

Quality Assessment of Some Imported and Local Canned Tuna Sold in Kafrelsheikh, Egypt

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Abstract

This study was conducted to evaluate and compare between some local and imported canned tuna products sold in supermarkets of Kafr El Sheikh Governorate, through physicochemical, bacteriological and sensory parameters assessment also, determining mercury and histamine level to ensure product safety. The results revealed that all examined tuna samples physicochemical parameters (pH, total volatile nitrogen (TVN), and thiobarbituric acid (TBA)) were compatible with the Egyptian specifications and considered safe for consumption. The microbial examinations indicated that samples show incidence of *S. aureus* was 44% for imported tuna. While for local chunk and shredded were 56% and 67%, respectively. In addition to detection of its enterotoxins, which were 11% and 22% for local chunk and shredded samples, respectively. The enterotoxins of isolated *S. aureus* were type A, detected from local chunk and types A, C, and D from local shredded samples. The incidence of anaerobic bacteria was 22% and 33% for local chunk and shredded samples, respectively, but not detected in imported samples with no detection of *Clostridium perfringens* in all local and imported samples. However, the imported and local samples were significantly different. All local tuna samples contained high level of mercury exceeded the permissible limits of 0.5 mg/kg while, the imported samples within the limit. Also, histamine levels were found within the Egyptian Standards of (20 mg/100 g) although there was a significant difference between imported and local samples. In conclusion, the results pointed out that all examined local and imported canned tuna samples agreed with the Egyptian and other standards making them considered safe for human consumption.

KEYWORDS

Canned tuna, physicochemical, bacteriological, Sensory parameters, Mercury, Histamine

INTRODUCTION

Consuming seafood products having a great nutritional importance owing to their high levels of food constituents such as protein, fat, and polyunsaturated fatty acids such as omega-3. The strong relation between the consumption of seafood products and health benefits, such as decreased risk of autoimmune and cardiovascular diseases, is well documented (Khalili Tilami and Sampels, 2018). However, due to the high concentration of amino acids, seafood quickly spoil when exposed to both microbes and chemicals (Muscarella *et al.*, 2013).

Canning is one of the most popular and common method for fish preservation as a food for human consumption, with extended shelf life of 1 to 5 years (Sampels, 2015). Canned fish is the most processed fish product consumed in both developing and developed countries around the world. Among canned seafood products, canned tuna is the most fish product consumed in the world (Storelli *et al.*, 2010). Tuna fish is one of the most popular types of fish used in canning production. The Egyptian Organization of Standardizations (ES, 2005) stated that canned tuna is a fish product made from tuna fish. This product is manufactured

by processing of fish flesh and preserved by canning with addition of edible oils and/or brine and undergoes commercial sterilization. Canned tuna should not have anaerobic spore-forming bacteria or clostridia species.

Spoilage of fish and its products is caused by microbial growth and/or activity resulting in physical changes in sensory parameters making the product organoleptically rejected by consumer (Özogul, 2004). Fish are constantly exposed to pollutants such as toxic heavy metals through contaminated waters; so, fish and fish products are the most food chain members affected by such pollutants (Altinok-Yipel *et al.*, 2022). Significant amounts of toxic metals such as mercury accumulates in tuna fish, especially when compared to other fish species. Because of their long life age and higher feed intake, they are more exposed to higher concentrations of toxic metals than other species (Kojadinovic *et al.*, 2007; Chen *et al.*, 2012). Mercury have great toxic effects on human health, especially on the nervous system and brain and also may cause allergies, muscle weakness, and even lead to death (FDA, 2017).

Seafood products are mainly associated with or scombroid poisoning or histamine fish poisoning (HFP). In this context, fish

from the families Scombridae like tuna are the most common types of fish affected by HFP due to their high concentrations of histidine amino acid in their flesh (Rahmani *et al.*, 2018). The FDA has set a maximum histamine concentration of 50 mg/kg in fish products (Tao *et al.*, 2011; FDA, 2012). The Codex Alimentarius stated that the maximum permissible level for histamine is about 200 mg/kg (Evangelista *et al.*, 2016).

