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Fate of Enterotoxigenic Staphylococci in Fish Subjected to Curing*

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Enterotoxin A,B,C,D and E producing strains of *Staphylococcus aureus* were innoculated into dressed Barracuda fish (*Sphyraena* sp.) salted (3:1) for 48 h and dried in the sun for 3 days. The total bacterial count, *S. aureus* count and moisture content decreased after 48 h salt curing and decreased further after sun drying. *S. aureus* could not be isolated after 3 days of sun drying from the fish.

Key words : ***Staphylococcus aureus*, fish, curing and drying**

Traditional fish preservation methods are based on salting, drying and smoking either singly or in combination. On a global basis about 14% of the marine fish landings are processed by curing, but in India utilization by this sector is of a higher order of around 32% (Thomas & Balachandran, 1989). In a developing country like India these processes have great significance and relevance, being very simple and cheap requiring least technical expertise.

Staphylococcal food poisoning due to the consumption of fish and its products has been reported from all parts of the world. In India, where a proper system of reporting of foodborne illness is non-prevalent there have been few reports of staphylococcal food poisoning all attributed to milk products (Ghosh & Chattoraj, 1965; D'Souza *et al.*, 1965; Narayanan & Sharma, 1979, Rajalakshmi & Rajyalakshmi, 1982).

Staphylococcus aureus is highly tolerant to low water activity (a_w) and can grow in media containing up to 18% salt (Marcy *et al.*, 1985). Although 10% salt inhibits production of enterotoxin B in the favourable pH range of 4.8 - 8.0 (Genigeorgis, 1974), enterotoxin A is produced at higher salt concentrations (Lotter & Leistner, 1978). Salt can also play a part in the growth of staphylococci by depressing the growth of competing microflora (Hobbs, 1973)

Cured fish collected from Tamilnadu coast were found to be free from *S. aureus* (Joseph *et al.*, 1986). Enterotoxigenic staphylococci were isolated from 20.5% of the cured fish samples collected from Cochin area (Sanjeev *et al.*, 1985). Though few studies have been carried out on the prevalence of enterotoxigenic staphylococci in fish products and among fish handlers (Sanjeev *et al.*, 1985, 1986, 1987) no information is available on the effect of fish curing operation on enterotoxigenic staphylococci. Hence the present study was undertaken.

Five enterotoxigenic strains of *S. aureus* A-100, BS-6, C-361, D-472 and E-326 which

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produce enterotoxins A, B, C, D and E respectively, obtained from Dr. M.S. Bergdoll, Food Research Institute, University of Wisconsin, U.S.A. were used for this study. Individual strains were grown in brain heart infusion broth (Difco) at 37°C for 48 h. Two ml of the broth culture of each strain were pooled into another sterile tube, centrifuged and the pellets washed twice with sterile normal saline. Final pellet was resuspended in saline and 2 ml of the cell suspension was added to 750 ml sterile saline.

Fresh Barracuda (*Sphyraena* sp.) was dressed, split open, washed in potable water, soaked in 10% brine for half an hour and drained. The fish were then dipped in *S. aureus* cell suspension for one second and arranged in a vat with sodium chloride in 3:1 ratio. After 48 h the fish were taken out, dipped in 10% brine, drained and dried in the sun for 3 consecutive days. The air temperature varied from 28-32°C.

Samples were drained and analysed for total bacterial count, *S. aureus* count (ICMSF, 1978) and moisture content immediately after inoculation, after 24 h and 48 h of curing and after one, two and three days of sun drying.

Presumptive *S. aureus* colonies on Baird-Parker medium were confirmed by tube coagulase test using rabbit plasma (Difco). The moisture content and the salt content of the samples were determined as per AOAC method (AOAC, 1975).

Changes in moisture content, total bacterial count and *S. aureus* count during salt curing and sun drying are given in Table 1. Enterotoxigenic staphylococci persisted in the fish up to 2 days of sun drying but could not be isolated after 3 days of sun drying. Salt content of the final product was 21.06%.

Table 1. Changes in moisture content, total bacterial count and *S. aureus* count during 48 h salt curing and 3 days sun drying

Period	Moisture content %	TBC g^{-1}	*S. aureus* g^{-1}
0 h	70.4	$3.2x10^4$	$1.6x10^4$
After curing			
for 24 h	50.3	$1.5x10^6$	$5.7x10^5$
48 h	49.7	$2.7x10^5$	$2.1x10^5$
After sundrying			
for 1 day	43.0	$1.4x10^6$	$1.0x10^6$
2 days	32.0	$2.4x10^3$	$2.0x10^3$
3 days	27.7	$1.8x10^2$	Nil

Total bacterial count of dried fish collected from Cochin were found to be less than 10^7 g^{-1}, *S. aureus* count was up to $5.3x10^3$ g^{-1} and moisture content ranged from 30 to 65% (Sanjeev, 1991). The cured fish collected from Tamilnadu coast were free from *S. aureus* (Joseph *et al.*, 1986). The mean total bacterial count and staphylococci count of dried beef and dried fish samples collected from Nigerian markets were $1.2x10^8$ g^{-1} and $4.6x10^6$ g^{-1} (Adesiyan, 1984). Fish flesh containing 100 million (10^8) bacteria g^{-1} is considered as unsuitable for food (Almas, 1981) and staphylococci count 10^6 g^{-1} is considered to be hazardous (Bergdoll, 1979).

Staphylococci, especially enterotoxigenic staphylococci do not form part of the normal bacterial flora of fresh marine fish, but they get contaminated either from the handlers or from the surface with which they come in contact. The studies of Sanjeev *et al.* (1987) have shown that 66.41% of the *S. aureus* strains isolated from fish processing factory workers were enterotoxigenic and they produced enterotoxins A, B, C, D and E either singly or in combinations. It is also known that *S. aureus* can grow vigorously in fish if conditions are favourable (Bryan, 1973; Liston, 1980) and produce toxin.

This study has shown that if salt curing and sun drying procedures are followed strictly, it is possible to get cured fish products which are free from enterotoxigenic staphylococci and its enterotoxins even from the raw material containing enterotoxigenic staphylococci as high as 10^4 g^{-1}.

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