

Mineral Composition of Elements in Red Lentil and Chickpea Cultivars Grown in Southeast of Turkey

Mehmet DÜZGÜN^{1*}, M. Zahir DÜZ², Murat KOÇ³, İrfan ERDEMÇİ⁴

^{1,3,4}GAP International Agricultural Research and Training Center, 21280, Diyarbakır, Turkey, ²Department of Chemistry, Science Faculty, Dicle University, 21280, Diyarbakır, Turkey

¹<https://orcid.org/0000-0003-4269-5544>, ²<https://orcid.org/0000-0002-3642-4206>, ³<https://orcid.org/0000-0002-4155-1963>

⁴<https://orcid.org/0000-0003-3066-410X>

✉: mehmetdzgn@yemail.com

ABSTRACT

Legumes such as red lentils and chickpeas, which are the most important legumes of the Southeastern Anatolia Region, have a rich content of many mineral substances. However, very little work has been done in this area in the region. In this study, contents of mineral elements such as K, Ca, Mg, Zn, Fe, Cu, Cd, Pb, Cr, As, Se, V, Sn, Mn, Si and Ni were determined by inductively coupled plasma optic emission spectrometry (ICP-OES) method in six red lentil and two chickpea cultivars registered by GAP International Agricultural Research and Training Center (GAPUTAEM). In addition, the element concentrations of the species and the comparison of the element amounts in lentils and chickpeas were evaluated. Element concentrations in lentil varieties were listed in descending order as follows K> Mg> Ca> Fe> Si> Zn> Sn> Mn> Cu> Ni> Cr> Se> V> Cd> Pb> As, while in chickpea cultivars are; K> Ca> Mg> Fe> Zn> Si> Mn> Sn> Cu> Ni> V> Cr> Se> Pb> Cd> As. The study results indicated that registered lentil and chickpea varieties were rich in potassium. On the other hand, calcium content of chickpea cultivars were higher than those of lentils. In addition, toxic heavy metals such as Cd, Pb and As concentrations were determined to be below the limits. The results showed that legumes were very important as mineral resources. Lentils and chickpeas were very similar in terms of macro and micro element contents. Additionally, it was observed that they had a nutritionally safe mineral structure.

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Türkiye'nin Güneydoğusunda Yetişen Bazı Kırmızı Mercimek ve Nohut Çeşitlerinin Mineral Bileşimi

ÖZET

Güneydoğu Anadolu Bölgesinin en önemli bakliyat ürünleri olan kırmızı mercimek ve nohut gibi baklagiller ayrıca mineral madde bakımından zengin bir yapıya sahiptir. Ancak bölgede bu alanda çok az çalışma yapılmıştır. Bu çalışmada, K, Ca, Mg, Zn, Fe, Cu, Cd, Pb, Cr, As, Se, V, Sn, Mn, Si ve Ni gibi mineral elementlerin konsantrasyonları GAP Uluslararası Tarımsal Araştırma ve Eğitim Merkezi (GAPUTAEM) tarafından tescilli altı kırmızı mercimek ve iki nohut çeşidinde indüktif eşleşmiş plazma optik emisyon spektrometresi (ICP-OES) ile belirlenmiştir. Ayrıca türlerin element konsantrasyonlarının belirlenmesi, mercimek ve nohuttaki element miktarlarının karşılaştırılması da değerlendirilmiştir. Mercimek çeşitlerinde element konsantrasyonları azalan sırayla K> Mg> Ca> Fe> Si> Zn> Sn> Mn> Cu> Ni> Cr> Se> V> Cd> Pb> As, nohut çeşitlerinde ise; K> Ca> Mg> Fe> Zn> Si> Mn> Sn> Cu> Ni> V> Cr> Se> Pb> Cd> As şeklinde olmuştur. Araştırmada tescilli mercimek ve nohut çeşitlerinin potasyum elementi yönünden zengin olduğu tespit edilmiştir. Nohut çeşitlerinde ise kalsiyum içeriği mercimek çeşitlerine göre daha yüksek bulunmuştur. Ayrıca toksik ağır metaller Cd, Pb ve As derişimlerinin sınır değerlerin altında olduğu belirlenmiştir. Sonuçlar bize baklagillerin çok önemli mineral

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kaynakları olduğunu göstermiştir. Mercimek ve nohutun, makro ve mikro element içerikleri bakımından birbirine çok benzer olduğu, ayrıca besin açısından güvenli mineral değerlere sahip oldukları görülmüştür.

