Journal of Environmental Health and Sustainable Development

A Comparative Study on Mineral Contents of Soybean by Two Methods of Digestion Using ICP-OES Technique: A Risk Assessment Study

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ARTICLE INFO

ORIGINAL ARTICLE

Article History:
Received: 10 February 2020
Accepted: 20 April 2020

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Keywords:
Soybean,
Digestion Method,
ICP-OES,
Risk Assessment.

ABSTRACT

Introduction: In recent years, the contamination of food with heavy metals has received much attention. Plants can absorb metal pollutants through contaminated water, soil, and air.

Materials and Methods: In the current study, accumulation of minerals in three types of soybeans was investigated by wet and dry digestion methods using ICP-OES technique. Thereafter, the metals' health risk was assessed by estimated daily intake, toxic hazard quotient (THQ), and hazard index (HI) values.

Results: According to the results, the concentrations of Cr, Se, Ca, Fe, Mo, Mn, and Mg in soybean seeds were 0.034-170.88 mg/kg, 0.21-243.79 mg//kg, 2.50-33.37 mg/kg, 0.05-0.86 mg/kg, 0.071-203.57 mg/kg, 0-0.47 mg/kg, and 2.69-19.31 mg/kg, respectively. The ashing method had a better performance in determining Ca, Fe, Mo, Mn, and Mg concentrations than the wet digestion method. The THQ rates were below 1 for the three varieties of each mineral element, but the HI values of variety 2 and variety 3 were higher than 1 in both methods.

Conclusion: Furthermore, continuous monitoring of the soybeans' mineral and heavy metal contents seems necessary.

Citation: Khalili N, Akrami Mohajeri F, Askari E, et al. ***A Comparative Study on Mineral Contents of Soybean by Two Methods of Digestion Using ICP-OES Technique: A Risk Assessment Study***. J Environ Health Sustain Dev. 2020; 5(2): 1010-5.

Introduction

Trace elements or essential micronutrients (manganese, iron, copper, zinc, cobalt, and selenium) are metals with different biochemical functions in living beings, but their excess amounts can cause toxicity [1]. Exposure to trace elements causes acute and chronic symptoms, such as cardiovascular disease, impaired fertility, and disorders in nervous and immune system. Transmission of metals to food chain has been considered as a crucial problem during the last decade [2]. Trace elements can be absorbed by plants through soils, fertilizer, air, and industrial activities [1]. Metals can be accumulated in crops grown in contaminated soils, which pose a risk to human health [3]. One of the most wide spread crops in the world is soybean (Glycine max) [4]. Considering the nutritional ingredients of soybeans, such as protein (40%), oils (20%), carbohydrates (35%), and essential elements, soybeans can be considered as an inexpensive and good source of protein, dietary fiber, and isoflavones, especially in developing countries [5-8]. To determine the metal content in foods, different analytical devices were proposed including atomic absorption spectrometry, inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS) [1, 9-10]. Prior to metal measurements, sample decomposition is necessary [1]. Wet ashing, dry ashing, and microwave digestion are among the most usual methods for sample decomposition [9, 11]. In wet ashing, a mixture of acids with or without oxidants (H_2O_2) was used along with heating in open or closed vessels [11-12]. This method needs constant monitoring and its applicability depends on the type of food [13]. Dry ashing is a convenient method in which thermal decomposition of samples occurs in thermal furnace at temperatures of 450 to 550 ˚C. Sample preparation is completed by dissolving the ash residues in diluted acids [13]. Although dry ashing is not suitable for volatile metals and pyrolytic organic materials (which is resistance to thermal decomposition), it is simpler, safer, and more applicable for large quantities than wet digestion [13-14]. The dried ash samples are completely free of organic matter and are suitable for determining low metal concentrations [13].

Consumption of foods contaminated with different metals can contribute to health problems. The potential health risk of metals can be estimated by Target Hazard Quotient (THQ) and Hazard Index (HI) for individual and multiple metals, respectively [15]. Thus, providing information about metal concentration of foods and the health risk evaluation of Iranian soybean consumers seem necessary in this area. In the present study, the THQ and HI of different metals were estimated after investigating the Cr, Mo, Mn, Mg, Se, Ca, and Fe contents of soybeans by two methods of digestion.

Materials and Methods

Reagents

All chemicals were of analytical reagent grade (Merck, Germany), all glass wares were soaked overnight in 10% HNO_3, and rinsed with distilled de-ionized water for three times [16].

Sample preparation

Three soybean cultivars were purchased from different local markets in Iran. The samples were transported to the laboratory. After cleaning and rinsing, the soybeans were milled with stainless steel blender [17].

Sample digestion

In dry digestion, one gram of each sample was placed in a crucible furnace until the temperature of 450 ˚C was reached slowly. After 16 hours, the white ash residue was treated with 10 ml HNO_3 (10% v/v). The filtered solution was transferred to 25 ml volumetric flask and diluted by distilled de-ionized water [16].

In wet digestion, the powdered soybean samples (1 g) were digested with a mixture of HNO_3 (65%), HCl (37%), and H_2O_2 (30%) in ratios of 5:2:1. The mixture was put on a hot plate (60 ˚C) until the digestion was completed [17].

