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RESEARCH ARTICLE

ASSESSMENT OF THE MICROBIOLOGICAL QUALITY OF FRESH FISH AT THE FISHERY HARBOUR OF LOMÉ IN TOGO

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Abstract

Microbiological contamination of fish can cause foodborne illness and constitute a threat to the consumer's health. This study was conducted to assess the microbiological quality of fresh fish landed at the fishery harbor of Lomé in Togo on 2016. The samples of fish, water and ice were collected by random sampling from fishermen, wholesalers and pond water. The observation of fishery stakeholder's reveals contamination due to insufficient knowledge of good hygiene practices, production and handling of fresh fish. The count of colonies was expressed by colony forming units (CFU/ml) according to the standard NF V08-051: 1999. The results show that the fishes, ices and waters were heavily contaminated with an average of TAFs (6.10^7 CFU/g), TTCs ($1,15.10^7$ CFU/g), E Coli ($1,2.10^3$ CFU/g), ASR ($1,1.10^4$ CFU/g); Staphylococci ($1,1.10^5$ CFU/g), FS ($3,8.10^3$ CFU/ml of water and $5,5.10^2$ CFU/ml of ice), CT ($6,6.10^3$ CFU/ml of water and $2,4.10^3$ CFU/ml of ice). Salmonella were not found in any sample. There is not a significant difference in each of the germs ($p > 0.05$). Contamination of fishing products from artisanal sector is a real problem of public health.

Introduction:-

Fish is very important for healthy diet due to the high quality of its protein and essential nutrients, vitamins and minerals (Stewart Anderson et al., 1993). It is an important food component for a large section of the world population (Pal et al., 2016). Fish has become an increasingly important animal commodity in the diet of people in West Africa (Belhabib et al., 2015). With an Atlantic coast nearly 4400 km, West Africa has a marine environment rich in fisheries resources which have allowed the emergence of two types of fishing: industrial fishery and small-scale fishery (Seck, 2014). These two types of fisheries contribute more than 25% of Gross Domestic Product (GDP) to coastal countries in West Africa. Small-scale fishery takes an important part in the fishing activities in developing countries and supplies about 30% of animal protein in population diets (Dieng et al., 2013).

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In Togo, fishery contributes to 3.6% of agricultural GDP on a narrow coastline of 50 km. Small-scale fishery unloads more than 99% of marine fish caught (Sedzro et al., 2016). The annual production of fish is estimated at 22000 tons, but this quantity doesn't supply the need of population (FAO, 2010). Seafood presents more risk of contamination because of many factors, including the nature of the environment from which they come, their mode of feeding, the season during which they are harvested, how they are handled and preserved (Safaeian and Khanzadi, 2018). Fish is becoming increasingly popular among consumers as a healthy food. The consumption of contaminated fish in food is responsible for 25% of foodborne diseases in the United States (Olsen et al., 2000). The microbial flora of fish causes a threat to the consumer's health and are responsible for gastric diseases (Kabré et al., 2003). Contamination affects both the organoleptic quality and the microbiological quality of fishes. Fishery products can have two possible origins of contamination. A primary or endogenous origin linked to the living environment (marine environment, fresh water) and a secondary or exogenous origin which relates to the contamination of the products after their capture (Rozier et al., 1985). In order to obtain knowledge on the fish contamination factors and their origin, this study was conducted to evaluate the microbiological quality of fresh fish landed at the fishery harbor of Lomé in Togo.

Material And Methods:-

Site and period of study

This study was conducted from August to November 2016 at Lomé fishery harbour in Togo. The harbour is composed of a landing stage or quay that have a length of 240 m, a water pond, and a hall for the sale of fishery products. Harbour is located at 6.14 latitude and 1.28 longitude on GPS.

