



CONSUMPTION AND EVALUATION OF THE MICROBIOLOGICAL QUALITY OF THE COMMON HORSE MACKEREL *TRACHURUS TRACHURUS* (LINNÉ, 1758) SOLD ON THE MARKETS OF THE CITY OF MAN (CÔTE D'IVOIRE).

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ABSTRACT

The present work aims at evaluating the microbiological quality of the common horse mackerel *Trachurus trachurus* sold in the markets of the city of Man (Côte d'Ivoire). Total Aerobic Mesophilic Flora, *Escherichia coli*, *Staphylococcus* sp and *Salmonella* sp were the main germs sought in the samples analyzed. The microbiological analysis was performed by the decimal dilution technique. A total of 45 samples were used. These analyses show an acceptable microbiological quality for the total aerobic mesophilic flora. On the other hand, the microbiological quality of the *Escherichia coli* contamination was not satisfactory for all the samples analyzed ($5.04.10^3 \pm 0.74$ and $1.12.10^5 \pm 1.1$ CFU/g). The contamination rates found in the samples from the three markets are worrying especially with the very significant presence of *Salmonella* sp and *Staphylococcus* sp respectively in the large market of Man ($1.27.10^3 \pm 1.27$ CFU/g), in the "Caca sport" market ($1.9.10^3 \pm 1.9$ CFU/g) and the Doyagouiné market ($5.83.10^3 \pm 5.83$ CFU/g). These results highlighted the existence of a potential health risk associated with the consumption of horse mackerel from these different markets.

KEYWORDS: Microbiological quality, *Escherichia coli*, *Staphylococcus* sp, *Salmonella* sp, *Trachurus trachurus*.

INTRODUCTION

Fish is a food of high nutritional value, but also a valuable supplement in diets that are low in protein, vitamins and essential minerals.^[1] Despite their high nutritional value, fish are very perishable foodstuffs. Indeed, with average temperatures between 25 and 30°C in tropical countries, fish deteriorate in less than 12 hours.^[2] High temperatures thus create favorable conditions for the development of fish spoilage microorganisms; this causes huge qualitative and quantitative losses.^[3] In addition, the WHO defines foodborne diseases as diseases caused by the consumption of contaminated food.^[4] In developing countries, more than 70% of diarrheal disease cases are foodborne.^[5] Very little work has been done on the sanitary quality of fish products sold on the market, yet fish provides nearly 60% of the protein consumed by human populations, especially in rural areas.^[6] This habit of breaking the cold chain of conservation of fish, handling them without taking into account the rules of hygiene and exposing them to high temperatures can be the basis of microbial contamination. Fish, after fishing,

are indeed exposed to subsequent microbiological contamination that can cause foodborne illnesses in consumers such as typhoid fever and shigellosis which are real public health problems.^[7] In addition, the actual incidence of diseases caused by the consumption of seafood and in particular fish, the main source of animal protein of the Ivorian consumer is unknown. Thus, the absence of a monitoring and control system can favor the appearance of numerous pathologies in humans, generating a real public health problem. These different aspects have led to the interest in carrying out a study whose main objective is to evaluate the microbiological quality of fish *Trachurus trachurus* (Linné, 1758) sold in the markets of the city of Man.

I-MATERIAL AND METHODS

1- Materials

1.1-Biological material

This study was conducted in the city of Man (Côte d'Ivoire) and more specifically in three markets in the city (the large market, the "Cacasport" market and the Doyagouiné market).

The biological material consists of fresh fish of the common horse mackerel of scientific name *Trachurus trachurus* (Linné, 1758) sold in the markets of the city of Man.

1.2 Technical material

The usual microbiology laboratory equipment was used for the realization of this work.

2 - Methods

2.1. Survey protocol

The surveys were carried out in the form of interviews with merchants and consumers but also visual observations of the points of sale.

The survey was carried out thanks to a questionnaire which were established from the software SphinxPlus.V5.TuiTe. The latter made it possible to collect on the one hand the information relating to the socioeconomic characteristics of the traders and the consumers and the food characteristics relating to the consumption of frozen fish.

Relevant information was collected from the vendors surveyed, including age, gender and level of education, compliance with good hygiene practices, mode of installation of the vendor, sources of supply of fish, mode of conservation of unsold fish, and sales capacities or volumes.

