

## Determination of histamine level in commonly consumed frozen fish in Mauritania

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### Article history:

Received: 3 December 2021

Received in revised form: 12 January 2022

Accepted: 8 February 2022

Available Online: 28 February 2023

### Keywords:

Marine fish,  
Histamine,  
HPLC-FLD,  
Quality,  
Scombroid fish,  
Food safety

### DOI:

[https://doi.org/10.26656/fr.2017.7\(1\).973](https://doi.org/10.26656/fr.2017.7(1).973)

### Abstract

The objective of the research was to evaluate the quality of most consumed fish products in Nouakchott-Mauritania, by quantifying histamine levels in frozen marine fish samples to assess the storage conditions and the handling methods for a fish product intended for exportation and local consumption. Histamine levels were determined by High-Performance Liquid Chromatography with Fluorescence detection (HPLC-FLD) method in 207 samples of frozen different fish species collected from different wholesalers at the Nouakchott fish market. According to the 200 ppm limit required by the FDA, our results showed that 17.77% of samples of non-scombroid fish (*Sardinella aurita*) analyzed had histamine levels above the limit of 200 ppm, while samples of scombroid fish (*Scomber japonicus* and *Sarda sarda*) were all below the limit of 200 ppm. Results showed that more attention should be carried out while handling fishery products from the landing until they are available to consumers to ensure food safety.

## 1. Introduction

Fish is an essential element in the diet of the Mauritanian population as the main ingredient for most meals and a source of protein. Marine fish species traded at Nouakchott come from artisanal fishery, mostly represented by pelagic (90% of species catches) (Ministère des Pêches et de l'Economie Maritime, 2006), with a predominance of species such as horse mackerel (42%) and clupeids (34%) (Ministère des Pêches et de l'Economie Maritime, 2015). Wholesalers are more interested in the export of fishery products since several international markets make demand from Mauritania. Among the different countries subject to export are those in Europe (France, Spain, Portugal), Asia (China and Japan) in Africa (Algeria, Tunisia, Benin and Ivory Coast) (Learoussy *et al.*, 2020). However, fishery product requires some safety handling such as adequate conservation, temperature check and good hygienic conditions, necessary to avoid product decomposition that can induce poisoning. Poisoning by scombroid fish, also known as histamine poisoning, is one of the most difficult food safety issues in the seafood industry (Hungerford, 2010). Histamine level in fish is considered an indicator of quality. Consumption of fish products

containing high histamine levels can induce histamine fish poisoning. Signs of symptoms of histamine fish poisoning occur from several minutes to several hours after ingestion of the toxic fish (Lehane and Olley, 2000). The primary symptoms are cutaneous, gastrointestinal, hemodynamic and neurological (Taylor, 1986).

Species from different families can be concerned, such as Clupeidae (*Sardina pilchardus*, *Sardinella aurita* and *Sardinella maderensis*), Carangidae (*Trachurus trachurus*), Scombridae (*Scomber japonicus* and *Sarda sarda*) and Engraulidae (Sidi, 2005). The so-called scombroid fish, which belong to the families Scomberesocidae and Scombridae, have been most frequently implicated in histamine poisoning. The various species of tuna, skipjack, bonito, albacore, mackerel, Spanish mackerel, Bluefin tuna, and saury are included in the scombroid fish category. Tuna, mackerel, and skipjack are most frequently involved, but this is partially due to the greater consumption of these fish worldwide. Some of the other scombroid fish are less commonly consumed and consequently are only infrequently implicated in outbreaks. However, certain

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scombroid fish may have less susceptibility to histamine formation (Taylor, 1985).

