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SAFETY OF CANNED TUNA MEAT AFTER OPENING AND STORAGE AT DIFFERENT TEMPERATURES

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Abstract
Canned tuna is widely consumed worldwide due to its palatability, nutritional value, and convenience. However, it may pose a health risk to consumers if not properly processed or improperly handled and/or stored by consumers. This study evaluated the microbial safety and histamine content of canned tuna meat and the effect of the storage at different temperatures (4, 28, and 31 °C) for 7 days after opening on the microbial safety and histamine content. Data were analyzed by the SAS program. The aerobic bacteria counts in tuna samples after 48 hours of storage at 4 °C, 28 °C, and 31 °C were 3.2, 2.75, and 5.09 log CFU/g, respectively, with no significant difference observed between 4 °C and 28 °C ($p > 0.01$). Similarly, the anaerobic bacteria counts were 3.3, 2.98, and 5.08 log CFU/g at 4 °C, 28 °C, and 31 °C, respectively, also showing no significant difference between 4 °C and 28 °C ($p > 0.01$). Storage of canned meat at 4 °C showed more significant ($p < 0.01$) microbial inhibition than storage at 28 °C, and 31 °C. No pathogenic bacteria were observed in all samples during storage at different temperatures. For the histamine test, the highest recorded concentrations were 3.53, 9.58, and 28.24 mg/kg in tuna samples stored at 4 °C, 28 °C, and 31 °C, respectively. The storage temperature influenced ($p < 0.01$) histamine formation in tuna meat during storage. Recording histamine concentrations at zero time indicates that histamine was formed before opening the can, which may be due to failure to apply good hygiene practices in handling fish, as histamine does not degrade once formed. However, it did not exceed the maximum permissible limit. Also, the results of the microbial count and histamine content indicate that holding canned tuna meat after opening at 4 °C contributes to maintaining the safety of the tuna during storage.

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Introduction

Canned tuna production is constantly increasing locally and globally [1], due to its high nutritional value and desirable taste. Canning is one of the preserving techniques that makes the food stable at room temperature for a relatively long period ranging from 1 to 5 years [2]. Thus, canned food is distinguished by the possibility of being easily distributed all over the world and needs relatively fewer requirements for storage and distribution [3]. The major steps of the canning tuna process include cleaning and preparing raw materials, precooking, cooling, cleaning, packing with a covering oil or vegetable broth, etc. in sealed cans, the thermal process (retorting), can cooling, labeling, casing, and storage [4].

Fish and fish products are an important nutritional source that is readily digestible and contains biologically highly valuable nutrients, including protein, polyunsaturated fatty acids, minerals, and vitamins [5–7]. Further, fish consumption is known to prevent diseases since fish is a source of omega-3 highly unsaturated fatty acids, which include eicosapentaenoic and docosahexaenoic acids [8]. On the other hand, it is considered a suitable environment for the growth of microorganisms due to the availability of moisture and nutrients, such as non-protein nitrogenous compounds that include free amino acids, peptides, amines, amine oxides, guanidine compounds, quaternary ammonium molecules, nucleotides, and urea [9–11], as well as the low acidity (pH > 6) of the meat [12]. Therefore, fish and fish products could be a cause of foodborne diseases (FBDs).

FBDs represent one of the most widespread public health problems [13,14]. FBDs associated with pathogenic microorganisms, such as bacteria and viruses, parasites, and chemical contaminants in food pose a serious threat to the health of millions of individuals, leading to conditions such as diarrhea, cancer, and even death [15,16]. Fish-borne

pathogenic bacteria include: 1) indigenous bacteria such as *Aeromonas hydrophila*, *Vibrio cholerae*, *Clostridium botulinum*, *Vibrio parahaemolyticus*, *Listeria monocytogenes* and *Vibrio vulnificus*; 2) non-indigenous bacteria present as a result of fecal contamination such as *Yerinia enterocolitica*, *Campylobacter* spp., *Escherichia coli*, *Shigella* spp. and *Salmonella* spp.; and 3) bacteria that are present as a result of contamination during processing such as *Clostridium perfringens*, *S. aureus*, *L. monocytogenes*, and *Bacillus cereus* [17,18]

The incidence of FBDs in some areas of developing countries may be attributed to poor hygiene practices, lack of access to safe adequate food storage facilities, and poorly enforced laws [19]. Food safety awareness, education, and promotion among consumers should be emphasized, as most FBDs outbreaks occur at home, in restaurants, and/or social events [20].

