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 °C por un periodo 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 8,0x10² a 9,0x10⁹ ufc/g. Las Enterobacteriaceae estaban en el rango de 2,0 x 10² a 6,5 x 10⁹ ufc/g, mientras las Pseudomonas spp. estaban entre 3,0 x 10² a 7,3 x 10⁹ 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 spectrophotometer (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 spectrophotometer 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 10⁶ 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 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 decarboxylase-positive spoiling microorganisms such as Pseudomonas, Micrococi 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
The number of total viable counts on the first day was 8.0 x 10^2, increasing to 9.0 x 10^6 cfu/g. *Enterobacteriaceae* increased from 2.0 x 10^2 on the 1st day to 6.5 x 10^9 cfu/g and that of *Pseudomonas* from 3.0 x 10^2 to 7.3 x 10^9 cfu/g by the 15th day. These results are comparable to those reported for tuna by Lopez-Sabater et al. (1995). 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.

**REFERENCES**


Bover-Cid S, M Izquierdo-Pulido, MC Vidal-Carou. 2001. Changes in 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 x 10^2 to 9.0 x 10^6 cfu/g, *Enterobacteriaceae* was in the 2.0 x 10^2 to 6.5 x 10^9 cfu/g range, while *Pseudomonas* spp. was in the 3.0 x 10^2 to 7.3 x 10^9 cfu/g range.

**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 x 10^2 to 9.0 x 10^6 cfu/g, *Enterobacteriaceae* was in the 2.0 x 10^2 to 6.5 x 10^9 cfu/g range, while *Pseudomonas* spp. was in the 3.0 x 10^2 to 7.3 x 10^9 cfu/g range.

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