Assessment of some Heavy Metals in Potato and Corn Chips available in Libyan Market

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Abstract

The current research was carried out to evaluate some heavy metal levels in potato and corn chips which purchased from Benghazi markets - Libya. The worldwide consuming of chips by children and young people around the world was the reason for performing the study. The collected samples were analyzed by Atomic Absorption Spectrophotometer (AAS) after wet digestion. Five metals; chromium (Cr), cadmium (Cd), manganese (Mn), zinc (Zn), and Lead (Pb), in twelve samples were evaluated. The heavy metal contents of the present study were compared with those reported in the literature. The concentration range of heavy metals in potato chips was found as follows: (2.325-6.950 mg/kg), (NE-0.722 mg/kg), (0.405-4.985 mg/kg), (1.580-2.545 mg/kg), and (2.325-6.950 mg/kg), for Cr, Cd, Mn, Pb, and Zn, respectively, whereas that in corn chips was (NE-0.716 mg/kg), (NE-0.421 mg/kg), (0.043-4.013 mg/kg), (0.450-2.900 mg/kg), and (1.325-7.500 mg/kg), for Cr, Cd, Mn, Pb, and Zn, respectively. Mostly, the results revealed that the potato chips were containing heavy metal levels higher than the corn chips except for Zn contents. The trend of metal levels for potato chips was: Zn>Mn~Cr>Pb~Cd, whereas for corn chips was: Zn>Pb>Mn>Cr~Cd. The levels of the selected heavy metals in the foods were comparable with data reported in studies carried out in other countries. The consumption of snacks with such metal loads over a long period of time may result in metal accumulation in the body beyond tolerable limit.

Keywords: Corn chips; Potato chips, Heavy metals; FAAS.

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1. Introduction

Artificial materials included in food as additives or which exist in foods unintentionally is a polluted soil exhibit a toxicity importance for the consumer [1,2]. Harvest cultivated for consuming on soil which has been polluted can likely lead to the existence of such contaminants, amongst which involves heavy metals, in the cultivated crop posing a health hazard to the consumer [3,4]. For the protection of consumers, accurate data on primary food constitution are subsequently imperative and also for convenient planning by health professionals in the evaluation of the sufficiency of nutrient intakes and assessment of toxic unnecessary components of food [5,6].

Snacks are small, packaged, ready-to-eat quick foods that are typically consumed as a stopgap rather than a normal meal. Snacks can be consumed to stave off hunger or to satisfy a craving. Snacks are typically ready-to-eating foods that are designed to be portable, quick, and filling. These foods, on the other hand, may contain both essential and harmful metals in varying amounts [7,8]. Countries all over the world have established a dietary guideline that calls for a range of foods to be included in the diet in order to achieve nutrient adequacy. For accurate food intake data, heavy metals in nutrition must be known, either for their nutritional worth or for their hazardous character. Metal contaminants include Pb, Cd, Zn, Hg, Mg, Mn, Cu, and Co. Iron, zinc, copper, chromium, cobalt, and manganese are classified as essential elements needed, and when present in the body above certain concentrations, they can become harmful, causing a variety of health conditions, whereas metals such as Pb and Cd have no known biological functions and may exhibit toxicological problems even at low or trace concentrations [6,9]. Toxic metals are well-known to be one of the oldest environmental issues. Pollution in the air, soil, and water all contribute to the presence of these harmful elements in food. Rapid industrialization, advances in agricultural chemical use, and urban activities have all resulted in metal pollution of the environment [10].