One of the FBDs is histamine (HIS) poisoning. HIS is one of the compounds named biogenic amines [21]. HIS is a toxic metabolite produced by bacteria [22]. It is worth noting that a review of published studies about biogenic amines, suggested indicative levels of HIS content in fish, pointing out that amounts of 5–20 mg/100 g are possibly toxic [23]. The formation of HIS depends on the type and amount of free amino acids, the presence of decarboxylase-positive bacteria, the availability of appropriate conditions for the growth of decarboxylase-positive bacteria and production of HIS, and the extent of application of hygienic practices and food safety standards [24]. HIS formation is most often caused by improper temperature control of fish after harvesting and the level of accumulation is influenced by the combination of time and temperature, with accumulation typically occurring rapidly after 12 hours of storage at 25 °C [25]. In addition, the formation of HIS is affected by the manufacturing process, and the conditions of transport and storage [26]. It is important to mention that HIS concentration may decrease with storage time because of its decomposition. Once it is produced, the HIS concentration does not depend only on the HIS but also on the presence of HIS decomposing bacteria within the flora [27]. Storage temperature and time can be used as the primary means to monitor and control the quality and safety of canned seafood [28]. Several studies were conducted to evaluate the safety of canned fish related to HIS [29–32]. In general, the results of these studies show that canned fish is safe for health [24]. However, the HIS may form after cans are opened due to improper storage practices by consumers, such as temperature abuse. A tuna sandwich is a significant and popular ready-to-eat food made from canned tuna. The HIS formation in opened canned tuna could rapidly increase if stored at 33 °C for 6 h [33].

It is worth mentioning that food patterns in Libya have changed as in other countries where the Libyans increasingly consume canned fish, especially canned tuna [34]. Consumers may not use all the contents of the can and might store the remainder in various ways, which could alter its characteristics. The various attributes of canned tuna that are important to the consumer, including safety, sensory and nutritional properties, are affected by storage temperature and time; therefore, proper storage of food is essential. Therefore, the main objectives of this study were to: 1) evaluate microbiological safety of canned tuna meat, including aerobic bacteria count (ABC), anaerobic bacteria count (AnBC), coliform bacteria counts, *E. coli*, *Salmonella* spp., *L. monocytogenes* and *S. aureus*; 2) determine the level of HIS in canned tuna meat; 3) and explore the effect of storage at different temperatures, including 4, 28, and 31 °C, on the microbial quality and HIS level in canned tuna meat after opening during 7 days of storage.

Objects and methods

Study plan

The study was conducted between September and December, 2024. One carton of Libyan-made tuna cans from the same brand and the same expiration date was purchased from the Al-Krimia market in Tripoli City, Libya. The carton contained 48 cans, and the filling media of tuna was a mixture of olive oil and brine. All cans were free of any leaky conditions or swelling. According to the product label, each tuna can had a net weight of 160 grams and a shelf life of three years from the date of production (July 2024 to July 2027).

Once the tuna cans arrived at the food microbiology laboratory of the Department of Food Science and Technology, Faculty of Agriculture, University of Tripoli, thirty cans were randomly selected and divided into three groups. Each group represented a trial and consisted of ten cans. The tuna cans were kept at room temperature until the opening. After the cans were opened and held in polyethylene bags, the first group was stored in the refrigerator at a temperature of 4 °C. The second and third groups were stored at 28 °C and 31 °C, respectively. The incubator (IN260) used to maintain temperatures of 28 °C and 31 °C was from Memmert GmbH +Co. KG (Germany). The temperature of the refrigerator and incubators was checked using a glass and digital thermometer (HTC2, CNWTC company, China). The storage temperatures tested in this study were chosen as follows: 4 °C is the recommended temperature for the refrigerated storage of fish, which is also available to consumers, while 28 °C and 31 °C were the average temperatures recorded in Tripoli and Sabha during the summer of 2023, respectively.