As a result, microbial, chemical and toxic hazards should be of concern to human health and because canned tuna fish is commonly consumed in Egypt, whether imported or locally produced, this study was aimed to evaluate the quality of some locally and imported canned tuna through physicochemical evaluation (pH, total volatile nitrogen (TVN), and thiobarbituric acid (TBA)), measurement of mercury and histamine, microbiological evaluation; isolation of *Staphylococcus aureus* with detection of their enterotoxins, anaerobic plate count and *Clostridium* and sensory evaluation of the products.

MATERIALS AND METHODS

Collection of samples

A total of 36 samples of imported and local canned tuna (18 for each divided into chunk and shredded tuna) were randomly collected from different shops and markets in Kafr El Sheikh Governorate, Egypt in the period from October 2021 to January, 2022. The samples were transferred to the Food Analysis Laboratory, Faculty of Veterinary Medicine, Banha University, Egypt without delay for physicochemical, microbiological, and sensory (organoleptic) evaluation.

Physicochemical evaluation

The pH of the examined tuna samples was determined according to the method of Pearson (2006). The Total volatile basic nitrogen (TVB-N) and thiobarbituric acid (TBA) concentrations were measured according to the method of ES: 63-9 (2006) and ES: 63-10 (2006), respectively.

Preparation of the samples for bacteriological examination

Ten grams of the sample were homogenized with ninety ml of sterile peptone water (0.1%) at 14000 rpm for 2.5 minutes then allowed to stand for 5 minutes at room temperature. One ml from the homogenized samples was transferred to tube containing 9 ml of sterile peptone water for preparation of tenfold serial dilutions (ISO, 2007).

Isolation and identification of *Staphylococcus aureus*

From previously serial dilutions, loopful was inoculated over Baird Parker agar plates and incubated at 37°C for 48 hours in inverted position. The black shiny colonies with narrow white margins are the characteristic morphological colonies of *S. aureus* (ICMSF, 1996). For further identification, the suspected colony from the Baird Parker agar plates were stated into semi-solid agar and incubated at 37°C for 48 hours. Further biochemical identification of *S. aureus* was performed by the method of MacFaddin (2000).

Detection and typing *S. aureus* enterotoxin

The *S. aureus* enterotoxins were detected and typed according to the method described by Shingaki (1981) using staphylo-

coccal enterotoxins kits A, B, C and D (SET-PRLA, Denka Seikei LTD, Japan).

Detection of anaerobic bacteria

One ml from each of previously prepared serial dilutions was spread over anaerobic agar plates. The inoculated plates were incubated anaerobically 35°C for 2 days. The ideal morphological colony of anaerobic bacteria was detected according to ISO (2013).

Detection of *Clostridium perfringens*

The technique recommended by Rhodehamel and Stanley (2001) was applied for *Clostridium perfringens* cultivation and detection. Appropriate one ml of dilution was spread over the surface of duplicate Tryptose Sulfite cycloserine (TSC) agar plates. The plates were overlaid with TSC agar and incubated in an anaerobic jar at 37°C for 24 hours. Accurately, *C. perfringens* appeared as small black colonies.

Determination of Mercury (Hg)

The tuna samples were firstly digested by the method of Staniskiene *et al.* (2006). The macerated tuna samples (0.5 g of each sample) were digested in 10 ml of concentrated H₂SO₄/HNO₃ solution (1:1). The tubes were sealed, shaken, and then left at room temperature overnight. The tubes heated for 4 hours, starting at 60°C and rising to 110°C with shaking every 30 minutes for complete digestion. The tubes allowed to cool at room temperature before being diluted with 0 mL of deionized water (30%) and warmed at 70°C. All organic matrices have been destroyed at this point. Each tube was diluted with deionized water till it reached 25 mL and filtered with Whatman filter paper. The filtrates were collected and stored at room temperature until analyzed for measurement of mercury concentration.

