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# Screening of Osmotolerant microorganisms in the dry fishes of Kerala

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## Abstract

Salting is traditional way of preserving fish and prawns all over the world.The present investigation was aimed to analyse the presence of osmotolerant microbes in the dry fishes and the safety of the product. Samples of dry fish varieties such as 'Mandhal', 'Ayila', 'Chemeen', 'Sravu' and 'Khozhuva' were collected from three main markets of Kerala, including Kollam, Talechery and Calicut in three different seasons. The bacterial pathogens such as *E.coli* (35%), *Bacillus cereus* (25%), *Klebsiella spp* (15%), *Micrococcus spp.*(10%), *Enterobacter spp.*(10%) and *Staphylococcus aureus* (5%) were found in almost all the dry fishes. In the case of dry prawns collected from Kollam and Calicut markets *E.coli (45%), Bacillus cereus pp.(20%), Vibrio cholera(15%), Vibrio parahemolyticus* (10%), *Shigella* spp.(5%) and *Staphylococcus aureus* (5%) were observed. The predominant fungi including *Aspergillus niger, Aspergillus flavus, Penicillium spp, Aspergillus fumigatus* and *Mucor sp.* were isolated from all the four samples whereas Ayila carried only *Aspergillus fumigatus*. No fungal contamination was seen in shrimps. The MPN readings for fecal indicators varied with the seasons. The fungal species *A. fumigates* and *A. flavus* were dominant in the dried sea foods of all the seasons. The presence of microorganisms in these salted fishes indicates the osmotolerance potential of microorganism. It finally leads to reduction in the quality of the dried fishes. The contamination may be due to improper drying, poor hygienic processing, inadequate salting and improper packaging and transportation of the dried fishes.

**Keywords:** Sun dried seafood's, Microbiological Analysis, Safety, Poor hygiene, fecal indicators

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## INTRODUCTION

Fish and fish products are considered as preferable source of high nutritional values and highly desirable food due to their high quality animal protein content as their exceptional richness in calcium and phosphorus and their generous supply of ß- complex vitamins (El Ahl, 2010). Fish and fish products constitute more than 60% of the total protein intake in adults (Adeleye, 1992). Fish flesh is one of the best sources of protein. Its flesh is tender due to bundles of muscle fibers, which are held together by fibrous material when heated it is better digested than beef or other types of protein (Fagade, 1992).

Fresh fish rapidly gets deteriorated unless it is preserved properly. Drying is a method of food preservation that works by removing water from the food, which inhibits the growth of microorganisms. Open air drying using sun and wind has been practiced since ancient times to preserve fish (Adebayo-Tayo *et al.* (2008).Water is usually removed by evaporation (air drying, sun drying, smoking or wind drying). Salting and drying are the ancient and simple methods used to preserve fish, and in India about 17% of the total catch is being used for salting and drying (Anon, 2001). Salting of fish followed by drying is a simple processing technique and it yields a product with relatively long shelf life (Chakrabarti and Varma, 2004).

Curing is a traditional method for preservation of fish especially in rural areas (Chakrabarti and Varma, 1999). The quality of salted and sun dried fishes are adversely affected by the occurrence of microorganisms. Determination of microbiological quality of such processed fishes from the market is very important for guarding consumer's health and hygiene (Lilabati *et al.*, 1999). In India, cured fishes are popular in the local markets and some commercially important species are exported to other countries. But, in recent years, the export of cured fish products has declined due to their poor quality (Sugumar *et al.*, 1995). Microbial contamination of fish is considered as the main cause of signs of spoilage off flavour and unpalatable taste and it may constitute a public health hazard as well as many of economic losses (Hassan *et al.*, 2007).

