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Global Journal of
Biology, Agriculture & Health Sciences

Search for thermotolerant coliforms in the fillets of frozen fish produced in cote d'ivoire and intended for export

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Abstract

This study is part of the search for solutions to the problems encountered in the manufacture and export of fish fillets. The production of fish fillets is mainly marked by a little mechanization. Thus, it is subjected to strong manipulation by the personnel, source of contamination. As a result, the search for thermotolerant coliforms, most of which are of fecal origin, makes it possible to assess the level of contamination of products and to judge the effectiveness of the hygiene measures taken by the fishnet production units.

190 preventive samples from 4 companies were studied. A survey was conducted to categorize the companies. Subsequently, the thermotolerant coliforms were counted on the violet crystal and neutral red (VRBL) bile lactose agar, inoculated in mass according to the French standard ISO 4832.

It appears that of the 190 samples; 97.4% (185) of the results are satisfactory while 2.6% (5) of the results are unsatisfactory. The evaluation of the manufacturers of these nets indicated that there was a positive correlation between the number of serious defects and the unsatisfactory results. The result is that the best organized firms have the best laboratory results.

Citation: Doutoum AD, Tidjani A, Somda MK, Traore AS, Kallo V, Ndiaye A, et al., (2018) Search for thermotolerant coliforms in the fillets of frozen fish produced in cote d'ivoire and intended for export, GJBAHS 7: 1. doi:

Recieved date: June 01, 2018; **Accepted date**: June 26, 2018; **Published date**: June 30, 2018

Competing interests: The authors have declared that no competing interest exist.

Sources of funding: No Funding.

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Keywords:

Fish fillets, Thermotolerant coliforms, Society hygiene, Quality assurance

Introduction

In coastal countries, fishery and aquaculture products play a significant role from both an economic and social point of view. It is a source of animal protein of high nutritional value as important as meat because of its low-fat content [1].

The fisheries sector of ECOWAS and Mauritania represents more than 2 million tons of fish in 2004, 3% of world production and employs about 1 million fishermen.

Member countries' imports reached almost 1 million tons in 2003, almost half of them by Nigeria. About 400,000 tons were exported, mainly by Senegal, Mauritania and Côte d'Ivoire (80% smoked) for a turnover of 350 billion FCFA (of which 40% for the processing segment). In Côte d'Ivoire, fishing represents, in all sectors,

(industrial, artisanal, lagoon, maritime, etc.) between 70,000 and 100,000 tons for a consumption of 300,000 tons. The gap is filled by imports.

Indeed, in Ivory Coast, the sector accounts for 3.1% of GDP and participates in food self-sufficiency, especially in terms of animal protein, employs a large workforce and contributes to the balance of income, payment due to exports. One of the main export markets is that of the European Union governed by a health legislation, which devotes the principle of prevention by ensuring the means and conditions of production, in addition to the quality of the safety of finished products. It is therefore essential to avoid any losses due to the withdrawal of products for non-compliance.

This is why the Ivorian health legislation lays down procedures obliging operators to fulfill the minimum conditions relating to the design of processing units and the quality of the finished product. Once the obligations of means have been respected, the controls must be carried out to assess the quality of the finished products. Thus, in Côte d'Ivoire, the Central Laboratory for Hygiene and Agro-Industry (LCHAI) performs this function through sensory analysis techniques, chemical and microbiological.

Fish fillets, which are commodities requiring many manipulations during their manufacture are very often contaminated by fecal germs. It is to contribute to the assessment of the level of contamination of these exported products that we chose this subject.

The ultimate goal of this work is to improve the hygienic quality of Ivorian fish fillets for export.

Since January 2006, the EU has requested that all laboratories analyzing fishery products have internationally recognized accreditation. This is not yet the case in Senegal and this should be a priority of the quality program.

In Ivory Coast, for example, each shipment of fish is controlled by LCHAI. Approximately 6,000 samples are analyzed each year. Controls are carried out on different forms of fish: crustaceans, fresh fish, canned fish, frozen fish, etc.

Analyzes differ by product type and standards: sensory (organoleptic) analysis for whole fresh fish, heavy metal analysis for canned tuna and chemical analysis for any product that is being handled. It should be noted that the rejection rate is less than 4%. In addition, the EU itself validates fishing establishments in Côte d'Ivoire. The fish processing and exporting sector to the EU is highly concentrated. There are three major operators in the fishing industry for canned tuna: CASTELLI, Peach and Cold (PFCI), and Scobi. Four artisanal processing workshops are also approved for export to the EU.

