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Estimation of heavy metals content, microbiological and physico-chemical characteristics of canned tuna fish from some local markets in Derna city

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ABSTRACT

Five distinct varieties of canned tuna, both foreign- and domestically-produced, were the subject of this investigation. Amid to: Estimation of heavy metals content, microbiological and physico-chemical characteristics of canned tuna fish from some local markets in Derna city. Material and Methods: The two main types of canned tuna in oil and brine (whole or slices) were the most common in Derna, and all samples were obtained in 2023 from supermarkets and shops in different neighborhoods of Derna. After wet digestion, the samples were analyzed with an Atomic Absorption Spectrophotometry (AAS) for six metals. According to the findings, the amounts of the heavy metals under study ranged from 0.192 to 0.387 g/g, 0.005 to 0.008 g/g, 0.0001 to 0.0055 g/g, (0.45 to 0.70 g/g), (0.010 to 0.20 g/g), and (0.17 to 0.54 g/g), respectively, for Hg, Pb, Cd, Zn, Cu, and Fe. Some metal concentrations were much below the acceptable limits established by the WHO and FAO. Additionally, a chemical analysis of the samples revealed that the tuna was in acidic conditions (pH 4.8–6.9), had a salinity range of 1.36–1.90%, a protein percentage of 18.58–20.45, and a moisture content of 55.5–69.3 percent. The overall bacterial count was determined by microbiological examination to be (1 101 - 3.7 101 cfu/g). But none of the examined samples had the dangerous diseases Escherichia coli or Salmonella.

1. INTRODUCTION

The term "canned tuna" describes tuna fish that has been prepared, packaged, and stored inside an airtight can or tin.Cleaning and cutting the tuna are usually the first steps in the procedure, and then it is cooked, frequently by steaming or boiling. A liquid medium, such as oil, water, or other sauces, is added to the tuna after it has been cooked and put into a can. To preserve the tuna for a long time and keep it fresh, the can is then sealed. Due to the lack of preparation or cooking required, canned tuna is a convenient food alternative. It can easily be eaten directly from the can, making it a quick and simple lunch or snack.

It provides a range of necessary nutrients. It is also a good source of high-quality protein, which is essential for maintaining and rebuilding tissues, creating hormones and enzymes, and supporting all other bodily processes (Maheswara *et al*., 2011). Additionally, tuna has a lot of omega-3 fatty acids, which are beneficial to the heart, reduce inflammation, and enhance cognitive function. The tuna also contains minerals e.g. (Se, K, Fe, Zn, Mg, etc.) as well as vitamins e.g. (A, B1, B6, B9, B12 and D), which increase the health benefits for humans, lowering obesity, weight gain, body mass index, insulin resistance, type 2 diabetes mellitus, inflammatory bowel diseases, and maintain harmony gut microbiota balance (Pittas *et al*., 2019). However, the canning are affected by the environment, which is polluted to a great extent. As a result of industrialization, urbanization, and vehicular pollution. When tuna is canned in a polluted environment, it may accumulate different toxic materials among which are heavy metals and microbiological contamination (Chekol and Ashenafi, 2009).

1.1. Microbial Spoilage and Chemical Changes

Spoilage can occur due to biological, chemical, or a combination of the two. The biological spoilage is mostly caused by microbial growth, whereas the chemical spoilage is caused by hydrogen produced by the action of acids in food and iron in cans. Swelling of canned products is occasionally observed, and this is usually due to high summer temperatures. Also, the presence of oxygen and moisture within the can, even in very small amounts, can create conditions suitable for microbial growth and spoilage. Proper sealing and complete removal of air during canning are vital to preventing oxygen and moisture ingress (Usydus *et al*., 2008). Therefore, Microbiological deterioration in canned goods persists despite thermal processing, as bacteria proliferation after leaks or improper preparation. Leakage is indicated by healthy mixed bacterial types, while under-processing can result from undercooking, incorrect thermometer, contamination, and viscous packing. These issues pose health risks (Ehrlich, 2007).

