Microbial Quality of Salted and Sun Dried Sea Foods of Tuticorin Dry Fish Market, Southeast Coast of India

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Abstract: The microbial quality of six sun dried seafood species available in Tuticorin dry fish market was analyzed during monsoon, post-monsoon and summer seasons. The microbial quality parameters varied with different seafood's in different seasons and the quality was found to be poor during monsoon season. The bacterial and fungal counts were increased with the increase of humidity of the environment and the moisture content of the dried seafood's. The spoilage indicators TMA-N and TVB-N of the samples didn't exceed the permissible limit except in *S. fimbriata*. The pathogenic bacteria occurred in different species of seafood's in different season. The MPN readings for fecal indicators varied with the seasons. The fungal species *A. niger, A. fumigatus* and *A. flavus* were dominant in the dried sea foods of all the 3 seasons and among them, *A. niger* and *A. flavus* showed 18% salt resistance. The poor quality of the dried fishes may be due to unhygienic processing, inadequate salting with poor quality salt and lack of air tight packing of the dried fishes

Key words:Sun dried seafood's • Quality • Total plate count • Total fungal count • Fungal species • Moisture

INTRODUCTION

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 [1]. Salting of fish followed by drying is a simple processing technique and it yields a product with relatively long shelf life [2]. Curing is a traditional method for preservation of fish especially in rural areas [3]. 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 [4]. 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 [5].

Fish must be dried quickly and hygienically in plenty of sunlight and moving air and this protects fish from insects and dirt. Fish is a reservoir of large number of microorganisms; one of the major factors contributing to poor quality of the fish in retail trade is unhygienic handling and storage leading to off smell, physical damage and contamination with dirt and objectionable

microorganisms [6]. The majority of these microorganisms are non pathogenic causing only spoilage to fish but some are pathogenic and causes food poisoning. Quality standards have been prescribed for fish and fishery products meant for export and they are being monitored strictly. Such control is not existing for the retail trade of fish and fishery products [7].

Fungal contamination is a common problem and it adversely affects the quality of cured fishes. The presence of fungi in dried fish along the west coast of India is prevalent [8] and the presence of different types of fungi in dried fishes has been reported by ealier [8-11]. The dominant fungus in salted and sun dried fishes varies with the place. The commonly occurring fungi in the south east coast of India are Aspergillus and Penicillium species [12]. The incidence of salmonella, vibrio and some faecal indicator bacteria in fishes sold in the retail markets of Cochin [13]. The quality deterioration of foods during processing, storage and distribution is mainly caused by microorganisms. The microorganisms present in foods are closely connected to the micro flora of the environment. Population densities of bacteria in seawater are ranging from 10³ to 10⁶ cells in 1 ml depending on the environmental conditions. In general, sea water in coastal

areas contains more bacterial cells than in open sea because micro flora of fish and shellfish are closely connected to the microbes of water and sediment [14].

Preservation of fish by salt curing has long been practiced in Tuticorin as a traditional technique, Different verities of fish are washed in the sea water and immersed in 20% brine for 24 hours and dried for 2-3 days on sea shore sandy area or on palm leaves. The same method is being followed till date with little modification. The ratios of salt used for salting of fish are too low to ensure adequate preservation [12]. Quality assessments are necessary to ensure the food safety of any processed product. This paper reported the microbial quality of dry fishes sold in Tuticorin dry fish market.

MATERIALS AND METHODS

The sun dried seafoods such as fin fishes Scomberoides lysan, Stolephores commersonii, Sardinella fimbriata and Scomberomores commerson, non-penaeid prawn, Acetes indicus and smoked and skip jack tuna, Katsuwonus pelamis meat product known as massi were bought from Tuticorin dry fish market and brought to the laboratory in clean polythene covers. The dried seafood samples were analyzed for Moisture content [15], total plate count [16], total fungal count [17], total coliform bacteria, fecal coliform bacteria, E. coli and fecal streptococci [18, 17], Salmonella [19], *Vibrio* [20] and spoilage indicators TMV-N and TVB-N [21].