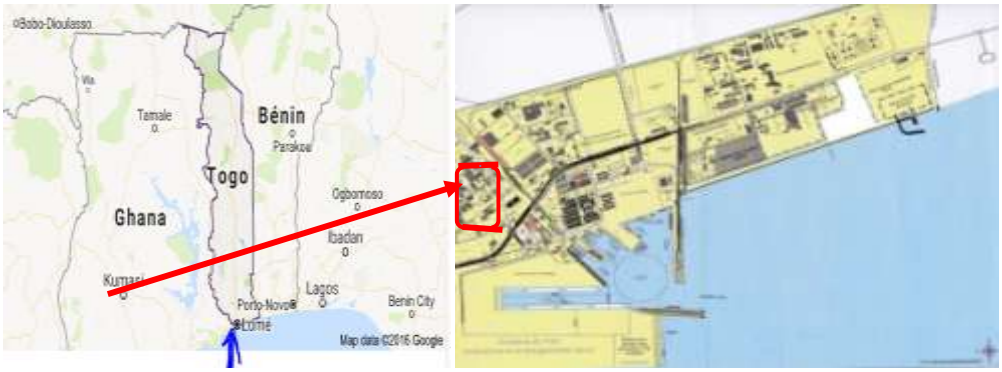

Figure 1:- View of the Fishing Port (Scheme of fishing port).

Consent and inclusion criteria

This study was carried out with the agreement of the authorities of the ministry in charge of the agriculture and fishery in Togo. The workshops have been organized with the supervisors of the fishermen and the security personal of the fishery harbour who have authorized its realization. Fishing activities include fishermen, wholesalers, and their helpers who were involved in this study.

Biological material

It is constituted to pelagic (surface to mid-water) fishes and demersal (bottom) or benthic fishes. The demersal fish samples are red carp (Lutjanus gorensis), bream (Sparus spp), gray bream (Sparus dentex), Pageot (Pagellus bellottii), gray grouper (Epinephelus tauvina), red grouper (Cephalopholis miniata) and damselfish (Chrysiptra parasema). The pelagic fish samples are anchovy (Engraulus encrasicolus), sardinella (Sardinella maderensis, Sardinella aurita) and bonito (Sarda sarda). In this paper, we will use the common names or trade names of the fish samples that were the subject of this study.

Survey

Five (5) interviewers are trained to follow by direct observation the hygiene practices at the Lomé fishery harbour. The data collected focused on the hygiene practices of fishery stakeholders. The semi-structured interview technique was used on the basis of a questionnaire that items were the identification of the respondent, organization and hygiene practices, health information, respect of hygiene conditions at the fishery harbour, work conditions and training on hygiene or production good practices. An observation grid was used to validate data collected. The grid

includes four items as hygiene staff, raw materials, work environment and equipment's used. Each item was scored by the interviewer from 0 to 3. 0 (No/Applicable), 1 (poor), 2 (fair) and 3 (good).

Sampling

Fish, water and ice samples were collected by random sampling from seining fishermen, anglers, wholesalers and pond water. To avoid change in the regular work habit, all the stakeholders were not informed on the day of data collection and sampling. The samples were taken on landing in sterile conditions. After mixing the fish contained in vessels or isolated boxes, a quantity of the fish species was taken at random with the hands protected by latex gloves. 1-3 kg of fish is taken and directly introduced into a sterile freezer bag kept cold into the cooler. Samples are sent to the microbiology laboratory of foodstuffs (LAMICODA) of the Higher School of Biological and Food Techniques of the University of Lomé (ESTBA / UL) for analysis. The samplings are composed to:

1. Ten (10) fish samples containing four (04) anchovy samples, three (03) sardinella samples and (03) bonito samples were collected at the landing of the canoes in the seining fishermen.
2. Ten (10) fish samples consisting of six (06) samples of anchovy, three (03) of sardinella and one (01) of bonito were taken from the wholesalers.
3. Ten (10) angling fish samples constituted to five (05) samples, two (02) red carp samples, one (01) Lutjanus sample, one (01) gray grouper and one (01) pink bream were directly taken from anglers and five (05) samples [one (01) damselfish, (02) sea bream, (01) red grouper and (01) pageot] were directly taken from the insulated boxes of wholesalers.
4. Three (03) ice cubes samples used to preserve fish and three (03) water samples were collected from the pond.