2.2. Consumer Selection

The consumer questionnaire was administered to 120 households. That is to say 40 consumers interviewed for each of the (3) housing levels (Low standard, Medium standard and High standard).

2.3. Methods of microbiological analysis of fish

The microbiological analysis of fish is based on the techniques of isolation, identification and enumeration. The identification of a germ is characterized by the absence or presence of the latter and is therefore a qualitative aspect. On the other hand, the enumeration, quantitative aspect, is carried out by counting the colonies followed by a calculation mode.

2.4 Sampling

Three stalls where *Trachurus trachurus* is sold were randomly selected for sampling. Three samples of fish were randomly collected from three traders for each market. A sample consisted of fish weighing an average of 250 g $\pm$ 36 g. A total of 09 samples were collected per week from the three markets. Thus, 15 samples were taken per market, for a grand total of 45 fish samples that were analyzed. These samples were collected in StomacherND bags and transported to the laboratory in coolers for analysis.

2.5. Preparation of the fish parts to be analyzed

2.5.1. Preparation of the skin surface

The method used is the one described by Adingra et al., (2010).^[6] Once in the laboratory, each fish sample was rinsed with Buffered Peptone Water (EPT) broth before being cleaned with alcohol in order to enumerate the germs present on the skin surface. The EPT solution collected in a sterile bottle was the stock solution.

2.5. 2. Preparation of the flesh

On a well-disinfected bench in front of a Bunsen burner, 25 g of flesh was collected and ground in a Stomacher bag. 225 mL of EPT was added to the grind and the resulting homogenate is the 10-1 stock solution.

Series of decimal dilutions in cascade were performed under aseptic conditions from 1 ml of each stock suspension until a 10-5 solution was obtained. These solutions were used for research and enumeration of microorganisms.

Search and enumeration of all FMAT colonies were done according to ISO Standard 4833: February 2003. PCA culture media were incubated at 37°C for 24 h. *E. coli* were tested on VRBL agar according to ISO 16649-2: April 2001. The enumeration was done after incubation at 44°C for 24 h. For *Staphylococcus* sp, the research was carried out according to ISO 6888-1: July 2003. The colony count was done on solid medium (Baird-Parker medium) after incubation at 37°C for 48 hours. The research of *Salmonella* was done according to the ISO 6579/A1: July 2007 standard.

After incubation the characteristic colony count for each dish containing less than 300 colonies and at least 30 colonies was performed.

The number N of colony forming units (CFU)/g present in the analyzed sample and considered as an average of successive dilution is given by the following formula.

$$N = \frac{C_1 + C_2}{V(n_1 + 0,1n_2)d}$$

C1+C2 = sum of the characteristic colonies of two successive dilution plates counted

V= volume of inoculum applied to each plate

d = dilution rate corresponding to the first dilution retained

n1 = number of plates retained at the first dilution

n2 = number of boxes retained at the second dilution

II- RESULTS AND DISCUSSION

1. Results of the survey

1.1.1 Survey carried out among the traders

After investigation among the traders, whose main objective was to assess the knowledge and implementation of good hygiene practices on the fish sold. It results from it the following report.

Fish marketing is 100% dominated by women; but 75% of these women do not practice hygiene rules “Fig 1”. Among these women, 100% do not wear professional sales clothing, although 6.8% know about it, compared to 93.2%; the percentage of women with no education is 45.5%, while the percentage of educated women is 54.5%.

In terms of the degree of healthiness of the fish, 68% were declared to be of poor healthiness while 32% were of acceptable healthiness “Fig 2”;

As for the unsold fish at the end of the day, 95% are returned to the cold room or put in the refrigerator “Fig 3”.

