Original Research Article

Microbiological compliance assessment of imported frozen fishes and local fresh chilled fishes marketed in northern Benin (West Africa)

ABSTRACT

Since the food safety and the foodborne diseases are becoming a main health concern in developing countries, the aim of this study is to determine, in accordance with the specific international standards, the compliance of the main frozen imported fish and fresh chilled fishes marketed and consumed in Northern Benin. From December 18 to March 5, 2019, the microbiological quality of the two main imported frozen fish (*Scomber scombrus* and *Trachurus trachurus*) and the two main freshwater fish locally produced (*Clarias gariepinus* and *Oreochromis niloticus*) and marketed in Benin were analysed in accordance with ISO standards specific to each germ counted as Colony-Forming Units (CFU). The Mesophilic aerobic flora and fecal coliforms loads in fresh chilled fishes (256,577 and 349.6 CFU, respectively) are significantly higher ($p<0.05$) than in the frozen fish (143,620 and 157.0 CFU, respectively) marketed in the northern Benin. *T. trachurus* seems more contaminated ($p<0.01$) by these germs than *S. Scombrus*, and *O. niloticus* showed significantly higher loads ($p<0.01$) of these bacteria than the *C. gariepinus*. No salmonella colony was observed in all the samples, and in the fresh and frozen fish, *Staphylococcus aureus* (11.27 and 10.77 CFU, respectively) and Sulfite-Reducing Anaerobes (0.38 and 0.38 CFU, respectively) loads showed no significant differences ($p>0,05$) both between origin and between species. However, the microbiological quality of all fish both imported frozen fishes and fresh fishes analysed during this study have not comply with the requirements of the standard AFNOR (2000) specific to frozen fish and fresh chilled fish. They are so classified as “unsatisfactory hygienic” due to their very high fecal coliforms loads. It would therefore be interesting to raise awareness among stakeholders in the marketing system for fish products on good hygiene practices and the HACCP approach.

Keywords: Frozen fish; fresh fish; microbiological quality; AFNOR 2000 standard; food safety

1. INTRODUCTION

Seafood plays an important role in human nutrition by contributing near 125 000 million tonnes per annum of finfish, shellfish, and other edibles, both from fisheries catch and from aquaculture production. Fisheries products, in particular fish, are foods of high nutritional value to humans and are one of the main and the best source of animal protein [1]. According to [2], the value of fish proteins is very important, better than meat protein, and has a stable composition of essential amino acids. It’s an easily digestible food and is often recommended to consumers by nutritionists and dieticians. [3] showed that eating fish, especially fatty fish, reduces the risk of muscle degeneration in old age.

However, fish is an easily perishable product due mainly to proteolytic reactions related to digestive, tissue and microbial enzymes [4, 5, 6]. Fishery products have been recognized as a major carrier of

foodborne pathogens [7, 8, 9, 10, 11, 12, 13]. According to [14], pathogenic bacteria associated with fishery product can be categorized into three main groups: *(i)* the indigenous bacteria that belong to the natural micro-flora of fish (*e.g., Clostridium botulinum, pathogenic Vibrio spp., Aeromonas hydrophila*); *(ii)* the enteric bacteria that are present due to fecal contamination (*e.g., Salmonella spp., Shigella spp.*, pathogenic *Escherichia coli*, *Staphylococcus aureus*); and *(iii)* the bacteria which contaminate during processing, storage or cooking (*e.g., Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Clostridium perfringens, Salmonella spp.*). Several authors [15, 16, 17, 10, 18, 19] have reported that improper storage and handling of fishery products can also increase growth of spoilage bacteria (*e.g. Lactobacillus* spp., *Proteus* spp., *Shewanella putrefaciens, Pseudomonas* spp).

