Research article

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Voltametric technique for measurement of heavy metals in canned fish

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Abstract

Background and objective: Heavy metals such as cadmium and lead do no degrade and/or metabolize in vital systems, and accumulate in different organs of the body such as muscles, adipose tissue, bones, and joints. In this study, we measured concentration of four elements of copper, zinc, cadmium, and lead in canned fish.

Materials and methods: At first, 54 canned fish samples were purchased from local market in Tehran (capital city of Iran). For analysis, 2 g of each sample was weighted and mixed with 65% nitric acid. The mixture was heated at 450 °C, and dry ash was used for voltametric analysis. In this work, direct determination of heavy metals in canned fish was carried out by differential pulse anodic stripping voltammetry (DPASV) technique at hanging mercury dropping electrode (HMDE).

Results and conclusion: Quantitative measurement limits were 0.045, 0.006, 0.004, and 0.013 mg/kg for zinc, cadmium, lead, and copper, respectively. The results showed that mean concentration was 1.41 ±0.948 mg/kg for zinc, 0.050 ±0.038 mg/kg for cadmium, 0.146 ±0.119 mg/kg for lead, and 0.082 ±0.065 mg/kg for copper. The mean value obtained for each heavy metal was compared with the values authorized by the National Standard Organization of Iran, World Health Organization, and Food and Agriculture Organization, through which all concentrations were below the maximum permitted values. Concentration of cadmium, copper, and zinc showed significant relationships with brand of the samples. This indicates significant differences in concentration of these elements among different brands, which needs to be investigated by the competent authorities.

Keywords: Heavy metals, Toxicity, Canned fish, Voltammetry

1. Introduction

Fish is a good source of omega-3 fatty acids such as EPA and DHA, which protect the body against heart disease, stroke, and other disorders [1]. High consumption of fish products has been recommended to improve human health in many countries [2]. Health benefits of fish consumption are mainly due to high content of unsaturated fatty acids, vitamins, and minerals in fish tissue [3]. Fish are defined by several authorities including various dictionaries and encyclopedias as a group of aquatic vertebrates with gills and fins, pointed snouts, and scale coverings [4]. Most fish are cold-blooded creatures whose bodies can accept the narrow

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temperature range of surrounding water [5]. There are 33,100 species of fish that have been discovered by humans, making them the most diverse group of vertebrates. Fish products have been important food source for human for a long time. They are of main diet of people living in areas near rivers, oceans, and lakes [6]. In the modern world, fishing is done both in wild lakes and rivers and in floating cages in the sea [7]. Industrial fishing has provided employment for millions of people, and fishing industry has made significant contribution to the world's food supply to provide an important source of animal protein [8]. Approximately, 154 million metric tons of seafood were caught worldwide in 2011. It is expected to increase to 186 million metric tons by 2030 [9].

Fish is a good source of protein, vitamins, and minerals, and it is beneficial for a healthy diet. Fish and fish oil contain omega-3 unsaturated fatty acids (PUFAs), which appear to contribute to human health in several ways [10]. However, some contaminants are accumulated in fatty tissues of fish [11]. The United States Department of Agriculture and the United States Department of Health and Human Services recommend eating at least eight ounces (about 228 g) of fish per week [12]. On the other hand, World Health Organization (WHO) recommends at least eight ounces of low-mercury seafood per week (less for children) for a 2,000-calorie diet and 8-12 ounces for pregnant and lactating women [13].

The methods used to qualify seafood products fall into three categories of physical, chemical, and biological approaches. These different methods are used to determine shelf life of marine products [14]. In general, food quality is determined by quality of ingredients, efficiency of food processing, type of distribution, efficiency of storage, and purity of final product [15]. Heavy metals pose a significant threat to aquatic life and safety of seafood products, and are considered as a potential health risk to human [16]. Therefore, it is necessary to determine how much of these trace metals are present in our foods. The main issue is whether seafood consumption poses a significant risk of contamination to humans. There is a need to accurately assess seafood consumption in general population. In addition, safety evaluation of food supply is of great importance. Unfortunately, some countries do not have adequate control over quality of seafood and marine products. With respect to the health risks associated with heavy metals, this study was conducted to determine concentration of copper, zinc, cadmium, and lead in canned fish by voltammetry that is a simple and cost-effective approach.