Laboratory analysis

The microbial analysis was performed according to AFNOR methods described by (Soncy et al., 2015). In a sterile vial containing 90 ml of Tryptone Salt (TS), 10 g of the fish sample was added to be analyzed. The mixture was homogenized with a ROBOR MAJOR Moulinex grinder. The suspension obtained constitutes the standard stock solution $1/10^{th}$ for the sample. The stock solution is kept at laboratory temperature during 45 minutes for germs regrowth. This stock solution is used for a series of decimal dilutions 1:10 with TS. The water and ice samples were considered directly as stock solutions. They were inoculated, with their decimal dilutions 1:10 with TS on the appropriate culture media. The count of colonies was expressed by colony forming units (CFU/ml) according to the standard NF V08-051: 1999

Statistical analysis of data

The results of the survey were processed by the Epi Info 7 software that allows to compare different statistical relationships existing between the data collected and the variables. The microbiological results were analyzed by Excel (ANOVA) and R (Wilcoxon test).

Results:-

Observation grid of the level of hygiene at the Lomé fishery harbour

The observation grid for hygiene practices at the Lomé fishery harbour (Table 1) give an average of 0.4765 closer to 0 than 1. The score for the staff and fishery community (fishermen, wholesalers, helpers) was 0 for the five (5) observers. Only the averages of the workplace (0.6) and the equipment (0.84) were closer to 1.

Table 1:- Rating criteria for the hygiene observation grid.

Criteria	Int 1	Int 2	Int 3	Int 4	Int 5	Average
Staff hygiene	0	0	0	0	0	**0**
Raw material	0,5	0,5	0,33	0,5	0,5	**0,466**
Working environment.	0,2	0,8	0,2	1	0,8	**0,6**
Equip. used	0,4	1,2	1	0,8	0,8	**0,84**
Average	**0,275**	**0,625**	**0,3825**	**0,575**	**0,525**	**0,4765**
EU standard	**1**	**1**	**1**	**1**	**1**	**1**

Int.: interviewer; Equip: equipment; EU: European Union

Analysis of fish taken from seining fishermen

The pelagic fishes were 100% contaminated by total aerobic flora (TAF) and thermo-tolerant coliforms (TTCs) (table 2). TAF count was ranged from 41.10^4 to 23900.10^4 CFU/g showing contamination levels from 66 to 4780 times higher than the EU standard (5.10^4 CFU/g of product). The lowest count of TAF was observed in bonitos that contain TAFs 8 times higher than EU standard. Thermotolerant coliforms (TTCs) count in anchovy samples were 5500 times higher than the EU standard ($10\text{-}10^2$ CFU / g of product). The anchovies count is acceptable according to EU standard in 33% of bonitos (60 CFU/g product) that present the lowest contamination rate. sardinellas have the higher contamination of Escherichia coli. The counts of E. coli reach the levels that were 840 times higher than the EU standard ($10\text{-}10^2$ CFU/g of product) and zero for 50% of the anchovy samples. The count of anaerobic sulphite-reducing (ASR) bacteria showed that 100% of the pelagic fishes were contaminated. 75% of sardinella and 25% of anchovies have acceptable levels for ASRs contamination. Bonitos samples were 100% contaminated by Staphylococcus aureus which were numbered 1190 above the EU standard. Salmonella (Sa) were not found in any fish samples.

Table 2:- Microbial contamination in fish taken from seining fishermen.

