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Monitoring the Bacteriological Contamination and Histamine Formation in Canned Tuna

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ABSTRACT

Canned tuna is a good source of nutrients and minerals that must be included in food. However, there are risks associated with canned fish, such as bacterial and chemical contamination causing risk hazards to consumers. 150 samples of various canned tuna brands (50 of each solid, chunk and crumbled tuna) were collected from different supermarkets in Elfaiyoum Governorate to determine bacteriological and chemical quality. The results showed that crumbled tuna's aerobic plate count was the highest ($9.70\times10^3\pm4.70\times10^3$). In contrast, the total anaerobic count was the highest in chunk tuna ($6.68\times10^3\pm3.16\times10^3$). 19.3% of samples were contaminated with *Vibrio* spp. Additionally, *V. parahaemolyticus* was detected in chunk and crumbled tuna at 12% and 18%, respectively, with no detection in solid tuna samples. Furthermore, 32% of samples have *Aeromonas* spp. However, *A. hydrophila* was found in 20% of the examined samples. Proximate analysis revealed that the crumbled tuna recorded the highest value of moisture content (68.12±0.5) and fat content (11.23±0.25); meanwhile, solid tuna had a higher protein value (24.10±0.33). Concerning the cholesterol content (mg/100gm), a high concentration was recorded in crumbled tuna and chunk tuna samples, with a mean value of 44.62± 1.3 and 42.3± 1.02, respectively, followed by solid tuna, with a mean value of 39.72 ± 1.02. Furthermore, solid tuna samples had the lowest histamine concentrations with a mean value of 48.33±1.12 ppm. In contrast, crumbled and chunk tuna had the highest concentrations of 62.66±1.59 and 56.00±1.86 ppm. In conclusion, canned tuna have accepted chemical parameters. However, they might be regarded as a risk for microbiological hazards; thus, competent authorities and food business operators should pay careful attention.

INTRODUCTION

Given that fish include macronutrients (proteins, fats, and ash) and micronutrients (vitamins and minerals), they represent a valuable component of the human diet. These nutrients are essential for human nutrition and have been shown to play a role in several metabolic processes **(Chandravanshi *et al.*, 2019)**. Canned tuna is widely recognized as one of the most common fishery products in the world **(Fattore *et al.*, 2015)**. Foodborne

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illnesses are considered a major public health challenge worldwide due to their incidence, associated mortality and negative economic impacts. Contamination can be caused by *Salmonella* spp., *Vibrio* spp., *Aeromonas* spp. and *Clostridia* spp., which are naturally present in all aquariums **(Sheng *et al.*, 2021)**. Consequently, fish form a frequent source of food poisoning and can lead to various ailments, from minor maladies to chronic or life-threatening conditions **(Dhanalakshmi *et al.*, 2014)**. Fish and fish products are highly sensitive foods that should be stored and handled carefully. The high portability of fish products is mostly caused by their particular content and structure although the length of storage period and temperature impact how well a product turns out **(Hassoun *et al.*, 2017)**. The main reason for perishable fish is due to the high composition of non-protein nitrogen contents and the low acidity (pH>6) of the meat, which are favorable environments for the growing of metabolites producing microorganisms, as well as affecting the quality of fish products **(Sivertsvik *et al.*, 2022)**.

The presence of biogenic amines in fish is directly associated with decarboxylase activity by microbes. These compounds are usually detoxified by oxidation within the human digestive tract, but some conditions may make food consumption unsafe. Because of its toxicity, histamine or scombrotoxin is a unique biogenic amine with regulatory limits on fish products. Biogenic amines are a measure of the hygienic quality of tuna as a result of relationships with microbial counts. Specifically, histamine has often been used as an indicator of tuna decomposition **(Visciano *et al.*, 2020)**. Therefore, this study was pointed to evaluate the quality of some canned tuna through the assessment of microbial quality by determining the bacterial load as well pathogens that cause food poisoning. *Aeromonas* spp., *Vibrio* spp. and their virulence genes were isolated and identified by PCR technique. Additionally, the estimation of the level of histamine and cholesterol contents were assessed to ensure the public health condition of canned tuna.

MATERIALS AND METHODS

Sampling

About 150 samples of various canned tuna brands (50 of each solid tuna, chunk tuna and crumbled tuna) were randomly selected from several supermarkets in the governorate of Elfaiyoum, Egyp during January 2023. Immediately after being collected, samples were taken to the lab for bacteriological and chemical evaluations.

