

SHORT COMMUNICATION

Histamine content in fresh and frozen pelagic species from the Mauritanian Atlantic Coast

Hana Youssef Learoussy^{1,2*}, Hasni Tfeil², Aly Yahya Dartige², Lotfi Aarab¹

¹Laboratory of Microbial Biotechnology and Bioactive Molecules, Faculty of Science and Techniques, Sidi Mohammed Ben Abdellah University, 2202 – Road d'Imouzzer, Fez, Morocco, ²National Office of Sanitary Inspection of Fisheries and Aquaculture Products in Nouakchott, 1416 -Nouadhibou, Mauritania.

ABSTRACT

Histamine is a security issue in food safety as an indicator of the freshness of consumed fish product; that is why we aimed in this study to determine the histamine content in 7 fish species (*Scomber japonicus*; *Sarda sarda*; *Sardinella aurita*; *S. maderensis*; *Ethmalosa fimbriata*; *Pomatomus saltatrix* and *Trachurus trachurus*). Hundred and eight frozen and fresh pelagic of scombroid and non-scombroid species were collected from the Nouakchott fish market (Mauritanian Atlantic coast) in different period of 2020 and 2021. Histamine was quantified by the technique of high-performance liquid chromatography with a fluorescence detector (HPLC-FLD). Histamine was detected in all analyzed fresh and frozen species and varied from 4.21 to 201.60 ppm. All evaluated fresh fish species (*S. aurita*; *S. maderensis*; *E. fimbriata*; *P. saltatrix* and *T. trachurus*) were conform to the FAO/WHO and the European commission regulations. Regarding investigated frozen fish species (*S. aurita*; *S. japonicus* and *S. sarda*); the scombroid species (*S. japonicus* and *S. sarda*) were all under the set limits; however, even its more likely for scombroid fish to develop histamine, our results showed that histamine exceed the limit in one sample of non-scombroid fish (*S. aurita*). No significant variation in histamine levels between scombroid and non-scombroid fish species was obtained; thereby, the study showed that fish product commercialized at the Nouakchott fish market have a good quality and safe for human consumption.

Keywords: Food safety; Histamine; HPLC-FLD; Pelagic; Mauritanian coast

INTRODUCTION

Histamine poisoning is a term used to describe human disease caused by eating foods high in the biogenic amine histamine. Fish poisoning due to histamine is a serious public health and safety hazard, as well as a trade issue. Histamine poisoning is one of the most commonly documented human illnesses connected with seafood around the world, with several outbreaks reported in numerous countries (Feng et al., 2015). Because of its prevalent relationship with fish product from the Scombridae family, the illness is often referred to as scombrototoxic fish poisoning. Fishery product requires safety handling such as adequate conservation; temperature check and good hygienic conditions to avoid product decomposition that afterwards induce fish poisoning. A consumption of fish product containing high histamine level can incite the appearance of symptoms such as (cutaneous; gastrointestinal; hemodynamic and neurological) that occur from several minutes to several hours (Taylor, 1986). These symptoms are likely to appear

while consumption some fish species from Scombridae family (*Scomber japonicus* and *Sarda sarda*). Nevertheless, symptoms may also occur when consuming fish species from non-Scombridae family (*Sardinella aurita*; *Sardinella maderensis*; *Trachurus trachurus*; *Pomatomus saltatrix*) (Taylor et al., 1986); (Sidi, 2005); in Mauritania these pelagic species account for the majority of commercialized fish products. Mauritania has a significant Atlantic coast that is considered one of the richest fishing areas in the world with increasing fisheries product exploitation (Ly et al., 1999); (MPEM, 2018). Fish products are important elements as essential for the Mauritanian population diet and have great exportation interest to international markets (Learoussy et al., 2020). Since there is a lack of publications on histamine content in fish species from the Mauritanian coast (Debeer et al., 2021), we aimed in the present work to accomplish a study to evaluate the quality of different commercialized pelagic species from Scombridae family (*Scomber japonicus*; *Sarda sarda*) and non-Scombridae family (*Sardinella aurita*; *S. maderensis*; *Ethmalosa fimbriata*; *Pomatomus saltatrix* and

*Corresponding author:

Hana Youssef Learoussy, Laboratory of Microbial Biotechnology and Bioactive Molecules, Faculty of Science and Techniques, Sidi Mohammed Ben Abdellah University, 2202 – Road d'Imouzzer, Fez, Morocco. **E-mail:** hena-net@live.fr

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Trachurus trachurus) intended for local consumption and worldwide exportation. The histamine limit¹ used in the current study is that set by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) (FAO/WHO, 2012). This is the first time a comparison of histamine level in fresh and frozen pelagic fish species from the Mauritanian coast is carried out.