Sampling

After opening the cans, the samples were taken immediately (zero time) and at different periods during 7 days: after 6 h, 24 h, 48 h, 72 h, 96 h, 120 h, and 168 h. After that, the samples were held at –18 °C until analyzed in the laboratory belonging to the food and drug control center/Tripoli branch. The samples were tested for ABC, AnBC, coliform count, *E. coli*, *Salmonella* spp., *S. aureus*, *L. monocytogenes*,

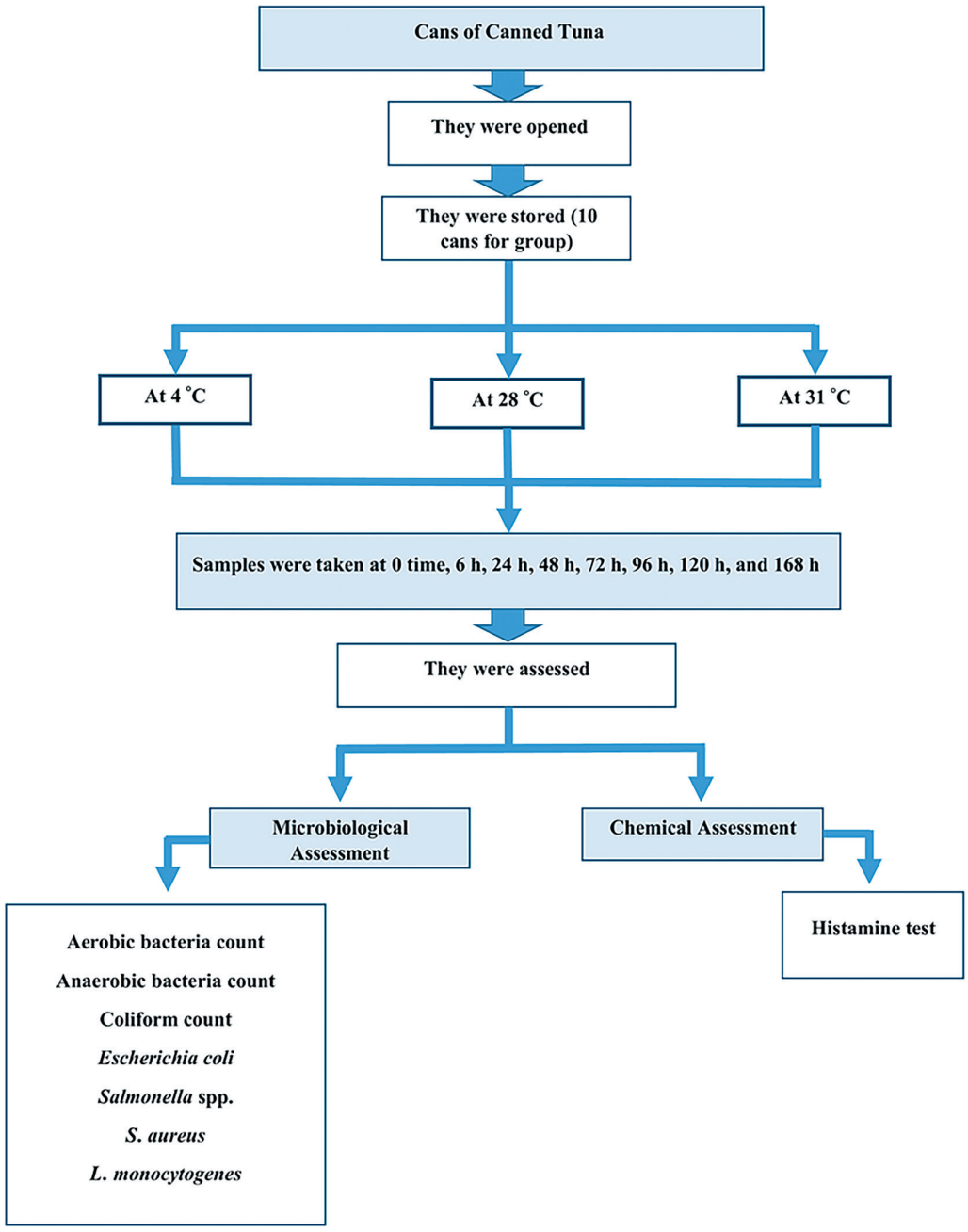

Figure 1. Diagram of the research methodology

and HIS content. All microbial experiments were carried out in duplicate, and the HIS experiment was in triplicate, and the mean values were recorded. The study sampling is displayed in Figure 1.

