Human Exposure Assessment to Potentially Toxic Elements (PTEs) from Tofu Consumption

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Abstract

Potentially toxic elements (PTEs) (V, B, Ba, Li, Sr, Cr, Ni, Al, Pb, Cd) were determined in 130 samples of different tofu types (natural, flavoured, smoked and fresh made) by ICP-OES (inductively coupled plasma optical emission spectrometry). Al was the most notable element found with the highest concentration (6.71 mg/kg ww) found in flavoured tofu. Ni level (0.38 mg/kg) stands out in smoked tofu. European tofu has higher PTEs levels than Chinese tofu. Organic produced tofu has higher PTEs concentrations than conventional produced tofu. 200 g/day of smoked tofu confers a contribution percentage of 39.6% of its TDI (tolerable daily intake). In addition, 200 g/day of flavoured tofu would mean a high Pb contribution with a 23.2% of the BMDL (benchmark dose level) set in 0.63 µg/kg bw/day to the development of nephrotoxicity. Mean consumption would not pose a risk to adults’ health. Considering the obtained results, it would be advisable to establish limits for certain metals such as Pb, Al and Ni in this type of product. Furthermore, it is recommendable to set consumer guidelines to some tofu types in order to avoid excessive intake of PTEs.

Chemical Compounds

Nitric acid (PubChem: CID 944)

1. Introduction

One the most important food in the diet of the Asian countries is the tofu. Tofu is a product made by the fermentation of soybeans. The nutritional composition of tofu is remarkably, for that reason, tofu has increasing its consumption in Western countries like USA and European countries.

Tofu is an important source of proteins, lipids, vitamins, amino acids and minerals (Serrazanetti et al., 2013; Xu et al., 2015) and, as a result, tofu is a key product in the vegetarians and vegans diets, where is used as a meat products substitute (Fang et al., 2005; Serrazanetti et al., 2013; Meng et al., 2016).

The diet is the main route of human exposure to pollutants from natural and anthropogenic sources (Bocio et al., 2005; Di Bella et al., 2020), especially due to the use of fertilizers and industrial activity. Potentially toxic elements (PTEs) such as aluminium (Al), cadmium (Cd) and lead (Pb) tend to accumulate, do not have any function in the human body and are harmful to health (Marini et al., 2021).

Al is a known neurotoxic agent that tends to accumulate mostly in the brain and relationships between high concentrations of this metal in the brain and neurodegenerative diseases such as Alzheimer’s disease have been reported (Arvand and Kermanian, 2012; Hardisson et al., 2017). Pb is one of the most well-known contaminants and, is another neurotoxic metal that causes serious damage to the central nervous system (Rubio et al., 2005; Nordberg et al., 2007). Cd is a toxic and bioaccumulative metal characterized by a long half-life (Barbier et al., 2005; Kumar and Sharma, 2019), and Cd competes in the organism with other essential elements such as Zn, Fe or Cu causing serious damage to the renal system (Rubio et al., 2017b). Cd levels are high in plant products such as tofu and diet is the main Cd exposure for non-smoker population (Kosečková et al., 2020).
Other PTEs such as B, Ba, Ni, Li, Sr, or V, are found naturally in the environment and, therefore, in food. In addition, these elements are mainly found in cereals, vegetables, and legumes. They do not have a known function in the human organism, but they are essential for other animal and plant organisms (IOM 2001; WHO 2010; SCHER 2012; González-Weller et al. 2015; Rubio et al. 2017b). Although they are usually found at trace levels, anthropogenic activities can increase their concentrations which would lead to different toxic effects. Although food poisoning due to the ingestion of these PTEs has not been described, it is necessary to consider their harmful effects on health.

Studies carried out with experimental animals show that B affects development, and the reproductive system and V can cause kidney damage (IOM, 2001). Ba is an element that acts as an antagonist of K and Ca and tends to accumulate in bones (Kato et al., 2020). Ni is an element that can cause dermatitis, especially harmful in individuals with hypersensitivity or kidney problems (Das et al., 2018). Sr is an element that can cause P deficiency, and, in addition, it accumulates in the bones (Pathak and Gupta 2020). High Li intakes could lead to kidney damage and blindness (Domínguez-Ortega et al., 2006).