In Benin, the most fish marketed and consumed are frozen fish imported from various origins [1]. In 2017, about 108,026 tons of frozen fish were imported, while national fish production is estimated to 52,251 tons (*Fisheries authority, Benin; unpublished national report 2019*). Among these species, Horse mackerel (*Trachurus trachurus*), Atlantic mackerel (*Scomber scombrus*) and Sardinella (*Sardinella aurita*) are mainly marketed and appreciated by the consumers. To ensure their preservation before sale, several methods (smoking, drying, salting, frying, refrigerating and freezing) are usually used to increase their safety [20, 6, 21, 22, 23, 24]. It's remarkable throughout the country that cold storage methods (freezing and refrigeration) are generally used by fish trader as well in the big cities, than in the remote villages. However, for several decades, the West African countries, in particular Benin, are facing an energy deficit that leads to a daily load shedding plan depriving households and traders of electricity for several hours (up to 48 hours sometimes). In addition, because of the high cost of electricity, many fish shops unplug their freezers during the night, which often leads to breakage of the cold chain during storage, accelerating the bacterial growth and physicochemical alterations of the products responsible for foodborne diseases [25, 26, 27, 21, 24]. Furthermore, locally produced fresh fish, including catfish (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*), are generally marketed fresh, either directly by fish farmers or fishmongers in various rural or urban markets. Nevertheless, storage times at ambient temperature from capture to markets or cuisine are often long which could lead to bacterial contamination of fresh fish marketed [28, 29].

It's therefore to determine, in accordance with the specific international standards, the compliance of the main frozen imported fish and fresh chilled fishes marketed and consumed in northern Benin that the present study was initiated to assess their microbiological quality, since the food safety and the foodborne diseases are becoming a global health concern.

2. MATERIALS AND METHODS

2.1. Study area

This study was conducted from December 18 to March 5, 2019 in the cities of Parakou, Kandi and Malanville located in the Northern Benin (Fig. 1). The microbiological analyses were carried out consecutively at the research unit of Food Processing and Quality Control (FPQC) of the laboratory of aquaculture and Ecotoxicology of the University of Parakou and at the Central Laboratory for the

Control of Food Safety (LCSSA) of the Ministry of Agriculture, Livestock and Fisheries (MAEP, Benin), an ISO/IEC 17025 : 2005 accredited structure since 2015.

Parakou (9°20'47'' N and 2°20'46'' E) is the main city of North Benin (about 500 km from the capital Cotonou) which covers an area of 441 km², of which 66% is urbanized with 225,478 inhabitants. Kandi (11°08'06'' N and 2°55'55'' E), is located at about 215 km of the North of Parakou and extends over 3,421 km² with about 179,290 inhabitants. Malanville (11°51'40'' N and 3°23'22'' E) is the border town located further north, along the Niger River, at about 800 km from Cotonou and 300 km from Parakou. Its covers 3,016 km² with about 168,641 inhabitants.

2.2. Sampling

Fish used for microbiological analyses were: *Scomber scombrus* (Atlantic mackerel) and *Trachurus trachurus* (Horse mackerel), the two main imported frozen fish marketed in Benin; and *Clarias gariepinus* (African catfish) and *Oreochromis niloticus* (Tilapia), the two main freshwater fish locally produced and marketed in Benin. These four fish species were also chosen because they are the most consumed in sub Saharan African countries, particularly in Benin and therefore of great interest for the current study.

The frozen fishes (Atlantic mackerel and Horse mackerel) were purchased from the three fish shops chosen per municipality. In each fish shop investigated, three samples (approximately 3 kg per sample) of each species were aseptically collected and placed in labelled sterile polyethylene bags and transported in an ice box (4 °C) with dry ice to the laboratory. Freshly caught local fishes were purchased from random fishermen. Three samples (about 1 kg per sample) of each freshwater species (African catfish and Nile tilapia) were collected in each town, *i.e.* a total of 18 samples taken and stored at 4 °C, as indicated above until the laboratory.