MATERIALS AND METHOD

Sampling

Samples were purchased from the Nouakchott fish market, which is the landing site of artisanal fishing practitioners (18°05'35"N; 16°01'34"W). Species sampling were conducted on most commercialized with great export interest from January 2020 to December 2021 (Table 1). Seventy-two samples of three frozen fish species (*Sardinella aurita*, *Scomber japonicus* and *Sarda sarda*) were collected during the hot dry period (February to June) and the cold dry period (October to December) of 2020. Hundred and eight samples of five fresh fish (*Sardinella aurita*, *S. maderensis*, *Ethmalosa fimbriata*, *Pomatomus saltatrix* and *Trachurus trachurus*) were collected during the cold dry period of 2021. Samples were transported in clean cool box to the National Office for Sanitary Inspection of Fishery and Aquaculture Products (ONISPA) laboratories for instant preparation and analysis performing. The temperature of frozen fish species varied between – 18 and – 20 °C and that of fresh fish varied from 4 °C to 6 °C.

Standards and chemicals

Reference standard of histamine dihydrochloride (≥ 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 PROLABO, Hydrochloric acid was purchased from Fluka. The o-phtalaldehyde (OPA) was purchased from Sigma. The water used is Ultra-pure water. The HPLC is of the brand SHIMADZU.

Histamine determination

Histamine was determined by the method of (Lerke and Bell, 1976) with some modification. Fifty (50) grams from

1 Histamine limit = 200 ppm

Table 1: Fish species collected during the study.

Specie	Scientific name	Common name
Fresh fish	<i>Sardinella aurita</i>	Round sardinella
	<i>Sardinella maderensis</i>	Madeiran sardinella
	<i>Ethmalosa fimbriata</i>	Bonga
	<i>Pomatomus saltatrix</i>	Bluefish
	<i>Trachurus trachurus</i>	Horse mackerel
Frozen fish	<i>Sardinella aurita</i>	Round sardinella
	<i>Scomber japonicus</i>	Spanish mackerel
	<i>Sarda sarda</i>	Atlantic bonito

fish sample's muscle was weighed to which 100 mL of 10 % trichloroacetic acid was added. After blending the solution is centrifuged for 15 minutes at 3000 rpm at 4°C in order to obtain a clear mixture and then filtered; after recovery of the supernatant, the complexation is carried out by adding 100 µl of OPA to the supernatant (Fig. 1); afterwards, 20 µl of the filtered solution was directly injected into the high-performance liquid chromatography (HPLC) system. The analysis was performed using a SHIMADZU HPLC system equipped with a binary pump, a degasser and an injection valve with a loop capacity of 20 µL. The column temperature was maintained at 40 °C and the detector used was a Scanning Fluorescence Detector with an excitation wavelength of 350 nm and an emission wavelength of 450 nm. Histamine compound was determined using a reversed phase 18 (250 X 4,6 mm);5 µm. The mobile phase (60:40) consisted of potassium dihydrogen phosphate and acetonitrile were used at a flow rate of 1 mL/min. Histamine was identified by comparison of the retention time of peaks in the sample in relation to standards. The concentration of histamine was determined by the direct interpolation in standard curves with $r^2=0.9986$ (Fig. 2).

