RESEARCH ARTICLE

SCREENING OF OSMOTOLERANT MICRO ORGANISMS IN THE DRIED SALTED FISH SOLD IN KANYAKUMARI DISTRICT, TAMILNADU
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ABSTRACT
Fish is one of the most important sources of animal protein available in the tropics and has been widely accepted as a good source of protein and other elements for the maintenance of healthy body. Salting and drying is an ancient and simple method to preserve fish and in India about 17% of the total catch is being used for salting and drying. The present investigation was aimed to analyse the presence of osmotolerant microbes in the dry fishes. The commercially important marine edible salted dry fishes were collected from the study area Pallam. Four common edible dry fishes like (Nethili, Sardines, Ribbon fish and Prawn) were selected for the screening of microbial population. The highest TFC value was reported in Sardine and Ribbon fish (5\times10^3) than the other fishes like Netthili and Prawn (4\times10^3 and 2\times10^3). The moisture content was higher (45%) in Prawn followed by Ribbon fish (40%), Sardines (29%), Neththili (26%). In this study, Prawn had high moisture content (45%) and high microbial load (5.3\times10^3 cfu/g). Total five fungal species were isolated from the selected dried fishes. The result of isolation of human pathogens such as \textit{Salmonella} and \textit{Vibrio} identified from the selected dry fish samples. The nutritive value of raw fish in found to be good. The sensory characteristics such as colour, odor, texture, insect infestation showed that the Sardine and Prawn was good in quality, while the netthili had decreased the quality. The study showed that salted and sundried fishes sold in study area are contaminated with pathogenic bacteria and fungal agents. Spoilage of dried fish products was found and this might be due to unhygienic handling of the fisher folks, improper processing and unhygienic vendors and vending areas.

Keywords: fishes, infestation, marketing, sundried and vendors

1. INTRODUCTION
Fish is one of the most important sources of animal protein in the tropics and has been widely accepted as a good source of protein and other elements for the maintenance of healthy body. Drying of fishes is susceptible to many types of spoilage which can affect the quality and shelf life. Physical and organoleptic qualities of many traditional sun-dried products are unsatisfactory for human consumption (Nowsad \textit{et al}., 2005). Damages occurring due to flies and insects are of great significance in open sun drying and this is a serious problem in traditional drying. Major problem with respect to distribution of seafood of fishery products is their susceptibility to spoilage, mainly due to the contamination of spoilage and pathogenic microorganism (Gram and Hus, 1996). The study area Pallam in Kanyakumari District situated in the Southern tip of peninsular India is under strategic location and has rich diversity of flora and fauna scattered over the hills and hillocks of the district. However, published data on assessment of the hygienic quality of dried salted fish sold in an around Pallam of Kanyakumari district, Tamil Nadu, South India are meagre. Reports on the assessment of the hygienic quality of dried salted fish sold in an around Kanyakumari district, Tamil Nadu, South India in Kanyakumari district is very scanty. Hence the present study was undertaken to assess the hygienic quality of dried salted fish sold in selected area.

2. MATERIALS AND METHODS
2.1. Study area
Pallam is a beautiful village with green coconut trees along with a beach worth spending sometimes and the churches that resembles the spirituality of the people. Pallam is a fishing village in Kanyakumari District of Tamil Nadu and is located 9 km away from Nagercoil. Villagers mainly depend on fishing for their livelihood. St Mathew church is here. Here is a wounderful beach that is known as Sanguthurai beach which is a very fine picnic place in Kanyakumari District. Pallam has a total...
population of 2,019 peoples. There are about 497 houses in pallam village.

2.2. Sample collection
The commercially important marine edible salted dry fishes were collected from the study area and transported to the laboratory (Suganthi Devadason Marine Research Institute Tuticorin) in clean polythene covers for further microbiological analysis. Four common edible dry fishes (Nethili, Sardines, Ribbon fish and Prawn) were selected for the screening of microbial population. The selective fishes were identified according to fish Base, 2010.

2.3. Preparation of sample
About 1 gm was taken from each fish homogenate was made in 10 ml distilled water. The solution was serially distilled ten folds. 0.1 ml of (10\(^{-10}\)) dilution was spread on the Zobell Marine Agar in duplicate and incubated for 18-24 hrs at 37°C.

