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Influence of physical damage and freezing on histamine concentration and microbiological quality of yellowfin tuna during processing

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Abstract

Yellowfin tuna has a high level of free histidine in their muscle, which can lead to histamine formation by microorganisms if temperature abuse occurs during handling and further processing. The objective of this study was to measure levels of histamine in damaged and undamaged thawed muscle to determine the effect of physical damage on the microbial count and histamine formation during the initial steps of canning processing and to isolate and identify the main histamine-forming microorganisms present in the flesh of yellowfin tuna. Total mesophilic and psicrophilic microorganisms were determined using the standard plate method. The presence of histamine-forming microorganisms was determined in a modified Niven's agar. Strains were further identified using the API 20E kit for enterobacteriaceae and Gram-negative bacilli. Physically damaged tuna did not show higher microbiological contamination than that of undamaged muscle tuna. The most active histamine-forming microorganism present in tuna flesh was *Morganella morganii*. Other decarboxylating microorganisms present were *Enterobacter agglomerans* and *Enterobacter cloacae*. Physical damage of tune during catching and handling did not increase the level of histamine or the amount of microorganisms present in tuna meat during frozen transportation, but they showed a higher risk of histamine-forming microorganism growth during processing.

Keywords: yellowfin tuna; histamine; processing; freezing.

1 Introduction

Tuna species are important globally because of their high market demand and high price. The main attributes of these fish species include their flavor, nutritional value, and high lipid content. These fishes have a high proportion of eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids, which are the longest n-3 fatty acids in foods. (SALES, 2010; ROY et al., 2011). Although currently almost all tuna is wild-caught, tuna aquaculture technology continues to develop; one additional objective of this technology is to prevent the depletion of natural stocks by overfishing (ROY et al., 2010; MIYAKE et al., 2011). Despite the nutritive aspect, the consumption of tuna and other fishes belonging to the *Scomberesocidae* and *Scombridae* families has been associated with histamine poisoning, a food-borne illness. These fish species have in common a high level of histidine in their muscle (TSAI et al., 2004; OVISSIPOUR et al., 2011); histidine can be transformed into histamine by decarboxylating microorganisms if microbiological risks are not avoided during processing (BEN-GIGIREY et al., 1999; TSAI et al., 2004; BJORNSDOTTIR-BUTLER et al., 2010; FERNÁNDEZ-NO et al., 2010). Although refrigeration and frozen storage can extend the shelf life of seafood products, there are various psychotrophic bacteria growing in the freezing brine that contaminate tuna. Tuna also have psychotrophic bacteria growing in their gut, which can contaminate the flesh during the gutting process and can increase in numbers if temperature abuse occurs during processing. If decarboxylating microorganisms are present in the flesh, they can induce the formation of high levels of histamine in the final product, and the level of histamine formed is affected by the combination of both time and temperature. The most frequently occurring microorganisms associated with fish histamine poisoning are *Enterobacteriaceae*, *Morganella morganii*, *Klebsiella pneumonia,* and *Hafnia* (KIM et al., 2000). However, a variety of bacteria capable of producing histamine has been identified in fish (KIM et al., 2000; ECONOMOU et al., 2007; CHEN et al., 2008; BJORNSDOTTIR-BUTLER et al., 2010; FERNÁNDEZ-NO et al., 2010). Some countries have established legal limits, or at least tolerable maximum contents for histamine in fish and fish products. The US Food and Drug Administration (FDA) has established a defect action level of 50 ppm for histamine in tuna, mahi-mahi (dolphin), and other fish species as an indication of potential health risk (FOOD..., 1996). The European Community has fixed 100 ppm for fish and fish products as a maximum average value in a group of 9 samples (VECIANA-NOGUES et al., 1995). Mexico has a maximum limit of 200 ppm of histamine for tuna and tuna products.

Frequently, tuna shows physical damage in the flesh because of shipboard handling operations. Some of this damage is associated with crushing due to the great volume caught. In addition, tuna is sometimes damaged by operators during boat offloading (GILL; PENNEY, 1977; CRAVEN et al., 2001). These damaged fish may contain or induce higher histamine

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levels. Currently, there is a lack of sufficient information about both the formation of histamine in yellowfin tuna (*Thunnus albacares*) processed in Mexico and the microorganism involved in the formation of histamine during processing. Yellowfin tuna Canning is the second most economically important fishing industry activity in Mexico.

The objective of this study was to measure levels of histamine in damaged and undamaged thawed tuna muscle to determine the effect of physical damage on the microbial count and histamine formation during the initial steps of canning processing and to isolate and identify the main histamine-forming microorganisms present in the flesh of yellowfin tuna.