Bacteriological examination (ISO, 2007)

The examined canned tuna samples were aseptically opened, and 10g of each specimen was added to 90ml of sterile peptone water (0.1%) before being homogenized at 14000 rpm for 2.5min to produce a homogenate of 1/10 dilution. One ml of this homogenate was transferred to a test tube containing nine ml of sterile peptone water (0.1%) to create 10^{-2}, from which tenth-fold serial dilutions up to 10^{-3} were produced.

1- *Total aerobic count* (Anihouvi *et al.,* 2019)

Two correctly labelled double Petri dishes received one ml of each serially made dilution, which was inoculated individually. A standardized agar was added after melting and cooling to 45ºC in each inoculated petri dish, then solidified before incubating at 37ºC for 48 hs.

2- *Total anaerobic count* (Youssef *et al*., 2021)

Two properly marked duplicated Petri plates containing reinforced clostridial agar were individually inoculated with one ml from each previously prepared serial dilution. These dishes were subsequently set in an anaerobic jar and incubated at 37°C for 48hs.

3- *Isolation and identification of Vibrio* spp. (Möller *et al*., 2021)

Ten g of each sample were incubated for 24hs at 37°C in 90ml of alkaline peptone water (A.P.W.). Thiosulfate citrate bile salts sucrose agar plates (TCBS, Hi-Media - India) were used to plate a looping alkaline peptone water, which was then incubated at 37°C for 24hs. The presumed *Vibrio* colonies were detected following collection, purification, and biochemical identification following ISO/TS 21872-1 and ISO/TS 21872-2 (2007). The colonies of *V. furnissii, V. cholera, V. alginolyticus* and *V. fluvialis* were smooth and yellow (sucrose positive), while typical colonies of *V. parahaemolyticus, V. vulnificus,* and *V. mimicus* were smooth and green (sucrose negative).

4- *Isolation and identification Aeromonas* spp. (Akmal *et al.*, 2020)

Alkaline peptone water (A.P.W.) was incubated with 10g of each sample individually in 90ml for 24hs at 37°C. An *Aeromonas* isolation medium agar plate treated with ampicillin (5 mg/L) was streaked with a looped A.P.W. before incubating at 37°C for 24hs. Green colonies with black centres suspected of *A. hydrophila* colonies were purified and biochemically identified **(Carnahan *et al.*, 1991)**.

5- *Molecular identification of V. parahaemolyticus and A. hydrophila virulence genes*

Biochemically identified colonies of *V. parahaemolyticus* were subjected to genetic identification of *tox*R and *L.tdh* virulence genes, while *A. hydrophila* isolates were subjected to molecular identification of *aerolysin* (*aerA*) and *haemolysin* (*ahh1*) virulence-associated genes. Bacterial D.N.A. was extracted according to GeneJETGenomic D.N.A. Purification Kit (Catalog No. #K0721, Thermo Scientific, U.S.A.) manufacturer's guidelines. The primers used are shown in Table (1), and the cycling conditions for *ToxR* are 94°C/10min, 20 cycles of 94°C for 60sec, 63°C/ 90sec, 72°C/ 90sec. For *L.tdh*, the cycling conditions are 94°C/ 5min, 30 cycles of 94°C for 60sec, 54°C/ 60sec & 72°C/ 60sec, followed by final elongation at 72°C/ 10min. While, for *aerA* and *ahh1*, conditions are 95°C/ 5min, 30 cycles of 95°C for 30sec, 59°C/ 30sec & 72°C/ 30sec, followed by final elongation at 72°C/ 7min.

Table 1. Oligonucleotide primers and amplified products used

Bacterium	Target gene	Oligonucleotide sequence (5′- 3′)	size (bp)	Reference
V. Parahaemolyticus	*ToxR*	GTCTTCTGACGCAATCGTTG	366	Lee (2018)
		ATACGAGTGGTTGCTGTCATG		
	L.tdh	CCATCTGTCCCTTTTCCTGC	373	Cohen *et al.* (2007)
		CCAAATACATTTTACTTGG		
A. hydrophila	*aerA*	CAAGAACAAGTTCAAGTGGCCA	309	Stratev *et al.* (2016)
		ACGAAGGTGTGGTTCCAGT		
	ahh1	GCCGAGCGCCCAGAAGGTGAGTT	130	
		GAGCGGCTGGATGCGGTTGT		

Chemical analysis

Proximate composition analysis. according to the procedures outlined by the Association of Official Analytical Chemists, AOAC (2016), this analusis was done on drained samples of canned tuna to evaluate moisture, crude protein, crude fat, ash and carbohydrate.