Potato and corn chips are two of the world's most popular foods. It serves as a carbohydrate source, a nutritionally complete protein source, and a source of essential nutrients [11]. A corn chip is a snack made from corn that has been fried or baked in a noodle or scoop shape. Corn chips are firm, crunchy, and thick. It has a strong aroma and flavor of roasted corn and is heavily salted. Corn chips can be eaten on their own or with a chip dip. Heavy metals play important roles in both positive and negative aspects of human life [12-18]. Potato and corn chips are now popular all over the world, particularly among young adults and children. In fact, they are consumed because they are readily available in markets and are easy for children to eat or swallow. However, their chemical risk has not yet been thoroughly investigated. Few studies have been conducted to assess the heavy metal content of various types of potato and corn chips [7].
Corn and potato chips are a well-known snack that is enjoyed by people of all ages. They are considered unhealthy due to their high heavy content. There are numerous corn chips products on the Libyan market. As a result, it is a significant food item on the market. Prices and nutritional values, on the other hand, can vary significantly. The study's goal was to evaluate the quality of corn and potato chips available on the Libyan market. Twelve corn and potato chips products offered by various producers for many food companies from various countries were chosen for this purpose. Chromium, cadmium, manganese, lead, and zinc ions were measured in samples taken from various production batches. The obtained results are then compared to other studies' results.

2. Materials and methods

2.1. Chemicals and Reagents

All reagents were of analytical grade. Deionized water was used throughout the study. Glassware and sampling bottles were cleaned by soaking in 10% (v/v) nitric acid and rinsed with deionized water and dried prior using. Nitric acid (65%) was purchased from Riedel-De Haën AG. Hydrogen peroxide (30%) was purchased from Merck India Ltd.

2.2. Sample collection

Twelve samples of both potato and corn chips were obtained, which were manufactured by more than one country with a variety of flavors, they are listed in the (Table 1). They are taken out of its bags, sorted and numbered, then dried and each type of it was stored in plastic containers and give them symbols from A to L. At this stage, the samples were placed in an oven for an hour at 60°C until complete dryness. After drying the samples, 2g were weighed from each sample using an analytical balance.

2.3. Analysis

Heavy metals (Cr, Cu, Mn, Pb, Zn and Cd) were determined using Atomic Absorption spectrophotometer [13,14] (Analytic Jena AA 300). The stock solution of 1000 ppm concentration for Cr, Cu, Mn, Pb, Zn, and Cd were purchased from Merck (Germany) and were used to prepare the working standard solutions (2.0-10 ppm Cr, 2.0-10 ppm Cu, 2.0-20.0 ppm Mn, 5.0-20.0 ppm Pb, 1.0-5.0 ppm Zn and 1.0-5.0 ppm Cd) in 1% HNO₃. The stock solutions were stored in plastic bottles and labeled appropriately. Working standards were freshly prepared from stock solutions by serial dilution. Calibration curves for each metal were prepared by determining the absorbance of various concentrations.
Table 1. Corn and potato chips samples collected from Benghazi markets.

<table>
<thead>
<tr>
<th>No.</th>
<th>Symbol</th>
<th>Corn chips</th>
<th>Country</th>
<th>Branded and flavored</th>
<th>Potato chips</th>
<th>Country</th>
<th>Branded and flavored</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>Egypt</td>
<td>Egypt</td>
<td>Lays—Cheese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>Egypt</td>
<td>Egypt</td>
<td>Fox-Cheese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>Libya</td>
<td>Egypt</td>
<td>Chipsy-Salt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>Libya</td>
<td>Egypt</td>
<td>Tiger-Cheese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>Libya</td>
<td>Poland</td>
<td>Pringles-Salt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>Jordan</td>
<td>Poland</td>
<td>Pringles-BBQ-Salt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>G</td>
<td>Jordan</td>
<td>Jordan</td>
<td>Snack Mix-Salt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>H</td>
<td>Turkey</td>
<td>France</td>
<td>Belin-Cheese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>I</td>
<td>Turkey</td>
<td>France</td>
<td>Crips the world-Cheese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>J</td>
<td>Malta</td>
<td>Turkey</td>
<td>Ciptos-Cheese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>K</td>
<td>Lebanon</td>
<td>Germany</td>
<td>Pomsticks-Salt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>L</td>
<td>Morocco</td>
<td>Germany</td>
<td>Chipsletten-Salt</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.4. Sample Digestion

The samples of chips were first digested with chemicals, which destroyed the organic matrix and left the element in a clear solution. In this study, the Wet Digestion method was used. 2.00 g of dried sample was treated with 15 mL (concentrated nitric acid) and 5 mL (30% hydrogen peroxide) and digested on an electric hot plate at 90°C, and the temperature of this mixture was gradually increased to 120°C until brown fumes appeared, indicating completion of organic matter oxidation. After destroying the organic matrix of the sample and leaving the metals in clear solution, the digested solution was filtered into a 100 mL volumetric flask and completed to the mark with double distillated water, and a blank digestion solution was made for comparison. Finally, the prepared samples and standard solutions are analyzed directly using a flame atomic absorption spectrophotometer [15].