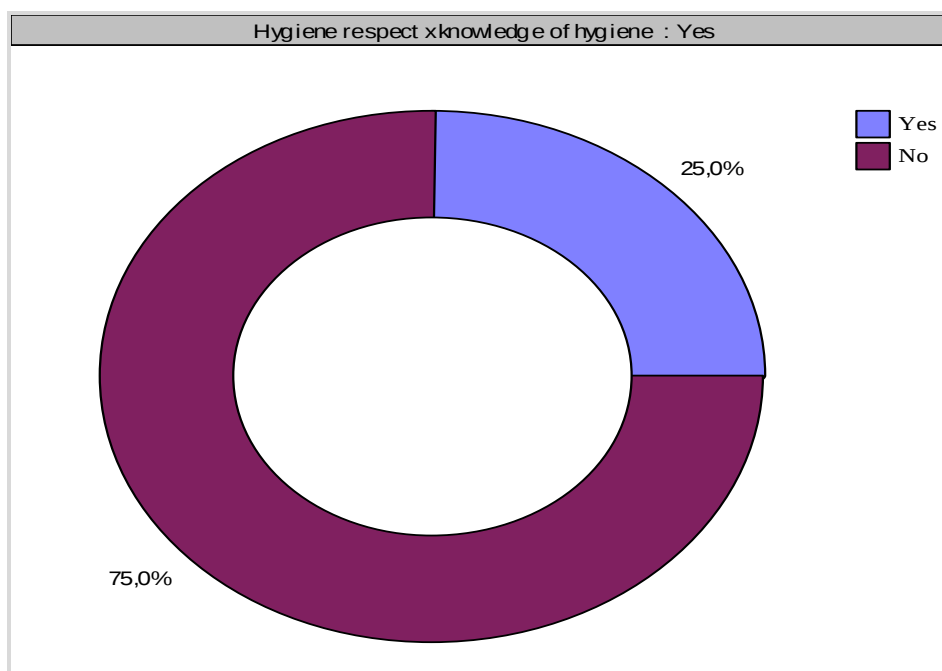


Figure 1: Results of the cross-tabulation between compliance with hygiene practices and hygiene knowledge.

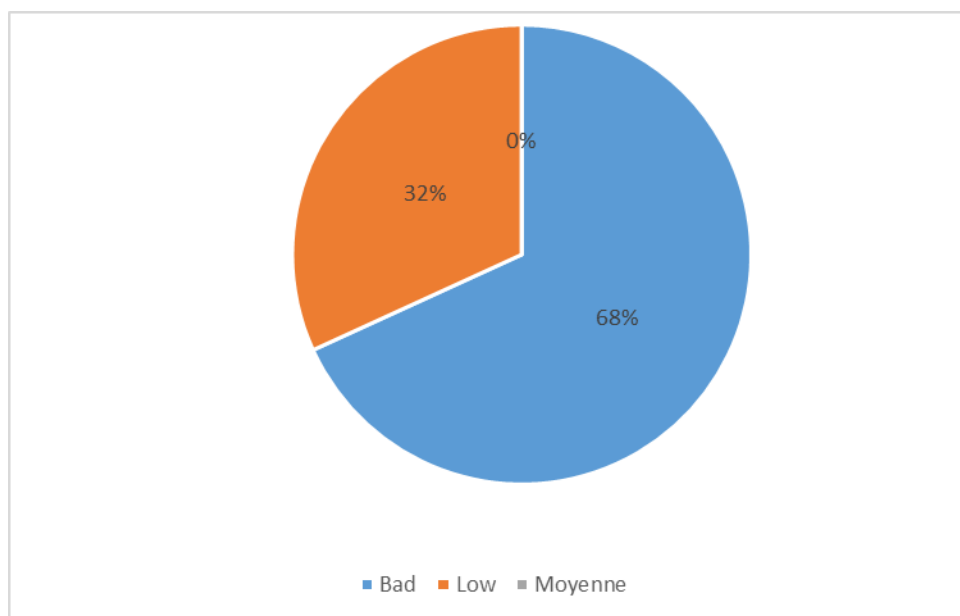


Figure 2: Fish safety results based on observation of fly presence, equipment and sales environment.