***Determination of caloric value of examined products*.** It was determined using the formula provided by **Merrill and Watt (1973)**.

Determination of cholesterol content (mg/100gm) by HPLC. It was evaluated based on the methodology and quantification used by **Kolaric and Imko (2020)**.

***Determination of histamine content (ppm) by HPLC*.** It was assessed based on the methodology and quantification used by **Pawul-Gruba *et al.* (2014)**.

Statistical study

Completely randomized design and general linear models were used in the statistical analysis, which was conducted using the SPSS program **(SPSS, 2009)**. Mean separation was determined using Duncan's multiple range test with $P \leq 0.05$.

RESULTS

Table (2) shows the mean value of total aerobic count (cfu/g) of solid tuna, chunk tuna and crumbled tuna were $4.35\times10^2\pm6.28\times10$, $5.05\times10^3\pm1.21\times10^3$ and $9.70\times10^3\pm4.70\times10^3$, respectively. In comparison, the mean of total anaerobic count (cfu/g) of solid tuna, chunk tuna and crumbled tuna were $5.25\times10^2\pm2.12\times10$, $6.68\times10^3\pm3.16\times10^3$ and $5.48\times10^3\pm2.42\times10^3$, respectively (Table 3).

Table 2. Total aerobic count (cfu/g) of the examined canned tuna (n=50, each)

Sample	Minimum	Maximum	Mean ± SD
Solid tuna	3.50×10^{2}	5.00×10^{3}	$4.35\times10^{2}\pm6.28\times10^{a}$
Chunk tuna	3.70×10^{3}	6.65×10^{3}	$5.05\times10^{3}\pm1.21\times10^{3b}$
Crumbled tuna	3.98×10^{3}	1.550×10^{4}	$9.70\times10^{3}\pm4.70\times10^{3c}$

$P\leq0.0001$ is considered extremely significantly different. Mean values with the same letters in each column have no significant difference.

Table 3. Total anaerobic count (cfu/g) of the examined canned tuna (n=50, each)

Sample	Minimum	Maximum	Mean ± SD
Solid tuna	2.33×10^{2}	7.46×10^{3}	$5.25\times10^{2}\pm2.12\times10^{a}$
Chunk tuna	2.21×10^{3}	9.56×10^{3}	$6.68\times10^{3}\pm3.16\times10^{3b}$
Crumbled tuna	5.48×10^{3}	10.52×10^{3}	$5.48\times10^{3}\pm2.42\times10^{3b}$

$P\leq0.05$ is considered extremely significantly different. Mean values with the same letters in each column are not significantly different.

The frequency and species of *Vibrio* in canned tuna displayed in Table (4) show that *Vibrio* spp. were present in 19.3% of the analyzed products, and the main *Vibrio* species isolated were *V. Parahaemolyticus* (10%), *V. alginolyticus* (0.67%), *V. cholera* (5.3%), and *V. mimicus* (3.3). Fig. (1) shows that the *ToxR* gene was present in 60% of the tested *V. Parahaemolyticus* isolates. However, the *L.tdh* virulence gene was found in 30% of the samples (Fig. 2).

Table 4. Prevalence and species of *Vibrio* spp. in canned tuna samples (no = 50/ each)

Canned tuna	*Vibrio* spp.		*V. Parahaemolyticus*		*V. alginolyticus*		*V. cholera*		*V. mimicus*	
	No.	%	No.	%	No.	%	No.	%	No.	%
Solid tuna	0	0	0	0	0	0	0	0	0	0
Chunk tuna	13	26	6	12	1	2	3	6	3	6
Crumbled tuna	16	32	9	18	0	0	5	10	2	4
Total	29	19.3	15	10	1	0.67	8	5.3	5	3.3