2.4. Microbiological analysis
Enumeration of bacterial load was done using plate count agar by using spread plate. Ten gm of the sample was mixed with 90ml saline water. Appropriate dilutions of fish homogenate were spread on plate count agar and incubated at 37°C for 24-48 hours and the colonies were counted for total bacterial count (TBC) (FDAABAM, 2001).

Fungal count was done (AOAC, 1998) by using Rose Bengal Chloramphenical (RBC) agar. Twenty five gm of the sample was blended with 225ml of 0.1% peptone water and 0.1ml of the appropriate dilutions of the sample was spread on the surface of the medium and incubated at room temperature (28 \(\pm\) 1°C) for 3-5 days. Fungal colonies were sub cultured on potato dextrose agar (PDA) and the fungal cultures were stained by using wet mount with lacto phenol cotton blue. Identification of the fungi is mainly based on morphology and can be carried out by standard keys. Sensitivity of the fungal isolates to sodium chloride was done by inoculating fungal colonies on PDA containing 0, 10, 14 and 18% NaCl.

The MPN Technique was used to determine the level of total coliforms, fecal coliforms, fecal streptococci and E.coli in dry fish samples. Dry fish homogenate was transferred to lauryl sulphate tryptone broth (LSTB) tubes and incubated and 37°C for 24 hours for the estimation of total coliforms. Samples from positive LSTB tubes were transferred coliform presence or absence broth tubes and incubated at 44.4 \(\pm\) 0.5°C for 18-24 hours for the estimation of fecal coliforms. Samples from positive PA broth tubes were transferred to EC broth tubes and incubated at 37°C for 24-48 hours for E.coli. Samples from positive EC broth tubes were treated on to eosine methylene blue agar plate to isolate E.coli culture of fecal streptococci was done in glucose azide broth and confirmed by KF agar.

For the isolation of Salmonella, 25g of sample was homogenized and enriched in 225 ml of alkaline peptone water (APW) at 36°C for 24 hours. Selective isolation of vibrio was carried out in thiosulphate citrate bile salt sucrose agar (TCBS). Presence of vibrio shows yellow closed colonies.

2.5. Estimation of biochemical composition
Biochemical parameters like proteins, lipids, carbohydrates and free fatty acids were estimated by following standard methods. The amount of proteins in the sample was estimated by Biuret Method (Raymont et al., 1964). Carbohydrate content was estimated by the phenol sulphuric acid method (Dubois et al., 1956), lipid was estimated according to the method of Bligh and Dyer (1959) and the free fatty acid, which is an indication of the quality of fat, was estimated by the method of Ke et al., (1976).

2.6. Physical characteristics
Physical characteristics such as colour, odor, texture and insect infestation of the traditionally sun-dried fishes were examined by sensory test on the basis of the method described by Roy, (2013) with help of five member panels of experts.

2.7. Proximate composition analysis
The compositions of moisture, protein, crude lipid and ash content of dried fish were analysed in triplicate according to the standard procedure given in association of official analytical chemists (AOAC, 1995).

2.8. Determination of lipid oxidation
To analyse the degree of lipid oxidation of dried fish, lipids was extended and then peroxide value and the acid value was determined.

2.9. Extraction of lipid
Total lipid was extracted from dried fish samples with a solvent combination of chloroform, methanol, distilled water according to the method of Bligh and Dyer, (1959) with slight modification making those final rations 10:5:3, v/v/v. Then the extracted lipid was dissolved in chloroform and stored at 20°C until further analysis.

2.10. Determination of pH
About 10g of samples was weighed in a beaker using electronic balance. Then the sample was homogenized using tissue homogenizer with 10
volumes of distilled water and the pH was measured by a pH meter.

2.11. Salt content
Salt content was determined as chloride where the ions are precipitated with silver nitrate and the excess silver ions are determined by titration with potassium thiocyanate (Pierson, 1999). All analysis are performed in duplicate.