The blank and standard solutions were prepared using the protocol of Andreji *et al.* (2005). The digest, blank and standard solution were aspirated, and mercury concentration was measured using the atomic absorption spectrophotometer (VARIAN, model AA240 FS, Australia) according to the equation:

$$C1 = (A1/A2) \times C \times (D/W) \text{ mg/kg}$$

Where,

C1=concentration of mercury (mg/kg) wet weight.

A1=Absorbency reading of sample solution.

A2= Absorbency reading of standard solution.

C=Concentration of mercury on the standard solution.

D=Dilution factor of sample.

W=weight of each sample.

Determination of histamine concentration

Histamine concentration was measured by ELISA according to the protocol described by Leszczyocha and Pytasz (2004) in combination with a supplementary kit (available for purchase separately, cat. no. BA E-1100). Ten grams tuna was homogenized, and 1 g of the mixture was transferred to a falcon test tube with 9 ml deionized water. The test tube was centrifuged for 5 minutes at 2500 rpm at room temperature. After then, three phases emerged, from top to bottom: oily, watery, and solid. The oily phase was removed, and 1 mL of aquatic phase was transferred to a new test tube with 9 ml deionized water. In the third tube, 200µl of the test tube contents were added to 9.8 ml deionized water as a final dilution. After adding the sample, the sample

was acylated with the kit and Acylated histamine was bound to the antibody coated on the well surface. The extra sample was then washed off. After washing, an enzyme-conjugated antibody was added. The stopper solution was added as the final step after the incubation time. It is recommended to use control.

Sensory evaluation

Sensory evaluation was conducted by three persons at the Food Control Department, Faculty of Veterinary Medicine, Kafrelsheikh University, based on the 9-point hedonic scale and hedonic according to Svensson (2012). The samples were evaluated for taste, texture, smell, color and overall acceptability. They score from 1 to 9; 1 identified as dislike extremely, 2 dislike very much, 3 dislike moderately, 4 dislike slightly, 5 neither like nor dislike, 6 like slightly, 7 like moderately, 8 like very much, and 9 like extremely.

Statistical analysis

All data were analyzed by Microsoft Excel 365 and expressed as mean±SD.

RESULTS

Physicochemical parameters of the examined tuna samples

The physicochemical quality parameters of the examined local and imported tuna samples were shown in Table 1 and Fig. 1. The obtained results declared that all examined tuna samples were within the normal pH value recommended by the Egyptian of Standardization (ES, 2005) which stated that pH value should not be more than 6.7 where pH value was 6.2±0.1 and 6.3±0.09 for imported chunk and shredded samples, respectively, while it was, 6.5±0.14 and 6.5±0.11 for local chunk and shredded samples, respectively. However, there was a significant difference be-

tween local and imported canned tuna samples. But there were no significant differences within imported (chunk vs. shredded) or local types (chunk vs. shredded).

Concerning the TVN and TBA values of examined tuna samples, a significant difference (P < 0.05) was recorded between imported and local canned tuna samples. There is a significant difference between chunk tuna of imported and local samples. For TVN, there is no significant difference (P > 0.05) within the same local or imported types (chunk vs. shredded) while a significant difference between shredded canned tuna (imported vs. local). While TBA significantly differed between imported tuna samples (chunk vs. shredded).

Incidence of S. aureus and its enterotoxin

Table 2 and Fig. 2, declared that the incidence of *S. aureus* in canned tuna samples was 44% for both imported chunk and shredded canned tuna samples. While those for local chunk and shredded were 56% and 67%, respectively. In addition to the detection of *S. aureus* enterotoxins, which were 11% and 22% for local chunk and shredded samples, respectively. The enterotoxins of isolated *S. aureus* were type A, detected from local chunk canned tuna samples and types A, C, and D from local shredded canned tuna samples. There was no significant difference between positive and negative samples in either local or imported canned tuna samples regarding incidence of *S. aureus* or detection of enterotoxins.