3. Results and discussion

Despite significant progress in the provision of healthy meals around the world in recent decades, the frequency of food contamination remains a cause of concern in terms of both health and economic effects. As a result, the current study attempted to examine the heavy metals in several varieties of potato and corn chips obtained from local markets in Benghazi city, Libya.
(Table 2) shows the heavy metal ranges in corn chips to be: Cr concentrations ranged from (not detected) in a few types of chips to 0.716 mg/kg in Bugles-Cheese, Mn concentrations ranged from 0.043 mg/kg in Checoo-Cheese to 4.013 mg/kg in Soho-Cheese, Zn concentrations ranged from 1.325 mg/kg in Lalone-Cheese to 7.500 mg/kg in Doritos-Sweet, Pb concentrations ranged from 0.450 mg/kg in Lalone-Cheese to 2.900 mg/kg in Bugles-Cheese, while Cd concentrations ranged from (not detected) in two types of chips to 0.421 mg/kg in Temmy-Vanillin.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Concentration of Cd²⁺± SD</th>
<th>Concentration of Cr²⁺± SD</th>
<th>Concentration of Mn²⁺± SD</th>
<th>Concentration of Pb²⁺± SD</th>
<th>Concentration of Zn²⁺± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.399±0.002</td>
<td>NE±0.007</td>
<td>1.773±0.014</td>
<td>2.710±0.010</td>
<td>7.500±0.003</td>
</tr>
<tr>
<td>B</td>
<td>0.421±0.005</td>
<td>NE±0.011</td>
<td>1.804±0.016</td>
<td>1.625±0.013</td>
<td>4.125±0.005</td>
</tr>
<tr>
<td>C</td>
<td>0.296±0.006</td>
<td>NE±0.007</td>
<td>0.043±0.009</td>
<td>1.375±0.006</td>
<td>5.350±0.009</td>
</tr>
<tr>
<td>D</td>
<td>0.199±0.020</td>
<td>NE±0.010</td>
<td>0.201±0.008</td>
<td>1.625±0.002</td>
<td>2.625±0.005</td>
</tr>
<tr>
<td>E</td>
<td>0.189±0.004</td>
<td>0.673±0.009</td>
<td>0.865±0.012</td>
<td>1.400±0.006</td>
<td>5.375±0.007</td>
</tr>
<tr>
<td>F</td>
<td>NE±0.005</td>
<td>0.288±0.003</td>
<td>1.319±0.005</td>
<td>2.663±0.014</td>
<td>3.643±0.007</td>
</tr>
<tr>
<td>G</td>
<td>0.392±0.000</td>
<td>0.716±0.0003</td>
<td>1.198±0.014</td>
<td>2.900±0.007</td>
<td>2.630±0.004</td>
</tr>
<tr>
<td>H</td>
<td>0.238±0.002</td>
<td>0.353±0.017</td>
<td>0.456±0.006</td>
<td>1.975±0.011</td>
<td>4.768±0.012</td>
</tr>
<tr>
<td>I</td>
<td>0.155±0.001</td>
<td>0.096±0.005</td>
<td>0.242±0.012</td>
<td>0.450±0.008</td>
<td>1.325±0.058</td>
</tr>
<tr>
<td>J</td>
<td>NE±0.002</td>
<td>0.701±0.004</td>
<td>0.323±0.008</td>
<td>1.625±0.013</td>
<td>7.175±0.010</td>
</tr>
<tr>
<td>K</td>
<td>0.215±0.003</td>
<td>0.292±0.011</td>
<td>1.488±0.006</td>
<td>2.795±0.004</td>
<td>5.750±0.002</td>
</tr>
<tr>
<td>L</td>
<td>0.213±0.001</td>
<td>NE±0.013</td>
<td>4.013±0.013</td>
<td>2.505±0.011</td>
<td>2.175±0.004</td>
</tr>
<tr>
<td>Min.</td>
<td>0.155</td>
<td>0.096</td>
<td>0.043</td>
<td>0.450</td>
<td>1.325</td>
</tr>
<tr>
<td>Max.</td>
<td>0.421</td>
<td>0.716</td>
<td>4.013</td>
<td>2.900</td>
<td>7.500</td>
</tr>
<tr>
<td>Ave.</td>
<td>0.257</td>
<td>0.260</td>
<td>1.144</td>
<td>1.971</td>
<td>4.570</td>
</tr>
</tbody>
</table>

