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Contamination Level of Histamine-Producing Bacteria in Fishery Products in Côte d'Ivoire

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ABSTRACT

Background and Objective: Histamine is the primary cause of fish and seafood-related illnesses. Identifying and characterizing histaminergic bacteria would allow to prevent their growth and thus avoid the production of histamine in fish products. The objective of this study was to evaluate the capacity of bacteria to produce histamine in fish products in Côte d'Ivoire. **Materials and Methods:** To do this, 18 samples (6 tuna samples, 3 mackerel samples, 3 tilapia samples, 3 crab samples and 3 shrimp samples) were collected. The enumeration of mesophilic aerobic germs, Enterobacteriaceae, *Staphylococcus* and lactic acid bacteria was carried out according to conventional microbiological methods. Then, 270 isolates from the different groups of microorganisms counted were tested for their ability to produce histamine in the Niven medium. **Results:** The results of this study showed that the average loads of the different microorganisms in the fish products varied significantly according to the species analyzed. Of the 270 isolates tested, 124 isolates produce histamine, i.e., 49.92%. Histaminergic Enterobacteriaceae showed a high percentage of histamine production with 47.58% in the different fish products. **Conclusion:** Enterobacteriaceae are therefore the most incriminated in the production of histamine in fishery products in Côte d'Ivoire. They would therefore represent a major health risk for consumers.

KEYWORDS

Histamine, histaminergic bacteria, fish products, Côte d'Ivoire

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INTRODUCTION

Fish products such as fish, cephalopods and crustaceans are a healthy source of high-quality protein, essential vitamins, minerals and polyunsaturated fatty acids. The beneficial effects of the consumption of these products on products on human health are the protection against coronary heart disease and cancers[1]. However, fish products are responsible for 10-20% of human poisonings. These poisonings are linked to the presence of chemicals, biological toxins, or allergens. Histamine poisoning is the leading cause of poisoning linked to the consumption of fish and other fish products[2]. Indeed, 30-40% of epidemics linked to the consumption of fish and other fish products are due to histamine[3,4]. This intoxication results in skin symptoms (redness, hives), neurological symptoms (headaches), gastrointestinal symptoms (diarrhoea, vomiting), palpitations and edemas that can sometimes lead to hospitalization and death of weakened patients.

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Histamine is a biologically active and thermostable molecule, belonging to the biogenic amines. The formation of histamine in fish and other fish products depends on two essential factors. The first factor is the histidine content, directly related to the animal species[5]. The second factor is the presence of bacteria capable of synthesizing histidine decarboxylase[6,7]. A very large number of bacteria are responsible for the formation of biogenic amines from free amino acids such as *Pseudomonas fluorescens*, *Morganella morganii*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter aerogenes*.

In Côte d'Ivoire, some authors established a relationship between bacteria and the formation of histamine in fish products in Côte d'Ivoire[8]. However, there is very little work on the control of fishery products marketed in Côte d'Ivoire and a lack of scientific data on the detection, early quantification and characterization of histaminergic bacteria in these fish products.

The objective of this study was to evaluate the histamine-producing bacteria isolated from fish products in Côte d'Ivoire to prevent their growth, avoid the production of histamine in fish products, reduce the risk of histamine poisoning and limit economic losses following the recall or withdrawal of lots.

MATERIALS AND METHODS

Sample collection and preparation: The sampling was conducted from June to September, 2021. Samples of tuna, mackerel, tilapia, crab and shrimp were collected respectively in Abobo-Doumé, Adzopé, Grand-Bassam and Motobé (Southern Côte d'Ivoire). The 18 samples (3 tuna samples from fishermen (tuna fish 1), 3 tuna samples from vendors (tuna fish 2), 3 mackerel samples, 3 tilapia samples, 3 crab samples and 3 shrimp samples) were collected. Three visits were made to the vendors and fishermen, with one sample of each type of fish product collected per visit. One sample consisted of approximately 1 kg of tuna, mackerel and tilapia and approximately 500 g of crab and shrimp. The preparation of the stock suspension and decimal dilutions was done according to ISO-6887-4[9].

