Dietary Intake of Essential Elements (Na, K, Mg, Ca, Mn, Zn, Fe, Cu, Mo, Co) from Tofu Consumption

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Received: 10 March 2020 /Accepted: 7 April 2020 / Published online: 20 April 2020 \odot Springer Science+Business Media, LLC, part of Springer Nature 2020

Abstract

Tofu is one of the most consumed soybean products. Currently, tofu is consumed in vegan and vegetarian diets to avoid meat. However, it is necessary to determine the content of essential elements to assess the dietary intake. Essential elements (Na, K, Mg, Ca, Mn, Zn, Fe, Cu, Mo, Co) were determined in 130 samples of tofu by ICP-OES (inductively coupled plasma optical emission spectroscopy). The highest element content was found in flavoured tofu; the most notable were Na (2519 mg/kg wet weight) and Fe (19.5 mg/kg ww). Consumption of 200 g/day of flavoured tofu by adults would mean a high contribution of Cu (46.9% women, 38.1% men), Fe (55.7% women, 65.0% men) and Na (25.2% adults) to its AI (adequate intakes) sets by the EFSA (European Food Safety Authority). Natural tofu would mean a remarkably Mn contribution (50% adults) to the AI. Tofu could be an important source of essential elements such as Mg, Mn, Na, Cu and Fe.

Keywords Tofu . Essential elements . Dietary intake . Organic crop

Introduction

Tofu is a staple food in the Asian diet and is a derivative of fermented soybeans. Tofu is an important source of proteins, lipids, vitamins, amino acids and minerals [[1,](#page-5-0) [2\]](#page-5-0). The consumption of soybean products has grown rapidly in the USA and Europe and is now commonly used in the diet of vegetarians and vegans as a substitute for meat products [\[1](#page-5-0), [3](#page-5-0), [4](#page-5-0)].

The main soybean producer countries are Brazil (126 million tons), USA (96.8 million tons) and Argentina (54 million tons). The European Union produces only 2.6 million tons of soybeans [[5\]](#page-5-0). Attending to environmental concerns, soybean cultivation is changing its cultivation techniques; intensive agriculture is decreasing amending for organic and sustainable crops $[6]$ $[6]$.

Soybeans contain numerous compounds such as isoflavones, essential fatty acids, antioxidants and tocopherols [\[1](#page-5-0), [7](#page-6-0)]. Thus, soybean products like tofu have several beneficial properties. In 1999, the Food and Drug Administration (FDA)

 \boxtimes Soraya Paz spazmont@ull.edu.es established that soybeans and their derivatives reduce the risk of heart disease [\[8](#page-6-0)]. These beneficial effects have been demonstrated in several studies conducted on Chinese women, in which there is an inverse relationship between the high con-sumption of soybean products and ovarian cancer [[9](#page-6-0)]. Therefore, the consumption of soybeans and their derivatives can decrease certain hormone-dependent diseases. Furthermore, the correlations between the consumption of soybean and their derivatives and the decrease in the probability of suffering from these diseases have been found in Asian populations [\[2](#page-5-0)], but important correlations have not been found in Western populations [[9\]](#page-6-0). Although a protective effect against various types of cancer has been established, other studies show an increase in breast cancer associated with a high consumption of soybeans and their derivatives, concluding that the protective effects against breast cancer may occur only when the consumption of these products starts at an early age [\[3](#page-5-0)].

However, the beneficial effects associated with the consumption of soybeans and their derivatives have led various countries to set a recommended intake of these, as is the case of the FDA (Food and Drug Administration) that recommends a daily consumption of 25 g of soybean protein to reduce the risk of heart disease [\[8](#page-6-0)], or the government of China, which recommends a consumption of at least 50 g per day of soybean products [\[10](#page-6-0)]. In Europe, although a recommended intake value of soybean products has not been established at present,

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the western population associates the consumption of this type of product with healthy lifestyle habits which explains the marked increase in the consumption of these foods, which have been become an ingredient staple in diets such as the vegan diet.

Some of which, such as the macroelements (Ca, K, Na, Mg) and the trace elements (Cu, Mn, Mo, Zn, Co, Fe), are essential for the human organism as they participate in multiple enzymatic reactions and vital processes as, for example Fe that is part of haemoglobin and participates in the transport of oxygen, or Co which is present in cobalamin [11]. Recommended daily doses of these elements have been set given their vital importance in the physiological processes of the organism [12].

