Evaluation of Histamine level in the Red Tuna (Thunnus thynnus) of the Mediterranean Sea in 2010–2015

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ABSTRACTS
Histamine is a biogenic amine present in fish species associated with a high amount of histidine, and it can cause the ‘scombroid poisoning’. The Commission Regulation (EC) No 2073/2005 govern the criteria to analyze histamine in fishery products and specifies that the high performance liquid chromatography (HPLC) is the reference method. In this work, 664 samples were presented in the 2010–2015 shine, of which 46 (6.9%) were positive. With Chart 1 it can be noted that the number of samples has increased substantially in recent years (2013–2015) due to intensified official controls. On the contrary, the number of positives has decreased progressively.

INTRODUCTION
Food safety is a very subject matter nowadays, in fact, the attention of consumers on the risks of ingestion of harmful compounds through food grows more and more every day. The fish is an essential food for a complete diet, but being particularly rich in protein and free amino acids, is more subject to decomposition processes that could lead to the formation of substances harmful to consumers. Among these, there is the Histamine, a biogenic amine, derived from the decarboxylation by fish proteolytic bacteria of the amino acid Histidine. Histamine causes the so-called scombroid syndrome (Hungerford, 2010). This syndrome is usually a mild illness characterized by rash, hives, nausea, vomiting, diarrhea, flushing, tingling and itching of the skin (Taylor, 1986), the severity of symptoms varies depending on the amount of histamine assumed and individual susceptibility. For these reasons the histamine levels should be monitored in particular in fish that contain high levels of histidine in their tissues, particularly fish species of the families: Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae, Scombresosidae, such as tuna, mackerel, anchovy and sardines. An inappropriate treatment of these fish, at temperatures above 4 °C, during the storage or processing stages does increase the histamine levels due to degradative processes. Once produced, histamine tends to remain unchanged in the food, since it is particularly resistant to heat and it is not destroyed by normal cooking temperatures.
The Commission Regulation (EC) No 2073/2005 of November 15th, 2005 on “Microbiological criteria for foodstuffs” and the subsequent Commission Regulation (EC) No 1441/2007, govern the criteria to analyze the Fishery products from fish species associated with a high amount of histidine. It specifies that the sampling plan includes nine aliquots and that the high performance liquid chromatography (HPLC) is the reference method that must be used to detect the histamine.

These regulation limit histamine content and evaluate the test results:
- satisfactory, if all the values observed are ≤ 100 mg/Kg,
- acceptable, if a maximum of 2 values are between 100 and 200 mg/Kg, and the rest of the values observed are ≤ 100 mg/Kg,
- unsatisfactory, if one or more of the values observed are > 200mg/Kg or more than 2 values are between 100 mg/Kg and 200 mg/Kg.

For products that have undergone enzymatic maturation in brine, fitness values are doubled. Analytical analysis is needed to evaluate the deterioration of fishery products, particularly fresh fish, where the possible use of new generation illicit additives such as Cafodos® that alters sensory evaluation (Muscarella et al., 2013; Piersanti et al., 2014). Histamine monitoring was accepted globally for the confirmation of the safety of fishery products (Tao et al., 2011). The purpose of this study was to evaluate the content of Istamine in the Red Tuna-Mediterranean Sea (Thunnus thynnus) of FAO 37.576 - 12.326 North latitude, received in the laboratories of the Department of Chemistry and Food Technologies of the Zooprofylactic Institute Experimental of Sicily in the years 2010–2015.

MATERIAL AND METHODS

A total of 664 Tuna samples from FAO 37.576-12.326 North latitude, were examined at the laboratories of the Department of Chemistry and Food Technologies of the “Istituto Zooprofilattico Sperimentale della Sicilia” (Italy) over the years 2015–2016.

The internal method was validated according to UNI CEI EN ISO / IEC 17025 standard by fortifying tuna samples on three levels of histamine (100, 200, 400 mg / kg) by performing ten replicas at each validation level to determine repeatability, uncertainty and robustness. Ten low fortified samples (10 mg/kg) were used for measuring the detection limit (LOD) and the quantification limit (LOQ). The linear response of the method was verified in histamine levels from 10 to 100 mg/L, with a determination coefficient (R2) equal to 0.9995.

Reagents and Equipment

Histamine dihydrochloride, sodium 1-decanesulfonate, potassium monophosphate, potassium hydrogenphosphate trihydrate, acetonitrile, perchloric acid were purchased from Sigma-Aldrich. All the chemical reagents and solvents were of analytical and chromatographic grade, respectively. Ultrapure water was obtained from a Millipore purification system.

A UHPLC Agilent 1290 series (Walldbronn, German) system and a chromocrophic column of Supelcosil LC-ABZ 15 cm, 4.6 mm. ID, 5 µm a was used for the analyzes

Sample preparation ad extraction

The samples were previously homogenized and weighed (10 g) in a 50 mL centrifuge test-tube. A sample was fortified by adding of 2 ml of the histamine standard solution at 1000 mg/L (final concentration 200 mg/kg). To the weighted sample was added 10 ml of 6% perchloric acid solution and the mixture was vortexed for 1 minute, then were added 30 mL of deionized water and the mixture was vortexed for 1 minute, centrifuged for 10 minutes at 3000 rpm and the supernatant was filtered on a 0.45 mm microfilter directly into vials.

High performance liquid chromatography conditions

The chromatographic separations were run with an UPLC Agilent 1290 with UV/DAD detector on a Supelcosil LC-ABZ column (15 cm, 4.6 mm. DI 5 mm). Injection volume was 20 µL, the flow rate was 1.2 mL/min at room temperature and the detector wavelength was set to 210 nm. The method involved an isocratic elution using a Mobile phase A consisted of the Phosphate buffer solution at pH 6.9 and mobile phase B consisted of acetonitrile (85:15, v/v).
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Standard solutions

Standard solutions were prepared according to Table 1.

RESULTS

The HPLC-DAD chromatogram of a histamine standard solution 200 mg/L is shown in Figure 1; you can see a unique and recognizable peak with a 4 minute retention time relative to histamine. While Figure 2 shows a typical HPLC-DAD chromatogram on a fortified sample, it is possible to note that there are other spikes associated with the matrix and, at 4 minute time, the peak of the analyte in question. The presence of the analyte can be further verified by superimposing UV-vis spectra. In Figure 3, a typical UV-Vis spectrum of histamine is shown, showing absorption between 200 and 240 nm with a maximum of $\lambda = 210$ nm

CONCLUSIONS

In this work, 664 samples were presented in the 2010–2015 shine, of which 46 (6.9%) were positive. With Chart 1 it can be noted that the number of samples has increased substantially in recent years (2013–2015) due to intensified official controls. On the contrary, the number of positives has decreased progressively.

Samples from the Mediterranean are fairly controlled and free from histamine, while cases of scombroid syndrome that are occurring are probably due to the poor conservation of fish by end consumers (and end-users).
REFERENCES


