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Dietary Element Assessment of Legumes Originated from Tunceli Province Using Different Dissolving Techniques

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Abstract: The objective of this study was to compare the mineral contents of chickpeas and haricot beans using two different analytical digestion methods, including dry ashing and microwave digestion method. The levels of micronutrients (Cu, Fe, Mn, and Zn) and macronutrient minerals (Ca, Mg, Na, and K) in two different legumes, as chickpea samples (*Cicer arientinum* L) and haricot bean (*Phaseolus vulgaris* L.) that are widely growing and consumed in Tunceli Province-Turkey were evaluated. The mineral composition of chickpea and haricot bean were determined by using FAAS after dissolving dry ashing and microwave digestion methods. In this way, both the mineral composition of legume samples and the dissolving methods were compared. Results obtained from each group and each method was analyzed statistically using the SPSS statistics program package. It was observed in chickpea and haricot bean samples that Ca, Cu, and Zn concentrations using microwave digestion method were higher than that of dry ashing method ($p<0.01$).

Keywords: Chickpea; Haricot bean; Mineral content; Dry ashing; Microwave digestion.

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INTRODUCTION

In human nutrition, especially children and young people, micro- and macronutrients such as iron, copper, and zinc have important roles for providing optimal health, growth, and development (1, 2). In Turkey, as well as all over the world, dietary intakes of vital micronutrients, especially iron and copper, are low. Because of inadequate intakes of these components among young children, some health problems occur and probably contribute to the mild, zinc deficiency and high prevalence of iron, deficiency anemia among this age group (3). Legumes especially chickpea, haricot and cereals are vital, because of their nutritional quality and food sources of micronutrients for infants and children in Turkey especially in rural areas, namely "Anatolia" (4, 5). Legumes contain several bioactive substances that play important roles in the metabolism of humans or animals who frequently consume these foods.

Chickpea (*Cicer arietinum* L.) is one of the oldest and most widely consumed food source in the world besides most important grain legumes and also a good source of macronutrients (Ca, P, Mg) and micronutrients (Fe, Zn and Cu). At the same time, it contains a variety of anti-nutritional factors, such as amylase inhibitors and protease, besides oligosaccharides and polyphenols, which disrupt nutrient absorption from the gastrointestinal tract; it also contains high levels of carbohydrates and proteins (6-8). Because of these reasons, chickpea can be considered as a nutraceutical or a functional food (9). Haricot beans (*Phaseolus vulgaris* L.) are rich sources of proteins, vitamins, carbohydrates, and especially minerals (10, 11). Conscious consumers are aware of the health benefits of foods and interest in foods that are rich in elemental content in their diets. The nutritional value of a food that given mineral depends not only on the variety mineral content and level, but also on its bioavailability for humans. In fact, the intakes of some vital elements are associated with reduced risk of several health problems such as stroke, cardiovascular disease, and various types of cancers like pharynx, mouth, esophagus, stomach, lungs, and colon (12, 13). When legumes are consumed frequently, 11% and 22% lower risk of cardiovascular disease and coronary heart disease are reported, respectively (14). Determination of mineral elements in foodstuffs such as chickpea and haricot bean is an important part of nutritional analyses. Iron, copper, and zinc are essential and critical micronutrients for human health as well as they play a critical role in human metabolism (15, 16). Copper can be found in the structure of many enzymes and is essential for iron in living metabolism. On the other hand, several studies have reported about direct correlation between the dietary Zn and Cu ratio and the incidence of cardiovascular disease. Due to the difficulties in establishing the recommended dietary intake of Cu, a safe range of copper for adults (1.5–3 mg/day) was reported by some organizations (17). Fe is also an essential and vital element because the body needs it, due to some reasons such as the type of Fe compound, the total amount in the diet, and the other dietary components (18). Zinc enzymes join in a wide variety of metabolic synthesis or degradation processes in addition it may also play a vital role in stabilizing plasma membranes. In addition, Zn has been recognized as a co-factor of some enzymes that protection against oxidative processes (19). The recommended daily intake for adults Zn and Fe are 12–15 mg and 10–15 mg were reported by some organizations, respectively (17, 20). Ca and Mg play an essential physiological role in many body functions. While Ca is the main component of bone and its function especially in cell membranes, muscles and regulation of blood pressure, are of great importance (21), Mg has an essential physiological role such as it has the ability to compete with calcium for binding sites on proteins and membranes (22). Na and K have great importance for many regulation systems besides ionic balance of the human body and maintain excitability of the tissue. Na has a vital role in the transport of metabolites while K is of importance as a diuretic. Body requires at least a daily intake of 2.4 g and 3.5 g are Na and K, respectively (23, 24).