Microbiological analysis: Mesophilic aerobic germ count was performed on PCA agar incubated at 30°C/72 hrs according to ISO 4833-2[10]. Enterobacteriaceae count was performed according to ISO-21528-2 on VRBG agar incubated at 37°C/24 hrs[11]. The enumeration of *Staphylococcus* was performed according to ISO 6888-1 on Baird Parker agar incubated at 37°C/24 and 48 hrs[12]. The enumeration of Lactic Acid Bacteria (LAB) was performed according to ISO 15214 on MRS agar incubated at 30°C/72 hrs in anaerobic conditions[13]. A Confirmation of LAB was performed by Gram stain and catalase test, followed by microscopic examination. Gram+ and catalase- bacteria were confirmed as lactic acid bacteria. The different microbial loads expressed in CFU g^{-1} have been calculated according to the formula ISO 7218[14]:

$$N(\text{CFU g}^{-1}) = \frac{\sum C}{(n1 + 0,1n2) \times d \times V}$$

Where:

N = Number

d = density

V = Volume

ΣC = Sum of all colonies

Isolation, purification and conservation of isolates: Colonies of each group of microorganisms (Enterobacteria, *Staphylococcus* and lactic acid bacteria) were collected. The purification of isolated colonies was performed by successive plating on MRS agar for lactic acid bacteria and nutrient agar for Enterobacteria and staphylococci. A total of 360 isolates were obtained, purified and preserved in Table 1.

Table 1: Total numbers of microorganisms isolated from the different samples

Samples	Enterobacteriaceae	*Staphylococcus*	Lactic acid bacteria	Total
Tuna fish 1	15	15	15	45
Tuna fish 2	15	15	15	45
Mackerel	15	15	15	45
Tilapia	15	15	15	45
Crab	15	15	15	45
Shrimp	15	15	15	45
Total	90	90	90	270

Search for histamine-producing bacteria: The culture medium used for the research of histamine-producing bacteria is the Niven medium[15,16]. Isolates maintained on the different agars were touch streaked using a Pasteur pipette onto Niven agar and incubated at 30°C for 24 hrs. Characteristic colonies of histamine-producing bacteria appear purple with or without a purple halo.

Statistical analysis: The Analysis of Variance (One-Factor ANOVA) was performed with the statistical software version 7.1 at the significance level (α = 0:05). In case of significant difference between the parameters studied, the ranking of the means is done according to the Newman-Keuls Test (https://en.wikipedia.org/wiki/Newman%E2%80%93Keuls_method).

RESULTS

Microbial loads of fish products

Mesophilic Aerobic Germ loads (MAG) of fish products: The average loads of mesophilic aerobic germs were between 4.68±0.03 and 8.21±0.13 log CFU g^{-1} in Fig. 1. The highest load was observed in the tuna samples from vendors (tuna fish 2) (8.21±0.13 log CFU g^{-1}) while the lowest load was observed in tilapia from vendors (4.68±0 .03 log CFU g^{-1}). The average GAM loads of tilapia, crab, mackerel and tuna samples collected from vendors showed a significant difference ($p<0.05$).

Enterobacteriaceae loads of fish products: The average Enterobacteriaceae loads in the different samples ranged from 2.59±0.16 to 7.45±0.01 (log CFU g^{-1}) in Fig. 2. Shrimp showed the highest load (7.45±0.01 log CFU g^{-1}) and tuna fish 2 showed the lowest load (2.59±0.16 log CFU g^{-1}). No significant difference was observed between tuna loads from fishermen (tuna fish 1) (2.66±0.26 log CFU g^{-1}), tuna loads from vendors (tuna fish 2) (2.59±0.16 log CFU g^{-1}) and mackerel (4 .36±0.06 log CFU g^{-1}).

***Staphylococcus* loads of fish products:** The average loads of *Staphylococcus* in the samples ranged from 6.85±0.01 to 3.04±0.06 (log CFU g^{-1}) in Fig. 3. Shrimp showed the highest load (6.85±0.01 log CFU g^{-1}) and tilapia showed the lowest load (3.04±0.06 log CFU g^{-1}). The average *Staphylococcus* load in tuna samples from fishermen (tuna fish 1) (3.77±0.10 log CFU g^{-1}) was lower than that of tuna samples from vendors (tuna fish 2) (5.24±0.03 log CFU g^{-1}).

Lactic acid bacteria loads of fish products: The average lactic acid bacteria loads ranged from 3.22±0.11 to 7.58±0.05 (log CFU g^{-1}) in Fig. 4. Shrimp showed the highest load (7.58 ± 0.05 log CFU g^{-1}) and tuna from fishermen showed the lowest load (3.22±0.11 log CFU g^{-1}). The average loads of mackerels and tilapias were not statistically different at the 5% threshold as well as those of crabs and tuna from vendors (tuna fish 2).

