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Research Article

Biochemical and Microbiological Characteristics of Fresh Sardines (*Sardinella maderensis*) from the Exclusive Economic Zone of Mauritania Waters

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Mauritania acquired the status of a lower-middle-income country in 2014. Economic development has relied primarily on fishing and mining. Fish is an important element in achieving adequate nutrition in these countries. But foodborne chemical and microbial hazards that may be present in fish are a major concern. The purpose of this study was to evaluate the microbiological and biochemical properties of fresh sardines (*Sardinella maderensis*). Totally, 135 samples were collected when boats arrived at the landing site and at the four main market sites. Microbiological and biochemical parameters were tested. Samples taken just after the arriving of the boats, almost always met quality standards. But when samples were taken on the markets, a high proportion of samples showed values of microbiological parameters higher than permissible limits. TVBN and TMA that result from the degradation of fish proteins, exceeded the authorized values in approximately 20% of the samples. Our findings suggest that postharvest contamination is the main health risk. Ensuring the cold chain, improving hygienic conditions in markets, and training vendors in food safety are the main challenges to be faced to reduce public health risks.

1. Introduction

Global fish production is estimated at about 179 million tons in 2018, with a total first sale value of USD 401 billion. Annual human consumption is around 20.5 kg per capita [1].

Fish are a valuable supplement in diets low in protein, vitamins, and minerals [2]. The chemical composition of fresh fish is 66-81% water, 16-21% protein, 0.2-1.5% fat, 1.2-1.5% minerals, and 0-0.5% carbohydrates [3, 4]. Fish are also a good source of amino acids, vitamins such as A, D, E, and K [5], and mineral salts, e.g., phosphorus, magnesium, iron, zinc, and lead [6]. Fish (sardines, tuna, mackerel, etc.) is one of the richest sources of ω-3 fatty acids [7].

Despite all these advantages, fish are extremely perishable foodstuffs, and the rate of spoilage is very high after

fishing [8, 9]. Indeed, if the temperature is between 25 and 30°C, as in tropical countries, fish can be spoiled within 12 hours [2, 10].

It is estimated that 35% of the world's fishery and aquaculture harvest is lost or wasted annually. Waste rates in North America and Oceania are almost half at the consumption stage. In Africa and Latin America, fish are lost due to inadequate conservation infrastructure [1].

The nonrespect of the rules of hygiene and appropriate temperature causes proliferation of microorganisms responsible for alterations of product and can cause food-borne pathogens increase [11].

Diseases caused by contaminated food were the most widespread health problem in the world. Each year, nearly 600 million people (more than 10% of the world's population) suffer from them. Of these, 420,000 die. This situation is more serious in African countries, as more than 91 million people are intoxicated, of whom 137,000 will die [12].

Storage on ice is one of the most effective ways to decrease the rate of spoilage and bacterial growth, which can extend the shelf life of fresh fish to 12 days. On the other hand, the addition of substances such as rosemary extracts can extend the shelf life by 3 to 4 days [13].

Mauritania lies at the border between the Arab Maghreb world and the western side of the Sahel, with over 3.5 million inhabitants. Fishing, together with mining, has been one of the sectors that has contributed most to the country's economic development in recent decades. The country acquired the status of a lower-middle-income country in 2014 [14].

The Exclusive Economic Zone of Mauritania (EEZ) is one of the fishiest regions in the world thanks to a coastal ecosystem characterized by the presence of shoals and mudflats that allows the proliferation of grass beds which are linked to the confluence of currents by upwelling of water from the depths (the Upwellings phenomenon), thus promoting the reproduction of marine species. These Mauritanian coasts extend nearly 720 km along the Atlantic Ocean, or about 200 nautical miles [15].

The exploitable potential of this resource is about 1.8 million tons annually, including about 1.4 million tons of small pelagic resources. The total catch can exceed 1.2 million tons per year, including more than 300 thousand tons of sardines. This sector generates 226,000 jobs and contributes 3.3% to the gross domestic product (GDP). The national consumption per capita is 12.6 kg [16].

Sardinella maderensis is a species of small pelagic or semi-pelagic oceanodromous fish which is found in the eastern Atlantic and south-eastern Mediterranean. They prefer warm waters, where they swim in massive schools. Small pelagic fish account for one-third of the world's marine fish production and make a huge contribution to the global economy, livelihoods, and nutrition, especially protein supply [17].

In Mauritania, this species was not targeted until the recent development of the fishmeal industry.

In previous works, we could show for the first time that the industrial processing is feasible from a microbiological and economic point of view. Now, we want to assess the sanitary quality of fish products for local consumption, and especially the case of *Sardinella maderensis*. This species is in great demand by the low-income population due to its affordable cost and high nutritional quality.