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INTRODUCTION

Food legumes grown in almost every region of our country constitute the protein source of Turkish cuisine and especially for low-income families. Lentil (*Lens culinaris*), which is among edible legumes, has an important place in human nutrition due to its high protein content. According to TURKSTAT data; in 2018, 51.5% of legume production was chickpea and 25.3% was red lentil (Anonymous, 2020). Turkey's south-eastern region is known as the motherland of gene center of legumes. It was reported that 630 thousand tons of chickpea and 310 thousand tons of red lentil were produced in Turkey (TÜİK, 2019). Overall, 77.5% of the production of red lentils is done in Şanlıurfa, Diyarbakır and Mardin, respectively. Chickpea has an important place in human nutrition thanks to 20-25% protein, 40-60% carbohydrate, 4.5-5.5% fat, phosphorus and calcium in their grains (Babaoğlu, 2013). Dried legumes are rich in minerals. One of the most important issues is the accumulation of toxic metals in the food structure. The concentrations of metals as a result of deposition may rise well above those in water and in the air. The human or animal who receives such a highly toxic metal-containing food can be poisoned. It also has the ability of the human body to accumulate some toxic metals. For example, the half-life of lead in the human body is 1460 days, that of cadmium is 200 days, that of zinc is 933 days (Gündüz, 2004). Minerals in the composition of foods cover a large and complex element group. Many of these are necessary for humans, and especially some trace level elements must be present in the body at certain concentrations. High levels of elements in the body are called macro elements, while those found in small amounts are called micro or trace elements. This distinction was made not by their physiological significance, but by their density. Basic elements provided through food; potassium, sodium, calcium, magnesium, chlorine, sulfur and phosphorus. Some other elements are: iron, copper, iodine, cobalt, selenium, fluoride and zinc. The elements whose nutritional values are not yet known are aluminum, boron, chromium, nickel and tin. Therefore, it is very important to analyze minerals and trace elements of all products. Food and Agriculture Organization (FAO) and World Health Organization (WHO), European Commission (EC) and other regulatory bodies of other

countries strictly regulate the allowable concentrations or maximum permitted concentrations of toxic heavy metals in foodstuffs (FAO/WHO 1984; EC, 1989). In the World there are a lot of studies about trace elements in foods. For instance, Ereifej at al. (2001); Salama and Radwan(2005); Momen at al. (2006); Erdoğan at al. (2006); Reyes at al.(2010) studied in this field. Few studies have been made in this field in Turkey. There has been almost no study about minerals related content of legumes grown in our region. In this study we aimed to determine mineral elements in registered red lentils and chickpea cultivars grown in Southeast of Turkey and varieties are established for nutrition and food safety. The function of some important elements can be summarized as follows (Table 1).

Calcium: An adult has 1.2-1.5 kg of calcium in the body of which about 99% is in the skeletal system. Although no side effects have been observed in adults taking up to 2.5 grams of calcium per day, doses above this cause various diseases (Baysal, 1996; Bilge, 1998; Keskin, 1987; Vaessen and Kamp, 1990; Erdoğan, 2002).

Magnesium: It is found together with calcium and phosphorus in the structure of bones and teeth.

Iron: The amount of iron in the body is 4-5 g. Most of this is found in hemoglobin and myoglobin pigments. When there is not enough iron in the body, the number of blood cells decreases in "iron deficiency anemia" anemia, the amount of hemoglobin is reported to be 15 mg per day for women and 12 mg for adult men (Bilge, 1998; Keskin, 1987; Levy, 1998).

Copper: The amount of copper in the body is around 100-150 mg. 2-5 mg of copper is taken with an average daily diet.

Chromium: Chromium (Cr) is among several essential trace elements. Chromium optimizes insulin function and can have a marked impact on the metabolism of carbohydrates, proteins, and lipids. Chromium has been found to improve in vivo insulin activity, which optimizes tolerance to glucose. In addition, Cr protects the body against arteriosclerosis. (Anonymous, 2021)

Manganese: It is an essential trace element commonly found in plant and animal cells. The human body contains 10-40 mg of manganese. It is at the level of 2-48 mg day⁻¹ that can be met by daily nutrition. Safe

and adequate intake of manganese is thought to be 2.0-5.0 mg day⁻¹ (Baysal, 1996; Bilge, 1998; Keskin, 1987). Selenium: Selenium and Vitamin E support each other

in terms of antioxidant effects. The recommended daily intake is 50-200 micrograms. 30-150 micrograms is sufficient for children (Özyılmaz, 1999).

