g (Trandafir et al., 2016), 1.606-2.615 mg/100 g (Rada et al., 2018). The best represented in terms of zinc content, without statistically significant differences, are the samples from sources S5 and S2. Lower concentrations of zinc, which can be considered statistically equal, have been identified in walnut kernels from sources S1, S3 and S4.

Copper is an essential element of several enzymes, such as cytochrome oxidase, monoamine oxidase, catalase, peroxidase, ascorbic acid oxidase, lactase, tyrosinase, and superoxide dismutase (Al-Fartusie and Mohssan, 2017). This essential micronutrient was determined in concentrates between 0.58-3.14 mg/100 g, values comparable to those reported: 0.5676-0.5494 mg/100 g (Ozgan et al., 2014), 1.173 mg/100 g (Kafaloglu, 2016), 0.65-1.43 mg/100 g (Polat, 2015), 0.77-2.44 mg/100 g (Simsek, 2016), 1.37-5.30 mg/100 g (Zhai, 2014), 0.93-2.47 mg/100 g (Ni Zhanglin et al., 2016), 0.521-2.08 mg/100 g (Cindrić, 2018), 1.41-3.223 and 2.93-347 mg/100 g (Cosmulescu et al., 2009; Cosmulescu et al., 2010), 0.964-1.007 mg/100 g (Rada et al., 2018), 2.14 and 2.03 mg/100 g (Trandafir et al., 2016).

The highest values of copper concentration, ensured statistically (significantly different statistically) were identified samples from sources S2 and S3. Lower statistically equal Cu concentrations were determined at sources S3 and S5. The walnut kernel from source S4 is the poorest in Zn.

Chromium, the essential microelement in the control of both blood glucose and fat levels (Marjan, 2013), was determined in the lowest concentrations of all the essential elements analysed. This trace element was determined in concentrations between 0.08-0.60 mg/100 g. Statistically, the walnut kernel samples richest in chromium, with no statistically significant differences, are those from S2 and S3 sources. Lower, statistically equal contents were identified in the walnut kernel from samples S1, S4 and S5.

Comparing the chromium concentrations obtained with those reported by: 0.1695-0.3323 mg/100 g (Özcan et al., 2010), 0.255-0.692 and 0.59-0.84 mg/100 g (Cosmulescu et al., 2009; Cosmulescu et al., 2010), 0.50 mg/100 g - walnut kernel with red pellicle (Trandafir et al., 2016) and 0.078-0.108 mg/100 g (Rada et al., 2018), no noticeable difference is observed.

The increased concentrations of mineral elements determined in the analyzed walnut kernel suggested the evaluation of the mineral intake of this valuable food. Practically, the degree of coverage of the daily mineral requirement corresponding to a consumption of 25 g of walnut kernel was determined (by calculation), for men and women aged between 19-50 years. 25 grams of walnut kernel were used on the recommendation of Seyit Mehmet Şen and Turan Karadeniz, who claim that in order to increase heart and brain health, 20-30 g of walnut kernel/day should be consumed from childhood (Şen and Karadeniz, 2015).

When assessing the mineral intake, the total content of the mineral elements present in 25 walnut kernels (mean values): 0.734 g Na, 99.70 g K, 22.45 g Ca, 52.80 g Mg, 1.473 g Mn, 0.858 g Fe, (0.588 g Zn, 0.434 g Cu, and 0.076 g Cr and the daily mining requirement recommended by the Food and Nutrition Board, Institute of Medicine, National (Food Academies, for people aged 19-50 years and Nutrition Board, Institute of Medicine, National Academies, for people aged 19-50 year) (Table 2).

To calculate the mineral intake, MI (Table 3) in the recommended daily diet, was performed using the relation: $MI\ [\%] = \frac{c}{a} \cdot 100$

where: MI - mineral intake, c-the amount of elements (mg) contained in the mass of walnut kernel consumed/day, a - the recommended amount of element (mg)/day.

Table 2. The recommended daily requirement and the upper tolerable limits for some mineral elements for the man and women 19-50 ages (Dietary reference intakes (DRIs): Recommended dietary allowances and adequate intakes, elements; Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels, Elements)

Specification	Raged People	Mineral element, mg/day								
		Na	K	As	Mg	Mn	Fe	Zn	Cu	Cr
Recommended values	Man	1500	3400	1000	420	2.3	8	11	0.9	0.035
	Women	1500	2600	1000	310	1.8	18	8	0.9	0.025
Tolerable values	Man	ND*	ND*	2500	350	11	45	40	0.010	ND*
	Women	ND*	ND*	2500	350	11	45	40	0.010	ND*

*Not determinable owing to a lack of a specific toxicological effect. ** WHO considered that chromium supplementation should not exceed 250 µg/day (WHO, 1996).