Histamine fish poisoning is a significant public health and safety concern (Sanchez-Guerrero *et al.*, 1997). A histamine level of 50 parts per million (ppm) is an indicator of decomposition (Food and Drug Administration (FDA), 2005) and several countries have set legal limits of histamine concentration that are regarded as safe for human consumption: Europe “100 ppm” (Journal officiel des Communautés européennes, 2003), USA “50 ppm” (FDA, 2005). In 2012, Food and Agriculture Organization (FAO) had set a limit of 200 ppm for histamine level, this limit is the one used in our research (Joint World Health Organization (WHO) and Food and Agriculture Organization (FAO), 2012).

In Mauritania the state requires the determination of histamine content in fishery products intended for export, however, there is a lack of systematic control of histamine level in fishery products intended for consumption by the local population. The Nouakchott fish market is the landing site for artisanal fishing practitioners, as well as the largest selling point for fish products, this is why it was chosen as the collection site. Evaluating the freshness of fish intended for consumption is a necessity in the aquaculture sector in order to ensure the quality of a good product. However, globalization encourages the freezing of fishery products during trade, to ensure product quality is maintained. This study aimed to evaluate the quality of commonly consumed fish species in Nouakchott, by quantifying histamine levels using the High Performance Liquid Chromatographic method.

## 2. Materials and methods

### 2.1 Sample collection

A total of 207 frozen marine fish samples were collected between 2019 and 2020, the samples included one non-scombroid fish species (*Sardinella aurita*) and two scombroid fish species (*Scomber japonicus* and *Sarda sarda*). Samples were taken from different wholesalers' containers located at the Nouakchott fish market. After collection, samples were transported in an ice pack to the National Health Inspection Office for Fisheries and Aquaculture Products Laboratory (ONISPA) in Nouakchott.

Fish species included 180 samples (86.96%) of *Sardinella aurita*, 18 samples (8.69%) of *Scomber japonicus* and 9 samples (4.35%) of *Sarda sarda*. In the first period, 135 samples of *Sardinella aurita* were collected and analyzed in the first semester of 2019. In the second period, seventy-two samples (72) of three species (*Sardinella aurita*, *Scomber japonicus* and *Sarda*

*sarda*) were collected and analyzed in 2020. All frozen fish samples were transported to the laboratory in ice packs and stored at 4°C for defrosting slowly then put at room temperature for analysis.

### 2.2 Temperature check

When samples arrive at the laboratory, the temperature of the ice pack is checked by a thermometer (SHIMADZU 826-T2 testo). For samples collected in 2019, the temperature of received samples was between -20°C and -19°C. Samples received in 2020 had a temperature range between -15°C and -20°C.

### 2.3 HPLC reagent and equipment

The reference standard of histamine dihydrochloride ( $\geq 99\%$ ) was purchased from Sigma. The trichloroacetic acid (TCA) was purchased from VWR, Sodium hydroxide was purchased from Sigma, Hydrogen potassium phosphate dibasic was purchased from PROLAB, and hydrochloric acid was purchased from Fluka. The o-phtalaldehyde was purchased from Sigma. The water used is Ultra-pure water. The HPLC is of the brand SHIMADZU.

### 2.4 Preparation of the calibration curve

An amount of histamine dihydrochloride is dissolved in a quantity of trichloroacetic acid 10%, to obtain a concentration of 0.2 mg/mL (200 ppm).

After the preparation of the solution of 200 ppm, five other solutions were prepared from it, respectively 20, 10, 5, 2 and 1 ppm. Those solutions allowed us to draw the calibration curve.

### 2.5 Sample preparation for histamine analysis

Approximately 50 g from the muscle of each sample is weighed in a 500 mL beaker, to which 100 mL of 10% trichloroacetic acid is added. The mixture is then blended until obtaining juice. The solution is then centrifuged for 15 mins at 3000 rpm at 4°C in order to obtain a clear mixture.

After recovery of the supernatant, the sample passes to the step of complexing with o-phtalaldehyde in order to pass it to HPLC.

### 2.6 Data processing

Data of measured samples are given in mean $\pm$ standard deviation, results were performed using Microsoft Excel and GraphPad-Prism version 6.01.