Therefore, and because tofu is the most popular soybean product, being a staple food in Asian, vegan and vegetarian diets, this study was conducted with the objective of determining the concentration of essential elements in various samples of organic and conventional tofu.

The aims of this study are to determine the concentration of essential elements (Na, K, Mg, Ca, Mn, Zn, Fe, Cu, Mo, Co) in samples of different types of ecological and conventional tofu from Europe and China through inductively coupled plasma atomic emission spectroscopy (ICP-OES), to evaluate the dietary intake of essential elements from the consumption of these products.

Material and Methods

Chemical reagents of analytical grade and deionised water of high purity obtained from a purification system of the trademark Milli-Q (Millipore, MA, USA) were used in the treatment of the samples, as well as in the subsequent analysis.

Samples

A total of 130 tofu samples were analysed, of which 60 samples were of tofu from organic European agriculture (20 samples of normal tofu, 20 samples of flavoured tofu and 20 samples of smoked tofu), 20 samples of conventional European tofu from Italy, 30 samples of conventional tofu from China and 20 samples of fresh homemade conventional tofu made in Chinese supermarkets on the island of Tenerife (Canary Islands, Spain) with ingredients from China.

The samples that were acquired in different commercial locations, local herb shops and Chinese supermarkets, were taken to the laboratory and stored at 4 °C. All samples were analysed before the expiry date recommended by the manufacturer.

Sample Treatment

A 10 g of each previously homogenised sample was weighed, in triplicate, in porcelain capsules (Staalich, Germany) and placed in an oven (Nabertherm, Gemany) at 70 °C for 24 h, for dehydration. Dehydration process is necessary to facilitate the acid digestion procedure.

The samples were subjected to acid digestion using 2–3 mL of 65% HNO₃ (Merck, Germany), evaporating the nitric acid by using a hot plate (Nabertherm, Germany). Subsequently, the incineration of the samples was carried out in a muffle furnace (Nabertherm, Germany) with a temperature-timeprogramme of 400 °C at 24 h with a progressive rise in temperature of 50 °C per hour, until obtaining white ashes [13, 14]. The resulting ashes were dissolved in nitric acid at 1.5% solution (HNO₃) (Merck, Germany) to a volume of 25 mL.

Method and Quality Control

The elements were determined using an inductively coupled plasma atomic emission spectrometer (ICP-OES) model ICAP 6300 Duo Thermo Scientific. This technique is accepted as the most suitable for the simultaneous analysis of elements in food due to its wide wavelength range from 166 to 847 nm, as well as its high automation and high precision [15]. The instrumental conditions of the ICP-OES were as follows: approximate RF power, 1150 W; gas flow (nebuliser gas flow, auxiliary gas flow), 0.5 L/min; injection of the sample to the flow pump, 50 rpm; stabilisation time, 0 s $[16]$.

Calibration curves were prepared every day before the sample analysis by using a multi-element stock solution Multi-Element Stds. SCP28AES of 100 mg/L of Cu, Co, Fe, Mn, Mo and Zn (SCP Science, Baie-d'Urfe, QC, Canada) and a stock solution IV-STOCK-2 of 10,000 μg/mL of Ca, Na, Mg and K (Inorganic Ventures, Christiansburg, VA, USA). These calibration curves were prepared with 1.5% nitric acid solution $(HNO₃)$ (Merck, Darmstadt, Germany).

The instrumental wavelengths (nm) of the analysed elements were the following: Ca (317.9), Co (228.6), Cu (327.3), Fe (259.9), K (769.9), Mg (279.1), Mn (257.6), Mo (202), Na (589.6) and Zn (206.2).

The instrumental quantification limits (LOQ) of the elements studied were calculated under conditions of reproducibility, such as ten times the standard deviation (SD) obtained from the analysis of 15 targets [17]; the values of the LOQs (mg/L) are as follows: Ca (1.955), Co (0.002), Cu (0.012), Fe (0.009), K (1.884), Mg (1.943), Mn (0.008), Mo (0.002), Na (3.655) and Zn (0.007).

The accuracy and precision of the analytical procedure was verified by performing quality controls based on the recovery percentage study obtained with the reference material under reproducible conditions (Table 1).

