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## *Physicochemical And Microbiological Parameters Associated With The Formation Of Histamine In Freeze Brine Of Commercial Yellowfin Tuna Fishing Boats*



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### ABSTRACT

Freeze brine used on board to freeze yellowfin tuna fishes is commonly used during several travels before being replaced. The aim of this work was to evaluate changes in the physicochemical and microbiological properties of freezing brine used in two fishing boats during two years, to determine the feasibility of forming histamine in the brine. Changes in pH, protein, chlorides, mesophilic and psychrophilic microorganisms and histamine level in freezing brine were determined. Changes in histamine and chlorides in yellowfin tuna muscle were evaluated too. In freezing brine the pH varied from 5.6 to 6.8, protein content increased near to 1.2%, chlorides varied from 15 to 18%. Maximum mesophilic and psychrophilic counts were  $4.6 \times 10^4$  UFC/g and  $4.5 \times 10^5$  UFC/g respectively. Freezing brine of the two fishing boats had a mean level of 19.68 (+/-13.51) ppm of histamine. In the flesh of the yellowfin tuna the value of chlorides varied from 0.86 to 1.57% and histamine mean level was 37.82 (+/-5.69) ppm. Results obtained indicated that halophilic microorganisms were able to grow in freezing brine. Histamine present in freezing brine was associated with growing of halophilic microorganisms.

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### KEYWORDS

Brine;  
 Freezing;  
 Yellowfin tuna;  
 Histamine.

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### INTRODUCTION

Seafood quality is affected by handling, gutting, processing, and storage temperatures. Although refrigeration and freeze storage can extend the shelf life of seafood products, proliferation of psychrotrophic bacteria at refrigerated temperatures still contributes to seafood spoilage. For the seafood industry, the monitor of indicator compounds such as histamine associated with seafood safety is as important as quality assurance<sup>[1]</sup>.

The consumption of fish belonging to the *Scomberesocidae* and *Scombridae* family (including yellowfin tuna, *Thunnus albacares*) is the most commonly cause of histamine poisoning foodborne illness. The production of histamine in foods has been associated with the presence of decarboxylant microorganisms which transform histidine into histamine. Tuna fish muscle contains high levels of histidine, thus is highly susceptible to show high levels of histamine if microbiological risks are not avoided during processing<sup>[2,3]</sup>. The temperature abuse of fish post-harvest induces the formation of high levels of histamine in the flesh, and the formation is affected by the combination of time and temperature. The production of histamine in fish has been associated to microbiologic contamination with bacteria possessing histidine decarboxylase. The most frequent microorganisms associated with fish histamine poisoning are *Enterobacteriaceae*, *Morganella morganii*, *Klebsiella pneumoniae* and *Hafnia alvaet*<sup>[4]</sup>.

The Food and Drug Administration (FDA) established a defect action level of 50 ppm for histamine in tuna, mahi-mahi (dolphin fish) and other fish species as an indication of potential health risk<sup>[5]</sup>. European Community has fixed 100 ppm histamine in fish and fish products as a maximum average value in a group of nine samples<sup>[6]</sup>. Mexico has a maximum limit of 200 ppm of histamine for tuna and tuna products.

Nowadays the origin of contamination of tuna fishes with histamine former bacteria remains unclear and to our knowledge there is not information about the level of histamine contained in freezing brine of tuna boats and little information is available concerning histamine formation in yellowfin tuna. The objective of this work was to determine

the presence of histamine in freezing brine, and its association with histamine level on the flesh of fresh tuna obtained directly from the freezing tanks of tuna boat.

### MATERIALS AND METHODS

#### Fishing boats

The fishing boats (FB) involved in this study had a capacity of 1,090 Ton (FB1) and 800 Ton (FB2) respectively. Before starting this study the freezing brine of FB1 was 14 months old, and the brine of the FB2 was 18 months old. After each fishing travel the brines were added with sodium chloride to adjust concentration to 18%, as a common practice of the industry.

