

The content of As, Cd, Pb, Cr, Ni and Sn in the meat of the samples were evaluated using ICP-OES (ICAD 6300 Duo view, Thermofisher, USA) (Table 1). Presence of As in fresh and frozen samples of super market was observed in 44.4% of samples with a maximum of 4.65 ppm which is well below the limit of 76 mg/kg (FSSR, 2011). Cd was detected in the 62.2% of the samples studied, with a maximum value of 0.235 mg/kg which is below the limit of 0.3 mg/kg for fishes (FSSR, 2011). While the Pb content crossed the limit of 0.3 mg/kg (FSSR, 2011) in 13% of fresh and frozen fish sample evaluated with a maximum of 3.82 (*Lutjanus gibbus*) and a minimum of 0.436 mg/kg (Frozen Pangasius). The lead content obtained in the current study is comparatively higher than the reports in fishes in and around markets of Cochin (Sivaperumal *et al.*, 2007). Cr and Ni were present in 37.78% and 35.55% of the samples analyzed, respectively but were very much below the regulatory limits of 12 mg/kg for Cr and 70 mg/kg (USFDA, 1993).

The higher level of Pb (Fig. 1) in the fresh and frozen fishes collected from super markets is pointing towards the risk coming out of high industrial activity to our environment. Stringent regulations and actions are required to regulate the industrial discharges to water bodies.

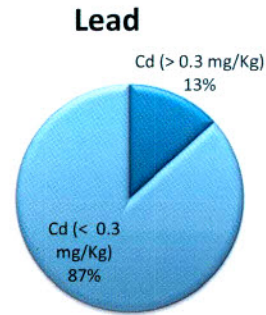


Fig. 1. Contaminaion level of lead in fresh and frozen fishes collected from super markets

### Referrences

- Food Safety and Standard Regulations, Amendment Regulations (2016) related to limit of Heavy Metals in food. Amendment in the Food Safety and Standards (Contaminants, Toxins and Residues)
- Sivaperumal, P., Sankar, T.V. and Viswanathan Nair, P.G. (2007) - Heavy metal concentrations in fish, shellfish and fish products from internal markets of India *vis-a-vis* international standards, *Food Chem.*, **102**: 612-620.
- USFDA (1993) - Food and Drug Administration. Guidance Document for Chromium in Shellfish. DHHS/PHS/FDA/CFSAN/Office of Seafood, Washington, DC.

## Growth kinetics and enterotoxin production of *Staphylococcus aureus* in fresh fish stored at 30°C

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*Staphylococcus aureus* is an enterotoxin producing pathogenic bacterium occurring as commensal flora of humans on nasal cavity and skin surfaces (Alves *et.al.*, 2014). The lack of proper hygienic practices during handling and processing may result in the contamination of fish with *S. aureus*. The most common means of fish to be contaminated with *S. aureus* is through contaminated food contact surfaces or by contact

with fish workers who are the carriers of this bacteria. This pathogen has a great importance to the food chain because of the ability of certain strains to produce heat-stable enterotoxins and other virulence factors responsible for Staphylococcal food poisoning (SFP), which is one of the most prevalent food-borne intoxication diseases. Staphylococcal enterotoxins (SE) are formed and secreted during late exponential or



post-exponential growth phase (Rosengren *et al.*, 2013). Many authors have reported the incidence of Staphylococcal enterotoxins in fish and fishery products (Chung *et al.*, 2010).

Usually, fresh fishes are displayed in retail or domestic markets at ambient temperatures without proper icing for several hours or kept for prolonged periods before the preparation in restaurants. During this course of time, fish proteins can breakdown into low molecular weight peptides and amino acids, which then support the growth of *S. aureus* (Simon and Sanjeev, 2007). As *S. aureus* is considered as a highly osmotolerant pathogen which can grow over a wide range of temperatures (10 to 45 °C), pH (4.5 to 9.3), and NaCl concentrations (up to 15%), complete elimination of this pathogen from food and contaminated surfaces is a difficult task. Even though normal cooking can kill this bacterium, the heat-resistant enterotoxins can persist and lead to SFP. The growth of enterotoxigenic *S. aureus* in fish and fishery products, causes a potential health hazard to consumers. Therefore, stringent good hygienic practices need to be followed to limit *S. aureus* growth and enterotoxin production.

In order to understand the growth kinetics and enterotoxin production potential of *S. aureus* in fish matrix, white sardine (*Escualosa thoracata*) was procured from the local market and analyzed for the absence of initial contamination with coagulase positive Staphylococci. Fish was weighed to 100±2g and packed separately in to sterile polythene pouches, spiked with six different concentrations (2, 3, 4, 5, 6 and 7 log

cfu/g) of enterotoxigenic *S. aureus* strain AVS1 isolated from the salted, dried shark collected from the local market. The spiked fish samples were kept at fixed exposure temperature of 30 °C for 8 h. *S. aureus* count and enterotoxin production was analyzed by withdrawing each pouches at different time intervals (0, 2, 4, 6 and 8 h) by surface plating in Baird parker agar and using commercial test kit (3MTecra™, Staph Enterotoxin visual Immunoassay kit, Australia). Results showed that in 2-7 log *S. aureus* spiked samples (1 log interval), the count reached to 3, 5.09, 5.85, 7.09, 7.29 and 8 log cfu/g, respectively after 8<sup>th</sup> h of incubation at 30 °C (Fig.1). There was no enterotoxin detected in 2 and 3 log spiked samples after 8 h of incubation at 30 °C (Fig 2). Studies have showed that this bacterium must have a density of at least 5 log cfu/g or mL of food to produce sufficient quantities of enterotoxins to cause food poisoning. The enterotoxin was detected in samples spiked with 4, 5, 6 and 7 log cfu/g after 8, 6, 4 and 2 h, respectively when the *S. aureus* count reached to 5.85, 6.03, 6.44, 6.98 log cfu/g, respectively. Present study also envisaged that when the population of *S. aureus* reached >5.8 log cfu/g, the enterotoxin production was detected. The time required to produce enterotoxin decreased linearly with the increase in inoculum size (Fig.2).