Table 3. Mineral content, in the recommended daily diet, for a consumption of 25 g walnut kernels

Source	People	Mineral supply (%)								
		Na	K	As	Mg	Mn	Fe	Zn	Cu	Cr
S1	Man	0.07	2.95	2.80	13.69	42.72	7.66	4.48	28.33	135.71
	Women	0.07	3.86	2.80	18.23	54.58	3.40	6.16	28.33	190.00
S2	Man	0.02	3.10	2.00	16.07	103.48	17.44	7.93	87.22	350.00
	Women	0.02	4.06	2.00	21.77	132.22	7.75	10.91	87.22	490.00
S3	Man	0.01	2.76	1.88	13.15	113.04	11.59	3.36	58.33	428.57
	Women	0.01	3.62	1.88	17.82	144.44	5.15	4.63	58.33	600.00
S4	Man	0.11	2.49	1.57	9.70	27.83	3.72	2.80	16.11	114.29
	Women	0.11	3.25	1.57	13.15	35.56	1.65	3.84	16.11	160.00
S5	Man	0.03	3.36	2.98	10.48	33.15	13.19	8.14	51.67	57.14
	Women	0.03	4.39	2.98	14.19	42.36	5.86	11.19	51.67	80.00
Mean	*Man*	*0.05*	*2.92*	*2.24*	*12.57*	*64.04*	*10.72*	*5.34*	*48.33*	*217.14*
	Women	*0.05*	*3.82*	*2.24*	*17.03*	*81.83*	*4.76*	*7.34*	*48.33*	*304.00*

As it can be seen from the table 3, a daily consumption of 25 g of walnut kernel contributes differently to ensuring the daily requirement of Na, K, Ca, Mg, Mn, Fe, Zn, Cu and Cr. The mineral intake, respectively the degree of coverage of the mineral requirement varies in wide limits between 0.01% (for Na - in men and women) - 600% (for Cr - in women), this being determined by the origin of the walnut kernel, the amount of kernel taken for consumption and its mineral concentration, the mineral requirement recommended by the category of consumers (men or women, age, pregnancy, lactation). Under the conditions of this experiment, the degree of coverage of the daily mineral requirement has the following values: 0.01-0.11% for Na, 2.49-3.36% for K, 1.57-2.98% for Ca, 9.70-16.07% for Mg, 27.83- 113.04% for Mn, 3.72-17.44% for Fe, 2.80-8.14% for Zn, 16.11-87.22% for Cu and 57.14-428.57% for Cr - in men and 0.01-0.11% for Na, 3.25-4.39% for K, 1.57 -2.98% for Ca, 13.15-21.77% for Mg, 35.56-144.443% for Mn, 1.65-7.75% for Fe, 3.84-11.19% for Zn, 16.11-87.22% for Cu and 80.00-600% for Cr.

Taking into account the average values of the concentrations of the analyzed mineral elements, it can be observed that the mineral contribution shows the following increasing trend: Na (0.05%) <Ca (2.24%) < K (2.92%) <Zn (5.34%) <Fe (10.72%) <Mg (12.57%) <Cu (48.22%) <Mn (64.04%) <Cr (217%) - for men and Na (0.05%) < Ca (2.24%) <K (3.82%) <Fe (4.76 %) <Zn (7.34%) <Mg (17.03%) <Cu (48.22%) <Mn (81.83%) < Cr (304%).

These values show that a consumption of 25 g of walnut kernel is not of interest from the point of view of Na, Ca and K, but can cover significant percentages of the need for Zn, Fe and Mg and high Cu and Mn. In addition, a consumption of 25 g of kernel ensures an amount of Cr three times higher than the recommended requirement. Because WHO considered that chromium supplementation should not exceed 250 µg/day (WHO, 1996) (Opinion of the Scientific Committee on Food on the Tolerable upper intake level of trivalent chromium), it can be observed that consumption of 25 g of walnut kernel does not present a risk of toxicity with Cr.