## 3. Results and discussion

Histamine level was examined in 207 samples of

various fish species in different periods of 2019 and 2020, the results are calculated in mg/kg (ppm) as presented in Table 1. Histamine level in non-scombroid fish (*Sardinella aurita*), was detected in all samples, during 2019 and 2020. In 2019, histamine levels in *Sardinella aurita* samples that are positive were 23% against 2.2% in 2020. Samples of *Sardinella aurita* analyzed in 2019 were collected in the first semester, the results of 135 samples are presented in Table 2.

In January, February and June of 2019, histamine content was under 50 ppm in all samples, thus in April and May, respectively 44.44% and 36.51% of samples were above the limit. The highest histamine level detected in April was 249.10 ppm and in May was 682.20 ppm. The distribution of histamine content during the first semester is exposed in Figure 1.

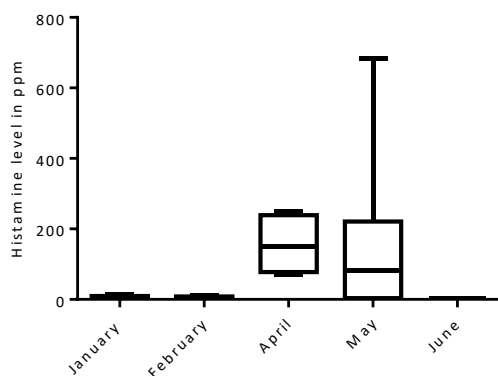


Figure 1. Distribution of histamine level in *Sardinella aurita* from January to June 2019

The Food and Drug Administration's experience with the preparation of standard packs of fish and the examination of many samples of seafood implicated in consumer poisonings suggest that fish are more likely to decompose and form scombrotoxic by-products when decomposition occurs at harvest or in the first stages of handling on the fishing vessels, rather than later in the distribution chain (Staruszkiewicz *et al.*, 2004). Accumulating histamine may be due to bacterial spoilage of fish tissue (Ching *et al.*, 2007), availability of free amino acid (histidine) and oxidation processes that are susceptible to the formation of histamine (Ababouch *et*

*al.*, 1991). Such spoilage is most likely to happen during handling of the catch on fishing vessels (Staruszkiewicz *et al.*, 2004; Poulouze *et al.*, 2013). High levels of histamine in non-scombroid fish species have been also reported by other authors (Auerswald *et al.*, 2006; Mejrhit *et al.*, 2018). Similar studies have shown a high level of histamine in frozen non-scombroid fish types (Ajayi *et al.*, 2014; Pavloc *et al.*, 2019).

The variation of histamine level in our samples can be caused by the period of heat where wholesales may not control the storage temperature of samples. Other factors can be incriminated such as the complicating factors in sampling that can include the wrong sample analyzed, variable histamine levels within the sample, and the presence of microbial toxins or other toxins or contaminants or metabolites (Lehane and Olley, 2000).

ONISPA is the national authority in charge of monitoring fishery products in Mauritania, it has been accredited since 2013 according to the ISO 17025 version 2005 by TUNAC (Tunisian Accreditation Council). When ONISPA detects histamine levels exceeding the limit, it notifies the service concerned to take the necessary measures about the batch of damaged fish products. The service ensures that the altered batch is intended for other than human consumption. Generally, they are either used as bait or intended for processing into fishmeal, which is a developed sector (around fifty fish processing factories on the Mauritanian coast).

In 2020, seventy-two samples of three frozen fish species were collected, the samples concerned non-scombroid species *Sardinella aurita* and two scombroid species *Scomber japonicus* and *Sarda sarda*, results are described in Figure 2.