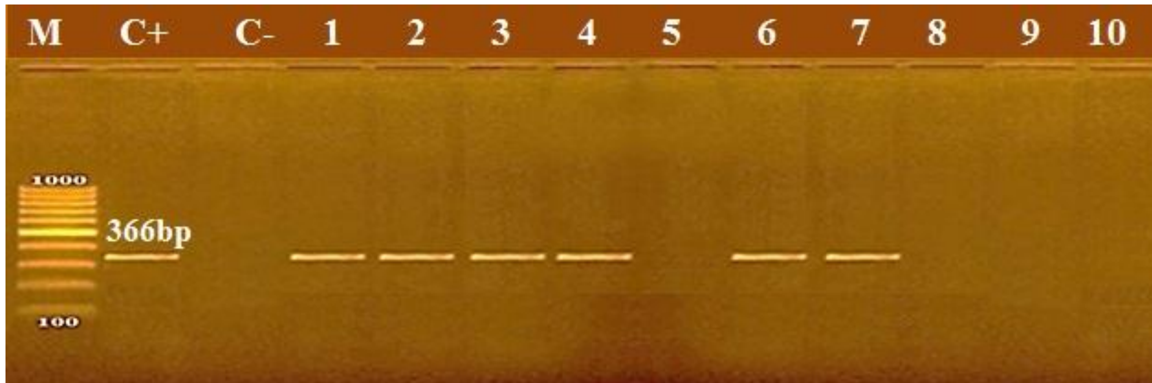

Fig. 1. Electrophoretic gel for detection of *ToxR* in *V. parahaemolyticus* at 366 bp. M= Marker (100bp); C+: Control positive; C-: Control negative. Lane 1, 2, 3, 4, 6 and 7 positive for *ToxR* gene.

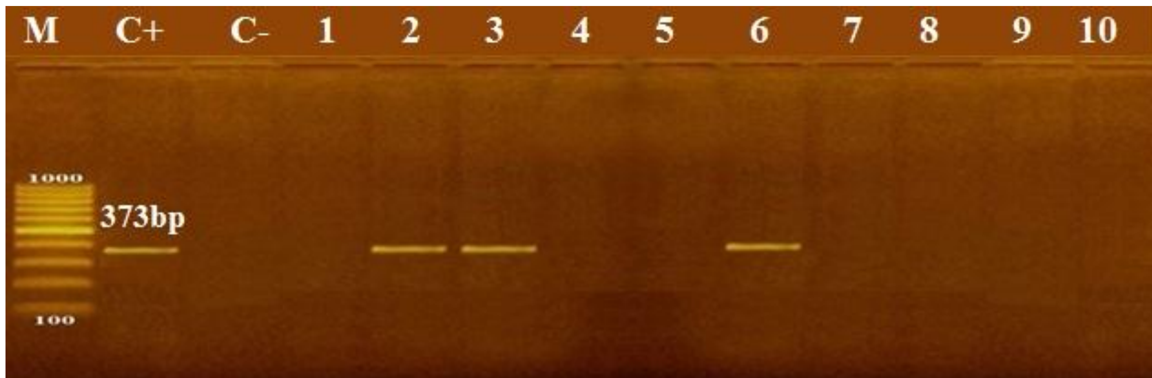

Fig. 2. Electrophoretic gel for detection of *L.tdh* in *V. parahaemolyticus* at 373 bp. M= Marker (100bp); C+: Control positive; C-: Control negative. Lane 2, 3 and 6 positive for *L.tdh* gene.

Aeromonas spp. identified with a proportion of 32% from all tested samples according to a bacterial analysis of the samples (Table 5). The most predominant isolated *Aeromonas* spp. were *A. hydrophila* (20%), *A. sobria* (3.3%), *A. fluvialis* (0.67%) and *A. caviae* (8%). It was found that, 70% of examined *A. hydrophila* isolates had *aerA* gene (Fig. 3). However, 30% had *ahh1* virulence genes (Fig. 4).