Fishes	**TAF CFU/g**	**TTC CFU/g**	**E. Coli CFU/g**	**ASR CFU/g**	**Staph CFU/g**	**Sa**
Anchovies 1	23900.10^4	2600.10^2	30.10^2	220.10^2	0	-
Anchovies 2	330.10^4	1600.10^2	20.10^2	1300.10^2	0	-
Anchovies 3	1400.10^4	5500.10^2	0	440	0	-
Anchovies 4	4200.10^4	15.10^2	0	40	0	-
Sardinellas1	16300.10^4	18.10^7	840.10^2	720	0	absence
Sardinellas 2	2500.10^4	900.10^2	20.10^2	20	0	-
Sardinellas 3	118.10^4	14.10^2	30	28	0	-
Bonitos 1	23100.10^4	3000.10^2	0	260	1190.10^3	-
Bonitos 2	41.10^4	60	5	21.10^2	170.10^3	-
Bonitos 3	56.10^4	1800.10^2	40	10^2	3.10^2	-
EU Standard	**5.10^4**	**$10 - 10^2$**	**$10 - 10^2$**	**$10 - 10^2$**	**10^2- 10^3**	**Tot abs./25g**

TAF: Total aerobic flora; TTC: Thermotolerant coliforms; E. coli: Escherichia coli; ASR: Anaerobic sulfite reducing bacteria; Staph: Staphylococcus aureus; Sa: Salmonella spp; Tot abs./25g: total absence in 25 g; EU: European Union

Analysis of seining fish taken from wholesalers

The fish samples are contaminated at 100% by TAFs and TTCs (Table 3). Bonitos have been the most contaminated by TAFs germs 21200 times higher than the EU standard. E. coli has been found in all samples except 40% of the anchovies that were conform to EU standard. The count of anaerobic sulphite-reducing (ASR) revealed that only 20% of anchovies have acceptable levels of ASR contamination according to the EU standard. The staphylococcus count was satisfactory for 80% of the fish samples. Salmonella were totally absent in all samples.

Table 3:- Microbial contamination in the fishes of wholesalers.

Fishes	**TAF CFU/g**	**TTC CFU /g**	**E. Coli CFU /g**	**ASR CFU /g**	**Staph CFU /g**	**Sa**
Anchovies 1	13000.10^4	10000.10^2	380.10^2	240	0	
Anchovies 2	15400.10^4	3900000.10^2	10.10^2	2200.10^2	0	
Anchovies 3	3500.10^4	3400.10^2	0	1500.10^2	0	
Anchovies 4	111.10^4	900.10^2	60.10^2	520	0	
Anchovies 5	360.10^4	60.10^2	80.10^2	80	500.10^3	absence
Anchovies 6	3600.10^4	250.10^2	230.10^2	60.10^2	0	
Sardinellas 1	4800.10^4	4500.10^2	70.10^2	140	0	
Sardinellas 2	4300.10^4	2100.10^2	10.10^2	120	0	
Sardinellas 3	69.10^4	29.10^2	180	320.10^2	0	
Bonitos 1	106000.10^4	200.10^2	40.10^2	220	1450.10^3	
EU Standard	**5.10^4**	**$10 - 10^2$**	**$10 - 10^2$**	**$10 - 10^2$**	**10^2- 10^3**	**Tot. abs/25g**

TAF: Total aerobic flora; CTT: Thermo-tolerant coliforms; E. coli: Escherichia coli; ASR: Anaerobic sulphite-reducing bacteria; Staph: Staphylococcus aureus; Sa: Salmonella spp.; Abs. tot./25g: total absence in 25 g; EU: European Union

Analysis of angling fishes collected from anglers and wholesalers

The angling fish were 100 % contaminated by TAFs germs and 90 % by CTTs (Table 4). The count of E. coli, ASR and Staphylococcus aureus revealed that 70 to 100 % of angling fishes were in compliance accordingly to EU standard. The contamination level of Staphylococcus aureus was very low and ranged from 3.10^2 to 4.10^2 CFU/g compared to the EU standard (10^2-10^3 CFU/g). Salmonella was not found in the angling sample.

