Journal of Food Technology 3 (1) 72-75, 2005

Microbiological Analysis of Retail Fresh Chalcalburnus Tarichi Caught from Van Lake in Turkey

Amil Ekici and Hunkar Avni Duyar[1]
Department of Food Hygiene and Technology,Veterinary College,University of Yüzüncü Yil,65080 Van/Turkey,[1]Department of Fisheries, Agricultur Faculty, University of Yüzüncü Yil, 65080 Van/Turkey

Abstract: In this study, it was aimed to analyze Chalcalburnus tarichi samples caught from Van lake of Turkey for microbiological quality. For this aim, the samples were analysed in term of total viable count, Lactic acid bacteria, Coliforms, Staphylococcus aureus, Salmonella spp. and yeasts. Total viable counts varied from 10^3 to $2.6x10^6$ cfu/g, Lactic acid bacteria varied from $<10^1$ cfu/g to $5.3x10^3$ cfu/g, Coliforms varied from $<10^1$ cfu/g to $6.4x\ 10^3$ cfu/g, yeasts varied from $<10^1$ cfu/g to of $5.3x10^3$ cfu/g, S. aureus varied from <10 cfu/g to $4.4x\ 10^3$ cfu/g. Only in one sample Salmonella spp., was detected as positive.

Key words:- Salmonella spp, Chalcalburnus tarichi, microbiological quality

Introduction

Indicator micro-organisms are used to evaluate the hygienic condition of foods, including fish, and the possible presence of pathogens. The coliforms group, E. coli and the total count of heterotrophic microorganisms may in some instances reflect the sanitary quality of food (Rompre *et al.* 2002; Falcao *et al.* 2002). Fish are of great importance for human nutrition worldwide. The microbial status of seafood after catch is closely related to environmental conditions and microbiological quality of the water, water temperature, salt content, distance between localisation of catch and polluted areas (human and animal faeces), natural occurrence of bacteria in the water, ingestion of food by fish, methods of catch, and chilling conditions. Nonpathogenic and pathogenic bacteria were found on the skin, the gills and in the intestines of fish (Feldhusen 2000). Live fish may be contaminated with a number of pathogenic bacteria normally found in the aquatic environment. They can also function as carriers of several microbial and other health hazards. Fish have also been recognized as carriers of health hazards such as disease causing microorganisms Salmonella spp. Several pathogenic microorganisms have been recognized to jeopardise the safety of fishes (Beckers *et al.* 1985; Koulikovski and Matyas 1985; Bean and Griffin 1990; D'Aoust 1994; Moosel *et al.* 1998; Feldhusen, 2000; Huss *et al.*, 2000).

The contamination of fish by these pathogens can occur prior to harvest, during capture, processing, distribution and/or storage. Understanding the profound influence of factors such as environment, process, and distribution conditions on fish, a need has been felt for quality assurance and adoption of standards in the fish industry in order to safeguard the health of the consumer. For example, a European Union Directive (91/492) has laid down rules that live bivalves must contain fewer than 300 faecal coliforms or fewer than 230 *E. coli* per 100 g shellfish flesh and contain no Salmonella spp. (Lyhs *et al.* 2001 and Venugopal 2002)

Inci kefali (Chalcalburnus tarichi, Pallas 1811) is an important species caught from the Van lake of Turkey. According to National Statistical Institute data, Inci kefali was caught from this lake as 14.1 ton (Odabasoglu 1993). Inci kefali are consumed most commonly as fresh, and less as frozen and salted in Turkey.

Chalcalburnus tarichi (Pallas, 1811) fish undergoes many processing steps, which can result in increased microbial contamination. This is significant from the hygienic point of view since this fish is eaten widely in Van area. In addition, the increasing popularity of Inci kefali emphasises the need for detailed microbiological quality and spoilage pattern research. No research work has been carried out dealing with the development of pathogen and bacterial counts during selling period.

The purpose of this study was to evaluate the microbiological quality of the Inci kefali caught from the Van lake of Turkey and as whole sold in Van retail seller. I twas purposed to determine the existing state of the samples in respect of their bacteriological quality and human health and to attract attention to the possible conditions that lead to the proliferation of bacteria.

Materials and Methods

Materials Fish samples caught from Van lake, eastern part of Turkey, were purchased randomly from lokal fish retail sellers about 3 kg every time in sterile bags and were directly transported to the laboratory. Sampling was performed two time every month for a period of 11 months starting March 2003 to January 2004. Assays were done on duplicate samples with the results being averaged.

Microbiological Analysis The test sample from whole Inci kefali was prepared by aseptically making incision and cutting this plug of tissue from the skeletal frame.

