Original Article

Journal of Food Safety and Hygiene

Journal homepage: http://jfsh.tums.ac.ir

Measuring the levels of Zn, Cu, Pb and Cd via the polarography method in fermentative pickled cucumbers purchased from Tehran market, Iran

Behrooz Jannat[1], Sara Mirshamsi[2], Naficeh Sadeghi[1,2], Mohammad Reza Oveisi[2], Mannan Hajimahmoodi[2,3] *

[1]*Halal Research Center of IRI, FDA, Tehran, Iran.*
[2]*Drug and Food Control Department, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.*
[3]*Food and Drug Administration, Tehran University of Medical Sciences, Tehran, Iran.*

ARTICLE INFO

Article history:
Received 10 Feb. 2021
Received in revised form
23 Mar. 2021
Accepted 28 Mar. 2021

Keywords:
Polarography;
Pickled cucumber;
Heavy metals

ABSTRACT

Pickled cucumber is frequently consumed by Iranians. According to the production process, there are two forms of pickled cucumber including industrial and fermentative. There is not an appropriate monitoring system available for the production of the latter form; therefore, high levels of heavy metals might be present in this type of pickled cucumber. Accordingly, so the levels of cadmium, zinc, lead and copper were measured in fermentative pickled cucumbers obtained from the Tehran market via the polarographic method. Polarography is a subclass of voltammetry where the working electrode is a dropping mercury electrode (DME) or a static mercury drop electrode (SMDE), which are useful for their wide cathodic ranges and renewable surfaces. Fifty different samples of pickled cucumbers were purchased from the market. Of each sample, dry ashes were produced. Measurement was repeated three times and the acquired data was then analyzed. The mean levels of zinc and copper in the evaluated samples were significantly lower than the standard limits respectively presented by the EOS and Codex (P value<0.001) while the mean level of cadmium and lead was slightly higher than the standard limit established by Codex with no significant differences observed (P values=0.450 and 0.246, respectively).

Citation: Jannat B, Mirshamsi S, Sadeghi N, Oveisi MR, Hajimahmoodi M**. Measuring the levels of Zn, Cu, Pb and Cd via the polarography method in fermentative pickled cucumbers purchased from Tehran market, Iran.** J food safe & hyg 2021; 7(1):52-59

1. Introduction

In recent 50 years ago, a remarkable increase in heavy metals exposure to human was observed (1). Despite the conflicts about the definition of heavy metals, generally the metals with specific gravity of more than 5 g/cm^3 are considered as heavy metals. Regarding the environmental health, sanitation and human healthcare, metals such as lead (Pb), mercury (Hg), copper (Cu), cadmium (Cd), nickel (Ni) along with

*Corresponding author. Tel.: +98 21 66954714
E-mail address: hajimah@sina.tums.ac.ir

their various compounds are considered as important threats which impose toxic effects on the environment and the human body (3). Since the heavy metals are a participant of enzyme structure, they are needed for suitable growth and development of human body. However, they can be detrimental in high doses of intake. Heavy metals have some disadvantageous effects in human body on nervous system, digestion, immunity, hormones, mental health, muscles, bones, and reflexes (1). Adverse effects of heavy metals on human health are due to slow elimination from the body, non- biodegradable properties, long biological half-life and high accumulation (4). The issue of environmental contamination with heavy metals has attained attention and concerns recently (5). Since different pathways may lead to the spread of heavy metals in the environment (2), it is notable that the vast industrial growth and the increased application of chemicals caused that pollutants have entered into the air, water, and soil and consequently our foods (6). The factors that can be lead to contamination of fruits and vegetables by heavy metals are mentioned following contaminated water used for irrigation, chemical fertilizers, and pesticides (4). It is generally believed that wherever the levels of heavy metals in soil are higher, more metals may be uptaken by plants (7). This is often evident in the food corps of the affected soils (8). Approximately 90% of consumers' exposure to heavy metals is related to polluted foods (4). So, one of the main routes from which these toxic elements enter the human body is nutrition the human body is the nutrition (6).

Due to this potential risk, most foods are closely monitored and evaluated for their levels of contaminants, especially heavy metals.

Cucumber (cucumis sativus) is from the gourd family cucurbitaceae. Three kinds of cucumber include slicing, burpless and pickling. While the originate of cucumber is southern Asia, these days it grows in the most of countries.

The pickled cucumber is frequently consumed by Iranians. Regarding the production process, there are two forms of the pickled cucumber including industrial and fermentative. While the industrial form of the pickled cucumber is produced in a standard way and is pasteurized in the production process, the fermentative form is produced in traditional workhouses and no proper monitoring system is available for their products. Therefore, high levels of heavy metals might be present in this type of the pickled cucumber. Because of the heavy metals toxicity, it is important that the analysis of vegetables and fruits contaminated by heavy metals are carried out in order to assess the amount of trace heavy metals and compare to the international standards (1).