1.2. Heavy Metals: Sources of Pollution and Effects on Human Health

In this regards, the term heavy metal refers to any metallic chemical element that has a relatively high density (greater than 5 g/cm^3), a high atomic weight, and is hazardous or poisonous at low concentrations. They include transition metals, metalloids, lanthanides, and actinides (Akane *et al*., 2010). In comparison to their physical features, the chemical properties of heavy metals are the most useful. Environmental toxicity that exceeds established maximum residue limits (MRL) has attracted increased attention from think tanks around the world. Mercury (Hg), cadmium (Cd), and lead (Pb) generate a frightening combination of environmental and health issues (Storelli, 2009). Copper, iron (Fe), manganese, and zinc, among other metals, are essential micro-nutrients for humans involved in important biological processes. These elements prevailingly plays functional and structural role in human body (Jaishankar, *et al* 2014). Generally, heavy metal sources were split into two main categories by scientists: anthropogenic and natural sources. Organic sources seawater contains naturally occurring heavy metals like mercury, lead, cadmium, and arsenic, which can build up in fish like tuna. This is especially true for larger, more enduring species of tuna that are found higher up in the food chain, such albacore or yellowfin.Through a number of different natural geologic processes, these metals get into the saltwater. While anthropogenic sources include industry, mining, and domestic effluents. Industries, mining, and wastewater are deemed anthropogenic sources of heavy metals (Emami *et al*., 2002). Naturally, heavy metal contamination can occur during the canning process due to improper maintenance and cleaning of equipment, water, and packaging materials. Can linings, made with tin or lead, can still transfer heavy metals into food, including canned tuna. Chemicals sush as mineral oil and solvents can also be used in manufacturing, posing risks to the food. Proper disposal and control of these materials are crucial to prevent contamination (Nader, *et al*., 2016). Diabetes, Alzheimer's disease, and various types of cancer are just a few of the illnesses that are becoming more prevalent as a result of heavy metal pollution. Acute metal poisoning results in substantial abnormalities in the nervous, reproductive, and renal systems in people. In addition to the specific symptoms of metal toxicity, chronic exposures to copper, lead, aluminum, zinc, mercury, cadmium, and arsenic have been linked to gastrointestinal (GI) dysfunctions, diarrhea, stomatitis, shivering, and hemoglobinuria that causes rust-red depression. Heavy metals can be harmful to human health even when present in small amounts in the environment (Appenroth, 2010). Fish consumption in Libya raises heavy metal toxicity in humans, especially children and pregnant mothers. Lack of scientific data on heavy metal levels in canned tuna products highlights the need for more information. According to a number of studies and scientific publications, in Iran, (Velayatzadeh and Askari, 2014) evaluated the buildup of heavy metals such as mercury, cadmium, tin, nickel, zinc, and iron in canned tuna samples. The results indicated that nickel, mercury, zinc, and tin levels in canned Hoover fish were higher than those authorized by the WHO and FDA, while cadmium levels were excessive. In another study, (Ababneh *et al*.,2013) researched heavy metals in canned tuna sold in Jordan in 2013. It was discovered that just a few of samples contain mercury and cadmium at levels slightly higher than authorized. The outcomes of these and other research suggest that ingesting tuna for a lengthy period of time may be harmful to one's health.

