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An Investigation on Microbial Screening on Salt Dried Marine Fishes

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ABSTRACT

Quality assurance of processed seafood is of utmost concern that has greater implications in health point of view. The conventional method employed in salt drying of fishes that are intended for human consumption are facing serious health hazards due to improper and unscientific methods. In this backdrop, an investigation has been carried out so as to analyze the quantum of microbial load in salt dried fishes processed by conventional methods. Samples of salt dried fishes were collected from drying yards in and around Parangipettai coastal village of Cuddalore district and were screened for microbial analyses. Presence of different group of faecal coliforms and *Vibrio* spp. is an alarming situation that warrants the need for incorporating hygienic and scientific ways of salt drying.

Key words: Dried fishes, salt drying, seafood spoilage

INTRODUCTION

Fish is one of the most important sources of animal protein and has been widely accepted as a good source of protein and other elements for the maintenance of healthy body (Ravichandran *et al*., 2012). Also it provides a good source of high quality protein and contains many vitamins and minerals. It is an extremely perishable commodity and quality losses can occur very rapidly after catch (Dewi *et al*., 2011; Musa *et al*., 2010; Khan and Khan, 2001). Fishes have a rich source of essential nutrients required for supplementing both infant and adult diets (Abdullahi *et al*., 2001; Fafioye *et al*., 2008). Fish salting and drying is one of the ancient and traditional methods that impart dietary food for human consumption. It decreases the water activity and consists of transporting salt into food structures and is governed by various physical and chemical factors such as diffusion, osmosis and a series of complicated chemical and biochemical processes (Turan *et al*., 2007). Sun drying of fishes is a simple and the oldest known method of fish preservation where fishes are dried under the sun. Drying method is considered as the least expensive method of fish preservation (Balachandran, 2001). Traditional drying is often rudimentary and good hygiene and it is rarely practiced too. During the monsoon, when the humidity is high, drying cannot be achieved by traditional methods. By this time, the fish can absorb the moisture and it serves as a habitat for microbial population such as bacteria, fungi and viruses even with insect attack (Azam, 2002). The common species which are subjected for dried fish production in India are oil sardines, lesser sardines, tuna, silver bellies, mullets, mackerels, ribbon fishes etc. Over the past few years, there has been an increasing trend towards the dried fish production. In Indian fisheries, totally about 17% of the total catch is used for the production of dry fishes (Shakila *et al*., 2003). Salted fish products have been shown to be safe for consumption.

Bacterial and fungal contaminations in the dried fishes are common issues and it severely affects the quality of cured fishes. The presence of these pathogenic loads in dried fishes is acquiring importance in the view of the safety and quality of the seafood (Patterson and Ranjitha, 2009). Fishes are susceptible to a wide variety of potentially pathogenic bacteria. Many of these bacteria capable of causing disease are considered to be saprophytic in nature but only become pathogenic when fishes are physiologically unbalanced, nutritionally deficient, or as a result of other stressors such as poor water quality, overstocking, which allow opportunistic bacterial infections to proceed and lead to considerable economic losses in aquaculture as a results of heavy mortalities in both culture and wild fishes throughout the world (Akinjogunla *et al*., 2011). The bacterial and fungal contaminations are mainly due to the improper and unscientific ways of salt drying. In the present study, an investigation has been attempted to screen the microbial pathogens from the sun dried fishes has been intended for human consumption, which are exposed to moisture due to unhygienic and unscientific practices.

MATERIALS AND METHODS

Oil sardines (*Sardinella longiceps*) which are subjected for drying were collected from the different drying yards of Parangipettai, Mudasalodai, Pudukuppam and Samiyarpettai (11.49°N, 79.76°E) of Cuddalore district, Tamil Nadu during monsoon season. The collection samples were brought to the laboratory and processed in aseptic conditions.

Isolation and enumeration of bacteria: Twenty five grams of fish tissues were taken and homogenized with 225 mL of sterile 10% physiological saline and serially diluted samples plated on nutrient agar by spread plate technique (Lakshmanan *et al*., 2002). The plates were incubated for 24 h at 37°C. The number of colonies developed on the plates were counted as total heterotrophs and expressed as CFU g^{-1}. Then the screening of pathogens was done using selective media such as mannitol salt agar, EMB (Eosin Methylene Blue) agar and TCBS (Thiosulfate Citrate Bile Salts Sucrose) agar.

Coliform analysis: Total coliform analysis was conducted using Most Probable Number (MPN) methods (FAO, 1982). *Escherichia coli* was determined by using LST (Lauryl Sulfate) broth and EC (*Escherichia coli*) broth.

Isolation and enumeration of fungi: For isolation and enumeration of fungi, samples were plated on Sabouraud's Dextrose Agar plates to which penicillin and streptomycin had been incorporated. The plates were incubated at 25°C for 3-5 days and colonies were enumerated, isolated and subcultured so as to obtain pure culture (ICFM, 2007). The growth rate, color, texture, colonial morphology and diffusible pigmentation of each sample were examined macroscopically. Tease mount using lactophenol cotton blue was adopted and microscopic features such as spore and hyphae morphology were observed and compared with the standard color atlas (Ochei and Kolhatkar, 2000).

