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Effect of Salt and Chlorine on Microflora of Fish at Erwadi Fish Landing Site, Ramanathapuram District

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Chemical agents are concerned with the death rate and the effect on it of antimicrobial agents (substance that kill microbes or inhibit their growth). The effectiveness of a chemical antimicrobial agent is affected by time, temperature, pH and concentration. The effect of salt solution and chlorine solution on the micro flora of fish were examined by dipping the fishes in different concentration of salt (5%, 10%, 15% and 25%) for 15 mins and chlorine solution (2ppm, 6ppm and 10ppm) for 2-3 mins at a temperature of below 4°C. And one fish was just preserved by ice only, which where served as a control for both of them. Thus dipping reduces the number of microorganisms in all culture conditions studied. For untreated fish (that is the control) highest number of bacteria were obtained on nutrient agar plates incubated at 30°C for 72 hours where as fishes that were dipped gets decreased as the concentration of the solutions were increased in both cases.

Key words: Fish, Microflora, Salt, Chlorine.

Many foods have complex micro habitat in which microorganisms can live. The safety and quality of foods depends on the ability to control microbial growth in these micro habitats. Many edible products are multi phase system, in which the chemical and physical conditions relevant to microbial growth can vary with position in the food microhabitat (Robinson and Wilson, 1994). The bacterial flora on a newly caught fish depends on the environment in which it is caught rather than on the fish species (Shewan, 1962). The fish flesh having a pH 6-7 in living condition, provides a most suitable place for their growth and multiplication (Shammi and Bhatnagar, 2006). Microbial fish spoilage is an area of global concern. An improved science based understanding of the growth and activity of spoilage of microorganisms in seafood and other foods is crucial for development of preservation techniques. Therefore, to control microbial growth in fish different physical and chemical agents are employed. More often chemicals like salts and chlorine are used to destroy and/or inhibit the microorganisms. The addition of salt (NaCl) to food stuffs reduces the amount of available water and alters the osmotic pressure. High salt concentration, such as that occurs in saturated brine solutions have bactericidal effect and cause shrinkage of microorganisms, there by many microbes are destroyed as salt has a toxic effect up on them (Atlas, 1997). Hypochlorous acid presents in aqueous chlorine solution is the biocidal active

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chlorine. The codex report considered that exposure of fishery products to solution of chlorine, significantly reduces over all bacterial counts of pathogens, or increase shelf live of chill-stored products and there is no cause to human health on exposure up to 10ppm. There are only a few studies on the formation of carcinogens or possible carcinogens from exposure of fishery products to chlorine solution. These are formed on contact of fishery products with high, greater than 100ppm, concentration of chlorine (CAC, 2000).

In Erwadi (Ramanathapuram District) most of the marine fish landed at one landing site and shifted for either marketed as fresh fish on ice or stored and shipped on ice prior to further processing. Processing methods used by fish processors in handling fresh fish are not very advanced. This lack of technology contributes to the loss of fish due to spoilage. In an attempt to delay spoilage by reducing the microbial levels on the fish, the application of brine salting and chlorine solution could minimize such problems through dipping the fish. The main objective of this paper is to determine the effect of adding salt (brine salt solution) and chlorine on the microbial flora on fish preserved in ice and to give recommendation based on the results on how to improve the current practice.

MATERIAL AND METHODS

Fish

Samples of fish–Tuna (*Euthynnus affinsis*) were collected from Erwadi fish landing site (Ramnathapuram Dist., Tamilnadu).

Water and ice

About 1000 ml of water and ice samples were collected from fisherman using ice plant, Erwadi.

Chlorine

Different chlorine solutions having a concentration of 2 ppm, 6 ppm and 10 ppm with a temperature of ranging 0^0C- 4^0C were prepared in three bowls. After that, three fishes were taken from the landing site just after landing of fishes and immediately dipped in each of the solution for 2-3 mins.

Salt

Different salt solutions having a concentration of 5%, 10%, 15% and 25% with a temperature of ranging 0^0C - 4^0C were prepared in four bowls. After that, four fishes were taken from the landing site just after landing of fishes and immediately dipped in each the solution for 15 mins.

Sampling

Sampling for Total Plate Counts (TPC) from fish that were dipped in the different concentrations of chlorine and salt solution were done twice at the landing site just after landing of the fishes.

Microbiological investigation

Sample preparation for fishes

The TPC of fish samples were analyzed by the standard reference method of ISO 4833:1991 (E). 10gm of sample was transferred aseptically into sterilized stomacher filter bags and diluted with 90ml of sterilized peptone water in an automatic dilute and then homogenized in stomacher for 60 seconds to get 10^{-1}dilution.