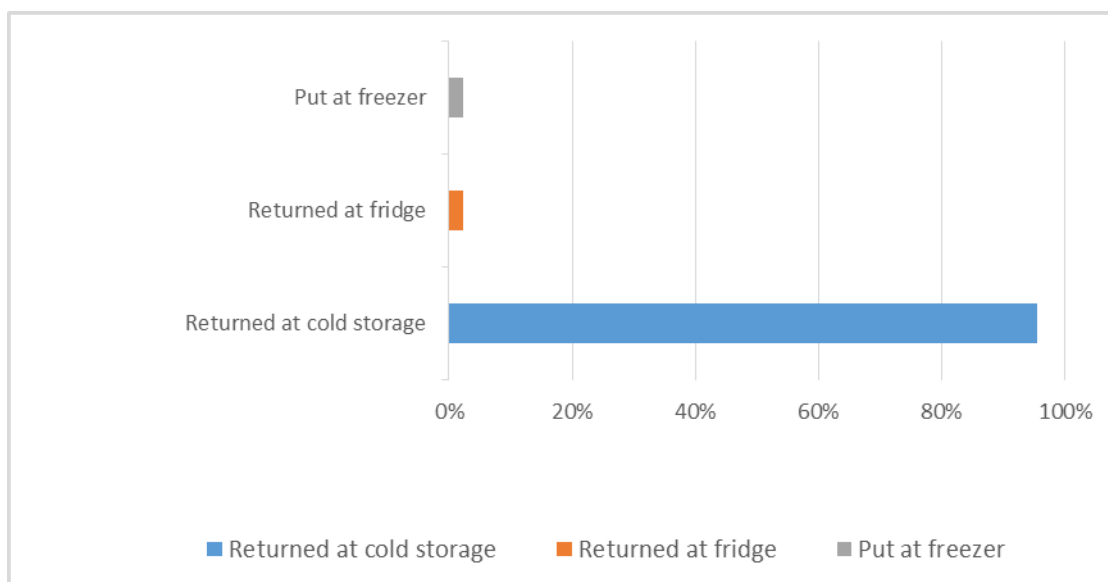


Figure 3: Method of storing unsold frozen fish at the end of the day.

1.1.2. Consumer survey

Among the fish consumed by households, the most consumed species is the common horse mackerel *Trachurus trachurus* with a rate of 46.7%, then comes the mackerel (*Scomber colias*) at 18.3%. Finally, Nile tilapia (*Oreochromis niloticus*) and mackerel (*Chrysichthys*

nigrodigitafus) follow with respective percentages of 11.7% and 10.8% "Fig 4".

Similarly, 52.5% of consumers affirm that the fish sold in the markets are of good quality, however 12.5% of them find it of poor quality and 1.7% of poor quality "Fig 5".

