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Effect of storage temperature on histamine formation in *Sardina pilchardus* and *Engraulis encrasicolus*

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Abstract

Histamine formation in scromboid fish species as *SardinaPilchardus*(Sardine) and *EngraulisEncrasicolus* (Anchovies) were measured in different storage temperature. Fresh fish caught off in Lezha and Durres coast were examined. A portion of dorsal muscle from each fish was analyzed immediately and two other parts were examined after storage in two different temperature 25 and 4^{o} for 24 and 72 h. Enumeration of *TVC and* E*nterobacteriaceae* were analyzed in the respective temperature. The analyses were carried out by high-performance liquid chromatography diode array. Histamine concentration was higher than the Albanian legislation admissible levels for samples stored in at 25C. In fish stored at 4C, histamine was lower than the level established in the legislation but was higher in Anchovies than in sardines.

Keywords: histamine, sardine pilchardus, engraulis encrasicolus.

1. Introduction

Histamine is a biogenic amine that can be found in decaying fish as well as a cause of food borne illness; specifically scombroid poisoning. Fresh caught fish does not contain histamine. However, once fish starts to decay or is subject of abuse temperature histamine begin to be accumulated. Histamine is formed when histidine(a naturally occurring compound in fish), is converted by certain bacteria. Histidine-decarboxylating bacteria are present in the fish normal microbial flora, but a part can be derived from post catching contamination on board fishing vessels, at the processing plant or in the distribution system [1]. Fish belonging to Scombridae and Scomberesocidae families are commonly involved in scombroid poisoning due to high content of free histidine in the muscle. But other species of non scombroid fish have been often implicated in these outbreaks [1, 2]. In many studies [2, 3] the optimum temperature reported is 25^{0}C, while low temperature (0^{o}C or below) can control effectively microbial growth and histamine production [4]. Histamine production is assumed to be formed as result of psychotropic bacteria growth [5, 6], usually at temperatures between 2 and 10^{0}C. In addition, once the enzyme histidine decarboxylase has been formed, it can be active near refrigeration temperatures [7]. Histamine production in fresh fish is extremely variable and is a function of species and individual fish, fish samples, time/temperature of storage, and types and numbers of present bacteria [8, 9].

Abuse bacterial growth couse histamine production within 3-4 hours [10]. However it is studied that histamine toxic levels take place before the sensory changes are instolled. Once the levels of bacteria have increased and started to produce histamine, residual enzyme activity will still occur at refrigerated temperatures (although bacterial growth ceases) [11]. Various regulatory bodies have defined the limits for histamine in fish. The European Community established that the average content of histamine in fish should not exceed 100 mg/100 g and no sample may contain more than 200 mg/100 g. US Food and Drugs Administration set 50mg/kg as an indicator of decomposition and 200mg/kg as hazardous. Codex defined 10mg/100g (100mg/kg) as

an indicator of decomposition and 20mg/100g (200mg/kg) as an indicator for poor handling and hygiene [12].

Many analytical methods are used for histamine determination. According to the Commission Regulation 2073/2005/EC 2005: "Examinations must be carried out in accordance with reliable, scientifically recognized methods, such as high-performance liquid chromatography [13].

The aim of this study was to evaluate the effect of abuse time/temperature on histamine formation in Sardine pilchardus and Engraulis encrasicolus after storaged at two different temperatures.

2. Materials and methods

Fish Sampling and Storage Conditions

S. pilchardus and E. encrasicolus were caught off the Adriatic Coast and were iced in portable coolers, (each layer of fish was alternated with ice layer). Samples were carried out from two different boats, and a total of 16 fish (for each species) were collected from each boat. The fish was transportated at the laboratory within 2 h and was kept at refrigeration temperature . The fish skin was removed carefully by three portions(dorsal muscle) with a sterile knife. One portion was immediately examined (time zero), while the remaining portions were placed in separate bags and were kept at two different temperatures (25°C and 4°C).The fish portions stored at 25°Cwere analyzed after 24 h, while those stored at 4°C were tested after 72 h.

Microbiological analysis

For estimating total viable counts (TVC) and E*nterobacteriaceae* were taken. 25 grams of fish muscle which were mixed with 225 ml of ringer solution and then stomached for 3 min. Samples belonged to three different fish stored in ice , (sardine and anchovies). Further decimal dilutions were prepared. From each dilution was taken 0.1 ml and was spread on Petri dishes media. The above procedure was used for the determination (ISO Standards 21528-2) of:

- TVC in count agar (Oxide) plates. They were incubated for 2 days at 30 _C.
- *Enterobacteriaceae* counts were determined by plating dilutions from 10 to 10^{-6} on Violet Red Bile Glucose agar (VRBGA; Oxoid CM 458, Basingstoke, Hampshire, England). The plates were incubated at 37^{o}C, for 24 hours.