2 Materials and methods

2.1 Tuna

Yellowfin tuna (*Thunnus albacar*es) were caught by a Mexican commercial fishing boat in the Pacific Ocean, frozen on board by immersion in freezing brine, and stored at –17 °C. Ten tuna measuring 12/20 were sampled directly from the boat immediately after their arrival by personnel working in the tuna canning industry quality control laboratory in Sinaloa, Mexico. In this study, only fish showing external physical damage on the upper loin of their body (gaps, perforations or cuts in their flesh) were selected.

2.2 Sampling

Using an electric saw previously sterilized with ethanol at 70%, samples of almost one kilogram were cut from the upper loin of each fish; these samples were placed in sterile bags before ice-storing, transported to the laboratory in less than 10 min, and stored at –15 °C until analysis (less than 24 h after the samples arrived at the laboratory). The samples were obtained from both a physically damaged region and an undamaged loin region of the same fish. The samples were stored at -15 °C until analysis.

2.3 HPLC analysis of histamine

The histamine-forming ability of isolated bacteria was determined using the HPLC technique previously described by Pacheco-Aguilar, Lugo-Sánchez and Robles-Burgueño (2000). Briefly, 25 g of fish flesh was homogenized with 100 mL 7.5% TCA at 4 °C for 2 min in a Waring blender. The homogenate was centrifuged at 5,000 × *g* for 15 min using a refrigerated centrifuge (0 to 4 °C). The supernatant was filtered through Whatman paper No. 1, and the filtrate was stored at 0 to 4 °C until use. Histamine was derivatized using 10 mg of OPA (o-phthaldialdehyde) dissolved in 250 µL of methanol, 37.5 µL of 30% Brij 35 solution, and 25 µL of 2-mercaptoethanol. This solution was diluted to 10 mL with 1 M sodium borate buffer (pH 10.4), mixed well, and stored under refrigeration in the dark for 24 h prior to use. Derivatization was performed immediately prior to loading the sample into the HPLC injection loop by mixing 0.25 mL of the OPA solution with a 0.25 mL aliquot of the extract. The solution was passed through a 0.2-µm millipore filter and was injected 2 min after derivatization. HPLC analyses were performed using a Varian high-pressure liquid chromatography equipment (Varian Assoc. Inc. Walnut Creek, Calif., U.S.A.) consisting of a Model 9102 pump, a Rheodyne Model 7125 injection valve with a 10 µL capacity loop (Rheodyne Inc., Catati, Calif., U.S.A.), and a Varian fluorescence detector Model 9070. Fluorescence was monitored at 350 nm for excitation and 450 nm for emission wavelengths. A Beckman Ultrasphere ODS RP 18 reverse-phase column (5 µm, 250 mm × 4.6 mm i.d.) (Beckman Instruments, Inc., Fullerton, Calif., U.S.A.) was used for the separation. The mobile gradient phase consisted of methanol and 0.1 M sodium acetate buffer pH 6.2 [(1L + 10 mL tetrahydrofuran (THF)]. Flow rate was set at 1.2 mL/min. Chromatogram recordings and integrations were calculated using Varian 3.4 software (Varian Assoc. Inc. Walnut Creek, Calif., U.S.A.). Quantification was done using a- aminobutyric acid (0.05 µmol/mL) as an internal standard.

2.4 Total viable count

Microbiological analysis was determined by blending 10 g of muscle with 90 mL peptone water (0.1%). An agar plate count was carried out in duplicate in accordance with 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 that had been supplemented with 0.5% NaCl at 50 °C. Total counts of psychrophilic microorganisms were obtained incubating at 5 °C for 10 days, and total counts of mesophilic microorganisms were obtained incubating at 37 °C for 48 h.

2.5 Histamine-forming microorganisms

A histaminic-forming population was determined using the technique described by Niven Junior, Jeffrey and Corlett Junior (1981). Ten grams of muscle were homogenized as previously described for total viable count, and 1 mL of suitable dilutions was inoculated in modified Niven's agar containing 0.5% triptone, 0.5% yeast extract, 2.0% L-histidine·2HCl, 0.5% NaCl, 0.1% Calcium carbonate, 3.0% bacteriologic agar (Bioxon), and 0.006% bromocresol purple; pH was adjusted to 5.5 with 0.1 N HCl. Petri dishes were incubated at 5 °C for ten days to determine the presence of psychrophilic microorganisms, and the presence of mesophilic microorganisms were determined by incubating at 37 °C for 48 h. Colonies showing a purple halo were considered to be histamine-forming bacteria.

2.6 Isolation and identification of histamine-forming bacteria

Colonies showing a purple halo with different morphological characteristics (form, elevation, color and halo intensity) were selected and inoculated into modified TSAH (tryptose-soy-casein agar) medium supplemented with 0.1% of L-histidine at pH 7.0, in accordance with López-Sabater et al. (1996a) and Du et al. (2002).