In this regard, the levels of cadmium, zinc, lead and copper were measured in fermentative pickled cucumbers obtained from the Tehran market via the polarographic method.

2. Materials and Methods

2.1. Materials

The used reagents were purchased from Merck Company (Darmstadt, Germany) including cadmium nitrate (211B981619), tartaric acid (819A135004), glacial acetic acid (K35377356546), sodium acetate

(K37186665742), lead nitrate (7397), zinc nitrate (8912071) and copper nitrate (6397182). 65% nitric acid was bought from Applichem (Germany).

2.2. Preparation of Standard Solution

Sodium acetate buffer was prepared by dissolving 17 g sodium acetate trihydrate in 30 mL distilled water and adding 0.75 g tartaric acid. A clear and uniform solution was produced using a sonicator device (Sonorex Rk52, Germany) via ultrasound waves. The pH of the solution was regulated to be in the range of 4.6 to 4.8 using glacial acetic acid solution and then the volume was brought to 100mL with distilled water.

Standard solutions of Cu, Pb, Zn and Cd were prepared by dissolving 0.1916 g $Cu(NO_3)_2$, 0.0799 g $Pb(NO_3)_2$, 0.2142 g $Zn(NO_3)_2$ and 0.1375 g $Cd(NO_3)_2$ in 100 mL double distilled deionized water, respectively.

The standard solution of Zn, Cu, Pb and Cd was prepared by adding 10 mL of Zn standard solution and 5mL from each of the Cu, Pb and Cd standard solutions followed by double distilled water up to 100 mL. The concentrations of Cu, Zn, Pb and Cd were then calculated to be 25.1974 µg/mL, 47.0856 µg/mL, 24.9921 µg/mL and 25.0546 µg/mL, respectively.

2.3. Sampling and Preparation

In the current study, 50 different samples of pickled cucumbers were purchased from the markets of different parts of Tehran. Two grams of each sample were heated to become dry ashes; then, 10 mL 65% nitric acid was added and the mixture was reheated to dry. Digestion was performed via heating in a furnace at a 4500°C temperature to produce dry ashes of the substance. One milliliter of 65% nitric acid and 9 mL deionized water were added and the solution was boiled. After cooling, it was filtered and the volume was brought in order to analyze the sample via the polarography. After adding 10 mL of sodium acetate buffer and 100 µL of three concentrations of standard solutions to the sample solution, 500 µL of it was analyzed by the polarograph. Measurements were repeated three times and the acquired data was then analyzed.

2.4. Measurement

10 ml of sodium acetate buffer was added to the polarograph's cell and stirred at 2000 rpm for 60 seconds. Measurement started with drawing a baseline using a Hanging Drop Mercury Electrode (HDME). The drop was 5 µL and a pulse with 50 mV height and 40 ms width was utilized. A potential of 800 mV was applied for 90 s for the primary electrolysis. Then, the stirring was stopped for 10 s as the resting phase so that the solution was ready for measurements. The voltage was changed with a scan rate of 60 mV/s and 6 mV steps from 800 mV to 100 mV. 500 µL of the prepared solution was added to the cell and stirring was done for 100 s after drawing the baseline. In order to obtain the voltamogram The next phases were performed similar to the aforementioned processes. Then, in three separate steps, 100 µL of the standard solution was added to the cell. The measurements were repeated after each addition from the top.

2.5. Statistical Analysis

Data were entered into SPSS v.20 and compared with standard limits presented by CODEX (9) and Egyptian Organization for Standardization and Quality Control (EOS) (10) via t-test.

3. Results

In the Differential Pulse Anodic Stripping Voltammetry (DPASV) method which is applied in this study, the limit of quantification (LOQ) of Zn, Cu, Pb and Cd were calculated and are shown in Table 1, also the precision that was calculated according to the three measurements for each element by using standard errors are shown in Table 1.

The concentration range, calibration formula, and the coefficient of determination of calibration through the standard addition method are presented in Table 2. The polarogram curves of the four elements are depicted in Figure 1.

The average amounts of the elements in pickled cucumber samples are demonstrated in Table 2 (in mg/kg). The standard limits of each element are shown in the fourth column of the table, of which the standards of Cu, Pb and Cd have been presented by Codex (9) and the standard for Zn has been presented by EOS (10).