The main objective of this study was to evaluate the microbiological and biochemical properties of fresh sardines (*Sardinella maderensis*) at the landing points and at the main distribution markets. A comparison of these results will allow us to draw the necessary steps for the improvement of the hygienic conditions of the locally consumed foodstuff.

2. Material and Methodology

2.1. Study Site, Design, and Sampling. This study was carried out in the region of Nouakchott (Mauritania, Figure 1), located on the Atlantic Ocean coast. Samples of fresh sardines were collected when boats arrived at the landing site (Market de Poisson Nouakchott, MPN, in English: Nouakchott Fish Market). This site was used as a reference for comparison. At the time of landing, the fish are brought by the fishermen in plastic bags, well filled, without any use of ice for their conservation.

Samples were also taken at the four main market sites (Cinquième Market, Eff Market, Teyarett Market, and MNP). The market MNP is in the same location as the landing site, but samples were taken the day after landing, when the fish were on sale. The fish are arranged in a hall. Cinquième Market (5.7 km) is a traditional market where everything is sold, and the fish are sold in the open air. Eff Market (6 km) is close to Cinquième Market (about 300 meters away); there is rubbish everywhere, and the fish is also sold in the open air. Teyarett Market (9 km) is the farthest. The fish are sold in a hall.

On all the markets, there is a lack of respect for the rules of hygiene: no toilets on the spot, no washbasins, and no soap. Each vendor has a bucket with water used both to clean the fish sold and to moisten the other fish exposed to the air. All these sellers are women with more than 20 years of experience in this trade, their educational level is low, and their financial situation is precarious. They have no subsidies, and when the days are hot, the price of ice is doubled. Only the saleswomen of the MPN site have had training in hygiene and food safety rules, but it is not applied due to a lack of financial means. For example, the fish are kept in plastic buckets or wooden boxes, and the quantity of ice used is not sufficient.

The study was conducted from April 2021 to March 2022. Three samples per month and per site were analysed. No samples were taken in August, September, and October due to the low presence of this species in those months.

A total of 135 samples of *Sardinella maderensis* were analysed. Twenty-seven samples were obtained at the landing site (MPN) and 108 samples from the four main market sites. At these markets, fish is sold by the pieces.

Each sample consists of 15 fish randomly picked up. Sardines are caught by small fishing boats that use purse seines. The fishing areas of these boats are generally located at 15-50 m depth and within 6 miles of the coast. Samples were collected around 4 to 5 p.m., about 2 to 3 hours after being fished. Samples were handled using sterile gloves, and

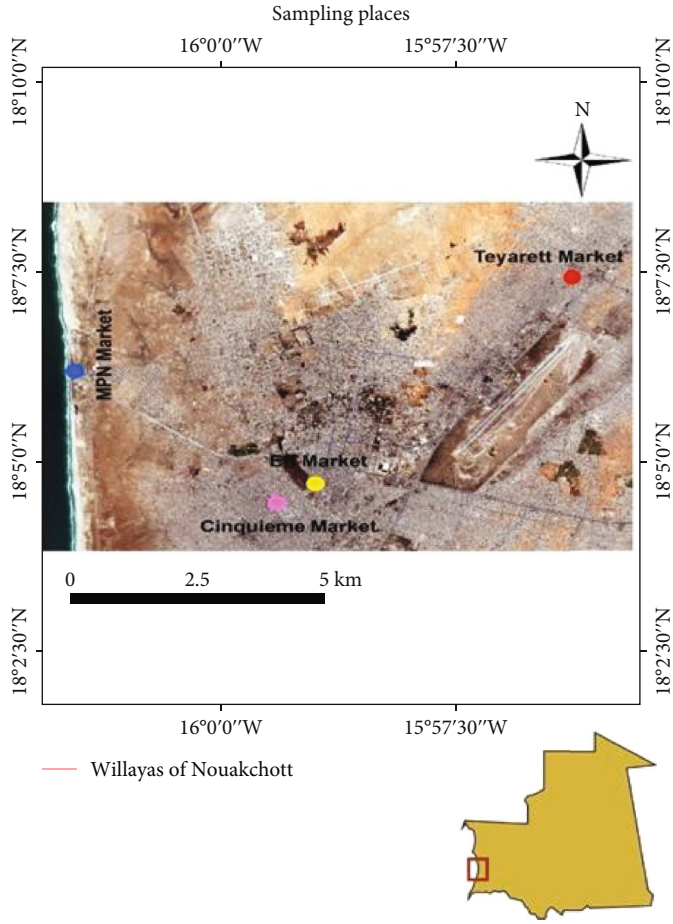

Figure 1: Map of sampling sites at Nouakchott (Mauritania).

temperature was measured immediately after being taken with a calibrated infrared thermometer (Testo, Germany). They were put in sterile bags that were sealed and transported in coolers to the Laboratory of Chemistry-Microbiology of the ONISPA (National Office of Sanitary Inspection of the Fishing and Aquaculture Products), which is the competent authority of the Mauritanian fisheries sector.

