International Journal of Microbiological Research 3 (2): 138-143, 2012
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2012.3.2.63119

Microbial and Biochemical Qualities of Salted and Sun Dried Sea Foods of Cuddalore, Southeast Coast of India

K. Saritha, K. Immaculate jayasantha, Velammal Aiyamperumal and Jamila Patterson

Suganthi Devadason Marine Research Institute, Tuticorin, India

Abstract: The biochemical and microbial qualities of salted and sun dried seafoods of Cuddalore coast were analysed. The spoilage indicators such as TMA-N, TVB-N and FFA were above the acceptable limit. The microbial load was high. Among the bacterial pathogens, only *E.coli* was isolated. Six fungal species coming under four genus were also detected.

Key words: Bacteria · Fungi · Spoilage Indicator · Salted and Sun Dried Fishes · Cuddalore Coast

INTRODUCTION

The salting process is considered as one of the oldest method of fish preservation and this process is still being used in several places around the world. Salted fish products are popular in many countries and it has been proved to be safe for the millenniums even in developed countries [1-3]. Fish is one of the best sources of protein, vitamins and nutrients required for both infant and adult diets [4]. In India about 17% of the total fishes caught are being used for salting and drying [5]. On the global basis, 74% of the marine landings are processed by curing [6].

Curing is a simple and cheap method of processing, requiring least technical expertise but it has a great significance and relevance in the socio-economic system of small-scale fisher folk [7]. The effect of salt is to destroy the growth of microorganisms, where in the end the fish meat gets its way to durability [8]. Salting is generally aimed at reducing water activity (a_w) which inhibits the growth of spoilage microorganism as well as to inactivate autolytic enzymes [9, 10].

Microbial action has been known to play an important role in the spoilage of fish [11]. Bacterial spoilage is characterized by softening of the muscle tissue and the production of slime and offensive odour [12]. Microbial and biochemical quality assessment are necessary to ensure the food safety of any processed product [13]. The aim of salting is not only to extend the shelf life of fresh fish but also to provide desirable sensorial changes [14].

Environmental conditions and microbial quality of the water affect the microbial status of seafood [2, 15]. The salt contains chloride ions and these chloride ions are toxic for some microorganisms [16]. The quality of such products is often adversely affected by the growth of fungi in dried fish, which has been reported by several workers [17-20]. The microbial safety of seafood is an important concern of consumers as the major share of the outbreaks related to consumption of fish are caused by bacteria such as *C. botulinum*, *E. coli, Salmonella, Staphylococcus, Vibrio sp* and *Bacillus cereus* [21]. Fishery products are also recognized as a carrier of food borne bacterial pathogens like *Salmonella, Vibrios* sp., *E. coli and Listeria* sp. [22, 23]. The pathogenic organisms are found in the internal and external surfaces of the fishes but in low concentration [24] and the absence of pathogenic microbes in salted fishes is reported [25].

The present investigation was carried out to determine the microbial and biochemical qualities of seven salted and sun dried sea foods which are highly preferred by the consumers of Cuddalore old town areas.

MATERIALS AND METHODS

Collection of Samples: Dry fish samples were bought from Cuddalore old town dry fish market and were packed in air tight polythene bags and brought to the laboratory. It was powdered and used for biochemical and microbial analysis.

Biochemical Analysis: The spoilage indicators such as total volatile base nitrogen (TVB-N) and Tri methyl amine nitrogen (TMA-N) were determined with Trichloro acetic acid extract of the meat by using Conway's, micro-diffusion method [26]. The free fatty acid was estimated by the method of Ke *et al*. [27].

Corresponding Author: Jamila Patterson, Suganthi Devadason Marine Research Institute, Tuticorin, India.

Determination of Moisture Content: Moisture content was determined by drying the sample in a hot air oven (Gallenkamp, HOTBOX; method OVB-306) at 105 ° C for 24 hours until contact weight [28].

Microbial Analysis: Total bacterial count (TBC) and total fungal count (TFC) were enumerated using nutrient agar and SDA (Sabouraud's dextrose agar) by the spread plate technique [29]. The fungal strains were isolated and stained with cotton blue in lacto phenol and identified according to Kohlmeyer and Kohlmeyer [30]. Pathogenic bacteria such as *Salmonella and Vibrio sp* were enumerated by the spread plate method recommended by the US Food and Drug Administration [31]. The presence of *E. coli*,was enumerated by 5 tube MPN method using APHA [32].