#### Sampling

Freezing brine and yellowfin tuna fishes (*Thunnus albacares*) were sampled directly from the fishing boats, immediately after their arriving by personal working in the control quality laboratory of a tuna canning industry. After each travel fishing, 2 L of freezing brine were sampled in sterile recipients (the same brine was used in all tanks of a same boat) and two tuna fishes were obtained from each boat. Freezing brine samples had  $-8^{\circ}\text{C}$  when sampled. Samples were ice-stored to transport at laboratory (less than 10 min) and were stored at  $-15^{\circ}\text{C}$  until their analysis (less than 24 hours). Tuna fishes were caught in the Mexican Pacific, had an internal temperature of  $-8^{\circ}\text{C}$  and a 5/12 size. Samples of almost one kilogram were cut out from the loin of each tuna fish using an electric saw previously sterilized with 70% ethanol and introduced in sterile bags before ice-storing, transported to the laboratory (less than 10 min), and stored at  $-15^{\circ}\text{C}$  until their analysis (less than 24 hours).

#### Histamine analysis

Histamine content was determined by duplicate following the method described by<sup>[7]</sup>. Briefly, ten grams of muscle (or brine) were homogenized with 100 mL TCA (2.5%) and filtered using a Whatman filter #4. The filtered solution was neutralized to pH 7 with KOH 1N. A 75 mL aliquot of this solution was introduced into an ionic exchange column

(Amberlite CG-50, mesh 100-200) and 150 mL of acetate buffer 0.2 N, pH 4.63 were added. The histamine was eluted with 25 mL HCl 0.2 N. An aliquot of the HCl 0.2 N eluted solution was mixed with 15 ml  $\text{Na}_2\text{CO}_3$  previously cooled and 2 mL of a diazo reagent were added. The mixture was kept at 0 °C for 10 min before measuring absorbance at 495 nm. A standard histamine solution (3-100 ppm) was prepared diluting histamine dihydrochloride (Sigma, St. Louis, Mo. U.S.A.) with HCl 0.1 N.

To evaluate the ion-exchange chromatography method fish samples were inoculated with one mL of 0 to 100 ppm of histamine and percent of recovery was measured. The recovery of histamine for the method was determined as 93 to 100%.

#### pH and chlorides

For pH measurement, 10 g of brine (or muscle) were mixed with 10 mL of distilled water and pH determination was obtained at 25°C by duplicate. Chlorides were determined in duplicate following the method described in the<sup>[8]</sup>

### MICROBIOLOGICAL ANALYSIS

Microbiological analysis was realized as following: blending 10 g of brine (or muscle) with 90 mL peptone water (0.1%). Agar plate count was carried out in duplicate according to the standard plate method. One-milliliter aliquots from each sample were serially diluted with peptone water (0.1%) and mixed with standard plate count agar supplemented with 0.5% NaCl cooled down to 50 °C. Total count of psychrophilic was obtained incubating at 5 °C for ten days and mesophilic was obtained incubating at 37 °C for 48 h.

#### Statistical analysis

Statistical analysis was performed using a Statgraphics 5.0 (Software Publishing Corporation, Bitstream Inc.). LSD's multiple range tests were used to determine significantly difference ( $p < 0.05$ ) among treatments.

### RESULTS AND DISCUSSION

Freezing brine from two tuna fishing boats was studied during two years to determine changes in

histamine concentration, physicochemical parameters (pH, protein and sodium chloride concentration) and microbiological quality (mesophilic and psychrophilic microorganisms). Tuna muscle from the same boats was measured to determine the concentration of histamine, sodium chloride, mesophilic and psychrophilic microorganisms. Freezing brine and tuna muscle were analyzed every time the fishing boats arrived to the industry port after the fishing trip.

#### Freezing brine

##### Protein concentration

Freezing brine from fishing boats one (FB1) and two (FB2) were 14 and 18 years old respectively at the start of the study. Thus, initial concentration of protein was relatively high (Figure 1).

During the first year, protein concentration increased from 1.0 to 1.5% (FB1) and from 1.16 to 1.41% (FB2). No significant difference ( $P < 0.05$ ) was found in protein concentration in freezing brine from both tuna boats. After the last fishing trip of 2003 (November month), the freezing brine of both fishing boats was totally exchanged. Thus initial protein concentration for the 2004 fishing period was zero.