Table 1 Concentration (mean \pm SD, $n = 3$) of the reference materials and recovery study (%) of the analysed elements

The reference materials used in this study were the following: Apple Leaves (NIST SRM 1515, Gaithersburg, MD, USA), Bovine Liver (NIST SRM 1577b, Gaithersburg, MD, USA) and Oyster Tissue (NIST SRM 1566b, Gaithersburg, MD, USA). The percentages of recovery obtained were higher than 93%; furthermore, no significant differences were found between the concentrations found and those certified by the manufacturer.

Statistical Analysis

The statistical study of the data was performed using the statistical programme IBM Statistics SPSS 22.0 for Mac®.

This analysis was carried out in order to determine the existence or not of significant differences between the following factors: type of crop (organic tofu and conventional tofu), type of tofu (normal, smoked and flavoured), origin of the tofu or its ingredients (Europe and China).

The distribution of the data was studied using the Kolmogorov–Smirnov and Shapiro–Wilk tests [18, 19]. Non-parametric tests such as the Kruskal–Wallis test were used for the groups of data that did not follow a normal distribution and the Mann–Whitney U test was applied in the case of significant differences [20, 21]. The parametric ANOVA test was used for data that followed a normal distribution and the Tukey's post-hoc test was applied in the case of the existence of significant differences.

The correlation study was performed using the Excel Analysis ToolPak of Microsoft Office 365 Pro Plus for Windows™. The study of the correlations was used to determine the correlation coefficients between the essential elements analysed.

Dietary Intake Assessment Calculations

The assessment of the dietary intake has been calculated by the EDI (estimated daily intake) value, obtained as the following:

 $EDI = Metal concentration (mg/kg ww)·Mean consumption$

Once the EDI value is obtained, it calculated the percentage of contribution to the guideline value for each analysed element:

Contribution $(\%) = [EDI (mg/day)/Guideline value] \cdot 100$

Results and Discussion

Concentration of Essential Elements in Tofu

Table 2 shows the mean average concentrations obtained (mg/kg wet weight) and the standard deviation (SD) of the tofu samples according to their origin (China, Europe), according to the type of crop (ecological, conventional) and depending on the type of tofu (natural, smoked, flavoured, homemade).

The macroelement content found in tofu from Europe was higher than that found in tofu from China. It is worth mentioning that K had the highest concentration $(1420 \pm$ 481 mg/kg wet weight), followed by Mg (1040 \pm 448 mg/kg ww) > Na (884 ± 1208 mg/kg ww) > Ca (821 ± 581 mg/kg ww). In addition, the statistical analysis confirmed the existence of significant differences ($p < 0.05$) in the content of macroelements between the tofu samples from China and Europe.

In the case of the essential trace elements in which concentrations are also higher in the European tofu than in the Chinese tofu, with Fe (17.7 \pm 4.86 mg/kg ww) being the most abundant trace element, followed by Zn $(9.64 \pm 1.99 \text{ mg/kg})$ ww) > Mn $(7.06 \pm 1.59 \text{ mg/kg} \text{ ww})$ > Cu $(2.34 \pm 0.85 \text{ mg/kg})$ ww). Significant differences ($p < 0.05$) were found between the different origins in the content of all the essential trace elements studied, except for Mo and Zn, which levels did not differ statistically between both origins.

In general, the tofu from Europe has higher element content than the tofu from China; this is clearly due to the differences of each region, environment, soil, irrigation water, climate or temperature. It is necessary to consider the differences in the elaboration process. European tofu will have the greatest nutritional value, from the point of view of essential elements.

Regarding the type of crop, the concentrations of macroelements were higher in the organic tofu than in the conventional tofu. The content of K in organic tofu is noteworthy, with a mean average level of 1483 ± 466 mg/kg ww followed by Mg $(1007 \pm 461 \text{ mg/kg} \text{ ww})$ > Na $(973 \pm$ 1244 mg/kg ww $>$ Ca (813 \pm 612 mg/kg ww). Likewise, the levels of the macroelements studied were significantly different ($p < 0.05$) between both types of crops.

Once again, the mean level of the essential trace elements in the organic tofu was higher than in the conventionally grown tofu. Fe $(16.8 \pm 4.15 \text{ mg/kg} \text{ ww})$ in organically grown tofu was found to be the most abundant essential trace element, followed by Zn $(9.50 \pm 2.04 \text{ mg/kg}$ ww) > Mn $(7.14 \pm$ 1.65 mg/kg ww $>$ Cu (2.35 \pm 0.90 mg/kg ww). The statistical analysis showed significant differences ($p < 0.05$) between the two types of tofu for all the essential trace elements studied except for the Mo.