Samples arrived at the laboratory within a maximum of 2 hours of being taken. On arrival at the laboratory, the sensory analyses were done immediately (5 individuals per sample; data not shown). The remaining fish were kept in the refrigerator, and microbiological [18] and chemical analyses were carried out the next day.

The ONISPA's laboratories have been accredited since 2013 to [19] standards by the TUNAC (Tunisian Accreditation Council) and still accredited until now. They also regularly participate in inter-laboratory tests, such as RAEMA (Network of Analysis and Exchanges in Food Microbiology, France) and FAPAS (Food Analysis Performance Assessment Scheme, UK), for biochemical analyses such as ABVT and TMA.

The results of participation in intercalibration networks are summarized in Table 1.

2.1.1. Microbiological Analysis. Nine different microbiological parameters were studied: total aerobic mesophilic bacteria count (TVC); enterobacteria (ENT); total coliforms (TC); thermotolerant coliforms (FC); *Escherichia coli* (EC); sulphite-reducing anaerobes (ANSR); *Salmonella* (SALM); coagulase positive *Staphylococcus* (SCP); and yeasts and moulds (YM).

Limit values for microbiological parameters are summarized in Table 2.

Sample preparation, initial suspension, and decimal dilutions were based on [20, 21].

Skin was removed, and then cubed pieces of the back muscles were taken and diced into a sterile bag. Ten grams of sample were weighed, diluted in 90 mL of triptone-salt diluent (Biokar, France), and homogenized between 1 and 3 minutes in a stomacher (Seward, England). For the preparation of the following decimal dilutions, 1 mL of the 10^{-1} dilution was transferred into a tube containing 9 mL of the diluent (1/100 or 10^{-2} dilution). The following dilutions were made in the same way, changing pipettes each time.

This procedure was applicable to all microbiological parameters except for *Salmonella* where 25 g of flesh were weighed and diluted in 225 mL of buffered peptone water (Biokar, France).

Enumeration of total aerobic mesophilic bacteria [22] was done using the pour plate technique. First, 1 mL of each dilution of the sample (from 10^{-1} to 10^{-5}) was placed in sterile Petri dishes. Then, a volume of 10 to 15 mL of Plate

Table 1: Results of participation in intercalibration networks.

Parameters	Abbreviation	Result	Intercalibration network	Number of the schemes and year
Total aerobic mesophilic viable count	TVC	Z-score[†]: 0.615	RAEMA[§]	73/2021
Enterobacteria	ENT	Z-score: 0.0356	RAEMA	73/2021
Total coliforms	TC	Z-score: 0.141	RAEMA	73/2021
Faecal coliforms	FC	Z-score: 0.976	RAEMA	73/2021
E. coli	EC	Z-score: 0.0623	RAEMA	73/2021
Sulphite-reducing anaerobes	ANSR	Z-score: 0.255	RAEMA	73/2021
Salmonella	Salm	Just[‡]	RAEMA	73/2021
Coagulase-positive Staphylococcus	*SCP*	Z-score: -1.37	RAEMA	73/2021
Yeasts and moulds	YM	Not realized	RAEMA	Not available
Total volatile basic nitrogen	TVBN	Z-score: 0.2	FAPAS[¶]	25204/2021

[†]Interpretation of score z values. (i) Satisfactory results ($-2 \leq z \leq 2$). Monitoring results ($-3 \leq z \leq 3$). Unsatisfactory results ($-3 \geq z \geq 3$. [‡]Qualitative values. (i) Just: detection of contaminated units). (ii) Not fair: contaminated units not detected (false-negative or false-positive). [§]RAEMA: Réseau d'Analyses et d'Echanges en Microbiologie des Aliments, France. [¶]FAPAS: Food Analysis Performance Assessment Scheme, UK.

Table 2: Limit thresholds for microbiological and biochemical parameters studied.

Parameters	Abbreviation	Limit thresholds	Regulations
Total aerobic mesophilic viable count	TVC	$<10^6$ cfu/g	FCD*, 2022
Enterobacteria	ENT	$<10^3$ cfu/g	Moroccan norms, 2019
Total coliforms	TC	<10 cfu/g	FAO**, [46]
Faecal coliforms	FC	<10 cfu/g	FAO, [46]
E. coli	EC	<10 cfu/g	MPEM***, 2006
Sulphite-reducing anaerobes	ANSR	<10 cfu/g	France microbiological criteria, 2012
Salmonella	Salm	Absence/25 g	MPEM-2006
Coagulase positive *staphylococcus*	SCP	$<10^2$ cfu/g	MPEM-2006
Yeasts and Moulds	YM	$<10^2$ cfu/g	AFNOR**** norms, 1996
Total volatile basic nitrogen	TVBN	<25 mg/100 g	National 2905, 2006
Trimethylamine	TMA	<10 mg/100 g	IFRIMER*****, 2008

*FCD: Fédération du Commerce et de la Distribution. **FAO: Food and Agriculture Organization of the United Nations. ***MPEM: Ministère de la Pêche et de lEconomie Maritime. ****AFNOR: Association Française de NORmalisation. *****IFRIMER: Institut Français de Recherche pour l'Exploitation de la MER.

