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Histamine and microbiological changes during storage of semi-preserved anchovies

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During the various stages of manufacture of semi-preserved anchovies, histamine shows a significant increase favored by the fragility of anchovies and wealth histidine, an amino acid precursor of histamine. The decarboxylation of histidine is present as a major problem in the industry of semi-preserved anchovies, especially during the ripening step. The objective of this work is to monitor the levels of alteration in a final product subjected to aging by incubation at 30°C ± 1. Accumulation of histamine, as well as microbial population in semi preserved anchovies were investigated. Enterobacteriaceae and Lactobacilli do not seem to resist to the salt applied to the product. They disappear within one month of storage. A total of 665 bacterial strains were selected from the prescreening step using various selective media. Only 20.6% of these selected isolates showed a positive reaction in Niven's differential medium, and 31.4% of the positive isolates were true histamine formers when confirmed by thin layer chromatographie. The values of histamine remain in close contact with the sanitary measures taken by each company. Regular monitoring and mastering of good practices are necessary for a good quality product.

Key words: Anchovy, bacteria, histamine, histidine, semi-preserved anchovies.

INTRODUCTION

Biogenic amine are basic nitrogenous compounds occurring in meat, fish, cheese and wine products, mainly due to amino acid decarboxylation activities of certain microbes (Hungerford, 2010). Histamine is a biogenic amine produced by decarboxylation of free histidine. Histamine is normally present at low levels in the human body, can be present in a variety of foods such as fish, cheese, meat, wine, and fermented foods. Since histamine is involved as a primary mediator in many allergic reactions, the increase in its levels to values greater than 500 mg/kg can be highly toxic giving symptoms that can be confused with alimentary allergies (Baross et al., 1992).

Histamine content in fish can rapidly increase during spoilage by bacterial histidine decarboxylases. High levels of histamine content have been found in various types of fish implicated in scombroid poisoning (Hwi-Chang Chen et al., 2008). A Scombroid fish (Teleostea,

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Scombroidei), such as mackerel and tuna, or clupeid fish (Teleostea, Clupeoidei) such as sardines, anchovies and herring, are frequently involved in histamine toxicity. These fishes have relatively high free histidine levels in their muscles when alive (Hernández-Herrero et al., 1999). Post-mortem proteolysis liberates additional histidine from muscle protein, and explains why histamine can reach high concentrations without the formation of organoleptic spoilage indicators. Significant relationship was observed between histamine content and Enterobacteriaceae count. Enterobacteriaceae species are the most important histamine-forming bacteria in tuna fish (Koohdar et al., 2011).

Anchovies, in brine or in oil, are semipreserved, obtained without any heating process to stabilize the final product. Changes during storage can occur, and even at low temperatures, enzymatic processes may lead to a product showing a high degree of proteolysis. Moreover, desalting and filleting increase the risk of secondary bacterial contamination in the final product packed immersed in oil. Previous studies showed that histamine levels increased during storage of semipreserved anchovies when they were not kept at refrigeration temperature (Yung-hsiang et al., 2005). Other biogenic amines such as tyramine, â-phenylethylamine, and tryptamine also seem to increase during storage (Veciana-Nogue's et al., 1997), but little information is available.

The preparation of semi-preserved anchovies *Engraulis encrasicholus* is a process of salting and ripening, (Hernández-Herrero et al., 1999). The ripening process is common in some Mediterranean areas and Argentina, where the species is *Engraulis anchoita*, (Triqui and Reineccius, 1995). Anchovy (*E. encrasicholus*) is primarily marketed and processed in Spain, Italy, Greece, France, and Morocco, (Hernández-Herrero et al., 1999).

The production of anchovy fillets for subsequent canning is a delicate job, usually requiring female hands. The process begins with salting and pressing of the fish which are then left to cure for several months until they acquire the right reddish color and aroma. Penetration of salt varies with the thickness of the muscle, temperature, the freshness and the fat content, (Clucas, 1982). After that, the fish are skinned, washed, trimmed to size then dried and filleted. Finally, they are placed in containers (cans or glass jars), and covered with olive oil before sealing. Unlike other preserves, in neither case are anchovies subjected to sterilization because heat would spoil them. This is why they are treated as semi-preserves. Cans must be stored at between 4 and 12°C, and the contents should be consumed between six months and one year after production.