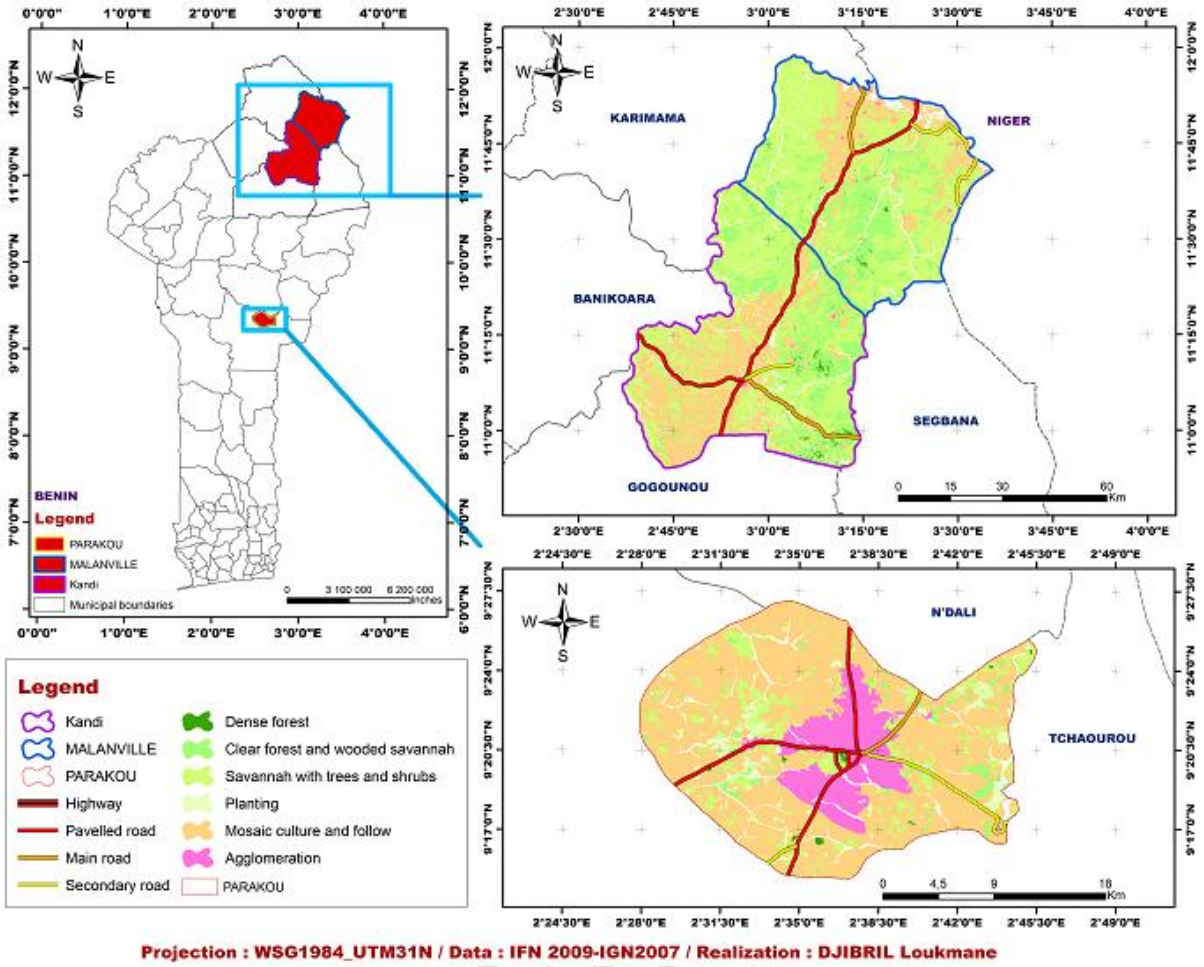

Fig. 1. Map showing the study area

2.3. Microbiological analysis

At the laboratory, the superficial and deep parts of the fishes were collected aseptically using sterilized knives and pliers. Each sample taken (about 25 g) was used to prepare the stock solution from which fecal pollution indicator germs and foodborne pathogens were searched. These include Mesophilic Aerobic Flora, fecal coliforms, *Escherichia coli*, suspected pathogenic staphylococci, *Salmonella spp*, and Sulfite-Reducing Anaerobes (ASR) germs (*Clostridium perfringens*). All the samples were analysed in accordance with French (NF) and/or European (EN) ISO standards specific to each germ counted as Colony-Forming Units (CFU), as follow:

- Mesophilic Aerobic Flora: *ISO 4833 : 2003*;
- Fecal Coliforms: *NF V 08-050 : 1999*;
- Presumed pathogenic staphylococci : *NF EN ISO 6888-2 : 1999*;
- *Salmonella spp*: *ISO 6579 : 2002*;
- *ASR germs*: *ISO 15213 : 2003*.