RESULTS AND DISCUSSION

Moisture: The results of the moisture content of samples is represented in Fig. 1. Among the seven salted and sun dried sea foods *Acetes* sp. contained low moisture (29.9%) content. The dry salting method produced considerable loss of water due to heavy uptake of salt [33]. *Scomberomorus* sp contained high (43.1 %) moisture content. The remaining five samples; *Chirocentrus dorab*, *Pomadays maculates*, *Leiognathus dussumieri*, *Stolephoru commersonnii* and *Parapeneus indicus* had 39.2, 42.4, 36.9, 32.7 and 33.7% of moisture content respectively. The standard moisture content for dried seafoods ranges from 15.9 to 16.2%. The moisture content is an exact indicator of the susceptibility of a product to undergo microbial spoilage [34]. It has a potential effect on the chemical reaction rate and microbial growth rate of the food product [35]. Moisture also affects the stability and shelf life of the food product. The moisture content plays an important role in the spoilage of fish and Stansby [36] reported that reduction of moisture content retards the spoilage of fish.

TMA-N: It is often used as an index to assess the quality of seafood products [37]. Total Volatile Base Nitrogen and Tri Methyl Amine Nitrogen results were shown in Figure 2 and 3. Connell [38] suggested that the acceptable limit of TMA-N for fishery products is 10-15 mg/100g. In the present study TMA-N in *Acetes* sp and *Stolephorus commersonnii were* within the acceptable limit (12.9 and 14.9 mg/100g). The other sun dried fishes contain high level of TMA-N content and were above the acceptable limit. Beatty and Gibbons [26] reported that the production of TMA-N is depend on the bacterial activity. All the samples had high moisture content for the proliferation of microbes which might be the reason for the high amount of Tri methyl amine nitrogen in the samples.