The Petri dish and test tubes were labelled unambiguously. 1ml of 10^{-1} was taken using Gilson pipette and poured into sterilized test tubes containing 9ml of peptone water to get 10^{-2} dilution and 1ml of the 10^{-2} was transferred in to a Petri dish labelled as 10^{-2} dilution. Further decimal dilutions were made up to 10^{-4} and then 1ml of each dilution was pipetted to the surface of the Petri dish each labelled with respective dilution factor. About 15-20ml of melted and cooled Nutrient Agar was used for the growth of bacteria to determine the TPC. Samples were swirled clock wise and anti clock wise for uniform distribution and media. The Petri plates were allowed to solidify and were incubated up side down in the thermostat incubator at 30^0C for 72 hours. The TPC were counted using cubic colony counter.

Sample preparation for ice and water

The TPC of ice and water were determined according to the standard reference method of NFEN ISO 6222. For the TPC, 1ml of ice and water were poured into the Petri plate using pipette and 15-20 ml of Nutrient Agar was added. The Petri plate swirled clock wise and anti clock wise to ensure uniform distribution of both bacteria and the media. The Petri plate then was incubated up side down for 48 hours at temperature of 22^0C and 37^0C. TPC was enumerated on Nutrient Agar by pour plate technique using cubic counter. Colony counter by the method of ISO 4833:1991.

RESULTS AND DISCUSSION

Total plate count from fish samples

The values of Total Plate Counts for Tuna at 30°C during the period of study are given in table 1 and 2. When we see in the case of fishes dipped in the different concentration of salt solution, in the first analyses, the fish that was served as a control was having a TPC of 2.5×10^4/g where as the fishes that where dipped in the different concentration salt showed that 3.60×10^3/g in 5% salt, 2.60×10^3/g in 10% salt and 2.30×10^3/g was found in 15% salt. When we see also in the second analyses, the fish that served as a control was having 5.10×10^4/g where as the fishes that were dipped in 5% salt 1.60×10^4/g, in 10% salt 5.7×10^3/g, in 15% salt 4.20×10^3/g and 2.40×10^3/g was found in fish that was dipped in 25% salt solution. In each of the analyses or results were showing that as the concentration of the salt increases the number of the microorganisms get decreased, this is in good agreement according to Fitzgerald, G.A. (1937), a brine of salt solution greater than 3.5% will normally reduce the number of microorganism presents in the fish. And also a brine of more than 10% will normally prevent all pathogenic bacteria. Particularly in 25% salt the number of microorganism reduces drastically, this finding was coherent as to the finding stated by Shammi and Bhatnagar (2006), as they have said that at 25% of salt concentration, multiplication of microorganisms are retarded significantly. The reason why these microorganisms retard their growth as the concentration of salt increases is due to the fact that the changing of the environment creates an enormous difficulty to adapt the situation. The salted water sets up a hypertonic environment; this cause water activity to reduce thereby the bacteria expose to death.

Table 1. Result of Total Plate Count of 1st and 2nd analysis of fish dipped in salt solution during the period of study

Parameter	Concentration of salt solution (%)				
	Control	5	10	15	25
TPC(CFU/g)	$2.50\times10^4\pm0.12$ $5.10\times10^4\pm0.20$	$3.60\times10^3\pm0.16$ $1.60\times10^4\pm0.23$	$2.60\times10^3\pm0.25$ $5.70\times10^3\pm0.15$	$2.30\times10^3\pm0.15$ $4.20\times10^3\pm0.15$	- $2.4\times10^3\pm0.15$

Table 2. Result of Total Plate Count of 1st and 2nd analysis of fish dipped in chlorine solution during the period of study

Parameter	Concentration of chlorine solution(ppm)			
	Control	2	6	10
TPC (CFU/gm)	$2.50\times10^4\pm0.15$ $5.10\times10^4\pm0.1$	$1.50\times10^4\pm0.26$ $1.40\times10^4\pm0.2$	$1.30\times10^4\pm0.07$ $4.80\times10^3\pm0.25$	$1.70\times10^3\pm0.2$ $1.80\times10^2\pm0.4$

Table 3. Result of Total Plate Count of 1st and 2nd analysis of water and ice from ice plant

Sample	Incubation Temperature(°C)	Total Plate Counts(/ml of sample)	
		1st result	2nd result
water	22	1	2
	37	1	2
ice	22	1	7
	37	2	2

