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COMMUNICATION

Histamine formation and microbiological changes in endemic *Chalcalburnus tarichi* Pallas 1811 (Inci Kefali) stored at 4 °C

Formación de histamina y cambios microbiológicos en *Chalcalburnus tarichi* Pallas 1811 (Inci Kefali) endémico almacenado a 4 °C

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RESUMEN

El objetivo de este estudio fue medir la formación de histamina y los cambios microbiológicos en *Chalcalburnus tarichi* fresco procedente del lago Van y almacenado a 4 ºC por un período de hasta 15 días. Muestras del músculo de los pescados fueron tomadas en intervalos de tiempo (días 1, 5, 7, 9, 11, 13 y 15) durante el almacenaje. El contenido de la histamina fue determinado usando un método espectrofluorométrico y el conteo total y características de las *Enterobacteriaceae* y *Pseudomonas* spp. presentes en las muestras fueron establecidas por procedimientos microbiológicos estándares.

La concentración inicial de histamina era de 27,5 mg/kg, aumentando gradualmente hasta 134,38 mg/kg en el día 15. El conteo bacteriano viable total varió de $8{,}0x10^2$ a $9{,}0x10^9$ ufc/g. Las *Enterobacteriaceae* estaban en el rango de $2{,}0 \times 10^2$ a $6{,}5 \times 10^9$ ufc/g, mientras las *Pseudomonas* spp. estaban entre $3{,}0 \times 10^2$ a $7{,}3 \times 10^9$ ufc/g.

Palabras clave: histamina, calidad microbiológica, *Chalcalburnus tarichi*

Key words: histamine, microbiological quality, *Chalcalburnus tarichi*

INTRODUCTION

The ingestion of foods containing substantial amounts of histamine is the cause of food poisoning episodes, a type of foodborne illness commonly associated to consumption of scombroid fish such as tuna and mackerel and some nonscombroid fish such as bluefish or mahi-mahi that have begun to spoil by the growth of particular types of bacteria (Merson *et al* 1974, Beling and Taylor 1982, Hughes *et al* 1991).

Biologically active amines have been found in many foods such as fish and fish products (Merson *et al* 1974, Shalaby 1994, Becker *et al* 2001). Biogenic amines are organic bases with an aliphatic, aromatic, or heterocyclic structure that has been found in many foods that include fish and fish products, cheese, wine, beer and other fermented foods (Stratton *et al* 1991, Hernández-Jover *et al* 1997).

Histamine is formed by bacterial enzymatic decarboxylation of histidine during the final steps of protein breakdown (Koehler and Eitenmiller 1978). Bacterial strains known to be capable of histamine production include *Escherichia, Enterobacter, Pseudomonads*, *Salmonella, Shigella, Clostridium perfringens, Streptococcus, Lactobacillus* and *Leuconostoc* (Edwards and Sandine 1981, Chang *et al* 1985, Stratton *et al* 1991, Santos *et al* 1998). Histamine may be involved in the onset of migraine attacks in susceptible subjects and may produce hypertensive crises in patients treated with monoamine oxidase inhibitor-type drugs (Khalid and Marth 1990).

In addition to its toxicological properties, histamine is of interest as an indicator of food quality (Bover-Cid *et al* 2001, Ekici *et al* 2004) and of food spoilage (Valsamaki *et al* 2000). The minimal concentration of histamine in foodstuffs that would elicit a toxic response has been estimated to be 100 mg/100g (Taylor *et al* 1978).

Fish are of great importance for human nutrition worldwide. Non-pathogenic and pathogenic bacteria have been found on the skin, gills and intestines of fish (Feldhusen 2000).

Lake Van is the largest soda lake and fourth largest closed lake on earth. It is situated at 1,648 m above sea level in Eastern Anatolia, Turkey. Many springs and freshwater rivers flow into this lake. Its high carbonate content makes it extremely alkaline, with a pH = 9,8 that is unsuitable for many fresh water species with exception of a fish locally known as "Inci Kefali" or Pearl Mullet, *Chalcalburnus tarichi*, a member of the cyprinidae family (Arabaci and Sari 2004).

To date, there is no detailed information available on histamine formation and microbiological quality of Inci Kefali. The purpose of this study was to measure the amount of histamine and microbiological changes in pearl mullet for 15 days while stored at 4 °C to determine if this is a safe practice for handling this product, in order

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to evaluate whether the level of histamine and microbial count would pose a health hazard.

MATERIAL AND METHODS

Inci Kefali were caught from Van lake. Whole and uneviscerated fresh fish were about 25 kg weight, placed immediately in an ice box and delivered to the laboratory approximately 3-5 h later, where they were immediately divided into eight lots weighing about 3 kg in sterile bags each. The bags were stored for 15 days at a constant temperature of 4 °C. At predetermined times, samples of five to six Inci Kefali were randomly drawn from each lot for analyses. Samples were periodically taken every other day (1st, 3rd, 5th, 7th, 9th, 11th, 13th and 15th) throughout the experimental period and used for the determination of histamine and bacteriological analysis. All assays were done in duplicate and the results are given as average of these two values.