Furthermore, the standards of AFNOR (12/12/2000), specific to frozen or deep-frozen fish and those relating to chilled fresh fish, were used to assess the compliance of products and their classification. The critical limits set by this standard are shown in Table 1.

Table 1. Critical limits of the standard AFNOR 2000 for fresh and frozen fish

Variables	Frozen fish	Chilled fresh fish
Mesophilic Aerobic Flora (Log CFU/g)	4/g	5/g
Fecal Coliforms (Log CFU/g)	0/g	1/g
Staphylococcus aureus (Log CFU /g)	2/g	2/g
Salmonella spp (Log CFU /25g)	Absence/25g	Absence/25g
ASR Germs (Log CFU /g)	0,3/g	1/g

CFU: Colony-Forming Units ; AFNOR : Association Française de Normalisation ;
ASR : Sulfite-Reducing Anaerobes (Clostridia)

2.4. Statistical analysis

The Statistical Analysis System software (SAS, 2006) was used for data analysis. The factors of variation considered were the region (Parakou, Kandi and Malanville) and the fish species (*Scomber scombrus; Trachurus trachurus*, *Clarias gariepinus;* and *Oreochromis niloticus*). The data were analysed according to General Linear Model procedure (GLM) of SAS (2006). The F test was used to determine the significance of the region and the fish species effects. Then, the least squares means were estimated and compared by the Student test.

3. RESULTS

3.1. Microbiological quality of frozen and chilled fresh fishes studied

For the imported frozen fishes (Table 2), only fecal coliforms loads showed a significant differences among species (*P<0.001*). The horse mackerel (*T. trachurus*) seems more contaminated (*p<0.01*) by these germs than the Atlantic mackerel (*S. Scombrus*) (Table 4), while no significant differences were observed among origin of fish samples studied (Table 2). No salmonella colony was observed, and *S. aureus* and Sulfite-Reducing Anaerobes (*Clostridia*) loads showed no significant differences both between origin and between species (Table 2 and 4).

For chilled fresh fishes investigated, the Mesophilic Aerobic Flora, fecal coliforms Staphylococcus loads showed significant differences among species and among origin (Tables 3 and 4). Nile tilapia (*O. niloticus*) showed significantly higher loads (*p<0.01*) of these bacteria than the African catfish (*C. gariepinus*), and fishes from Parakou city are less contaminated by these germs than those from Kandi

and Malanville (Table 3). *Salmonella spp* and ASR germs loads also showed no significant differences, both between origin and between species (Table 3 and 4).
Overall, chilled fresh fishes analysed were more contaminated by the enterobacteria than the imported frozen fishes (Table 5).

3.2. Microbiological compliance of imported frozen fish and fresh chilled fish studied

The microbiological quality of all imported frozen fishes (*S. scombrus* and *T. trachurus*) and fresh chilled fish (*C. gariepinus* and *O. niloticus*) collected during this study have not comply with the requirements of the standards AFNOR (2000) specific to frozen or deep-frozen fish (Table 6) and fresh chilled fish (Table 7). They are therefore classified as “unsatisfactory” because of their high enterobacteria loads, particularly fecal coliforms, germs indicator of hygiene. However, it’s important to note the absence of salmonella (0 CFU / 25g) in 25 grams of frozen and fresh chilled fishes analysed, and the staphylococci and ASR germs, despite their no less important loads in these fishes (Tables 1, 2, 3, 4 and 5), remain below the “unsatisfactory” limit set by the standard AFNOR (2000) specific to frozen or deep-frozen fish and fresh chilled fish.