Test methods

Microbiological methods

Aerobic bacteria count was determined using the ISO method (ISO 4833-1:2013)[1]. Using a stomacher (Stomacher 400 Circulator Lab Blender, Seward Ltd., UK), 10 grams of tuna sample was homogenized with 90 ml of sterile buffered peptone water (BPW). A series of decimal dilutions was prepared using test tubes containing 9 ml of sterile BPW. One milliliter was transferred aseptically from the homogenate to the first test tube using a sterile pipette, resulting in a 10^{-1} dilution. This procedure was repeated sequentially to achieve further dilutions up to 10^{-5}. From the 10^{-5} dilution, 1 mL was aseptically transferred into sterile, labeled Petri dishes. Approximately 15 mL of Plate Count Agar (Liofilchem, Italy), previously melted and cooled to 45 °C, was then poured into each dish. The plates were gently swirled to mix the contents. After solidification, the plates were incubated in an inverted position at 30 °C for 72 hours. After the specified incubation period, visible bacterial colonies on the plates were counted, and the results were expressed as colony-forming units per gram (CFU/g).

Anaerobic bacteria counts were determined according to ISO 15213:2003[2]. The sample and serial dilutions

[1] ISO 4833-1:2013. Microbiology of the food chain — Horizontal method for the enumeration of microorganisms. Part 1: Colony count at 30 °C by the pour plate technique.

[2] ISO 15213:2003. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions.

were prepared in the same manner as for aerobic bacteria. From the 10^{-4} dilution, one milliliter was aseptically transferred into sterile labeled Petri dishes. Approximately 15 mL of Iron Sulfite Agar (Liofilchem, Italy) was then added to each dish. Anaerobic conditions were established using an oxygen removal system and carbon dioxide generation. Then, the inoculated plates were placed in an anaerobic jar (IQ2000® GasPak System, BD Company, USA) and incubated (Incubator B50, Memmert GmbH +Co. KG, Germany) for 48 hours at 30 °C. Colonies were subsequently counted using the same principles applied to aerobic bacteria.

Coliform bacteria counts were determined using the ISO method (ISO 4832:2006)[3]. Ten grams of the sample was placed in 90 ml of sterile saline solution to prepare a 1:10 dilution, and the sample was shaken thoroughly to prepare a homogeneous solution. One milliliter of an appropriate dilution was transferred into sterile Petri dishes, and approximately 15 mL of Violet Red Bile Agar (VRBA) (Liofilchem, Italy), cooled to 44–47 °C, was poured into each dish. The contents were gently mixed and allowed to solidify. After solidification, an overlay of VRBA was added to suppress surface spreading of colonies. The plates were incubated (Incubator IN750, Memmert GmbH +Co. KG, Germany) at 37 °C for 18–24 hours. After incubation, red to dark red colonies with a precipitated bile zone were counted as presumptive coliforms.

Escherichia coli were determined using the ISO method (ISO 16649–2:2001)[4]. The sample and dilutions were prepared in the same way as for aerobic bacteria. One milliliter of 10^{-3} dilution was transferred to sterile labeled petri dishes. Approximately 15 ml of Tryptone Bile X-glucuronide (TBX) Agar (Liofilchem, Italy) was then poured. The contents were gently mixed and allowed to solidify. The plates were incubated (Incubator IN750, Memmert GmbH +Co. KG, Germany) at 44 °C for 24 hours. After the incubation period, the plates were examined to identify the characteristic colonies. Positive colonies of *E. coli* produce distinct colonies that appear blue or green on the TBX agar due to β-glucuronidase activity. Non-target bacteria may form colorless or different-colored colonies or may be inhibited entirely.

Salmonella spp. was detected according to the ISO method (ISO 6579: 2002)[5]. A 25 g portion of the sample was aseptically transferred into 225 mL of sterile BPW for non-selective pre-enrichment. The sample was incubated at 37 °C for 16–20 hours. Following pre-enrichment, 0.1 mL of the culture was inoculated into 10 mL of Rappaport-Vassiliadis Soya Peptone (RVS) broth (Liofilchem, Italy) and incubated (Incubator INB200, Memmert GmbH +Co. KG, Germany) at 42 °C for 24 hours for selective enrichment. Presumptive positive colonies of *Salmonella* spp. appear as red colonies with black centers on XLD agar, due to hydrogen sulfide production and the inability to ferment lactose or sucrose.