(Table 3) shows that the heavy metal ranges for potato chips were as follows: Cr concentrations range from 2.325 mg/kg in Chipsy-Salt to 6.950 mg/kg in Fox-Cheese, Mn concentrations range from 0.405 mg/kg in Chipsy-Salt to 4.985 mg/kg in Lays—Cheese, and Zn concentrations range from 2.325 mg/kg in Chipsy-Salt to 6.950 mg/kg in Fox-Cheese, Pb concentrations ranged from 1.580 mg/kg in Pringles-Salt to 2.545 mg/kg in Crips the world-Cheese, while Cd concentrations ranged from (not detected) in two types of chips to 0.722 mg/kg in Fox-Cheese. In general, the amounts of Cr and Mn in potato chips samples were higher than those in corn chips. While, the levels of Zn in corn chips were higher than...
those in potato chips. The amounts of Cd and Pb in the two types of chips, however, were nearly identical. This is evident in (Tables 2 and 3), where the mean metal concentrations in corn chips were found to be 0.257, 0.260, 1.144, 1.971, and 4.570 mg/Kg for Cd, Cr, Mn, Pb, and Zn, respectively. Cd, Cr, Mn, Pb, and Zn mean concentrations in potato chips were 0.164, 2.325, 2.238, 1.962, and 3.758 mg/Kg, respectively.

### 3.1. Cadmium Contents

Cadmium levels in several types of chips (corn and potato chips) ranged from NE (not found) to 0.722 mg/kg, as shown in (Fig 1). The Fox-Cheese variety from Egypt has the greatest mean level. Cadmium levels in food are limited at 0.05 mg/kg [19]. The mean cadmium levels in this investigation were higher than the allowable limits in all corn chip samples (except in Bugles-Ketchup and Crix-Cheese). Only five of potato chips samples had cadmium levels that exceeded the allowable limits. Jaradat and Tarawneh [16] recorded cadmium levels in Jordanian potato chips ranging from 0.14-0.41 mg/kg and corn chips ranging from 0.03-0.37 mg/kg, which are comparable to the values identified in this investigation. Similarly, Harmankaya et al. [20] recorded cadmium levels below the limit of detection.
in Turkish *corn* and *potato chips* (0.001-0.007 mg/kg), and Gopalani et al. [21] discovered cadmium levels below the limit of detection in Indian *potato chips*.

Cadmium can accumulate with metallothioneins; a daily dose of 3.0–330.0 mg/day is lethal, and a daily dose of 1.5–9.0 mg/day is lethal to humans [22]. Cadmium damages the kidneys and causes chronic poisoning symptoms such as impaired renal function, decreased reproductive capacity, hypertension, tumors, and hepatic dysfunction [23].