Quality assurance

ONISPA laboratories have been accredited by the Tunisian Council for Accreditation (TUNAC) since

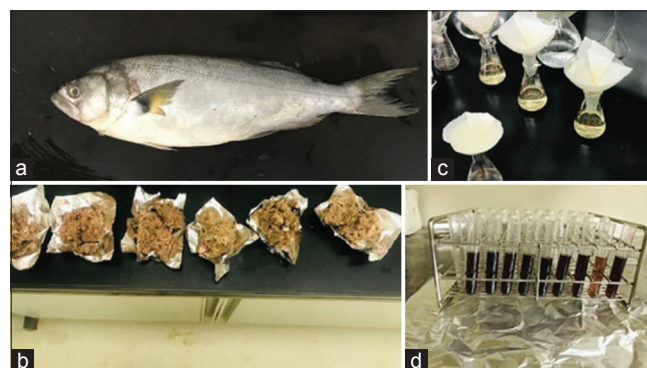


Fig 1. Preparation procedure. a: sample of *P. saltatrix*; b: muscle of sample after grinding; c: filtration phase; d: complexation phase.

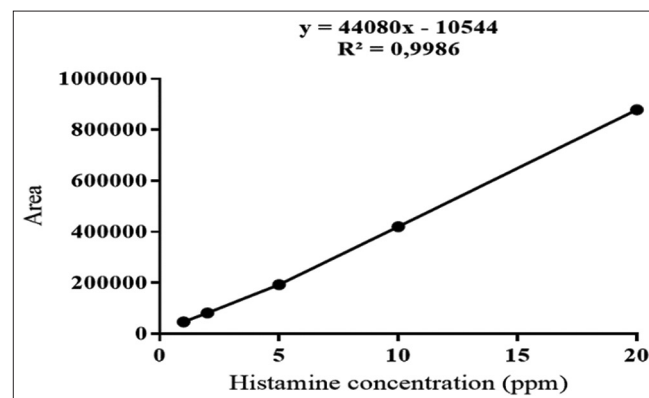


Fig 2. Calibration curve.

2021 for the analyses of histamine in fish according to international standard 17025:2017. Its laboratories participated in worldwide inter-laboratory comparison on the determination of histamine.

Statistical analysis

Results were analyzed using GraphPad-Prism version 6.01 software. Descriptive statistics of means, standard deviation and analysis of variance (ANOVA) and Mann-Whitney U were applied when analyzing results. A significance level of 5% was used ($P < 0.05$).

RESULTS AND DISCUSSION

Histamine level in fresh fish

Histamine in freshly caught fish are typically low; different authors have reported histamine content below 1 ppm in scombroid and non-scombroid fish species (Auerswald et al., 2006); (Ruiz-Capillas and Moral, 2004); (Poulose et al., 2013). In our study, histamine was detected in all 108 samples of analyzed fresh fish species, but none surpassed the limit of 200 ppm. Histamine contents varied in the range of (34.08 – 77.62), (30.75 – 42.43), (37.29 – 40.62), (35.92 – 72.86), (36.13 – 62.77) (ppm) for *S. aurita*; *S. maderensis*; *E. fimbriata*; *Pomatomus saltatrix* and *T. trachurus* respectively (Fig. 3), with the following ascending order: *S. maderensis* < *E. fimbriata* < *S. aurita* < *T. trachurus* < *P. saltatrix*. The difference of histamine variation was statically significant ($P = 0.0001$) for these five species. The highest histamine mean value was obtained with *P. saltatrix* (59.02 ppm), this concentration was higher than that reported in Bulgarian market ranged from 2.51 to 13.51 (ppm) (Bangieva et al., 2020). Regarding *T. trachurus* species, our results were way lower than that reported in Portuguese markets (480.25 ppm) (Diniz et al., 2021); our results were higher than that reported in Bologna, Italy (10 ppm) (Mancusi et al., 2010).

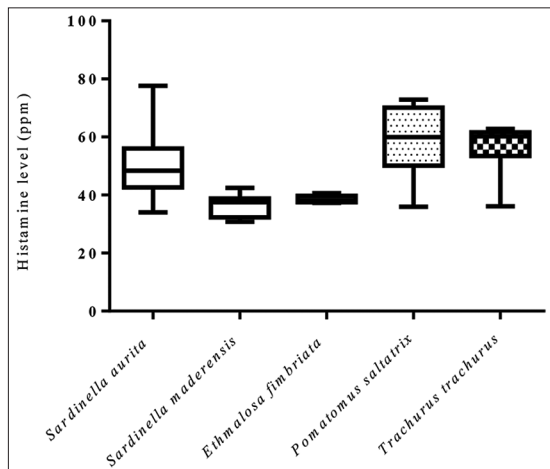


Fig 3. Histamine content in fresh fish species.