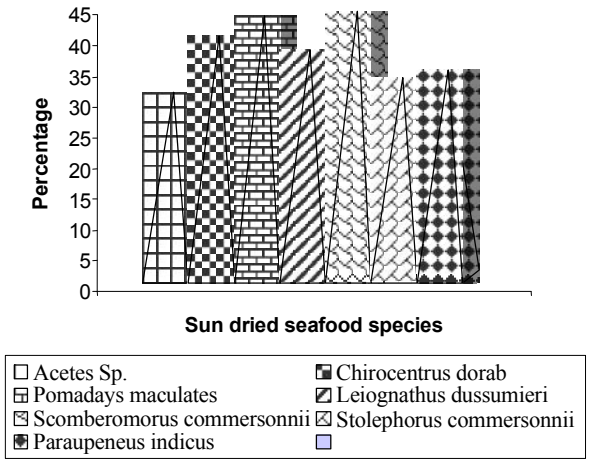

Fig. 1: Moisture content of the sun dried seafoods

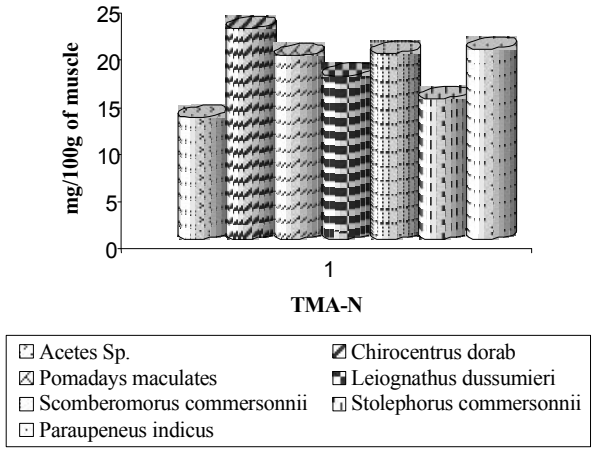

Fig. 2: TMA-N in sun dried seafoods

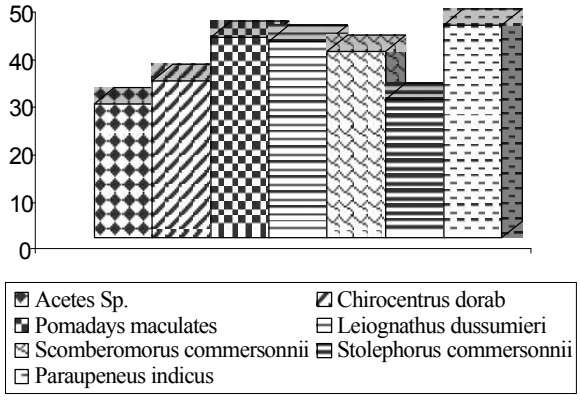

Fig. 3: TVB - N in sun dried seafoods

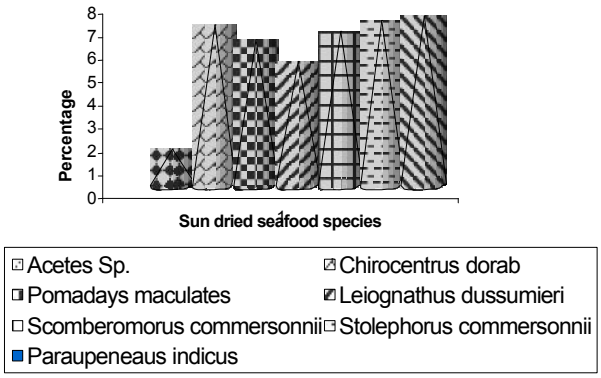

Fig. 4: FFA in sun dried seafoods

TVB-N: TVB-N is accepted universally as an indicator of quality that uses ammonia. Lannelongue [39] and Sikorski [40] reported 30mg/100g of TVB-N as the upper limit, above which fishery products are considered unfit for human consumption. In the present study, *Acetes* sp. and *Stolephorus commersonnii* had 28.2 and 29.3mg/100g respectively and it was within the acceptable limit. Remaining species contained high level of TVB-N content and were above the acceptable limit (Fig. 3). Level of total volatile nitrogen in fish is commonly used as a spoilage indicator [41, 42]. TVB-N measurement indicates the extent of the breakdown of protein due to bacterial and enzymatic action leading to amine production. In the present study since the dry fish samples had high moisture content it will help the microbes to proliferate for the production of total volatile nitrogen.

FFA: Free Fatty Acid (FFA % of Oleic acid) is one of the quality indicators of seafood products. The result of FFA content was shown in figure 4. Huss, [43] reported that the acceptable limit of FFA in seafood is about 0.5-1.5%. The Free fatty acid contents of all the seven salted and sun dried sea foods were high and were above the acceptable limit. The increase amount of FFA is directly proportional to the degree of spoilage [7].

Microbiological analysis also showed variations among the samples. The total bacterial count (TBC), total fungal count (TFC) and pathogenic bacterias *E. coli, Salmonella* and *Vibrio* were assessed and the results were presented in tables 1 and 2. Quality levels are based on the plate counts with representative sample unit not less than 5 X 10^5 CFU/g are good quality between 5 X 10^5-10^7 marginally accepted quality and plate count at or above 10^7 are considered unacceptable in quality(ICMSF, 1986-44 p). In our present study higher bacterial count such as 2.13 X 10^6 was observed for the sun dried fish *Paraupeneus indicus* which was not above the permissible limits. For In the case of fungal count lower count was observed for *Acetes* sp. In sun dried fish *Pomadays maculates* visible fungus was observed and it had higher fungal count of 2.2 X 10^4.

In the present study bacterial and fungal colonies were observed in the commercial sun dried seafood and it is having contamination. This may be due to post harvest delay, improper transportation, unhygienic handling and processing during the salting and sun drying process, contaminated working floor, salt and water.