Soybeans, a key ingredient in tofu, can accumulate potentially toxic elements from environmental pollution, irrigated water, pesticides, fertilizers, and contaminated soils (Tóth et al., 2016; Gu et al., 2016; Shaheen et al., 2016). Therefore, it is necessary to determine the content of PTEs in this type of products and, if were necessary, to set some recommendations to avoid the risks.

Considering the growing consumption of tofu in Western countries, the great absorption capacity of soybean plants and the possible presence of high PTEs levels in derivatives such as tofu, this study has been conducted with the objective of determining the concentration of potentially toxic elements (PTEs) (V, B, Ba, Li, Sr, Cr, Ni, Al, Pb, Cd) in different tofu types (European vs Chinese, organic vs conventional, types) by inductively coupled plasma atomic emission spectroscopy (ICP-OES) to assess its toxic risk.

2. Material And Methods

During the samples treatment and the analysis were used chemical reagents of analytical grade and deionized water of high purity obtained from a purification system of the trademark Mili-Q (Milipore, MA, USA) (Paz et al., 2020).

2.1. Samples

A total of 130 tofu samples were analysed. The samples were acquired in local organic food shops, hypermarkets, and Chinese superstores. The samples were stored at 4ºC in a conventional fridge. The characteristics of the analysed samples are in the Table 1.
Table 1
Characteristics of the analysed samples

| Type         | Origin | Production | No |
|--------------|--------|------------|----|
| Natural      | EU     | Organic    | 20 |
| Flavoured    | EU     | Organic    | 20 |
| Smoked       | EU     | Organic    | 20 |
| Natural      | EU     | Conventional | 20 |
| Natural      | China  | Conventional | 30 |
| Fresh homemade | China  | Conventional | 20 |

*Fresh homemade conventional tofu made in Chinese supermarkets with ingredients from China.

2.2. Sample treatment

10 g of each previously homogenized sample were weighed in porcelain crucibles (Königliche, Germany). The samples were placed in an oven (Nabertherm, Germany) for dehydration at 70°C – 24 h. Then, the samples were subjected to acid digestion with 65% nitric acid (HNO₃) (Merck, Germany). Subsequently, the samples were subjected to incineration in a muffle furnace (Nabertherm, Germany) with a temperature – time program of 400°C – 24 h, with a progressive rise 50°C per hour (Hardisson et al. 2001; Gutiérrez et al., 2008; Paz et al., 2020). The ashes were dissolved in nitric acid (HNO₃) (Merck, Germany) at 1.5% to a volume of 25 mL.

2.3. Method and quality control

The PTE content was determined using an inductively coupled plasma atomic emission spectrometer (ICP-OES) model ICAP 6300 Duo Thermo Scientific. ICP-OES is a technique commonly used in the determination of trace and toxic elements (Chaves et al., 2010; Karasakal, 2020). The ICP-OES instrumental conditions were: RF power, 1150 W; gas flow (nebulizer gas flow, auxiliary gas flow), 0.5 L/min; injection of the sample to the flow pump, 50 rpm; stabilization time, 0 s (Rubio et al. 2018; Paz et al. 2020). The instrumental wavelengths (nm) of the analysed PTEs were Al (167), B (249.7), Ba (455.4), Cd (226.5), Cr (267.7), Li (670.8), Ni (231.6), Pb (220.3), Sr (407.7), V (310.2).

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 Al, B, Ba, Cd, Ni, Li, Pb, Sr and V (SCP Science, Baie-D’Urfe, QC, Canada). The calibration curve was prepared using a solution of 1.5% of nitric acid (HNO₃) (Merck, Darmstadt, Germany).

The LOQ (limits of quantifications) of the PTEs were calculated, under conditions of reproducibility, such as ten times the standard deviation (SD) obtained from the analysis of 15 targets (IUPAC, 1995), the values of the LOQs (mg/L) were Al (0.012), B (0.012), Ba (0.005), Cd (0.001), Li (0.013), Ni (0.003), Pb (0.001), Sr (0.003), V (0.005).
The analytical procedure accuracy and precision was verified by performing quality controls based on the recovery percentage study obtained with the reference material under reproducible conditions (Table 2). The reference materials used in this study were Tomato Leaves (NIST SRM 1573a, 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.