Table 1. A person weighing an average of 70 kg per day the amount of elements to be taken (Erdoğan 2002).
Çizelge 1. Günde ortalama 70 kg ağırlığında bir kişinin alması gereken element miktarları (Erdoğan 2002).

<i>Elements</i>	<i>Amount (mg day⁻¹)</i>	<i>Elements</i>	<i>Amount (mg day⁻¹)</i>
Zn	15	Co	0.04
Mn	2.8 (2-5)	Ni	0.025
Fe	15 (10-28)	Cr	0.05-0.20
Cu	2.5 (2-3)	Pb	0.415
Sr	1.6 (0.98-2.2)	Mg	300
Ba	1.1 (0.65-1.7)	Ca	500

Zinc: The amount of zinc contained in tissues in the human body varies between 2-4 g. The requirement met by a normal diet is 6-22 mg day⁻¹. Zinc is an important part of many enzymes and is found in more than 200 enzymes. Zinc requirement is determined as 12 mg day⁻¹ for an adult woman and 15 mg day⁻¹ for a man (Bilge, 1998; Onianwa et al., 2001).

Potassium: The concentration of this element in the body is 2 mg g⁻¹. It regulates the osmotic pressure in the cell as it is usually found inside the cell. The amount of potassium taken with a normal diet is 2-5.9 g day⁻¹ and the minimum requirement of potassium varies between 1.6-2.0 g per day (Baysal, 1996; Bilge, 1998; Keskin, 1987; Erdoğan, 2002).

Sodium: The sodium content in the body is 1.4 mg g⁻¹. Sodium, together with potassium, is involved in the regulation of the osmotic pressure of the body fluid. Sodium absorption by the body is very fast. Sodium intake of the body under normal conditions varies between 1.7-6.9 g day⁻¹ (Bilge, 1998; Keskin, 1987; Erdoğan, 2002).

Silicon: In animal experiments, silicon deficiency causes growth retardation, bone, cartilage and connective tissue disorders. However, no determination has been made regarding silicon deficiency in humans so far.

Vanadium: Firstly, vanadium was found to be essential for bone and tooth development. It is on record that vanadium plays an important role in carbohydrate metabolism.

MATERIAL AND METHOD

Materials

Registered six red lentil varieties (Çağlı, Altıntoprak, Seyran-96, Fırat-87, Yerli Kırmızı, Tigris) and Chickpea cultivars (Diyar-95, ILC-482) were taken from Food Legumes Unit of GAP International Agricultural Research and Training Center. Samples were stored in polypropilen cups until analysis time.

Methods

Digestion procedure: About 0.25 g dried and ground

sample was put into burning cup and 8 ml 65% HNO₃, 2 ml 30% H₂O₂ added. The samples were dissolved in a microwave oven (Milestone Smart D) according to program showing at table 2. Samples dissolved and diluted 25 ml volume with ultra pure water. Concentrations were determined with ICP-OES (Thermo ICAP 6300).

Table 2. Sample digestion program for lentil and chickpea cultivars

Çizelge 2. Mercimek ve nohut çeşitleri için örnek çözünürleştirme programı

<i>Step</i>	<i>Time(min)</i>	<i>T (°C)</i>	<i>Power(W)</i>
1	20:00	180	1200
2	20:00	180	1200

Instrumentation

Parameters related to the device and method are shown in the tables below table 3, table 4 and table 5.

Statistical Analysis

The Mann-Wihtney Test, which is one of the nonparametric tests applied between two independent groups to determine whether there is a statistically significant difference between the element concentrations contained in the lentil and chickpea samples. As a result of the test, it was seen that there was a difference between the groups in terms of the mean of Ca, K, Mn, Cu, Ni and Se according to the level of significance (P <0.05).

Correlation between elements was determined by considering the Spearman rho coefficient.