Count Agar (PCA; Biokar, France) was poured into each dish and homogenized by circular movements by hand in one direction and then in the other. The mixture of inoculum and culture medium was left to solidify, and the inoculated plates were incubated at 30°C for 72 hours. Colonies were counted, and this result was multiplied by the dilution factor and expressed as colony-forming units per gram (cfu/g).

For enumeration of presumptive *Enterobacteriaceae* at 30°C [23], the inoculations were carried out from dilutions 10^{-1} to 10^{-3} in the same way as the previous standard but using Crystal Violet, Neutral Red, and Glucose Bile Agar (VRBG; Biokar, France) and incubating at 30°C for 24 hours. Enterobacteria colonies are pink or red, with or without a halo of precipitate.

Enumeration of total coliforms [24] was carried out using dilutions from 10^{-1} to 10^{-3} in the same way as the previous sections with the use of Crystal Violet-Neutral Red-Lactose Bile Agar (VRBL; Liofilchen, Italy). Incubation was done at 30°C for 24 hours. Characteristic colonies are purplish and sometimes surrounded by a reddish zone.

The procedure for enumeration of Faecal coliforms [25] is the same as the one for total coliforms, except for the incubation temperature (44°C).

For enumeration of *Escherichia coli* [26], the inoculations were made from 10^{-1} to 10^{-2} dilutions in the same way as the previous standard with the use of Tryptone-Bile-Glucuronide Agar (TBX; Biokar, France) and incubated at 44°C for 24 hours. Blue colonies are suspicious of being *E. coli*.

Enumeration of sulphite-reducing anaerobes at 46°C [27] was done by inoculation of 10^{-1} to 10^{-2} dilutions in tubes containing 20 mL of tryptose-sulphite cycloserine agar (TSC; Biokar, France), supplemented with 0.4 mg/L D-cycloserine (Biokar, France). They were incubated at 46°C for 20 hours under anaerobic conditions. Black colonies were suspicious of being ANSR.

The detection of *Salmonella* [28] included the following steps: preenrichment was done using buffered peptone water (EPT; Biokar, France). Later, enrichment on Rappaport Vassiliadis medium with soy (RVS; Biokar, France) and Muller-Kauffmann with tetrathionatenovobiocin (MKTTn;

Biokar, France) was done. For isolation, both xylose lysine deoxycholate (XLD; Biokar, France) and *Salmonella-Shigella* (SS; Biokar, France) were used. Incubation was done at all stages at 37°C, except for the RVS which was at 41.5°C. The incubation time was 24 hours, except for the preenrichment which was 18 hours. Typical *Salmonella* colonies growing on XLD agar have a black centre and are surrounded by a clear, transparent red halo. H2S-negative *Salmonella* variants (e.g., S. Paratyphi A) are pink with a dark pink centre. Lactose-positive *Salmonella* are yellow without blackening. On the other hand, when growing in SS, presumptive *Salmonella* colonies are colourless with a black centre. Presumptive *Salmonella* colonies were isolated on nutrient agar (Difco, France). Biochemical confirmation was done by API 20 E (BioMérieux, France). *Salmonella* spp. Results are expressed as presence/absence.

For enumeration of coagulase-positive *Staphylococcus* at 37°C [29], 0.1 mL of the sample was spread on the surface of the plate of Baird Parker medium with egg yolk and potassium tellurite (BP; Biokar, France). The characteristic colonies are shiny black or grey, convex, and surrounded by a halo of clearing. Presumptive colonies were isolated on brain heart broth (BCC; Biokar, France). Gram staining was done to confirm Gram-positive cocci forming grape-like clusters. The presence of coagulase was tested using rabbit plasma (Biokar, France).

The [30] method was the followed for enumeration of yeasts and moulds. Samples (0.1 mL) were spread on the surface of one plate of dichloran agar with an 18% mass fraction of glycerol (DG18; Liofilchem, Italy). They were incubated at 25°C for five to seven days, and YM colonies were counted. When necessary, plates were examined using a binocular loupe or microscope to differentiate yeast or mould cells from bacterial colonies.

2.1.2. Biochemical Analysis. Two biochemical parameters were studied: total volatile basic nitrogen (TVBN) and trimethylamine (TMA). Limit values for biochemical parameters are summarized in Table 2.

Total volatile basic nitrogen [31] results from the degradation of nitrogenous compounds (proteins) during the alteration of fish products.