Table 2
Recovery percentage found for the reference materials used

| Reference material                  | Metal | Certified C. (mg/kg) | Obtained C. (mg/kg) | R (%) |
|------------------------------------|-------|----------------------|---------------------|-------|
| SRM 1577b Bovine Liver             | Sr    | 0.136 ± 0.001        | 0.138 ± 0.002       | 101.3 |
|                                    | Al    | 3                    | 3.1 ± 0.12          | 103.7 |
|                                    | Pb    | 0.129 ± 0.004        | 0.122               | 94.4  |
|                                    | Cd    | 0.5 ± 0.03           | 0.47                | 93.1  |
| SRM 1566b Oyster Tissue            | B     | 4.5 ± 1.9            | 4.35 ± 1.2          | 96.7  |
|                                    | Ba    | 8.6 ± 0.3            | 8.15 ± 0.9          | 94.8  |
|                                    | Ni    | 1.04 ± 0.09          | 0.99 ± 0.12         | 95.3  |
|                                    | V     | 0.5389 ± 0.09        | 0.577               | 93.4  |
| Method of standard addition        | Li    | 1.81                 | 1.804 ± 0.087       | 99.7  |
| SRM 1573a Tomato Leaves            | Cr    | 1.95 ± 0.09          | 1.99                | 98.1  |

2.4. Statistical analysis

The statistical study of the data was performed using the statistical program IBM Statistics SPSS 22.0 for Mac®. The aim of the statistical analysis is to determine the significative differences (p < 0.05) considering the different factors: production (conventional vs organic), type (natural, smoked and flavoured), origin (Europe vs China).

The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to study the data distribution (Rubio et al., 2017b). Then, the Kruskal-Wallis non-parametric test was applied to data that did not follow a normal distribution. In case of significative differences, was applied the Mann-Whitney U test (Gutiérrez et al., 2008; Paz et al., 2020). The parametric ANOVA test was used for data that followed a normal distribution and the Tukey Post Hoc test was applied in the case of the existence of significant differences.

A correlation study was carried out to detect positive or negative correlation between the PTEs analysed.

2.5. Risk assessment calculation
The risk assessment was conducted by the calculation of the estimated daily intake (EDI). The EDI is the quantity of potentially toxic elements ingested with a serving of tofu.

\[ \text{EDI (mg/day)} = \text{PTE concentration (mg/kg)} \times \text{Tofu consumption (kg/day)} \]

Then, the contribution percentages (%) to the guideline values were obtained by the following expression:

\[ \text{Contribution (\%)} = \left( \frac{\text{EDI}}{\text{Guideline values}} \right) \times 100 \]

### 3. Results And Discussion

#### 3.1. Concentration of metals in tofu

Table 3 shows the mean average concentrations obtained (mg/kg wet weight) and the standard deviation (SD) of the tofu samples by its origin (China vs Europe), production type (conventional vs organic) and by tofu type (natural, smoked, flavoured, homemade).

| Origin     | Type     | Culture |
|------------|----------|---------|
|            | China    | Europe  | Homemade | Flavoured | Smoked | Natural | Organic | Conventional |
| Al         | 1.52 ± 1.14 | 3.56 ± 3.47 | 1.54 ± 1.09 | 6.71 ± 5.65 | 2.09 ± 1.81 | 2.96 ± 1.48 | 3.52 ± 3.65 | 2.00 ± 1.42 |
| Cd         | 0.01 ± 0.01 | 0.01 ± 0.00 | 0.01 ± 0.01 | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.01 ± 0.01 |
| Pb         | 0.01 ± 0.00 | 0.03 ± 0.01 | 0.01 ± 0.00 | 0.04 ± 0.02 | 0.02 ± 0.00 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01 |
| B          | 0.61 ± 0.21 | 1.07 ± 0.33 | 0.62 ± 0.22 | 1.17 ± 0.44 | 0.92 ± 0.19 | 1.11 ± 0.32 | 1.08 ± 0.35 | 0.70 ± 0.26 |
| Ba         | 0.50 ± 0.25 | 1.24 ± 0.59 | 0.51 ± 0.26 | 1.44 ± 0.67 | 0.79 ± 0.29 | 1.39 ± 0.55 | 1.24 ± 0.62 | 0.64 ± 0.36 |
| Li         | 0.26 ± 0.20 | 0.33 ± 0.25 | 0.26 ± 0.19 | 0.48 ± 0.26 | 0.30 ± 0.26 | 0.27 ± 0.20 | 0.35 ± 0.25 | 0.24 ± 0.19 |
| Ni         | 0.18 ± 0.15 | 0.34 ± 0.20 | 0.18 ± 0.15 | 0.31 ± 0.12 | 0.38 ± 0.23 | 0.33 ± 0.20 | 0.36 ± 0.20 | 0.18 ± 0.13 |
| Sr         | 1.38 ± 0.92 | 1.95 ± 1.22 | 1.43 ± 0.97 | 1.52 ± 1.69 | 2.26 ± 0.80 | 1.97 ± 1.12 | 1.92 ± 1.29 | 1.56 ± 0.89 |
| V          | <LOQ*     | 0.03 ± 0.09 | <LOQ*     | 0.05 ± 0.05 | 0.01 ± 0.03 | 0.03 ± 0.12 | 0.03 ± 0.10 | <LOQ* |