A 10-g portion of minced flesh was aseptically weighed into 90 ml of 0.9% NaCl (w/v) and 0.1% (w/v) peptone water (Difco, 0118-17-0) in a sterile plastic bag, and then blended in a Stomacher (IUL Instruments Masticor) for 30 s. Ten fold serial dilutions were used for microbiological analysis. From the 10^{-1} dilution, other decimal dilutions were prepared (Lyhs *et al.*, 2001). Total viable count was determined by using pour plate method. Plate Count Agar (Difco, 0479-17) was used as medium (Harrigan and McCance 1976). Plates were incubated at 30°C for 24-48 h.

For *Salmonaella* analysis 25 g of a sample and 225 ml buffered peptone-water were homogenized using a Stomacher (IUL Instruments Masticor) for 30 s. The analysis were performed by depends on the principles of preculturing for 18–24 h in Buffered Peptone-Water at a temperature of 35–37°C, the selective culturing for 24 h in Selenite Cystine Broth at a temperature of 35 °C, and the identification of the suspected colonies through the current biochemical tests (FDA 1998; Harrigan 1998).

Lactic acid bacteria were determined using overlaid plates of MRS agar (Oxoid) and Staphylococcus aureus: 10 g of sample and 90 ml peptone-water were homogenized using a Stomacher(IUL Instruments Masticor) for 30 s. Further 10-fold dilutions (10^2, 10^3) were prepared and analysed. The analysis depends on the principles of incubation for 24–48 h in Baird Parker Agar at a temperature of 35–37 °C. Then suspected colonies were Gram stained, and tested for catalase activity, fermentation of glucose and mannitol, coagulase and thermonuclease production. (Gonzalez-Rodriguez *et al.* 2002). *Yeasts:* 10 g of sample and 90 ml peptone water were homogenized and further 10-fold dilutions were made. The analysis depends on the principles of incubation for 5 days in Potato Dextrose Agar used for at a temperature of 25 °C, yeasts colonies were counted separately (Altug and Bayrak 2003). Coliforms: The analysis of Coliforms were carried out in accordance with the reference of Harrigan, 1998

Results and Discussion

The limitation of the microbiological development and the optimization of the factors that prevent this development are of great importance in respect of the maintenance of food safety related with the food preservation. The analysis findings for the samples studied with the analysis for total viable count, *Coliforms*, *Salmonella* spp. *S. aureus*, *Lactic acid bacteria* and *yeasts* are summarized in Table 1.

In the course of the study *Chalchalburnus tarichi* caught from Van lake in term of microbiological quality were examined. In our study, total viable count has been found at levels of 7.6×10^6 cfu/g for the total samples. This level does not exceed the maximum level (10^7/g) of the acceptibility of fresh or frozen fish, as recommended International Commission of Microbiological Standarts for Foods (ICMSF 1978). Quality assesment by means of total viable count is an important matter. It has been stated that the total viable count is found below 10^7 cfu/g for the foods leading to food-poisoning and the relevant count is generally found at the level of 10^6–10^7 cfu/g (Altug and Bayrak 2003). Notwithstanding that the is considered as a quality criterion for the food samples. It has been known that the total viable count is an indicator for the lifecycle of the fish and the potential for growth of the microorganism present. It constitutes a criterion in the determination of the general microbiological quality of the product (FAO 1992). The total viable count values obtained as a result of the study indicate that the Inci kefali samples are on the verge of exceeding the critical limits specified in respect of microbiological quality.

Coliform organisms and S. aureus are good indicators of the standard of hygiene and handling. The mean value obtained in samples in relation with the existence of Coliform bacteria that showed a variation within the range of minimum <10 cfu/g and maximum of 6.4×10^3 cfu/g and with an average level of 2.3×10^3cfu/g. Since maximum level of fecal coliform bacteria in fresh or frozen fish was given by ICMSF, it is not possible to put forward an idea for coliform bacteria count in fresh inci kefali. According to Harrigan and McCance (1976), since coliform bacteria count should be less than 200/g in salt-water fish, it was found that coliform bacteria count of fresh inci kefali exceeded the maximum level. The existence of the *Coliforms*, has been considered as leading to the fact that the product was subject to process under inefficient hygiene conditions (Harrigan and McCance, 1976; Altug and Bayrak,2003). The mean value obtained in samples in relation with the existence of *S. aureus* that showed a variation within the range of minimum <10 cfu/g and maximum of 4.4×10^3 cfu/g and with an average level of 1.3×10^3 cfu/g. The results obtained, indicates that the fish samples subjected to inspections have had the characteristic that are detrimental for the bacteriological quality durind selling and caucghing processes.