Table 2: Microbiological quality of main imported frozen fishes (*Scomber scombrus* and *Trachurus trachurus*) marketed in northern Benin

Variables	PARAKOU				KANDI				MALANVILLE				Zone effect	Species effect
	Scomber scombrus		*Trachurus trachurus*		*Scomber scombrus*		*Trachurus trachurus*		*Scomber scombrus*		*Trachurus trachurus*			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
Mesophilic Aerobic Flora (CFU/g)	142,087	21,221	130,258	19,182	118,005	3,528	165,289	8,914	118,738	14,878	187,342	10,848	***NS***	***NS***
Fecal coliforms (CFU/g)	155.00	16.50	151.30	32.20	116.67	6.89	187.00	15.40	124.33	9.84	207.67	8.01	***NS***	*****
Staphylococcus aureus (CFU/g)	10.67	1.33	10.33	0.88	13.00	1.53	10.33	0.88	10.33	0.88	10.00	0.58	***NS***	***NS***
Salmonella spp (CFU/25g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***NS***	***NS***
ASR germs (CFU/g)	0.33	0.33	0.67	0.33	0.33	0.33	0.00	0.00	0.33	0.33	0.67	0.33	***NS***	***NS***

*CFU: Colony-Forming Units; ASR: Sulfite-Reducing Anaerobes (Clostridia); NS: Not significant; SE: Standard Error; **: P<0.01.*

Table 3: Microbiological quality of chilled fresh *Clarias gariepinus* and *Oreochromis niloticus* marketed in northern Benin

Variables	PARAKOU				KANDI				MALANVILLE				Zone effect	Species effect
	Clarias gariepinus		*Oreochromis niloticus*		*Clarias gariepinus*		*Oreochromis niloticus*		*Clarias gariepinus*		*Oreochromis niloticus*			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
Mesophilic Aerobic Flora (CFU/g)	151,777	5,386	228,895	10,227	269,419	9,519	208,380	4,899	230,559	20,661	450,435	29,899	**	*
Fecal coliforms (CFU/g)	208.67	8.69	299.00	8.39	329.00	23.70	304.70	15.60	309.00	18.10	647.30	19.90	**	**
Staphylococcus aureus (CFU/g)	7.33	0.33	10.00	1.00	10.33	1.20	14.00	1.15	10.33	0.88	15.67	0.33	**	***
Salmonella spp (CFU/25g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***NS***	***NS***
ASR germs (CFU/g)	0.00	0.00	0.33	0.58	0.33	0.33	0.00	0.00	0.67	0.33	1.00	0.33	***NS***	***NS***

*CFU: Colony-Forming Units; ASR: Sulfite-Reducing Anaerobes (Clostridia); NS: Not significant; SE: Standard Error; *: P<0.05; **: P<0.01; ***: P<0.001.*

Table 4: Comparative microbiological quality of frozen fish and chilled fresh fishes studied

Variables	Fish Species	Mean	SE Mean	Variation Coefficient	Species effect
Mesophilic Aerobic Flora (CFU/g)	*Clarias gariepinus*	196,905a	23,455	20.63	*
	Oreochromis niloticus	316,249b	68,105	37.30	
	Scomber scombrus	126,277c	7908	10.85	
	Trachurus trachurus	160963a	16620	17.88	
Fecal coliforms (CFU/g)	*Clarias gariepinus*	282a	37.2	22.84	*
	Oreochromis niloticus	417b	115.0	47.83	
	Scomber scombrus	132c	11.7	15.36	
	Trachurus trachurus	182d	16.5	15.68	
Staphylococcus aureus (CFU/g)	*Clarias gariepinus*	9.3a	1.00	18.62	*NS*
	Oreochromis niloticus	13.2a	1.69	22.11	
	Scomber scombrus	11.3a	0.84	12.86	
	Trachurus trachurus	10.2a	0.10	1.70	
ASR germs (CFU/g)	*Clarias gariepinus*	0.3a	0.203	105.36	*NS*
	Oreochromis niloticus	0.4a	0.296	118.42	
	Scomber scombrus	0.3a	0.000	0.00	
	Trachurus trachurus	0.5a	0.233	86.60	

*CFU: Colony-Forming Units; ASR: Sulfite-Reducing Anaerobes (clostridia). *: P<0.05; NS: Not significant; values in the same column followed by different letters are significantly different (p<0.05).*