Prevalence of histaminergic bacteria in fish products: Table 2 presents the isolation percentages of histaminergic bacteria in fish products. Of the 270 isolates tested, 124 isolates produce histamine, i.e., 49.92% of the isolates tested. These are 59 Enterobacteriaceae isolates, 39 *Staphylococcus* isolates and 26 lactic acid bacteria isolates which showed their ability to produce histamine, i.e., respectively 21.85, 14.44 and 9.62%.

Figure 5 presents the prevalence of histaminergic bacteria in fish products. Of the 124 histaminergic bacteria obtained, Enterobacteriaceae showed the highest prevalence (47.58%) followed by *Staphylococcus* (31.45%). The lowest prevalence was observed in lactic acid bacteria (20.97%).

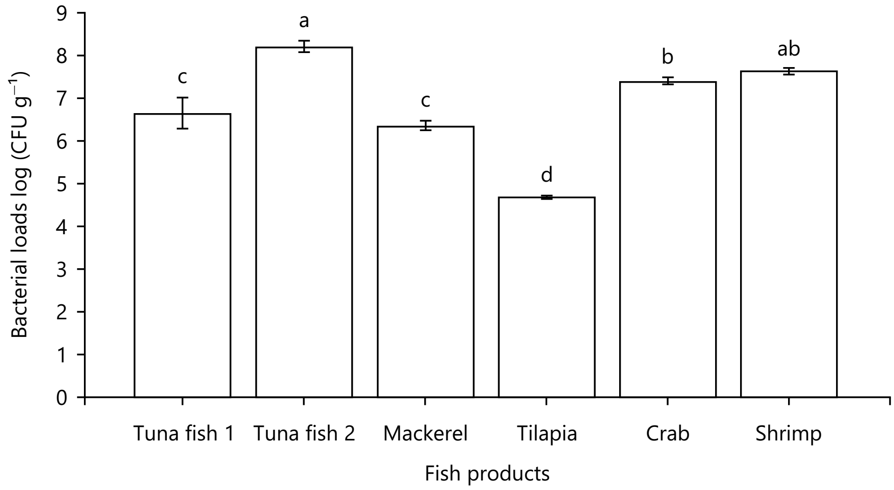

Fig. 1: Average loads of mesophilic aerobic germs in fish products

Mean values bearing the same letters are not statistically different at the threshold α = 5%

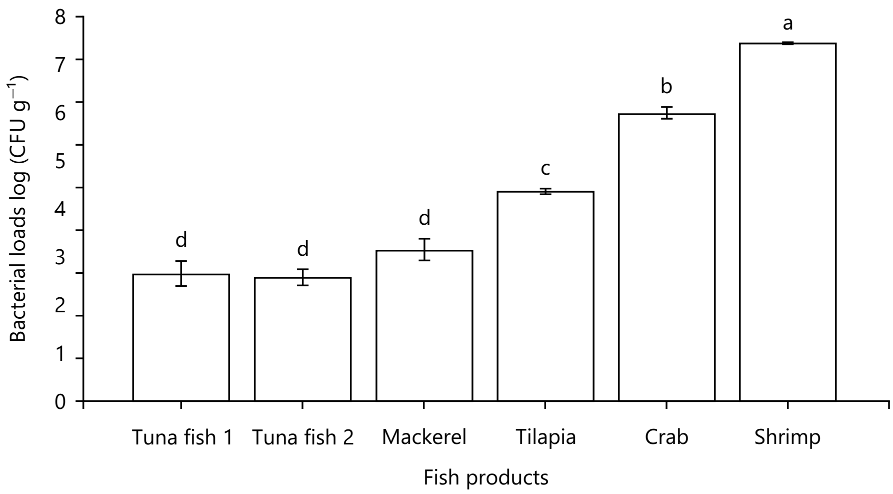

Fig. 2: Average loads of Enterobacteriaceae germs in fish products

Mean values bearing the same letters are not statistically different at the threshold α = 5%