## MATERIALS AND METHODS

Samples of dry fishes such as 'Mandhal', 'Ayila', 'Chemeen', 'Sravu' and 'Khozhuva' were collected and

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brought to the laboratory in clean polythene covers. The dried seafood samples were analyzed for moisture content and to determine the total plate count, total fungal count, total coliform bacteria, fecal coliform bacteria, *E. coli*, *Vibriosp*, *Salmonella*, *Shigella sp.*,*Staphylococcus aureus*, *Bacillus cereus*, etc.,

**Total Bacterial count**

Enumeration of bacterial load was done by using plate count agar following the spread plate technique. 10gms of sample was mixed with 90 ml saline water, serially diluted up to seven dilutions and last three dilutions of fish homogenates were spread on plate count agar and incubated at 37°C for 24-48 hours. The colonies were counted to determine the total bacterial count.

**Fungal count**

From the serially diluted samples dilutions$10^{-3}$, $10^{-4}$, $10^{-5}$ were taken and spread over molten sabrouds dextrose agar plate for the enumeration of fungi and the plates were incubated at 28 °C for 4 days.

**Isolation of *Vibrio Sp* (Smith, 1970) :**

25g of sample was homogenized and enriched with 225 ml of alkaline peptone water and inoculated in to thiosulphate citrate bile salt sucrose agar (TCBS agar) plate for the enumeration of *Vibrio* sp. Plates were incubated at 37 °C for 24-48 hours.

**Isolation of *Salmonella* sp. (Taylor *et al.*, 1958):**

25g of sample was homogenized and inoculated in to Salmonella-Shigella agar plate for the enumeration of *Salmonella* sp. The plates were incubated at 37 °C for 24-48 hours.

**Isolation of Coliforms (Grasso *et al.*, 2009):**

25g of sample was homogenized and inoculated in to Mac Conkey agar and EMB agar plates for the enumeration of *Klebsiella* and *E.coli*. Plates were incubated at 37 °C for 24-48 hours.

## RESULTS AND DISCUSSION

**Moisture content:**

Dry fishes are dried, salted and salted fish products and consumed as the source of protein, calcium, phosphorus and vitamins. Neverthless improper drying, processing, salting and packaging lead to contamination and spoilage of dry fishes and sometimes become carrier of pathogenic micro organisms.

**Table 1.** Qualitative and quantitative analysis of dried sea foods of Kollam Market:

| Sample | Moisture Content % | TPC X 10-7 | TFC X10-5 | E.coli % | Klebsiell a sp.% | B.cereus % | Micrococ cus% | Enteroba cter% | S.aureus % |
|---|---|---|---|---|---|---|---|---|---|
| Mandhal | 30 | 51 | 3 | 37 | 3 | 6 | 0 | 1 | 2 |
| Ayila | 30 | 60 | 4 | 42 | 4 | 5 | 2 | 6 | 2 |
| Chemeen | 15 | 42 | 3 | 33 | 2 | 5 | 0 | 2 | 0 |
| Sravu | 40 | 54 | 5 | 31 | 6 | 8 | 7 | 2 | 0 |
| Kozhuva | 12 | 35 | 4 | 32 | 0 | 1 | 1 | 0 | 1 |

**Table 2.** Qualitative and quantitative analysis of dried sea foods of Calicut Market:

| Sample | Moisture Content % | TPC X 10-7 | TFC X 10-5 | E.coli % | B.cereus % | Vibrio cholera | V.parahe molyticus | Shigella % | S.aureus % |
|---|---|---|---|---|---|---|---|---|---|
| Mandhal | 33 | 58 | 3 | 47 | 3 | 5 | 2 | 0 | 1 |
| Ayila | 29 | 57 | 1 | 51 | 4 | 3 | 3 | 3 | 0 |
| Chemeen | 20 | 62 | 0 | 42 | 3 | 7 | 5 | 0 | 1 |
| Sravu | 36 | 59 | 3 | 55 | 8 | 0 | 0 | 2 | 2 |
| Kozhuva | 15 | 47 | 3 | 34 | 2 | 0 | 0 | 0 | 1 |