There is no comprehensive study in the literature about comparing the element composition of chickpeas and haricot bean in terms of dry ashing and microwave digestion methods. The present study is focused on two different digestion processes; compared in terms of some micro and macroelement contents of the chickpea and haricot bean samples were determined by flame atomic absorption spectrometry (FAAS) method. Because of the beneficial effects and nutritional properties, the growing popularity and frequent consumption of legumes requires a quality control in terms of mineral composition.

MATERIALS AND METHODS

Apparatus

In order to determine element contents such as Ca, Cu, Fe, K, Mg, Mn, Na, and Zn using FAAS (Perkin Elmer AAnalyst 800) equipped with a lamp and an air-acetylene flame/N_2O-acetylene, single slot-burner head. The instrumental operating parameters are listed in Table 1. For dry ashing and microwave digestion method, a model Protherm Furnace and a model microwave digestion method (Coolpex, China) were used, respectively.

Table 1. Instrumental operating conditions for FAAS*.

Element	Wavelength (nm)	Slit width (nm)
Ca	422.7	0.7
Cu	324.8	0.7
Fe	248.3	0.2
K	766.5	0.7
Mg	285.2	0.7
Mn	279.5	0.2
Na	589.0	0.2
Zn	213.9	0.7

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

Reagents and standard solutions

In all experiments, all chemical reagents were of analytical reagent grade and all solutions were prepared using ultrapure water (Milli-Q, Millipore 18.2 μΩ cm^{-1} resistivity). HNO_3 and H_2O_2 (E. Merck, Darmstadt) were of ultrapure quality. All glassware and reaction vessels were cleaned using 10% HNO_3 solution before use, then rinsed with deionized water. 1000 mg L^{-1} of metal stock solutions were prepared. All standard solutions were diluted these stock solutions and they used for calibration graphs.

Legumes sampling and analytical procedure

In this study, chickpea and haricot bean samples grown in Tunceli were purchased from different local markets of Tunceli province-Turkey in 2015. These samples were washed firstly by tap water then with ultrapure water. After washing step, samples are dried at 105 °C temperature in a drying oven about 24 h and homogenized using a porcelain mortar. Finally, samples were put into plastic bags and preserved until analysis.

For dry ashing method, dried homogenized chickpea and haricot bean samples and standard reference material (NIST-SRM 1547 peach leaves and NIST1549a milk powder) were weighed about 0.50 g. The furnace was heated gradually using the following sequence: at 250 °C for 30 minutes, at 300 °C for 5 minutes, at 350 °C for 5 minutes, at 400 °C for 5 minutes, at 450 °C for about 20 minutes to finish gas formation, and ashed at 480 °C for 2-4 hours. The mixture of concentrated HNO_3/H_2O_2 (1:1) (2 mL for 0.5 g of dried sample) was added to the ashed sample and then evaporated to near dryness with occasional stirring. The same operation was repeated once more. After cooling, 15 mL of 1.0 M HNO_3 was added, and the samples were centrifuged, if necessary. Analyses were prepared at least five times. The clear solutions were analyzed by FAAS for micro and macro element contents.