L. monocytogenes detection was performed according to the ISO method (ISO 11290-1:2004)[6]. Ten grams of tuna were aseptically transferred into sterile incubation bags. Then, 90 ml of Half Fraser Broth (Liofilchem, Italy) supplemented with Listeria Fraser Supplement, was added. The sample was homogenized thoroughly and incubated at 30 °C for 24 hours for primary enrichment. Following this, 0.1 mL of the primary enrichment was transferred into 10 mL of Fraser Broth (Fraser Broth Base supplemented with Listeria Fraser Supplement) and incubated at 37 °C for 48 hours for secondary enrichment. After incubation, aliquots of the secondary enrichment were streaked onto PALCAM Agar (Listeria Agar Base PALCAM, Condalab, Spain) using the spread plate method after solidification of the medium. The plates were incubated (Incubator IN750, Memmert GmbH + Co. KG, Germany) at 37 °C for 24–48 hours. Presumptive *L. monocytogenes* colonies on PALCAM agar appear gray-green with a black center and are often surrounded by a red or dark halo, due to esculin hydrolysis and mannitol fermentation inhibition.

S. aureus enumeration was performed according to the ISO method (ISO 6888-1:2003)[7]. The sample was prepared and the dilution series was prepared in the same way as used for aerobic bacteria. One milliliter of the 10^{-3} dilution was transferred to the labeled petri dish. Then, approximately 15 ml of Baird-Parker Agar Base (Liofilchem, Italy) supplemented with Egg Yolk Tellurite Emulsion, was poured into the dish. After the agar solidified, the plates were incubated (Incubator IN750, Memmert GmbH +Co. KG, Germany) at 37 °C for 48 hours. Positive results for *S. aureus* colonies appear in a shiny black or gray color and are surrounded by a clear zone due to the activity of lipase on the egg yolk. Non-*S. aureus* colonies typically lack this appearance and do not produce a clear zone.

Histamine method

HIS content was analyzed using the AOAC-approved method (RIDASCREEN® HIS (enzymatic) (Art. No. R1605) [35]. It is an enzymatic test in microliter plate format for the quantitative determination of HIS in fresh fish, canned fish, fish meal. The test kit is sufficient for a maximum of 96 determinations (including standards). Each test kit contains components as displayed in Table 1.

[3] ISO 4832:2006. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique.

[4] ISO 16649-2:2001. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli*. Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.

[5] ISO 6579:2002. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella* spp.

[6] ISO 11290-1:2004. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp.

[7] ISO 6888-1:2003. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Technique using Baird-Parker agar medium.

Table 1. Reagents and volumes used for HIS standard curve preparation

Component	HIS concentration	volume
Microtiter plate		96 wells
Buffer		15 mL
Standard 1	0 mg/L	1.3 mL
Standard 2	1 mg/L	1.3 mL
Standard 3	5 mg/L	1.3 mL
Standard 4	10 mg/L	1.3 mL
Standard 5	15 mg/L	1.3 mL
Standard 6	20 mg/L	1.3 mL
Enzyme solution		1 mL
Spiking solution	500 mg/L	3 mL
Catalase Catalase to remove ascorbic acid		1 mL

Sample preparation: 5 g of the homogenized sample was placed in a 50 ml polypropylene screw cap vial and 20 ml of distilled water was added. The vial was closed and shaken by using a Vortex device until the sample was evenly suspended. Then, the sample was heated in a boiling water bath at 100 °C for 20 minutes. Every 10 minutes, the vial was removed using protective gloves and shaken for 3 seconds. After that, the vial was placed in an ice bath incubated (Bionics Scientific Technologies (P) Ltd, India) for at least 2 minutes to reach room temperature and was placed in a centrifuge (Hettich Universal 32R Centrifuge, Germany) for 10 minutes at a speed of not less than 2500 × g at a temperature of 4 °C. The lower layer was withdrawn carefully with a pipette and delivered to a new vial. After that, the new vial contents were filtered and centrifuged again and 100 µL of the undiluted clear extract was used per well. The extracted sample was stable at room temperature (20–25 °C) for 2 h. **Analysis steps**: Using a multi-stepper (815, Socorex, Switzerland), 150 µL of buffer was added to the wells and the plate was shaken manually for 3 sec. 100 µL of standards, controls, or samples was added to separate wells in duplicate. Thereafter, the plate was carefully shaken manually for 3 seconds. After 3 min., the absorption (A_1) was measured at 450 nm using a Microtiter plate spectrophotometer (ELx808, BioTek Instruments, USA). Then, 10 µL of the blue-dyed enzyme solution was added to each well using a multi-stepper. The plate was then carefully shaken manually for 3 seconds. After 10 minutes, the absorbance (A_2) was measured at 450 nm. The HIS concentration was calculated according to the manufacturer's instructions.