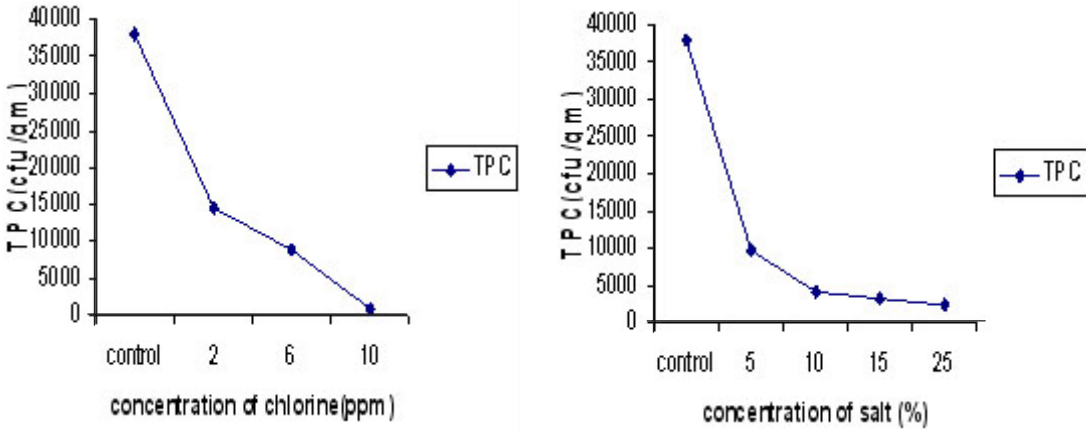
In the case of fishes were dipped in the different concentration of chlorine solution, was also conducted by two analyses. When carefully studied in the first analyses, the fish that was served as a control was having a TPC of $2.5x10^4$/g where as the fishes that were dipped in the different concentration of chlorine showed that a TPC of $1.50x10^4$/g in 2ppm, $1.30x10^4$/g in 6ppm and in 10ppm was having a TPC of $1.70x10^3$/g. In the second analyses, the fish that served as a control was having $5.10x10^4$/g whereas in 2ppm the TPC was $1.40x10^4$/g, in 6ppm $4.80x10^3$/g and $1.80x10^2$/g was in 10ppm. Based on the above results, the concentration of chlorine increases then the total bacterial count was significantly decreased. The above finding of the present study was in good agreement with the findings of CAC (2000), they reported that exposure of fishery products to solution of chlorine, significantly reduces over all bacterial counts of pathogens.

Total Plate Count of water and ice

Total Plate Counts (48 hours) of the water and ice from ice plant used in icing of fish is given in table 3. In the case of water and ice, it was observed that very less growth of microorganisms were observed after 48 hours of incubation. Since the observed growth is below the standards given, this implies that the ice and water were free from bacteria with good standard and hygienic. Water used for processing fish, washing and making of ice should meet drinking water standards (Huss, 1994). The microbiological parameters which are suggested by WHO and EEC that, the microbiological criteria for drinking water in 100ml of water sample contains total coliform, fecal coliform and the total bacterial count should not be greater than 10^3 cfu/100 ml (EEC, 1980).

We conclude that microorganisms are the most dangerous agents in spoiling the quality of fish and fish products, therefore it is very important to reduce the total number of microorganisms in order to maintain the quality and lengthen the shelf life of fish and fish products. However had it been studied the effect of salt and chlorine on the specific microorganisms in addition to the total plate count, it would have given that a clear picture to know which specific microorganism is killed or disrupted at what concentration.

Finally we recommended hygiene is the main criteria in any fishing and fish processing plant including hygiene of fisher men, boat, workers, fish boxes, landing site and every room in the processing and mode of transportation. Since most fishes can be loaded with bacteria from the time of harvesting to the time of landing or to the time of delivering the fishes that are caught to the fish processing plant, so to reduce the amount of bacteria that can be loaded on the fishes, we recommend that, any fish processing can apply dipping method by dipping the fishes in chlorine solution or salt solution to reduced the microbial count to the lesser extent as a result the amount of bacteria entering seafood before and during processing will be minimized.



(A) (B)

Fig. 1. Effectiveness of chlorine (A) and salt (B) on reduction of microflora of fish

Another important point is that the cost of chlorine and salt is very cheap any fish processing plant can prepare solution that can be used for dipping the fishes that are landed. However the only difficulty with salt solution is that to stir the solid salt in water is labour intensive and also the time of dipping the fish is some how long, so further study is needed on how to minimize the labour intensive into simple work not only this but also further study is required what if the time of dipping is reduced.

There should be sufficient time during chlorination for complete dissociation of chlorine in the water. Due to its effectiveness, low cost and simple to operate chlorine is advisable to use in fish processing plant at the landing site. But the maximum concentration of chlorine in contact with fish or fish product should not be more than 10 mg/l, this is because when the concentration is beyond this level there is a possibility of carcinogenic effect.

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