Histamine analysis. Histamine was analyzed spectrofluorimetrically following the procedure of Lerke and Bell (1976). The fluorescence of the compound was measured by means of a Luminescence spectrofluorometer (Perkin Elmer LS 50 B Wellesley, USA), using an excitation wavelength of 350 nm and an emission wavelength of 450 nm. Standard curves were automatically obtained by the spectrofluorometer from known solutions. The results were expressed as mg/kg wet weight of fresh fish muscle sample.

Microbiological analysis. For microbiological analysis 10 g fresh muscle fish samples were weighed in stomacher bags to which 90 ml sterile saline solution with peptone (0.85% NaCl + 0.1% peptone) were added. The samples were then homogenized in the bags for 2 min. After homogenization, serial decimal dilutions were prepared up to 10^9 that were used for inoculating the growth media in Petri dishes. For the total viable count, the drop plate technic on the plate count agar (PCA) (Oxoid CM325) at 35 °C for 72 hours, *Enterobacteriaceae* violet red bile agar (VRBA) (Oxoid CM485) pour plate technic at 37 °C for 48 hours, *Pseudomonas spp*. the drop plate technic on pseudomonas agar base (PAB) (Oxoid CM559), at 25 °C for 48 hours was used. After inoculation, the colonies formed were counted (Pichhardt 1993).

RESULTS AND DISCUSSION

The results are shown in figures 1 and 2. As can be seen from figures the concentration of histamine and microorganisms increased steadily during the two weeks storage. Formation of biogenic amines depends on the concentration of amino acids or peptides which act as precursors in food (Voight *et al* 1974, Leuschnert and Hammes 1998). Recent studies have shown that *Morganella morganii* can be a contaminant of fish tissue during spoilage and that few

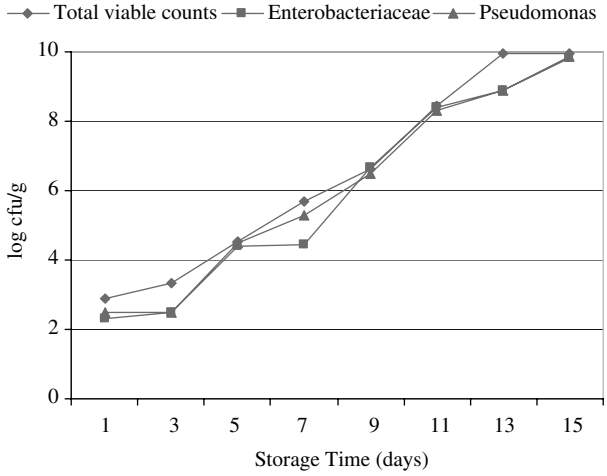

Figure 1. Microbiological analyses of *Chalcalburnus tarichi* stored 1-15 days at 4°C.

Análisis microbiológicos de *Chalcalburnus tarichi* almacenado 1-15 días a 4 °C.

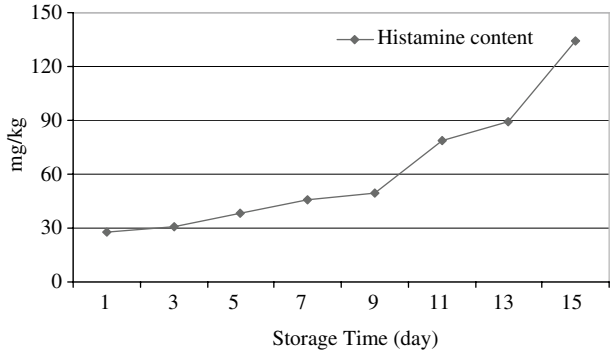

Figure 2. Histamine content of *Chalcalburnus tarichi* stored 1-15 days at 4°C

Contenido de histamina en *Chalcalburnus tarichi* almacenado 1-15 días a 4 °C.

other bacteria have as great a capacity to form histamine (Eitenmiller *et al* 1981). Amine-degrading activities were found in *Brevibacterium linens* and coryneform bacteria (Leuschnert and Hammes 1998). The presence of some decarboxilase-positive spoiling microorganisms such as *Pseudomonas*, *Micrococci* and *Enterococci* that can result in histamine formation have been cited as possible cause of this increase (Santos *et al* 1998).

There are several studies linking bacterial contamination to histamine formation in aquatic species. Tekinsen *et al* (1993) investigated histamine formation in three aquatic species. After 3 days of storage at 4 °C, the histamine content in mackerel, bonito and anchovy was 6.28, 3.70 and 3.25 mg/kg, respectively, well below toxic or dangerous levels. Edmunds *et al* (1975) also reported that histamine formation in Spanish mackerel, white shrimp, common

Table 1. Results of microbiological analyses of *Chalcalburnus tarichi* stored 1-15 days at 4°C.
Resultados de los análisis microbiológicos de *Chalcalburnus tarichi* almacenado 1-15 días a 4 °C.