Moisture content determination: Moisture content was determined by oven drying at 105°C for 4½ h as described by Adebayo-Tayo *et al*. (2008).

Statistical analysis: Statistical analysis was performed with Origin Pro-8 SR0 version 8.0724, to evaluate the total heterotrophic count on salted fishes at different stations on different season.

RESULTS AND DISCUSSION

The Total Plate Count (TPC) was enumerated month wise and the results are given in Fig. 1-4. Higher values of TPC were recorded in the month of November for all the stations. Maximum TPC was found in Parangipettai (5.3×10^6 CFU g^{-1}) followed by Mudasalodai, Samiyarpettai, respectively, whereas minimum was found at Pudukuppam (3.2×10^6 CFU g^{-1}) during the month of December. Since total plate count was found higher in the month of November for all the stations, most probable number test was carried out for samples of this month Table 1. MPN studies revealed that among the coliforms, *E. coli* were dominant followed by *Vibrio* sp., *Salmonella* sp. and *Staphylococcus* sp., respectively. Total Fungal Count (TFC) showed maximum during the month of November at Mudasalodai (13×10^2 CFU g^{-1}) and minimum was found at Pudukuppam in September month Table 2. Totally 6 fungi were recorded, namely *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Penicillium* sp., *Rhizopus* sp. and *Mucor* sp. Moisture content of the dried fishes were recorded and are given in Table 3. High moisture content