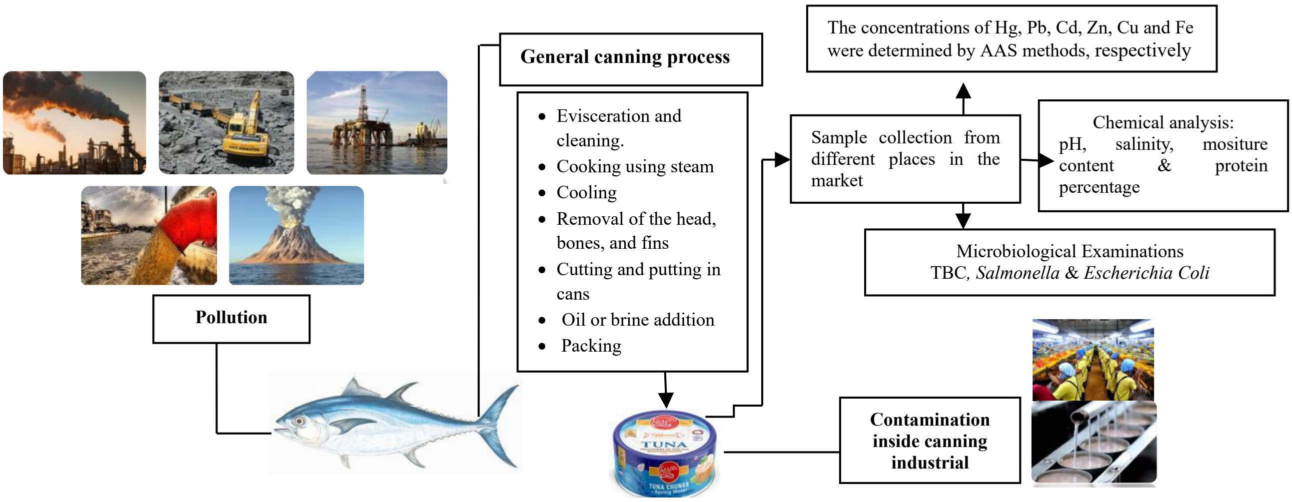

Figure 1: Scheme of the process followed for detection of heavy metals, microbiological and chemical characteristics of canned tuna fish samples

2. MATERIAL AND METHODS

2.1. Study Area

The area of study is located in the north-east of Libya in an important locality on the eastern coast of Libya, which is also part of the south shoreline of the Mediterranean basin. The markets are spread out over a large region of the city and due to the openness of the Libyan market in recent years, canned food was imported without controls and from Different and unknown markets and the loss of health control by the concerned agencies over what is imported and produced locally from canned food. Five local markets were selected at city Derna is a coastal city with a population of (170,289) ref as the city is boarded from the south by a chain of rocky hills the city has expanded along the shore line. Figure (2).

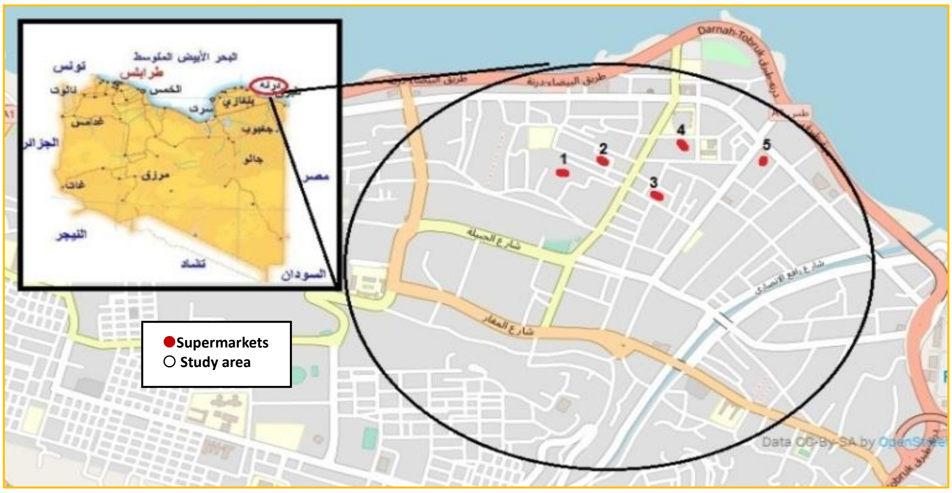

Figure 2: Map of the locations of the studied area (Google Earth)

2.2. Sampling

Five varieties of canned tuna (three replicates for each type) were gathered from Derna city center stores. The collected Tuna fish canned samples were transported to Alqumma Libya Aloula laboratory, Al-Bayda to determine the levels of heavy metals and microbiological characteristics given in Table 1.

Table (1): Tuna fish canned samples.

S. no.	Types	Product country
1	Alwafa tuna	Libya
2	Americana Tuna	Thailand
3	Aya Tuna	Thailand
4	Elwafeer tuna	Algeria
5	Qorina Tuna	Libya

2.3. Chemical Analysis

2.3.1. Measurement of PH

The pH-value of tuna sample was measured in the site immediately after collection using Bench type (JENWAY, 3410 Electrochemistry Analyzer pH-meter).