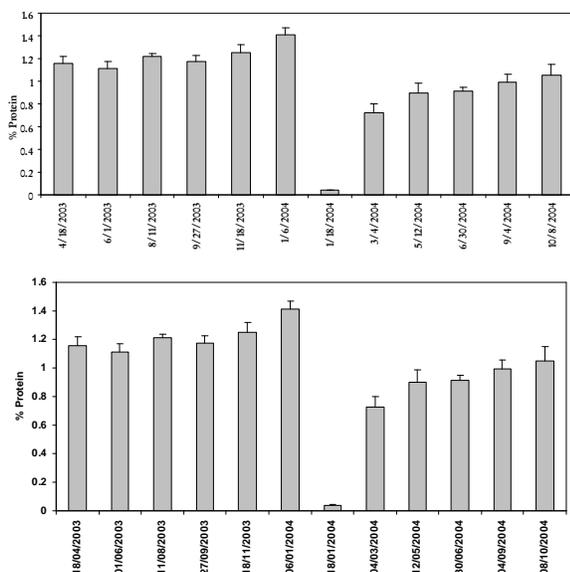
Protein concentration for the second year increased from 0.03 to 1.11% (FB1) and from 0.04 to 1.05% (FB2). No significantly difference ( $p < 0.05$ ) was found in protein concentration in freezing brine from both tuna boats for the second year.

The presence of protein is needed for the growing of microorganisms. Freezing brine of both fishing boats contained protein from tuna fishes. Proteins came from external mucosa, excreted feces, blood, and muscle. The two last origins are associated with damage in the integrity of the tuna fishes associated with operations on board. Although tuna industry has implemented good handling practices on board, is not feasible to avoid totally the physical damage in some fishes, because of the high volume of catching.

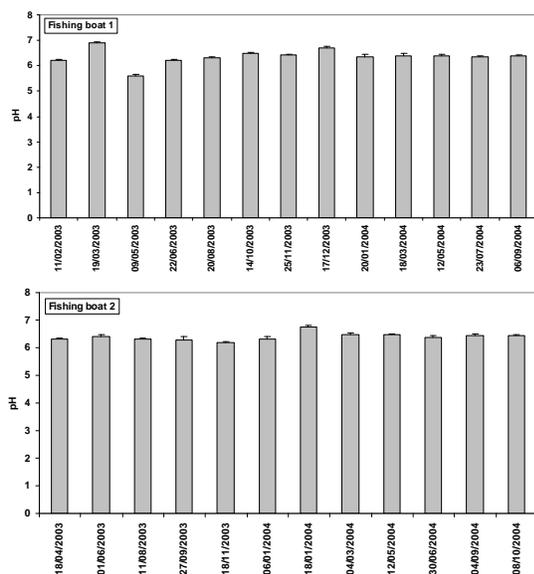
#### pH

The pH varied from 5.6 to 6.8 in the FB1 and from 6.4 to 6.8 in FB2 (Figure 2). There were not significantly differences in pH values ( $p < 0.05$ ) between freezing brine of both fishing boats. The total

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**Figure 1:** Changes in protein concentration in freeze brine used for two yellowfin tuna fishing boats. Mean values of two samples by duplicate. Bars indicate one standard deviation.



**Figure 2:** Changes on pH of the freezing brine used in two yellowfin tuna fishing boats. Points are the mean value from two replicates. Bars indicate standard deviation.

exchange of freezing brine after the first year of fishing period had not effect on the pH measured in the brines of both boats. This result indicates that the concentration of protein present in brines did not

affect their pH. The pH range found in brine of both fishing boats might be appropriated for growing of different microorganisms.

### Sodium chloride

The sodium chloride in the freezing brine of both fishing boats varied from 15 to 18% during the two years of this study (Figure 3). The content of chloride in the brine was according with the level of salt used to prepare the freezing brine (18% NaCl w/v). No difference in the amount of salt between freezing brine of both boats was found ( $p < 0.05$ ).

### Mesophilic microorganisms

As was to mention above, freezing brine from fishing boats one (FB1) and two (FB2) were 14 and 18 years old respectively at the start of the study (Figure 4). Thus the initial microbial count was high in both boats:  $1.4 \times 10^4$  UFC/g for FB1 and  $2.25 \times 10^4$  UFC/g for FB2.