3. RESULTS AND DISCUSSION

3.1. Total plate count (TPC)
In the fish sample, the highest total plate count (TPC) (5.3×10^4 CFU/g) was observed in Prawn followed by Netthili, Sardines and the lowest TPC in ribbon fish (2.5×10^3 CFU/g) (Table 2). In fresh fish, the acceptable limit is 5×10^2/g at 37°C but for cooked or dried fish, the permissible limit is 1×10^4/g at 37°C (Surendram et al., 2006). In this study, prawn had the highest TPC 5.3×10^4 which exceed the permissible limit. Similar works carried out in dried fishes of Tuticorin market have recorded high bacteria count in Prawn 3.5×10^3 (Ashok Kumar, 2008). In Cochin market, the bacterial count in dried fishes was found to be less than 4.1×10^3/g (Sanjeev, 1997). In Nigerian market count of dried fish samples was 4.6×10^6 g⁻¹ (Adesiyan and Kaminjola, 1992). The present study results revealed high bacterial load and the mean temperature of the period was 27±3°C. In this season, the dried sea foods absorb moisture from the atmosphere and this leads to the spoilage of the products. The least bacterial load was observed during summer season and this is due to high temperature, low moisture and adequate drying. This results agrees with the direct relationship between the microbial count and moisture content of the sample (Lilabati et al., 1999). The highest TFC value was reported in Sardine and Ribbon fish (5×10^3) than the other fishes like Netthili and Prawn (4×10^3& 2×10^3) (Table 2).

3.2. Moisture
The moisture content was higher (45 %) in Prawn followed by Ribbon fish (40%), Sardines (29%), Netthili (26%). Moisture content of seafood’s plays on important role in spoilage and lowering of moisture relards the spoilage (Shanshy, 1963). In the present study, high moisture content and microbial load were observed during monsoon season. There was a direct relationship between the microbial counts and moisture content of the sample. The seasonal variation in moisture content of dried seafood could be the results of variable in drying time, environmental changes and level and type of salt used for curing (Anihouvi et al., 2006). However, the moisture content seems to be an exact indicator of the susceptibility of a product to undergo microbial spoilage. In the present study the moisture was increased during monsoon season and it was considerably lowered in summer season and this result agrees with the result of earlier reports (Chakrabasri et al., 1999).

In this present study, Prawn had high moisture content (45%) and high microbial load (5.3×10^4 CFU/g). Visible fungal colonies appeared on the sea foods during monsoon season due to high moisture content of the samples and high relative humidity of the atmosphere. In this present study also high fungal count noted with high moisture in monsoon season.

3.3. Fecal indicator bacteria
Pathogenic or indicator bacterial may not be present insufficiently in large numbers in water of food to be detected by planting methods. In such cases, MPN methods are used, where large volumes of samples can be used for inoculation. MPN is only a statistical approximately on the test bacteria in the given sample and not the actual number. MPN method is used to detect the *coliform* bacteria in water or food (Surendran et al., 2006).

In the present investigation, the MPN value of the seafood samples varied with different season. The total *coliforms* and fecal coliforms during summer varied from 95-45 and 70-20/100 ml respectively (Table 2). Total coliforms group can be sub grouped as fecal and non fecal coliforms. The fecal coliform subgroup is derived from feces of human and other warm-blood animals such as cows, sheep, poultry, etc. The non fecal subgroup is frequently found on vegetation and in the soil; some are plant pathogens. The presence of fecal coli-fron organisms indicates recent and possibly hazardous fecal pollution. The most common fecal coliform species is *Escherichia coli* (Kabler et al., 1960, Sherman 1937). Fecal *streptococci* are non pathogenic organisms but commonly occur in the intestines of man and other warm-blooded animals which make them a useful group of indicator of fecal contamination. (Litsky et al., 1955). Washing the catches in polluted coasted water definitely add the fecal indicator bacterial. Drying done in unhygienic way also added fecal indicator bacteria in fish and coastal waters has already been reported to be high along Tuticorin fish landing centres (Sugumar, 2002). However, the fecal pollution at Bhuranagar coast was reported to be of human origin based on the fecal diseases.

Sewage imparts considerably to the fecal microorganisms which are considered as a good
indicator of the extent of fecal pollution in the environment. In our present study high MPN values were observed in the monsoon season and it may be due to unhygienic handling in processing and inadequate drying. In our present study also E.coli count showed variations, high in monsoon season. Levels of focal indicator bacteria were also reported to be high both in fish and dehydrated fish from Cochin fisheries and retail markets of Mumbai (Kimura and Kiamukura, 1934).