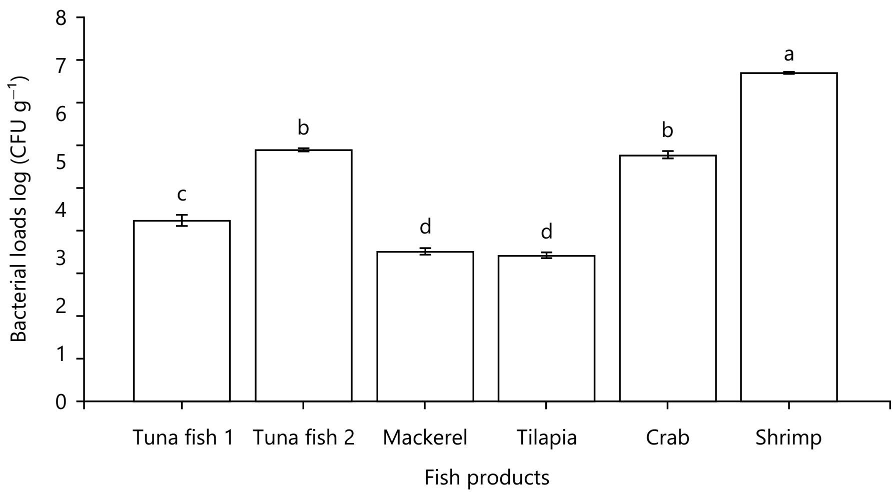

Fig. 3: Average loads of *Staphylococcus* germs in fish products

Mean values bearing the same letters are not statistically different at the threshold α = 5%

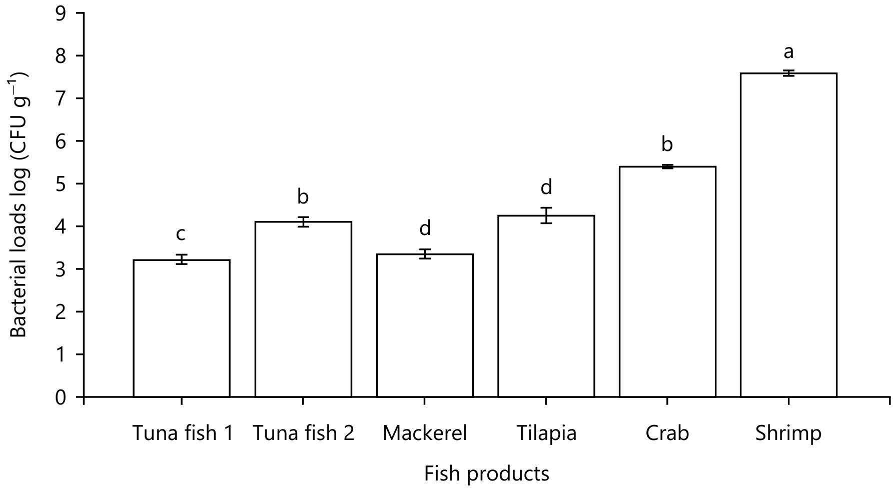

Fig. 4: Average loads of lactic acid bacteria germs in fish products

Mean values bearing the same letters are not statistically different at the threshold α = 5%

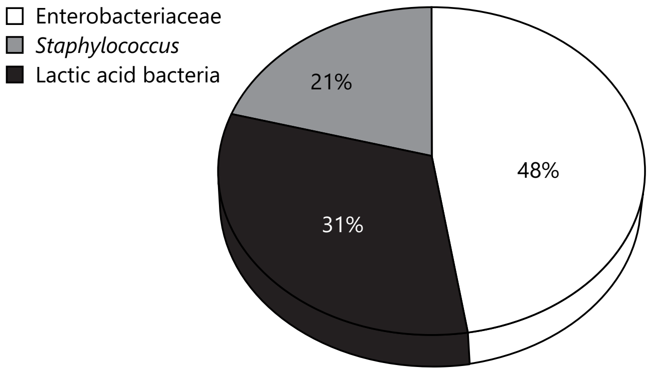

Fig. 5: Prevalence of histaminergic bacteria in fish products

Table 2: Percentage of histaminergic bacteria in fish products

Samples	Enterobacteriaceae	*Staphylococcus*	Lactic acid bacteria	Total
Number of positive isolates/total number of isolates tested	59/270	39/270	26/270	124/270
Percentage (%)	21.85	14.44	9.62	49.92

Table 3: Distribution of histaminergic bacteria in fish products

Samples	Enterobacteriaceae (%)	*Staphylococcus* (%)	Lactic acid bacteria (%)
Tuna fish 1	4.44	7	0.00
Tuna fish 2	5.56	8	2.22
Mackerel	7.78	2	1.11
Tilapia	14.44	10	14.44
Crab	16.67	9	6.67
Shrimp	16.67	8	4.44