For microwave digestion method, dried homogenized chickpea and haricot bean samples and standard reference material (NIST-SRM 1547 peach leaves and NIST1549a milk powder) were weighed about 0.50 g and taken into a Teflon vessel. For this process, 7 mL of concentrated HNO_3 and 1 mL of H_2O_2 were added and re-dissolved in the microwave oven. The following program was run, Step 1: 2 min to reach 80 °C at 15 atm, Step 2: 2 min to reach 120 °C at 20 atm, Step 3: 2 min to reach 160 °C at 25 atm, Step 4: 4 min to reach 200 °C at 30 atm, then cooled at room temperature (10 min). Analyses were prepared at least five times. The obtained clear solutions' final volume was completed to 15 mL with ultrapure water and analyzed by FAAS for micro and macro element contents.

RESULTS AND DISCUSSION

Mineral composition of chickpea and haricot bean were determined using dry ashing and microwave digestion method and presented in Tables 2-3, respectively. Comparison of dry ashing and microwave digestion method in chickpea and haricot bean were given in Tables 4 and 5, respectively. There are significant ($p < 0.01$) differences of both food sources' mineral contents were observed. In chickpea samples, Ca, Cu, and Zn concentrations obtained by microwave digestion method were statistically higher than that of dry ashing method, Fe and Na concentrations obtained by dry ashing method were statistically higher than that of microwave digestion method ($p<0.01$). In haricot bean samples, Ca, Cu, and Zn concentrations obtained by microwave digestion method were statistically higher than that of dry ashing method, K and Na concentrations obtained by dry ashing method were statistically higher than that of microwave digestion method ($p<0.01$). These observations are in agreement with literature reported by several researchers (9, 10, 20, 25-28) for chickpea and haricot bean. However, obtained results from this study suggest that there are significant differences between dry ashing and microwave digestion method, for both chickpea and haricot bean.

All results are the mean value of the at least five separate portions of the same sample. All elements' average concentrations of chickpea and haricot bean samples using dry ashing and microwave digestion were evaluated. The method's accuracy was tested with NIST-1547 peach leaves and NIST-1549a milk powder standard reference materials. NIST-1547 peach leaves was used for Ca, Cu, Fe, Mg, Mn, and Zn and NIST-1549a milk powder was used for Na and K. The obtained recoveries of NIST-1547 peach leaves and NIST-1549a milk powder standard reference materials using dry ashing method were 99% for Ca, 100% for Cu, 92% for Fe, 100% for Mg, 96% for Mn, 95% for Zn and 102% for Na, 96% for K. The obtained recoveries of NIST-1547 peach leaves and NIST-1549a milk powder standard reference materials using microwave digestion method were 94% for Ca, 100% for Cu, 99% for Fe, 98% for Mg, 95% for Mn, 96% for Zn and 102% for Na, 97% for K.

Table 2. Mineral composition of chickpea and haricot bean using dry ashing method (mg kg^{-1} dry weight basis).

Dry ashing method	**Microelements**				**Macroelements**			
Element	**Cu**	**Fe**	**Mn**	**Zn**	**Ca**	**K**	**Mg**	**Na**
Chickpea	7.6±0.1**	43.6±1.0	26.7±0.4**	35.3±0.3**	724±12	8888±561	1437±97	92±3**
Haricot bean	6.7±0.1	55.2±1.3**	14.1±0.2	29.6±0.6	1193±29**	11446±487**	1561±55	68±2

Means±standard deviation of five determinations.
**Significant at $p<0.01$.

Table 3. Mineral composition of chickpea and haricot bean using microwave digestion method (mg kg^{-1} dry weight basis).

Microwave digestion method	**Microelements**				**Macroelements**			
Element	**Cu**	**Fe**	**Mn**	**Zn**	**Ca**	**K**	**Mg**	**Na**
Chickpea	9.2±0.1	36.3±1.5	27.1±0.1**	37.5±0.9	887±22	8074±187	1280±20	68.3±2.3**
Haricot bean	9.2±0.1	53.4±1.6**	14.0±0.8	34.3±0.2	3050±75**	8943±713**	1702±69**	52.7±3.7

Means±standard deviation of five determinations.
**Significant at $p<0.01$.

Table 4. Comparison of dry ashing and microwave digestion method in chickpea samples.