Incidence of Anaerobic bacteria and Clostridium perfringens

The results showed in Table 3 and Fig. 3, recorded the incidence of anaerobic bacteria of examined canned tuna samples where the incidence percent were 22% and 33% for local chunk and shredded samples, respectively, and not detected in imported canned tuna samples. While *Clostridium perfringens* can't be detected in all examined samples.

Table 1. Statistical analysis of pH, TVN and TBA of examined canned tuna samples.

Test		Imported canned tuna (n=18, 9 each)		Local canned tuna (n=18, 9 each)	
		chunk	Shredded	chunk	shredded
pH	Mean±SD	6.2±0.1	6.3±0.09	6.5±0.14	6.5±0.11
	P-Values	P1= 0.001* ^a , P2=0.001* ^a , P3= 0.002* ^a , P4=0.119, P5=0.18			
TVN (mg/100g)	Mean±SD	6.3±1	9.1±1.2	12.3±3.5	13.7±1.9
	P-Values	P1= 0.001* ^a , P2=0.002* ^a , P3= 0.001* ^a , P4=0.055, P5=0.7			
TBA (mg/kg)	Mean±SD	0.9±0.4	2±0.4	2.1±1.0	2.1±0.85
	P-Values	P1= 0.027* ^b , P2=0.019* ^b , P3= 0.4, P4=0.015* ^b , P5=0.45			

(*) Statistically tests used: (a): T-test, (b): Mann-Whitney, P1: Imported Vs. Local, P2: Chunk tuna (Imported vs. Local), P3: Shredded tuna (Imported vs. Local), P4: Imported Tuna (Chunk vs. Shredded), P5: Local Tuna (Chunk vs. Shredded)

Table 2. Incidence of *Staphylococcus aureus* and its enterotoxin in examined canned tuna samples.

Types of samples (n.=36, 9 each)	Incidence of <i>S. aureus</i>		Detection of enterotoxin		Type of enterotoxin
	Positive no. (%)	Negative no. (%)	Positive no. (%)	Negative no. (%)	
Imported Chunk	4 (44)	5 (56)	0 (0)	9 (100)	-
Imported Shredded	4 (44)	5 (56)	0 (0)	9 (100)	-
Local chunk	5 (56)	4 (44)	1 (11)	8 (89)	A
Local Shredded	6 (67)	3 (33)	2 (22)	7 (78)	A, D, C
X ²	1.23		4		N/A
P-Value	0.75		0.26		

X²: Chi- square

Determination of mercury and histamine levels of examined canned tuna samples

The mercury (Hg) and histamine levels in examined canned tuna samples were summarized in Table 4 and Fig. 4. In contrast the examined local tuna samples exceeded the permissible limit. The results showed that the mean±SD was 0.3±0.26 and 0.4±0.26 mg/100 g for imported chunk and shredded samples, respectively. While it was 0.5±0.3 and 0.62±0.3 mg/100 g for local chunk and shredded samples, respectively. No significant difference between all examined samples.

For histamine level, the mean±SD for imported chunk and shredded tuna was 4.6±2.8 and 8.2±2.7, respectively. Whereas for local chunk and shredded tuna, it was 9.4±4.3 and 10.8±3.63, respectively. There was a significant difference between imported and local canned tuna samples, but no significant difference

between imported (chunk vs. shredded) and local types (chunk vs. shredded).

Sensory evaluation

The organoleptic parameters of local and imported canned tuna samples depending on a 9-point hedonic score seen in table 5. The results revealed imported and local canned tuna samples were significantly different, with a higher hedonic score of imported chunk samples (above 7) in all sensory parameters (taste, texture, smell, color and overall acceptability).