Table 1. Calibration table of Zn, Pb, Cd and Cu

element	coefficient of determination	calibration formula	concentration range	LOQ	precision
Zn	0.9901	y = 0.0166x + 1.919	47_188.3	0.05	5.6%
Cd	0.9822	y = 0.0154x + 1.513	25_100.2	0.005	4.5%
Pb	0.9838	y = 0.0215x + 0.6955	24.9_99.9	0.005	3.5%
Cu	0.9875	y = 0.0413 + 0.612	25.1_100.7	0.01	3.2%

Table 2. The average amount of each element measured in pickled cucumber samples in mg/kg

element	Number	Mean ± SD	Standard Limit	p value
Zn	50	1.9805 ±1.7435	5	<0.001
Cd	50	0.0578 ±.0656	0.05	0.45
Pb	50	0.1135 ±0.0746	0.1	0.246
Cu	50	1.9018 ±1.1440	10	<0.001

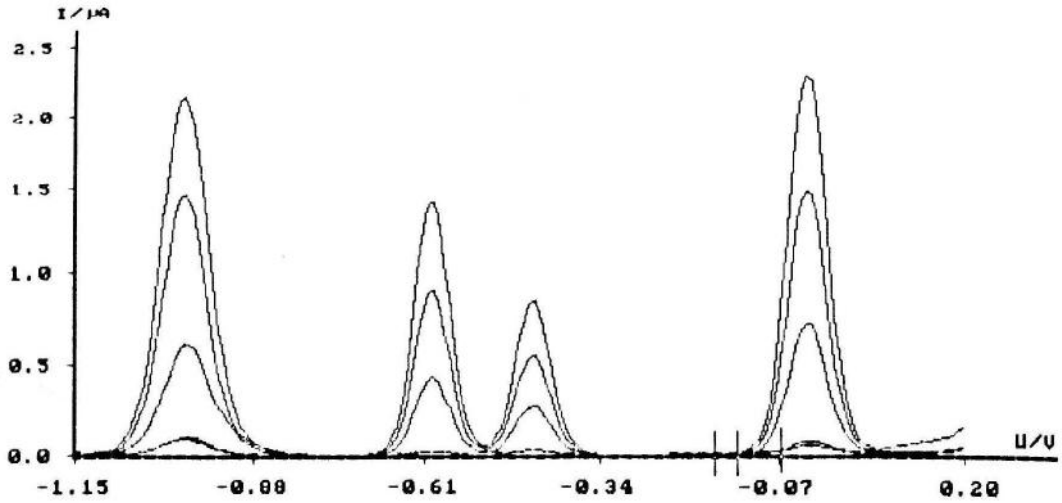

Figure1. Polarogram curve of Zn, Cd, Pb, and Cu in order from left to right

4. Discussion

As a result of the toxic chemical compounds and elements (especially heavy metals) entrance to the food chain, humans as the greatest part of these chains might be prone to the accumulation of these toxins (6). Precise monitoring of foods has been developed. Moreover, many organizations have assessed the safe amounts of these elements and have recommended guidelines for their standard limits of which Codex (9) and EOS (10) are two of the most referred ones. Most foods must undergo strict health monitoring in every country according to their health infrastructures and regulations, but some particular foods are produced at workhouses or homes without proper health monitoring. The fermentative pickled cucumber is one of these foods produced under no supervision.

As shown in table 2, the level of Zn was significantly lower than the standard limit recommended by the EOS (10) ($p < 0.001$). Similarly, compared to the 10 mg/kg standard limit proposed by Codex (9), the amount of Cu was also found to be significantly lower ($p < 0.001$).

On the other hand, the concentrations of Pb and Cd were found to be slightly higher than the recommended standard limits for cucumber proposed by Codex (9) but the difference was not significant (p value: 0.450 and 0.246, respectively). But these amounts are within the recommended standard limits for canned pickled cucumber.

To the best of our knowledge, this is the first study evaluating the levels of Cu, Cd, Zn and Pb in fermentative pickled cucumber samples distributed in the Tehran market. However, in order to assess the concentration of heavy metals in various products, similar studies have been carried out. For instance, in 2010, the DPASV method was utilized by Jannat et al. to measure the Pb, Cd, Cu and Zn contents of infant formula samples. They reported the concentrations of Pb and Cu to have exceeded the standard levels presented on the label of one of their samples (11). In 2011, Ramezani et al. measured the levels of Cd and Pb in samples of dill and onion cultivated in Khuzestan, Iran. They used flame atomic absorption spectroscopy and found the levels of Pb and Cd to be 0.208 mg/kg and 1.972 mg/kg in dill samples, respectively, both exceeding the safe limits established by the Codex. Pb was not detected in their onion samples but the concentration of Cd was reported to be 0.0475 mg/kg which was in the normal range (12).

The concentrations of Pb, Zn, Cu and Cd were measured in fish samples in two different studies conducted by Sobhanardakani et al. (13) and Jafari et al. (14), both in 2011. The levels were found to be lower than the upper limits of normal in both surveys for all the assessed heavy metals.