![Cadmium concentration (mg/Kg) in corn and potato chips samples](image)

**Fig 1.** Cadmium concentration (mg/Kg) in *corn* and *potato* chips samples

### 3.2. Chromium Contents

It is widely acknowledged that chromium is a necessary element for humans [24]. Chromium is a mineral that is required in animal nutrition and is utilized in feed additives for a variety of purposes, including glucose homeostasis, growth performance, and antidepressant effects [25]. Metal alloys, paint pigments, cement, paper, rubber, and other materials contain chromium. Low levels of exposure might cause skin irritation and ulceration. Long-term exposure can harm the kidneys and liver, as well as the circulatory and nervous systems [26]. Consuming chromium-contaminated food can result in stomach problems, ulcers, convulsions, and even death [27]. It has been estimated that human requires nearly 1 µg per day [21]. As indicated in (Tables 2 and 3), the concentrations of chromium in the different chip's types (*potato* and *corn chips*) ranged between NE (not detected) and 6.950 mg/kg. The highest chromium level was observed in *potato chips* samples. The highest mean concentration of chromium detected in *potato chips* was for *Fox-Cheese* from Egypt and the lowest concentration in *corn chips* was observed for *Lalone-Cheese* from Turkey. The main concentrations of Cr levels in *potato* and *corn chips* were 6.950 and 2.424 mg/Kg dry weight, respectively. Harmankaya et al. [20] reported chromium concentrations ranging from 0.351 mg/kg to 0.451 mg/kg in *potato* and *corn chips* from Turkey. Also, Gopalani et al.
reported Cr levels ranging from not detected to 1.6 mg/kg. The levels of chromium found in the present study for potato chips were higher than reported in the literature. The levels of chromium in potato and corn chips are illustrated in (Fig 2).

**Fig 2.** Chromium concentrations (mg/Kg) in corn and potato chips samples.

### 3.3. Manganese Contents

Manganese is an essential trace element that may be found in a variety of foods and can also be purchased as a dietary supplement. Many enzymes, including manganese superoxide dismutase, arginase, and pyruvate carboxylase, require manganese as a cofactor [28,29]. Manganese is involved in amino acid, cholesterol, glucose, and carbohydrate metabolism; reactive oxygen species scavenging; bone formation; reproduction; and immunological response through the activity of these enzymes [30-34]. Manganese, in conjunction with vitamin K, has a role in blood coagulation and hemostasis [32]. Manganese can be found in whole grains, clams, oysters, mussels, almonds, soybeans and other legumes, rice, green vegetables, coffee, tea, and many spices, including black pepper [35].

Manganese was found in all of the samples examined (potato and corn chips). The Mn content of chips ranged from 0.043 mg/kg (Libyan Checoo-Cheese corn chips) to 4.985 mg/kg (Egyptian Lays—Cheese potato chips) (Tables 2 and 3). Furthermore, the Mn content of potato chips (avg. 2.238 mg/kg) was found to be higher than that of corn chips (avg. 1.144 mg/kg). In terms of potato chips, Lays—Cheese from Egypt had the greatest manganese level, while Chipsy-Salt from Egypt had the lowest. However, the highest manganese concentration corn chips sample was identified in Moroccan Soho-Cheese, while the lowest was reported in Libyan Checoo-Cheese. Gopalani et al. [21] found that the Mn concentration of potato chips samples ranged from 0.499 to 8.249 mg/kg, which was greater than the current study. Manganese concentrations in potato chips samples (0.0006-0.0147 mg/kg) were found to be less than 1
mg/kg, according to AL-rajhi [36]. Our findings differed from those seen in the literature. Manganese contents in potato and corn chips samples are presented in (Fig 3).

Fig 3. The manganese concentration (mg/Kg) in corn and potato chips samples.

3.4. Lead Contents

Lead and inorganic lead compounds can be found in a wide range of commercial and industrial items, including paints, plastics, storage batteries, bearing alloys, pesticides, and ceramics [37]. Lead is one of the most important and widely dispersed contaminants in the environment, causing a variety of ailments [38]. Lead has toxicological and neurotoxic effects, including irreparable brain damage [39].

Lead exposure in humans can have a variety of biological effects depending on the degree and duration of exposure. Over a wide range of dosages, various effects occur, with the developing fetus and babies being more sensitive than adults. Lead poisoning has toxic biochemical effects on the kidneys, gastrointestinal tracts, joints, and reproductive systems, as well as issues with hemoglobin synthesis and acute and chronic nervous system impairment [39].