The steam distillation method was used to determine TVBN content. It consists of an extraction of basic nitrogen by a solution of trichloroacetic acid 7% (Lobachemie Pvt. Ltd., India), followed by the distillation in a basic medium (solution of sodium hydroxide 10% (LCH Chimie, France)) with the help of distiller KJELDHAL. Boric acid (Emprove, Germany) is used to capture the ammonia resulting from the distillation. The [(B(OH)3NH3)]+complex was titrated with 0.1 N sulfuric acid (VWR, France) to determine the nitrogen content. Results are expressed as mg of nitrogen per 100 g of muscle.

Trimethylamine [31] is a volatile amine with a strong odour. The determination of TMA was done by the same method as of TVBN, but formalin (LCH Chimie, France) was added at the distillation step to prevent the components of TVBN other than TMA. Results are expressed as mg of TMA per 100 g of muscle.

2.1.3. Statistical Analysis. Data have been classified by date (nine months have been considered, from April to July 2021 and from November 2021 to March 2022) and site. Furthermore, the values of the different variables have been codified as yes/no according to whether or not they meet the health requirements. Association between compliance with requirements and date and between compliance with requirements and site have been tested by chi-squared tests. When a significant association was detected, post hoc analysis using Fisher exact tests, with Bonferroni adjustment for multiple contrasts [32] was carried out to determine which months were significantly different from the rest and which markets showed significant differences from the relevant reference MPN market. The association between the different variables and the temperature was assessed by using Spearman correlation. The significance level was set at the value 0.05 for all the statistical tests. Statistical analysis was performed with the R Statistical Language and Environment version 4, 2, 1 [33].

3. Results

3.1. Microbiological Parameters. The values obtained for 3 of the microbiological parameters studied (coagulase-positive *Staphylococcus*, yeasts and moulds, and sulphite-reducing anaerobes) met the required levels, being under the limit values in all the samples analysed, irrespective of date or sampling area. Table 3 shows the percentage of samples meeting permitted levels for every variable in each of the observation months. Table 4 shows that percentages for every location. Figures 2(a) and 2(b) depict graphically the same information.

Salmonella was detected only in two samples, collected at Cinquième Market in July 2021 and February 2022.

Results for total viable counts ranged from 1.5×10^2 to 2×10^7 cfu/g. No statistically significant differences were found for sampling dates (chi-square p values 0.0624) or sampling sites (chi-square p value = 0.3091), with percentages of compliance greater than 80% in the different dates and greater than 88.9% on the different places.

Enterobacteria levels were studied only on 5 of 9 sampling dates. Results ranged from <10 to 1.4×10^5 cfu/g, with no significant differences between any of the sampling dates ($p = 0.0527$) in the level of compliance, ranging from 33.3% in March 2022 to 80% on December 2021.

Teyarett Market presented the worst results for this parameter, with 86.7% noncompliant samples compared to only 20% in the reference market, being this difference statistically significant ($p = 0.0027$). The rest of the markets showed noncompliance percentages ranging from 53.3% to 60% of the samples, although these percentages did not differ significantly from the reference market ($p > 0.18$).

There was a high percentage of samples (88.9% or higher) exceeding the permitted levels of TC in all studied areas (except in the reference area). The differences between the values obtained in these markets and those obtained in the reference one are all statistically significant ($p < 0.0001$ in all cases). Even so, there was a relatively high percentage (25.9%) of samples from the reference

Table 3: For each variable and each observation month, the percentage of samples NOT meeting the quality standard as well as the q/n values (absolute number of samples not meeting the quality standard/total number of available samples) are shown. For each variable, the p value of the chi-square tests for testing whether the differences between months are significant is also shown.

Variable	2021-4	2021-5	2021-6	2021-7	2021-11	2021-12	2022-1	2022-2	2022-3	P
TVC	13.3% (2/15)	0.0% (0/15)	0.0% (0/15)	0.0% (0/15)	0.0% (0/15)	0.0% (0/15)	6.7% (1/15)	20.0% (3/15)	0.0% (0/15)	0.0624
ENT					66.7% (10/15)	20.0% (3/15)	60.0% (9/15)	60.0% (9/15)	66.7% (10/15)	0.0527
TC	80.0% (12/15)	80.0% (12/15)	73.3% (11/15)	93.3% (14/15)	80.0% (12/15)	53.3% (8/15)	100.0% (15/15)	80.0% (12/15)	80.0% (12/15)	0.1318
FC	73.3% (11/15)	20.0% (3/15)	13.3% (2/15)	33.3% (5/15)	46.7% (7/15)	40.0% (6/15)	33.3% (5/15)	46.7% (7/15)	26.7% (4/15)	0.0413
EC	26.7% (4/15)	6.7% (1/15)	6.7% (1/15)	26.7% (4/15)	13.3% (2/15)	0.0% (0/15)	6.7% (1/15)	0.0% (0/15)	6.7% (1/15)	0.1358
TVBN35	13.3% (2/15)	0.0% (0/15)	6.7% (1/15)		0.0% (0/15)	0.0% (0/15)	0.0% (0/15)	0.0% (0/15)	0.0% (0/15)	0.3197
TVBN25	66.7% (10/15)	33.3% (5/15)	20.0% (3/15)		13.3% (2/15)	0.0% (0/15)	13.3% (2/15)	6.7% (1/15)	0.0% (0/15)	0.0002
TMA	73.3% (11/15)	60.0% (9/15)	13.3% (2/15)		20.0% (3/15)	0.0% (0/15)	20.0% (3/15)	6.7% (1/15)	0.0% (0/15)	0.0002