2.3.2. Measurement of salinity

Salinity can be measured as a percentage using various methods, such as the Gravimetric method or Salt-Meter Pal-Salt Probe.

2.3.4. Measurement of moisture content

To determine the content of moisture in canned fish fillets, 10 g of the sample was weighed and placed in a container. The prepared samples were heated in an oven at 103 ° C until they reached a consistent weight. After exiting the oven, the container was put in a desiccator for 30 minutes to cool before being weighed (AOAC, 2005). The moisture percentage was calculated using the following equation: % dried matter = (weight of dried sample / initial weight of sample) × 100
Content of moisture = % wet matter − % dried matter

2.3.3. Measurement of protein percentage

The Kjeldahl technique was used to determine protein percentage in samples. 1 g of each sample was digested with sulfuric acid and catalyst, and then distilled water was added to each balloon. Titration with 1% sulfuric acid was performed. Crude protein percentage was calculated by estimating total nitrogen using 25.6 × % N, and nitrogen percentage was calculated using the following equation: % N = amount of acid consumption 0.1 normal / sample weight × 100

2.3.5. Heavy Metals Analysis

The heavy metals were determined by Atomic absorption spectroscopy (AAS) in the Alqumma Libya Aloula laboratory, Al-Bayda, which represents the concentrations of Pb, Cd, Zn, Fe, and Cu successively. While the mercury concentration was determined by cold vapor atomic absorption spectrophotometry at the Food and Drug Control Center in Tropli. For the determination of selected heavy metals, about 0.5 g of homogenized sample was weighed and transferred into a (100-ml) beaker then 5 ml of concentrated HNO_3 was added. Canned tuna fish samples were digested according to the procedure used by (APHA, 2017).

2.3.6. Microbiological Examination

Total bacterial count (TBC) was determined using nutrient agar according to the method described by (Ragab, 1997). *Salmonella* was determined on *Salmonella* Agar (SS Agar) modified oxoid *Escherichia. Coli* determined on Macconkey agar according to (Bryan, 1991).

2. RESULT AND DISCUSSION

3.1. Physico – Chemical Characteristics

Results given in (Table 2 and Figure 3) indicated that the pH values ranged between a minimum value of (4.8) in sample 4 (Elwafeer tuna) and a maximum value of (6.9) in sample 4 (Qorina tuna). The pH values tended to increase at some cans. Generally the results showed that the values of pH showed slight variations. PH levels can have various effects on canned tuna, including its taste, texture, and shelf life. Also, pH plays a crucial role in preventing microbial growth in canned tuna. By maintaining a low pH (acidic) environment. According to the Codex Alimentarius, which is an international food standards organization jointly established by WHO and FAO, the pH of canned seafood should be below 4.5 to prevent the growth of harmful bacteria (Zeinab, 2012).

There was no variation in salinity (1.3 - 1.9%) between all samples. Salting is a common preservation method in the canning process as it helps inhibit bacterial growth and maintain the quality of the canned tuna, also the salinity levels in canned tuna can vary based on manufacturer specifications and consumer preferences. According to Table 2, the moisture content of all canned tuna samples differed slightly from one another. The highest moisture content (69.3%) is observed for Libyan canned fish. Higher moisture content may dilute the concentration of proteins, fats, and minerals present in the meat. Therefore, canned tuna with lower moisture content may have a higher concentration of these essential nutrients. The chemical analysis of the samples also revealed that, despite a decrease in the protein content of the canned samples, the nutritional value of canned fish was kept at a respectable level, giving the body the nutrients it requires. The percentage of protein in canned tuna can vary depending on the brand and type of tuna. On average, canned tuna contains around 25-30 % protein. However, it is always recommended to check the nutrition label on the specific brand of canned tuna for accurate information.

Table (2): The values of Physico-chemical characteristic of the studied canned tuna fish samples.