3.4. Total fungal count

The results of fungal counts in different sun dried sea food were presented in table 4. Total five fungal species were isolated from the dried sea food. The dry fish samples were free from visible fungal colonies during post-monsoon and summer seasons while visible fungal colonies were noted on the fishes during monsoon season. In monsoon season, visible fungal colonies appeared quickly due to the moisture content of the fish samples and high relative humidity of the atmosphere. Even through visible fungal colonies were not noted in post monsoon and summer season but enrichment in RBC broth and plating on RBC agar recovers almost all the fungal flora. The antibiotic chloramphenicol was added to the media to arrest the growth of bacteria.

3.5. Pathogenic bacteria

The result of isolation of human pathogens such as Salmonella and Vibrio from the selected dry fish samples are shown in Table 2. Both the bacteria are noted in netthili, sardine, prawn except ribbon fish. Vibrio is a halophilic bacterium usually present in the marine environment, but in the case of Salmonella, it does not occur naturally in marine waters and is presence is usually due to unhygienic handling carries or polluted costal water (Christolite, 2004). Contamination of fish and fishery products with salmonella and vibrio has been reported in different parts of India. Incidence of pathogens in the samples of fish market may be attributed to external contamination (Lyer and Shrivastava, 1989) and poor handling at ambient temperature (Jedah et al, 1998).

The study showed that salted and sun dried fishes in Pallam sample markets were contaminated with pathogenic bacterial and fungal agents. Spoilage of dried fish products was food and this might be due to the unhygienic handling of the fisher folks, importance of quality products and to avail products by hygienic processing of the fishes and air tight packing of the final products up to marketing of the products.

Physico-chemical parameters like proteins, fatty acids Carbohydrates, ph, Sodium chloride and lipid were estimated and the results are given in Table 3. The nutritive value of raw fish in found to be good. But the salt dry fishes had a reduced values Protein 8% (netthili), 7% (sardiae), 9% (ribbon fish) and 6% (prawn). Low fatty acid content (0.015% netthili, 0.010% sardine, 0.011% ribbon fish and 6 % prawn). Carbohydrate is also reduced (13% netthili; 15% aardine; 12 % ribbon fish, 11% prawn). pH also loss in Sardine (6), ribbon fish (6.5) and prawn (6) of 7 in netthili. Low nutritive value in commercially sun dried fish samples is due to improper handling, inadequate preservation and unhygienic mode of drying the fish samples. The lowest pH values of dried products may enhance microbial inhibition and contribute to extend the shelf life of dried fish by inhibiting the activity of the endogenous proteases. On the other hand, an increase in pH indicates the loss of quality in fishes (Farid et al., 2014). Sensory characteristics of sea dried selected samples are depicted in Table 1. The sensory characteristics such as colour, odor, texture, insect infestation showed that the Sardine and Prawn was good in quality, while the netthili had decreased the quality. Ribbon fish lost their colour, texture, and odor from their original characteristics. Neththili and Prawn are free from insect infestation and Sardine and ribbon fish are slightly infected. High quality of broken pieces might be the results of using poor quality raw material, excess drying or improper drying of handling of due to moisture reconstitution (Mansur et al., 2013).

In the quality assessment of salted dry fishes, the sensory evaluation is most important. As quality deterioration progresses, several off-odours can be noticed. Many different odour compounds can be perceived by some are having very low odour. The microbiological analyses also showed variations among the samples. The changes in enumeration of total viable bacteria (Total plate count, TPC), Total fungal count (TFC), E.coli and pathogenic bacteria, were assessed and the result are given in Table 5. The pathogenic bacteria colonies were tested using the methods of APHA (1992). The highest count of TPC and E.coli in commercially sun dried fish sample revealed the unhygienic condition prevailed during the drying process.

Fish acts as protein supplement for the people living in 63% of countries (Bangladesh DOF, 2001). The increase in drying time and loss and moisture content leads to protein de-naturation. The protein content was higher in sew fish than the sun dried fish and it is mainly due to protein de-naturation of reducing moisture level.
The free fatty acid levels (1% oleic acid) of all the sun dried fish samples were higher than the raw fish. In the present study, commercially sun dried sample (Prawn) shows high fatty acid content due to long storage period. The lower fatty acid in raw fish (Sardines) was due to the less degree of oxidation.