*LOQ of V (0.005 mg/L).

#### 3.1.1. PTEs concentration by origin (EU vs China)
The concentration of PTEs was higher in European tofu than Chinese tofu. The most remarkable PTEs found in EU tofu were Al (3.56 mg/kg ww), Sr (1.95 mg/kg ww), Ba (1.24 mg/kg ww) and B (1.07 mg/kg ww). In addition, Pb (0.003 mg/kg ww) level was higher in the tofu from Europe than in the Chinese tofu. The statistical analysis shown significant differences between both origins for all the potentially toxic elements studied, except for Li.

In summary, the European tofu content high PTEs levels than the tofu from China. The differences found between both origins are due to several factors like environment, soil, irrigation water, etc. This fact is not positive, because European countries have more legal control over the pollution than China, which is an industrialized country. So, it is necessary to establish maximum limits for these metals, especially Al, Pb and Ni in tofu.

### 3.1.2. PTEs concentration by production type (conventional vs organic)

According to the type of production of the tofu, the PTEs levels found in the organic tofu were higher than the conventional tofu. The levels of Al (3.52 mg/kg ww), Sr (1.92 mg/kg ww), Ba (1.24 mg/kg ww), and B (1.08 mg/kg ww) were notable. Significant differences were also detected between the two types for all the trace metals studied, except Li.

Organic produced tofu has a stronger metal profile than conventionally produced tofu. This is a disadvantage because organic products should have lower levels of pollutants like PTEs than conventional products. Although, organic products are subject to lower amounts of pesticides and/or chemical agents, it is necessary to bear in mind other factors such as soil, environment, irrigation water, tofu water, etc. (Hornick, 2009). Normally, the control under organic products is focused on the levels of pesticides, but, considering the results obtained in the present study, it is necessary to consider other factors that could increase the levels of pollutants like PTEs.

### 3.1.3. PTEs concentration by type of tofu (natural, flavoured, smoked, homemade)

Al stands out in the four types studied and the highest mean Al level (6.71 mg/kg ww) was found in the flavoured tofu. The Pb level (0.04 mg/kg ww) is also noteworthy in the flavoured tofu, which was the highest in all the types of tofu. Likewise, the tofu containing algae have the highest concentrations of Al, Cd and Pb. This is also due to the high metal absorption capacity of algae (Khan et al., 2015; Rubio et al., 2017b). In addition, the content of Al and Pb found in the flavoured tofu differs statistically from the content found in the other three types.

Is remarkably the Sr content found in the four types of tofu studied and it is worth mentioning the level of Sr (2.26 mg/kg ww) found in the smoked tofu. On the other hand, the level of Ni (0.38 mg/kg ww) found in smoked tofu is also noteworthy, which could be due to the wood used in the smoking process of this type of tofu, which, according to the manufacturer, is beech and oak wood. Studies have shown that beech and oak trees can accumulate metals from the absorption of these metals through the soil (Queirolo et al. 1990; Pastircakova 2004), which, when the wood is heated, could pass to the tofu.
After performing the statistical analysis, significant differences were detected in the Li content found in the flavoured tofu, which differ significantly from the other three types (homemade, smoked, and natural). Besides which, the level of Ni is statistically significant between the homemade tofu and the other types, with the lowest Ni content (0.18 mg/kg ww) being found in the flavoured tofu.