Storage time Days	Total viable counts cfu/g	Enterobacteiaceae cfu/g	Pseudomonads spp. cfu/g
1	8.0×10^2	2.0×10^2	3.0×10^2
3	2.2×10^3	3.0×10^2	3.0×10^2
5	3.4×10^4	2.6×10^4	3.3×10^4
7	5.2×10^5	2.8×10^4	2.0×10^5
9	4.2×10^6	4.5×10^6	3.0×10^6
11	2.8×10^8	2.4×10^8	2.0×10^8
13	9.0×10^9	8.1×10^8	8.2×10^8
15	9.0×10^9	6.5×10^9	7.3×10^9

mullet, speckled trout and channel catfish did not increase in any of these species after 14 days storage even though extensive psychrophilic spoilage had occurred judged by appearance and odor evolution. Lopez-Sabater *et al* (1995) found that under some conditions the level of histamine was higher than the 50 mg/100 g level established by the FDA for tuna fish after 21 days storage at 0, 8 and 20 °C. *Enterobacteriaceae* and *Pseudomonas* ssp. are not part of the normal microflora in fresh fish, but they grow rather quickly in aerobically stored fish (Lougovois *et al* 2003, Behling and Taylor 1982). *Pseudomonas* can be the dominant spoilage bacteria in fish from warmer waters and the maximum histidine decarboxylase activity was observed during the late logarithmic phase of growth (Falcao *et al* 2002).

Establishment of a precise toxicity threshold of histamine in individuals is extremely difficult because the toxic dose is strongly dependent on the efficiency of the detoxification mechanisms of each individual. Nevertheless, toxicological levels have been proposed, such as 10-100 mg of histamine per 100 g of food (Stratton *et al* 1991, Hernandez-Jover *et al* 1997). The FDA has set the hazardous level for histamine in tuna at 50 mg histamine/100g (Sumner *et al* 1990, Stratton *et al* 1991). The histamine content of Inci Kefali in our study was lower than the FDA's hazardous level for histamine in tuna at 50 mg histamine/100g. At present, there is no limitation of histamine for fish in theTurkish food codex.

The initial quality of the pearl mullet in our study was good, as indicated by a low initial bacterial count before storage. All counts increased during storage and spoilage was mainly due to psychrotrophic bacteria.

The number of total viable counts on the first day was 8.0×10^2, increasing to 9.0×10^9 cfu/g. *Enterobacteriaceae* increased from 2.0×10^2 on the 1st day to 6.5×10^9 cfu/g and that of *Pseudomonas* from 3.0×10^2 to 7.3×10^9 cfu/g by the 15th day. These results are comparable to those reported for tuna by Lopez-Sabater *et al* (1995).

Total viable count is an important tool to assess the quality of foodstufffs. It is considered an indicator for the lifecycle of fish, constituting a criterion in the determination of the general microbiological quality of the product (Altug and Bayrak 2003). It has been stated that a total viable count above 10^8 cfu/g may lead to food spoilge in smoked fish (Pichhardt 1993). The total viable count values here reported indicate that the pearl mullet samples were on the threshold of critical limits at the 11th day of storage.

CONCLUSIONS

It can be concluded that the histamine levels in Inci Kefali samples hygienically stored for up to 15 days at 4°C do not seem to pose a health hazard. As microorganisms readily propagate in fish, proper sanitary care should be taken during handling. There is, however, a potential risk depending from individual susceptibility to histamine, some gastrointestinal conditions, alcohol consumption or the use of certain medicines that may influence the biogenic amines detoxification routes.

SUMMARY

Histamine accumulates in food via microbial decarboxylation of histidine. Small amounts of histamine naturally occurring in food under normal circumstances do not pose a public health hazard. Certain microbial species such as *Enterobacteriaceae* and *Pseudomonas spp*. have considerable capacity for histamine formation and can proliferate during handling or processing of foodstuffs, possibly elevating the histamine content to a harmful level. The objective of this study was to measure the formation of histamine and microbiological changes in fresh *Chalcalburnus tarichi* from Van lake and stored at 4 °C for up to 15 days. Fish muscle samples were taken on day 1, 3, 5, 7, 9, 11, 13 and 15 of experiment, during storage. Histamine content was determined using a spectrofluorometric method and the total count and features of *Enterobacteriaceae* and *Pseudomonas spp.* present in the samples were established by standard microbiological procedures. The initial concentration of histamine was 27.5 mg/kg, increasing gradually up to 134.38 mg/kg on day 15. Total viable bacterial count varied from 8.0×10^2 to 9.0×10^9 cfu/g. *Enterobacteriaceae* was in the 2.0×10^2 to 6.5×10^9 cfu/g range, while *Pseudomonas spp.* was in the 3.0×10^2 to 7.3×10^9 cfu/g range.

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