Histamine level in frozen fish

Histamine quantification was conducted on non-scombroid (*S. aurita*) and scombroid (*S. japonicus* and *S. sarda*) frozen fish species during different period of 2020. To assess the formation of histamine according to the storage period, a comparison was carried out with *S. aurita* species during the hot dry and cold dry season of 2020 (Fig. 4).

During the hot dry season, histamine ranged from 32.40 to 46.89 ppm with a mean of 44.13 ± 3.35 ; while in cold-dry season, it ranged between (4.21 and 204.60 ppm) with a mean value of 92.46 ± 66.51 . One samples among all, collected in the cold dry period exceeded the limit of 200 ppm (204.60 ppm). The statistical analysis using Mann-Whitney U indicated a significant difference in histamine variation during the two seasons with P value = 0.006.

Regarding histamine formation in species from scombroid (*S. japonicus* and *S. sarda*) and non-scombroid (*S. aurita*) family, an assessment was carried out during the cold dry season of 2021 (Fig. 5); respectively, 45; 18 and 9 samples

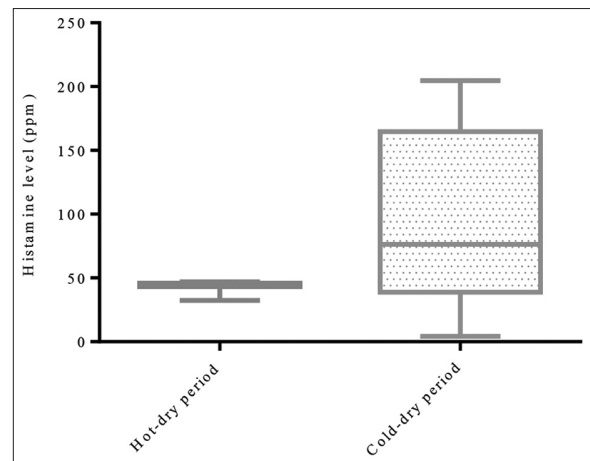


Fig 4. Histamine variation in frozen *S. aurita* during two seasons of 2020.

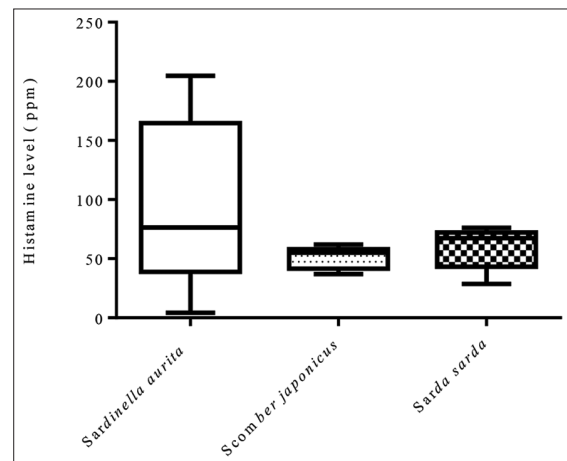


Fig 5. Histamine content in different frozen fish during the cold dry season.

of *S. aurita*, *S. japonicus* and *S. sarda* species were analyzed with histamine means values was as follow: 73.13 ± 56.49 , 50.84 ± 8.74 , 60.02 ± 17.23 for *S. aurita*; *S. japonicus* and *S. sarda* respectively. The statistical analysis using One way ANOVA showed no significant difference between the three species.

In literature high levels of histamine in frozen non-scombroid fish species have been also reported by other authors (Auerswald et al., 2006); (Mejrhit et al., 2018). Similar studies have showed a high level of histamine in frozen non scombroid fish type (Pavloc et al., 2019). The variation of histamine level in frozen samples can be caused by the period of heat where wholesales may not control the storage temperature of samples. Frozen temperature (-18°C or below) can stop the growth of bacteria and prevent any preformed histidine decarboxylase from producing histamine. Conversely histamine production is greater at high abusive temperatures (21.1°C or higher) particularly at temperatures near 32.2°C (FDA, 2011). 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 toxin or other toxins or contaminant or metabolites (Lehane and Olley, 2000).