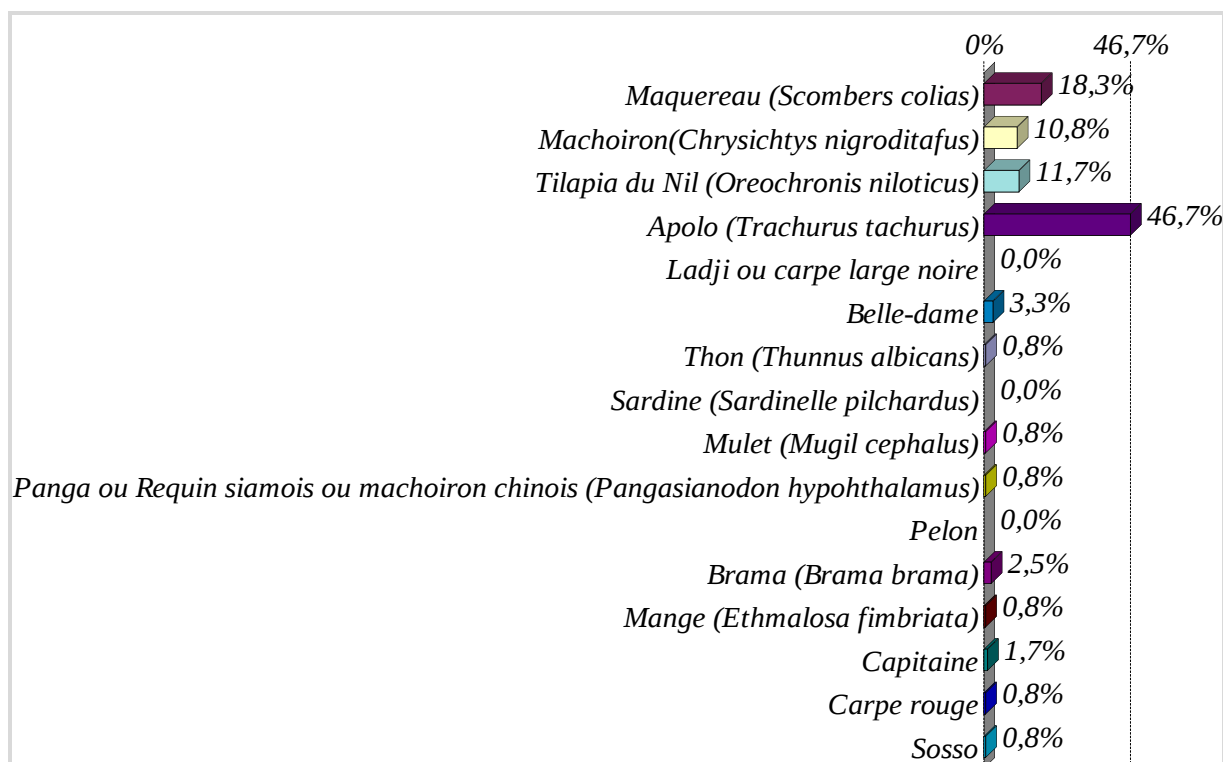


Figure 4: Percentage of consumption preference for fish species.

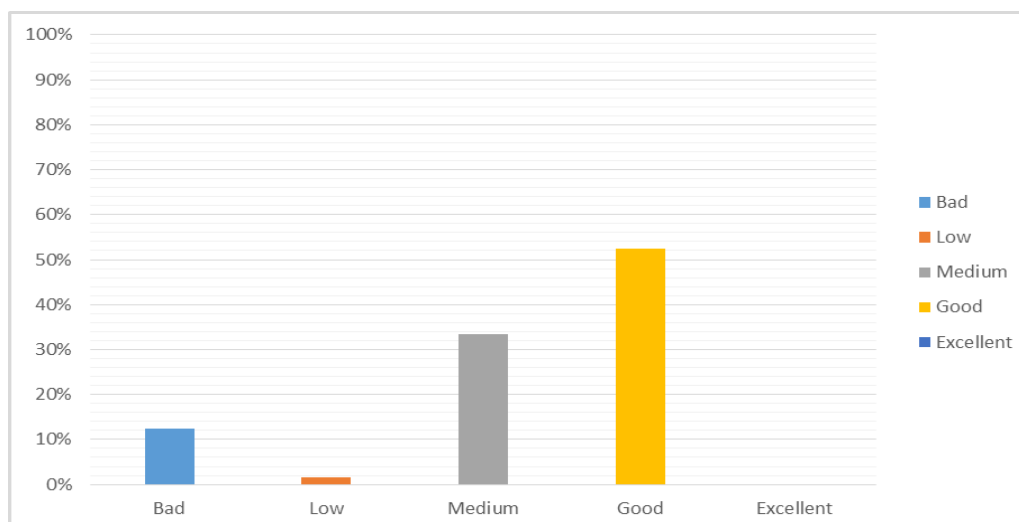


Figure 5: Consumers' assessment of the quality of marketed fish.

At the level of consumption, 91.7% of the people interviewed stated that they had felt uncomfortable after eating poor quality fish "Fig 6".

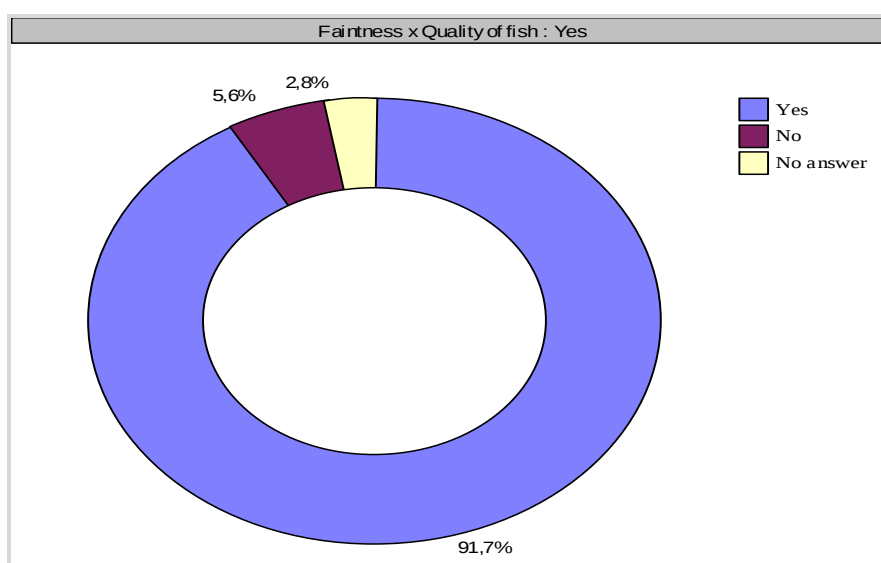


Figure 6: Result of the cross-tabulation between the frequency of having experienced discomfort knowing that the fish was rotting.