The total plate count and E.coli counts were found to be higher in commercially sun dried fish which is available in Tuticorin local market than the experimentally sun dried fish. High counts of TPC and E.coli in commercial same was due to high content of moisture and humidity in the environment and unhygienic method of preparation.

The biochemical and microbial analysis showed that the quality of experimentally sun dried fish sample was good than the commercially sun dried fish sample. Experimentally sun dried fish was properly handled and well exposed to sun light and moving air and it dried quickly and the end product was clean hygienic.

Water reconstitution of dried fish products the water reconstitution of dried fish products are presented in generally, water holding capacity of dried fish products are increased with the increase of water temperature and soaking time. The rehydration ability of dried fish products was depended on elevated soaking temperature and extended period of time. It has been reported that there was a positive relationship between rehydration ability and physical properties of dried fish products (Reza et al., 2005). This might be due to the fact that increased temperature of water opens the internal structure of fish muscle which maximizes the scope of rapid rehydration (Tunde-Akinlud, 2008). Moreover, rehydration ability of dried fish products depends on the variation of species beside time and temperature (Nurullah, 2005).

The pH values were ranged from 6-7. The highest pH value was found in Netthili while the lowest was observed in Ribbon fish. The lowest pH values of dried products may enhance microbial inhibition and contribute to extend the shelf life of dried fish by inhibition the activity of the endogenous proteases. On the other hand an increase in pH indicates the loss of quality in fishes (Farid et al., 2014).

The moisture content of the dried fishes was observed in the range of 29%-44% with the highest value obtained from Prawn and the lowest value from netthili. The variation of moisture content among the dried fishes may occur due to improper storage, improper drying, unawareness of processors etc. According to Kamruzaman (1992), when salt is added to the fish before drying, less water needs to be removed to achieve the same effect, and the product with a water content of 35% 45%, depending on amount of salt present, is often dry enough to inhibit the growth of moulds and bacteria under most climatic condition. It has been reported that the water activity increases with the water absorption from environment, which enhances the microbial growth and reduces the loss of nutrient and shelf life of dried products (Nowasad, 2005). Sometimes fish processors keep comparatively higher moisture content in the dried fish products to gain more weight for economic benefit. The crude protein content ranged from 8%-8.5% with the highest value Ribbon fish and Prawn and the lowest in Netthili.

According to Edema and Agbon (2010), the most common sources of fish deterioration is fungal, which have the ability to grow on substrates with low water activity down to 0.6 (Thiam, 1993) and are thus important in determining fish quality. Geetha et al. (2014) isolated Staphylococcus aureus in all dried fish samples and concluded Staphylococcus was the most predominant organisms. Staphylococcus aureus can grow in the presence of salt. Staphylococcus aureus counts in this study was lower than those reported by Goja (2013) about fresh fish. Staphylococcus aureus has also been detected during the process drying and subsequent smoking of eels in Alaska in 1993 (Etlund et al., 2004). Salmonella sp. was no detected in the sample collected from Bandar torkman. But Salmonella contamination was detected in other samples and Shigella was observed in all of the samples. The absence of Salmonella was similar to the results Oulai et al. (2007); Dodds, (1992). However, Djinou (2001) found that 0.8% of their samples had Salmonella.

E.coli is responsible for the production of histamine in the dried fishes (Loges et al., 2012). In rare cases Samonella and Staphylococcus species produce histamine residue (Iduang et al., 2010). So safety measures should be taken to reduce the contaminations and insect infestations. The presence of vibrio sp., in the fish can cause pathogenic infection to the consumer. In the present investigation, Vibrio sp., was studied qualitatively and found in all the samples. According to recommendation of International Association of Microbiology Societies, fresh and frozen fish should be free of Vibrio (0/gm). The present study revealed that microbial quality was not good due to presence of Vibrio sp., in all the samples.