DISCUSSION

In addition to being a healthy fish product with high nutritional benefits, Tuna may not be safe food for human consump-

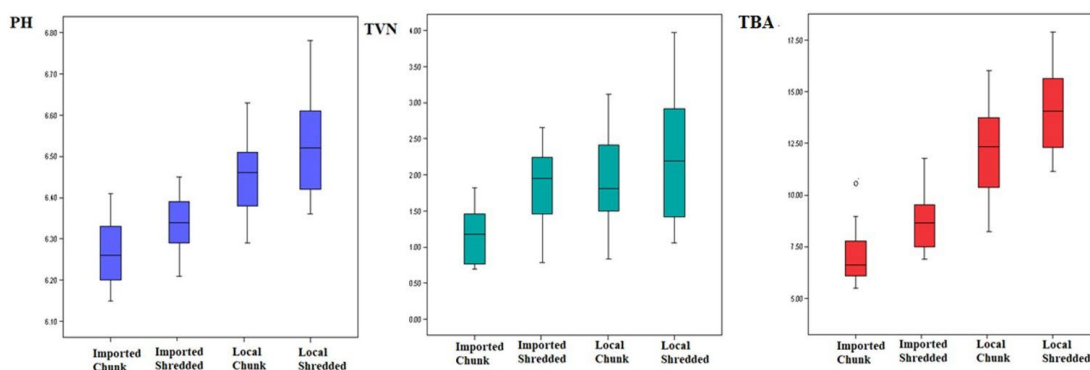


Fig. 1. Boxplot; pH, TVN and TBA of examined canned tuna samples.

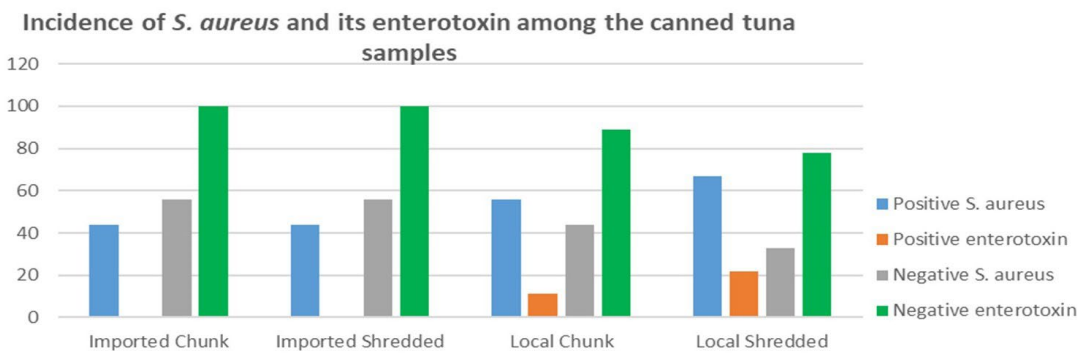


Fig. 2. Incidence of S. aureus and its enterotoxins among canned tuna samples.

