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Survival and Growth of *Listeria monocytogenes* in Refrigerated Fish

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The ability of *Listeria monocytogenes* to survive and grow in fish at refrigerated condition was studied. Fresh, white sardine *(Kowala coval)* were washed and inoculated with *L. monocytogenes* (NCTC 1994) at a level of 104 cfu.g^{-1}. The inoculated fish were kept at room temperature (28±2°C) for 6 h and then stored at refrigerated condition (4±1°C). The fish was found to have a load of 10^5 cfu.g^{-1} of *L. monocytogenes* after the temperature abuse. Though there was a minimal increase in the count during the first two days of refrigerated storage, a slight reduction in counts was observed on the 4th day of chilled storage and thereafter the counts did not show appreciable change. The results suggest that chilling does not have a significant effect on the control of *L. monocytogenes* in fish and there is a possibility of contaminated seafood acting as a vehicle of infection.

Key words: ***Listeria monocytogenes*, refrigeration, survival, growth, white sardine, *Kowala coval***

Listeria monocytogenes has been recognized as an important food-borne pathogen since 1981 and seafoods have been implicated in several sporadic cases of listeriosis (Facinelli *et al.*, 1989; Frediksen, 1991; Baker *et al.*, 1993) and in two outbreaks (Lennon *et al.*, 1984; Reido *et al.*, 1990). Buchanan *et al.* (1989) isolated *L. monocytogenes* from 15% of uncooked seafood samples. *L. monocytogenes* was found to be present in 24% of raw fish samples (Hartemink & Georgsson, 1991). Walker *et al.* (1990) reported the growth of *L. monocytogenes* at refrigerated temperatures. Harrison *et al.* (1991) determined the fate of *L. monocytogenes* in packaged and iced fish. Against this background, the effect of chilling on the survival and growth of *L. monocytogenes* in fish stored under refrigerated condition was studied.

Material and Methods

Fresh white sardine *(Kowala coval)* were procured from Mangalore fish market, washed and kept in ice till they were used for the inoculation studies. They were initially tested for the presence of listeriae. The standard *L. monocytogenes* (NCTC 1994) bath was prepared by growing the culture overnight at 37°C in 300 ml of trpticase soy broth (TSB) containing 0.6% yeast extract. Then, the culture was centrifuged at 5000 rpm for 10 min at 4°C to remove the cell pellet. The cell pellet was washed twice in sterile physiological saline and suspended in 5 ml saline and the whole cell suspension was added to 1 l sterile saline and the load of listeriae was tested immediately after the addition of *L. monocytogenes* by plating serial dilutions on to modified McBrides listeria (MML) agar plates and incubating at 37°C for 24-48 h. Typical colonies were confirmed by Gram reaction as well as catalase and motility tests (Jones & Seeliger, 1992).

The fish were immersed in the inoculation bath for 1 min and then drained. The inoculated fish were kept at room temperature (28±2°C) for 6 h, so as to simulate the fish handling conditions practised by the local fishermen, and then, stored at refrigerated temperature (4±1°C). The load of *Listeria* in the treated fish was determined immediately after the dip treatment and after 6 h at room temperature. The survival and

growth of *L. monocytogenes* on the refrigerated fish was monitored at regular intervals until the fish was apparently spoiled.

Results and Discussion

The fish samples were initially free from listeriae. On experimental inoculation, they were found to have *L. monocytogenes* load of 2.64×10^4 cfu.g^{-1} (Table 1). After the temperature abuse, the fish was found to have a count of 9.84×10^5 cfu.g^{-1} of *Listeria* which was higher than the initial load. A marginal increase in counts of *Listeria* was noticed in fish during the first two days of refrigerated storage. Later, a slight reduction in the count was observed on the fourth day of storage and thereafter it remained almost at the same level, till the fish was totally spoiled.

The results showed that *L. monocytogenes* could survive and grow in fish under refrigerated condition. It also demonstrated that exposure of infested sample to ambient temperature even for 6 h could lead to a 10 fold increase in counts of *Listeria* (Table 1). On the second day of storage at refrigerated condition, one log increase in the count was noticed, but on the 4th day, one log reduction in the count was observed. Thereafter, no significant change in the counts was observed till the fish was totally spoiled. However, Lovett *et al.* (1990) found that there was a five log increase in the count when *L. monocytogenes* was artificially inoculated into white fish and surimi and stored at 7°C for 14 days. In present study, the storage time was only 8 days and the initial counts were also high. Nevertheless, there was a 1-2 log units increase in the counts during the storage.

Table 1. Survival of *Listeria monocytogenes* in chilled fish

Sample	Storage period	*L. monocytogenes* counts (cfu.g^{-1})
Fresh	-	Nil
Inoculated	0	2.64×10^4
Stored at room temperature	6 h	9.84×10^5
Stored at 7°C	1 day	7.96×10^5
	2 day	1.34×10^6
	3 day	1.66×10^6
	4 day	7.60×10^5
	5 day	2.38×10^5
	6 day	3.68×10^5
	7 day	6.16×10^5
	8 day	6.16×10^5

Farber (1991) also reported that *L. monocytogenes* grew fairly well on salmon, increasing about 2-3 logs within 7 days at 4°C. But, Wang & Shelef (1992) reported that *L. monocytogenes* load in refrigerated cod fish fillets remained unchanged during the first 10 days of storage at 5°C, and the numbers increased by more than one order of magnitude at the end of 17 days of storage. The results are in agreement with the findings of Dorsa *et al.* (1993) who reported that a temperature of 6°C would support the growth of *L. monocytogenes* and short term temperature abuse at 12°C would induce rapid growth of the organism. The results of the study suggest that low numbers of *L. monocytogenes* present in raw seafoods might multiply and reach dangerous levels during storage at refrigerated temperatures.

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