2-Results of microbiological analyses

For all the samples analyzed at the different markets, it is noted that all the germs tested have a higher average load on the surface of the skin than in the fish flesh "Fig 7."

2.1. Total aerobic mesophilic flora (TAFF)

The analysis of the FMAT of *Trachurus trachurus* revealed an average load N of acceptable microbiological quality (AMQ) reference made to the microbiological criterion (3.10^5). These average loads vary between $1.77.10^5 \pm 1.3.10^5$ and $5.95.10^5 \pm 1.1.10^5$ respectively for the Doyagouiné Market and the "Cacasport" Market.

2.2. *Escherichia coli*

For *E. coli*, the level of contamination is very high. The loads obtained were $1.12.10^5 \pm 1.1$ on average for the

samples from the "Cacasport" market, $2.83.10^4 \pm 1.35$ for those from the Doyagouiné market and $5.04.10^3 \pm 0.74$ for the samples from the Grand Marché de Man. The microbiological reference criterion is 102. The values obtained are well above (10) times the reference criterion. The fish samples analyzed in these three markets are therefore of unsatisfactory microbiological quality (UMSQ).

2.3. *Staphylococcus* sp

The results of the microbiological analysis carried out show the absence of *Staphylococcus* sp contamination in the Grand Marché and the "Cacasport" Market. The samples taken at these markets are therefore of satisfactory microbiological quality (QMS).

On the other hand, the Doyagouiné market has a very high level of contamination (higher than the microbiological criterion of 10) with an average load of $5.83.10^3 \pm 5.83$ found only in the samples of the skin surface of *T. trachurus*. This indicates an unsatisfactory microbiological quality.

2.4. *Salmonella* sp

The results of the *Salmonella* sp test revealed an absence of this germ in the *T. trachurus* samples from the Grand Marché de Man, which indicates QMS.

On the other hand, *Salmonella* sp was found only on the skin surface of *T. trachurus* samples collected at the Grand Marché de Man and at the Marché de Cacasport with average loads of $1.27 \times 10^3 \pm 7.78$ and $1.9 \times 10^3 \pm 1.9$ respectively. The presence of this germ on the fish samples collected shows that these samples have an unsatisfactory microbiological quality.

The results of the analysis of the microbiological quality of the samples from the different markets are presented in Table I.