In summary, organic tofu contains higher essential element levels than conventionally grown tofu. It has been demonstrated that sustainable and organic agriculture improves the soil fertility and the productivity of the crop [22].

Depending on the type of tofu (flavoured, homemade, natural and smoked), the highest concentrations of the analysed essential elements were observed in the flavoured tofu, with Na having the highest mean level $(2519 \pm 1085 \text{ mg/kg} \text{ ww})$ followed by K $(1852 \pm 489 \text{ mg/kg} \text{ ww}) > Ca$ $(1276 \pm$ 640 mg/kg ww). The Mg level $(1135 \pm 437 \text{ mg/kg}$ ww) was the highest in the natural tofu. Regarding the different types of flavoured tofu, those containing algae had the highest

concentration of Ca (1877 \pm 159 mg/kg ww). Previous studies conducted by Paz et al. [23], concluded that edible seaweeds have high Ca levels with mean contents of 3118 mg/kg dry weight in European edible seaweeds and 2272 mg/kg dw in Asian edible seaweeds [23]. Then, seaweeds could contribute to the Ca concentration in this kind of tofu.

The Na concentration $(3117 \pm 167 \text{ mg/kg}$ ww) is worth mentioning in the tofu with quinoa. According to the USDA Food Nutrient Database, quinoa contains a mean Na level of 7 mg per 100 g of quinoa, which means a content of 70 mg/kg of quinoa [24]. The K concentration $(2029 \pm 641 \text{ mg/kg}$ ww) in the tofu with olives and Mg (1096 ± 66.1 mg/kg ww) in the tofu containing sesame is higher than the other flavoured tofu samples. Mo levels were higher in the tofu containing sesame. Sesame is a cereal that stands out for the Mo levels [25].

The statistical analysis showed that the Na content of natural tofu statistically differs ($p < 0.05$) from the Na content in the flavoured tofu.

The Fe levels found in the four types of tofu analysed (homemade, flavoured, smoked and natural) are noteworthy. The highest mean level of Fe $(19.5 \pm 4.83 \text{ mg/kg} \text{ ww})$ was found in the flavoured tofu, with the maximum levels found in tofu that contained algae $(25.6 \pm 2.77 \text{ mg Fe/kg}$ ww). This higher content of Fe is because algae are known to have high concentrations of this metal [19, 23, 26].

The other notable trace elements are $Zn (10.2 \pm 2.66 \text{ mg/kg})$ ww) which the highest level was recorded in the smoked tofu and the Mn level $(7.51 \pm 1.45 \text{ mg/kg} \text{ ww})$ in natural tofu. In a study conducted by Noël et al. [27], the Mn level (8.88 mg/kg ww) found in tofu samples, was like the level found in the present study [27]. Likewise, the content of trace elements differs significantly $(p < 0.05)$ among the four types, except for Mo.

The differences found in the essential element content of the four types of tofu studied may be due to several factors,

		Ca	Na	K	Mg	Fe	Cu	Co	Zn	Mn	Mo
Correlation coefficient $(n=130)$	Ca										
	Na	$0.177*$									
	K	0.003	$0.485*$								
	Mg	$-0.264*$	$-0.111*$	$0.170*$							
	Fe	$0.634*$	$0.120*$	$-0.086*$	$0.338*$						
	Cu	$0.264*$	$0.422*$	$0.471*$	$0.268*$	$0.358*$					
	Co	$0.669*$	$0.389*$	$0.511*$	$0.062*$	$0.444*$	$0.639*$				
	Zn	$0.069*$	-0.001	$0.244*$	$0.142*$	$0.239*$	$0.199*$	$0.151*$	1		
	Mn	$0.257*$	$-0.186*$	$0.256*$	$0.585*$	$0.419*$	$0.383*$	$0.513*$	$0.406*$	$\mathbf{1}$	
	Mo	$0.088*$	$-0.150*$	$-0.230*$	$-0.016*$	$0.319*$	$0.023*$	$-0.253*$	$0.267*$	$-0.165*$	

Table 3 Correlation coefficient between the studied essential elements in tofu

*The correlation is significant at the 0.01 level (bilateral)

such as other ingredients (cereals, algae, quinoa, additives) in the case of flavoured tofu or, for example in the case of smoked tofu, the smoking process can significantly influence the element content [28, 29], as well as the water or the coagulating agent used in its production.