Table 5: Effect of preservative method used on microbiological quality of fish marketed in northern Benin

Variables	Type of fish preservation	Mean	Standard Error	Variation Coefficient	Effect of preservation type
Mesophilic Aerobic Flora (CFU/g)	Fresh fish	256,577a	41,831	39.94	*
	Frozen fish	143,620b	11,310	19.29	
Fecal coliforms (CFU/g)	Fresh fish	349.6a	61.9	43.40	*
	Frozen fish	157.0b	14.4	22.43	
Staphylococcus aureus (CFU/g)	Fresh fish	11.27a	1.24	27.02	*NS*
	Frozen fish	10.77a	0.46	10.37	
ASR germs (CFU/g)	Fresh fish	0.38a	0.16	103.58	*NS*
	Frozen fish	0.38a	0.11	70.80	

*CFU: Colony-Forming Units; ASR: Sulfite-Reducing Anaerobes (clostridia). *: P<0.05; NS: Not significant; values in the same column followed by different letters are significantly different (p<0.05).*

Table 6. Microbiological compliance of frozen *Scomber scombrus* and *Trachurus trachurus* marked in northern Benin

Variables	*Scomber scombrus*			*Trachurus trachurus*			*Standard AFNOR (2000)*	*Classification by germ*
	Parakou	**Kandi**	**Malanville**	**Parakou**	**Kandi**	**Malanville**		
Mesophilic aerobic flora (Log CFU/g)	>5	>5	>5	>5	>5	>5	*4/g*	***Unsatisfactory***
Fecal coliforms (Log CFU /g)	>1	>1	>1	>1	>1	>1	*0/g*	***Unsatisfactory***
Staphylococcus aureus (Log CFU /g)	1	1	1	1	1	1	*2/g*	***Satisfactory***
Salmonella spp (Log CFU /25g)	<1	<1	<1	<1	<1	<1	*Absence/25g*	***Satisfactory***
ASR Germs (Log CFU /g)	<1	<1	<1	<1	<1	<1	*0,3/g*	***Satisfactory***
Overall quality	***Non-compliant***	***Non-compliant***	***Non-compliant***	***Non-compliant***	***Non-compliant***	***Non-compliant***	*-*	***Unsactisfactory***

CFU: Colony-Forming Units; AFNOR: Association Française de Normalisation; ASR : Sulfite-Reducing Anaerobes (Clostridia)

Table 7. Microbiological compliance of fresh chilled *Clarias gariepinus* and *Oreochromis niloticus* marketed in northern Benin

Variables	*Clarias gariepinus*			*Oreochromis niloticus*			*Standard AFNOR (2000)*	*Classification by germ*
	Parakou	**Kandi**	**Malanville**	**Parakou**	**Kandi**	**Malanville**		
Mesophilic aerobic flora (Log CFU/g)	>5	>5	>5	>5	>5	>5	*5/g*	***Unsatisfactory***
Fecal coliforms (Log CFU /g)	>1	>1	>1	>1	>1	>1	*1/g*	***Unsatisfactory***
Staphylococcus aureus (Log CFU /g)	1	1	1	1	1	1	*2/g*	***Satisfactory***
Salmonella spp (Log CFU /25g)	<1	<1	<1	<1	<1	<1	*Absence/25g*	***Satisfactory***
ASR Germs (Log CFU /g)	<1	<1	<1	<1	<1	<1	*1/g*	***Satisfactory***
Overall quality	***Non-compliant***	***Non-compliant***	***Non-compliant***	***Non-compliant***	***Non-compliant***	***Non-compliant***	*-*	***Unsatisfactory***

CFU: Colony-Forming Units ; AFNOR : Association Française de Normalisation ; ASR : Sulfite-Reducing Anaerobes (Clostridia)

4. DISCUSSION

From this study, it appears that the overall quality of imported frozen fish (*S. scombrus* and *T. trachurus*) and fresh chilled fish (*C. gariepinus* and *O. niloticus*) marketed in the northern Benin does not comply with the requirements of the AFNOR (2000) Standards specific to fresh chilled or frozen fish. These results matched those reported on frozen, chilled and artisanal smoked fishes by previous studied in west Africa countries [30, 31, 24].

