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RESEARCH ARTICLE

SMOKING EFFECT ON BACTERIOLOGICAL QUALITY OF ATLANTIC MACKEREL (*Scomber scombrus*) AND HORSE MACKEREL(*Trachurus trachurus*) IN SOUTH BENIN.

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Abstract

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Fish is one of the most important sources of protein in South Benin. The fainting of hygiene in the processing and preservation process constitutes a potential danger to the consumer by the appearance of pathogenic germs. This study aimed to control the sanitary quality of smoked fish in the Littoral Department (Benin). Thus, data were collected on the flesh quality of *Scomber scombrus* and *Trachurus trachurus* from June to December 2015 at Wlacodji in South Benin. No difference was observed between bacterial loads of *Scomber scombrus* and *Trachurus trachurus*. The TMAF load was higher (P<0.05) in fresh fish (39.56 x 10^3 CFU/g) than in smoked fish (0.752 x 10^3 CFU/g). Total coliform load was higher in fresh fish than in smoked fish (0.705 x 10^3 *vs* 0.0025 x 10^3 CFU/g, P<0.001). Similarly, fecal coliform load was higher in fresh fish (0.365 x 10^3 CFU/g) than in smoked fish (0.0026 x 10^3 CFU/g, P<0.05). Staphylococcal contamination rate was 0.17% and 0.06%, respectively, in fresh and smoked fish. Contrary to fresh fish, no cases of salmonella and SRA were counted in smoked Horse mackerel and Atlantic mackerel. The smoking performed in good hygienic conditions reduces the bacteriological load of fish.

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Introduction:-

Halieutic products in general and fish in particular are foods of high nutritional value and very perishable. In order to guarantee their quality after fishing, several processing and preservation methods or techniques are used. These are refrigeration, freezing or deep-freezing, smoking, drying, salting and fermentation. These preservation methods

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help to manage post-harvest losses which range from 25% to 50% in Africa (FAO, 2016). In Benin, they are estimated to 20% (Anihouvi, 2005) and among the causes is the tropical temperature which contributes to the degradation of fish quality after fishing. To reduce these losses, smoking is one of the most widely used methods of fish preservation and processing in Benin (Djessouho, 2015) and is usually traditionally performed. This preservation method requires an intensive products handling by workers which is a source of frequent contaminations by the ubiquitous and pathogenic germs. Also, the poor preservation of these processed products favors the development of spoilage germs (Adedeji*et al*., 2011, Salifou *et al*., 2013).

Studies by Degnon *et al*. (2013), Chabi et *al*. (2014) and Kpodékon *et al*. (2014) showed that on processing sites, the production and sales conditions of smoked fish don't respect good hygiene practice rules. However, Wlacodji is a large smoking site in Cotonou. Fish processing is practiced from generation to generation in the processors family (Djessouho, 2015). Processors acquired raw material for processing from marine and lagoon fisheries and imported frozen products. Processed products are dumped in local (Dantokpa, Ouidah, Kpassè, and Comè) and regional (Togo and Nigeria) markets. In view of this site importance, a quality approach adoption is required in the fish handling in order to guarantee not only the sanitary safety, but also the organoleptic and nutritional values. The objective of the study was to evaluate the smoking effect on the bacteriological quality of Atlantic mackerel and Horse mackerel traditionally smoked at the Wlacodji smoking site in Benin. Both species are chosen because of their socio-economic importance and their importance in the processing sector in Benin (El Ayoubi and Failler 2013, Kpodékon et *al*.,2014, Djessouho 2015).

Material and Methods:-

Study area

Data collection on the flesh quality of *Scomber scombrus* (Atlantic mackerel) and *Trachurus trachurus* (Horse mackerel) was carried out from June to December 2015 at Wlacodji in South Benin.

The study area of Wlacodji is located at 8 meters of altitude between 6° 2' North latitude and 2° 26' East longitude. It is in the Littoral Department and is part of the 5th district of the Township of Cotonou. It covers about 10 hectares, and lies in a sandy angle formed by the sea and the Cotonou lagoon channel. The climate is of subequatorial type characterized by two rainy seasons and two dry seasons. Wlacodji is located near the fishing port and the commercial port of Cotonou. By its geographical position, in the center of Cotonou and especially near the economic activities, many of the area inhabitants work at the fishing port, the commercial port and in the big markets. This area shelters one of the major smoking sites of Littoral. Lagoonary and artisanal marine fishing and fish processing activities are developed there.

The sampled fish analyses were carried out at the Laboratory of Animal Biotechnology and Meat Technology of the Animal Production and Health Department of the Polytechnic School of Abomey-Calavi of the University of Abomey-Calavi.