The differences found in the metal content of the four types of tofu studied may be due to several factors, such as other ingredients (cereals, algae, additives, etc.) in the case of flavoured tofu or, for example, in the case of smoked tofu, the smoking process can significantly influence the metal content, as well as the water or the coagulating agent used in its production etc.

3.2. Correlations between PTEs

Table 4 shows the correlation values between the studied PTEs. The correlations study have revealed some negative and positive correlations between almost of the PTEs analysed. The strong negatives correlations were found between Cd-Ni with a Spearman's Rho correlation of -0.531, that is interesting because it shows a possible competition among Cd and Ni. This correlation value means that when Cd levels arise the Ni levels decrease and vice versa.

|     | Al  | B   | Ba  | Cd  | Li  | Ni  | Pb  | Sr  | V   |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Al  | 1   |     |     |     |     |     |     |     |     |
| B   | 0.459* | 1   |     |     |     |     |     |     |     |
| Ba  | 0.329* | 0.441* | 1   |     |     |     |     |     |     |
| Cd  | 0.147* | -0.145* | -0.194* | 1   |     |     |     |     |     |
| Li  | 0.218* | 0.078* | 0.039* | 0.011* | 1   |     |     |     |     |
| Ni  | -0.291* | 0.149* | 0.291* | -0.531* | 0.005 | 1   |     |     |     |
| Pb  | 0.884* | 0.574* | 0.557* | -0.019* | 0.237* | -0.061* | 1   |     |     |
| Sr  | -0.222* | 0.103* | -0.065* | 0.212* | -0.107* | 0.185* | -0.144* | 1   |     |
| V   | -0.008 | 0.020* | 0.218* | -0.171* | 0.004 | 0.170* | 0.033* | -0.048* | 1   |

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

According to the positive correlations, it is remarkably the Spearman's Rho correlation found between Al-Pb (0.884). This value, near to 1, shows that both elements does not compete and when Al levels increasing, the Pb levels too.

3.3. Human exposure assessment

Table 5 shows the guide values of the maximum admissible intakes set by different institutions. The estimated daily intakes (EDIs) were calculated considering a mean consumption of 200 g of tofu daily, which is equivalent to one portion. The average weight of an adult was 68.48 kg (AESAN, 2006) (Table 6).
Table 5
Maximum reference intake values set by different institutions

| Parameter | Value               | Reference     |
|-----------|---------------------|---------------|
| Al        | TWI 1 mg/kg bw/week | EFSA, 2011a   |
| Cd        | 2.5 µg/kg bw/week   | EFSA, 2011b   |
| Sr        | TDI 0.13 mg/kg bw/day | WHO, 2010  |
| Ba        | 200 µg/kg bw/day    | SCHER, 2012   |
| Ni        | 2.8 µg/kg bw/day    | EFSA, 2015    |
| Pb        | BMDL<sub>Nephrotoxicity</sub> 0.63 µg/kg bw/day | EFSA, 2011 |
| Pb        | BMDL<sub>Cardiovascular</sub> 1.50 µg/kg bw/day |    |
| B         | UL 17–20 mg/day     | IOM, 2001     |
| V         | 1.8 mg/day          |               |

TWI, tolerable weekly intake; TDI, tolerable daily intake; UL, upper level intake; BMDL, benchmark dose level

Table 6
Estimated daily intakes (EDI) and percentages of contribution to the reference values set by different institutions