It is important to state that majority of the fungal agents isolated were of medical significance. The occurrence of Aspergillus spp., Penicillium spp., and Candida spp could lead to mycotoxin
elaboration and when consumed, they induce gastrointestinal and metabolic disturbances (Martin,
Table 1. Sensory characteristic of sun dried salty fishes collected from Pallam

<table>
<thead>
<tr>
<th>Samples</th>
<th>Colour</th>
<th>Odour</th>
<th>Texture</th>
<th>Insect infestation</th>
<th>Overall quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netthili</td>
<td>Brownish</td>
<td>good</td>
<td>firm &amp; flexible</td>
<td>Slightly</td>
<td>acceptable</td>
</tr>
<tr>
<td>Sardines</td>
<td>Brownish</td>
<td>good</td>
<td>firm &amp; flexible</td>
<td>Slightly</td>
<td>acceptable</td>
</tr>
<tr>
<td>Ribbon fish</td>
<td>Slightly dark</td>
<td>characteristic</td>
<td>Loss of firmness</td>
<td>Nil</td>
<td>good</td>
</tr>
<tr>
<td>Prawn</td>
<td>whitish</td>
<td>Firm and flexible</td>
<td>Firm and flexible</td>
<td>Slightly</td>
<td>good</td>
</tr>
</tbody>
</table>

Table 2. Microbial characteristic of selected sun dried salty fishes collected from Pallam

<table>
<thead>
<tr>
<th>Samples</th>
<th>TPC Fu/g</th>
<th>TFC cfu/g</th>
<th>Moisture</th>
<th>Total coliform</th>
<th>Fecal coliform MPN/100ml</th>
<th>E.coli MPN/100ml</th>
<th>Fecal Streptococci MPN/100ml</th>
<th>Salmonella 25g</th>
<th>Vibrio 25g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netthili</td>
<td>$3.9 \times 10^3$</td>
<td>$4 \times 10^3$</td>
<td>26%</td>
<td>60</td>
<td>25</td>
<td>8</td>
<td>95</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Sardines</td>
<td>$3.8 \times 10^3$</td>
<td>$5 \times 10^3$</td>
<td>29%</td>
<td>65</td>
<td>30</td>
<td>6</td>
<td>90</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Ribbon fish</td>
<td>$2.5 \times 10^3$</td>
<td>$5 \times 10^3$</td>
<td>40%</td>
<td>45</td>
<td>20</td>
<td>11</td>
<td>115</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Prawn</td>
<td>$5.3 \times 10^4$</td>
<td>$2 \times 10^3$</td>
<td>45%</td>
<td>95</td>
<td>70</td>
<td>15</td>
<td>75</td>
<td>Present</td>
<td>Present</td>
</tr>
</tbody>
</table>

Table 3. Physicochemical analysis of selected sun dried salt fishes collected from Pallam

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Netthili</th>
<th>Sardines</th>
<th>Ribbon fish</th>
<th>Prawn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates (%)</td>
<td>13</td>
<td>15</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>pH</td>
<td>7</td>
<td>6</td>
<td>6.5</td>
<td>6</td>
</tr>
<tr>
<td>Fatty acid content (%)</td>
<td>0.015</td>
<td>0.010</td>
<td>0.011</td>
<td>6</td>
</tr>
<tr>
<td>Protein content (%)</td>
<td>8</td>
<td>7</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Sodium chloride (%)</td>
<td>15</td>
<td>16</td>
<td>16.5</td>
<td>17</td>
</tr>
<tr>
<td>Lipids (%)</td>
<td>1.5</td>
<td>1.00</td>
<td>1.20</td>
<td>1.60</td>
</tr>
</tbody>
</table>
Aspergillus niger a) on identification media at 25°C for 7 days, b) light microscopy of condial heads on MEA, c) conidia showing characteristic thick and rough, spiny walls, and d) deep brown reverse colouration on AFPA agar

Orange yellow reverse colouration of A. niger b) deep brown reverse colouration of A. niger, on AFPA agar

Fig. 1. Identified fungi isolation and identification of fungi
Fungi isolated from the present study are in consonance with findings by other authors however, Rajafal et al. 2010 reported the Penicillium spp., Aspergillus spp., and Rhizopus spp. are normal mycoflora present in most fish. Not with standing, many fungal genera have virulence factor which cause toxin elaboration under favourable predisposing environment. Ecology is also an important factor which influences the diversity of fungus genera on fish and their eggs. According to Pallwal et al., diversity of water moulds depends upon the interaction of physicochemical factors.