During the study, the microbial count increased in FB1 from  $1.4 \times 10^4$  to  $4.6 \times 10^4$  UFC/g after 5 months (February to June 2003) (Figure 4). During the month of July, freezing brine was renewed, but only 80% of total volume was replaced. This partial replace had been a common practice for this industry. In August 2003, the renewed freezing brine had a microbial count of  $1.5 \times 10^4$  UFC/g, which increased to  $3.1 \times 10^4$  UFC/g after 4 month. In December 2003, the freezing brine was totally renewed. The microbial count of the new freezing brine was very low, starting with  $5.5 \times 10^2$  UFC/g in January and increasing to  $3.2 \times 10^4$  in September.

Microbial count of FB2 freezing brine increased from  $2.3 \times 10^4$  UFC/g in April 2003, to  $1.2 \times 10^4$  UFC/g for November 2003. After renewing of the freezing brine, microbial count initiated from  $7.5 \times 10^2$  UFC/g in January to  $3.9 \times 10^3$  UFC/g in October.

The freezing brine, contained microorganisms capable of tolerating high level of salt (halophiles) and low temperatures (psychrophiles or psychrotrophic), which could grow in because of the protein content and the pH of the brine. Partial renewing of freezing brine, a common practice for the industry, producing a short life of the freezing brine, associated with the growing of microorganisms. Although free

zing brine is conserved at  $-10^{\circ}\text{C}$ , this temperature can be increased during the initial freezing process of the tuna fishes, allowing the growing of halophilic psychrophilic microorganisms.

### Psychrophilic microorganisms

Freezing brine of the two fishing boats showed higher microbial counts for psychrophilic microorganisms in comparison to mesophilic microorganisms, except for renewed freezing brines (Figure 5).

Freezing brine from FB1 increased from  $1.2 \times 10^5$  in February to  $4.5 \times 10^5$  UFC/g after 5 months (June 2003) (Figure 5). After partial renewing (80%) of freezing brine, microbiological count of psychrophilic organism decreased to  $1.9 \times 10^5$  UFC/g and increased to  $3.1 \times 10^5$  UFC/g after 4 months. After the total replace of freezing brine in the second fishing year, the microbiological count increased from 1.0 UFC/g in January to  $1.4 \times 10^4$  UFC/g in September.

Freezing brine from FB2 increased during the first fishing year from  $1.2 \times 10^5$  UFC/g in January to  $2.3 \times 10^5$  UFC/g in November. After total renewing of the freezing brine, microbiological count increased from 1.0 UFC/g in January to  $1.7 \times 10^4$  UFC/g in September.

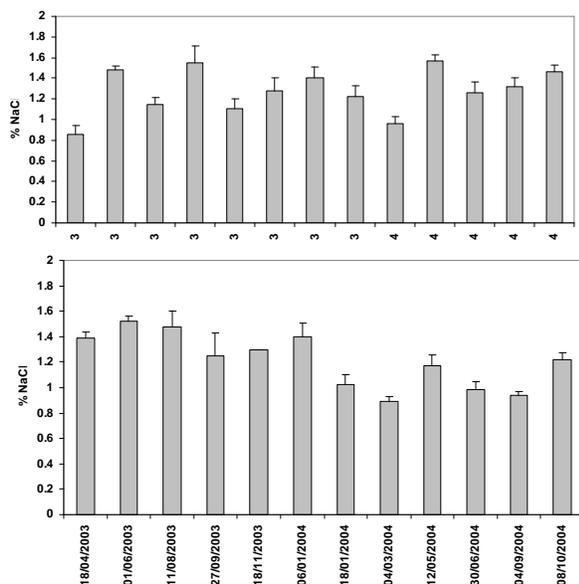


Figure 3 : Changes on sodium chloride concentration in freezing brine. Points are the mean value from two replicates. Bars indicate standard deviation.

The origin of microorganisms present in freezing brine remains unclear, but they can have different origins, such as the salt and the sea water used to prepare freezing brine, as well as microorganisms present in the skin, gills, feces and exposed guts of damaged tuna fishes caught.

Results of this study, indicates that microorganisms present in the freezing brine of the two fishing boats studied, had their origin in the tuna fishes, more than in salt and sea water used for the freezing brine. It seems that microorganisms contaminating freezing brine required a long period of adaptation before growing in the extreme conditions of salt and temperature of the freezing brine. Thus, is important to replace totally the freezing brine after having high microbiological counts, to avoid a fast growing of such microorganisms.