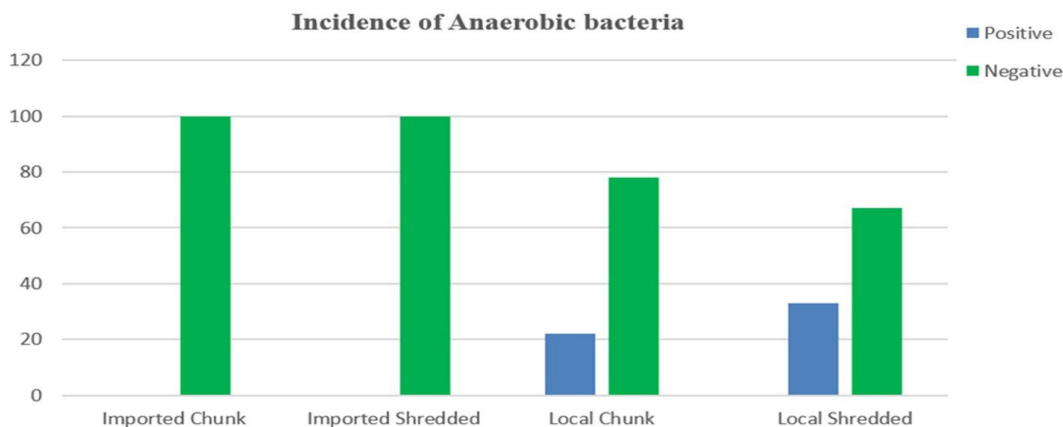


Fig. 3. Incidence of Anaerobic bacteria among the examined canned tuna samples.

tion and act as a source of food-borne intoxications and pollutants (Bosch et al., 2016).

Total volatile Nitrogen is regarded as fish quality indicator as well as index of freshness and spoilage degree (Wong and Gill, 1987). TVN values of the canned tuna samples were within the permissible limit of Egyptian Organization of Standardizations ES (2005) of not more than 40 mg/100g and within the acceptable limit (30–45 mg/100g) stated by EC (1995). The TVN results in the present study were nearly similar to the result of ElShehawy and Farag (2019). The TBA index is a measurement of malonaldehyde (MDA) level, which is one of the lipids hydroperoxide degradation products produced during the oxidation of polyunsaturated fatty acids (Gomes et al., 2003). According to the permissible limit of TBA value (4.5 mg MDA/kg) recommended by ES (2005), all examined samples were accepted for human consumption. The higher value of TVN and TBA of shredded tuna may be due to

the endogenous enzyme activity and spoilage bacteria activity (Hosseini et al., 2016). This also may be attributed to that chunk tuna being obtained usually from high grade cuts of fish whereas shredded tuna was obtained from low grade cuts or trimming of main parts of the fish, which may lead to protein and lipid degeneration (Murthy et al., 2012).

For *S. aureus*, the obtained results were not compatible with the Egyptian Organization of Standardization (ES, 2005), which stated that canned fish products shouldn't have *S. aureus* in canned fish. Since humans represent the first reservoir of *S. aureus* (FDA, 2011), handling and manipulation during preparation and/or processing steps may contaminate tuna. Furthermore, frozen storage for a long time before industry doesn't inhibit *S. aureus* count as it persists in frozen tuna loins at -20°C for 4 weeks and rapidly increases population up to 3 log cfu/g when thawed (Wu and Cheng, 2014). Therefore, the presence of *Staph-*

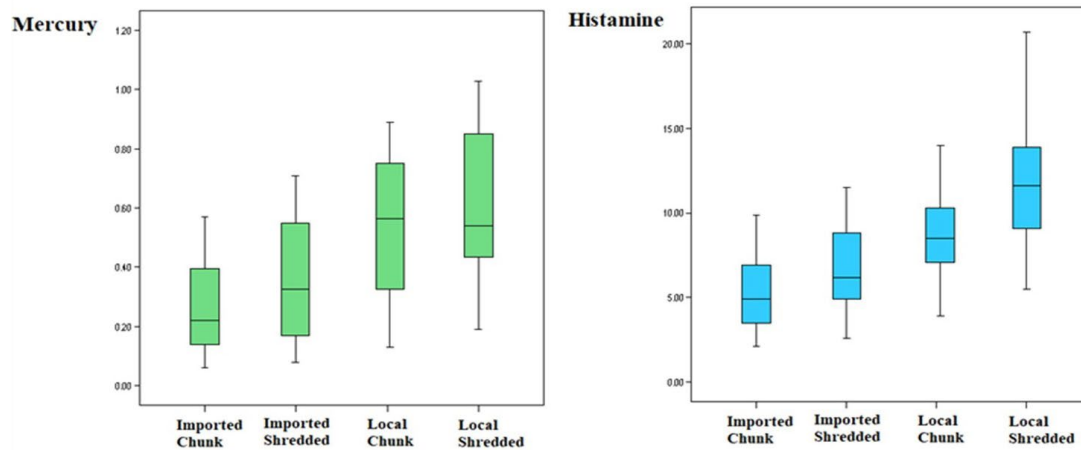


Fig. 4. Boxplot; mercury and histamine levels of examined canned tuna samples.