Distribution of histaminergic bacteria in fish products: Histaminergic Enterobacteriaceae in the different samples showed the highest percentage in the crab and shrimp samples (15/90 or 16.67%). The percentage of histaminergic Enterobacteriaceae in those tuna from fishermen (tuna fish 1) (4/90 or 4.44%) was lower than that of tuna from vendors (tuna fish 2) (5/90 or 5.56%). The percentage of histaminergic *Staphylococcus* in the tilapia samples was the highest (9/90 or 10%). The lowest percentage of histaminergic *Staphylococcus* was found in mackerel (2/90 or 2%). The percentage of histaminergic lactic acid bacteria varied from 0-14.44%. The highest percentage was observed in tilapias (13/90 or 14.44%). No histaminergic lactic acid bacteria were determined in tuna from fishermen in Table 3.

DISCUSSION

The general objective of this study was to search for histamine-producing bacteria in fish products to prevent their growth and thus limit the spread of the histamine they produce. The bacteriological analysis of the five selected fish products revealed the presence of a very diverse microbial population. Indeed, microbial contamination of fish products is caused by several dominant populations of bacteria found in fish, mollusks and crustaceans. The level of contamination of these products by these groups of bacteria can vary considerably from one fish to another as shown by the different microbial loads observed in this study. These results are similar to various loads of fish products[17].

The presence of Enterobacteriaceae in samples with average loads as high as those of GAM were observed. According to the authors, this group of bacteria is mainly responsible for the production of histamine in fish and other seafood[18]. The presence of Enterobacteriaceae is also an indicator of faecal contamination and a lack of hygiene. The presence of these organisms seems to be the result of the pollution of the surrounding water. The high loads of *Staphylococcus* in the shrimps could be due to the high pollution of the river in which they were fished. It is also an indicator of human contamination and possibly poor handling practices and inadequate hygiene of the handlers[19]. Lactic acid bacteria were isolated in the different samples. This could be explained by the fact that we find them in different ecological niches such as milk and dairy products, plants, meat, fish, human and animal mucous membranes and the digestive tract and are also recognized as spoilage agents in many food products[20].

Isolations of these bacteria on the Niven medium showed that the percentage of histaminergic bacteria in all samples was higher than that of non-histaminergic bacteria. Niven's method is considered a suitable and presumptive method for the detection of biogenic amines in environmental samples[21]. The presence of histaminergic bacteria poses a very high health risk to consumers. Indeed, histamine is the first cause of food poisoning linked to the consumption of fish and other fish products in several countries. It is a thermostable molecule so neither cooking, canning, nor freezing destroys it. The risk is correlated to the number and histidine decarboxylase activity of contaminating bacteria that grow in the flesh of fish rich in free histidine, such as tuna and mackerel[8,22]. Histaminergic Enterobacteriaceae also showed high percentages in fish products. According to several authors, the main bacteria responsible for the formation of histamine belong to the family of Enterobacteriaceae[6,7]. Ekici and Alisarli[23] recorded that during handling or processing of fresh *Chalcalburnus tarichi* Enterobacteriaceae and *Pseudomonas* species can proliferate and have considerable capacity for histamine formation. The percentages of histaminergic Enterobacteriaceae found in this study corroborate those of many authors and who found that the majority of Enterobacteriaceae present in fish samples are histaminergic[24-26]. Histaminergic *Staphylococcus* and histaminergic lactic acid bacteria showed lower percentages than those of histaminergic Enterobacteriaceae. Indeed, histaminergic Gram-positive bacteria are generally found in fermented fish products[27].

CONCLUSION

This study was carried out to research histamine-producing bacteria to prevent their proliferation in fish products and thus limit histamine poisoning.

The average loads of MAG, Enterobacteriaceae, *Staphylococcus* and lactic acid bacteria in fish products showed that the level of microbial contamination of fishery products varied according to the species analyzed.

The ability to produce histamine of the various isolates tested on the Niven medium showed that out of 270 isolates tested, 124 or 49.92% of the isolates produced histamine. Gram-negative bacteria, namely Enterobacteriaceae, were the most incriminated in the production of histamine in fish products in Côte d'Ivoire. They would represent a very great health risk for consumers.

SIGNIFICANCE STATEMENT

This study discovered that December, 2021 can be beneficial for the fisheries sector and consumers. This study will help the researchers to uncover the critical areas of the microbiology of fish products that many researchers were not able to explore. Thus a new theory on the relationship between histamine level and histaminergic bacterial load in fish products may be arrived at.

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