| EDI (mg/day)          | Contribution (%) |
|-----------------------|-------------------|
|                       | Homemade | Flavoured | Smoked | Natural | Homemade | Flavoured | Smoked | Natural |
| Al                    | 0.31     | 1.34      | 0.42   | 0.59    | 3.16     | 13.7      | 4.27   | 6.10    |
| Cd                    | 0.003    | 0.002     | 0.002  | 0.002   | 11.0     | 8.07      | 9.26   | 8.10    |
| Pb*                   | 0.002    | 0.01      | 0.004  | 0.01    | 4.64     | 23.2      | 9.28   | 23.2    |
| Pb**                  | 0.002    | 0.01      | 0.004  | 0.01    | 1.95     | 9.74      | 3.90   | 9.74    |
| B                     | 0.12     | 0.23      | 0.18   | 0.22    | 7.28     | 13.8      | 10.8   | 13.1    |
| Ba                    | 0.10     | 0.29      | 0.16   | 0.28    | 0.75     | 2.11      | 1.15   | 2.03    |
| Li                    | 0.05     | 0.10      | 0.06   | 0.05    | -        |           |        |         |
| Ni                    | 0.04     | 0.06      | 0.08   | 0.07    | 18.7     | 32.4      | 39.6   | 34.2    |
| Sr                    | 0.29     | 0.30      | 0.45   | 0.39    | 3.22     | 3.41      | 5.07   | 4.43    |
| V                     | 0.001    | 0.01      | 0.002  | 0.01    | 0.05     | 0.52      | 0.10   | 0.35    |

*BMDL to nephrotoxicity **BMDL to cardiotoxicity

The Ni contribution (39.6%) from the consumption of smoked tofu stands out, this contribution percentage to the TDI of Ni (2.8 µg Ni/kg bw/day) can pose a particular risk for people with sensitivity to this element (EFSA,
The TDI of Ni would be overpassed in cases of 3 portions of smoked tofu (600 g/day).

In addition, the percentage of contribution of Pb from the consumption of flavoured tofu is noteworthy, with it a 23.2% of the BMDL (benchmark dose level) set in 0.63 µg/kg bw/day to the development of nephrotoxicity (EFSA 2011). It is necessary to consider this contribution percentage, because the total intake of Pb from the whole diet could contribute to the 100% of the BMDL. However, a study conducted by Chen et al. (2001) a negative correlation between tofu consumption and blood Pb levels in young Chinese adults was found.

The percentage of contribution of Al (13.7%) also stands out in flavoured tofu. It should be due to the presence of some food additives (Ogimoto et al., 2016). However, the absorption of Al through the digestive system is not elevated (Oliveira et al., 2017), it is necessary to consider this contribution percentage. An alternation between flavoured and other tofu types should be recommended in order to avoid an elevate Al intake.

The Cd contribution percentage from homemade tofu is 11.0% of its TWI set in 2.5 µg/kg bw/week. Cd is an element that should be monitored and controlled in tofu and other soybeans products. A study conducted by Adams et al. (2011) concluded that tofu consumption is one of the specific foods that is associated with the urine Cd concentration. Considering that cigarette smoking is one of the major Cd source, the consumption of tofu by smokers could be a severe risk for the health.

However, considering the consumption of 200 grams of tofu per day, the intake of PTEs would not pose a risk to the health of adults. Nevertheless, is necessary to develop more studies about the PTEs in tofu and other soybean products to set limits, in special, to Ni, Al, Cd and Pb levels, to avoid risks to the consumers. In addition, is necessary to set consumer guidelines to some tofu types to avoid excessive intake of PTEs.

### 3.4. Recommendations for tofu consumers

The recommendations about the tofu consumption depends on different factors like type of diet (omnivorous, vegetarian or vegan), life styles (smokers or non-smokers), physiological factors (age, gender, illness, etc). However, considering the adult population, general recommendations could be done.

Figure 1 shows a proposal of recommendation guidelines for tofu consumers to avoid a overexposure to PTEs. The recommendation guidelines proposed for tofu are according to the PTEs levels found in the present study. As previously commented, the risks of tofu consumption are due to Ni, Pb, Al and Cd levels recorded.

Two different scenarios were proposed, 1–4 servings per week which means between 200–800 g per week, and more than 4 serving per week that means more than 800 g per week.

In cases of omnivorous diet, the consumption of 1–4 servings of tofu per week does not represent a health risk because in the limit case of 4 servings per week, it means a 800 g/week of tofu or 114 g/day of tofu. The contribution percentages of PTEs like Ni (22.7%), Pb (10.6%), Al (7.83%) and Cd (4.67%) does not exceed the limit.

However, in the case of the same consumption (800 g/week) by a vegetarian or vegan diet, the total diet intake of Ni, Pb, Al or Cd could overpassed the limit intake value. A vegetarian and/or vegan person normally
consumes high amounts of cereals, vegetables, soybean drinks, edible seaweeds, etc. This foods contain high PTEs. As previously published by Krajčovičová-Kudláčková et al. (2006) found that vegan group have the high Cd levels in blood. The recommendations to the vegetarian and vegan people is to vary among the different tofu types and reduce the consumption of other soybean product like soybean drinks, etc.