Table 3. Incidence of Anaerobic bacteria and *Clostridium perfringens* in examined canned tuna samples.

Types of samples (n.=36, 9 each)	Anaerobic bacteria		Detection of <i>Clostridium perfringens</i>
	Positive no. (%)	Negative no. (%)	
Imported Chunk	0 (0)	9 (100)	ND
Imported Shredded	0 (0)	9 (100)	ND
Local chunk	2 (22)	7 (78)	ND
Local Shredded	3 (33)	6 (67)	ND
X ²	6.3		N/A
P-Value	0.10		

X²: Chi- square, ND: non-detected

Table 4. Sensory evaluation of imported and local canned tuna samples.

Parameters Score 9 points each	Imported canned tuna				Local canned tuna			
	Chunk		Shredded		Chunk		Shredded	
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median
Taste	7.3±0.7	7	5.6±1.0	5	5.6±1.3	7	4±1.3	4
P-Values	P1= 0.0001*, P2=0.0001*, P3= 0.0001*, P4=0.0001*, P5=0.0001*							
Texture	7.7±0.8	8	4.1±1.8	4	6±0.8	6	4±1.9	4
P-Values	P1= 0.012*, P2=0.0001*, P3= 0.0001*, P4=0.0001*, P5=0.0001*							
Smell	7.3±1.1	7	4.8±1.4	5	5.7±1.4	6	3±1.6	3
P-Values	P1= 0.0001*, P2=0.0001*, P3= 0.001*, P4=0.0001*, P5=0.0001*							
Color	7.3±1.2	7	4.5±1.7	4	5.7±1.3	6	3±1.8	3
P-Values	P1= 0.001*, P2=0.002*, P3= 0.078, P4=0.0001*, P5=0.0001*							
Overall acceptability	29.5±2.4	29	19±4.9	18	23±3.5	22	15±5.7	15
P-Values	P1= 0.0001*, P2=0.0001*, P3= 0.004*, P4=0.0001*, P5=0.0001*							

P1: Imported vs. local; P2: Chunk tuna (Imported vs. Local); P3: Shredded tuna (Imported vs. Local); P4: Imported Tuna (Chunk vs. Shredded); P5: Local Tuna (Chunk vs. Shredded)

Table 5. Statistical analysis of mercury and histamine levels of examined canned tuna samples.

Test		Imported canned tuna (n=18, 9 each)		Local canned tuna (n=18, 9 each)	
		Chunk	shredded	Chunk	shredded
Mercury mg/100g	Mean±SD	0.3±0.26	0.4±0.26	0.5±0.3	0.62±0.3
	P-Values	P1= 0.077, P2=0.4, P3=0.13, P4=0.48, P5=0.79			
Histamine mg/100g	Mean±SD	4.6±2.8	8.2±2.7	9.4±4.3	10.8±3.63
	P-Values	P1= 0.002*, P2=0.024*, P3=0.016*, P4=0.25, P5=0.16			

(*) Statistically tests difference.

P1: Imported Vs. Local; P2: Chunk tuna (Imported vs. Local); P3: Shredded tuna (Imported vs. Local); P4: Imported Tuna (Chunk vs. Shredded); P5: Local Tuna (Chunk vs. Shredded)

Y. enterocolitica in all the examined samples can be due to the manual handling of pre-cooked tuna fish prior to processing or during a faulty processing step of the canning operation. *S. aureus* enterotoxin detection in the examined local tuna samples could be attributed to that it is a thermostable toxin, normally produced when *S. aureus* reaches count of 5-6 Log cfu/g and able to resist the canning process (Le Loir et al., 2003).