The digested samples were filtered and diluted to 25 ml with distilled de-ionized water and injected to Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, SPECTRO GENESIS model). The multi element calibration standard was also used as the standard solution.

Health risk assessment

Regarding the Estimated Daily Intake (EDIs), the concentration of metals in soybean is considered important to estimate the daily intakes. The following equation was used to determine the EDI [15, 18].

$$EDI = (C \times CR)/BW$$

Where, C is the metal concentration (μg/kg), CR is the average daily consumption (which is evaluated 1.36 g/person/day for an Iranian adult), and BW is the body weight (which is considered 65 kg for an Iranian adult) [18].

The Target Hazard Quotient (THQ), is determined as the ratio of metal dose to a reference dose (RfD). The proportion of less than 1 represents that the population is exposed to adverse effect of these pollutants [18].

$$THQ = EDI/RfD$$

The reference dose (RfD) for Cr, Fe, Mn, Se, Ca, Mo, and Mg were 1.5, 0.7, 0.014, 0.005, 0.001, 0.005, and 0.14 mg/kg/day, respectively [19]. To assess the non-carcinogenic effects of more than one metal, the Hazard Index (HI) was used [4].

$$HI=\sum THQ= THQ_1+THQ_2+THQ_3+\ldots+THQ_n$$

Results

The concentrations of metals by two digestion methods are presented in Table 1. It was found that Ca, Fe, Mn, and Mg concentration of dry digestion was estimated higher than that of wet digestion. The highest Cr concentration was found in variety 2 of wet digestion (170.88 mg/kg). The lowest Se and Mo were determined in variety 1 of dry digestion with concentration of 0.21 and 0.071 mg/kg, respectively. The concentrations of Cr, Se, Ca, Fe, Mo, Mn, and Mg in soybean seeds ranged from 0.034 to 170.88 mg/kg, 0.21 to 243.79 mg/kg, 2.50 to 33.37 mg/kg, 0.05 to 0.86 mg/kg, 0.071 to 203.57 mg/kg, 0 to 0.47 mg/kg, and 2.69 to 19.31 mg/kg, respectively.

Table 1: Concentration of different mineral elements in different soybean varieties by two methods of digestion (mg/kg dry weight)

Elements	Dry digestion			Wet digestion		
	variety 1	variety 2	variety 3	variety 1	variety 2	Variety 3
Cr	0.034^{f}	25.56^{e}	51.65^{c}	163.41^{b}	170.88^{a}	33.36^{d}
Se	0.21^{e}	40.42^{d}	190.56^{b}	189.25^{b}	171.19^{c}	243.79^{a}
Ca	19.11^{b}	33.37^{a}	30.14^{a}	2.50^{d}	16.67^{b}	8.06^{c}
Fe	0.26^{b}	0.81^{a}	0.91^{a}	0.05^{b}	0.86^{a}	0.20^{b}
Mo	0.071^{f}	203.57^{a}	160.21^{b}	9.33^{e}	109.16^{c}	33.66^{d}
Mn	0.072^{cd}	0.25^{bc}	0.47^{a}	ND^{d}	0.33^{ab}	0.04^{cd}
Mg	15.41^{b}	16.5^{b}	19.31^{a}	4.16^{d}	8.77^{c}	2.69^{d}

Different words in each row show significant difference at p value < 0.05
LOD: Ca: 0.002 ppm, Fe: 0.001 ppm, Mo: 3.202 ppb, Mn: 0.0001 ppm, Mg: .0.0001 ppm, Cr: 4.980 ppb, Se: 15.795 ppb.
Wave length: Cr: 284.325, Mo: 202.095, Mn: 257.611, Mg: 285.213, Se: 196.090, Ca: 317.933, Fe: 238.204 (nm)

Health risk assessment

The estimated dietary intakes and target hazard quotients of the mineral elements are given in Tables 2 and 3, respectively. The EDIs of the mineral elements different pattern in different varieties and methods. According to EDIs, only Fe of all samples was lower than the RfD value. Since the EDIs of Ca, Se, Mo, Mn, and Mg were higher than RfD in most samples, attention should be paid in soybean consumption.

According to Table 3, the THQ of individual mineral elements for three varieties were below 1, but the HI values of Variety 2 and Variety 3 were higher than 1 in both methods. Samples in wet digestion showed higher HI than dry digestion method.

Table 2: Estimated daily intakes of mineral elements in different soybean varieties by two methods of digestion

EDI Elements	Dry digestion			Wet digestion		
	variety 1	variety 2	variety 3	variety 1	variety 2	Variety 3
Cr	0.000711	0.697994	3.575335	3.41904	1.080677	0.534794
Se	0.004394	5.100837	3.581822	3.959692	3.987102	0.845711
Ca	0.39984	0.16864	0.348788	0.052308	0.630622	0.698203
Fe	0.00544	0.004185	0.017994	0.001046	0.01904	0.016948
Mo	0.001486	0.704271	2.283963	0.195212	3.352086	4.259311
Mn	0.001506	0.000837	0.006905	--------	0.009834	0.005231
Mg	0.322425	0.056283	0.183495	0.08704	0.404025	0.345231

The HI value or additive effects of contaminants in 2 varieties of soybean were higher than 1 by both wet and dry digestion methods. The variety 2 in wet digestion method had the highest HI value (2.10) followed by variety 3 of wet digestion (1.72). The lowest HI value was reported in variety 1 in the dry digestion method.