Enumeration of bacterial load was done using plate count agar by using spread plating. Ten g of the sample was mixed with 90 ml 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) [16],

Fungal count was done [17] by using Rose Bengal Chloramphenicol (RBC) agar. Twenty-five g of the sample was blended with 225 ml of 0.1% peptone water and 0.1 ml of the appropriate dilutions of the sample was spread on the surface of the medium and incubated at room temperature (28±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 ^[22]. Sensitivity of the fungal isolates to sodium chloride was done by inoculating fungal colonies on PDA containing 0, 10, 14 and 18% Na Cl.

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 at 37°C for 24 hours for the estimation of total coliforms. Samples from positive LSTB tubes were transferred to Coliform presence or absence broth tubes and incubated at 44.4±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 streaked 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 lactose broth at 37°C for 24 hours..Selective enrichment of *Salmonella* was carried out in tetrathionate (TT) broth and Rappaport vassilidis (RV) medium in thermostatically controlled water bath. Each of these enriched cultures was streaked in XLDA. Typical *Salmonella* exhibit pink colonies with or without black centers.

For isolation of *Vibrio*, 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 colored colonies.

The spoilage indicators, trimethylamine nitrogen (TMA-N) and total volatile base nitrogen (TVB-N) were determined from trichloroacetic acid extract of the seafood by the micro diffusion method.

RESULTS AND DISCUSSION

Total Plate Count (TPC): During monsoon season, the highest total plate count (TPC) (of 5.7×10^4 CFU/g) was observed for *S. fimbriata* followed by *K. pelamis*, *S. lysan*, *S. commersonii* and *S. commerson*, where the lowest TPC $(3.0 \times 10^4$ CFU/g) was observed in nonpenaeid prawn *Acetes Indicus* (Table 1). During post monsoon season, the highest plate count $(4.0 \times 10^4$ CFU/g)) was observed in *S. fimbriata* and the lowest TPC $(2.5 \times 10^4$ CFU/g) was noted in *A. indicus* (Table 2).

In fresh fish, the acceptable limit is 5×10^5 /g at 37° C but for cooked or dried fish, the permissible limit is 1×10^5 /g at 37° C [17]. In this study, *Sardinella fimbriata* had the highest TPC 5.7×10^4 in monsoon which did not exceed the permissible limit. Similar works carried out in dried fishes

Table 1: Qualitative and quantitative analysis of dried seafood's for monsoon season (Temp. 27°C)

	TPCC	TFC	Moisture	Total coliform	Fecal coliform	E. Coli	Fecal streptococci	Salmon-		TMA-N	TVB-N
Samples	FU/g	Cfu??/g	(%)	MPN/100g	MPN/100g	MPN/100g	MPN/100g	ella25g	Vibrio25g	(Mg%)	(Mg%)
S. lysan	5.3×104	4×10³	45	95	75	15	1100	Absent	Present	15.01	26.26
A.indicus	3.0×104	2×10^3	35	65	30	14	115	Absent	Absent	7.1	18.4
K.pelamis	5.5×104	4×10^3	40	65	25	7	200	Absent	Absent	8.15	26.12
S.commersonii	4.0×104	3×10^3	30	65	30	11	150	Aresent??	Absent	9.1	23.14
S.fimbriata	5.7×104	8×10^3	55	150	95	16	200	Aresent??	Present	16.34	30.00
S.commerson	3.8×104	5×10^3	50	75	65	20	450	Present	Present	10.4	25.79

Table 2: Qualitative and quantitative analysis of dried seafood's for post -monsoon season (Temp. 28°c)

	TPCC	TFC	Moisture	Total coliform	Fecal coliform	E. Coli	Fecal streptococci	Salmon-		TMA-N	TVB-N
Samples	FU/g	Cfu/g	(%)	MPN/100g	MPN/100ml	MPN/100ml	MPN/100ml	ella25g	Vibrio25g	(Mg%)	(Mg%)
S. lysan	3.8×104	3×10³	30	95	75	16	150	Absent	Absent	6.74	14.81
A.indicus	2.5×104	1×10^3	25	45	20	11	115	Absent	Absent	6.01	16.95
K.pelamis	3.0×104	2×10^3	35	65	15	11	95	Absent	Absent	6.49	17.02
S.commersonii	2.8×104	2×10^3	25	75	35	7	65	Absent	Absent	7.2	18.40
S.fimbriata	4.0×104	5×10 ³	40	115	95	20	200	Absent	Present	7.15	19.42
S.commerson	3.5×104	4×10^3	45	65	40	6	160	Absent	Present	6.11	18.46