In 2012, Saei-Dehkordi et al. applied the stripping chronopotentiometry method to assess the levels of Pb, Cd, Cu, and Zn in commercial Iranian vinegars. They found the levels of this contaminant to be in the normal range in all the vinegar samples evaluated (15). Jafarian-Dehkordi et al. assessed the heavy metal contamination of vegetables in Isfahan, Iran and reported that the level of Pb was the highest among the evaluated heavy metals. They also found that the concentrations of Cd, Cr and Pb exceeded the safe limits in some samples of vegetables (16).

In 2014, Sadeghi et al. conducted a study to measure the concentration of Zn, Cu, Pb, and Cd in the baby weaning food and powder milk through the DPASV method. They reported the levels of these four elements to be in the normal range in the evaluated samples (17).

These were only some examples of the studies determining the amounts of heavy metals and contaminants in nutritional products. Various methods can be used to measure the concentration of trace elements in target samples, of which we chose the DPASV method for our survey.

5. Conclusion

According to our limited findings, any serious hazard was not found but further studies with more samples are recommended for precise conclusion.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgements

This work was student thesis and supported by the research council of Tehran University of Medical Sciences, Tehran, Iran

References

1. Mustapha BU, Kanada YB, Goni Chamba B, et al. Evaluation of some heavy metals in fruits. J Biolog Sci & Bioconserv 2016; 8: 57-72.

2. Järup L. Hazards of heavy metal contamination. Br Med Bull 2003; 68: 167-182.

3. Fatima N, Khan M, Kabeer MS. Evaluation of heavy metals content in the canned/packed fruit juices from local and imported origin in Lahore, Pakistan. J Food Safe & Hyg 2020; 6: 183-197.

4. Abbasi H, Shah MH, Mohiuddin M, et al. Quantification of heavy metals and health risk assessment in processed fruits' products. Arabian J Chem 2020; 13: 8965-78.

5. Tchounwou PB, Yedjou CG, Patlolla AK, et al. Heavy metal toxicity and the environment. Molecul Clinic & Environ Toxicol 2012; 101: 133-64.

6. Peralta-Videa JR, Lopez ML, Narayan M, et al. The biochemistry of environmental heavy metal uptake by plants: implications for the food chain. Int J Biochem Cell Biol 2009; 41: 1665-77.

7. Voutsa D, Grimanis A, Samara C. Trace elements in vegetables grown in an industrial area in relation to soil and air particulate matter. Environ Pollut 1996; 94: 325-35.

8. Khan S, Cao Q, Zheng Y, et al. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China Environ Pollut 2008; 152: 686-92.

9. Food and Agriculture Organization of the United Nations WHOF. Codex Alimentarius: fresh fruits and vegetales F. . Food and Agriculture Organizationof the United Nations, World Health Organization. FAO; 2007.

10. Control EEOfSaQ. EOS Egyptian Organization for Standardization and Quality Control 1993.

11. Jannat B, Sadeghi N, Oveisi MR, et al. Simultaneous determination of lead, cadmium, copper and zinc in Infant formula by anodic stripping voltammetry. Iran J Pharm Res 2010; 20: 159-62.

12. Ramezani Z, Aghel N, Shiralipour R, et al. Determination of lead and cadmium content of dill (Anethum graveolens) and onion (Allium Cepa L.) cultivated in Khozestan/Iran. Iran J Pharm Sci 2011; 7: 197-203.

13. Sobhanardakani S, Tayebi L, Farmany A. Toxic metal (Pb, Hg and As) contamination of muscle, gill and liver tissues of Otolithes rubber, Pampus argenteus, Parastromateus niger, Scomberomorus commerson and Onchorynchus mykiss. World Appl Sci J 2011; 14: 1453-6.

14. JafarI SM, SobHanarDakanI S. Determination of heavy metal (Cu, Pb and Zn) concentrations in muscle tissue of Hypophthalmichthys molitrix, Cyprinus carpio and Ctenopharyngodon idella caught from Zarivar Wetland, Western Iran. Curr World Environ 2014; 9: 923.

15. Saei-Dehkordi SS, Fallah AA, Ghafari E. Determination of lead, cadmium, copper, and zinc content in commercial Iranian vinegars using stripping chronopotentiometry. Food Anal Methods 2012; 5: 767-73.

16. Jafarian-Dehkordi A, Alehashem M. Heavy metal contamination of vegetables in Isfahan, Iran. Res Pharm Sci 2013; 8: 51.

17. Sadeghi N, Oveisi MR, Jannat B, et al. Simultaneous measurement of zinc, copper, lead and cadmium in baby weaning food and powder milk by DPASV. Iran J Pharm Res 2014; 13: 345.