Here we studied the evolution of the contents of histamine throughout the shelf life of ripened anchovies from the Morrocan coast. Changes in histamine during storage of anchovies packed immersed in oil and ready to eat from three different manufacturers were studied during 12 months of storage of the final products and stored under two temperatures, 4 and 30°C ± 1. The assigned shelf-life period for these anchovies is 6 months. Total mesophilic, halophilic, lactic flora and enterobacterial counts were also determined to check their possible relationship with histamine production linked to the handling process needed before the final packaging of anchovies immersed in oil.

Table 1. Characterization of samples used.

Company	Description	Packaging
S1	Fillet anchovy caper rolled	Canned product
S2	Anchovy fillet	Glass jars
S3	Anchovy fillet	Canned product

MATERIALS AND METHODS

Sampling

Samples of semi-preserved anchovies are from three different companies S1, S2 and S3 (Table 1). Anchovies are caught in the coast of Morocco and prepared in the same way (heading and gutting, ripening, washing, threading, oil immersion and packaging). Samples of each company were incubated at two temperatures, 4°C ± 1 in a refrigerator (SANYO MPR 311D) as recommended storage temperature of the final product, and 30°C ± 1 in an incubator (SANYO MCO 175), which is the temperature that causes aging of the product. Histamine departing values are 3, 12 and 70 ppm for the S1, S2 and S3 companies. Each month, samples are taken for analysis, a mixture of five cans to determine the histamine content and perform a bacterial count.

Chemical analysis

The histamine assay is done according to the spectrofluorometric method of Lerk and Bell (1978). 10 g of sample is homogenized in 90 ml of 10% trichloroacetic acid filtered buffer. 200 µl of the filtrate is transferred with 150 ml of acetate buffer in an ion exchange column. Histamine is then eluted with hydrochloric acid. Reading the DO at excitation wavelength of 350 nm and emission wavelength of 450 nm after complexation of 20 µl filtrate with Ortho-phthaldehyde. To measure the pH, a sample of 10 g is homogenized in 10 ml of distilled water and the pH is measured using a Toa DKK pH meter HM- 20J.

Microbiological analysis

From a mixture of 5 can of each company, 25 g were removed aseptically and homogenized with 225 ml of buffered peptone water in a stomacher bag for a count of the total flora of PCA medium (Plate Count Agar) at 30°C ± 1 for 72 h, the total halophilic flora in PCA medium based on sea water at 25°C for 5 days (SANYO MIK-153). Sea water is filtered before to a porosity of 0.45 µ. Enterobacteriaceae on VRBG medium (Glucose Agar with Crystal Violet, the Red neutral and Bile) at 30°C ± 1 for 24 h (ISUZU FR-114S). The lactic flora in MRS media (De Man, Rogosa and Sharpe) and M17 respectively bacilli and shells at 30°C ± 1 for 48 h (ISUZU FR- 115S). Seeding is double layer for VRBG, MRS and M17.

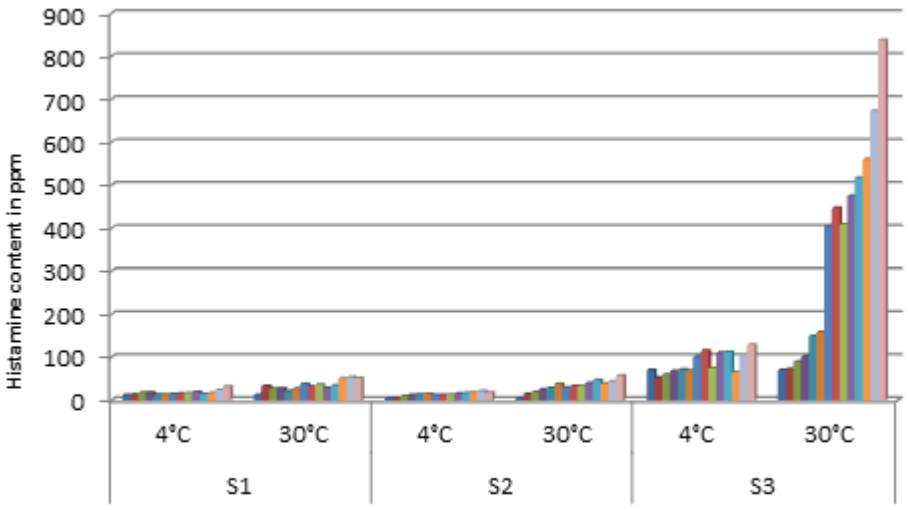

Figure 1. Influence of storage temperature on the histamine formation in semi preserved anchovies