Strains were further identified by the use of the API 20E kit for enterobacteriaceae and Gram-negative bacilli (Biomerieux, Marcy L'Etoile, France) and biochemical tests (Gram-negative,

catalase production, oxidase activity, motility, nitrate reduction, and nitrogen production).

2.7 Histamine production

The capability of producing histamine was determined by inoculating isolated histamine-forming microorganisms in a medium containing 400 g/L of tuna flesh, 10 g/L of peptone and 25 g/L of histidine. The medium was sterilized before inoculation at 121 °C for 15 min (NIVEN JUNIOR; JEFFREY; CORLETT JUNIOR, 1982).

2.8 Statistical analysis

Statistical analysis was performed using Statgraphics 5.0 (Software Publishing Corporation, Bitstream Inc.). LSD's multiple range tests were used to determine significant differences ($P < 0.05$) among the treatments.

3 Results and discussion

3.1 Microbiological analysis

The influence of physical damage in the content of total viable count and histamine-forming microorganisms in the flesh of thawed tuna just before processing was determined. These results are shown in Table 1.

The damaged flesh showed a higher level of mesophilic microorganisms (1.7×10^5 CFU/g) than that of the undamaged flesh (9×10^4 CFU/g); however, this difference was not significant ($P \leq 0.05$). Guillén-Velasco et al. (2004) found that bluefin tuna skin showed a higher total viable count than that of the muscle. The origin of the external contamination of tuna is associated with the contamination of the seawater, freezing brine, handling operations, and fresh water used for thawing tuna (GILL; PENNEY, 1977; CRAVEN et al., 2001; LORCA et al., 2001).

Damaged and undamaged tuna flesh samples showed high levels of mesophilic histamine-forming bacteria (5×10^3 and 5.3×10^3) (Table 1). Psychrophilic histamine-forming microorganisms were not detected in any sample. Histamine-forming bacteria are mesophilic microorganisms whose histamine-forming ability is affected by storage temperature. These microorganisms have been reported to reach minimal activity at temperatures lower than 4 °C, with no activity at 0 °C (LÓPEZ-SABATER et al., 1996b, SILVA; DA PONTE; ENES-DAPKEVICIUS, 1998; KIM; AN; PRICE, 1999; DU et al., 2002; BERMEJO et al., 2003). The histidine decarboxylase enzyme is fully inactivated at temperatures below –8 °C (BURNS, 1985).

Table 1. Microbiological count and histamine level in tuna muscle.

Parameter	Undamaged flesh	Damaged flesh
Mesophilic aerobic (CFU/g)	$9 \times 10^{4\,a}$ (4.9×10^4)	$1.7 \times 10^{5\,a}$ (9.7×10^4)
Mesophilic histamine-forming bacteria (CFU/g)	$5 \times 10^{3\,a}$ (2.3×10^3)	$5.3 \times 10^{3\,a}$ (2.5×10^3)
Psychrophilic histamine-forming bacteria (CFU/g)	0	0
Histamine level (ppm)	40 (8.8)[a]	49 (9.6)[a]

[a,b]letters indicate significant differences between treatments ($P \leq 0.05$). Mean values of analysis of 9 tuna samples in duplicate. Values in parentheses show the standard deviation.

The flesh of damaged tuna showed similar total viable count but equal histamine-forming bacteria levels when compared with undamaged tuna. Aside from these findings, there was no significant difference ($P < 0.05$) in the concentration of histamine between the damaged (36-53 ppm) and undamaged tuna muscle (34-59 ppm) (Table 1). These results indicated that physically damaged muscle was not more susceptible to microbiological contamination during frozen transportation, thus indicating that at low temperature there is a minimal formation of histamine. Three of the tuna fish samples showed levels of histamine higher than the upper limit established for the FDA (50 mg-kg) (FOOD…, 1996).

3.2 Identification of histamine-forming bacteria

Colonies of microorganisms with different morphological aspects forming a purple halo in Niven's medium were selected and identified with the API 20E kit system used to detect enterobacteriaceae and Gram-negative bacilli. Niven Junior, Jeffrey and Corlett Junior (1981) found that histamine-forming microorganisms isolated in his study were mesophilic bacteria from the *Enterobacteriaceae* family, and according to MacFaddin (1990), Enterobacteriaceae bacteria are Gram-negative, catalase-positive, and oxidase-negative. Further biochemical tests indicated that all isolated strains were Gram-negative, catalase-positive, oxidase-negative, and showed no motility. A total of twelve different colonies were analyzed, and the results obtained are shown in Table 2. Identified colonies included three *Enterobacter agglomerans,* two *Enterobacter cloacae,* three *Morganella morganii* colonies, and four other unidentified colonies.