Microbiological analyzes

Seventy-two (72) samples of Horse mackerel and Atlantic mackerel species were collected from 18 women fish processors as two fresh fish and two smoked fish per fish wholesaler. By species, 18 fresh fish and 18 smoked fish samples were collected. The fish were sampled when they were smoked and exposed for sale. Each sample (fresh or smoked) was collected in a Stomacher bag and then sealed and stored in a cooler at an average temperature of 4° C to the laboratory. The superficial and deep parts of smoked fish were aseptically collected with sterile knives and pincers near the flame of a bunsen burner for microbiological germs investigation. Each collected sample weight 25 g per fish. The sampled fraction is used for the stock solution preparation (ISO 7218: 2007 standards).

From the stock solution, decimal dilutions were performed. One milliliter of the stock solution was taken and put into a test tube containing 9 ml of buffered Sodium Chloride Peptone Solution (BPW OXOID CM0509) to obtain the 10^{-2} solution. From this tube, 1 ml was taken and introduced into another test tube containing 9 ml of TS in order to have the 10^{-3} solution. The isolation and enumeration of the Total Mesophilic Aerobic Flora (TMAF) were performed in accordance with ISO 4833 (2003). Fecal coliforms were counted in accordance with ISO 4831 (2006) and suspected pathogenic staphylococci (*Staphylococcus aureus*) according to ISO 6888- (1999). Sulfite-Reducing Anaerobes (SRA) and Salmonella were counted respectively according to ISO 15213 (2003) and ISO 6579 (2007). The microbiological results were interpreted according to a two classes plan for SRA and Salmonella, and a three classes plan for TMAF, Coliforms, and Staphylococci taking into account the microbiological criteria defined by the

Regulation N° 2073/2005 of the European Union and of the Commerce and Distribution Federation Companies (CDFC, 2014; 2016), setting the minimum tolerance thresholds (m) and the maximum (M). A sample is qualified of satisfactory microbiological quality if the flora (F) is less than or equal to 3m, of acceptable microbiological quality if F is between 3m and 10M and of unsatisfactory microbiological quality if F is higher than 10M.

Statistical analysis

Data were analyzed using SAS (2013). A two-way variance analysis was used to test the significance of effects of species and processing mode on the flesh hygienic characteristics of both fish species. The means were calculated and compared paired by the student t-test. The frequencies of counted SRA and salmonella were calculated by the *Procfreq* procedure and compared by the Chi-square and the Z bilateral tests.

Results:-

Total Mesophilic Aerobic Flora

The TMAF load didn't significantly vary between Horse mackerel and Atlantic mackerel (Table 1). It was 25.66 x 10^3 CFU/g and 14.65 x 10^3 CFU/g, respectively in Horse mackerel and Atlantic mackerel. The TMAF load was higher (P<0.05) in fresh fish (39.56 x 10^3 CFU/g) than in smoked fish (0.752 x 10^3 UFC/g), both species together. Within the same species, it was higher (P<0.05) in fresh Horse mackerel (50.68 x 10^3 CFU/g) than in smoked Horse mackerel (0.63x10^3 CFU/g) and also higher (P<0.05) in Atlantic mackerel in fresh state (28.43 x 10^3 CFU/g) than in smoked state (0.87 x 10^3 CFU/g).

Total coliforms

The total coliform load didn't vary with species (Table 1). However, it was higher (P<0.001) in fresh fish than in smoked fish (0.71 x 10^3*vs* 0.0027 x 10^3 CFU/g). Within the same species, the total coliform load was higher (P<0.05) in fresh Horse mackerel (0.63 x 10^3 CFU/g) than in smoked Horse mackerel (0.0042 x 10^3 CFU/g). The same trend was observed for fresh and smoked Atlantic mackerel (0.77 x 10^3*vs* 0.0122 x 10^3 CFU/g).

Fecal coliforms

Fecal coliform load was 0.19 x 10^3 and 0.18 x 10^3 CFU/g, respectively, in Horse mackerel and Atlantic mackerel. No significant difference was observed between the loads of both species. Similarly, for the same species, fecal coliform loads didn't vary with treatment status. Finally, fecal coliform load was higher (P<0.05) in fresh fish (0.37 x 10^3 CFU/g) than in smoked fish (0.0026 x 10^3 CFU/g).