Table 4:- Microbial flora in angling fish for anglers and wholesalers.

Fishes	TAF CFU /g	TTC CFU /g	E. Coli CFU /g	ASR CFU /g	Staph CFU /g	Sa
Red carp A1	110.10^4	0	0	100.10^2	0	-
Red carp A2	68000.10^4	117.10^2	0	0	0	
Gray grouper A3	170.10^4	86.10^2	0	0	0	
Red carp A4	1500.10^4	300.10^2	350	0	5.10^2	
Bream A5	82.10^4	14.10^2	0	400	0	absence
Fish damsel W1	2550.10^4	11.10^2	0	0	0	
Bream W2	3800.10^4	110.10^2	0	0	0	
Gray bream W3	2130.10^4	1100.10^2	0	140	0	
Red grouper W4	32.10^4	94.10^2	120	0	0	
Pageot W5	83.10^4	43.10^2	20	0	4.10^2	
EU Standard	**5.10^4**	**$10 - 10^2$**	**$10 - 10^2$**	**$10 - 10^2$**	**10^2- 10^3**	**Tot. abs/25g**

A: angler; W; wholesalers; TAF: Total Aerobic Flora; CTT: Thermo tolerant coliforms; E. coli: Escherichia coli; ASR: Anaerobic sulphite-reducing bacteria; Staph: Staphylococcus aureus; Sa: Salmonella spp.; Tot abs./25g: total absence in 25 g, EU: European Union

Water analysis of the fishery harbour pond

The microbial flora counted in the pond water showed the presence of all the germs. The water didn't contain salmonellas (Table 5). The water samples were non-compliant to EU standard for E. coli (275 to 13.10^3 CFU / ml) and fecal streptococci (73.10^2 CFU/ml). The level of Staphylococci (22 - 520 CFU / ml) was acceptable according to the EU standard (10^2-10^3 CFU/ml)

Table 5:- Microbial flora counted in the pond Water.

Water	TAF CFU/ml	TTC CFU/ml	E. Coli CFU/ml	ASR CFU/ml	Staph CFU/ml	FS CFU/ml	Sa CFU/ml
Basin water 1	2.10^4	450	390	286	520	257	-
Basin water 2	10^4	220	215	210	22	256	-
Basin water 3	14.10^4	10^2	13.10^3	32.10^2	140	73.10^2	-
EU Standard	**5.10^4**	**$10 - 10^2$**	**NC**	**$10 - 10^2$**	**10^2- 10^3**	**Abs. tot./25g**	**Tot. abs/25g**

TAF: Total Aerobic Flora; CTT: Thermo tolerant coliforms; E. coli: Escherichia coli; ASR: Anaerobic sulphite-reducing bacteria; Staph: Staphylococcus aureus; Sa: Salmonella spp.; Tot abs./25g: total absence in 25 g Staph: Staphylococcus aureus; FS: Fecal Streptococci. ; Abs. tot./25g: total absence in 25 g; NC: non-compliant

Microbial flora counted in the ice sample collected at the fishing port of Lomé.

The count of bacteria in the ice (table 6) showed that ASRs were completely absent in all samples as well as staphylococci that had a satisfactory level of contamination (54 CFU/ml) compared to the EU standard (10^2-10^3 CFU / ml). All samples were non-compliant for E. coli and Faecal Streptococci.

Table N° 6:- Microbial Flora Counted in ice Sample.