Two companies are approved for the export of noble fresh fish. Companies are evaluated for equipment and hygiene conditions, and then for annual evaluations. The list of companies authorized to export is defined by ministerial decree.

The procedure is then set up at the initiative of the operator: it is the operator who must approach the state services and submit a file to present its activities. After a documentary audit and then in the field, the competent authority decides on the certification.

Once the operator is certified, the inspection services bring samples for each fish shipment to the Central Laboratory for Food Hygiene and Agro-Industry/National Laboratory for Agricultural Development Support (LCHAI/LANADA).

Thus, to meet international standards and reduce discards, fish fillet factories do self-checks but also other controls by accredited laboratories, which is why we were asked to study at the school's HIDAOA laboratory, Interstate of Sciences and Veterinary Medicine of Dakar.

Material and Methods

Material

Survey material: It consists of fact sheets.

Products analyzed: They consist mainly of frozen fish fillets from the four plants in the square. These are finished products already packed and stored in a cold room while waiting for their shipment.

Laboratory equipment: It includes one commonly used in microbiology laboratories. These elements are:

1. Sampling equipment: scissors, forceps, scalpels;

2. Dehydrated culture medium;
3. Oven covering the temperature of 44°C;
4. vortex agitator;
5. Bunsen burner with tripods;
6. Rack for test tube;
7. Sterilization equipment: autoclave;
8. Glassware;
9. Petri dish for single use;
10. Pallet homogenization equipment: STOMACHERND.

Methods

This study was carried out in three stages:

Survey at the level of sites or processing units of fish in net

Choice of processing units: We have chosen the units that have submitted samples to the laboratory during the last four years. These factories are four in number.

Assessment of sites by degree of non-compliance by Ababouch [1]: We used two cards; the first relates to the evaluation of construction and operating equipment, while the second is related to the assessment of industrial hygiene and operating conditions. Each of these sheets is divided into several elements to be evaluated.

These elements are in turn divided into sub-elements whose severity in case of non-compliance is already indicated in the form [2-10]. When visiting the facility, note for each case of non-compliance, a cross in the boxes reserved for it. If the sub-element is compliant, we will not notice anything.

Once all cases of non-compliance have been noted and their relative severity assessed, the overall assessment of the facility will be conducted. To do this, we will add all the cases of non-compliance marked in the boxes.

To get the coast of the plant, only the most severe cases will be considered. Once the factory coast is determined, we can refer to the classification table of the plants to define its category.

Retrospective study: This study consisted of collecting all the results concerning the fish fillets analyzed in the microbiology department and classifying them by company.

Sample analysis

Choice of the sample: In the course of this work, we used as some sample cartons of fillets of one kilogram or two kilograms. During the analysis, for each sample, the fillets are taken at random.

Analysis

Sampling for analysis (ISO 6887-1, 1999): Superficial and deep portions of the fish fillet were removed using sterile knives and forceps near the lit Bunsen burner.

This operation consists in taking aseptically a fraction of the flesh of fillets chosen at random until a weight of 25 g is obtained. The fraction thus taken is used for the preparation of the stock solution.

Stock solution and decimal dilution: The preparation of the stock solution consists of introducing the 25 g fraction of fillet into a sterile STOMACHERND bag. 225 ml of buffered peptone water (PTE) are then added to obtain a stock solution (MS) titrating 1/10. Grinding and homogenization of the contents of the bag is done for 3 minutes at STOMACHERND.

This solution is allowed to stand for 40 minutes to ensure revivification. The title of this mother solution is obtained by realizing the ratio: weight of the food/total volume (diluent+food). Moreover, for very dehydrated foods we consider that their density is close to 1, and therefore 1 g of food equals a volume of 1 ml.

Enumeration of fecal coliforms (I.S.O 1978)

Preparation of the culture medium (VRBL): 38 g of powder of the complete medium dehydrated in 1 L of distilled water previously heated to 100°C for 10 minutes and then brought to the laboratory temperature. After 5 minutes, we mix until a homogeneous suspension is obtained. Then it is heated slowly, adding frequently, until boiling and complete dissolution. Then, pH is adjusted to 7.4. The medium is used in the hours following its preparation. Before use, the medium is in a bath at 48°C + or -1°C.