**Table 3.** Qualitative and quantitative analysis of dried sea foods of Talechery Market:

| Sample | Moisture Content % | TPC X 10-7 | TFC X 10-5 | E.coli % | B.cereus % | Vibrio cholera | V.parahe molyticus | Shigella % | S.aureus% |
|---|---|---|---|---|---|---|---|---|---|
| Mandhal | 30 | 59 | 5 | 45 | 3 | 4 | 1 | 1 | 2 |
| Ayila | 20 | 46 | 1 | 52 | 3 | 3 | 4 | 2 | 1 |
| Chemeen | 27 | 54 | 0 | 43 | 3 | 7 | 5 | 1 | 0 |
| Sravu | 35 | 50 | 3 | 51 | 7 | 1 | 0 | 1 | 1 |
| Kozhuva | 10 | 36 | 2 | 37 | 4 | 0 | 0 | 0 | 1 |

Moisture content of the dry fishes plays an important role in dry fishes. Low moisture content in the dried fishes prevents microbial colonization and spoilage (Stansby, 1963). Seasonal changes in the atmospheric humidity and the moisture content in the storage environment, variable time and the quality of salt used for curing are the causes that determine the moisture content of the dry fish (Anihouvi et al., 2006). Thus the moisture content becomes the exact indicator of the susceptibility of the product to undergo spoilage (Troller and Christian, 1978).

**Total Fungal Count**

The results of the fungal counts in different sun dried sea foods are presented in Tables 1 to 3. Visible fungal colonies appeared quickly due to the moisture content of the fish samples and high relative humidity of the atmosphere. The predominant fungal isolates namely *Aspergillus niger, Aspergillus flavus, Penicillium spp, Aspergillus fumigatus.* And *Mucor sp.* were isolated from all the four samples, whereas Ayila carried only *Aspergillus fumigatus.* No fungal contamination was seen in shrimps. The quality of salted and sun dried fishes was adversely affected by the occurrence of fungi (FDA, 1982). Also, the dominant fungi in salted and sun dried sea food vary with the place. The commonly occurring fungi in the west coast of India were *Aspergillus, Fusarium, Rhizopus,* and *Mucor* (CIFT. 1994).

**Total Plate count:**

In the total plate count assay all the samples showed a good count of cfu (colony forming units) in $10^{-7}$ dilutions, the value ranges from $6.2x10^{-7}$ to $4x10^{-7}$ cfu/ml (Table 1 to 3).whereas in Cochin market, the bacterial count in dried fishes was found to be less than 107 $g^{1}$ (Sanjeev, 1997]). In Nigerian market, the total bacterial count of dried fish samples was $4.6\times10^{6}g^{-1}$ (Adesiyan, and Kaminjolo, 1992),

**Isolation of Enterobacteriaceae:**

In the present study Enterobacteriaceae such as *E.coli, Klebsiella* sp, *Shigella, Salmonella* sp, *Vibrio cholera* and *Vibrio parahemolyticus* were also isolated from Calicut and Talechery market samples (Table 2 and 3), where as *Vibrio* sp. was not seen in Kollam market samples (Table 1). But the number varied with the place based on the climatic condition. *Vibrio* is a halophilic bacterium usually present in the marine environment, but in the case of *Salmonella*, it did not occur naturally in marine waters and its presence is usually due to unhygienic handling, carriers, or polluted costal water (Clucas and Ward, 1996).

**Isolation of gram positive organisms:**

In the study Gram positive bacteria such as *Staphylococcus aureus, Bacillus cereus, Micrococcussp* and very few fecal streptococci were observed as given in Table 1to 3.

**Summary and Conclusion:**

The study showed that salted and sun dried fishes sold in Kerala fish markets were contaminated with pathogenic bacteria and fungal isolates in different seasons. Spoilage of dried fish products was found and this might be due to the unhygienic handling of the fisher folks, improper processing and unhygienic vendors and venting area. So, public awareness is required regarding the importance of quality products and to avail products by hygienic processing of the fishes and air tight packaging of the final product up to marketing of the products, inorder to avoid the infections and intoxication by the pathogens.

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