Not all fungi which recur in fish are considered deleterious. Moulds are one of the important cause of spoilage of salted dried fish products and they produce mycotoxins and they are able to grow in salt concentrations between 5 and 26% (Reilly, 1986). It is clear from the results than an increasing trend was shown during the storage period. TFC was more in gunny bugs stared products as compared to corrugated boxed. A significant fungal growth was recorded after 4 the month of storage and this may be due to increase in aw, moisture content and salt content. Products deteriorate by growth of moulds if the water content is approximately 15% (Gandotra et al., 2012). These observations were in close agreement to the present study. The rapid reduction in the water activity (aw<0.75) is the most important factors in controlling fungi/mould contamination of the fishery products during storage (Kolakowska, 2002).

### Table 4. Identified fungal species in salted dried fishes of selected samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Name of the fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netthili</td>
<td>Aspergillus flavus, A. niger, Mucor</td>
</tr>
<tr>
<td>Sardine</td>
<td>Penicillium spp, A. niger</td>
</tr>
<tr>
<td>Ribbon fish</td>
<td>A.niger, A. oryzae, A. flavus</td>
</tr>
<tr>
<td>Prawn</td>
<td>A.niger, A.flavus, A. oryzae</td>
</tr>
</tbody>
</table>

The observed results indicated that dry fishes are prone to contamination by pathogenic microorganisms. As fishes to be dried are usually handled with bared hands and drying process is carried out in the open atmosphere, this unhygienic practice might cause the contamination of dried fishes posing a risk of food born disease. The level of microorganisms associated with these foods can be decreased by irradiation that depends upon the absorbed dose of radiation. Ashokkumar (2008) studied the total heterotrophic bacterial count from the dried fishes of tuticorin drying yards. Azam et al. (2003) studied the total coliform count in the monsoon season as well as summer and they found more number of coliform in the monsoon because of moisture. The fungus Aspergillus flavus is responsible for the products 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. Present of different types of fungi and bacteria in dried fishes has been reported by several workers. (Ashok-kumar, 2008; Gupta and Samule, 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 issue and infested with pathogenic microbes leads to the dry fish unit 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 depends upon their moisture content (Scott, 1957; Waterman, 1976; Troller and Christian, 1978). When the moisture is high during the drying of fishes, if favors the growth of microbes and there is a change of infestation with files. Khan and Khan (2001) studied the insect infestation in the dried fishes and control measures using the saturated brine solution. Using the pesticide on the dried fish to control the files, leads to the health hazards to the dry fish consumes, 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 scobroid 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 residence (Hyanga et al., 2010). So safety measures should be taken to reduce the contaminations and insect infestations.
4. CONCLUSION

In a general note, health education/enlightenment will be of great significance of fishermen, fish handlers, sellers and buyers that good processing and availability of storage facilities are crucial to minimize general microbial contamination. The study showed that salted and sundried fishes sold in four places (study area) were contaminated with pathogenic bacteria and fungal agents in the different seasons. Spoilage of dried fish products was found and this might be due to unhygienic handling of the fisher folks, improper processing and unhygienic vendors and venting area.

Hence control measures such as used of good quality raw material, hygienic handling practices, potable water, good quality packaging material, hygienic processing practice may be considered to improve the microbial quality of the dried fish product. Proper cooking procedures should be emphasized to climate or reduce the microorganisms to an acceptable level.

To the best of our knowledge this is the first study of the shelf life of salt treated sun-dried fish stored at refrigeration temperature. Based on the presented data (TVB-N, FFA, pH values, and microbial load counts) the optimal shelf life of sun-dried salted fishes is approximately 24 to 32 months for refrigeration (4°C) storage. The results also indicated that dried fish have greater nutritive value in terms of percentage crude protein for maintain human health. Also the effect of heat and dryness associated with open sun-drying reduces the water activity of the fish thereby limiting microorganisms, a prerequisite of spoilage. Fish processing by the combination of salting and drying is recommended for used because it gives relatively greater percentage protein and fat. Hence, it is suggested that low storage temperature (4°C) and the traditional preservatives like NaCl (Table salt) which are easily available and chapter cost wise, along with traditional sun drying process can be used by the fisher folk to arrest the growth of bacterial in fishes, thereby avoiding fish poisoning.

REFERENCES

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