Table 3: Qualitative and quantitative analysis of dried seafood's for summer season (Temp. 37°c)

	TPCC	TFC	Moisture	Total coliform	Fecal coliform	E. Coli	Fecal streptococci	Salmon-		TMA-N	TVB-N
Samples	FU/g	Cfu/g	(%)	MPN/100ml	MPN/100ml	MPN/100ml	MPN/100ml	ella25g	Vibrio25g	(Mg%)	(Mg%)
S. lysan	3.2×10³	3×10³	25	65	20	7	95	Present	Present	4.21	12.97
A.indicus	1.0×10^{3}	-	15	40	15	11	75	Absent	Absent	ND	9.31
K.pelamis	1.1×10^{3}	3×10^3	20	35	15	7	40	Absent	Absent	3.89	12.34
S.commersonii	2.1×10^{3}	1×10^3	15	40	20	6	45	Absent	Absent	4.04	13.46
S.fimbriata	2.2×103	1×10^3	20	30	10	4	75	Absent	Present	5.00	14.14
S.commerson	2.0×10^{3}	2×10^3	20	45	30	15	115	Absent	Absent	4.38	11.06

of Tuticorin market have recorded high bacterial count in *S. fimbriata* at 3.5×10³ [23]. In Cochin market, the bacterial count in dried fishes was found to be less than 10⁷ g⁻¹ [24]. In Nigerian market, the total bacterial count of dried fish samples was 4.6×10⁶g⁻¹ [25]. The present study results revealed high bacterial load in monsoon season and the mean temperature of that 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 (Table 3) 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 [4].

TMA-N and **TVB-N**: The presence of spoilage indicators TMA-N and TVB-N in sun dried sea foods in different seasons is shown in tables 1 to 3. TMA-N and TVB-N values indicate freshness of the fish¹26]. TMA-N value during the monsoon season ranged between 7.1-16.34 mg / 100 g and the highest value was recorded in *S. fimbriata* and the lowest level was recorded in *A. indicus* (Table 1). During post-monsoon season, (6.01-7.2 mg / 100g) the highest and lowest values were observed in *S. fimbriata* and *A. indicus*, respectively (Table 2). In case of summer

season (3.89-5.00 mg / 100g), *S. fimbriata* also hasd the highest value of TMA-N where *A. indicus* showed the lowest value (Table 3).

There was also wide variation in critical values suggested for individual species concerning TVB-N evidenced by the highest and lowest TVB-N values of the dried seafood's during monsoon season (30-18.4 mg / 100g) (Table 1) followed by post-monsoon season (19.42-14.81 mg / 100g) (Table 2) and summer season (14.14 mg / 100g-9.31 mg/100g) (Table 3), respectively.

Enzyme from the spoilage microorganisms can metabolize the aminoacid of the fishmuscle producing total volatile base nitrogen and it is used for the estimation of spoilage. The production of TMA is dependent on the bacterial activity as well as from endogenous enzyme [27]. The recommended level of the TMA-N value for human consumption is 10-15 mg/100 g [28]. Bacterial putrefaction of spoilage bacteria is the reason for the sudden increase of TMA-N in fish muscle [29, 30]. TVB-N level in fish has also been used to indicate spoilage growth of microorganism [31]. The acceptable level of TVB-N in fishes is 35-40 mg/ 100 g [32].

Higher results TVB-N level of fish in retail market were obtained by several investigators. The TVB-N level of fish in retail market was high as 98 mg / 100 g [33]. TVB-N level of *S. fimbriata* stored at 20°C for 24 hours was 23.9 mg / 100 g and it increased to 53.6 mg / 100 g during 4 days of storage [34] but normally TVB-N value increased during storage at ambient temperature [35]. Visible fungal colonies were noted in fish sample even before TVB-N value reached the maximum permissible limit [28]. The sudden increase of TMA-N and TVB-N (>10Mg%) values to be concurrent with the onset of bacterial putrefaction [29].

Moisture: The results of moisture content in different types of salted and dried seafood samples in different seasons are presented in Tables 1 to 3. The moisture content was higher (55%) in *S. fimbriata* followed by *S. commerson* (50%), *S. lysan* (45%), *K. pelamis* (40%) *A. indicus* (35%) and *S. commersonii* (30%), in monsoon season. In the post-monsoon season, high moisture content was observed in *S. commerson* (45%) followed by *S. fimbriata* (40%), *K. pelamis* (35%), *S. lysan* (30%) and *A. indicus* & *S. commersonii* (25%). During summer season, the highest and lowest moisture contents were observed in *S.* lysan (25%) and *S. commersonii* (15%) & *A. indicus* (15%).