Type of tuna sample	pH	salinity %	Moisture content	Protein percentage
Sample 1	5.4	1.80	55.5	18.95
Sample 2	5.9	1.37	58.5	20.45
Sample 3	5.8	1.90	69.3	19.76
Sample 4	4.8	1.80	53.2	18.58
Sample 5	6.9	1.36	60.5	20.33

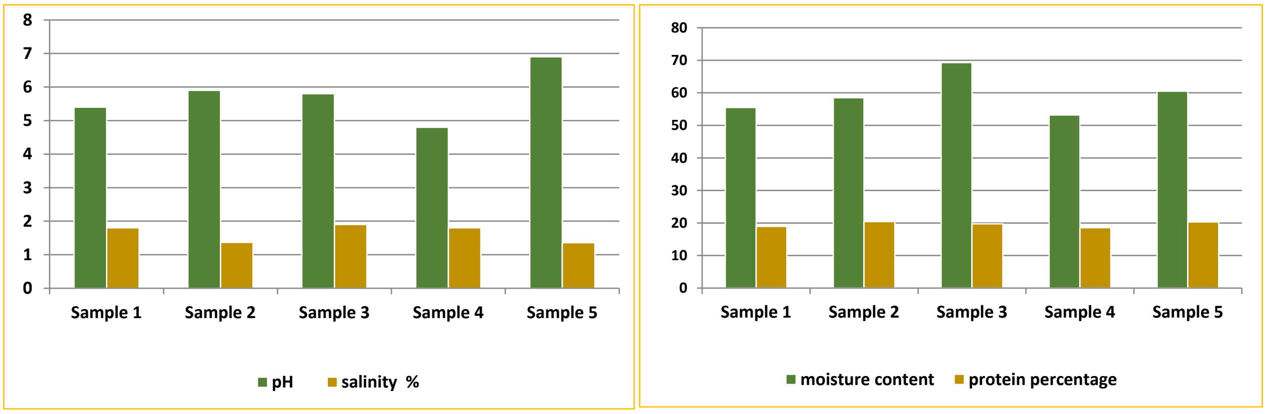

Figure 3: Comparison of pH, salinity, moisture content, and protein percentage values of the samples

3.2. Heavy Metals Content

The concentrations of (Hg, Cd, Pb, Zn, Cu, and Fe) in canned tuna are depicted in Table (3) and Figure (4) Among the samples, the highest Zn and Fe content (0.70 µg/g) was found in the Libyan-made canned tuna and in the Thai-made canned tuna. Most of the iron and zinc concentration levels recorded were above maximum permitted concentrations by FAO/WHO. Human health implications of heavy metals are determined by accumulated concentration levels from ingestion through food. The food intake is related to body weight and age. Safe values for (Fe) and (Zn) in cans tuna recommended by the WHO/FAO are (40.0 µg/g) (Hosseini, *et al*., 2015). As shown by chemical analysis, presence of slight proportions of heavy metals (Hg, Cd,and Pb), the concentration of mercury varied from 0.192 to 0.0387 µg/g, while in all samples, cadmium concentrations ranged from 0.0001 to 0.0055 µg/g whereas lead contents ranged from 0.005 to 0.008 µg/g. According to the WHO/FAO, the maximum allowable limit for total mercury and lead is (1.0 µg/g) of canned tuna. This limit applies to the edible portion of the fish and is intended to ensure that the consumption of fish and seafood does not pose a risk to human health. Likewise, the concentration of copper in the present study was 0.20 µg/g in Algerian canned tuna and 0.017 µg/g in Libyan made canned tuna. The FDA reports that copper levels in canned tuna fish are in the range of 10 ppm. Copper contamination in canned tuna is a severe problem. It can induce anemia, cold sweat, a weak pulse, and liver and kidney damage at large dosages.

Table (3): The concentrations (µg/g) of heavy metals different types of the tuna cans samples.

Type of tuna sample	Hg	Pb	Cd	Zn	Cu	Fe
Alwafa tuna	0.0342	0.007	0.0005	0.45	0.015	0.60
Americana Tuna	0.0192	0.006	0.0055	0.45	0.016	0.45
Aya Tuna	0.0355	0.005	0.0001	0.70	0.010	0.22
Elwafeer tuna	0.0298	0.006	0.0019	0.59	0.20	0.70
Qorina Tuna	0.0387	0.008	0.0021	0.70	0.017	0.17