According to [32], microbial flora in fish is mainly depend of the environment in which they are been caught or processed rather than on the fish species. Fish caught in very cold or clean waters carry the lower numbers whereas fish caught in warm waters have slightly higher load of bacteria. Contamination of hands and surfaces during cleaning and gutting of fish is a common route of infection of fisheries products. In the present study, Mesophilic Aerobic flora, particularly Fecal Coliforms, hygienic indicator germs, were observed in all of the frozen and chilled fishes samples collected and analysed. However, fresh fishes analysed were more contaminated ($p<0.05$) than frozen fish, although both did not comply with AFNOR (2000) standard. [24] also reported that Total Mesophilic Aerobic Flora loads were significantly higher ($p<0.001$) in chilled *S. scombrus* and *T. trachurus* samples than in frozen fish in the southern Benin. This is in accordance with [32] who reported that in warmer waters, higher numbers of mesophiles bacteria can be isolated comparatively to temperate waters. Because Enterobacteria are the host of the digestive tract of humans and animals consequently their presence is due to contamination of fecal origin. Enterobacteria are usually considered as hygiene indicators and therefore used to monitor the preventive pre-requisite measures such as Good Manufacturing Practices and Good Hygiene Practices (GMP/GHP) (Cox et al., 1988). In this study, all of the fish shop investigated do not have hand washing and disinfection facilities. Thus, according to the standards, the requirement to wash hands before each resumption of work is not met. Furthermore, since these shops do not have a fence, stray pets can also contaminate equipment and products through their faeces that they leave behind during their visit to the site. As reported by many authors in frozen and fresh fish in Benin [31, 24], fortunately no salmonella was observed in the frozen and fresh fish analysed during our study. The absence of this potentially pathogenic germ was also reported by [31] when assessing the microbiological quality of *T. trachurus* during the traditional smoking process.

The presence of *Staphylococcus aureus* in our samples indicates non-compliance with good hygiene practices by producers and sellers during distribution operations. Indeed, *S. aureus* is a highly pathogenic germ; through its enterotoxins, it can cause foodborne illness in humans, resulting in nausea, headache, abdominal pain, severe, uncontrollable and repeated vomiting, often accompanied by diarrhea. However, despite the presence of *S. aureus* in our samples, their loads (9.3 to 13.2 CFU/g) remain below the limit of non-compliance defined by the AFNOR standard for fresh and frozen fishes. No significant difference was observed between frozen fish and the fresh fish analysed, and all the samples collected were compliant with the AFNOR (2000) standard defined for this germ. Similar results are reported in frozen, fresh and traditionally smoked fish [33, 31, 24].

Overall, all fish collected and analysed both frozen and fresh do not comply with the AFNOR 2000 standard. [11], also found that 66.6% of fresh and frozen fish caught off the Adriatic coast of Croatia

were unacceptable according to the Croatian microbiological standards for foods. Our results show that, both in the fish shops investigated and in the environment where the local fishes are caught, the hygiene procedure for handling does not comply the standard required since Thermo-tolerant coliforms are a sign of poor hygiene conditions. In general, as reported by many authors for tropical fish species [34, 35], Gram-negative bacteria (Mesophilic aerobic flora and fecal coliforms) dominate the microflora of fish marketed in the northern Benin.

5. CONCLUSION

The microbiological quality of all fish both imported frozen and (*S. scombrus* and *T. trachurus*) and fresh chilled fish (*C. gariepinus* and *O. niloticus*) analysed during this study have not comply with the requirements of the standards AFNOR (2000) specific to frozen fish and fresh chilled fish. All these fish are therefore classified as "unsatisfactory hygienic" due to their very high Mesophilic aerobic flora and Fecal Coliforms loads for which hands and surfaces hygiene during cleaning and gutting of fish is a common route of contamination. It would therefore be interesting to raise awareness among stakeholders in the marketing system for fish products on good hygiene practices and the HACCP approach.

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