Staphylococci

The number of staphylococci found in Horse mackerel and Atlantic mackerel (P> 0.05) was the same. It was also the same between fresh and smoked fish (Table 1). The interaction between species and treatment state was not even significant. Staphylococcal load was on average 0.03 and 0.19 x 10^3 UFC/g, respectively, in Horse mackerel and Atlantic mackerel. It was 0.17 and 0.06 x10^3 CFU/g in fresh and smoked fish.

Salmonella

The counted Salmonella frequencies are presented in the table 2. No Salmonella was counted in smoked Horse mackerel and Atlantic mackerel. However, 33.33% and 66.67% of Salmonella cases were observed respectively in fresh Horse mackerel and Atlantic mackerel. The Salmonella frequency obtained in fresh Horse mackerel was not different from that obtained in smoked Horse mackerel. But, the Salmonella frequency obtained in fresh Atlantic mackerel was significantly higher (P<0.001) than that of smoked Atlantic mackerel.

Sulphite-Reducing Anaerobes (SRA)

The SRA frequencies counted are presented in Table 2. No SRA was also obtained in smoked Horse mackerel and Atlantic mackerel. In contrast, one case (11.11%) of ASR was found in the fresh Horse mackerel and Atlantic mackerel. No significant difference was observed between the counted SRA frequency of fresh and smoked Horse mackerel on the one hand and between fresh and smoked Atlantic mackerel on the other hand.

Table 1:- Bacterial load of fish by species and by treatment state

Variables	Species		State		Interaction				RSD	Acceptability limit threshold		Significance test		
					H Mackerel		A Mackerel							
	H Mackerel	A Mackerel	Fresh	Smoked	Fresh	Smoked	Fresh	Smoked		Fresh (m)	Smoked (m)	Species	State	Interaction
TMAF (10^3CFU/g)	25.66a	14.65a	39.56a	0.75b	50.68a	0.63b	28.43a	0.87b	34.62	10^6	10^6	NS	*	*
Total coliforms (10^3CFU/g)	0.32a	0.39a	0.71a	0.0027b	0.64a	0.0042b	0.77a	0.0012b	0.45	10^2	10^2	NS	***	*
Fecalcoliforms (10^3CFU/g)	0.19a	0.18a	0.37a	0.0026b	0.37a	0.0039a	0.36a	0.0012a	0.39	10	Absence	NS	*	NS
Staphylococci (CFU/g)	0.03a	0.19a	0.17a	0.06a	0.06a	0a	0.28a	0.11a	0.26	10^2	10^2	NS	NS	NS

NS: P>0.05; *: P<0.05; ***: P <0.001; RSD: residual standard deviation; TMAF: total mesophilic aerobic flora; H: Horse; A: Atlantic; the intra-class means of the same row followed by different letters differ significantly at the threshold of 5%; m = threshold below which all results are considered satisfactory

Table 2:- Comparison of Salmonella and SRA loads of fresh and smoked Horse mackerel and Atlantic mackerel

Variables	Horse mackerel				Atlantic mackerel				Acceptability limitthreshold		Significance test	
	Fresh (N=18)		Smoked (N=18)		Fresh (N=18)		Smoked (N=18)		Fresh (m)	Smoked (m)		
	Positif	%	Positif	%	Positif	%	Positif	%			H mackerel	A mackerel
Salmonella	6	33.33a	0	0a	12	66.67a	0	0b	Absence	Absence	NS	**
SRA	2	11.11a	0	0a	2	11.11a	0	0a	30	Absence	NS	NS

NS: P>0.05; **: P<0.01; N: Number; SRA: Sulphite-Reducing Anaerobes. H: Horse; A: Atlantic; Intra-class means of the same row followed by different letters differ significantly at the threshold of 5%; m = threshold below which all results are considered satisfactory

Discussion:-

Total Mesophilic Aerobic Flora

All the samples in this study were in accordance with the TMAF microbiological criteria according to the 2073/2005/EC regulation. The TMAF load didn't vary significantly in fresh Horse mackerel and Atlantic mackerel. In a previous study, the species effect on TMAF load was also not observed between *S. melanotheron* and *C. guineensis* randomly bought in the market and at the Abomey-Calavi embankment (Assogba et *al*., 2018). Similar contamination cases were reported in Benin (Wabi, 2010), in Togo (Abochi, 2010) and in Nigeria (Adedeji*et al*., 2011) on traditionally smoked fish.