Element	Dry ashing method	Microwave digestion method
Ca	724±12	887±22**
Cu	7.6±0.1	9.2±0.1**
Fe	43.6±1.0**	36.3±1.5
K	8888±561	8074±187
Mg	1437±97	1280±20
Mn	26.7±0.4	27.1±0.1
Na	92±3**	68.3±2.3
Zn	35.3±0.3	37.5±0.9**

Means±standard deviation of five determinations.
**Significant at $p<0.01$.

Table 5. Comparison of dry ashing and microwave digestion method in haricot bean samples.

Element	Dry ashing method	Microwave digestion method
Ca	1193±29	3050±75**
Cu	6.7±0.1	9.2±0.1**
Fe	55.2±1.3	53.4±1.6
K	11446±487**	8943±713
Mg	1561±55	1702±69
Mn	14.1±0.2	14.0±0.8
Na	68±2**	52.7±3.7
Zn	29.6±0.6	34.3±0.2**

Means±standard deviation of five determinations.
**Significant at $p<0.01$.

Micro- and macro-element concentrations such as Fe, Cu, Ca, Mg, and K in chickpea that grows under different farming regimes were examined using Wavelength-Dispersive X-Ray Fluorescence Spectrometry (WDXRF) by Akbaba *et al.* (9). They evaluated about 30 elements' concentrations in chickpea and they presented results as average (n=10). They found that Ca, Cu, Fe, Mn, P, and Zn concentrations as 0.1123%, 0.0007%, 0.0258%, 0.0025%, 0.1884%, 0.0088%, respectively. Another presented study by Alajaji and El-Adawy (25) shows the effects of some cooking methods with microwave cooking and autoclaving on the anti-nutritional such as elemental composition of chickpea (*Cicer arietinum* L.) samples. After wet ashing processes using acid mixing (HNO_3 and $HClO_4$), some minerals such as Ca, K, and Na were determined by a flame photometer, other minerals such as Fe, Mg, Cu, and Zn were determined using an AAS. They found that Na, K, Ca, Mg, P, Mn, Zn, and Cu and concentrations in the chickpea (dry weight basis) as 114-121 mg 100 g^{-1}, 341-870 mg 100 g^{-1}, 124-176 mg 100 g^{-1}, 165-176 mg 100 g^{-1}, 195-226 mg 100 g^{-1}, 1.80-2.11 mg 100 g^{-1}, 3.42-4.32 mg 100 g^{-1}, 0.73-1.10 mg 100 g^{-1} and 6.81-7.72 mg 100 g^{-1}, respectively. In another study, Cabrera *et al.* (20) evaluated the levels of some essential and vital elements like Cu, Fe and Zn in different legume and nut samples. After samples were homogenized by a blender and weighed, using a temperature controlled microwave, samples were mineralized in a Teflon vessel and elements were determined by ETAAS. The Cu, Fe, and Zn levels were found in haricot sample varied from 2.00 to 3.30 µg g^{-1}, 64.60-74.70 µg g^{-1} and 45.10-70.20 µg g^{-1}, respectively. They calculated these levels in chickpea samples varied from 3.20 to 4.90 µg g^{-1}, 65.00-70.20 µg g^{-1} and 37.40-42.80 µg g^{-1}, respectively. Chan *et al.* (4) found that the Fe, Zn, and Ca levels in cereals and legumes consumed by Indonesian infants using FAAS. They found the highest level of Zn in tofu (Lombok, boiled) as