Ice	TAF CFU/ml	TTC CFU/ml	E. Coli CFU/ml	ASR CFU/ml	Staph CFU/ml	FS CFU/ml
Ice W1	38.10^4	850	26.10^3	0	54	497
Ice W2	19.10^4	70	400	0	0	125
Ice W3	10^4	830	44.10^2	0	0	980
EU Standard	**5.10^4**	**$10 - 10^2$**	**NC**	**$10 - 10^2$**	**10^2- 10^3**	**Tot. abs/25g**

TAF: total aerobic flora; TTC: Thermo-tolerant coliforms; E. coli: Escherichia coli; ASR: Anaerobic sulphite-reducing bacteria; Staph: Staphylococcus aureus; SF: Fecal Streptococci; Abs. tot./25g: total absence in 25 g. NC: non-compliant

Discussion:-

Live fish is normally free of microbes, but microorganisms can be found in the skin and the gills due to polluted environment and human handling (Pal et al., 2016). The results (Table 1) have shown that staff hygiene is very unsatisfactory at Lomé Fishery Port. The fishing community does not apply the rules of hygiene required for the handling of foodstuffs. This was reflected by the rating of 0 assigned by the 5 observers for the staff (Table 1). These results corroborate Rather et al. (2017) studies showing that man is the most frequent source of contamination of foodstuffs of animal origin. The hygienic standards of production were not applied in Lomé fishery harbour. The same observations have been done in Cotonou fishery port by Abotsi (2010). Most of the fish species sold by seining fishermen and wholesalers are pelagic fish (anchovies, sardinellas, bonitos) that live in waters near the surface or between the surface and the bottom. The results of the microbial analysis (Table 2 and 3) have shown that pelagic fishes are highly contaminated by the TAFs, TTCs, E. coli and ASRs both among fishermen (7194.10^4 CFU/g for the TAFs) and wholesalers ($15\ 114.10^4$ CFU / g for TAFs). Similar results have been found at the Cotonou fishery port where the fishes landed by artisanal fishing were highly contaminated by TAFs and TTCs (Babadjide et al., 2015). However, the fishes landed in Cotonou industrial fishery port present the acceptable level of TAFs according to EU standards (Degnon et al., 2012).

For demersal or bottom fishes (Table N°4), the germs count have shown that E. coli, ASRs and staphylococci are acceptable according to the EU standard for both angling fishers and their wholesales. High level of contamination of demersal fishes by TAFs and TTCs indicate that the contamination of fish has an exogenous origin linked to the fishery stakeholders, the materials, the transport, the sale of the products. Human handling is a major source of fish contamination at fishing port of Lomé. This confirm the previous studies proving that the normal microbial flora makes their home on or in some part of the human body bound to microbe forever (Pal et al., 2016). The fish that is not polluted in the sea has become polluted for sale and this pollution is due to the behaviour of the different actors (Babadjide et al., 2015).

The count of microbial flora in the pond water (Table 5) has shown a high contamination by the different germs that contaminated fishes except salmonella which were not found in any samples. The pond water is the main source of water used by fishermen and wholesalers. It serves to clean fishes and materials (basket, bowl, utensils) and causes fish contamination by contact. The same thing was observed by authors who pointed out that contaminated surfaces and unclean, equipment's inadequately cleaned are at the origin of high bacteria count in vendors (Rabia et al., 2017). The ice was contaminated by TAFs, TTCs and E. coli with rates above the EU standard (Table N°6). These results are contrary to those found at Cotonou fishery port where the level of the contamination of water and ice was satisfactory according to EU standard (Babadjide et al., 2015).

Conclusion:-

it appears from all these results that most of the bacteria flora sought were present in the fishes, in the water and ice. At Lomé fishery harbour, the contamination is linked to hygiene practices of the actors involved in on-site activities. Hygienic conditions in artisanal fisheries do not always meet the required quality standards. Contamination of artisanal fishery products is a real problem of public health in Togo.

Author's Contribution

This paper was a collaborative work among all authors. VIB designed the study, collected specimen on field and carried out laboratory work. Author ECB performed statistical analysis and wrote the manuscript. Authors KMS assisted on study process and analysis of raw data. Author MS, KA, DK performed laboratory analysis. YA and MG played a role in paper review

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