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Histamine Contents in Tuna Loins (*Thunnus* sp.) Produced in Côte d'Ivoire and its Relation with Bacterial Load

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Abstract: ***The aim of the present study was to determine the occurrence of histamine and its relation with the bacterial load in tuna loins of four tuna species from two production sites in Côte d'Ivoire. Histamine concentration was measured by High Performance Liquid Chromatography (HPLC). pH, humidity and histamine content ranged from 6.4 to 6.8, 67.98% to 71.19% and 13.34 to 28.19 mg/kg respectively. Aerobic plate count, total coliform, fecal coliform, Escherichia coli and anaerobic sulphite reducers in all samples ranged from 4.35 to 6.29 log CFU/g, 1.41 to 3.21 log CFU/g, 0 to 3.04 log CFU/g, 0 to 2.80 log cfu/g and 0 to 1.94 log CFU/g respectively. The means concentrations of histamine varied not depending on the industries (Industry A and B) and on the tuna species (Skipjack, Longfin, Yellowfin and Bigeye). These concentrations were lower than the maximum permissible values in European and Ivoirian regulation. Data of the co-inertia analysis revealed that aerobic plate count, fecal coliforms and anaerobic sulphite reducers were correlated with the formation of histamine in tuna loins***

Keywords: tuna loins, histamine, microbial load, corrélation, Côte d'Ivoire

1. Introduction

Fish is an extremely perishable food commodity. Its deterioration occurs as a result of enzymatic and microbial activities which lead to loss of quality and spoilage (1). Deteriorative changes in fish are indicated by several physicochemical characteristics. Among them is the production of biogenic amines such as histamine. Histamine is the causative agent of scombroid poisoning, a foodborne chemical hazard. Scombroid poisoning is usually a mild illness with a variety of symptoms including rash, urticaria, nausea, vomiting, diarrhea, flushing and tingling, and itching of the skin (2). Factors affecting histamine production are availability of free histidine, presence of microorganisms that can decarboxylase histidine and favorable conditions for the growth of such microorganisms and production of decarboxylase enzyme (3). *Clostridium* species and Enterobacteriaceae such as *Proteus morganii*, *Klebsiella pneumonia* and *Hafnia alvei* are the most important histamine producing bacteria (4). Scombroid poisoning (scombrotoxicosis) is a worldwide problem of foodborne intoxication caused by the consumption of seafood containing large quantities of histamine (5). This amine is mainly cause by consumption of Scombroid fish such as tuna, mackerel, bonito and saury (6). Recently, a case of histamine intoxication associated with tuna was reported in Senegal (7). Food and Drug Administration (FDA) has set the maximum action level of histamine as 50 ppm for scombroid like fish (8). However, a level of 100 mg/kg of food was fixed by the European Community and Ivoirian ministry (9), (10).

Côte d'Ivoire through the processors and exporters of fish products has become one of the largest exporters of tuna products to the global level. The exports of products tuna boats in 2009 amounted to 24 994 tons for 135 million USD (11). There are 2 types of tuna products exported in Côte d'Ivoire: finished products (canned) and semi-finished products (tuna loins, tuna flakes, tuna skin and tuna pulp). The tuna loins are portions of the tuna flesh usually skinless and boneless and ready to be used. Beforehand precooked in a temperature about 65°C, packed and stored in a lower temperature, the tuna loins are likened to semi-canned tuna so to products semi-finished tuna. They are intended for canning factories and for fast food. The world production of tuna loins is 11 903 tons (12). In Côte d'Ivoire, this sector produces 2 200 tons (13) and employs 70 % of women; that constitute a livelihood for several families (14).

No reports are available on the occurrence of histamine in tuna loins produced in Côte d'Ivoire. The present study aimed to determine concentrations of histamine in this product and its relation to bacterial load.

2. Materials and methods

2.1 Samples

Tuna loins were obtained from two production sites (A and B) located in Abidjan (economical capital of Côte d'Ivoire) from September 2011 to September 2013. Per sampling day, samples of about 500 g frozen tuna in a polyethylene bag were aseptically collected from each industry. Per month, the number of samples collected and analyzed depends on the importance of the tuna loins production. Quantities of 134 samples were collected from Industry A and 337 from industry B. Quantities of 370 samples were collected from specie *Skipjack* (*Katsuwonus pelamis*), 57 from *Yellowfin* (*Thunnus albacares*), 32 from *Bigeye* (*Thunnus obesus*) and 12 from *Longfin* (*Thunnus alalunga*). A total of 471 samples were collected from both industries. Each sample was labeled, stored in an ice box and sent to the laboratory.