Table 3: Target hazard quotients and hazard index of the mineral elements in different soybean varieties by two methods of digestion

THQ Elements	Dry digestion			Wet digestion		
	variety 1	variety 2	variety 3	variety 1	variety 2	variety 3
Cr	4.74×10^{-7}	4.65×10^{-4}	2.38×10^{-3}	2.27×10^{-3}	7.2×10^{-4}	3.57×10^{-4}
Se	8.78×10^{-4}	1.02	7.16×10^{-1}	7.91×10^{-1}	7.97×10^{-1}	1.69×10^{-1}
Ca	3.99×10^{-1}	1.68×10^{-1}	3.48×10^{-1}	5.23×10^{-2}	6.30×10^{-1}	6.98×10^{-4}
Fe	7.77×10^{-6}	5.97×10^{-6}	2.57×10^{-5}	1.49×10^{-6}	2.72×10^{-5}	2.42×10^{-5}
Mo	2.97×10^{-4}	1.40×10^{-1}	4.56×10^{-1}	3.90×10^{-2}	6.70×10^{-1}	8.51×10^{-1}
Mn	1.07×10^{-3}	5.97×10^{-4}	4.93×10^{-3}	---------	7.02×10^{-3}	3.73×10^{-3}
Mg	2.30×10^{-3}	4.02×10^{-4}	1.31×10^{-3}	6.22×10^{-4}	2.88×10^{-3}	2.46×10^{-3}
HI	0.40	1.33	1.53	0.88	2.10	1.72

Discussion

Industrialization, economic developments, metal accumulation in soil, and its entrance into food chain caused public concern about the safety of food [20]. Soybean is one of the most important crops used in different food products. In previous studies, soybean and other beans were reported as crops which can strongly accumulate metals [18]. Some metals, such as magnesium, manganese, chromium, cobalt, calcium, iron, potassium, copper, nickel, and zinc have an important role in biological processes of the microorganisms, which are called micronutrients [21].

The comparison between these two digestion methods indicated different results for each element. According to the achieved results, concentrations of all elements, except for Cr and Se, were higher in dry digestion than the wet digestion methods. The highest Cr and Se concentrations were found in variety 2 and variety 3 of soybeans in wet digestion method, respectively. In Zhuang study, the Cr content of soybean was estimated within the range of 1.14 - 1.75 mg/kg [18], which is lower than our findings in the present investigation, except for variety 1 in dry digestion method. Metal accumulation by plants can be affected by metal content of soil, level of soil fertility, soil organic matter, soil acidic-alkaline and reductive-oxidative conditions, climatic conditions such as rain and temperature, and genetic differences between varieties [21-22].

Akinyele reported no significant differences between dry ashing and wet digestion in determination of manganese, zinc, cadmium, and lead in the studied food samples. However, these mineral contents of food in dry ashing were slightly higher than wet digestion, which was probably due to recovery rate. Dry ashing is recommended for food analysis because it is cost effective, has less risk due to chemical application, needs simple equipment, and has a better recovery. Dry ashing method can be useful in determining manganese, copper, iron, chromium, zinc, lead, and cadmium in legume and cereals [23]. In the current study, the ashing method performed better than wet digestion in determining Ca, Fe, Mo, Mn, and Mg, which is in agreement with the results reported by Akinyele et al. [23]. Saracoglu et al showed no significant differences in the levels of manganese, zinc, chromium, and nickel by dry ashing and wet digestion method in baby food samples [24]. In another study, the comparison among dry, wet, and microwave digestion of the dried fruits' elements showed no significant differences. However, microwave digestion performed better due to its accurate, simple, and fast procedures [16].

It was shown that the THQ value of all samples were lower than 1 which is compatible with results of Zhang et al [25]. According to the achieved results, HI value or non-carcinogenic effects of the samples except for variety 1 were higher than 1, which are important in public health. In the Zhang study the HI value of all studied metals were lower than 1 [25], which is not in agreement with current study. Therefore, it is likely for consumers to be hurt by metals in soy bean. As a result, the metal concentration of soybean should be monitored using different processing methods.

Conclusion

The findings demonstrated that the two methods of wet and dry digestion had different trends in determination of each metal. Except for Cr and Se, other metals had higher concentrations in dry digestion method than wet digestion. The THQ of all metals in three varieties were lower than 1. However, the HI values of variety 2 and variety 3 were estimated higher than 1 in both methods. Therefore, it is necessary to monitor the metal concentration of consumed foods.

Acknowledgment

We are grateful to School of Public Health, Shahid Sadoughi University of Medical science for their support.

Funding

The article was supported by Shahid Sadoughi University of medical sciences, Yazd, Iran.

Conflict of interest

There is not any conflict of interest.

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