Table 4: For each variable and each market, the percentage of samples not meeting the quality standard as well as the q/n values (absolute number of samples not meeting the quality standard/total number of available samples) are shown. For each variable, the p value of the chi-square tests for testing whether the differences between markets are significant is also shown.

Variable	MPN (reference)	Market Cinquième	Market eff	Market Teyarett	MNP	P
TVC	0.0% (0/27)	7.4% (2/27)	0.0% (0/27)	11.1% (3/27)	3.7% (1/27)	0.3091
ENT	20.0% (3/15)	53.3% (8/15)	60.0% (9/15)	86.7% (13/15)	53.3% (8/15)	0.0084
TC	25.9% (7/27)	92.6% (25/27)	92.6% (25/27)	100.0% (27/27)	88.9% (24/27)	<.0001
FC	3.7% (1/27)	48.1% (13/27)	40.7% (11/27)	77.8% (21/27)	14.8% (4/27)	<.0001
EC	0.0% (0/27)	14.8% (4/27)	11.1% (3/27)	22.2% (6/27)	3.7% (1/27)	0.0644
TVBN35	0.0% (0/24)	4.2% (1/24)	0.0% (0/24)	4.2% (1/24)	4.2% (1/24)	1.0000
TVBN25	12.5% (3/24)	29.2% (7/24)	12.5% (3/24)	29.2% (7/24)	12.5% (3/24)	0.2855
TMA	16.7% (4/24)	33.3% (8/24)	20.8% (5/24)	29.2% (7/24)	20.8% (5/24)	0.7095

Figure 2: This figure graphically depicts the percentages of samples that fulfill requirements for every variable in each month as shown in Table 2 (a) and in each site as shown in Table 3 (b).

zone that did not meet the standards either, with values ranging from $<4 \times 10$ to 8.7×10^2 cfu/g. For the remaining sampling areas, TC levels ranged from <10 to 4×10^4 cfu/g.

The compliance rate for faecal coliforms showed appreciable variability over the study period, from only 26.7% in April 2021 to a maximum of 86.7% in June 2021. In the remaining months, this percentage ranged from 53.3% to 80%. Although the differences are globally significant (chi-square test p value = 0.0413), this significance can be attributed to the presence of the two extreme values cited, as when considering only the rest of the values, this significance disappears ($p = 0.7321$). When we analysed results obtained for different markets, Cinquième Market ($p = 0.0010$), Eff Market ($p = 0.0043$), and Teyarett Market ($p < 0.0001$) showed statistically significant differences with MPN. While the compliance percentage was 96.3% in MPN, it remained below 59% in the above-mentioned markets.