RESULTS and DISCUSSION

Results

In this study, the results are presented in Table 6. As a result of our research on different varieties of red lentil and chickpea varieties the amount of Ca was found in the lentils and chickpeas between 601±5.4-950±41 and 1038±21-1499±15 mg kg⁻¹ respectively. The results correspond to those reported previously for cowpea (Harmankaya et al., 2016), bean (Ceyhan, 2001; Ceyhan et al., 2008; Harmankaya et al., 2009;

Table 3. Determination of Analytical Wavelengths (λ), Detection Limits (LOD) and Quantitation Limits (LOQ), Accuracy Assessment Through the Analysis Element Contents of CRM (GBW10011 wheat flour-trace elements) After Shredding (mean \pm S.D, n = 3, $\mu\text{g g}^{-1}$ dry weight)

Çizelge 3. Analitik Dalga Boylarının (λ), Tespit Limitlerinin (LOD) ve Kantitasyon Limitlerinin (LOQ) Belirlenmesi, Çözünme Sonrası SRM Analiz İçeriği (GBW10011 buğday unu-iz elementler) aracılığıyla Doğruluk Değerlendirmesi (ortalama \pm SD, n = 3, $\mu\text{g g}^{-1}$ kuru ağırlık)

Chemical Elements	Wavelengths (λ nm)	LOD ($\mu\text{g g}^{-1}$)	LOQ ($\mu\text{g g}^{-1}$)	Certified values ($\mu\text{g g}^{-1}$)	Confidence Interval Measured ($\mu\text{g g}^{-1}$)	Recovery (%)
Ca	317.933	0.0078	0,0259	340 \pm 20	355 \pm 18	104.40
K	766.440	0.0084	0.0279	1400 \pm 60	1380 \pm 85	98.58
Mg	279.553	0.0052	0.0173	450 \pm 70	435 \pm 62	96.65
Fe	259.940	0.0150	0.0499	18.5 \pm 3.1	17,7 \pm 4.8	95.60
Zn	206.200	0.0004	0.0013	11.6 \pm 0.7	12.1 \pm 0.51	104.31
Sn	189.989	0.096	0.319	-	7.356 \pm 1.36	-
Mn	257.610	0.0023	0.312	5.4 \pm 0.3	5.68 \pm 0.40	105.18
Cr	267.716	0.028	0.093	0.096 \pm 0.014	0.093 \pm 0.02	96.87
Cu	327.393	0.0012	0.0039	2.7 \pm 0.2	2.64 \pm 0.127	97.70
Cd	228.802	0.0018	0.0060	0.018 \pm 0.04	0.0193 \pm 0.004	107
Si	251.611	0.085	0.283	80	75.6 \pm 5.31	95
Ni	221.647	0.0056	0.0186	0.06 \pm 0.02	0.065 \pm 0.01	108.33
V	292.402	0.0098	0.0326	0.034 \pm 0.012	0.036 \pm 0.018	105.88
Pb	220.353	0.0083	0.0276	0.065 \pm 0.024	0.070 \pm 0.032	107.69
As	193.696	0.0012	0.0040	0.031 \pm 0.005	0.029 \pm 0.006	93.54
Se	196.090	0.0095	0.0316	0.053 \pm 0.007	0.056 \pm 0.012	105.66

Table 4. Instrumental Operating Conditions Using Thermo ICAP 6300 ICP-OES

Tablo 4. Thermo ICAP 6300 ICP-OES Enstrümantal Çalışma Koşulları

Parameter	Normal	Hydride System
Power	1150 W	1350 W
Pump speed	50 rpm	30 rpm
Purge gas	Argon	Argon
Coolant Gas		
Flow	12 L/min.	16 L/min.
Auxiliary gas		
Flow	0.5 L/min.	0.5 L/min.
Torch	Axial, Radial	Axial
Auto sampler	Cetac ASX-260	

Table 5. Corelation between variables

Çizelge 5. Değişkenler arasındaki korelasyon

	Ca	K	Mg	Zn	Fe	Si	Mn	Sn	Cu	Ni	Pb	V	Cd	Cr	Se
Ca	1														
K	-0.214	1													
Mg	0.393	-0.250	1												
Zn	-0.310	0.429	-0.393	1											
Fe	-0.667	0.048	-0.214	0.667	1										
Si	-0.405	0.024	0.286	0.095	0.548	1									
Mn	0.500	-0.524	0.536	-	-	-0.357	1								
Sn	-0.095	-0.452	0.143	0.833*	0.714*		0.071	1							
Cu	-0.548	0.738*	-0.429	0.071	0.119	0.095	-0.262	0.524	1						
Ni	-0.500	0.619	-0.393	0.571	0.571	-0.024	-0.619	-0.119	0.690	1					
Pb	0.317	-0.610	0.00	0.122	0.146	0.024	0.195	-0.415	-0.537	-0.268	1				
V	-0.024	-0.476	-0.107	-0.119	-0.238	-0.048	0.286	-0.095	-0.476	-0.714*	0.293	1			
Cd	-0.443	0.587	-0.607	0.024	-0.168	-0.299	-0.275	0.216	0.635	0.371	-0.798*	-0.084	1		
Cr	-0.494	0.602	-0.631	0.458	0.446	0.012	-0.542	0.060	0.795*	0.916**	-0.136	-0.136	0.394	1	
Se	-0.619	0.619	-0.750	0.095	0.143	0.095	-0.357	0.452	0.929**	0.619	-0.439	-0.436	0.707	0.807*	1