Low limit of histamine in scombroid samples were found with other authors (Gonzaga et al., 2009; Pavloc et al., 2019; Marilena et al., 2013; Feng et al., 2015). Fish are more likely to decompose and form toxic by fish products when decomposition occurs at harvest or in the first stages of handling on fishing vessels, rather than later in the distribution chain, according to FDA experience with the preparation of standard packs of fish and the examination of many samples of seafood implicated in human poisonings (Staruszkiewicz et al., 2004). Histamine concentrations can increase substantially of bacterial deterioration of fish tissue tissue (Ching et al., 2007) or, availability of free amino acids (histidine) and oxidation processes (Ababouch et al., 1991; Hardy and Smith, 1976). This type of spoiling is most likely to occur during the transfer of the catch to fishing vessels or due to inadequate storage temperatures (Staruszkiewicz et al., 2004); Poulouse et al., 2013; Yuko et al., 2012). The effectiveness of postharvest holding conditions on board the fishing vessel, at the dock, or in transportation in keeping unfrozen fish safe will be determined by the original temperature and time to which the fish were exposed at harvest postmortem. Although freezing the fish as soon as possible after catch is the most effective control, high levels of biogenic amines in unfrozen products can be avoided if fish are chilled as quickly as possible at harvest and low temperatures are maintained in storage vessels (Staruszkiewicz et al., 2004).

Table 2: Distribution of histamine content (ppm) in analyzed species

	FDA		EC		FAO/WHO
	Acceptable				Unacceptable
	1-35	1-100	100-200	≥ 200	
Fresh fish					
<i>S. aurita</i>	2	66	-	-	
<i>S. maderensis</i>	3	9	-	-	
<i>E. fimbriata</i>	-	9	-	-	
<i>P. saltatrix</i>	-	13	-	-	
<i>T. trachurus</i>	-	11	-	-	
Frozen fish					
<i>S. aurita</i>	6	36	8	1	
<i>S. japonicus</i>	-	27	-	-	
<i>S. sarda</i>	-	9	-	-	

In the present study the occurrence of histamine level was detected in all samples, though, the highest concentration was observed in a frozen fish sample, this shows that a requirement for more attention during the treatment, and freezing of samples. To avoid the increase number of histamine detected samples among frozen as well as fresh fish, further systematic control should be carried out from the boarding of fishes to various stage of freezing process.

Oleya et al, reported that histamine poisoning is less likely to develop after consuming frozen fish products than it is after consuming fresh fish from *Sardinella* species or other fresh pelagic fish (Oleya et al., 2018); however, our results showed that all fresh fish samples (108) are under the limits set by FAO/WHO and the European Commission regulations. Regarding frozen fish samples, all scombroid species (*S. japonicus* and *S. sarda*) are also compliant with the FAO/WHO and the European Commission regulations; however, for the non-scombroid fish species (*S. aurita*) one sample surpassed the FAO/WHO limit.

Comparison of histamine content according to different regulations

In the current study, histamine content was detected in all analyzed species; yet, one sample of *S. aurita* surpassed the limit set by FAO/WHO. A comparison of our results with other regulations such as that of the European Commission (EC) (EC, 2003) and the United State Food and Drug Administration (US FDA) (FDA, 2021) was conducted as described in Table 2.

CONCLUSION

From a marketing perspective, this paper is the first of its kind to assess the quality of fresh and frozen fish species in Mauritania by determination of histamine content. All samples were under the limit of the FAO/WHO and the European Commission except of one sample of frozen *S.*

aurita species (201.60 ppm), indicating that these products are safe for commercialization; although, more monitoring should be carried out by the relevant authorities especially on frozen fish species to ensure their food safety.

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Author's contribution

Hana Youssef Learoussy (HYL) wrote the manuscript and perform analysis. Hasni Tfeil (HT) perform data analysis. Aly Yahya Dartige (AYD) and Lotfi Aarab (LA) revised the final manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

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