On the other hand, the anaerobic bacteria detected in the local tuna in contrast with the results obtained by ElShehaw and Farag (2019) with no incidence *C. perfringens* in all examined tuna samples. *Clostridium perfringens* can't be detected in all examined samples as recommended by ES (2005) and GSO (2016) which stated that canned tuna shouldn't have *Clostridium*. Proper handling of fish after catching and further processing steps could reduce the chance of presence and growth of *C. perfringens* (Lalitha and Lyer, 1986).

Mercury has been identified as a potential human carcinogen (Occupational Safety and Health Administration, 2004). Because of the pollution of the aquatic environment, heavy metals accumulate in fish and cause health problems. As fish are more susceptible to toxins than invertebrates, they are a useful indicator of pollution in the water (Moiseenko et al., 2008; Mendil et al., 2010). All examined imported tuna samples within the permissible limit according to CIFA, (1992); EC, (2005); ES, (2010) which stated that Mercury level should not be more than 0.5 mg/kg. The obtained results for imported tuna samples were nearly similar to the study conducted on tuna samples in developing countries by Ulusoy (2023). Ashraf, (2006) recorded that mercury levels were ranged from 0.20-0.66 mg/kg in Libya, 0.043-0.25 mg/kg in Iran and 0.18-0.86 mg/kg in Saudi Arabia, respectively. The study conducted by Hajrić et al. (2022) reported the mean value of mercury concentration in the tuna samples was 0.165 mg/kg. The decrease of mercury level can be done selection of smaller, younger tuna fish during processing and the strict heat treatments during canning (Jinadasa et al., 2021). The higher mercury values in local canned tuna may be attributed to the geographical area from which tuna fish is obtained (Silva and Lima, 2020).

Fish and fish products mainly associated with histamine fish poisoning (HFP) especially tuna fish and canned tuna due to the presence of high levels of histidine amino acid in its muscle (Rahmani et al., 2018) in presence of histamine producing bacteria which produce histidine decarboxylase enzyme (López-Sabater et al., 1996; Kim et al., 2001). Although the histamine levels of the canned tuna samples were found within the Egyptian Standards according to ES (2010), which recommended that the maximal permissible limits for histamine be (20 mg/100 g), there was a significant difference between local and imported canned tuna samples. Similar results were obtained by Naficeh et al. (2019) who examined 56 samples of canned tuna in Tehran and found that histamine concentration was 5.75 mg/100 gm. The lower levels of histamine in the examined local and imported tuna samples might be attributed to HACCP and GHP application during the various stages of processing of canned tuna starting from harvesting until storing (Peivasteh-Roudsari et al., 2020).

For sensory evaluation, High hedonic score of imported chunk tuna samples may be due to their prevalence in the previous evaluations, either physicochemical or microbial. ElShehaw and Farag

(2019) reported that the average scores of sensory properties of canned tuna samples were high. These results agreed with Maheswara et al. (2011) who recorded that the organoleptic quality of canned tuna products preserved in curry packed in free-steel and tin cans was good with score of 8 /10 even after storage of 5 months at room temperature.

CONCLUSION

Generally, from this study, it can be concluded that the investigated imported and local canned tuna are compatible with Egyptian specifications from the physicochemical view. However, canned tuna are polluted with mercury metal. Microbiologically, the incidence of *S. aureus* is detected in all canned tuna. The detection of *S. aureus* enterotoxins is limited to local canned tuna. Anaerobic bacteria are detected in local but not in imported canned tuna. While *Clostridium perfringens* can't be detected in all examined tuna. Therefore, human exposure to the danger of mercury toxicity increases when large amounts of canned tuna are consumed. Consumers should consume tuna in moderation to avoid the possible risk of mercury toxicity.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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