Freezing brine showed appropriated conditions for growing of microorganisms and might be a source of contamination of damaged fishes, as well as for contaminating external surface of tuna fishes, which might be a risk of contamination for tuna flesh during processing. The surviving of psychrophilic amine-forming bacteria in fish and shrimp stored in ice at  $0^{\circ}\text{C}$  has been reported<sup>[9]</sup>.

### Histamine

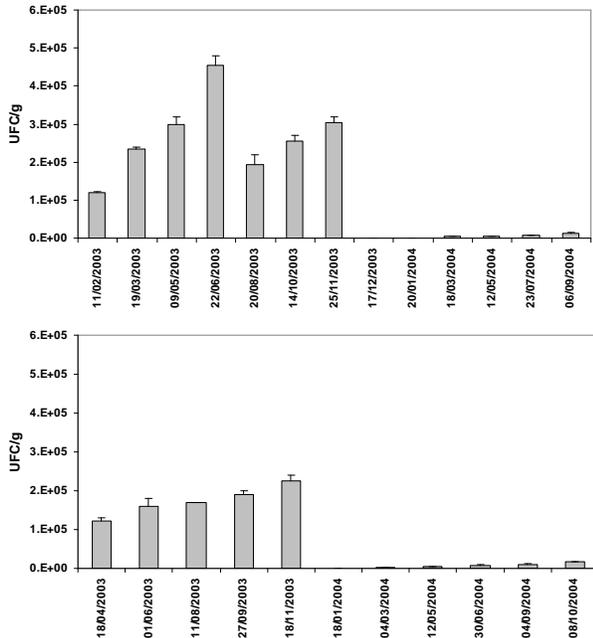
Histamine was present in the freezing brine of both fishing boats (Figure 6). Histamine mean level for the freezing brine of both fishing boats was of 22.5 (+/-17.0) ppm for 2003 and 16.58 (+/-7.84) ppm for 2004 year. Histamine mean level for the experimental period was 19.68 (+/-13.51) ppm.

Histamine level for the freezing brine of the FB1 varied from 63.8 to 25.5 ppm during the first fishing year. Recent renewed freezing brine did not showed the presence of histamine, but histamine level increased with time, associated with an increase in protein content and microorganism growing. Nine months older freezing brine had 15.2 ppm of histamine.

Histamine level for the freezing brine of the FB2 varied from 21.4 to 22.6 ppm during the first year. The histamine level in renewed freezing brine varied from 0 to 15.1 ppm after 9 months.

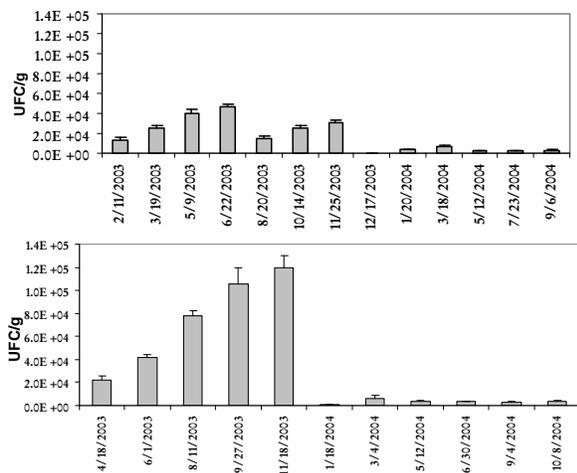
Histamine present in freezing brine seems to have its origin in the presence and growing of decarboxilant

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**Figure 4 :** Changes on the concentration of mesophilic microorganisms. Points are the mean value from two replicates. Bars indicate standard deviation.