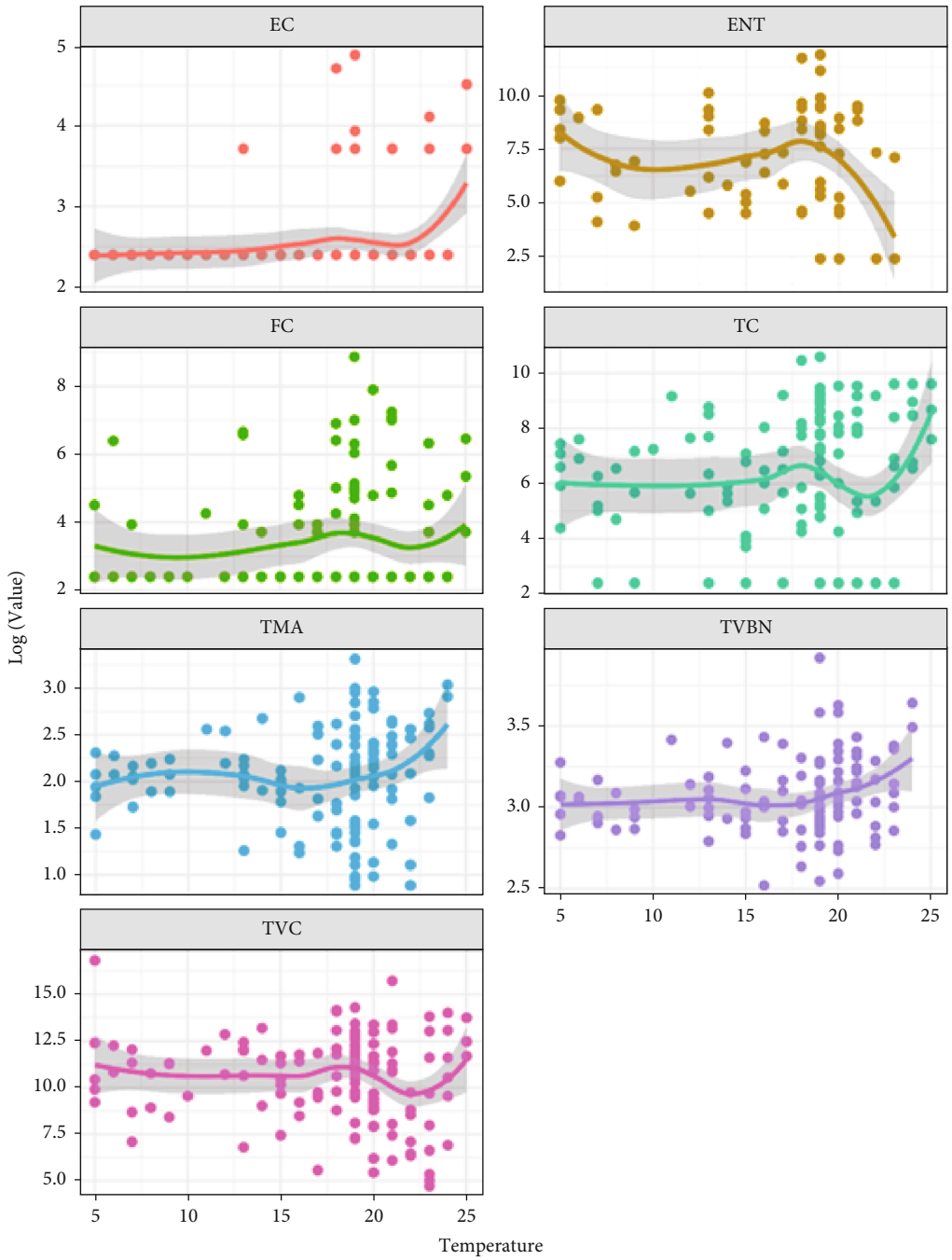

Figure 3: Scatterplot of the observed values of the variables in the samples (logarithmic scale) represented against temperature. In all cases, a smoothing spline has been fitted to the points to better visualize possible associations.

Levels of *E. coli* ranged from <10 to 1.3×10^2 cfu/g. Teyarett Market presented worst results than the rest of the sampling zones, but these differences were not statistically significant (chi-square test p value = 0.0644). Also, no statistically significant differences in results for different sampling dates were observed (chi-square test p value = 0.1358).

3.2. Biochemical Parameters. Due to technical reasons, all the TVBN and TMA results from July 2021 were discarded.

TVBN values ranged from 11.42 to 49.31 mg/100 g. Statistically significant differences were found for TVBN values when comparing sampling dates (chi-square test p value = 0.0002), with April, May, and June 2021 having the worst results than the rest of the sampling months ($p < 0.0311$). No differences among sampling sites were observed (chi-square test p value = 0.2855).

TMA values ranged from 1.43 to 19.82 mg/100 g. Again, for this parameter, statistically significant differences were found when comparing sampling dates (chi-square test p value = 0.0002). In this case, compliance rate values

Table 5: Spearman correlation between each variable and temperature. p values are shown for testing significance of these correlations.

Variable	Spearman Corr.	p
TVC	-0.08	0.3389
ENT	-0.16	0.1801
TC	0.07C	0.3915
FC	0.02	0.8531
EC	0.19	0.0277
TVBN	0.21	0.0196
TMA	0.20	0.0305

obtained in April and May 2021 (less or equal than 40%) presented statistically significant differences with the ones obtained in the rest of the sampling months that were greater than 80% ($p < 0.0277$). There were no significant differences between markets ($p = 0.7095$) with the compliance rate ranging from 66.7% in Cinquième Market to 83.3% in the reference site (MPN).

3.3. Correlation between Compliance with Standards and Sample Temperature. According to the International Standard [21], the temperature of fresh fish should be between 0 and 10°C. For all markets, the mean storage temperature of samples was 18°C (median 19°C (5°C to 25°C)). For the MPN (reference), the lowest temperature recorded was 15°C. Regarding all markets, the average value of nonconformity was 87.4%. The market with the highest rate of nonconformity was the MPN Référence, where all samples were stored at a temperature over 15°C. In the MNP market, we had the best rate of conformity, where 9 temperatures records were under 10°C (33%).