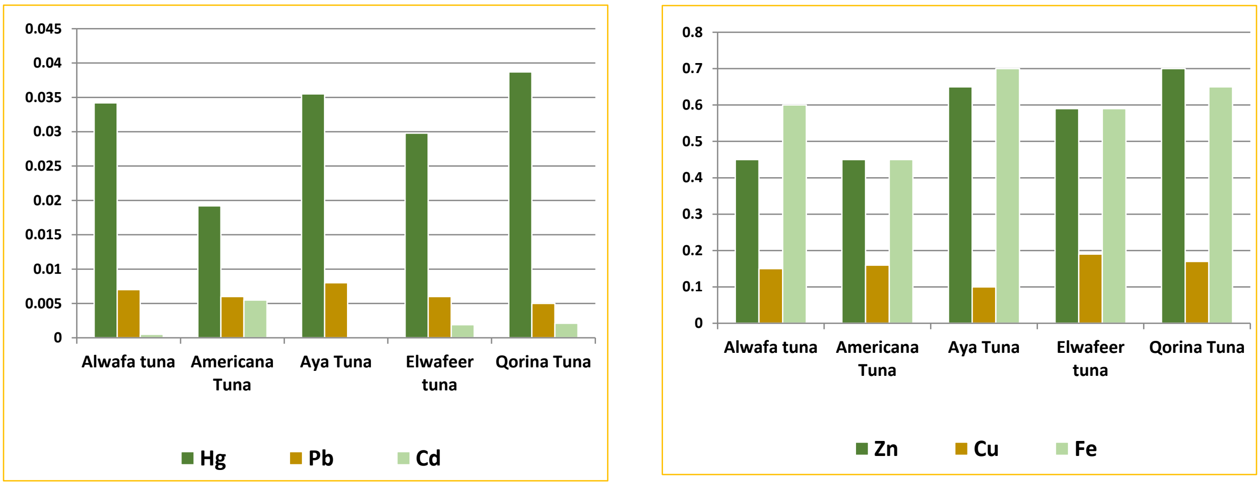

Figure 4: Comparative levels of selected toxic (Hg, Pb, &Cd) and essential (Zn, Fe, & Cu) metals in canned tuna fish

3.3. Microbiological Characteristics

Values of microbiological examinations of canned fish samples are presented in Table (4). The microbial examinations indicated that canned tuna samples had the least value of total viable bacterial count, but Elwafeer Tuna Canned (Algerian-made canned tuna) had the highest count being 3.7×10^1 cfu/g . The FDA recommends that the total bacterial count in canned seafood, including tuna, should not exceed 1000 cfu per gram or milliliter (Francesco *et al*., 2015). This limit helps ensure the safety and quality of the canned tuna. *Salmonella sp.* and *Escherichia. Coli* aureus was not detected in all samples analyzed.

Table (4): Microbiological analysis of canned tuna fish samples.

Type of tuna sample	TBC (cfu/g)	*Salmonella*	*E. Coli*
Alwafa tuna	1×10^1	- ve	- ve
Americana Tuna	1×10^1	- ve	- ve
Aya Tuna	2×10^1	- ve	- ve
Elwafeer tuna	3.7×10^1	- ve	- ve
Qorina Tuna	1×10^1	- ve	- ve

TBC = Total bacterial count - ve = Negative result

4. CONCLUSION

Based on the results of this study, the presence of certain heavy metals for analysis was proven in samples selected from different producing countries in relatively good concentrations compared to the Libyan standard specifications for imported and local tuna cans. Because these concentrations were below permissible levels and international standards, restricting their intake would not be harmful for human consumption. There are slight differences between the concentrations of heavy metals in local and imported canned tuna, as well as an increase in the concentrations of iron and zinc in canned tuna, which sometimes approach the upper limit compared to other regions of the world.

This study has shown the presence of microbial contamination in the tested canned fish product, and The microbial examinations indicated that canned tuna had a high total bacterial count of 3.7×10^1 cfu/g.This bacteria does not depend on the species or the season variation but the hygienic conditions during manufacturing. The Tuna products countries, at the time of their marketing have to answer standards been imperative by the Libyan specifications and WHO\FAO. It is thus necessary that the fish cannery display more effort in the improvement of the hygienic quality so that their products made the competition on the international markets.

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