5.80±1.0 mg 100 g^{-1} (DW), Fe in the tofu (Lombok, boiled) as 17.4±4.7 mg 100 g^{-1} (DW) and Ca in the tempe (Lombok, raw) as 435.7±260.4 mg 100 g^{-1} (DW). According to Gunes *et al.* (26), they carried out a study to determine N, P, K, Fe, Zn, and Mn in mixed wheat/chickpea and wheat/lentil samples that were grown under different conditions. After the ashing step, ashes were dissolved in HNO_3 and diluted to 25 mL using ultrapure water. Obtained extracts were filtered and stored in plastic vials until analyzed. While micro elements (Zn, Mn and Fe) were determined by AAS, K was measured using flame photometer. They reported that K, Fe, Zn, and Mn concentrations in wheat/chickpea mix were found as 46.18 mg kg^{-1}, 79.21 mg kg^{-1}, 34.45 mg kg^{-1} and 41.03 mg kg^{-1}, respectively. Oomah *et al.* (27) investigated some haricot cultivars grown in Southern Manitoba and evaluated in terms of phenolic content and antioxidant properties besides mineral contents. After dry-ashed samples were dissolved in 1.2 N HCl, mineral contents (Zn, Fe and Mn) were determined FAAS and also Ca, Cu, Mn, P, and K were measured by inductively coupled spectrophotometry (ICP, Spectro Flame). The means level of Ca, Mg, K, P, Cu, Zn, Fe, and Mn were calculated 1300 mg kg^{-1}, 1804 mg kg^{-1}, 17070 mg kg^{-1}, 1.5 mg kg^{-1}, 23.5 mg kg^{-1}, 47.5 mg kg^{-1} and 13.6 mg kg^{-1} as dry weight, respectively. In another study, the mineral contents of Desi chickpea and Kabuli chickpea were compared using AAS (28). While Ca, K, Mg, Fe, P, Zn and Mn amount of Desi chickpea were presented as 165.0 mg 100 g^{-1}, 994.5 mg 100 g^{-1}, 169.0 mg 100 g^{-1}, 4.59 mg 100 g^{-1}, 451.5 mg 100 g^{-1}, 4.07 mg 100 g^{-1} and 3.81 mg 100 g^{-1}, mineral content of Kabuli chickpea were calculated as 81.7 mg 100 g^{-1}, 1060.0 mg 100 g^{-1}, 147.0 mg 100 g^{-1}, 5.5 mg 100 g^{-1}, 394.0 mg 100 g^{-1}, 3.4 mg 100 g^{-1}, 3.28 mg 100 g^{-1}, respectively. In another study, Zn composition was investigated varieties of haricot bean grown in Ethiopia as well as some another antinutrient such as phytic acid, and tannins trypsin inhibitors (10). Because of their Zn content and other beneficial nutritional parameters especially lower antinutritional factors, according to results of this study, Awash, Roba, Redwolaita and Mexican were found to be the best food types among eight haricot beans in Ethiopia. Zn levels were found 17.21±0.23 mg kg^{-1}, 15.99±0.00 mg kg^{-1}, 28.22±0.00 mg kg^{-1}, 17.91±0.16 mg kg^{-1}, respectively.

The presented study's results compared with the literature is generally compatible, but some micro- or macro-element levels are slightly higher than the literature. Soil is the best important parameter on mineral contents of plant products because it affects significantly to the pH and organic matter content. The differences in element levels in plant samples may be attributed from measurement methods as well as other factors, especially environment, soil composition, and climatic conditions.

CONCLUSIONS

It is important to ensure the adequate intake of micro and macro minerals such as Ca, Mg, Cu, especially Fe and Zn during early childhood period to maintain optimum health, growth, and development. Therefore, determination of food content with accurate and sensitive analytical methods is important. On the other hand, it will contribute towards defining the magnitude of dietary inadequacies of mineral especially in childhood period. Obtained and presented data in this study can also be used to identify legumes that provide relatively large amounts of macro and micro elements per person portion, which will thus assist in the development of food-based strategies to improve the micronutrient intakes of rural Turkish people.

As a conclusion, both chickpeas and haricot beans are potential sources of both for macro- and micro-nutrients according to obtained data from different digestion methods as dry ashing and microwave digestion method. Determined concentrations of Ca, Cu, Fe, K, Mg, Mn, Na, and Zn elements that are essential and vital for human health were higher in the both legume samples. Thus, we could suggest that this food source is crucial for human nutritional regime. In the light of this information, from obtained results from present study, the usage of different two digestion methods and FAAS analysis are efficient and useful techniques for food science which deserves attention for interdisciplinary studies.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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