The correlation between percentages of samples meeting microbiological and biochemical requirements and sample temperature was studied using the Spearman correlation coefficient. Results are shown in Figure 3. For TVBN, two different calculations were done using 25 and 35 mg/100 g, respectively. A statistically significant correlation was found for *E. coli*, TVBN, and TMA values ($p \leq 0.05$), as shown in Table 5.

4. Discussion

This study analyses the level of regulatory compliance for several biochemical and microbiological parameters in samples of freshly caught fish and then in samples of fish from the same batches on sale in several markets in Mauritania.

When samples were taken just after arriving on boats, microbiological standards were almost always met. From our results, it seems that contamination happens postharvesting and during handling and selling on markets. The lack of a cold chain and noncompliance with hygiene measures produce an increase in microbiological load [34]. In contrast to what has been described in other studies [35], the results do not seem to be due to microbial contamination of the waters where fishing takes place.

Some authors [36] propose that this impact will be higher during the summer season, when temperatures are higher. However, we did not find statistically significant differences for different sampling months during the year, with percentages of compliance for TVC greater than 80% on the different dates, a bit lower than the ones described by other authors in Brazil [37]. No significant differences between any of the sampling dates in the level of compliance were found for enterobacteria or other microbiological parameters.

A study by [36] found very few samples contaminated with *E. coli*, *Salmonella* spp., or *S. aureus*. Similar results were obtained in Eritrea [17]. This agree with our results for *Salmonella* spp. or *S. aureus*, but in our study, noncompliance with *E. coli* standards was found in 14 samples (10.37%). In a study in Morocco [38], in an area close to the one we have studied, 17.5% of fish samples harboring *E. coli* were found.

Salmonella presence has been described in 10% of seafood samples from markets in Egypt [39] a higher prevalence than ours (1.48%). Maramarque Nespolo et al. [37] found *S. aureus* in one sample (3%) however did not found *E. coli* in any samples of salmon *(Salmo salar)* tested in Brazil. In that study, TC levels were higher than the limit in 20% of samples and FC in 6.4%.

FC and *E. coli* are indicators of faecal contamination. The presence of *E. coli* reflect contamination with faecal matter or sewage [40]. Lázaro *et al.* [41] examined the water, environment, and sanitation characteristics of open-air markets in Malawi, correlating them with food safety. As in our results, the need for safe water, functional toilets, and handwashing stations with soap at every market, as well as food hygiene education of vendors, is remarked.

In a study at an inland city in Turkey [42], many fish samples were unacceptable in terms of loads of diverse indicator bacteria. *Salmonella* was isolated in 39.3% of samples, *S. aureus* in 28.6%, and all the samples were unacceptable according to the critical limit for *E. coli*. But the author did not compare his results with the ones of fresh-caught fish.

Regarding markets, the worst results were obtained for Teyarett Market, but *Salmonella* was detected only in samples from Cinquième Market.

N'Guessam et al. [43] used Ishikawa's method to identify the causes of sanitary insecurity in the frame of a study about fresh fish sold in Ivory Coast. Their results support the need for qualification, training, and/or awareness of good hygiene practices at the transport, processing, and marketing of fish. When analysing Norwegian pelagic fisheries sector, Smith Svanevik *et al.* [44] did 41 samplings, which included 628 fish samples and 533 contact point samples. They found that 21 of 41 samplings were not in compliance concerning hygiene, and 9 of 41 were not in compliance concerning food safety.

A statistically significant correlation between percentages of samples meeting microbiological and biochemical requirements and sample temperature was found for *E. coli*, TVBN, and TMA values. For TVBN and TMA, noncompliance was found in 18.7% and 23.57% of samples, respectively. Jeyasekaran et al. [45] investigated the effect of delayed freezing on the bacteriological quality of barracuda

(Sphyraena barracuda). The fish were divided into two batches: one was frozen immediately (II), and the other was left at room temperature for 6 hours and then frozen (DI). Immediate freezing extended the shelf life of the barracuda *(Sphyraena barracuda)* by 6 days. In II fish, total coliforms were very low, while in DI total coliforms were high. These results reinforce our idea about the relevance of sample temperature in microbiological criteria compliance.

5. Conclusion

Fishing is one of the main economic resources in Mauritania, and the exported product complies with the sanitary legislation required by the many countries that purchase it. It would be necessary to make an effort at the governmental level to extend this compliance to the domestic fish market, especially for areas far from the coast, given the large size of the country.

From our results, we consider that the main problem for meeting microbiological and biochemical standards in fish's samples is the postharvesting contamination. The absence of enough ice to avoid fecal bacteria growth aggravates the problem. Great efforts are needed to implement a cold chain and to improve hygiene measures in markets, but also to increase formation on food safety. This will contribute to protect the health of consumers.

Data Availability

Data are available from corresponding author upon reasonable request.

Consent

Corresponding and all the co-authors are willing to participate in this manuscript. All authors are willing for publication of this manuscript.

Conflicts of Interest

Authors declare that they have no conflict of interest.

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