Table 5. Prevalence and species of *Aeromonas* sp. in canned tuna samples (no=50/ each)

Canned tuna	*Aeromonas* spp.		*A. hydrophila*		*A. sobria*		*A. fluvialis*		*A. caviae*	
	No.	%	No.	%	No.	%	No.	%	No.	%
Solid tuna	9	18	6	12	0	0	0	0	3	6
Chunk tuna	17	34	9	18	3	6	1	2	4	8
Crumbled tuna	22	44	15	30	2	4	0	0	5	10
Total	48	32	30	20	5	3.3	1	0.67	12	8

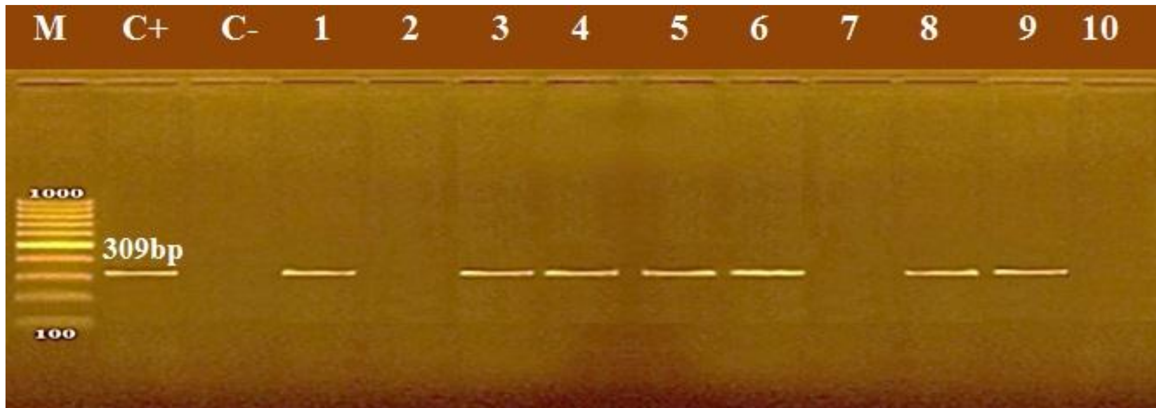

Fig. 3. Electrophoretic gel for detection of *aerA* in *A. hydrophila* at 309 bp. M= Marker (100bp); C+: Control positive; C-: Control negative. Lane 1, 3, 4, 5, 6, 8 and 9 positive for *aerA* gene.

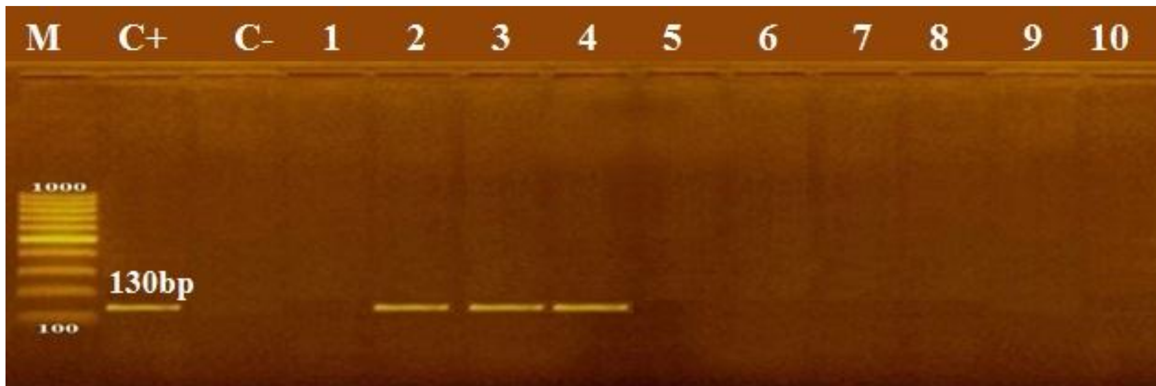

Fig. 4. Electrophoretic gel for detection of *ahh1*in *A. hydrophila* at 130 bp. M= Marker (100bp); C+: Control positive; C-: Control negative. Lane 2, 3 and 4 positive for *ahh1*gene.

The data presented in Table (6) show the statistical findings of the proximate analysis of the tested samples of canned tuna. While, Table (7) revealed that the caloric value (kcal/100gm) of the examined solid tuna, chunks and crumbled tuna were 188.63 ± 4.6, 184.36 ± 4.52 and 197.18 ± 3.25, respectively. Furthermore, Table (8) exhibits the cholesterol content (mg/100gm) with values of 39.72 ± 1.02, 42.3± 1.02 and 44.62± 1.3 in solid tuna, chunks tuna, and crumbled tuna, respectively. Meanwhile, the histamine concentrations "ppm" were 48.33±1.12, 56.00±1.86 and 62.66±1.59 in solid, chunks, and crumbled tuna, respectively (Table 9).