An eventual addition of the consumption of walnut kernels in order to increase the mineral intake may be possible, but with caution respecting tolerable upper intake levels elements corresponding to each element (Table 2). A supplement brings with it other compounds, which above certain concentration limits may have unwanted side effects.

CONCLUSIONS

The walnut kernel sold in agri-food markets in Timisoara (Romania) contains increased amounts of K, Mg, Ca, as well as significant amounts of Na, Fe, Mn, Zn, Cu and Cr unevenly distributed depending on the origin of the walnut kernel and the nature of the element analyzed.

The results obtained when evaluating the mineral intake show that a consumption of 25 g walnut kernel contributes differently to ensuring the daily mineral requirement depending on the nature of the element and the consumer

The analyzed walnut kernel is not of interest in terms of intake of Na, Ca and K, but can cover significant percentages of the need for Mg, Zn, Fe and high percentages of the daily requirement of Cr, Cu, and Mn.

Finally, it can be stated that the analyzed walnut kernel can be considered as an additional source especially of Cr, Mn, Cu, Mg, and to a lesser extent as a source of Zn and Fe.

REFERENCES

Al-Fartusie, F.S., & Mohssan, S.N. (2017). Essential Trace Elements and Their Vital Roles in Human Body, *Indian Journal of Advances in Chemical Science, 5(3),* 127-136.

Al-Snafi, A.E. (2018). Chemical constituents, nutritional, pharmcological and therapeutic importance of Juglans regia-A review, *IOSR Journal of Pharmacy, 8(11).*

Arpadjan, S., Momchilova, S., Elenkova, D., & Blagoeva, E. (3013). Essential and toxic microelement profile of walnut (*Juglans regia* L.) cultivars grown in industrially contaminated area – Evaluation for human nutrition and health*, Journal of Food and Nutrition Research, 52(2),* 121–127.

Aryapak, S. & Ziarati, P. (2014). Nutritious Value of Persian Walnut (Juglans *regia* L.) Orchards, *American-Eurasian J. Agric. & Environ. Sci., 14(11)*: 1228-1235.

Cindrić, I.J., Zeiner, M., & Hlebec, D. (2018). Mineral Composition of Elements in Walnuts and Walnut Oils, *Int. J. Environ. Res. Public Health, 15*, 2674.

Cosmulescu**,** S., Baciu, A., Achim, G., Botu, M., & Trandafir, I. (2009). Mineral Composition of Fruits in Different Walnut *(Juglans regia* L.) Cultivars, *Not. Bot. Hort. Agrobot. Cluj 37 (2)***,** 156-160.

Cosmulescu, S., Botu, M., & Trandafir, I. (2010). Mineral Composition and Physical Characteristics of Walnut (Juglans regia L.) Cultivars Originating in Romania, *Selçuk Tarım ve Gıda Bilimleri Dergisi, 24(4***), 3**3-37.

Ebrhimi, S., Jamei, R., Nojoomi, F., & Zamanian, Z. (2017). Persian Walnut Composition and its Importance in Human Health, *Int J Enteric Pathog., 6(1),* 3-9.

Fatima, T., Showkat U., & Hussain, S.Z. (2018). Nutritional and health benefits of walnuts, *Journal of Pharmacognosy and Phytochemistry, 7(2),* 1269-1271.

Kafaoglu, B. (2016) Determination and evaluation of element bioaccessibility in some nuts and seeds by in-vitro gastrointestinal method*, J. Food Comp. Anal., 45*, 58-65.

Kalkişim, Ö., Ozdes, D., & Onaran, A. (2014). Assessment of mineral elements and heavy metal contents of walnut samples *(Juglans regia L.)*, *Advances in Food Sciences, 36(1),* 24 – 29.

Leahu, A., Damian, C., Oroian, M., & Hreţcanu, C.E. (2013). Estimation of biochemical properties of walnuts from the region of Suceava – ROMANIA, *Journal of Faculty of Food Engineering, Stefan cel Mare University of Suceava, Romania, XII(2),* 169 – 175.

Mehri A., & Marjan, R.F. (2013). Trace Elements in Human Nutrition: A Review, *Int. j. Med. invest., 2(3),* 115-128.

Momchilova, Sv., Arpadjan, S., & Blagoeva, E. (2016). Accumulation of microelements Cd, Cu, Fe, Mn, Pb, Zn in walnuts *(Juglans regia* L.) depending on the cultivar and harvesting year, *Bulgarian Chemical Communications,* 48(1), 50 – 54.