Table 6. Concentrations of Elements in Lentil and Chickpea Cultivars (mean±standart deviation, mg kg⁻¹, dry weight n=3)

Çizelge 6. Mercimek ve Nohut Çeşitlerinde Element Konsantrasyonları (ortalama ± standart sapma, mg kg⁻¹, kuru ağırlık, n = 3)

Elements	Çağıl	Lentils				Chickpeas		
		Altıntoprak	Seyran-96	Fırat-87	Yerli Kırmızı	Tigris	Diyyar-95	ILC-482
Ca	601±5.4 ^b	783±17	606±7	609±6.2	879±14	950±41	1038±21	1499±15
K	4851±77	5350±13	4996±95	4891±37	5052±4	5441±33	4235±154	3285±102
Mg	863±10	880±24	855±9	848±15	867±21	854±16	-	901±14
Zn	32.4±0.05	34.1±0.07	38.03±0.2	38.2±0.04	40.8±0.03	33.4±0.1	33.7±0.2	29.6±0.02
Fe	73.1±0.4	70.3±8	96.4±10	73.2±9	86±3.3	84.3±0.5	49.0±0.98	52.1±7
Si	33.5±0.7	36.8±3	50.3±1	34.2±2.7	32.5±7	29.7±2	23.3±0.3	35.4±5
Mn	16.3±0.08	14.4±2	14.2±2	12.3±3	12.5±0.5	14.8±0.6	22.2±0.4	20.1±6
Sn	20.0±1.5	30.2±0.3	22.1±4	19.4±4	17.4±1.4	23.4±0.5	18.7±0.4	21.2±1.9
Cu	11.1±0.05	10.4±1.9	11.3±1.7	8.6±2	9.5±1	11.7±0.6	8.3±0.1	7.2±2.5
Ni	2.10±0.01	1.8±0.008	2.27±0.03	2.09±0.03	2.51±0.04	2.12±0.003	1.06±0.03	0.94±0.02
Pb	<0.0025	<0.0025	0.131±0.07	0.070±0.01	0.062±0.02	<0.0025	0.310±0.03	0.080±0.02
V	0.470±0.1	0.710±0.1	0.100±0.006	0.89±0.2	0.054±0.003	0.071±0.01	1.48±0.14	0.20±0.02
Cd	0.031±0.006	0.022±0.006	0.020±0.007	0.030±0.001	0.021±0.004	0.032±0.003	0.020±0.001	0.011±0.006
As	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
Cr	0.52±0.007	0.51±0.006	1.63±0.006	0.52±0.005	0.59±0.007	0.71±0.01	0.51±0.01	0.50±0.0036
Se	0.552±0.1	0.475±0.11	0.558±0.06	0.480±0.17	0.437±0.07	0.650±0.02	0.410±0.09	0.316±0.15

a: mean

b: standart deviation

Ceyhan et al., 2014), chickpea (Kahraman et al., 2015) and pea (Harmankaya et al., 2010; Ada et al., 2019; Ceyhan and Simsek, 2021). On the other hand, according to Ereifej et al. (2001), the amount of Ca is given as 423 ± 40, 979 ± 48, 710 mg kg⁻¹ in Jordan and Morocco for lentils, respectively. These values are close to our findings in this study. At the same time, Mg and K contents were found in the lentils and chickpeas between 848 ± 15 to 880 ± 24 mg kg⁻¹, 901±14 mg kg⁻¹, and 4851±77-5441±33, 3285±102 to 4235±154 mg kg⁻¹ respectively. Significant genotype effects were observed for Mg and K contents in bean (Ceyhan, 2001; Ceyhan et al., 2008; Harmankaya et al., 2009; Ceyhan et al., 2014), chickpea (Kahraman et al., 2015) and pea (Harmankaya et al., 2010; Ada et al., 2019; Ceyhan and Simsek, 2021) grown in Konya, Turkey. However, According to Ereifej et al.(2001) the amount of Mg and K has been reported as 129 ± 20, 1190 ± 10 mg kg⁻¹, 381 ± 30, 5480 ± 210 mg/kg in Jordan and Morocco lentils, respectively.