2.2 pH value and humidity determination

The pH was measured according to the NF standard ISO 11289 with a pH-meter (Milwaukee, USA) in 30 g of the sample homogenized with 6 mL of distilled water. The humidity was determined according to the NF standard V 04-348 by calculating the difference between the fresh and the dry weight of the samples; dried at 103±2°C during 4 h until constant weight.

2.3 Microbiological assays

One in the laboratory, twenty five grams of the tuna loins were suspended in 225 mL of 0.1% sterile buffer peptone (Oxoid CM 509) in a plastic stomacher and mixed together for 30 sec at normal speed. The homogeneous were used for the microbiological analysis. Total aerobic counts were performed using Plate Count Agar according to the NF standard ISO 4833. The plates were incubated for 72 hours at 30°C. Coliforms counts were carried out on according to the NF standard V08-17 on the Violet Red Bile Lactose (VRBL) incubated during 24 hours at 30°C for the total coliforms and 44°C for fecal coliforms. *Escherichia coli* were enumerated according to the NF standard IN ISO 16140 on Rapid e'coli 2 and incubated for 24 hours at 44°C. Anaerobics sulphite reducers (ASR) were enumerated according to the standard ISO 7937 on Tryptone Sulfite Neomycin (TSN) and incubated for 24 to 48 hours at 46°C.

2.4 Histamine analysis

Tuna loins samples were homogenized and 10 grams of homogenized tuna loins were blended with 20 mL of 10% trichloroacetic acid for 3 min using warring blender (Turmix, Switzerland). The homogenates were centrifuged (4000 PPM during 5 min) and filtered through No. 127 filter paper (Durieux, France). An aliquot of the filtrate was diluted to one-tenth in ultra pure water according to the method (15). A volume of 100 µL of the new filtrate were then placed in volumetric flask with 900 µL of ultra pure water; 200 µL of 2N NaOH and 100 of 1% OPA for 4 min. The complexing reaction was blocked by adding 150 µL of 3N HCl. The 5 µL aliquots were used for HPLC injection. Histamine was determined with HPLC (Shimadzu, Prominence, Japan) consisting of a Model LC-20AD pump, a Model SIL-20AC loading sample injector, a model RF-10AXL fluorescence detector (excitation wavelength (350 nm) and emission wavelength (450 nm)) and a CTO-20A column oven and spherical silica column (Supelcosil LC 18(150 cm X 4.6 mm)). The elution time was 2.3 min for 1 mL/min.

2.5 Statistical analysis

Data were expressed as mean ± standard deviation (SD). Data were analyzed by ANOVA at $\alpha = 0.05$. Comparison of means was performed by Duncan test and difference was considered significant at $p < 0.05$. The co-inertia analysis was performed using ADE 4 software. It was carried out to determine relationships between histamine content and the bacterial load.

3. Results

3.1 Influence of different tuna species on histamine content in tuna loins

The concentrations of histamine in *skipjack*, *bigeye*, *yellowfin* and *longfin* species are shown in **table 1**.

Table 1: pH, humidity and histamine content according to tuna fish species in tuna loins

	Tuna fish species			
Parameters	*skipjack*	*bigeye*	*yellowfin*	*longfin*
Histamine (mg/kg)	19.39^a ± 6.75	21.70^a ± 9.23	20.91^a ± 8.69	20.78^a ± 8.37
pH	6.05^a ± 0.07	6.07^a ± 0.04	6.06^a ± 0.05	6.06^a ± 0.05
Humidity (%)	68.89^a ± 1.78	69.47^a ± 1.84	69.22^a ± 1.93	69.17^a ± 1.73

Mean ± SD. Value in the same column with the same letters are not statistically different ($p > 0.05$)

The concentrations (mg/kg) of histamine in *skipjack*, *bigeye*, *yellowfin* and *longfin* species were: 19.39 ± 6.75; 21.70 ± 9.23; 20.91 ± 8.69 and 20.78 ± 8.37 respectively. Values of pH and humidity (%) for skipjack, yellowfin, longfin and bigeye were 6.05 ± 0.07 and 68.89 ± 1.78; 6.06 ± 0.05 and 69.22 ± 1.93; 6.06 ± 0.05 and 69.17 ± 1.73; 6.07 ± 0.04 and 69.47 ± 1.84 respectively.