Statistical analysis

The experiment was conducted using a Complete Randomized Design (CRD). Analysis of variance and statistical tests were performed to study the effects of storage temperature, storage time, and their interaction, utilizing the Statistical Analysis System (SAS-2002) program. The results obtained were expressed as means with standard deviation (±SD). Duncan's multiple range test was used to determine the significance of differences between the means of different treatments at a probability level of ≤ 0.01. Microsoft Excel 2010 was used to prepare tables and graphs.

Results and discussion

Fish can carry bacteria from the environment naturally or as a result of contamination due to improper handling, processing, storage, distribution, or preparation for consumption. However, under carefully controlled conditions at processing, commercially canned fish is safe [36]. ABC reflects bacterial contamination and give an indicator of the application of hygiene standards in the factory [37]. As shown in Table 2, the results of this study reveal that aerobic bacteria were not detected at zero time in the three trials. On the contrary, the number of microorganisms exceeded the maximum limit in a study carried out by Alhafeth et al. [36], where the average total number of bacteria in 20 samples of canned fish was $23.25 \times 10^7 \pm 3.42 \times 10^7$ CFU/g.

During storage at 4 °C, the highest ABC (3.2 log CFU/g) was found after 48 h, i. e., on the third day of the storage period. Then, ABC decreased to 0.5 log CFU/g after 120 h, i. e., on the sixth day of the storage period, and was not detected after 168 h, i. e., on the seventh day of storage. Low-temperature storage slowed down the growth of ABC. The results of the statistical analysis showed a significant effect ($P \leq 0.01$) of temperature and storage time on the ABC. The ABC in all samples during storage at 4 °C did not exceed the maximum level set by the International Commission on Microbiological Specifications for Foods (ICMSF) of 1.0×10^6 CFU/g [38].

Canned tuna can be contaminated if consumers abuse the storage temperature after opening. The ABC increased gradually during the storage period at 28 °C and 31 °C, reaching the highest values of 6.09 and 6 log CFU/g, respectively, after 120 h and 96 h, i. e., on the sixth and fifth days of the storage period. The highest ABC values at 28 °C and 31 °C reached the maximum level set by the ICMSF [38]. However, the tuna exhibited significant spoilage signs and notable changes in its organoleptic characteristics, including smell and appearance, after 3 and 2 days at 28 °C and 31 °C, respectively.

Table 2. Aerobic bacteria count in canned tuna meat at different storage temperatures

Storage Time	Aerobic Bacteria Counts (log CFU/g ±SD)		
	4 °C	28 °C	31 °C
Zero time	n.d.	n.d.	n.d.
After 6 h.	n.d.	2.86 ± 0.13d	1.59 ± 0.14e
After 24 h.	n.d.	1.54 ± 0.08ef	2.09 ± 0.12e
After 48 h.	3.2 ± 0.00d	2.75 ± 0.00d	5.09 ± 0.00b
After 72 h.	0.5 ± 0.70gh	5.07 ± 0.00b	5.03 ± 0.11b
After 96 h.	1 ± 0.00fg	6.08 ± 0.00a	6.0 ± 0.00a
After 120 h.	0.5 ± 0.70gh	6.09 ± 0.00a	6.0 ± 0.00a
After 168 h.	n.d.	4.47 ± 0.02c	5.13 ± 0.00b

n.d. — not detected.
Means that share one letter within a column are not significantly different ($p \leq 0.01$).