Seeding: 1 ml of the stock solution is transferred to the center of each petri dish using a pipette, bringing the end of the pipette into contact with the dry surface of the can.

Agar distribution: About 12 ml of VRBL agar at 45°C + or -1°C is poured into the seeded dish. It is mixed immediately after pouring the agar, by rotating the petri dish to obtain a regular distribution of colonies after incubation. It is allowed to solidify on a cool, horizontal surface. Then, a control box with 1 ml of the medium is prepared to control its sterility. After complete solidification of the medium, the surface of the seeded medium will flow to about 4 ml of the medium VRBL at 45°C + or -1°C. and allowed to solidify.

Incubation: During this phase the boxes are put in the oven, in the inverted position. The temperature of the oven is set at 44°C. This operation lasts 24 hours.

Counting (ISO 4832) (5): Dark red colonies with a diameter of at least 0.5 mm characteristic of coliforms are counted.

Expression of results: The number of coliforms per gram is equal to:

Σ c=sum of colonies of successively counted boxes

V=dilution volume used

n1=number of boxes in the first dilution

n2=number of boxes in the second dilution

d=dilution factor

For counting, keep the boxes containing more than 10 and less than 150 colonies. If the number is less than 10, it is indicated that the number of coliforms per gram is "less than 10Xd". If it is greater than 150, the number of colonies is multiplied by the inverse of the division factor. This result is expressed in "estimated number of coliforms per gram". The standard used is m=20 coliform per gram.

The quality of a lot is considered as:

For the counting, the satisfactory when will be retained when the observed value is less than or equal to 3 m;

Acceptable when the observed value is between 3 m and 10 m; Unsatisfactory when the observed value is greater than 10 m.

Results and discussion

Results

Assessment of companies by degree of non-compliance

Table 1: Classification of facilities by degree of non-compliance.

Companies	Critical defects	Serious faults	Major defects	Minor defects	Categories
I	0	6	14	3	D
II	0	1	3	0	B
III	0	3	9	2	C
IV	0	11	12	3	D
Total	0	21	38	59	

Table 1 indicates that there are no critical faults. However, major, minor and serious defects vary from one society to another. In general, the most important defects in number are represented by the major defects followed by the serious defects. The serious defects vary between 11 for company IV and 01 for company II. Major defects vary between 14 (company I) and 03 (company II).

The largest number of defaults is found at company IV followed by company I. This makes it possible to classify these 2 companies in category D. Societies II and III for their part are respectively in categories B and C according to Ababouch, [1].

The factory code is obtained by adding the cases of non-conformity marked by a cross in the boxes of Tables 2 and 3. This operation allowed us to classify the factories according to the number of serious defects and to categorize them.

Result of bacteriological analyzes

Overall results: The results are expressed in "estimated number of coliforms per gram". The standard used is m=20 coliform per gram according to the International Commission on Microbiological Standards.

The quality of a lot is considered as:

Satisfactory when the observed value is less than or equal to 3 m; Acceptable when the observed value

is between 3 m and 10 m; Unsatisfactory when observed value is greater than 10 m.

Number of samples per year: Over the period from 2011 to 2014, the central laboratory for hygiene and agribusiness registered 190 samples (Table 4).

Table 2: Evaluation of construction and operating equipment.

Elements to inspect	Major				Minor				Serious			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
1. **Design of fish workplaces**	*											
- Sufficient surface to work			*									
- No-intersecting circuits				*					*			*
2. **Fish workplaces**												
2.1 Soils: Flow and evacuation				*			*					
2.2 walls												
2.3 ceilings												
- easy to clean	*		*	*								
2.4. Doors												
- easy to clean				*	*							
2.5. Ventilation	*											
- Adequate			*						*			*
- Good evacuation steam		*	*									*
2.6. Lighting								*				
2.7. No-hand disinfection												
- Devices in sufficient numbers	*		*	*								
3. Fish workplaces												
3.1. Floors: Easy drainage and evacuation				*	*		*					
3.2. walls												
- Clear, smooth surfaces, easy to clean												
3.3. ceilings												
- easy to clean	*		*	*								
3.4. Doors												
- easy to clean					*			*				
3.5. Lighting								*				
3.6. Sufficient cooling capacity				*					*			
4. Protection against vermin												
- Appropriate devices									*			*
5. Work tools												