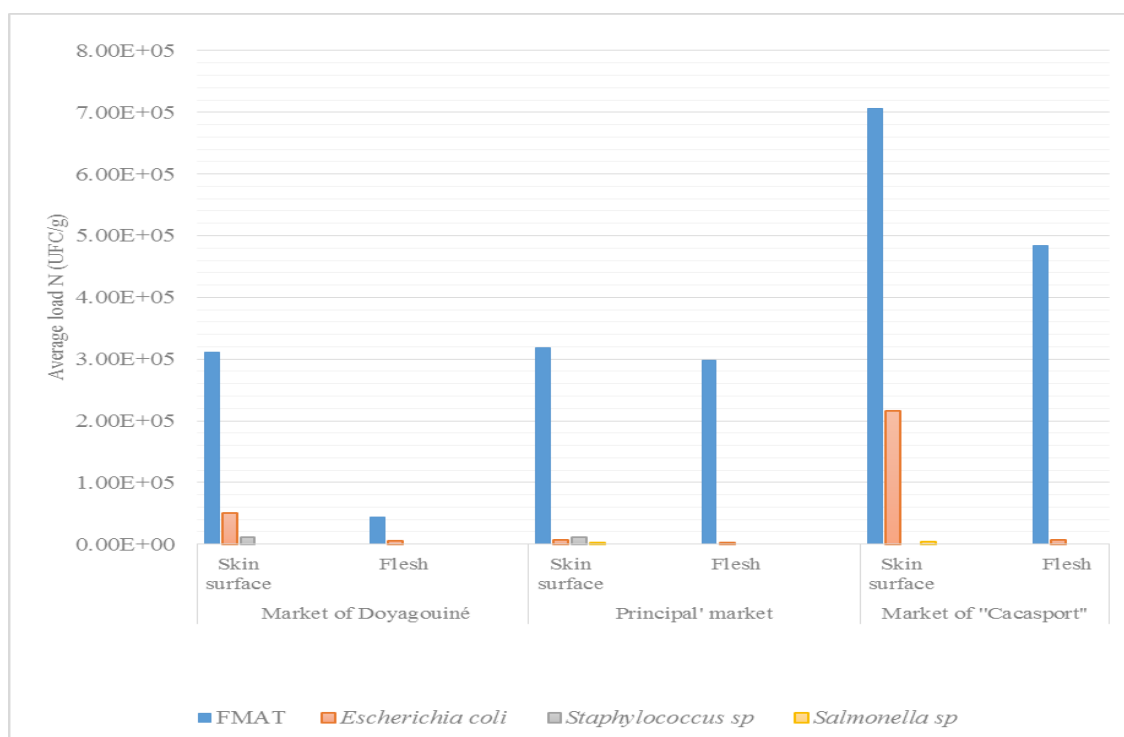


Figure 7: Average germ load (FMAT, *E. coli*, *Staphylococcus* sp and *Salmonella* sp) per market.

Table I: Microbiological quality of *Trachurus trachurus* samples collected from the three markets.

Germs studied		Samples taken in the markets			Microbiological criteria
		Market of Doyagouiné	Principal market Man	Market of "Cacasport"	
FMAT	Load in UFC/g	$1,77.10^5 \pm 1,3$	$3,08.10^5 \pm 0,01$	$5,95.10^5 \pm 1,1$	3.10^5
	Quality	QMA	QMA	QMA	
<i>Escherichia coli</i>	Load in UFC/g	$2,83.10^4 \pm 1,35$	$5,04.10^3 \pm 0,74$	$1,12.10^5 \pm 1,1$	10^2
	Quality	QMNS	QMNS	QMNS	
<i>Staphylococcus aureus</i>	Load in UFC/g	$5,83.10^3 \pm 5,83$	Absent	Absent	10^2
	Quality	QMNS	QMS	QMS	
<i>Salmonella</i> sp	Load in UFC/g	Absent	$1,27.10^3 \pm 7,78$	$1,9.10^3 \pm 1,9$	Absent/25g
	Quality	QMS	QMNS	QMNS	

QMS: Satisfactory microbiological quality, QMA: acceptable microbiological quality
QMNS: unsatisfactory microbiological quality, UFC/g: Colony Forming Unit per gram

DISCUSSION

The results of the survey revealed that the marketing of frozen fish is 100% dominated by women. This finding

can be explained by the fact that women generally resort to tertiary sector activities, particularly fish trade, to ensure their survival and to support the household

financially. This observation is similar to that of Abotchi^[9] and Oulaï *et al.*^[1] On the other hand, according to the results reported by Baryeh^[10], the sale of fish is mostly carried out by men (88.8%) compared to 11.2% of women.

Of all the women interviewed, all stated that they did not have a work certificate and had never received training in basic hygiene. This observation is linked to a lack of involvement of the competent authorities in the development of a system of supervision and monitoring in the fish trade sector. The present observation is similar to that of Itongwa *et al.*^[3] but 33.34% different from that of Abdi *et al.*^[11] who conducted a similar study on fresh and frozen fish in the city of Guelma, Algeria.

In addition, the cross-tabulation shows that 75% of the women do not follow hygiene rules. On this basis, the majority (68%) of the fish were found to be of poor quality. This tendency to respect hygiene measures is not surprising in view of the above-mentioned arguments. Moreover, 45.5% of these women have no education. This is justified by the fact that in developing countries these women are often less educated.^[3]

Horse mackerel *T. trachurus* is the most sold fish species at a rate of 70.5%. The majority of traders emphasized the preference of consumers for this species of fish over others. This implies that it is a relationship between demand and supply that influences the choice of traders to offer one or more species of fish to their clients. The MIRAH-DR MAN^[12] report does reflect the veracity of this survey in that the amount of *T. trachurus* marketed was 614.9 tons.

The discomfort observed after consumption of the fish is declared by 30% of the consumers interviewed. Of these, 91.7% stated that the fish was rotten. Public health officials consider that good quality means the absence of harmful agents such as parasites, pathogenic bacteria, chemical poisons etc.^[11] Therefore the discomfort of these investigated people may be due to the presence of pathogenic bacteria or their toxin or parasites.