The results of the present study showed that the exposure of fish to the inoculum level of 2 and 3 log cfu/g for 8 h at 30 °C does not appear to present the risk of causing *S. aureus* intoxication. This study also demonstrates that enterotoxin

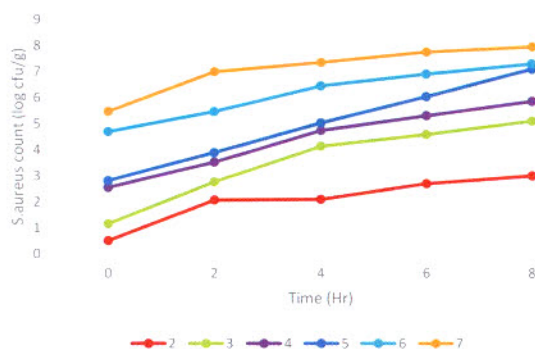


Fig. 1. Growth of *Staphylococcus aureus* (log cfu/g) in fresh fish at different inoculum level

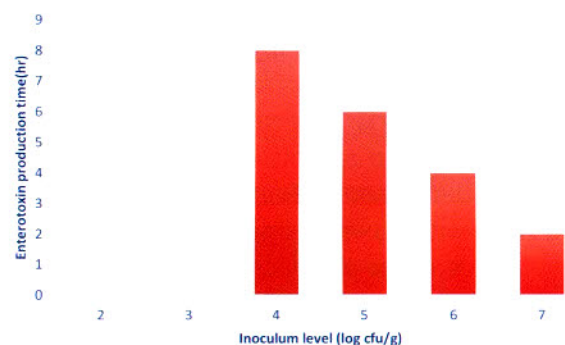


Fig. 2. Staphylococcal enterotoxin production in fresh fish at different inoculum levels



production in fresh fish is initiated when background load of *S. aureus* increased to 5.8 log cfu/g. Therefore, proper icing is necessary to prevent the growth of these organism and its enterotoxin production in the fish and also prevent prolonged exposure of fish to ambient temperature before undergoing processing.

#### References

- Alves, D.R., Gaudion, A., Bean, J.E., Perez Esteban, P., Arnot, T.C., Harper, D.R., Kot, W., Hansen, L.H., Enright, M.C., Tobias, A. and Jenkins, A. (2014) - Combined use of bacteriophage K and a novel bacteriophage to reduce *Staphylococcus aureus* biofilm formation, *Appl. Environ. Microbiol.* **80**: 694-703.
- Chung, S.I., Kim, S.Y., Nam, Y.J. and Kang, M.Y. (2010) - Development of surimi gel from King oyster mushroom and cuttlefish meat paste, *Food. Sci. Biotech.* **19**: 51-56.
- Rosengren, A., Lindblad, M. and Lindqvist R. (2013) - The effect of undissociated lactic acid on *Staphylococcus aureus* growth and enterotoxin A production. *Intl J. Food Microbiol.* **162**: 159-166.
- Simon, S.S. and Sanjeev, S. (2007) - Prevalence of enterotoxigenic *Staphylococcus aureus* in fishery products and fish processing factory workers, *Food Control* **18**: 1565-1568.

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## Antibiotic resistant profile of *Escherichia coli* isolated from the seafood samples of Veraval coast, Gujarat

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India is bestowed with diversified aquatic resources with great potential of capture and culture fisheries. Seafood is an important source of nutrient-rich diet to humans in many industrialized countries. Aquaculture production is progressing and seafood trade is gaining more importance across the world. Indian seafood market is well established in the international seafood trade in terms of supplying quality fish and shellfish products. The present trend in the seafood trade lies with demand and supply of the processed aquatic products by importing countries. Till date, seafood trade barriers are of major concern to the industry and more attention should be given to overcome the barriers. Many consignments from India were withdrawn by importing countries over the years due to the presence of microbial hazards, banned antibiotics etc. The importance of the antibiotics as a growth promoter and bacterial suppressors are no way encourageable in aquaculture and seafood trade. Use and misuse of antibiotics contributed to the development of

antibiotic resistance in bacteria and industrialized countries are combating hard to overcome this critical scenario. *Escherichia coli* is a known bacteria related to water contamination and unhygienic conditions during the handling process. Currently, six categories of diarrheagenic *E. coli* has been recognized: Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enterohemorrhagic *E. coli* (EHEC, Shiga toxin-producing *E. coli* or STEC), Enterococcal *E. coli* (EAEC or EAggEc), and diffusely adherent *E. coli* (DAEC) (Costa, 2013). In the present study, antibiotic resistance pattern in *E. coli* isolates from the seafood was carried out. A total of 31 *E. coli* were isolated from fish and shellfish of retail markets of Veraval coast, Gujarat, India as per the United States Food and Drug Administration Bacteriological Analytical Manual (Peter Feng *et al.*, 2011). All the 31 isolates were purified and characterized biochemically as rod shaped, catalase positive, oxidase negative, indole positive, methyl red positive, voges-