On the contrary, the TMAF load was higher in fresh fish than in smoked fish. The same trends were reported by Abochi (2010) after the evaluation of the microbiological quality of traditionally smoked fish in Togo, by Goueu (2006) in traditionally smoked fish in Ivory Coast and by Chabi*et al*. (2014) on Sardinella (*Sardinapilchardus*) and

Horse mackerel (*Trachurus trachurus*) smoked in Benin. Indeed, the study of Chabi*et al*. (2014) showed that smoked *S. pilchardus* (17 x 10^1 CFU/g) and *T. trachurus* (41 x 10^1 CFU/g) had lower TMAF loads than fresh *S. pilchardus* (62 x 10^1 CFU/g) and *T. trachurus* (45 x 10^2 CFU/g).

The decrease in the TMAF load is due to the smoke and heat effect on fish flesh. Smoking slightly acidifies the flesh, which slows down microbial growth because of the inhibitory effect of phenolic compounds and other molecules, such as organic acids on microbial growth (Knockaert*et al*., 2009). In addition, ANSES (2010) evoked a bacteriostatic effect of smoke and heat constituents of which the purpose is to destroy micro-organisms. Moreover, the TMAF loads of both species in this study were lower than those found by Kpodékon *et al*. (2014) in smoked *T. trachurus* in Calavi (9.0 x 10^6 CFU/g) at Godomey (8.0 x 10^6 CFU/g) and at Cocotomey (4.5 x 10^6 CFU/g) in Benin, by Dodds*et al*. (1992) in smoked fish (10^5 CFU/g) in Toronto, by Thiam (1993) in braised-dried fish (3.4 x 10^8 CFU/g) in Dakar, by Djinou (2001) in smoked fish (5,4 x 10^5) in Ivory Coast, by Goueu (2006) in smoked fish (<10^6 CFU/g) in Ivory Coast, by Chabi*et al*. (2014) in smoked *Trachurus trachurus* in traditional oven (14 x 10^3 CFU/g) at Aplahoué-Benin, by Degnon *et al*. (2013) in smoked *T. trachurus* (1.4 x 10^6 CFU/g) in Cotonou and by Olaleye and Abegunde (2015) in smoked *T. trachurus* (4.2 x 10^6 CFU/g) and *Scomber scombrus* (7.5 x 10^6 CFU/g) in Nigeria. However the TMAF loads of species in the current study are higher than that of Kolodziejska*et al*. (2002) in industrially smoked *T. trachurus* (2.4 x 10^2 CFU/g) in Poland and of Chabi*et al*. (2014) in *Sardinapilchardus* (31 x 10^1 CFU/g) in Aplahoué-Benin. The differences in the observed loads in the various studies after smoking could be due to the products contamination following handling. Indeed, most of the fish processors don't apply the basic hygiene rules. The construction plan of the smoking workshop doesn't allow the application of good manufacturing practices (clean and soiled sectors separation, forward march or chains non-intercrossing).

Total coliforms:-

The total coliform load didn't vary according to the species. However, it was higher in fresh fish than in smoked fish. Similar results were reported in Benin on total coliform loads of *Sarotherodon melanotheron* and *Coptodon guineensis* (Assogba et *al*., 2018) in Ivory Coast on traditionally smoked fish for export (Goueu, 2006) and in Togo on traditionally smoked fish (Abochi, 2010). By contrast, total coliform loads of smoked fish were lower than those found by Chabi*et al*. (2014) in smoked *S. pilchardus* (<40 CFU/g) and in smoked *T. trachurus* (3x10^2 CFU/g) using an improved smoking device (Chorkor oven). The smoking of Horse mackerel and Atlantic mackerel leads to a product of satisfactory microbiological quality because the microbial loads of total coliforms: 4.2 x 10^{-3} and 1.2 x 10^{-3} CFU/g are below the microbiological criteria (<10^2 CFU/g). An unsatisfactory smoked fish quality was found by Chabi*et al*. (2014) in smoked *S. pilchardus* and *T. trachurus* in the Township of Aplahoué in South-eastern Benin. The observed differences could be due to the smoking temperature variations and to the various handling that could contaminate the smoked products.

Fecal coliforms:-

No significant difference was observed between fecal coliform load of Horse mackerel and Atlantic mackerel. However, smoking of Horse mackerel and Atlantic mackerel leads to a product of unsatisfactory microbiological quality because the microbial loads of fecal coliforms (3.9 x 10^3 and 1.2 x 10^3 CFU/g) are above the microbiological criteria (0 CFU/g). The conclusions were the same for fresh Horse mackerel and Atlantic mackerel. These results are similar to those of Kpodekon *et al*. (2014) who found that all the *T. trachurus* samples weren't in accordance with the 1996 AFNOR standards due to the presence of fecal coliforms. Moreover, fecal coliform load was higher in fresh than in smoked fish. This reduction shows a significant improvement in the respect of hygiene rules and Good Manufacturing Practices (GMP) by fish wholesaler after smoking which leads to a lower contamination level of smoked fish by these germs.