As a result of the correlation analysis, some significant positive and negative relationships were found between the investigated element concentrations in lentil and chickpea seeds. Seed Mn content was negatively correlated with Zn and Fe (respectively r=-0.833, r=-0.714 respectively, P<0.05 for both). In addition to, seed V content with Ni, seed Cd content showed a negative statistically significant correlation with Pb (r=-0.714 and r=-0.798 respectively, P<0.05 both). Seed Se content had a strong positive correlation with Cu (r =0.929, P < 0.01) while seed Cr content had a strong positive correlation with Ni (r=0.914, P < 0.01). Also, Seed K content showed a positive correlation with Cu, and seed Cr content showed a positive correlation with Se and Cu (r=0.738, r=0.807 and r=0.795 respectively, P<0.05 for all three)

(Table 5).

The values which was found in this study quite higher than Jordan lentils, whereas to be lower than those in Morocco lentils.

The amount of Fe was found in lentils and chickpeas between 70.3±8-96.4±10 mg kg⁻¹, 49.0±0.98-52.1±7 mg kg⁻¹, respectively. The results were similar to previous reports for cowpea (Harmankaya et al., 2016), bean (Ceyhan, 2001; Ceyhan et al., 2008; Harmankaya et al., 2009; Ceyhan et al., 2014), chickpea (Kahraman et al., 2015) and pea (Harmankaya et al., 2010; Ada et al., 2019; Ceyhan and Simsek, 2021) in Turkey. On the other hand, Ereifej et al.(2001) stated the amount of Fe as 133 ± 20, 119 ± 14, 78 mg kg⁻¹ in Jordan, Morocco and FAO lentils, respectively. In this study Fe values was high than Jordan and Morocco lentils. However, It was found to be in line with FAO 's values. The amount of Zn was found in lentils and chickpeas between 32.4±0.05-40.8±0.03 mg kg⁻¹, 29.6±0.02-33.7±0.2 mg kg⁻¹, respectively. Whereas Zn values is given as 62±0.3, 37.3±0.8 mg kg⁻¹ in Jordan and Morocco lentils, respectively, according to Ereifej et al.(2001), Mn concentrations were found in lentils and chickpea between 12.5±0.5-16.3±0.08 mg kg⁻¹, 20.1±6-22.2±0.4 mg kg⁻¹, respectively. On the other hand Cd, Cu, Fe, Mg, Mn, and Zn values in lentils is given as 0.50 ± 0.03, 7.7 ± 0.4, 129 ± 5, 2229 ± 59, 19.0 ± 0.8, 58 ± 3 mg kg⁻¹, respectively according to Momen et al. (2006). In the study, heavy metal concentrations such as Cd, Cu, Cr, Ni ve Pb ve Se were found to be a parallel the indicated quantity in the literature such as Salama and Ridwan, (2005), Akinyele and Shokunbi, (2015), Çiçek et al. (2017). At the same time in the study As concentrations were found below the detection limit.

CONCLUSION

As a result, the fact that consumption of lentils and chickpea provides many benefits to human health in terms of mineral balance has been revealed in this study. In general, it can be said that lentils and chickpeas are rich in macro and micronutrient elements, especially elements such as calcium and potassium, and the deficiency of these minerals can be eliminated by consuming them. This study will benefit in terms of food safety both in Turkey and to the people of our region by determining the mineral content in registered lentils and chickpeas. Lentil seeds had higher mean values for K, Zn, Fe, Si, Sn, Cu, Ni, Cr and Se elements. However, chickpea seeds had higher average values in terms of Ca, Mg and Mn. The amount of Ca, K and Se of Tigris cultivar was higher than others. The amount of Cr, Fe and Si in Seyran-96 cultivar, on the other hand, was found to be higher compared to other types. The amount of Mg was found to be close to each other in all varieties. In the products analyzed weren't found concentrations of the toxic levels of elements according to the FAO/WHO and Turkish food Codex.

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Statement Contribution of the Authors

Authors declares the contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

Ethics Committee Decision Statement

The article does not need an ethics committee decision.

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