Table 6. Proximate analysis of examined canned tuna samples

Product	Mean ± SD*				
	Moisture	Protein	Fat	Ash	Carbohydrate
Solid tuna	64.13^{a} ± 0.54	24.10^{a} ± 0.33	5.52^{a} ± 0.31	1.07^{a} ± 0 .05	3.42^{a} ± 0.25
Chunk tuna	66.19^{a} ± 0.38	22.29^{b} ± 0.46	7.55^{b} ± 0.33	1.53^{a} ± 0.07	4.87^{a} ± 0.35
Crumbled tuna	68.12^{b} ± 0.5	20.52^{c} ± 0.26	11.23^{c} ± 0.25	1.92^{a} ± 0.1	4.73^{a} ± 0.64

*Mean and standard deviation. $P \leq 0.0001$ is considered significantly different. Mean values with the same letters in each column do not have significant difference.

Table 7. Caloric value (kcal/100gm) of the examined canned tuna samples

Product	Minimum	Maximum	Mean ± SD
Solid tuna	168.37^{a}	217.46^{a}	188.63 ± 4.6^{a}
Chunk tuna	162.72^{b}	224.52^{b}	184.36 ± 4.52^{b}
Crumbled tuna	177.21^{b}	257.14^{c}	197.18 ± 3.25^{b}

$P \leq 0.0001$ is considered significantly different. Mean values with the same letters in each column are not significantly different.

Table 8. Cholesterol content (mg/100gm) in the examined canned tuna samples

Product	Minimum	Maximum	Mean + SD
Solid tuna	31.5	54.32	39.72 ± 1.02[a]
Chunk tuna	33.64	55.92	42.3± 1.02[a]
Crumbled tuna	34.28	59.47	44.62± 1.3[a]

$P \leq 0.0671$ is considered not significantly different.

Table 9. Histamine concentrations "ppm" in the examined canned tuna samples

Canned tuna	Minimum	Maximum	Mean ± SD
Solid tuna	30	60	48.33±1.12[a]
Chunk tuna	41	70	56.00±1.86[b]
Crumbled tuna	44	83	62.66±1.59[b]

$P \leq 0.001$ is considered significantly different. Mean values with the same letters in each column are not significantly different.

DISCUSSION

Bacteriological analysis

Fish can become contaminated with bacteria directly from dirty water or indirectly via secondary contamination during handling, processing, storage, distribution or preparation. When fish are eaten raw or with minimal processing, this contamination is more important. *Aeromonas* is one of the most common bacterial species causing fish deterioration **(Yemmen & Gargouri, 2022)**. Aerobic plate counts can detect bacterial contamination and hygienic measures during fish production (**Møretrø *et al.*, 2019**). Contamination by microbiological infections is the most important concern, with respect to the security of fish products. Additionally, fish products are extremely susceptible to spoiling because of their high water content, neutral pH, high quantities of amino acids, and naturally existing autolytic enzymes **(Jeyasekaran *et al*., 2006)**.

It is clear from the results of this study (Table 2) that the highest aerobic plate count (cfu/g) in the inspected canned tuna was found in crumbled tuna ($9.70\times10^3\pm4.70\times10^3$), followed by chunk tuna ($5.05\times10^3\pm1.21\times10^3$), while the solid tuna recorded the lowest, with a mean value of $4.35\times10^3\pm6.28\times10^2$. In addition, the differences in the count among