3.2 Histamine content in tuna loins from two production sites

The results obtained for the histamine content in tuna loins from sites A and B are presented in **table 2**. The histamine content for site A and B were 19.55 ± 6.39 mg/kg and 20.57 ± 6.90 mg/kg respectively. Values of pH and humidity (%) for site A and B were: 6.06 ± 0.05 and 69.23 ± 1.87; 6.07 ± 0.04 and 69.39 ± 1.85 respectively.

Table 2: pH, humidity and histamine content in tuna loins from two production sites

Parameters	*Site A*	*Site B*
Histamine (mg/kg)	$19.55^a \pm 6.39$	$20.57^a \pm 6.90$
pH	$6.06^a \pm 0.05$	$6.07^a \pm 0.04$
Humidity (%)	$69.23^a \pm 1.87$	$69.39^a \pm 1.85$

Mean ± SD. Value in the same column with the same letters are not statistically different (p> 0.05)

3.3 Chemical properties of the tuna loins

The histamine content of the tuna loins studies are presented in **table 3**. The results indicated that the histamine content in all samples varied from 13.34 to 28.19 with a mean value of 20 ± 7.55 mg/kg. Values of pH varied from 6.4 to 6.8 with a mean value of 6.06 ± 0.05. Those for humidity in all samples ranged from 67.98 to 71.19 with a mean value of 69.35 ± 1.85%. These histamine concentrations were lower than the maximum limit values in European (9) and Ivoirian (10) regulation.

Table 3: pH, humidity and histamine in tuna loins

Parameters	*Range*	*Mean*	*European regulation*	*Ivorian regulation*
Histamine (mg/kg)	13.34-28.19	20 ± 7.55	100	100
pH	6.4-6.8	6.06 ± 0.05	-	-
Humidity (%)	67.98-71.19	69.35 ±1.85	-	-

(-): no regulation

3.4 Bacteriological characteristics of tuna loins

Table 4 shows the level of bacterial contamination of tuna loins. Total aerobic count (log CFU/g) of the tuna loins varied from 4.35 to 6.29 with a mean value of 5.97. That of the total coliforms (log CFU/g) ranged from 1.41 to 3.21 with a mean value of 3.03 followed by the fecal coliforms from 0 to 3.04 with a mean value of 2.64. The level of *Escherichia coli* (log CFU/g) varied to 0 to 2.80 with a mean value of 2.27. Anaerobic sulphite reducers load varied from 0 to 1.94 with a mean value of 1.25 log CFU/g.

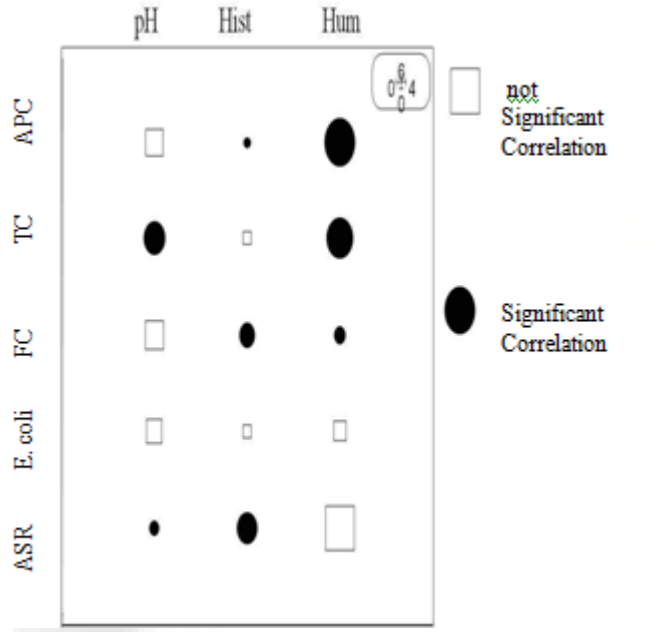
Table 4: Bacterial counts among examined tuna loins

Parameters (log CFU/g)	*Min*	*Max*	*Mean ± SD*
Aerobic plate count	4.35	6.29	5.97 ± 5.02
Total Coliforms	1.41	3.21	3.03 ± 2.06
Fecal Coliforms	0	3.04	2.64 ± 1.71
Escherichia coli	0	2.80	2.27 ± 1.66
Anaerobic sulphite reducers	0	1.94	1.25 ± 0.70