2. Materials and methods

2.1. Sampling

For this study, 54 canned fish samples were purchased from supermarkets in Tehran. In the laboratory, 2 g of each sample was weighed and placed on a heater until it was completely burned. Then, 10 ml of 65% nitric acid was added, and the mixture was heated to be dried followed by heating at 450 ºC. In the next step, 1 ml of 65% nitric acid and 9 ml of deionized water were added to the ash, and the mixture was heated to boiling point. After cooling, it was filtered by a filter paper (150 µm), and filtrate was made up to 25 ml by deionized water. To examine the sample in polarograph, 10 ml of sodium acetate was first added to the cell. Then, 500 µl of the prepared sample was injected to the device, and 100 µl of standard solution of four heavy metals (lead, cadmium, copper, and zinc) were added three times. The measurements were performed twice for each sample.

2.2. Measurement of heavy metals by polarograph

10 ml of sodium acetate was added to the device cell, stirred at 2,000 rpm for 60 sec, and deoxidized with nitrogen gas. Then, measurement stage began and the baseline was drawn. The electrode used for measurement of heavy metals was an HMDE Hanging Drop Electrode with size of 5 µl, pulse height of 50 mV, and pulse duration of 40 ms. Time of current measurement was 20 ms, and total time for applying the voltage was 0.1 s in each step. The potential used in this phase (initial electrolysis or concentration phase) was -1,500 mV for 90 sec. The next phase was

the rest phase, where stirring was stopped for 10 sec, and the environment was kept static for the measurement. In the quantification and dissolution phase (sweeping), the voltage was changed from -1,500 to 200 mV at a rate of 60 mV/s and in 6 mV steps. Then, a prepared sample of 500 ml was added to the device's cell, stirred over 100 sec, and deoxygenated with nitrogen. Measurement was then performed, and voltammogram was obtained as described above. 100 µl of the four standard solutions at specific concentration was then added to the device cell in three separate steps. A same analytical procedure was used after addition of standard solutions. The experiments were done in triplicate for each sample.

2.3. Statistical analysis

The results were analyzed by SPSS software version 16. The means were compared by analysis of variance (ANOVA), and differences were considered as significant at $p \leq 0.05$.

3. Results and discussion

3.1. Figure of merits

3.1.1. Calibration curve results

To prepare calibration curve by standard addition method, four standard mixtures were prepared at concentrations of 23.54, 5.01, 8.29, and 6.29 mg/l for zinc, cadmium, lead, and copper, respectively. Calibration curve was prepared after injection of standard solutions to the device, and used to determine concentration of four heavy metals in each canned fish sample. Figures 1 to 4 show calibration curves of the four heavy metals in the samples.

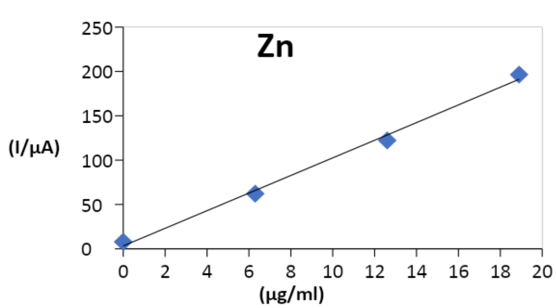

Figure 1- Calibration curve of zinc

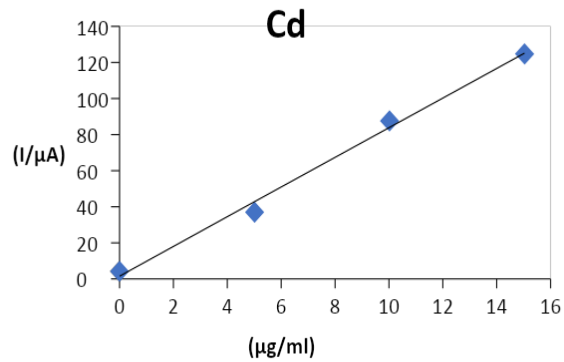

Figure 2- Calibration curve of cadmium

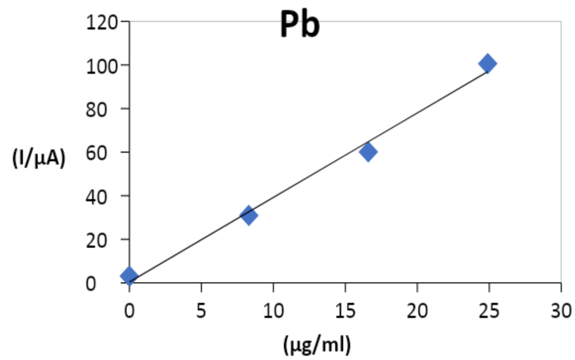

Figure 3- Calibration curve of lead

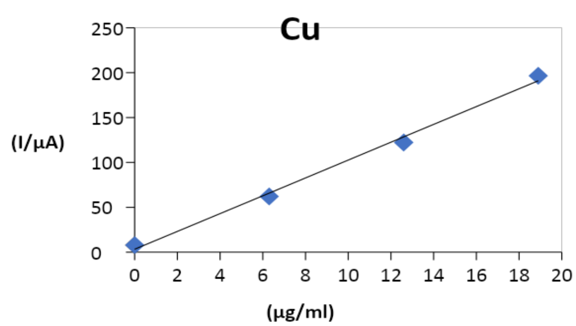

Figure 4- Calibration curve of copper

3.1.2. Precision and reliability of data

Repeatability of data was 5.6% for zinc, 3.5% for cadmium, 4.5% for lead, and 3.2% for copper based on RSD%. Reproducibility was 11.9% for zinc, 14.8% for cadmium, 16.2% for lead, and 10.6% for copper based on RSD% in same sample. Method recoveries were 97, 96, 99, and 103% for lead, cadmium, copper, and zinc, respectively.

3.1.3. Limit of detection

There are several methods for determination of detection limit, of which three times use of blank standard deviation is the best. For this purpose, blank solution was scanned 10 times with a voltage between -1550-100 mV. Detection limit was set at 0.011, 0.0017, 0.0014, and 0.007 mg/kg for zinc, cadmium, lead, and copper, respectively.

3.1.4. Limit of quantitation

To determine quantification limit, blank solution was scanned 10 times using the analytical technique, and standard deviation of the blank criterion was used 10 times to calculate limit of quantification. Thus, limit of quantification was calculated as 0.033, 0.005, 0.004, and 0.021 mg/kg for zinc, cadmium, lead, and copper, respectively.

3.1.5. Heavy metal contamination

This study determined the concentration of zinc, cadmium, lead, and copper in 54 canned fish samples by voltammetry. Figure 5 shows polarogram curves of the elements found in the canned fish samples. The minimum measurable levels using the differential pulse anodic stripping voltammetry (DPASV) method were 0.003, 0.001, 0.0001, and 0.001 mg/kg for zinc, cadmium, lead, and copper, respectively. Table 1 shows the mean concentration of copper, cadmium, lead, and zinc in 54 canned fish samples. The table also includes the values approved by Food and Agriculture Organization (FAO), WHO, and the National Standard Organization of Iran (INSO) [17, 18] for comparison.

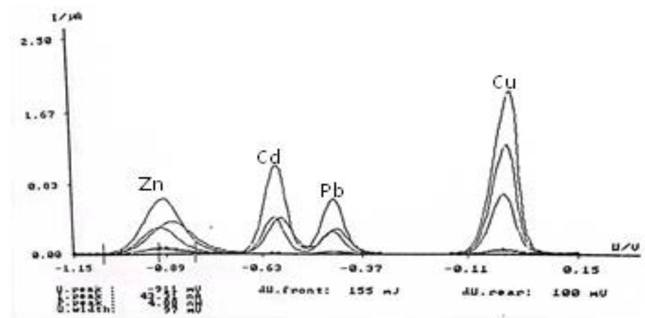

Figure 5- Polarogram curve of the heavy metals in canned fish samples

Table 1- Concentration of heavy metals in canned fish (mg/kg) compared to permitted levels in Iran and International agencies

Element	Concentration	Iran national standard (mg/kg)	FAO/WHO (mg/kg)	p-value
Zinc	1.41 ±0.948	50	50	0.0001
Cadmium	0.050 ±0.038	0.1	0.5	0.0001
Lead	0.146 ±0.119	0.5	0. 5	0.0001
Copper	0.082 ±0.065	20	30	0.0001

The results showed no significant relationship between mean concentration of each element in different types of canned fish. Concentration of cadmium, copper, and zinc showed significant relationship with brand of the samples. Mean of zinc, copper, cadmium, and lead were compared with the values determined by FAO, WHO, and INSO. The results showed that concentration of these elements in canned fish was significantly lower than the acceptable levels, indicating the healthiness of the all samples. The highest zinc concentration was found in the canned fish that did not contain vegetable oil (1.58 ±1.03 mg/kg). Concentration of lead, cadmium, and copper was not significantly different among canned fish samples containing saltwater, vegetable oil, and non-vegetable oil. Average concentration of copper was 0.039 ±0.074, 0.07 ±0.088, and 0.065 ±0.077 in saltwater, vegetable oil, and non-vegetable oil containing samples. Zinc concentration varied significantly among different brands, but the difference was less significant in the case of the other elements. Mean concentration of copper was 3.98 ±4.29 and 3.03 ±2.70 mg/kg for the brands Shabab and Galaxy, but 0.139 ±0.174 and 0.253 ±0.384 mg/kg for the brands Eita and Kadous, respectively. For lead, the mean concentration was 0.12 ±0.31mg/kg in Shabab and 0.03 ±0.03 mg/kg in Eita canned fish. Mean concentration of copper was 0.069 ±0.205 mg/kg in Shabab and 0.033 ±0.024 mg/kg in Bandar Abbas canned fish. The test results indicated that there was no significant relationship between mean concentration of each element in different types of canned fish, while there was significant relationship between concentration of cadmium, copper, and zinc, and brand of canned fish. Heavy metal contamination might be related to the raw materials used in different factories as well as method of preparing canned goods in factories which leads to entrance of heavy metals into the products. Various studies have been done about heavy metals' contamination (Table 2) [19-24]. Rahimi et al. studied 60 canned fish samples in Iran to measure their mercury, cadmium, and lead content. Mean concentration of lead and cadmium in their study was 0.085 ±0.096 and 0.039 ±0.050 mg/kg, respectively. In addition, they compared their results with those reported by other scientists in other countries, as shown in Table 3 [25].

Table 2- Comparison of mean concentration of cadmium and lead (µg/g) in canned fish examined in different countries

Country	Cadmium	Lead	Reference
Australia	0.01-0.12	0.02-1.0	19
Libya	0.09-0.32	0.18-0.40	20
Malaysia	0.06-0.14	0.40-0.76	21
Turkey	0.06-0.25	0.09-0.40	22
Saudi Arabia	0.08-0.66	0.14-0.82	23
USA	0.0-0.05	0.0-0.03	24

Table 3- Mean concentration of heavy metals in different canned fish samples prepared from different species (mg/kg) [18]

Metals	Yellowfin tuna	Kilka	Kawakawa	Longtail tuna
Lead	0.19 ±0.015	0.95 ±0.88	0.28 ±0.23	1.59 ±1.56
Copper	5.77 ±4.17	30.47 ±29.82	6.77 ±5.21	7.44 ±6.11
Cadmium	0.15 ±0.12	0.07 ±0.05	0.12 ±0.09	0.06 ±0.04

Andayesh et al. analyzed 54 canned fish samples from Tehran domestic market for their average concentration of cadmium, arsenic, lead, and mercury. Accordingly, 49 out of 54 samples had cadmium content below limit of quantification (0.6 µg/kg), while other five samples had mean concentration of 0.015 ±0.013 mg/kg for cadmium. Average concentration of lead was below limit of quantification (3 µg/kg) in 87% of samples, while it was 0.05 ±0.053 mg/kg for remaining 13% of the samples [26]. Sobhanardakani et al. studied several popular brands of canned fish for their heavy metal content and reported that the mean concentration of copper in the samples was 0.19±0.92 µg/g, which was below the permitted limit determined by WHO (30 µg/g) [17]. Zareii et al. studied concentration of histamine and six heavy metals in canned fish and found that mean concentrations of lead, cadmium, copper, and zinc were in the ranges of 0.007-0.51, 0.002-0.07, 0.17-8.001, and 0.124-27.001 µg/g, respectively (all of which were below the permitted level of WHO) [27]. In agreement, average concentration of zinc, cadmium, lead, and manganese in different brands of canned fish in Tehran market were below the permitted level of WHO in study of Hosseini et al. [18]. Finally, Novako et al. measured the concentration of seven heavy metals in 57 canned fish samples collected from Serbian market. Their results showed that mean concentration of lead, cadmium, copper, and zinc were 0.15 ±0.06, 0.03 ±0.03, 2.6 ±1.51, and 21.96 ±9.24 mg/kg, respectively. All heavy metals' concentrations were below the permitted level of WHO [28].

In general, heavy metal contamination in canned fish is strongly dependent to the species used and method of preparation. However, level of contamination in the samples evaluated in our study was as low as not posing health concern in the consumers.

4. Conclusion

This study showed that mean concentration of copper, zinc, cadmium, and lead in canned fish samples was below the permitted levels determined by INSO, FAO, and WHO. It indicated that the canned fish samples were safe for the consumers with respect to these four contaminants. However, we found that mean concentration of cadmium, copper, and zinc was significantly correlated with brand of the samples, which needs to be monitored strictly by regulatory agencies, especially in the case of cadmium.

5. Acknowledgements

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6. Conflict of interest

The authors declare that there is no conflict of interest.

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