The correlation study showed a positive correlation between almost the study elements (Table 3). The coefficients of correlation obtained for Ca–Co (0.669), Cu–Co (0.639) and Ca–Fe (0.643) are notable indicating a strong positive correlation. The obtained data confirm that these elements do not compete.

^a Taking a mean daily consumption of 200 g/day of tofu

^b Considering a phytate level intake of 300 mg/day

^c Considering a phytate level intake of 600 mg/day

 d Considering a phytate level intake of 900 mg/day

e Considering a phytate level intake of 1200 mg/day

However, the correlation study found negative correlation coefficients between Ca–Mg (− 0.264), Co–Mo (− 0.253), K– Mo (− 0.230), Na–Mn (− 0.186), Mn–Mo (− 0.165), Na–Mo (− 0.150), Na–Mg (− 0.111), K–Fe (− 0.086) and Mg–Mo (− 0.016). Several studies confirm interactions among the essential elements in plants. As for example, Mg uptake by soybean is inhibited when K and Ca are present in high concentrations [30, 31].

Dietary Intake Assessment

Table 4 shows the values of the EDIs calculated assuming a mean average consumption of 200 g/day of tofu, which is equivalent to one serving and, a mean average weight of 68.48 kg of an adult set by AESAN [32].

In terms of macroelements, the contribution of flavoured tofu to the adequate intakes (AI) values set by the EFSA for adult population of Na (25.2%) , Ca (34.0%) and K (10.6%) stands out, while the consumption of natural tofu offers the largest contribution of Mg (75.7% for women, 64.9% for men) to the AI, set by the EFSA [12] at 300 mg/day for women and 350 mg/day for men.

Regarding the essential trace elements, the consumption of 200 g/day of natural tofu assumes a Mn contribution percentage of 50% to the AI set by the EFSA [12] at 3 mg/day for adults. On the other hand, the consumption of smoked tofu contributes significantly to the AI of Zn set by the EFSA at 6.2 mg/day for women and 7.5 mg/day for men with percentages of 32.7% and 27.1% for women and men, respectively. The contribution percentage of the AI values of Cu (46.9% for women, 38.1% for men) and AI values of Fe (55.7% for women, 65.0% for men) from flavoured tofu consumption is worth mentioning.

Therefore, the mean average consumption of 200 g a day of tofu would provide essential elements such as Na, Mg, Mn, Cu and Fe. However, it is necessary to take into account the bioavailability and the absorption of elements such as Fe, since Fe is found in a nonheme form in plant foods, which absorption varies between 1 and 15%, whereas iron is found in a heme form in products of an animal origin, with an approximate absorption of 40% depending on the type of meat [33]. However, several studies concluded that the consumption of vitamin C, citric acid or other organic acids could increase the absorption of nonheme Fe [34, 35].

On the other hand, the Na contribution only from the consumption of tofu is noteworthy. As this macroelement is found in most foods, its AI could be exceeded. This is important because a high intake of Na is directly related to hypertension [36]. It is necessary to consider this fact in cases of hypertensive individuals.

Conclusions

The results obtained in the study show that flavoured tofu containing other ingredients, such as seaweed, sesame, quinoa and olives, have higher nutritional value. Likewise, organic tofu was found to have higher essential element contents than conventionally grown tofu. It is necessary to consider this fact, because, attending to the obtained results, the consumption of tofu from organic crops provides an essential element profile better than tofu from conventional crops.

Positive correlations were found among almost of the studied elements, showing that these elements do not interfere with each other. However, negative correlations were found between Ca–Mg, Co–Mo, K–Mo, Na–Mn, Mn–Mo, Na–Mo, Na–Mg, K–Fe and Mg–Mo. These negative correlations show a possible interference among these elements.

In general, the consumption of 200 g/day of tofu provides a notable contribution of essential elements such as Na, Mg, Mn, Cu and Fe to their AI values. The consumption of flavoured tofu contributes greatly to cover the recommended intake to almost the analysed elements because this kind of tofu contains other ingredients (seaweeds, quinoa, carrots etc.) that improve the tofu nutritional profile. However, it is necessary to control the intake of Na from the consumption of tofu, since this element is present in all foods, as there is a risk of hypertension in cases of prolonged consumption of tofu.

Funding Information This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Compliance with Ethical Standards

Conflict of Interests The authors declare that they do not have any conflict of interest.

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