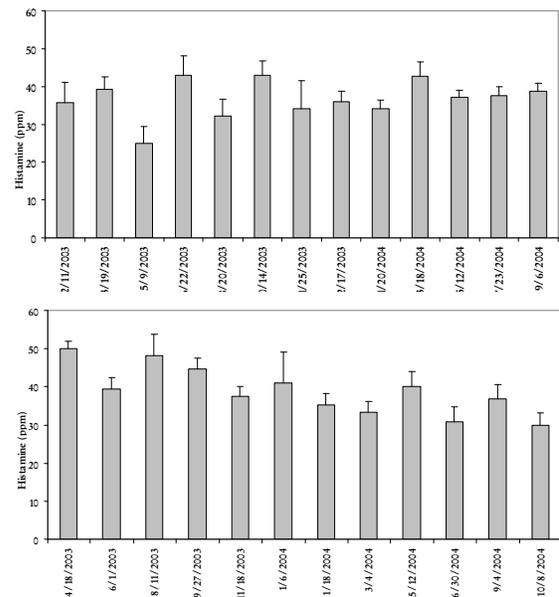
microorganisms, associated with histamine formation. Freezing brine contained protein, which is important for the growing of decarboxilant microorganisms. Tuna muscle is rich in protein and yellow-



**Figure 5 :** Changes on the concentration of psychrophilic microorganisms in freezing used in two yellowfin tuna fishing boats. Points are the mean value from two replicates. Bars indicate standard deviation.

fin tuna (*Thunnus albacares*) has high levels of histidine, required amino acid for histamine production.<sup>[10]</sup>, reported a concentration of 956.6 ( $\pm$  53.9) and 378.7 ( $\pm$  29.8) mg of histidine in white and red yellowfin tuna muscle respectively.

Although micro-organisms do not grow at temperatures of freezing (-8 to -10) present in freezing brine, well adapted psychrophilic or psychrotrophic decarboxilant microorganism might grow and to produce histamine, during fluctuation of freezing brine temperature, associated with increasing of tempera-



**Figure 6 :** Changes on histamine concentration in freezing brine used in two yellowfin tuna fishing boats. Points are the mean value from two replicates. Bars indicate standard deviation.

ture of the brine after introducing great volumes of tuna recently caught. Freezing brine temperature might increase to 2 to 4 °C for 12-24 hour according with the register of the fishing boats.

The pH range found in brine of both fishing boats might be appropriated for growing of decarboxilant bacteria and for the production of histamine<sup>[11]</sup>. Reported that histidine decarboxilant enzyme required a pH from 5.9 to 6.8 for activity<sup>[12]</sup>. Found that 25 different decarboxilant bacteria were able to grow and produce histamine in a pH range from 5.8 to 6.5<sup>[12]</sup>. Found that decarboxilant bacteria can grow at pH range from 5.3 to 6.3.

Results obtained in this study suggest that decarboxilant microorganisms, responsible of histamine production, can grow and produce histamine in the freezing brine.<sup>[13]</sup> Reported that histamine was produced in salted makarel even when fish muscle reached 15% NaCl after 6 days of storage at 37°C. Forming of histamine by decarboxilant microorganisms has been reported in Korean salted and fermented products<sup>[14,15]</sup>. This result indicates that decarboxilant bacteria might grow in salt concentration present in the freezing brine of yellowfin tuna fishing boats. Thus freezing brine might be a source of contamination for histaminogenic bacteria associated with histamine formation in tuna fish during further processing. To our knowledge there is not information about this subject in the scientific literature.

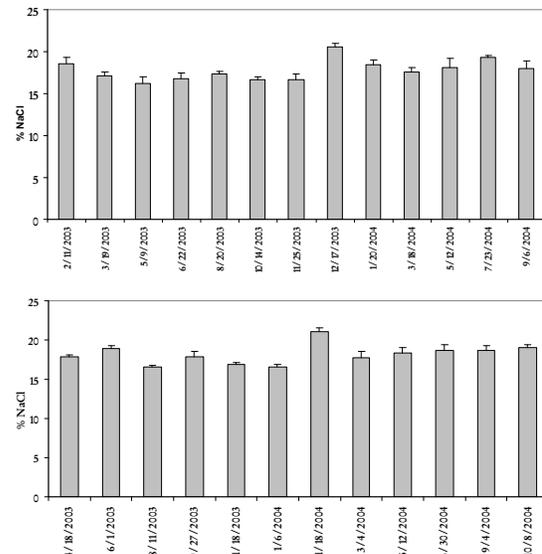
### Tuna muscle Sodium Chloride

The sodium chloride in the freezing brine of both fishing boats varied from 0.86 to 1.57% during the two years for the two fishing boats (Figure 7). No difference in the salt content in tuna flesh of both fishing boats was found ( $p < 0.05$ ). The mean level of salt determined was of 1.25% (+/-0.22%). Salt content in fish muscle must be lower than 1.5%. This results no showed evidence of extensive osmotic exchange of salt into fish muscle. The absence of high osmotic exchange between freezing brine and tuna muscle indicate that histamine formed in freezing brine has a different origin than histamine formed in tuna muscle.