6. Disposal of fishery products not intended for human consumption	*											*
- Watertight waste containers made of corrosion-resistant materials	*			*								
- Adequate local												
7. Water supply												
8. Waste water												
9. Cloakrooms and cabins of ease												
- Number of appropriate locker rooms	*		*									*
- Suitable number of washbasin	*	*	*									*
- Appropriate number of lavatories	*										*	*
10. Premises for inspection service												
11. ND means of transport												
- Appropriate equipment	*			*								
12. Installation for living products												
13. Freezing and storage facility												

Table 3: Assessment of industrial hygiene and operating conditions.

Elements to inspect	**major**				**Minor**				**Serious**			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
1. **General conditions of hygiene**												
1.1. Premises and equipment												
- Maintained in good condition of ownership and maintenance									*			*
1.2 Staff hygiene												
- Prohibit any person from contaminating the product from handling									*		*	*
- Wearing clothes												
2. **Container and fresh products**												
- Protects products against contamination	*			*								
3. **Fresh products**												
- Iced products are regularly regulated										*		*
- Threading and slicing in a different location than for heading and gutting											*	
4. **Thawed products**												
- Hygienically	*											
- Appropriate temperature	*	*	*	*								

Of the 190 samples, 185 have an average number of germs of less than 10 (6) germs per gram (7) or 97.4%.

The other 5 have an average number of seeds greater than 10 per gram (2.5×10 2), therefore unsatisfactory.

Table 4: Samples analyzed by company.

Society	Number of samples	Percentage (%)
I	41	21,6
II	59	31,1
III	44	23,2
IV	46	24,1
Total	190	100

The fish species used: The laboratory recorded 16 different species over the 4 years (Table 6).

According to Table 5, Company II sent to the laboratory the largest number of samples, ie 31% (59), followed by companies IV, III and I.

Table 5 shows that red mullet is the most widely used fishmonger with 27.9% (53). It is followed by sole with a percentage of 25.8% (49). Then comes the mostelle with a percentage of 16.3% (31). Species such as scorpionfish, saint-pierre and tilapia are used very little (less than 1%).

Table 5: Overall Results of Microbiological Analyzes.

Society	Satisfactory results	Unsatisfactory results
I	40	1
II	58	1
III	43	1
IV	44	2
Total	185	5

Table 6: Fish species used for net manufacturing in Ivory Coast.

Especie	Number of fish	Percentage (%)
Triggerfish	7	3.7
Bar	12	6.3
dolphinfish	4	2.1
Swordfish	7	3.7
poleaxe	5	2.7
Grouper	2	1.0
Mostelle	31	16.3
scorpion fish	1	0.5
Shark	4	2.1
Red mullet	53	27.9
Sole	49	25.8
Saint Pierre	1	0.5
Tuna	6	3.1
tilapia	1	0.5

Tom	2	1.1
Zebra	5	2.7
TOTAL	190	100

Results by company

Company 1: This company sent 41 samples for 2 years and the results are shown in Table 7.

Table 7: Samples and results of company I.

Year	Quantity	Percentage %	Results in CFU / gram	
			Satisfactory	Not satisfying
2012	40	97.6	6 (40)	0
2013	1	2.4	0	2,6.102(1)
Total	41	100	40	1

Table 7 shows that, the average values of their faecal coliform abundance are 6 and 2.6.102 ufc/ g successively in the samples of the year 2012 and 2013.

Or

97.6% (40) of the results are satisfied and 2.4% (1) unsatisfactory.

Company 2: This company sent 59 samples for 3 years and the results are shown in Table 8.

According to Table 8, it was in 2012 that Company II sent the largest number of samples (55.9%) while it was absent from the register in 2013. All these samples are satisfactory to the except for 2012.

Table 8: The samples and the results of the company II.

Year	Quantity	Percentage %	Results in CFU/gram	
			Satisfactory	Not Satisfying
2011	11	18,6	6 (11)	0
2012	33	55,9	7 (32)	2,5.102(1)
2014	15	25,4	5 (15)	0
TOTAL	59	100	58	1

The average values of faecal coliforms are 6, 7 and 5 ufc / g respectively in the samples of 2011, 2012 and 2014.