Table 3 presents the results of the AnBC. Anaerobic bacteria were not detected at zero time in the three trials. This finding was not comparable to a study conducted by Alhafeth et al. [36], which reported that the mean AnBC of 20 samples of canned fish was 3.6 x 10^3 CFU/g. In this study, after 48 h of storage at 4 °C, the AnBC recorded the highest count of 3.3 log CFU/g, which decreased to 1 log CFU/g by the end of storage. At 28 °C and 31 °C, the AnBC increased during storage, reaching 6.05 and 5.21 log CFU/g after 120 h and 168 h, respectively. According to statistical analysis, there was a significant difference in the effect of storage temperature at 4 °C on AnBC compared with 28 °C and 31 °C, while there was no significant difference between 28 °C and 31 °C ($p \leq 0.01$). In addition, there was a significant effect ($p \leq 0.01$) of the storage time on the AnBC.

Table 3. Anaerobic bacteria count in canned tuna meat at different storage temperatures

Storage time	Anaerobic Bacteria Count (log CFU/g) ±SD		
	4 °C	28 °C	31 °C
Zero time	n.d.	n.d.	n.d.
After 6 h.	n.d.	2.56± 0.10^{de}	1.82± 0.04^{fg}
After 24 h.	n.d.	2.22± 0.86^{ef}	1.98± 0.18^{ef}
After 48 h.	3.3± 0.04^{d}	2.98± 0.02^{d}	5.08± 0.02^{b}
After 72 h.	0.65± 0.91^{hj}	4.08±0.01^{c}	4.52± 0.08^{bc}
After 96 h.	1.15± 0.21^{gh}	5.09±0.00^{b}	5.14±0.00^{b}
After 120 h.	1.24± 0.33^{gh}	6.05±0.01^{a}	5.19±0.00^{b}
After 168 h.	1± 0.00^{h}	4.33±0.04^{c}	5.21±0.00^{b}

n.d. — not detected.
Means that share one letter within a column are not significantly different ($p \leq 0.01$).

The growth of pathogenic bacteria leads to economic losses, as products are excluded if they are not compliant with regulations, and if these products reach consumers, they can cause FBDs [17]. Proper handling, preparation, and processing steps lead to the safety of canned tuna. Table 4 shows that the pathogenic bacteria considered in this study, including *coliform count*, *E. coli*, *S. aureus*, *L. monocytogenes*, and *Salmonella* spp., were not detected. Thus, the results correspond with the Libyan Standard [39], which states that canned tuna should be free from pathogenic bacteria and/or their toxins. The absence of pathogenic bacteria in the studied canned tuna may be attributed to good manufacturing practices and good hygiene practices, as well as operators' care about the tuna source and contracting with reliable suppliers. Moreover, the absence of pathogenic bacteria in the studied samples may reflect the characteristics of the fish environment [40]. On the contrary, Dhinesh et al. [2] reported the presence of various pathogenic bacteria such as *E. coli*, *S. aureus*, *Salmonella* spp., *Vibrio* spp., and *Listeria* spp. in canned tuna meat of different brands.

The consumption of canned tuna can cause FBDs due to the activity of pathogenic bacteria, including HIS-producing bacteria [41]. Since the HIS is a thermostable compound, cooking, smoking, or freezing will not eliminate it when forming. Thus, keeping the HIS at low levels from capture to consumption is an important key to fish safety [24]. Table 5 reports the results of HIS concentration in canned tuna meat at different storage temperatures. All tuna meat samples in the three trials contained HIS ranging between 2.69 to 4.46 mg/kg at zero time, which was below the maximum limit of 100 mg/kg established by Libyan standard [39] and also below the safety level of 50 mg/kg established by the Food and Drug Administration [42]. Because low temperatures inhibit the growth of HIS-producing bacteria during fish processing [21], keeping fish cool from the moment of capture until it is eaten serves as an essential step in lowering the incidence of HIS poisoning. Furthermore, the use of food safety systems such as HAZARD ANALYSIS AND CRITICAL CONTROL POINT (HACCP) SYSTEM in the processing of canned tuna may be the reason for the low HIS contents in the samples under examination [43]. On the same line, in Libya, a study of tuna sandwiches being sold to pupils and students determined HIS content in 19 tuna sandwiches col-

Table 4. Pathogenic bacteria in canned tuna meat at different storage temperatures

Pathogenic bacteria	Storage temp.	Storage Time							
		Zero time	After 6 h.	After 24 h.	After 48 h.	After 72 h.	After 96 h.	After 120 h.	After 186 h.
Coliform count	4 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	28 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	31 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
E. coli	4 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	28 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	31 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S. aureus	4 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	28 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	31 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
L. monocytogenes	4 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	28 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	31 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Salmonella spp.	4 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	28 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	31 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. — not detected.