Moisture content of seafood's plays an important role in spoilage and lowering of moisture retards the spoilage [36]. 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 [37]. However, the moisture content seems to be an exact indicator of the susceptibility of a product to undergo microbial spoilage [38]. In the present study, the moisture content was increased during monsoon season and it was considerably lowered in summer season and this result agrees with the result of earlier reports [3, 39]. The total bacterial count of dried fishes obtained from cochin was less than $10^7 g^{-1}$ while the moisture content ranged from 80-65% [24]. In the present study, S. fimbriata also had high moisture content (55%) and high microbial load (5.7×10⁴cfu/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.

Fecal Indicator Bacteria: Pathogenic or indicator bacteria may not be present insufficiently in large numbers in water or food to be detected by plating 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 [17].

In the present investigation, the MPN value of the seafood samples varied with different seasons. The total coliforms and fecal coliforms during summer varied from 30-65 and 10-30 / 100 g, respectively (Table 3). In post-monsoon, the total coliforms and faecal coliforms ranged between 45-115 and 15-95 / 100 g, respectively (Table 2), while during monsoon, the values were between 65 -150 and 25 -95 / 100 g, (Table 1). E. coli is a commensal bacterium which colonizes the intestinal tract of humans. However, some are pathogenic causing diarrhea and are termed as enteropathogenic E. coli. In the present work, MPN of E. coli values recorded during summer were between 4 -15 / 100 g. In post monsoon and monsoon, the values varied between 6-20 and 7 -20 / 100 g. Fecal streptococci values were ranging from 40-115 / 100 g in summer, 65-200 / 100 g in the post-monsoon. In the monsoon period, the values varied from 115-1100 / 100 g.

Total coliform group can be sub grouped as fecal and non fecal coliforms. The fecal coliform subgroup is derived from feces of human and other warm-blooded animals such as cows, sheep, poultry, etc. The non fecal subgroup is frequently found on vegetation and in the soil; some are plant pathogens[40]. The presence of fecal coli- form organisms indicates recent and possibly hazardous fecal pollution. The most common fecal coliform species is *Escherichia coli* [41, 42]. Fecal streptococci are non pathogenic organisms but commonly occur in the intestines of man and other warm-blooded animals which makes them a useful group of indicator of fecal contamination [43, 44].

Washing the catches in polluted coastal water definitely add the fecal indicator bacteria. Drying done in unhygienic way also added faecal bacteria to the fishes [45]. Seasonal variation of fecal indicator bacteria in fish and coastal waters has already been reported to be high along Tuticorin fish landing centers [46]. However, the fecal pollution at Bhuvanagar coast was reported to be of human origin based on the fecal index [47]. 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 handing in processing and inadequate drying. MPN *E.coli* count showed more variation between samples collected at the same time and at different seasons, which ranged from 9/100 ml to over 1400/100 ml[46]. In our present study also *E.coli* count showed variations, high in monsoon season. Levels of fecal indicator bacteria were also reported to be high both in fish and dehydrated fish from Cochin fisheries and retail markets of Mumbai [32].

Total Fungal Count: The results of the fungal counts in different sun dried sea foods were presented in tables 1 to 3. 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 though 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.

Fungal counts were high in sun dried sea foods during monsoon seasons. *S. fimbriata* had the highest fungal count $(8\times10^3\text{cfu/g})$ followed by *S. commerson* $(5\times10^3\text{cfu/g})$, *S. lysan & K. pelamis* $(4\times10^3\text{ cfu/g})$, *S. commersonii* $(3\times10^3\text{ cfu/g})$ and *A. indicus* $(2\times10^3\text{ cfu/g})$. In post-monsoon period, fungal colonies were slightly decreased in all the sea foods where as in summer season again fungal counts were low in all the sea foods.