Ni, Z., Tang, F., Yu, Q., & Liu, Y. (2016). *Toxic and essential elements in five tree nuts from Hangzhou market, China, J. Nutr. 144*, 561S–566S.

Ozyigit, I.I., Uras, M.E., Yalcin, I.E., Severoglu, Z., Demir, G., Borkoev, B., Salieva, K., Yucel, S., Erturk, U., & Solak, A.O. (2018). Heavy Metal Levels and Mineral Nutrient Status of Natural Walnut (Juglans regia l.) Populations in Kyrgyzstan: Nutritional Values of Kernels, *Biological Trace Element Research, 189(4),* 1-14.

Özcan, M.M., İman, C. & Arslan, D. (2010), Physico-chemical properties, fatty acid and mineral content of some walnuts *(Juglans regia* L.) types, *Agricultural Sciences 1(2),* 62-67.

Polat, M., Okatan, V., & Güçlü, S.F. (2015). Determination of some physical and chemical properties of walnut *(Juglansregia L.*) genotypes grown in the central district of Bitlis/Turkey, *Scientific Papers. Series B, Horticulture. Lix,* 2015. Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653.

Rada, M., Berbecea, A., Alda, L.M., Cozma, A., Zippenfening, S.E., Nemeş, O.F., Alda, S., & Gogoasa, I. (2018). Preliminary Research Regarding The Mineral Intake Of Walnut Kernel, *Proceedings Of The International Conference On Life Sciences, Timisoara, May, 2018*, 921-928, ISBN 978-88-85813-24-3 First Edition July 2018 © Copyright 2018 Filodiritto Publisher *filodirittoeditore.com.*

Ros, E. (2010). Health Benefits of Nut Consumption, *Nutrients, 2*, 652-682.

Ros, E., Izquierdo-Pulido, M., & Sala-Vila, A. (2018). Beneficial effects of walnut consumption on human health: role of micronutrients, *Curr Opin Clin Nutr Metab Care, 21.* 498–504.

Simsek, M., (2016). Chemical, mineral, and fatty acid compositions of various types of walnut *(Juglans regia* L.) in Turkey, *Bulgarian Chemical Communications, 48 (1),* 66 – 70.

Soetan, K. O., Olaiya, C. O., & Oyewole, O. E. (2010). The importance of mineral elements for humans, domestic animals and plants: A review, *African Journal of Food Science, 4(5),* 200-222.

Suliburska J., & Krejpcio, Z. (2014). Evaluation of the content and bioaccessibility of iron, zinc, calcium and magnesium from groats, rice, leguminous grains and nuts, *J Food Sci Technol,* 51(3), 589–594.

Şen, S.M., & Karadeniz, T. (2015). The nutritional value of walnut, *Journal of Hygienic Engineering and Design, 11,* 68-71.

Trandafir, I., Cosmulescu, S., Botu M., & Nour, V. (2016). Antioxidant activity, and phenolic and mineral contents of the walnut kernel *(Juglans regia L.)* as a function of the pellicle color, *Fruits,71(3),*177-184.

Yilmaz, S., & Akça, Y. (2017). Determination of Biochemical Properties and Fatty Acid Composition of New Walnut *(Juglans regia*) Genotypes, *JAFAG, 34 (2)*, 74-80.

Yin, L.L., Qing, T., Zhang, S.X., Yin, K.X., & Qin, J. Y. (2015). Determination, Of Trace Elements in Edible Nuts in the Beijing Market by ICP-MS**,** *Biomed Environ Sci, 28(6),* 449-454.

Zhai, M., Wang, Z., Wang, D., Xu, J., SHI, G. (2014). Comparative Analysis of Mineral Elements and Essential Amino Acids Compositions in *Juglans sigillata* and *J. directed walnuts* kernels, *Not Bot Horti Agrobo, 42(1),* 36-42.

*** Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Elements Food and Nutrition Board, National Academies, https://www.ncbi.nlm.nih.gov/books/NBK545442/table/appJ_tab3/?report=objectonly.

***Dietry Reference Intakes (DRIs): Tolerable Upper Intake Levels, Elements. https://www.ncbi.nlm.nih.gov/books/NBK278991/table/diet-treatment-obes.table18die/

*** EFSA Journal 2010;8(12):1882

***IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA)