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Original research

Canning process effects on heavy metals (lead, cadmium, tin) contents in skipjack tuna (*Katsuwonus pelamis*) in oil

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A B S T R A C T

Certain heavy metals can contaminate fish during the processing and packaging of the fish. This study sought to determine how enamel, cooking, storage duration, and canning affect the levels of heavy metals. An atomic absorption spectrometer was used to perform the measurements. The study's findings demonstrated that the average levels of heavy metals following the storage period were within the allowable limits set by the FDA, WHO, and ISIRI. The canning process did not substantially change the heavy metal content in the final product. Additionally, no heavy metals moved from the can or enamel to the contents, and the metal elements of the product did not alter considerably over time. The pH and histamine levels of the product remained steady ($p \geq 0.05$), but the peroxide value changed significantly. The pre-cooking process was the only one that significantly increased the concentration of these elements ($p < 0.05$). We may infer that the increase in heavy metal concentration was not significantly affected by the canning procedure or the 30-day storage of canned fish at 45 °C.

Keywords: *Skipjack tuna*; Canned tuna fish; Heavy Metal; Oil; Enamel coating.

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1. Introduction

The increasing hazardous metal content in marine products and the associated risks to human life have become a global burden (Hajrić et al., 2022). With rising concern about the risks and health benefits of food consumption, significant attention has been given to identifying the presence of heavy metals in food. Although contamination levels vary across different food items, food materials can be tainted with toxic metals in various ways. Some metals, such as Cd, Cr, Hg, and Pb, are harmful even in trace amounts, while other metals are essential due to their important roles in biological systems. Nonetheless, excessive intake of crucial metals may lead to toxicity (Hosseini et al., 2013).

Heavy metals are primary pollutants in aquatic environments. The toxicity of these pollutants, coupled with their ability to accumulate in marine organisms, makes them particularly hazardous. Factors such as the concentrations of metals in water, exposure duration, and environmental conditions (including salinity, pH, hardness, and temperature) affect heavy metal accumulation in tissues. Additionally, metal accumulation in the tissues of marine animals is influenced by their ecological needs, seasonal changes, size, sex, and molting (Ashraf et al., 2012).

Foods like fish are of high quality for consumers. Worldwide, fish is recognized as a nutritious food because it contains essential proteins, polyunsaturated fatty acids, and fat-soluble vitamins (Rana et al., 2023). Tuna is one of the most commonly consumed fish globally. Heavy metals accumulate in large amounts in tuna, known for being predators. The livers of tuna concentrate heavy metals significantly due to their rapid metabolism and nutrient intake rates (Khansari et al., 2005; Storelli et al., 2010). Moreover, canned tuna is a popular part of the human diet because of its convenience, affordability, taste, and health benefits (Gerstenberger et al., 2010). An estimated 80% of Iranians consume canned tuna: 32% less than once a month, 28% once a month, 25% three to four times a month, and 4% more than once a week.

Most pollutants enter the human body through the consumption of contaminated food (Tuzen & Soylak, 2007). Several studies have reported a positive correlation between increased fish consumption and elevated levels of heavy metals in hair and blood (Oken et al., 2005). Additionally, canned food can be contaminated with heavy

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Table 1. Raw material properties.

Sample	Parameter	Values	Permitted Levels	Reference
Salt	Moisture content (g/100g wet wt.)	0.030 ± 0.002	Max 0.1	(ISO 2483-1973)
	Purity (g/100g dry wt.)	99.6 ± 0.1	Min 99.2	(CODEX 150-1985)
Soybean oil	Acidity (oleic acid%)	0.050 ± 0.003	Max 0.1	(ISO 660: 1996, amended 2003)
	Peroxide value (meq/kg)	0.40 ± 0.01	Max 1.5	(ISO 3960: 2001)
	soap content (mg/kg)	0.00	Max 5	(Codex BS 684 Section 2.5)
	Iodine value (wt./wt.%)	125 ± 4	118-141	(ISO 3961: 1996)
	Moisture content (wt./wt.%)	0.030 ± 0.001	Max 0.1	(ISO 662: 1998)
Fish	Total nitrogen (mg/100g)	8.0 ± 0.5	Max 20	(National Aquaculture Legislation Directive 7/98)
	Peroxide value (meq/kg)	N. D*	Max 5	(ISO 3960:2007)
	Histamine (mg/100g)	2.8 ± 0.2	Max 10	(CAC/RCP 52-2003)
Can	Lacquer resistance and adhesion	Acceptable	Without Cracks, Corrosion, and pinholes	(CAC/RCP 23-1979)

*N. D: Not Detected

metals during the canning process, along with polluted raw materials (Malakootian et al., 2011). The canning process and storage time influence the concentration of heavy metals in canned tuna. Solder used to manufacture cans is a known source of lead contamination during canning (Voegborlo et al., 1999). This study examined the effects of processing steps on the levels of lead, cadmium, and tin. Furthermore, the migration of heavy metals from the can wall to the contents and other ingredients was also studied.

2. Materials and Methods

2.1. Materials

Skipjack tuna fish (*Katsuwonus pelamis*) samples were collected from canned tuna fish factories in Tehran, Iran, which source their fish from the Indian Ocean (African coast). The other raw materials are common materials that the factory receives from suppliers. The characteristics of the raw materials are listed in Tables 1 and 2.

Table 2. Mean heavy metal levels in raw materials (in mg/kg wet wt.).

Sample	Average concentration ± SD*		
	Pb	Cd	Sn
Raw Fish	0.026 ± 0.002	0.002 ± 0.001	2.60 ± 0.07
Salt	0.040 ± 0.010	0.011 ± 0.000	0.400 ± 0.008
Soybean Oil	N.D**	0.002 ± 0.001	0.080 ± 0.005

*SD: Standard Deviation; **N. D: Not detected

2.2. Preparing and storing test samples

After being defrosted and cleaned, the frozen fish was cooked for around an hour at 100 degrees Celsius and 0.3 to 0.6 bar of pressure on a tray. After separating the skin, dark meat, and bones, it was filled into cans, and other raw materials, including salt and oil, were added, and then the capping and autoclaving steps were performed. Tests were run on raw materials, cooked, and stored. To compare the heavy metal content during storage and after production, two circumstances were created. 30 days were spent in an incubator set at 45 °C, and 15 days were spent in the environment (Beldi et al., 2012). All tests were performed in triplicate.

2.3. Measuring device

An atomic absorption spectrometer (Varian Spectra AA-220, USA) equipped with a graphite furnace, atomizer, deuterium lamp as a background corrector, sample dispenser, wavelengths of 217 and 326.1 nm, and a spectral bandwidth of 1 and 0.5 nm monochromator was used for the determination of Pb and Cd, with detection limit < 0.01 ppm and 0.001 ppm respectively. Tin was determined by aspirating the sample solution into a NO_2/acetylene flame with a detection limit of < 0.02. All glassware used in the analytical work was soaked in 10% (v/v) HNO_3 overnight, followed by rinsing with double-distilled water, and then kept in an oven at 110 °C until needed (Khansari et al., 2005). According to the Standard methods mentioned in Table 1, salt, edible oil, and fish meat were analyzed for trace metal concentrations (Pb, Cd, and Sn) and other physicochemical properties. The raw material properties can be found in Table 2. To examine the interaction between the product and the metal package and determine the stability of the protective varnish applied to the inside of cans, the preserved products were periodically analyzed physicochemically throughout the 30-day storage period at 45 °C (Olmedo et al., 2013).

2.4. Sample preparation and digestion for detection

All reagents were of analytical grade (Merck, Germany). Lead, Cd, and Sn standard stock solutions were prepared from Titrasol (1000 mg/l) and diluted to the corresponding metal solutions. The working solution was freshly prepared by diluting an appropriate aliquot of the stock solutions using 10% HNO_3 for diluting lead and cadmium solutions, and 5% HCl for diluting tin solution 3.00 ± 0.001 g of homogenized sample was weighed into a 100 ml Erlenmeyer flask and 30 ml of concentrated HCl was added, followed by 10 ml of concentrated HNO_3 were slowly added. The flask was then placed on a hot plate to complete the dissolution (Khansari et al., 2005).

2.5. Histamine measurement

The histamine concentration in canned tuna fish samples was determined by an ELISA method using a histamine kit. The test was done following the manufacturer's instructions. Histamine was quantitatively converted to N-acetylhistamine using an acylation reagent after sample preparation. Peroxidase-conjugated antibodies were added for binding to the antibody-histamine complex. The unbound antigen is then removed by washing. The substrate (urea peroxide) and chromogen were added to the wells of the microtitration plate and incubated. The bound enzyme conjugate converts a colorless chromogen into a blue product during incubation, and the blue color changes to yellow after adding the solution. On the ELISA plate reader, the optical density is measured at 450 nm after the substrate reaction. Inversely proportional to the concentration of histamine in the sample are the amount of complexes bound and the optical density (Rahimi et al., 2012).

2.6. Statistical Analysis

One-way analysis of variance (ANOVA) and Duncan's test were used to determine significant differences, and values less than 0.05 ($p < 0.05$) were considered statistically significant. Statistical calculations were performed using the SPSS 15.0 version (SPSS Inc., Chicago, IL, USA) statistical package.

3. Results and Discussion

3.1. The influence of the canning process on heavy metal concentration

Significant differences ($p < 0.05$) were found in the lead, cadmium, and tin concentrations before and after cooking in *skipjack* raw fish, but sterilization by autoclave didn't cause any significant differences in comparison with cooked meat ($p \geq 0.05$). Heavy and toxic metals are found in relatively high levels in canned food compared to fresh food samples, according to Al-Thagafi et al. (2014). As shown in Table 3, the highest concentrations were found in canned tuna fish. Metal residues in meat are affected by time, temperature, and cooking environment (Hajeb et al., 2009). According to some authors, cooking and defrosting decrease heavy metal levels in meat because heavy metals leach with serum during cooking and defrosting (Hashemi-Moghaddam et al., 2011).

Samples of canned tuna fish tested for lead, cadmium, and tin contained lower concentrations than the permitted levels, according to standard limits. Hussein et al. (2024) reported similar results from canned fish in Mansoura, Egypt, as did Bartels et al. (2023) in Ghana. According to the literature, tin, cadmium, and lead concentrations in canned tuna samples collected from Iran ranged from 0.0046 to 0.072 with a mean of 0.0223 $\mu g.g^{-1}$ for cadmium, 0.0726 to 0.0162 with a mean of 0.0366 $\mu g.g^{-1}$ for lead, and non-detectable for tin. Metal concentrations were well below FAO/WHO permissible levels for these toxic metals (Khansari et al., 2005; Pourjafar et al., 2014). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) revised the acceptable monthly intake of cadmium (Cd) to 25 μg/kg body weight (bw) in 2013 (kosher et al., 2023). Cadmium and lead concentrations of *skipjack* raw fish in the literature have been reported in the range of 0.060-0.064 and 0.187-0.249 μg/g dry weight, respectively (Ganjavi et al., 2010).

Table 3. Heavy metal contents in raw, cooked, and canned tuna (μg/g wet wt.)

Sample	Average concentration ± SD		
	Pb	Cd	Sn
Raw Fish	0.026 ± 0.002^b	0.0023 ± 0.001^b	2.60 ± 0.07^b
Cooked fish	0.231 ± 0.002^a	0.006 ± 0.001^a	5.50 ± 0.50^a
Canned fish	0.233 ± 0.000^a	0.006 ± 0.000^a	6.31 ± 0.57^a

Values within the same column with different letters are significantly different ($p < 0.05$).

3.2. Heavy metals migration from can

Migration tests were performed to determine whether heavy metals migrated from the can walls. The corrosion of tinplate and trace metal release can be influenced by storage duration, temperature, deformation, and chemical composition of canned food (Kassouf et al., 2013). Interaction product/packaging occurs due to discontinuities in internally lacquered cans (Buculei et al., 2012). Two conditions were designed to compare the heavy metal content after production and during storage. 15 days were spent in ambient conditions, and 30 days in an incubator set at 45 °C (Beldi et al., 2012). Deionized water was used as a simulant to measure the migration from the cans and enamel. The pH of the simulant was 5.9; therefore, citric acid N/10 was used to adjust the pH. The storage results revealed no migration, and the heavy metal content of the simulant did not change significantly. Buculei et al. (2014) reported that the migrated metal concentrations increased during storage. Other factors that influence the internal corrosion of cans include product composition, pH, and organic acid type.

3.3. Effect of storage duration on heavy metal concentration

Heavy metal concentrations in canned tuna were measured to evaluate the interaction between the package and its contents. Additionally, the pH of the canned product, quality of lacquer coatings, oxygen concentration in the headspace, and storage place can affect the metal content of canned fish (Boadi & Twumasi, 2011). Based on Table 4, there were slightly higher tin concentrations during storage intervals, but there were no significant changes in heavy metal concentrations. Through the use of lacquered walls and mechanical seams, new packaging technologies reduce or eliminate the leaching of heavy metals into food (Khansari et al., 2005).

Table 4. Heavy metals levels during storage time (μg/g wet wt.).

Storage time	Average concentration ± SD		
(Day)	Pb	Cd	Sn
15	0.233 ± 0.000^a	0.006 ± 0.001^a	6.31 ± 0.57^a
30	0.233 ± 0.004^a	0.006 ± 0.001^a	6.33 ± 0.57^a

Values within the same column with different letters are significantly different ($p < 0.05$).

3.4. Effect of storage duration on the physicochemical properties of canned tuna fish

The peroxide value significantly changed during the 30-day storage period at 45 °C ($p < 0.05$). The peroxide value measures the amount of fat oxidation that occurs during processing and storage. Throughout the storage and heating processes, the oil sample oxidizes, resulting in higher peroxide values (Alhibshi et al., 2016). Table 5 indicates that the pH and histamine levels did not change with storage time. Buculei et al. (2014) reported that the pH of canned meat remained constant during one year of storage.

Histamine is produced from free histidine found in spoiled fish by histidine-decarboxylating bacteria. The guideline levels for histamine content in fish are as follows: (i) < 5 mg/100 g (safe for consumption), (ii) 5-20 mg/100 g (possibly toxic), (iii) 20-100 mg (probably toxic), and (iv) 100 mg/100 g (toxic and unsafe for human consumption) (Mahmoudi and Norian, 2014). Thus, the samples produced were safe for human consumption. The histamine content of Iranian canned tuna samples ranged from 4-236 mg/kg, and 18.9% of the samples contained more than 50 mg/kg, the level suggested by the FDA (Zarei et al., 2010). In a study of canned tuna fish samples in Iran, the histamine content in canned tuna samples was determined using the ELISA method. Histamine was present at concentrations between 17 and 210 mg/100 g in 69.8% of the canned tuna samples. (Rahimi et al., 2012). The overall mean level of histamine found in canned tuna collected from Qazvin province, Iran, was 8.59 ± 14.24 ppm, and the concentration ranged from 2.51 to 74.56 ppm. The histamine content of 2.50% of the positive samples exceeded 50 ppm (Mahmoudi & Norian, 2014).

Table 5. Canned tuna samples' physicochemical properties during storage time.

Storage time (Day)	Average concentration ± SD		
	Histamine (mg/100g)	**Peroxide value (meq/kg)**	**pH**
15	2.93 ± 0.02[a]	N.D*	5.91 ± 0.01[a]
30	2.92 ± 0.01[a]	0.020 ± 0.002[a]	5.94 ± 0.01[a]

*N. D: Not detected.
Values within the same column with different letters are significantly different ($p < 0.05$).

4. Conclusion

Metals (Cd, Pb, and Sn) were detected in raw materials, cooked meat, and end products during storage. Among the three metals studied, tin showed the highest level. It was also found that the concentrations of Pb and Cd in canned tuna fish were lower than the permissible levels established by the FAO, WHO, and ISIRI. Therefore, the consumption of canned tuna fish is safe for human health despite possible contamination with heavy metals. According to the results of this study, cooking significantly increased the concentration of heavy metals in tuna meat. The canning process slightly increased the heavy metal concentrations, but the difference was not significant. Heavy metal levels did not increase significantly during 30 days of storage at different temperatures. Additionally, metal migration from the inner walls of lacquered cans did not occur in their contents.

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Authorship contribution statement

Shima Nikfallah: Methodology, Data curation, Writing Original draft, Visualization, Investigation, and Validation, Sepideh Bahrami: Conceptualization, Reviewing, Editing, Supervision, and Maryam Moslehi Shad: Supervision.

Conflict of interest

The authors declare that there is no conflict of interest.

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