Histamine analysis

Samples, of 5 g, were homogenized in 50 mL of 0.4-M perchloric acid with Ultraturrax blender (Janke& Kunkel KG, IlkaWerke, Staufen, Germany) (at room temperature), then centrifuged for 5 min at 1,280 x g, and supernatants were filtered through Whatman no. 41 paper (Whatman International Ltd., Maidstone, UK). A sample extract aliquot, 1mL, was transferred in 7-mLvials, where 0.5-mL, 0.4-M perchloric acid was added. This solution became alkaline by adding 200-mL, 2-N sodium hydroxide solution and buffered with a 300-mL saturated sodium bicarbonate solution. Derivation was obtained by adding 1-mL dansyl chloride solution in acetone (1%, w/v) and transferring the reaction mixture to a 40C incubator for 45 min. Residual dansyl chloride was removed by adding 150-mL ammonium hydroxide 30% (w/v). After 60 min the solution was adjusted to 5 ml with acetonitrile and was centrifuged (model Megafure, Hearaeus Instruments GmbH, Harau, Germany) for 10 min at 1,280 x g. Dansyl derivatives of calibration standard solutions (0.5–4 mg/L) were prepared together with the samples. For this method, the limit of detection (LOD) was 0.418 mg/kg, and the limit of quantification (LOQ) was 1,379 mg/kg. These values were calculated from 20 blank samples derived from the considered species. The LOD was calculated as the average of noise plus three times standard deviation (SD), while the LOQ was calculated as the average of noise plus six times SD. Recovery tests were prepared by adding specific amounts of histamine to arrive at a concentration of 5 and 10 mg/100 g of histamine-free samples of fish. The fish samples were tested to verify that they were "histamine-free" before the fortification,.

Statistical Analysis

The mean± SD values (n = 10) were calculated for each species using the Microsoft Excel data analysis tools.

3. Results and discussion

Microbiological results

Enumeration of *TVC* and E*nterobacteriaceae* were analyzed in respective temperature 25°C and 4°C for both types of fish. The histamine mean values in 4^{o}C storage were $2.8x10^{3}$ cfu/g for anchovies and $1.4x10^{3}$cfu/g for sardine. It is evident that the anchovy's values are higher than for sardine. Similar results were also reported in literature [14].

Histamine results

Histamine was never detected in all the samples examined at their arrival to the laboratory. The samples stored at 25°C for 24 h revealed different amounts of the amine depending not only on species but also on single fish Table 1. A similar variability of histamine concentrations in fish belonging to the same batch was observed by Lorca [15]. E. encrasicolus showed the highest levels of the amine compared with Sardine pilchardus. It could be due to the natural micro flora spreading into the muscle more quickly. Kim [16] showed that bacterial proliferation at ambiental temperature (25°C) with consequent quality deterioration of Scomber scombrus progressed faster than that of larger fish such as Thunnus alalunga and Coryphaen ahippurus.

Table 1. Histamine concentrations (range, mean±sd; mg/100 g) in samples stored at 25°C for 24 h and 4°C for 72 h.

		Sardinapilchardus	***Engraulisencrasiculus***	***Sardinapilchardus***	***Engraulisencrasiculus***
	Sampling	**Range (min-max)**	**Mean±STDV**	**Range (min-max)**	**Mean±STDV**
25°C	First	17.89 - 65.65	39.45 ± 19.094	54.79 - 126.87	77.921 ± 29.23
	Second	23.76 - 92.12	50.450 ± 23.387	64.43 - 106.39	84.592 ± 17.91
4°C	First	d.l	d.l	1.55 -7.98	5.028 ± 2.28
	Second	d.l	d.l	3.78 - 9.95	6.05 ± 2.37

d.l, detection limit (0.5 mg/kg).

Our results are consistent with other studies which take in consideration histamine formation in fish at similar storage conditions. Veciana-Nogués [17] detected high levels of histamine – above 100 mg/100 g – in E. encrasicolus stored at room temperature (22°C) after 24 h. In our study, all samples of E.encrasicolus examined after 24 h, with the exception of one, exceeded this value, up to a maximum of 146.5 mg/100 g. In S. pilchardus, histamine ranged from 17.89 to 92.12 mg/100 g. El Marrakchi [11] found histamine levels of 30 mg/100 g in Moroccan sardines (S.pilchardus) stored at ambiental temperature (25°C) for 24 h. In a similar study, histamine was accumulated to a value of 235 mg/100 g [10]. Histamine concentrations in E. encrasicolus and S. pilchardus stored at +25°C were higher than the health hazard level of 50 ppm, fixed by the U.S.FDA, but lower the legal limit of 200 ppm. The guidance level of 50 ppm has been established because "histamine is not uniformly distributed in a decomposed fish. So if one section has 50 ppm, exists the possibility that other sections may exceed 500 ppm". Shakila [18] found histamine content above the U.S. FDA maximum allowable limit of 50 ppm after 12 h in mackerel and after 15 h in sardine stored at ambient temperature (32°C).

Histamine formation after 72-h Storage at 4°C on S. scombrus stored in polystyrene boxes with alternating layers of fish and ice for different times related to ice/fish ratio showed low levels of histamine [6].Compared to Moroccan sardines(S. pilchards) caught and stored under the same conditions [11], S. scombrus had a lower histamine content. At rejection time, the histamine content averaged 8 mg/100 g in S. pilchards, while it was only 2 mg/100 g in S.scombrus. In the study, the fish were kept at refrigeration temperature in bags without ice, but no histamine was detected after 72h storage, except for E. encrasicolus, which showed concentrations ranging from 1.55 to 9.95 mg/100 g. Veciana-Nogués [17] found higher values (>20 mg/100 g) in E. Encrasicolus stored at 6°C after 73 h, even if in that study, the samples were mechanically triturated and then stored.

4. Conclusions

S. pilchards and E. encrasicolus demonstrated histamine concentrations in the limits of being toxic when the fish was stored at 25°C for 24 h. Fish should be placed in ice or in refrigerated seawater or brine at 4.4°C or less within 6 h from death. Any exposure time above 4.4°C significantly reduces the expected "safe shelf life". Fish that have not been previously frozen should not be exposed to temperatures above 4.4°C for more than 4 h, cumulatively, if any portion of that time is at temperatures above 21°C or for more than 8 h, cumulatively, as long as no portion of that time is at temperatures above 21°C after chilling onboard the harvest vessel. The safety of these limits is dependent upon proper sea handling .

5. References

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