The seasonal variation of fungal species in dried sea foods are shown in Table 4. In dried sea foods, 10 fungal species belonging to 4 genera were

present. Out of 10 species, 9 species were Deuteromycetes and 1 species was Phycomycetes. The fungal species Aspergillus niger, A. fumigatus and A. flavus were dominant in the seafood samples in all the three seasons. Presence of different fungal species in dried seafood's in different seasons were reported [8, 9, 11, 39, 48]. The quality of salted and sun dried fishes is adversely affected by the occurrence of fungi [48]. Also, the dominant fungi in salted and sun dried seafood vary with the place. The commonly occurring fungi in the west coast of India are Aspergillus, Fusarium, Rhizopus, and Mucor [8]. In cochin market, A. Flavus and A. ochraceus are dominant [49] where as in Tuticorin south east coast of India Aspergillus and Penicilium are dominant [12].

Total ten fungal species were isolated from the dried sea foods and among that the dominant seven fungal species were grown in different NaCl concentrated PDA medium to identify halo tolerant fungi and the results are shown in Table 5. The fungal species such as *A.niger* and *A.flavus* were grown on up to 18%, of NaCl concentrated PDA medium. The *A. fumigatus* was grown on 14% Na Cl PDA medium where *A. orazae*, *Mucor* and *A.nidulants* were grown only on 0% Na Cl. In case of *Pencilium* species, it was grown on 0 and 10% NaCl but it was not grown on 14 and 18% NaCl PDA medium. Among the seven fungal species, only A. *niger* and *A. flavus* were halo tolerant fungi in the salted and sun dried sea food samples and this report coincide with the earlier reports [3].

Pathogenic Bacteria: The results of isolation of human pathogens such as *Salmonella* and *Vibrio* from salted and sun dried sea foods in different seasons are shown in tables 1 to 3. During summer season, *Salmonella* and *Vibrio* were present in *S. lysan* but in in *S. fimbriata*, only *Vibrio* was present. In the case of other seafood's, both the pathogenic bacteria were absent in this season.

Table 4: Seasonal variation of fungal species in salted and sun dried seafood's

	May-July	Aug-Oct	Nov-Feb
Samples	Summer	Monsoon	Post monsoon
S. lysan	Aspergillus orazae, A.niger	A.niger, A.flavipes	A.flavus, A.orazae, Rhizopus
A.indicus	A.flavus, A.niger	Penicillium, A. flavus, A. fumigatus	A.flavus, A.niger
K.pelamis	A. flavus, White sterile mycelia	A.fumigatus, A.niger	A.flavus, A.orazae
S.commersonii	Mucor, Penicillium	A.flavus, Rhizopus, A.niger	A.sydowi, A.flavus, A.niger
S.fimbriata	A.niger, A.flavus, A.sydowi	A.niger, A.nidulans, Penicillium	A.flavus, A.orazae, A.fumigatus
S.commerson	A.niger, A.f umigatus	A.flavus, Afumigatus, Mucor, A.niger	A.niger, A.flavus

Table 5: Salt tolerance of fungi isolated from saltedand sun dried seafood's

	Concentration of sodium chloride							
Fungal species	0%	10%	14%	18%				
Aspergillus niger	+	+	+	+				
A.flavus	+	+	+	+				
A. fumigatus	+	+	+	-				
A. orazae	+	-	-	-				
A.nidulants	+	-	-	-				
Mucor sp.	+	-	-	-				
Penicillum sp.	+	+	-	-				

During post-monsoon season, none of the samples was contaminated with *Salmonella* but *Vibrio* species was found in *S. fimbriata* and *S. commerson*. In monsoon season, only *S. commerson* was infected by *Salmonella* but *Vibrio* was present in *S. lysan, S. fimbriata and S. commerson*.

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 its presence is usually due to unhygienic handling, carriers, or polluted costal water [50]. The dry fishes of dry fish markets were basically collected and processed in Therespuram fishing village. Extensive contamination of coastal water along Thirespuram has been reported [51]. Contamination of fish and fishery products with Salmonella and Vibrio has been reported in different parts of India [52-57]. Incidence of pathogens in the samples of fish market may be attributed to external contamination [53]. and poor handling at ambient temperature [58].

The study showed that salted and sun dried fishes sold in Tuticorin fish markets 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 the unhygienic handling of the fisher folks, improper processing and unhygienic vendors and venting area. So, public awareness about the importance of quality products and to avail products by hygienic processing of the fishes and air tight packing of the final product upto marketing of the products.

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