Morganella morganii has been reported as a short bacilli Gram-negative, catalase-positive, and oxidase-negative (KIM et al., 2001) bacterium frequently found in tuna and tuna products, including yellowfin tuna, associated with the presence of histamine. *Morganella morganii* is considered one of the most efficient histamine-forming bacteria and has been associated with several histamine outbreaks resulting from tuna consumption (BJORNSDOTTIR-BUTLER et al., 2010, 2011; FERNÁNDEZ-NO et al., 2010).

Enterobacter cloacae has been frequently reported as a histamine-forming bacterium present in different tuna species, including yellowfin and bluefin tuna. *Enterobacter agglomerans* has rarely been reported as a histamine-forming bacterium associated with tuna, but it has been found in yellowfin tuna (DU et al., 2002), bluefin tuna (LÓPEZ-SABATER et al., 1994), and commercial tuna products (TAYLOR et al., 1979).

3.3 Histamine-production ability

The histamine-production ability of the three identified bacteria was determined by inoculating each of them in a medium containing 25 g/L of histidine (tuna flesh, water, histidine, and peptone) and by incubating them at 37 ± 2 °C for 24 hours. The changes in concentration of histidine and

Table 2. Microorganisms identified using the API 20E kit and biochemical tests.

No	Microorganism	Grammm	Motility	Form	Catalase	Oxidase	NO_2	N_2
1	*Enterobacter agglomerans*	–	–	Bacilly	+	–	+	–
2	*Enterobacter agglomerans*	–	–	Bacilo	+	–	+	–
3	*Enterobacter agglomerans*	–	–	Bacilo	+	–	+	–
4	*Enterobacter cloacae*	–	–	Bacilo	+	–	+	–
5	*Enterobacter cloacae*	–	–	Bacilo	+	–	+	–
6	*Morganella morganii*	–	–	Bacilo	+	–	+	–
7	*Morganella morganii*	–	–	Bacilo	+	–	+	–
8	*Morganella morganii*	–	–	Bacilo	+	–	+	–
9	Not identified	–	–	Bacilo	+	–	+	–
10	Not identified	–	–	Bacilo	+	–	+	–
11	Not identified	–	–	Bacilo	+	–	+	–
12	Not identified	–	+	Bacilo	+	–	+	–

Table 3. Histamine-production ability of strains isolated from yellowfin tuna.

Presumptive strain	Histamine production (ppm in 24 h)
Enterobacter agglomerans	446.90
Enterobacter agglomerans	420.38
Enterobacter agglomerans	435.12
Enterobacter cloacae	499.35
Enterobacter cloacae	418.20
Morganella morganii	5802.91
Morganella morganii	6034.89
Morganella morganii	6245.42

the increased histamine in the medium were determined using HPLC. Results are shown in Table 3. Although all the microorganisms studied produced histamine, their ability to transform histidine into histamine varied. All three species produced a high level of histamine during the first 24 hours. *Enterobacter agglomerans* and *Enterobacter cloacae* were less efficient than *Morganella morganii* in transforming histidine into histamine under the conditions used in this study (Table 3). *Enterobacter agglomerans* transformed only 5.1 to 5.8% of the histidine contained in the medium (10,000 ppm) into histamine, and *Enterobacter cloacae* transformed 4.4 to 5.2%. *Morganella morganii* transformed 61.5 to 73.7% of the histidine into histamine. The higher ability of some microorganisms to convert histidine into histamine depends strongly on their enzymatic activity and their ability to grow in different environmental systems (EDMUNDS; EITENMILLER, 1975; KIM et al., 2002).

Morganella morganii has been reported as the most efficient decarboxylating microorganism in different studies (LÓPEZ-SABATER et al., 1994; LORCA et al., 2001; KIM et al., 2001, 2002; RODRÍGUEZ-JEREZ et al., 1994: DU et al., 2002)

4 Conclusion

The flesh from physically damaged tuna showed higher total viable counts but an equal number of mesophilic histamine-forming bacteria than the flesh of undamaged tuna, indicating that the inappropriate handling of tuna during the processes of catching, freezing, transporting, and discharging greatly influences microbiological contamination. The histamine level of just-thawed tuna was lower than 50 ppm, and there was no presence of psychrophilic histamine-forming bacteria in any of the tuna samples investigated, and there was no difference in histamine level between damaged and undamaged muscles right after thawing. Therefore, histamine levels higher than 50 ppm in final products can be associated with temperature abuse conditions during processing. *Morganella morganii* was the most active histamine-forming bacterium found in yellowfin tuna.

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