Min= minimum; Max= maximum

3.5 Correlation between histamine and bacterial load

Histamine content was significantly correlated to aerobic plate count, fecal coliforms and anaerobic sulphite reducers as shown by **figure 1**. Humidity content was also significant correlated to aerobic plate count, total coliforms and fecal coliforms. pH was significant correlated to total coliforms and anaerobic sulphite reducers



APC= aerobic plate count; TC= total coliforms; FC=fecal coliforms; ASR= anaerobic sulpthite reducers

Figure 1: correlation between histamine and the bacterial load

4. Discussion

The average pH of tuna loins was 6.06. This result could be explained by the fact that after the death of fish, lactic acid resulting decrease in the pH of muscle tissue is formed by biochemical processes (16). According to (17), the pH of muscle tissue generally range from 7 to 7.5 but can drop to 5-6 after death. The results obtained in this study were similar to that (18) who found a pH range from 5.85 to 6.0 in steaks and fresh tuna loins.

The average humidity (%) of tuna loins was 69.35. Water usually represents 80% of the weight of fresh fish. According to (19), humidity in tuna (*Thunnus* sp.) is 71%. The results of this study were closed to those (20). These authors found humidity content (%) of 63.9 and 51.6 to 76.8 respectively in tuna fish before canning in the U.S. and the flesh of tuna bought in Taiwan.

Concentrations (mg/kg) of histamine in tuna loins found in this work were 20.2. These results could be due to the handling of fish without hygienic practices throughout the manufacturing. According to (21), the high histamine content in tuna loins could be due to non-compliance with the waiting period between the steps of cooling and trimming. For (22), the stage of cooling after precooking is a critical point if it is not controlled. For several authors (23), the temperature is a key factor in the synthesis of histamine. Thus, (24) found that leaving a sample of yellowfin without bacteria for one day at room temperature of 30°C, the histamine content increased up to 500 mg/kg. Also, (25) showed that a rate of histamine (8.9 mg/kg) measured at 25°C increased to 331 mg/kg at 31°C. During the step of thawing, tuna is exposed to ambient temperature resulting in a temperature increase of the tuna. Time and temperature are

the key factors to control tuna loins in the manufacturing process since these factors can limit the growth of histamine producing bacteria and the formation of histidine decarboxylase (26).

Histamine production in fish depends also to the type of muscle (white or dark), slices (the histamine levels near the tail differs from histamine levels near the back), the environmental temperature, the fishing season, the degree of pollution and bacterial infection (27).

However, the histamine content in the tuna loins in this study does not exceed that recommended by the Ivorian and European regulations. (28) consider an histamine levels greater than 50 mg/kg as indicative of decomposition; an histamine levels greater than 500 mg/kg associated with disease. The histamine content determined in this study was significantly higher than those determined in the pre-cooked and canned tuna by several authors (29). These authors found histamine values between 1.6 and 10 mg/kg.

No significant difference ($p > 0.05$) between histamine content in tuna loins from the two production sites and the four tuna fish species was noted. This result could be explained by the origin of tuna capture. Indeed, industries A and B stock up with the same French and Spanish ships. These ships go fishing in the Atlantic East-Central Ocean and land 53 % of the tunas in the fishing port of Abidjan (30)
A strong correlation was observed between the aerobic plate count, fecal coliforms, anaerobic sulphite reducers and histamine. According to (31), most of the histamine producing bacteria belongs to the family Enterobacteriaceae. *Clostridium* spp. is also identified like histamine forming bacteria (32). The results of this study were closed to those of (33) which showed strong correlation between aerobic plate count and histamine in smoked herring and molouha, and between fecal coliforms and histamine in fish fillet in Egypt.

5. Conclusion

This aim of this study was to assess the concentrations of histamine in tuna loins of four tuna fish species from two production sites in Côte d'Ivoire. All values of histamine were lower than the limits of concentration set up by the European and Ivoirian legislation. The means concentrations of the histamine did not depend on the production sites or the tuna fish species. The production of histamine in tuna loins was correlated to their bacterial load. The data obtained in this work did not show evidence risk of histamine for the consumers. Histamine in tuna loins will not be a public health risk if some good hygienic practices are taken in the process of manufacturing tuna loins. This study will be opportunities to isolate and identify histamine-forming bacteria.

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