Company 3: This company brought 44 samples for 3 years (Table 9).

Table 9: The result and samples of society III.

Year	Quantity	Percentage %	Results in CFU/gram	
			Satisfactory	Not Satisfying
2012	21	47,7	7 (20)	2,4.102(1)
2013	16	36,4	6 (16)	0
2014	7	15,9	5 (7)	0
Total	44	100	43	1

The largest number of samples was recorded in 2012 with 47.7%. Out of a total of 44 samples, we obtained 97.7% (43) satisfactory results and 2.3% (1) unsatisfactory results.

Company 4: This company brought 46 samples during 4 years (Table 10).

Table 10: The results and samples of the company VI.

Year	Quantity	Percentage %	Résults in CFU/gram	
			Satisfactory	Not Satisfying
2011	4	8,7	6 (4)	0
2012	8	17,4	5 (7)	2,6.102 (1)
2013	24	52,2	7 (24)	0
2014	10	21,7	6 (9)	2,5.102 (1)
Total	46	100	44	2

Table 10 shows that of the 46 samples analyzed, 95.7% (44) were satisfactory and 4.3% (2) unsatisfactory.

In general, all 4 companies have fairly satisfactory products. But given the competition, these products must be treated in a more hygienic way by avoiding as much as possible the critical points (Table 11 and Figure 1).

Table 11: Relationship between number of defects and unsatisfactory results.

Society	Serious faults	Satisfactory results	Unsatisfactory results
I	6	40	1
II	1	58	1
III	3	43	1
IV	11	44	2
Total	21	185	5

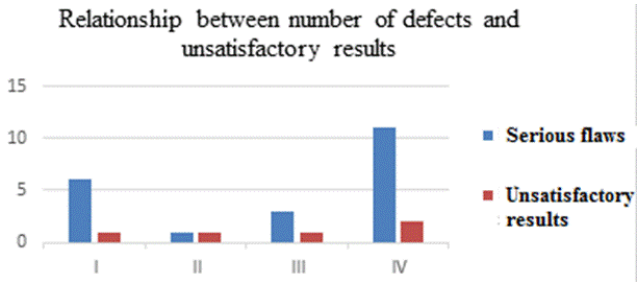

Figure 1: Graph showing positive correlation between the number of serious defects and unsatisfactory results.

We find that there is a positive correlation between the number of serious defects and unsatisfactory results. Companies with the highest number of serious defects have the highest percentages of unsatisfactory results. Thus, the best organized companies have the best laboratory results.

Discussion

As for the total number of samples for the 4 years, it is 190. The sample size is very large given the packaging that contains many fish fillets [11-13]. Then, the four Ivorian companies often practice self-monitoring to meet the standards of the European Union. Finally, they also send samples to regional microbiological fish testing laboratories [14]. Collected 600 samples in a single year in Senegal, where this sector has grown significantly [15-22]. The species used in making fish fillets are mainly red mullet, sole and broth. Our results are similar to those of Cote d'Ivoire except that the brotules are replaced by the mostelles [23-37]. Our results are comparable to those found by Seydi, et al. [38] in Senegal. Because these authors use mainly soles and brotules the two main fish species exploited in the making of frozen fillets. They used only one type of fish for their study, namely Mugil cephalus, and obtained a high level of contamination of 180 germs per gram of coliforms. Our results gave a satisfaction rate of 97.4% and a non-satisfaction rate of 2.6%. Our results are in line with those of Baer, et al. [7] who found a contamination rate of between 1 and 10 germs per gram of product. Five samples out of 90, which is 2.6% are unsatisfactory but are in deca of those