There was detected for one sample *Salmonella spp.* The isolation of *Salmonella spp.* from fish samples that sold in retail seller was of significance. The disease caused by *Salmonella spp.* is related to a growing global epidemic. A series of studies demonstrated the ability of these bacteria to survive at low temperatures (Beckers *et al.*, 1985; Koulikovskii and Matyas 1985; D' Aoust 1994; Falcao *et al.*, Dias *et al.*, 2002; Huss *et al.*, 2000). It is important to point out that the fish sold in retail seller to fish and, therefore, it is possible that the source of contamination was a improperly cleaned equipment involved in the caughting and selling process. The presence of *Salmonella spp.* demonstrates the importance for public health of caughting and selling fish under strict hygienic conditions. These

Table 1:Microbial Quality of Chalchalburnus Tarichi According to $\log_{10}$ Unit

Months	n = 11	Total Variable counts	Coliforms	Lactic acid bacteria	S. aureus	Salmonella spp.	Yeasts
March	1	4.50	2.0	<1.00	<1.3	Nd	<1.00
April	1	6.04	1.5	<1.00	<1.3	Nd	<1.00
May	1	3.85	<1.0	2.43	<1.3	Nd	<1.00
June	1	5.83	3.6	3.49	<1.30	Nd	3.07
July	1	6.64	3.8	3.72	<1.30	Nd	3.20
August	1	4.47	3.4	1.81	2.00	+	2.66
September	1	3.47	3.4	2.47	1.14	Nd	2.15
October	1	3.36	2.7	2.17	<1.30	Nd	1.95
November	1	4.47	3.4	3.47	1.60	Nd	3.47
December	1	6.44	3.0	3.65	<1.30	Nd	3.53
January	1	5.43	3.5	2.50	<1.30	Nd	3.64
Max.		6.64	3.8	3.72	2.00	-	3.64
Min.		3.36	<1.0	<1.00	1.14	-	<1.00
Mean		4.95	2.8	2.51	1.37	-	2.42

Nd: Not determination

results demonstrate that Chalchalburnus tarichi is an important potential source of pathogenic enterobacteria presenting a risk to consumers

Microorganisms are the major cause of spoilage of most seafood products. During storage, the microflora changes owing to different abilities of the microorganisms to tolerate the preservation conditions. Gram-negative, fermentative bacteria (such as Vibrionaceae) spoil unpreserved fish and lactic acid bacteria (Gram and Dalgaard, 2003).

The existence of Lactic acid bacteria that showed a variation within the range of minimum <10 cfu/g and maximum of $5.3x10^3$ cfu/g and with an average level of $1.5x10^3$ cfu/g. The LAB counts were samblable than the other bacterial counts determined at the time of selling period. LAB and yeast are the remaining organisms in semi-preserved fish products. Different Lactobacillus spp., including Lactobacillus alimentarius have been identified as spoilage organisms of fish (Gram *et al.* 2002).

The yeasts are considered among the micro-organisms causing food spoilage (Loureiro and Querol 1999). The existence of yeast with the count of within the range of minimum <10 cfu/g and maximum of $5.3x10^3$ cfu/g and with an average level of $1.3x10^3$ cfu/g has been detected in our samples in our study. The yeasts causing organoleptic spoilage that was visually observed depended on the time and processing intensity and affect the commercial quality of the fish a negative manner.

In our study, the fish samples with the high counts for the existence of total viable count, Coliforms bacteria and yeasts lead to the conviction that these fish are selled under deficient hygiene and sanitation. There has not been any negative aspects observed among these samples that can prevent their sales in respect of macroscopic aspects. However, taking into account that there can be spoilage and deterioration in sensorial characteristics of the fish with high counts for total viable count, *Coliforms* and yeasts until they are delivered to the consumers, the problems of possible contamination gains much more importance in respect of future prospects. It has been well known that there are several environmental factors that influence the micro-organism development in the natural environment (Huss *et al.* 2000). Fish may carry bacteria on the surface of their bodies if the water they live in is microbiologically contaminated. To protect the fish from bacteriological spoilage, abiding to hygiene and sanitation continual control via bacteriological analyses throughout the flow of the sold and caught process are important.

Since we have not found any studies that were carried out in this field, we are not able to evaluate our findings through comparisons. However, the microbiological analysis findings in this study have displayed that the total viable count and yeast counts were beyond the desired levels in respect of the analysed samples.

Conclusion

High bacterial counts have a major significance in sensory assessment of Chalchalburnus tarichi. Critical counts of = 10^6 and = 10^7 cfu/g for samples sold in retail seller, together with beginning sensory faults indicate that the quality of the fish is decreasing and that the remaining shelf-life may be short. Further studies on identification of the specific spoilage and pathogen organisms are necessary to understand their role in the spoilage process and for public health.

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