To check the hability of bacteria to produce histamine, 665 strains were isolated and tested on Niven medium (L- histidine 2 HCl 27%, 5% tryptone, 5% yeast extract, sodium chloride 5%, 1% Calcium Carbonate, Agar 30%, 0.6% bromocresol purple). and Yamani and Unterman medium (2% peptone, Lab Lemco powder 1%, 5% sodium chloride, L- histidine HCl 10%, 0.1 % bromocresol green, chlorophenol red 0.2%). The incubation is done at 30°C ± 1 for 24 h (ISUZU FR- 114S) strains are considered positive if they have a blue purple halo on agar Niven, or broth turn from green to blue in the Yamani & Unterman medium.

RESULTS

Histamine content recorded during the storage of the final product evolves differently for the three companies. The company S3 displays the highest values exceeding 800 ppm after one year of storage at 30°C ± 1. However, histamine values stored at 4°C ± 1 increase lower than samples kept at 30°C ± 1. Therefore, we would point out that storing semi preserved anchovies under refrige-ration, as recommended, reduces but does not prevent histamine formation.

Both S1 and S2 companies have similar histamine values and remain low compared to standards. The highest and earliest formation of histamine was observed in samples from S3 (Figure 1). Histamine contents after 6 months of storage at 30°C ± 1 surpassed the maximum average value (200 ppm) permitted for ripened fish product. Histamine levels remained constant in S1 and S2 samples stored at 4°C ± 1 and increase at 30°C ± 1 were, in general, lower than recommendation.

The average bacterial count in three companies studied is almost similar in the three culture media PCA, PCA in sea water and M17. There was a slight increase during the first two months which exceeded 10^6 cfu/g, followed by a gradual decline until the end of the storage period (Figure 2). Note however that counts of bacteria belonging to the *Enterobacteriaceae* family on VRBG and Lactococcus on MRS media disappear after the first month of storage. Most frequently isolated profilic histamine-formers fish under controlled storage conditions are mesophilic enteric bacteria such as *Morganella morganii*, *Proteus vulgaris*, *Hafnia alvei* and *Citrobacter freundii* (Chiara et al., 2012).

When the Niven, Yamani and Unterman medium was used, only 20.6% strains were found to be positive and forming histamine. The majority of these bacteria is represented by the *Enterobacteriaceae*, halophilic total flora, and lactic bacteria (Figure 3).

DISCUSSION

Biogenic amines are formed mainly through decarboxylation of specific free amino acids by exogenous decarboxylases released by the microbial species associated with seafood. Many bacterial species are known to possess histidine decarboxylase and have the ability to produce histamine (Yi-Chen Lee et al., 2016). Histamine formation in fish is related to the environmental conditions, histidine content, and the presence of histamine forming bacteria. Traditionally, histamine formation in fish has primarily been prevented by limiting microbial growth with chilling and freezing (Jia-Wei et al., 2015). High levels of histamine can induce symptoms similar to allergic reactions and ingestion 100-1000 mg once can cause histamine poisoning, (Rossano et al., 2006).

It seems well established that histamine formation increases with temperature during storage, but remains dependent on the initial quality of the final product. The results indicate that both S1 and S2 record small