According to the results of this study, the range of lead concentration in corn chips samples were from 0.450 mg/kg for sample Lalone-Cheese produced by Turkey as the lowest concentration, to 2.900 mg/kg for Bugles-Cheese produced by Jordan as the highest concentration. Both samples are with cheese flavor, as indicated in (Table 2). The average concentration of lead in potato chips samples was equal to that of corn chips samples (1.962 mg/kg) as shown in (Table 3) and their concentrations ranged from 1.580 mg/kg for Pringles-salt from Poland to 2.545 mg/kg for Crips the World-Cheese from France. The levels of Pb in corn and potato chips are illustrated in (Fig 4). The concentrations reported in this study are higher than that of AL-rajhi (0.0026 mg/kg) [36], Harmankaya et al. (0.012-0.427 mg/kg) [20], and Gopalani (NE-0.13 mg/kg) [21].
Zinc is an important nutrient that is vital to people, plants, and animals. Superoxide dismutase deserves special note because it plays a critical role in protecting the body against oxygen free radicals [40]. Zinc can be found in the air, soil, and water, as well as in all foods [41]. It is a necessary component of certain digestive enzymes and proteins. Zinc deficiency in the human diet frequently results in poor reproduction, development, and immunity, as well as hair loss. Excessive amounts in diet can cause heart disease in humans [41]. Excessive ingestion of zinc salts, whether unintentionally or as a dietary supplement, causes critical toxicity. Yomiting commonly occurs after consuming more than 500 mg of zinc sulfate [42]. High zinc concentrations can produce adult respiratory distress syndrome, acute renal tubular necrosis, chemical pneumonitis, interstitial nephritis, gastrointestinal irritation, and corrosion [43].

From the results presented in (Tables 2 and 3), the concentrations of zinc varied from 1.325 mg/kg in Lalone-Cheese corn chips from Turkey to 7.500 mg/kg in Doritos-Sweet pepper corn chips from Egypt. The levels of zinc in the various corn chips samples were higher than those in potato chips samples. AL-rajhi [36] reported zinc levels ranging between 0.0008-0.0641 mg/kg in potato chips from Saudi Arabia which are less than the present study results. However, Gopalani et al. [21] reported zinc concentrations ranging from not detected to 15.503 mg/kg in potato chips from India which are higher than our results. Zinc concentrations of corn chips ranged between 1.3250 mg/kg in Lalone-Cheese sample from Turkey to 7.500 mg/kg in Doritos-Sweet pepper sample from Egypt. The mean concentration of zinc in corn chips samples was found to be 4.570 mg/kg. Also, from (Tables 3), the lowest concentration of zinc in potato chips samples was found in Chipsy-Salt sample from Egypt was equal to 2.325 mg/kg and the highest concentration was found in Fox-Cheese sample from Egypt which was equal to 6.950 mg/kg.
The mean zinc concentration in *potato chips* samples was 3.758 mg/kg. The current study results were comparable with to those reported by Harmankaya et al. [20]. The zinc concentrations of *potato* and *corn chips* samples are displayed in (Fig 5).

**Fig 5.** Zinc concentrations (mg/kg) in *corn* and *potato chips* samples.

**Conclusion**

Among the chip's samples, Zn had the highest mineral content. The lowest Zn content was found in Lalone-Cheese corn chips sample from Turkey while highest content was found in Doritos-Sweet pepper sample corn chips sample from Egypt. The total mean heavy metal concentration of potato chips samples in this study revealed an order of Zn>Cr~Mn>Pb>Cd while the order for corn chips samples was Zn>Pb>Mn>Cr~Cd. The results in this study demonstrated that potato and corn chips purchased in Libyan markets were contaminated with heavy metals; especially with Cd and Pb. The regular monitoring of heavy metal concentrations in Snacks is necessary to prevent heavy metal risks to human health.

**Conflict of Interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**References**