lected from food vendor premises in March and April, 2016. The HIS concentrations ranged between 0.52 to 4.85 mg/kg and were below the Libyan maximum permitted levels and FDA [44]. The HIS formation exhibited varying patterns at different temperatures, highlighting the significant impact of storage temperature on HIS production. After 24 hours, the greatest HIS concentrations in the present investigation were 3.53 mg/kg for samples kept at 4 °C and 9.58 mg/kg for samples kept at 28 °C. For the samples stored at 31 °C, the HIS increased with the storage. The results of the statistical analysis showed a significant effect ($P \leq 0.01$) of temperature and storage time on the HIS. None exceeded the maximum limit established by the Libyan standard of 100 mg/kg [39] and FDA safety level of 50 mg/kg [42]. Although HIS levels were below the maximum limit, the tuna exhibited significant spoilage signs and notable changes in its organoleptic characteristics including smell and appearance after 3 and 2 days at 28 and 31 °C, respectively. On the same line, Altafini et al. [45] found that no HIS formation was detected in tuna samples stored at room temperature for six days. However, the tuna showed marked spoilage and changes in organoleptic characteristics after five days. These results agree with findings by Lehane and Olley [46], who observed that decarboxylase-positive bacteria growing at refrigeration temperatures typically produce HIS in lower quantities than species that grow at warmer temperatures, making it less likely for toxic levels to be reached. In a similar study, Altafini et al. [45] found that storing canned tuna in sunflower oil, to which certain types of vegetables were added after opening, at 4 °C, 12 °C and 20 °C for 8 days, did not result in HIS formation in the samples collected daily during storage. Also, Kordiovská et al. [47] found that the HIS was not recorded in carp fish at 3 ± 2 °C during the 7-days storage period. The HIS concentration not significantly rising during storage may be attributed to the fact that the presence of carboxylase-positive bacteria is necessary for HIS formation and that temperature alone is not sufficient to stimulate this process [45]. Furthermore, the low HIS concentrations during storage may be attributed to the brine used as a filling medium in tested tuna that prevents the growth of HIS [48]. In addition, it was reported that among the factors that affect the HIS formation is a salt concentration [49,50].

Table 5. HIS concentration in canned tuna meat at different storage temperatures

Storage time	HIS concentration mg/kg ± SD		
	4 °C	28 °C	31 °C
Zero time	2.69 ± 0.12^{k}	4.46 ± 0.06^{fgh}	4.03 ± 0.26^{fghj}
After 6 hours	2.85 ± 0.37^{k}	4.61 ± 0.49^{fg}	4.24 ± 0.21^{fgh}
After 24 hours	3.53 ± 0.35^{ghjk}	9.58 ± 0.72^{d}	3.58 ± 0.20^{ghjk}
After 48 hours	3.14 ± 0.18^{jk}	6.26 ± 0.05^{e}	4.24 ± 0.06^{fghj}
After 72 hours	2.80 ± 0.18^{k}	4.9 ± 0.05^{f}	7.31 ± 0.05^{e}
After 96 hours	2.39 ± 0.22^{k}	4.62 ± 0.20^{fg}	11.53 ± 0.75^{c}
After 120 hours	2.89 ± 0.67^{k}	4.40 ± 0.13^{fgh}	21.35 ± 1.67^{b}
After 168 hours	3.38 ± 0.34^{hjk}	4.44 ± 0.13^{fgh}	28.24 ± 1.22^{a}

Means that share one letter within a column are not significantly different ($p \leq 0.01$).

Conclusion

Prioritizing proper storage is the key to ensuring the safety of the canned tuna meat. Storage at a refrigeration temperature of 4 °C effectively suppressed microbial growth and HIS formation. The storage of canned tuna meat at 28 °C and 31 °C provided a suitable environment for microbial growth and accelerated the process of HIS formation, resulting in higher HIS levels, although the concentration did not exceed the maximum level established in the Libyan standard. Thus, fish should be stored at temperatures of 4 °C or below to maintain the safety of the tuna. Consumers should be aware of the proper storage of canned tuna after the opening. Depending on the results of this study, it is recommended to conduct broader studies on the safety of canned tuna comparing locally manufactured brands and on the extent, to which the filling medium affects the safety of canned tuna.

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