As for the microbiological criterion, it refers to the high presence of spoilage germs or the absence of pathogenic and spoilage germs that are responsible for food poisoning and organoleptic quality alteration, respectively. Spoilage can be defined as a change in the product, which makes it less acceptable, unacceptable or dangerous for human consumption.^[13]

The average FMAT load of *T. trachurus* samples collected from different markets indicates an acceptable microbiological quality of *T. trachurus* in FMAT.

FMAT is an indicator of the general level of hygiene that may reflect the "history" of the product (break in the cold chain, poor time/temperature management). It can be an indicator of spoilage, its average load in a product gives

information on the state of freshness of a product or the effectiveness of a heat treatment or conservation.

These germs do not have a great impact on the health of the consumer, however, they cause significant economic losses due to product spoilage. Indeed, it has been shown that fishery products with a FMAT level higher than 106 CFU/g are likely to be at an unacceptable stage from the microbiological point of view and can be considered unfit for human consumption.^[14]

The acceptable quality of the analyzed samples is consistent with that of Abotchi^[9] in Togo, who observed FAMT contamination of fresh fish used in the artisanal smoked fish production process of *Trachurus trachurus*, obtaining a microbial load of 5, 95.105 CFU/g for the raw material in Togo. However, it differs from that found by Abdi *et al.*^[11] and Itongwa *et al.*^[3] who obtained satisfactory and unsatisfactory microbiological quality respectively.

For faecal coliforms, especially *E. coli*, the quality of *Trachurus trachurus* is not satisfactory with respect to the standard in force for frozen fish. It should be noted that the high contamination by fecal coliforms in the fish samples indicates the poor hygienic conditions in which the fish are sold but also the lack of respect for hygiene practices. The fish would therefore be infected by the germs of faecal origin generally found in the digestive tract of humans and animals. These results present a contrast with those of Abdi *et al.*^[11] who arrived after analysis of their samples at an average load of less than 10 CFU/g when working on canned sardines. Conversely, they are similar to those obtained by Oulaï *et al.*^[1] (4.8.104 CFU/g), Abotchi^[9] (1.87.102 CFU/g) and N'Guessam *et al.*^[15] (7.1 102 CFU/g) who worked on the distribution circuit of fresh and frozen fish.

The high rate of *Staphylococcus* sp is explained by the handling during the sale and the absence of professional sales clothing for fishmongers such as gloves, cap and nose cover. This presence observed on the surface of the skin and not in the flesh is justified by the higher risk of contamination for the surface of the skin compared to the flesh because it is in permanent and direct contact with the environment and subject to possible handling. This microorganism is not competitive with other organisms present in the fish, so it cannot multiply (Huss, 1988). Our results are supported by Dione^[16] and are dissimilar to those of N'Guessam *et al.*^[15] and Oulaï *et al.*^[1]

The presence of *Salmonella* sp observed on the samples from the main market and "Cacasport" attests to a microbiological quality that is not satisfactory in relation to the standard. The presence of this germ is associated with fecal contamination of human or animal origin. The contamination can be direct (staff hygiene) or indirect (contact with soiled material). This observation is related to the unhealthy environment of the sales sites, but also to the uncontrolled health of the saleswomen.

The absence of this germ in the flesh of *T. trachurus* for the samples of the three (3) markets results from the fact that the first barrier encountered by the bacteria is constituted by the integuments and mucous membranes, whose mechanical resistance is reinforced by a mucus covering the entire body of the fish. This mucus, especially abundant on the surface of the skin, contains many components including lysozyme, which exerts lytic activity on bacterial walls.^[8] The presence of *Salmonella* is contrary to the results reported by Djinou,^[17] and Oulaï et al.^[1] In contrast, Dione^[16] also noted the presence of *Salmonella* in 10 of these samples of braised-dried fish.

CONCLUSION

The present work was carried out at the level of marketing, consumption and microbiological analysis. In view of the above, it appears that the most popular source of animal protein among the population of the city of Man is fish, particularly the common horse mackerel *Trachurus trachurus*. It is therefore found on most of the stalls of fish traders in various markets in the city.

Regarding the results of analysis of the microbiological quality of *Trachurus trachurus* sold in the markets of the city, it has revealed a potential contamination of this species of fish by various germs; the germs of alteration and pathogenic. This contamination is correlated with the poor hygiene practices of the sellers and the lack of adequate marketing infrastructure.

These results highlighted the existence of a potential health risk associated with the consumption of horse mackerel from these different markets.

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