the examined products were considered significant at $P<0.05$. All counts fell below the acceptable threshold set by the ICMSF (International Commission on Microbiological Specifications for Foods) of 1.0 10^6 CFU/g. Quality, shelf life, and post-heat processing contamination can all be determined using the aerobic plate count **(Ibrahim *et al.*, 2016)**. Moreover, the aerobic bacteria were detected by **Saleh *et al.* (2007)** in 53.3% of tuna with mean values of 2.16 x $10^2 \pm 7.2$ x 10/g. Higher counts of A.P.C. values were achieved in the studies of **Ibrahim *et al.* (2016)** and **Agwa *et al.* (2018)**. Whereas, **Stratev *et al.* (2015)** and **ElShehawy and Farag (2019)** reported low results. An important category of anaerobic bacteria is to blame for many health risks posed to people who ingest canned fish. As a result, the information obtained in Table (3) shows the average value of the total anaerobic plate count (cfu/g) of the samples that were analyzed, revealing that the chunk tuna was the highest total anaerobic count ($6.68\times10^3\pm3.16\times10^3$), followed by crumbled tuna ($5.48\times10^3\pm2.42\times10^3$). Meanwhile, solid tuna had a mean anaerobic count of $5.25\times10^2\pm2.12\times10$. The difference between the analyzed samples was statistically significant at $P<0.05$. Additionally, all examined products did not match the **E.O.S. (808, 2005)** and **G.S.O. (1817, 2016)** standards based on the anaerobic count, which stated that the canned tuna should be free from anaerobic microbes. **Saad *et al.* (2021)** obtained higher results; they stated a mean of 1.7 x$10^3\pm9.2$ x10^2/g for the examined canned tuna. *Vibrio* spp. are microbiological water-borne diseases that are mostly found in various types of seafood and make people more vulnerable to dangers regarding their health **(Semenza & Paz, 2021)**.

The current study identified 19.3% of *Vibrio* species in the examined products. *V. parahaemolyticus* was identified in 10%, *V. alginolyticus* in 0.67%, *V. cholera* in 5.3%, and *V. mimicus* was found in 3.3%. Furthermore, all samples of solid tuna were free from *V. parahaemolyticus*; however, 16% and 12% of chunk and crumbled tuna were contaminated with *V. parahaemolyticus*. The results match with those of **Refai *et al.* (2020)** who recorded that, the prevalence of *V. parahaemolyticus* in the examined products was 10%. Lower isolation rates were reported in the studies of **Ahmed *et al*. (2018)**, **Suresh *et al*. (2018)** and **Yan *et al*. (2019)**, recording values of 0.9%, 6.9%, and 3.89% for *V. parahaemolyticus*. Contrarily, **Morshdy *et al.* (2022)** recorded a percentage of 42.3% for all examined samples containing *Vibrio* spp. The variation in *V. parahaemolyticus* percentages may be attributed to improper handling, lack of hygiene, variations in storage temperature and cross-contamination **(Letchumanan *et al*., 2015)**. The *toxR* gene, which is highly conserved amongst *V. parahaemolyticus*, is the target of a PCR-based test that has gained popularity for the molecular detection and identification of *V. parahaemolyticus* in seafood samples **(Zaafrane *et al.*, 2022)**. According to the findings of the current investigation, 60% and 30% of the tested isolates possessed the regulator toxin (*toxR*) and the *L.tdh* virulence gene (Fig. 1, 2). The *toxR* genes were found in every *V. parahaemolyticus* isolate, as recorded in the studies of **Yen *et al.* (2021)** and **Morshdy *et al.* (2022)**. However, **Almejhim *et al.* (2021)** discovered that only 26 of 120

isolates of *V. parahaemolyticus* (21.7%) tested positive for the *toxR* gene, which is a lower percentage than previously reported.

In addition to being a fish pathogen, *A. hydrophila* is a zoonotic pathogen that can infect humans and cause illnesses such as gastroenteritis, septicemia, and infections of traumatic and aquatic wounds (**Stratev & Odeyemi, 2016**). The data achieved in the current study illustrate that 32% of examined samples were contaminated with *Aeromonas* spp. Additionally, 20%, 8%, 3.3% and 0.67% of the samples were *A. hydrophila*, *A. caviae, A. sobria* and *A. fluvialis*. The highest incidence of *A. hydrophila* was observed in crumbled tuna (30%) and chunk tuna (18%), followed by 12% for solid tuna, respectively. Additionally, **Dasilva *et al.* (2010)** and **Praveen *et al.* (2014)** recorded lower results, adding respectively that, 28%.3% and 18.89% of samples examined were *A. hydrophila*. Likewise, higher results recorded in the studies of **Elsheshtawy *et al* . (2019)**, **Nhinh *et al.* (2021)** and **Ahangarzadeh *et al.* (2022)** revealed that, *A. hydrophila* was isolated and identified from 80%, 47%, and 47.2% of the examined samples. The results of the PCR shown in Figs. (3, 4) indicate that, 7 (70%) and 3 (30%) out of 10 examined biochemically identified *A. hydrophila* isolates harbored *aerA* and *act* virulence genes. The results coincide with those of **Emeish *et al.*, (2018)**, **Hafez *et al.*, (2018) and Morshdy *et al.* (2022)** who reported that, 64.3%, 60%, and 75% of *A. hydrophila* isolates harbored *aerA*. On the other hand, the current findings disagree with those of **Elsheshtawy *et al.* (2019)**, **Nhinh *et al.* (2021)** and **Ahangarzadeh *et al.* (2022)** regarding *aerA* and *act* genes. The various species, sampling periods, and geographical areas could explain these variations.