### Histamine level

The level of histamine measured in tuna fish muscles during the two fishing years involved in this study, varied from 25 to 42.9 ppm in FB1 with a mean level of histamine of 36.79 (+/-4.98) ppm (Figure 8). In FB2 the histamine level varied from 29.8 to 50.1 ppm with a mean level of 38.94 (+/-6.40) ppm (Figure 8). In this study tuna fish muscle had a histamine mean level of 37.82 (+/-5.69) ppm for all fishing period.

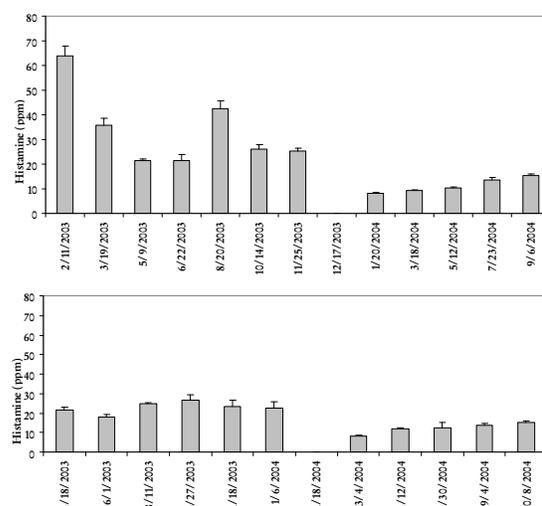
The presence of low values of histamine in tuna fishes has been reported previously<sup>[16]</sup>. Reported 0 to 9.5 ppm of histamine in fresh tune from Spanish



**Figure 7 : Sodium chloride concentration of yellowfin tuna flesh from two yellowfin tuna fishing boats. Points are the mean value from two replicates. Bars indicate standard deviation.**

markets<sup>[17]</sup>. Reported 0 to 17.3 ppm in fresh frozen albacore captured in the US Northwest from 1994 to 1996<sup>[1]</sup>. Reported 10.2 (+/- 6.0) for fresh yellowfin tuna stored at 4°C (analyzed the same day).

Histamine level in yellowfin tuna from this study was obtained from not damaged fishes, thus muscle



**Figure 8 : Histamine concentration of yellowfin tuna flesh from two yellowfin tuna fishing boats. Points are the mean value from two replicates. Bars indicate standard deviation.**

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was sterile and histamine must be formed from biochemical reactions associated with the stress of being fished. Histamine is released during muscle contraction playing a role in the regulation of the microcirculation causing vasodilatation and increasing permeability of capillary vessels<sup>[18,19]</sup>, and might be involved in a defensive response during induced fatigue in skeletal muscle, stimulating sensory nerve endings to produce pain and gastric acid secretion, as part of a defense mechanism preventing damage to the muscle<sup>[20]</sup>.

The histamine content in yellowfin tuna obtained from this study was lower than tolerable maximum contents for histamine established for different countries (50 ppm, USA; 100 ppm European Community; 200 ppm Mexico). Thus, fresh frozen yellowfin tuna, processed appropriately might satisfactorily meet any international regulation for histamine level.

## CONCLUSIONS

Freezing brine used in yellowfin tuna fishing boats contained enough protein level and appropriated pH to allow halophilic decarboxilant microorganism to grow up during temperature fluctuations and to produce histamine. Partial replace of freezing brine (near to 80%) induced a quickly growing of microorganism and forming of high levels of histamine in a short time. Total replace of freezing brine allowed reducing the growing of microorganisms and histamine formation. Thus the practice of a total replace of the freezing brine should be a common practice for fishing tunides. Histamine formed in tuna muscle seemed to have a biochemical origin, associated with the stress of fishing. The level of histamine present in fresh frozen tuna fish was lower than all international quality standards for final products derived from tuna fishes. According with this result, levels of histamine higher than 50 ppm might be associated with problems in quality assurance during processing.

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