found by Seydi, et al. [38] who found results much higher on the order of 31.87% [39-41]. Toure [42] found results of noncompliant nets of the order of 9.49%, these results are similar to ours. It is noted that in Senegal microbiological analyzes have no doubt been improved with the introduction of the HACCP system at the level of all companies processing fish products from 1996 by Baer [7]. Other authors, such as Ndiaye [31] in 1996 and 1997 and Sitti [40] from 1997 to 2000 on the evolution of the bacteriological quality of fishery products for export showed that there is a significant decrease in thermotolerant coliforms over the years, which indicates a significant improvement in compliance with hygiene rules and good manufacturing practices in processing plants for fish products. Indeed, according to the following authors Ababouch [1], Borner [9], Chandraval, et al. [10], Degnon, et al. [12], Dib amira [13], Fleming [16], Tsamba [43], thermotolerant coliforms, which are present in the food, are witnesses of the non-respect of the rules of hygiene (faecal contamination of the products). This favorable evolution of food quality is undoubtedly the result of the introduction of the Hazard Analysis Critical Control Points (HACCP) or Hazard Analysis and Critical Control Point (ADMPC) system at the level of all processing companies. fishery products from 1996. Have an average of 69 germs/ g against 236 germs/ g depending on the culture medium used. These authors, whatever the medium, have found results far superior to ours. They explain these high values by the fact that the products came into contact with many critical points. For Ndiaye [31] 111 samples out of 112 gave numerical results with an average of 236 germs per g of net. This average is much higher than ours and as well as that obtained by Baer quoted by Seydi, et al. [39] or between 1 and 10 germs 1 g of product. The average we obtained, however, is much lower than that obtained by Seydi et al. [38] or 625 seeds per g of net. In addition, Seydi, et al. [39], Toure [42] and also found higher values of the order of 275 sprouts/ g net. These results were obtained before the actual implementation of the HACCP system. Azibe [5], Ouattara [32] find very different results. The first reports an average contamination of 625 germs / g while the second finds an average of 12 germs/ g, have shown in their work that the fertility of coliforms depends on the culture medium used: some are very fertile and allow a large count while others are not very fertile and cause a low rate of coliforms, which is a bias that explains in part the disparity in the results obtained. In contrast, the processing units we evaluated did not implement any HACCP system and therefore did not develop a real sampling plan. These producers may have prepared special samples for analysis and this will not reflect the microbiological quality of the lot. To this end, Huss [21,22] states that the number, size and nature of the samples greatly influence the results obtained. Numerous faecal coliforms have been enumerated massively in the Fresco lagoons Kouadio, et al. [27]. Many authors around the world, whatever the measures implemented, have also observed thermos tolerant coliforms on fish analyzes [Alves Guerreiro [4], Badri, et al. [6], Dib Chandraval, et al. [10], Amira [13], El Afly [15], Fleming, et al. [16], Ghanem, et al. [17], Grant, et al. [19], Ip [23], Polimetla, et al. [34], Teophilo, et al. [41,44], Tsamba [43]]. Thermos tolerant coliforms are not always adapted to the high temperature of 44°C because many authors have identified them in fish incubated at very low temperatures of times below 30°C by Albuquerque [2], Chapot-Chartier, et al. [11], Wounter, et al. [45]. The results obtained on fish fillets vary from one technique to another. Current methods based on genetics make it possible to detect bacteria that would have gone unnoticed by culture, biochemical and microscopic methods [Almeida, et al. [3], Badri, et al. [6], Bennani [8], Chapot-Chartier, et al. [11], Gourmelon [18], Kumar, et al. [28], Mayo, et al. [30], Park [33], Ryu, et al. [35], Sauvageot, et al. [36], Wounter, et al. [44]]. Finally, some thermos tolerant coliforms are insensitive to certain drug substances [Ip [23], Jeyasanta, et al. [26], Park, et al. [33].

Conclusion

In Ivory Coast, as in most coastal countries, fish production plays a significant role both economically and socially. It participates in the balance of the trade balance thanks to the inflows of currencies consecutive to exports. Many companies have started this activity, given the growing demand. Also, among fish commodities, the net is one of the most popular products on the international market. However, its entry into this market is dependent on the satisfaction of certain microbiological criteria. Its production is especially marked by a mechanization can push factories. The

consequence is a strong manipulation of the products by the staff, a source of frequent contamination by thermos tolerant coliforms. That's why we started looking for these microbes in fish fillets for export. The purpose of this work was to assess their level of contamination. To do this, we conducted technical visits to four companies producing these commodities, and we conducted a retrospective analysis of the results of the analyzes for these germs in fish fillets from 2011 to the first half of 2014. From this study bacteriological, it appears that 97.4% of the samples are satisfactory and that 2.6% are unsatisfactory. Thus, to get closer to the requirements of the European Union in terms of quality assurance and preserve or even acquire new markets for products for export; it would be essential to undertake a set of actions to acquire the legal, human and technical means necessary for the establishment of a national inspection and quality assurance program.

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