In cases of omnivorous diet, when the consumption of tofu is higher than 800 g/week a reduction of cereals and, specially, rice, should be advisable. In addition, it is recommendable to reduce the consumption of smoked and flavoured tofu, and consequently, a reduction of marine food products could be necessary. The same scenario of consumption but by vegan or vegetarian population could represent a high risk to the health, and consequently it is necessary to avoid the consumption of flavoured and smoked tofu, reduce the tofu consumption, avoid the consumption of other soybean products and avoid the consumption of edible seaweeds, which have high Cd, Pb and Al levels. In the case of smokers, the Cd levels from tofu consumption with the Cd levels from cigarettes, should represent an extreme risk for the consumers health.

3.5. Future perspectives

Food production systems contribute to the environmental pollution and climate change. As a future perspectives of several countries is the reduction of the environmental impact and, as a part of this policies, the food production is a key factor. The greenhouse gas emissions and the energy demand are the best way to classified the different food production systems.

Tofu is a staple food in Asian countries, but in the last decades, the consumption of tofu by Western populations is high. This food have beneficial and nutritional properties. About the future perspectives of tofu in Western countries, it is necessary to consider that tofu is not only a valuable food from the nutritional point of view, tofu could be a sustainable food.

Heller et al. (2018) concluded that the U.S. consumption of meat and animal milk are the main producers of greenhouse gas emissions. Hendrie et al. (2016) concluded that the consumption of core foods like vegetables, dairy and grains, provide a nutritional benefit and are more environmental friendly than other food types. A study conducted by Mejía et al. (2017) evaluated the greenhouse gas emissions generated by the tofu production. The results obtained by this authors concluded that the emissions of greenhouse gas emissions from tofu production were lower than other food products.

4. Conclusions

The results obtained in the study show that flavoured tofu that containing other ingredients, such as seaweed, sesame, quinoa, and olives, have higher PTEs than the other tofu types. Except for Ni, whose highest level was found in smoked tofu. Likewise, organic produced tofu was found to have a higher PTEs concentrations than conventional produced tofu.

The correlation study revealed a strong negative correlations between Cd-Ni, it shows a possible competition among both elements because when Cd levels arise, the Ni levels decrease. The strong positive correlations found were between Al-Pb, Pb-Ba and Pb-B, that's interesting because it shows that when Pb levels arise, the Al, Ba and B levels decrease.
The maximum limits are not exceeded, although, in the case of Ni, there is a contribution percentage of 39.6% of the TDI of this metal from the consumption of smoked tofu, and this percentage should be borne in mind because individuals with sensitivity to Ni can be affected. Just 600 g/day (3 portions) of smoked tofu contributes more than the 100% of the TDI. The Pb contribution percentage from the flavoured tofu consumption with a 23.2% of the BMDL (benchmark dose level) set in 0.63 µg/kg bw/day to the development of nephrotoxicity. It is necessary to consider this contribution percentage, because the total intake of Pb from the whole diet could contribute to the 100% of the BMDL. The percentage of contribution of Al (13.7%) also stands out in flavoured tofu. The Cd contribution percentage from homemade tofu is 11.0% of its TWI set in 2.5 µg/kg bw/week. Considering that cigarette smoking is one of the major Cd source, the consumption of tofu by smokers should be controlled to avoid a health damage.

Considering the obtained results, it would be advisable to establish limits for certain metals such as Pb, Al, Cd and Ni in this type of product. Furthermore, it is recommendable to set consumer guidelines to some tofu types in order to avoid excessive intake of PTEs.

**Declarations**

**Conflict of interests**

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

**Ethical Approval**

Not applicable.

**Consent to Participate**

Not applicable.

**Consent to Publish**

Not applicable.

**Authors Contributions**

All authors contributed to the study conception. Design of the study was performed by CR and AH. Material preparation, data collection and analysis were performed by SP, ÁJG and DGW. The first draft of the manuscript was written by SP and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Competing Interests**
The authors declare that they have no conflicts of interest.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article

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