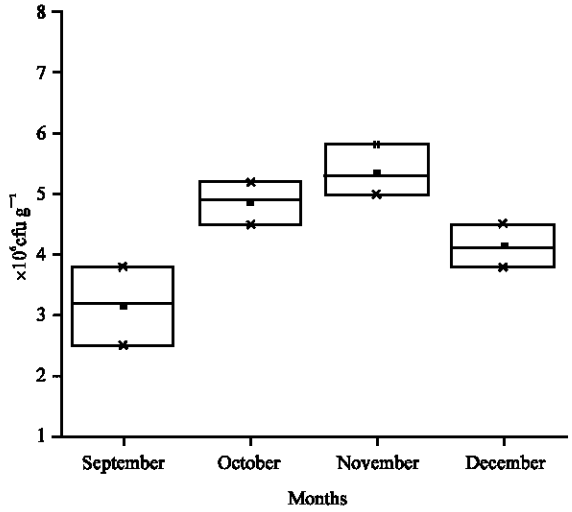

Fig. 1: Total plate count from Parangipettai

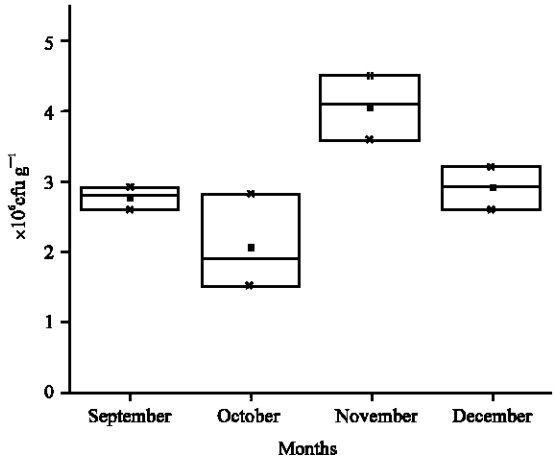

Fig. 2: Total plate count from Mudasalodai

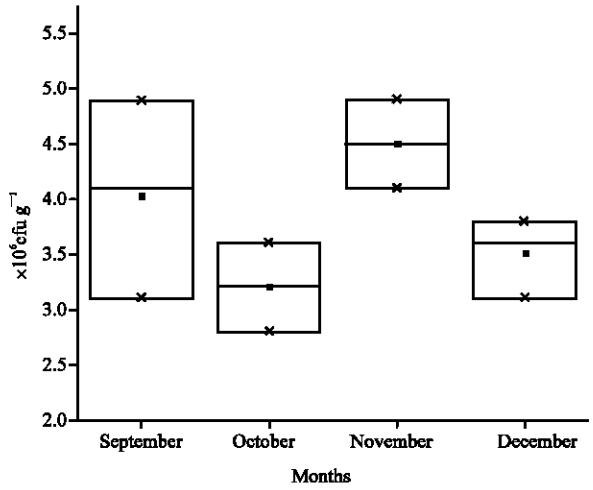

Fig. 3: Total plate count from Pudukuppam

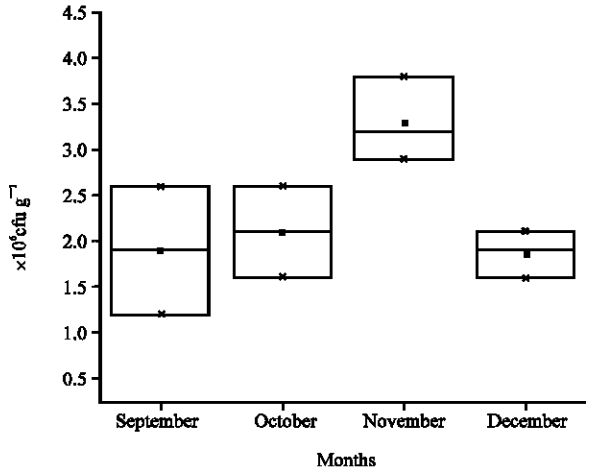

Fig. 4: Total plate count from Samiyarpettai

Table 1: Most probable number test for November month

Locations	*E. coli*	*Vibrio* sp.	*Salmonella* sp.	*Staphylococcus* sp.
Parangipettai	5.2	1.9	0.12	Absent
Mudasalodai	1.3	0.23	Absent	Absent
Pudukuppam	2.5	2.1	Absent	0.42
Samiyarpettai	4.9	0.7	Absent	Absent

Table 2: Total fungal count (CFU g^{-1})

Locations	September	October	November	December
Parangipettai	4×10^2	5×10^2	8×10^2	1×10^2
Mudasalodai	6×10^2	9×10^2	13×10^2	8×10^2
Pudukuppam	1×10^2	3×10^2	8×10^2	9×10^2
Samiyarpettai	2×10^2	5×10^2	12×10^2	11×10^2

was recorded during the month of November which might be due to the exposure and soaking of dried fishes to heavy downpour prevailed in November. This findings unravel the fact that though the fishes subjected for drying were properlysalted but the unwanted exposure of the fishes to

Table 3: Moisture level (%) in different months at different stations

Locations	September	October	November	December
Parangipettai	28.7	33.1	59.30	55.8
Mudasalodai	22.3	38.6	52.50	46.9
Pudukuppam	33.4	42.9	63.60	58.3
Samiyarpettai	34.1	51.8	49.21	42.8

heavy rainfall, due to poor storage facilities might revealed in dilution of salt concentration triggered secondary infestation and contamination with bacteria and fungi. Maximum number of bacteria and fungi recorded in the month of November evinced that the moisture leads to the contamination of dried fishes. Further enumeration of maggots to the tune of 150-200 per fish proved that the fish intended for drying were putrefied and not intended for human consumption.

Patterson and Ranjitha (2009) enumerated TPC and TFC from commercially and experimentally dried fishes showed that total plate count and Total fungal count seemed to be high in the commercially dried fishes than the experimentally dried. Ashok-Kumar (2008) studied the total heterotrophic bacterial count and total fungal count from the dried fishes of the Tuticorin drying yards. Azam *et al.* (2003) studied the total coliform count in the monsoon season as well the summer and they found more number of coliform in the monsoon season because of moisture. The fungus *Aspergillus flavus* is responsible for the production of aflatoxin and it is also found that it cause food borne intoxication which leads to serious health hazards. Hashem (2011) have studied the mycotoxins from the fishes and recorded that *Aspergillus* is the main genus that commonly involved in the production of mycotoxins. Presence of different types of fungi and bacteria in dried fishes has been reported by several workers. (Ashok-Kumar, 2008; Gupta and Samuel, 1985; Philips and Wallbridge, 1976). Moisture level of fish also plays an important role in the spoilage and lowering of moisture retards the spoilage (Ashok-Kumar, 2008). This issue is not common throughout the year. During the monsoon season, this problem occurs very severely. This leads to the quality issues and infested with pathogenic microbes leads to the dry fish unfit for consumption. For the large scale drying, bamboo made racks of 0.6-1.2 m height from the floor should be used (Samad *et al.*, 2009). During the monsoon season, bamboo splits made mat is used on the rack where the raw fishes were spread for drying. The microbial stability of dried fish products during processing and storage is depend upon their moisture content (Scott, 1957; Waterman, 1976; Troller and Christian, 1978). When the moisture is high during the drying of fishes, it favors the growth of microbes and there is a chance of infestation with flies. Khan and Khan (2001) studied the insect infestation in the dried fishes and control measures using the saturated brine solution. Using of pesticide on the dried fish to control the flies, leads to the health hazards to the dry fish consumers, so fishermen should be aware of these things. The requirement of the satisfactory dried product is highly desirable and to achieve this scientific drying method should be practiced in all the drying process (Samad *et al.*, 2009). In some of the cases, the food borne illness such as scombroid poisoning is observed in dry fishes mainly due to the chemical agent, histamine. It is also called as histamine poisoning, *E. coli* is responsible for the production of histamine in the dried fishes. In rare cases, salmonella and staphylococcus species are also produce histamine residue (Huanga *et al.*, 2010). So safety measures should be taken to reduce the contaminations and insect infestations.

CONCLUSION

Dried fish samples stored for sale in drying yards of Cuddalore District were heavily contaminated with bacteria, fungi and insects due to high moisture level and it is found unfit for

edible purpose. The water being used to clean the fishes is not that of good quality that leads to severe health issues. As discussed above, for conventional drying on bamboo made racks should be used or else the fishes can be dried over the concrete floor to reduce the microbial and insect infestations. Landing sites should be maintained clean and the domestic sewage and agricultural run of which flow into the sea could be at least partially can be treated before discharge to avoid hazards to marine biotopes.

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