Chemical analysis

The nutritional profile comprises the closest components of the fish flesh and provides a preliminary indication of the fish's commercial standards, which are necessary for food regulations (**Marichamy *et al.*, 2012**). Table (6) shows the mean of the proximate analysis of examined canned tuna. Crumbled tuna recorded the highest value of moisture content (68.12±0.5) and fat content (11.23±0.25); meanwhile, solid tuna had a higher protein value (24.10±0.33) than other examined products. Such findings concur with those recorded in the studies of **Manthey-Karl *et al.* (2014)**, **ElShehawy and Farag (2019) and Hassan *et al.* (2022)**. Furthermore, **USDA (2011)** reported that, the percentage of moisture, protein, fat and ash for tuna canned in oil are 59.83, 29.13, 8.21, and 2.76%, respectively, and accordingly, the examined samples nearly matched with USDA regulations. On the other hand, the results in Table (7) elucidate that crumbled tuna and solid tuna samples had the highest caloric value (Kcal/100gm) among the investigated products, with mean values of 197.18 ± 3.25 and 188.63 ± 4.6, respectively, followed by chunk tuna (184.36 ± 4.52). The results are nearly parallel to that detected in the work of **Roe *et al.* (2013)** and **Hassan *et al.* (2022)**. Generally, the environment, season, sex and age all impact the chemical makeup of fish (**Mahaliyana *et al.*, 2015**).

Regarding nutritional and health considerations, it is crucial to quantify the amount of cholesterol present in foods. Many countries' legal requirements require that food labels provide nutritional information **(Sharmin *et al.*, 2016)**. Concerning the findings in Table (8), the cholesterol content (mg/100gm) was detected with a high concentration in crumbled tuna and chunks tuna samples, with a mean value of 44.62± 1.3 and 42.3± 1.02, respectively, followed by solid tuna, with a mean value of 39.72 ± 1.02. Furthermore, no statistically significant differences between the investigated items were identified at $P<0.05$. The World Health Organisation **(WHO, 2007)** claims that the maximum cholesterol should be 300mg/ day. Thus, all samples were approved based on cholesterol content. These findings match with the results of **Roe *et al.* (2013)**, **USDA (2020)** and **Hassan *et al.* (2022)**. While, they disagree with those of **Donmez (2009)** and **Manthey-Karl *et al.* (2014)**.

Histamine, or scombrotoxin, is a biogenic amine produced due to time and temperature abuse. Furthermore, bacterial species producing the enzyme histidine decarboxylase can convert histidine in fish into histamine **(Lehane *et al.*, 2000)**. It was clear from the most recent data listed in Table (9) that, solid tuna samples had the lowest histamine concentrations with a mean value of 48.33±1.12 ppm. In contrast, crumbled and chunk tuna had the highest concentrations of 62.66±1.59 and 56.00±1.86 ppm. These results do not match with previous studies of **Cicero *et al.* (2020)**, **Mamdouh *et al.*, (2022)** and **Sulfiana *et al.* (2022)**. In addition, the maximum acceptable histamine limit for tuna with respect to **FDA (2011)** and **EOS (804/2005)** regulation must not exceed 100ppm; therefore, all examined products are accepted according to **EOS** measures. To produce canned fish, a storage procedure (chilling or freezing) is required to keep the raw material safe before canning. Using a heating step (cooking, smoking or frying) is typical to reduce water content and inactivate endogenous enzyme activity. To render microorganisms inactive and provide a lengthy shelf life, a thorough thermal treatment (sterilization) is applied. Proteins, vitamins, lipids, and minerals that are labile and necessary in raw fish are subjected to various processing conditions that can lower the nutritional and sensory attributes of the finished product **(Aubourg, 2001)**.

CONCLUSION

Based on the present findings, canned tuna that is fit for human consumption has acceptable chemical quality but may provide a significant risk of microbiological dangers, thus responsible authorities and food industry operators should take particular care.

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