It's important not to ignore the bacteriostatic effect and the heat during smoking effect which can reduce the microbial load. According to ANSES (2010), heat affects microorganisms differently depending on the species, the strain and even their number. The thermotolerant coliforms are proof of poor hygienic conditions especially personnel hygiene (Abochi, 2010). Then, they are host of humans and animals digestive tract. Their presence is due to fecal contamination. Smoking workshops don't have washing and hands disinfecting material. Thus, the hands washing exigency before each work resumption is not observed. In addition, since these workshops don't have a fence, stray animals can through their feces contaminate the smoking material and products.

Staphylococci:-

No significant difference was observed between the amount of staphylococci counted in Horse mackerel and Atlantic mackerel (P>0.05). Smoked Horse and Atlantic mackerels had a satisfactory load in accordance with the microbiological criterion (<100 CFU/g). Similar results were obtained by Kpodekon *et al*. (2014) in *T. Trachurus* sold in the markets of Abomey Calavi. On the contrary, cases of non-conformity were found by Djinou (2001) and Goueu (2006) in smoked fish in Ivory Coast. Although *S. aureus* is not a thermoresistant bacterium, there was no difference between staphylococcal loads of fresh and smoked fish in this study. Normally, the smoking temperature required for smoking would drastically reduce the bacterial load or ensure this germ absence. The presence of these germs is explained by their ability to develop at a low water activity and their salt high concentration tolerance. According to Ababouch (1995), the *S. aureus* strains are ubiquitous. It is possible that *S. aureus* from soil or contaminated material soiled fresh Horse mackerel and Atlantic mackerel (fresh and smoked). Moreover, *S. aureus* is a pathogenic germ and by its enterotoxins, it can cause in people a food poisoning resulting in nausea, headaches, abdominal pains, violent, incoercible and repeated vomiting often accompanied by diarrhea. Its presence in fresh and smoked fish poses serious health problems for the consumer.

Salmonella:-

The analysis results of smoked fish samples didn't reveal the presence of Salmonella in the products. The results of the smoked fish (Atlantic mackerel and Horse mackerel) are all considered in accordance with the standard (absence in 25g). Our results are similar to those reported in traditionally smoked fish (*Sardasarda*, *Trachurus spp*) for export in Togo (Abochi, 2010) and in *Ehtmalosafimbriata* and *Sardinellaaurita* traditionally smoked in Ivory Coast (Oulai*et al*., 2007) but opposite to those obtained by Djinou (2001) in smoked fish in Ivory Coast (0.8% of cases of salmonella). This result obtained in our study is explained by the fact that fecal germs of the genus *Salmonella* didn't contaminate smoked fish. However, the salmonella frequency obtained in fresh Atlantic mackerel was significantly higher than that in the smoked ones. The health risk therefore exists for consumers. Indeed Salmonella is a germ of fecal origin and its presence in fresh fish indicates a failure in hygiene rules practice with the raw material before processing. Salmonella poisoning due to fish is serious in view of the patients number as well as the developed symptoms severity. All serotypes can be involved; they vary according to the country and the period.

Sulfite-Reducing Anaerobes:-

Sulfite-Reducing Anaerobes are bacteria of genus *Clostridium* characterized by thermoresistance. No SRA was observed in smoked Horse mackerel and Atlantic mackerel. Similar results were found by Dodds *et al*. (1992) in smoked *T. trachurus* and by Oulaï*et al*. (2007) in *Ethmalosafimbriata* and *Sardinellaaurita*. However, these results are different from those of Goueu (2006) who obtained 0.88% of sample not in accordance with the standard. These germs presence in fresh Horse mackerel and Atlantic mackerel proves a contamination by soil or soiled equipment. In fact, they are telluric bacteria frequently found in the nature (soil) and in organic matter in putrefaction. Their absence in this study samples is proof that the fish processors respect certain hygiene rules. The Sulphite-Reducing Anaerobes germs secrete enterotoxins responsible for serious food poisoning, which imposes their absence in foodstuffs intended for human consumption.

Conclusion:-

The TMAF, total coliforms, fecal coliforms and staphylococci loads of Atlantic mackerel and Horse mackerel flesh are higher in the fresh than in the smoked fish. Contrary to fresh fish, no case of salmonella and SRA was registered in smoked Atlantic or Horse mackerel. The smoking performed in good hygienic conditions reduces fish microbiological load. The development of standards on the sanitary quality of smoked fish in Benin is necessary to ensure the health safety of the population.

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