Of the 45 samples of *Sardinella aurita*, one had a level of histamine above the limit of 200 ppm (204.61 ppm), it was the maximum limit found in all samples in 2020. In the same period, our results showed that 2.22% of non-scombroid species had histamine values more than 200 ppm, against 0% for each other scombroid

Table 1. Overview of histamine level in different fish species

Year	Fish species	Sample size	Mean±SD (ppm)	Positive samples (> 200 ppm)
2019	Round sardinella ( <i>Sardinella aurita</i> )	135	62.53±78.33	23%
	Round sardinella ( <i>Sardinella aurita</i> )	45	73.13±56.49	2.20%
2020	Spanish mackerel ( <i>Scomber japonicus</i> )	18	50.84±8.74	0%
	Atlantic bonito ( <i>Sarda sarda</i> )	9	60.02±17.23	0%

Table 2. Histamine level in *Sardinella aurita* samples during the first semester of 2019

	January	February	April	May	June
Histamine range (ppm)	4.77 – 14.30	1.55 – 11.27	70.43 – 249.10	0.84 – 682.20	2.28 – 2.94
Mean±SD	7.91±3.15	5.89±2.79	156.60±75.53	139.50±160.50	2.74±0.18

species.

A low limit of histamine in those scombroid samples was found by other authors (Gonzaga *et al.*, 2009; Marilena *et al.*, 2013; Feng *et al.*, 2016; Pavloc *et al.*, 2019). Storage of fish product above 0°C induces the formation of histamine (Hardy and Smith, 1976). The risk of histamine poisoning following the consumption of frozen fish products is lower compared to that which may occur following the consumption of fresh sardine or other fresh pelagic fish (Oleya *et al.*, 2018). Histamine accumulates in fresh fish during storage at low temperature when psychrophilic histamine-producing bacteria are present (*Photobacterium phosphoreum* and *Photobacterium iliopiscarium*). Further, inadequate storage temperatures cause the accumulation of histamine in a short time (Yuko *et al.*, 2012).

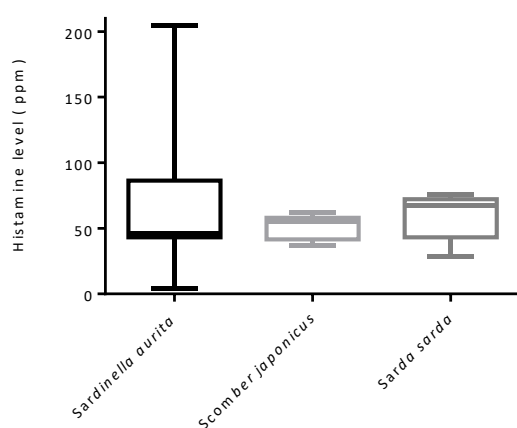


Figure 2. Histamine level in different fish species in 2020

The relative effectiveness of postharvest holding conditions on board the fishing vessel, at dockside, or in transit in maintaining unfrozen fish in a safe condition will depend on the initial temperature and time to which the fish were exposed at harvest postmortem. Although the most effective control is to freeze the fish immediately after capture, high concentrations of the biogenic amines in unfrozen products can be avoided if fish are chilled as rapidly as possible at harvest and if low temperatures are maintained in storage vessels (Staruszkiewicz *et al.*, 2004).

#### 4. Conclusion

This report is the first of its kind on the levels of histamine in frozen fish samples in Mauritania from a commercial view, in this study histamine was detected in all samples. Histamine concentration has exceeded the limit of 200 ppm in few samples of non-scombroid species (*Sardinella aurita*). However, in samples of scombroid fish species (*Scomber japonicus* and *Sarda sarda*) the histamine contents were all below the limit of 200 ppm. Even its more likely for scombroid fish to develop histamine, our results showed that histamine at a

high level was found in non-scombroid fish samples. Our results indicate good quality of fishery products; thus, the risk of histamine poisoning remains and can occur. The systematic control of histamine level and adequate storage during the handling of fish products are recommended when collecting and handling fish products intended for human consumption.

#### Conflict of interest

The authors declare no conflict of interest.

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