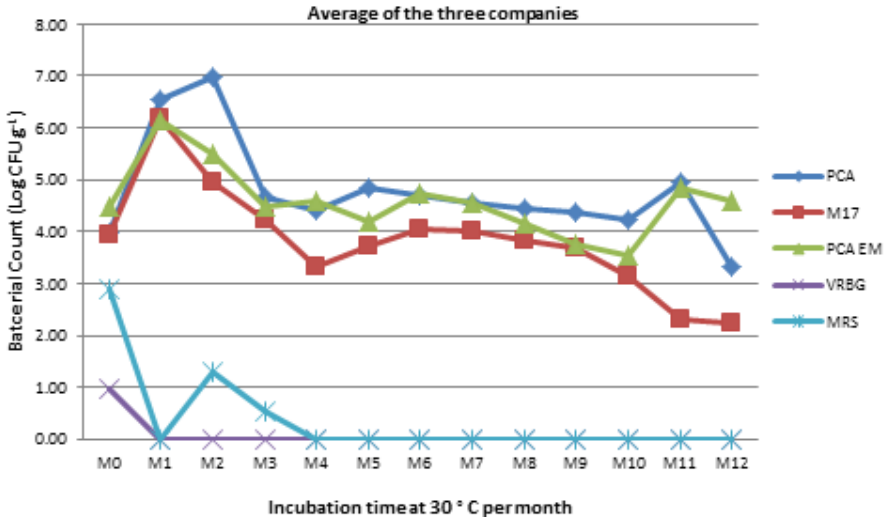

Figure 2. Average bacterial flora during the incubation of semi preserved anchovy at 30°C ± 1.

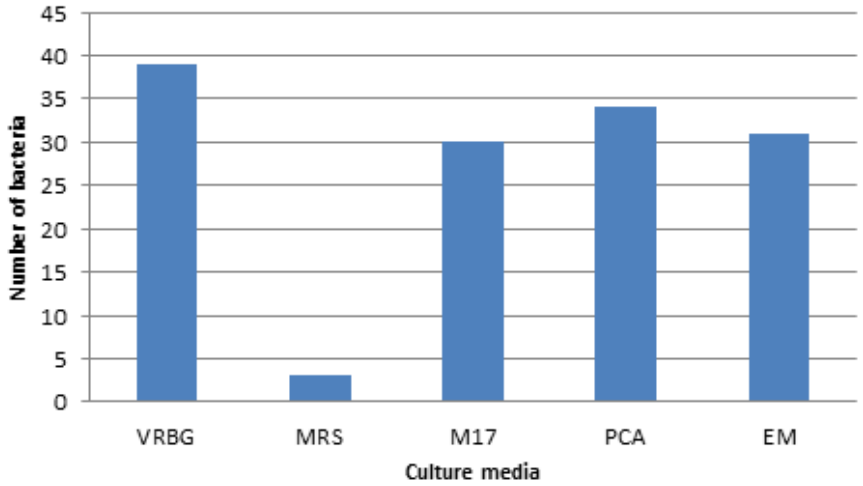

Figure 3. Histamine forming bacteria from semi preserved anchovies incubated at 30°C ± 1 isolated in different culture media.

increases histamine content even at storage of 30°C ± 1, 52.03 and 57.65 ppm respectively. However, the S3 show a high level on histamine 837.93 ppm knowing that the initial value is 70.69 ppm. Low temperature delayed the growth of bacteria, resulting in minimal changes of histamine.

High levels in histamine in anchovies, even before the shelf life, may be related to certain toxic effects. The dose of 10 mg histamine was related to symptoms of histamine poisoning, (Veciana-Nogue's et al., 1997). Anchovies are usually consumed in small quantities as a food ingredient in many dishes, and it can be difficult to surpass the levels previously mentioned.

However, taking into account the weight of 60 g anchovy in a final product, the histamine content recorded in all these stored samples at 4°C ± 1 for one year remains insufficient either to cause histamine poisoning or headache. After 12 months of storage at 30°C ± 1, the consumption of 12 g of the anchovy from S3 could provide more than 10 mg of histamine. Both S1 and S2 companies stay away from the dose adverse effect. The histamine toxicitiy level was exceeded in S3 after only 6 months of storage at 30°C ± 1.

Conclusion

The results of this study show that the semi-preserved anchovies deteriorate easily and can be transformed into dangerous products during storage, especially if the store does not meet the conditions recommended by the

manufacturer. The fragility of the anchovies and persistence of histamine-forming bacteria in the product contribute significantly in this alteration. Good control of the manufacturing process and good hygiene practices is needed to prevent the introduction of Enterobacteria in the product at any stage of its manufacturing.

Conflict of Interests

The authors have not declared any conflict of interests.

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