Table 1: TBC and TFC determination of salted and dried fishes

S. No	Name of the species	TBC (CFU/g)	TFC (CFU/g)
1	*Acetes* sp.	1.3 X 10^6	1.3 X 10^4
2	*Chirocentrus dorab*	1.94 X 10^6	1.9 X 10^4
3	*Pomadays maculates*	1.43 X 10^6	2.2 X 10^4
4	*Leiognathus dussumieri*	2.02 X 106	1.9 X 10^4
5	*Scomberomorus commersonnii*	1.82 X 106	2.1 X 10^4
6	Stolephorus *commersonnii*	1.13 X 10^6	1.5 X 10^4
7	*Paraupeneus indicus*	2.13 X 10^6	2.1 X 10^4

Table 2: Presence of pathogenic bacteria in salted and dried fishes

S.No	Name of the species	*E. coli* (MPN/100 ml)	*Salmonella* (25g)	*Vibrio* (25g)
1	*Acetes* sp.	11	Absent	Absent
2	*Chirocentrus dorab*	3	Absent	Absent
3	*Pomadays maculates*	21	Absent	Absent
4	*Leiognathus dussumieri*	26	Absent	Absent
5	*Scomberomorus commersonnii*	22	Absent	Absent
6	Stolephorus *commersonnii*	3	Absent	Absent
7	*Paraupeneus indicus*	9	Absent	Absent

Table 3: Presence of fungi in salted and dried fishes

S. No	Name of the species	Name of the Dominated Fungi
1	*Acetes* sp.	*Aspergillus flavus, Aspergillus ustus,*
2	*Chirocentrus dorab*	*Aspergillus awamori, Mucor* sp, *Fusarium* sp.
3	*Pomadays maculates*	*Aspergillus ustus, Aspergillus nidulans, Mucor* sp..., *Penicillium* sp.
4	*Leiognathus dussumieri*	*Penicillium.*sp, *Fusarium* sp, *Aspergillus niger, Aspergillus candidus, Hemicola* sp.,
5	*Scomberomorus commersonnii*	*Aspergillus flavus, Sterile mycelium, Verticilium* sp, *Fusarium* sp.
6	*Stolephorus commersonnii*	*Aspergillus candidus, Penicillium* sp.
7	*Paraupeneus indicus*	*Aspergillus awamori, Mucor* sp., *Penicillium* sp.

The above results coincide with the result of Jamila Patterson and Govindan Ranjitha [7] and they observed higher bacterial and fungal contamination in commercial sun dried fish of Tuticorin dry fish market.

The dry fish samples such as *Pomadays maculates, Scomberomorus commersonnii and Paraupeneus indicus* had visible fungal growth at the time of purchasing. But all of other samples there is no visible fungal growth but after enriched with Sabouraud's Dextrose Agar we observed different fungal species belonging to 6 genera such as *Penicillium* sp., *Fusarium* sp., *Aspergillus* sp., *Mucor* sp., *Hemicola* sp. and *Verticilium* sp with immature fungal growth (Sterile mycelium). *Aspergillus* sp was detected in all the seven sun dried sea foods and *Penicillium* sp. in four sun dried sea foods, *Fusarium* sp. and *Mucor* sp. were found in three sun dried sea foods. But *Hemicola* sp was detected only in *Leiognathus dussumieri* and sterile *mycelium*. Chakrabarti and Varma [45] reported that, *Aspergillus sp* and *Mucor* sp. are the common fungi in the sun dried fishes of Visakhapatnam and Kakinada coast.

The fungal species such as *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp. and *Fusarium* sp. are pathogenic to human beings [46]. Sharma [47] reported that *Aspergillus* sp., *Mucor* sp and *Penicilium* sp. are known to cause food spoilage. Thus the presences of these fungi are of great significance in view of seafood safety and quality. Marine fungi in the dried fishes may have entered the fishes through the seawater used for washing or salt used for the preparation of brine [46]. The presence of high fungal count in the present study may be due to the brine used in the fishing curing yard of Cuddalore. Sugumar *et al*. [48] reported high level of fungal counts in the brine used in fish curing place. Fish and fishery products are in the forefront of food safety because of their indispensable role as cheap protein supplement and their significance as one of the most internationally traded foodstuffs. In recent times the number of food borne disease out break are rising [49].

In the present study, *E.coli* was detected in all the sun dried sea foods. The presence of *E. coli* indicates the seafood samples contaminated with total and faecal coliforms. Normally coliforms are normal flora of human and animal intestine [25]. Faecal contamination in the landing center, washing the catches in polluted coastal water with the disposal of sewage, reused water and improper disposal of faecal materials are the possible sources for coliform contamination in commercial seafood samples. Our results coincide with the earlier results [50,51]. But, *Samonella* and *Vibrio* sp. which are frequently involved in the spoilage of fishes were not found in all the seven seafood samples used in the present study. Azam *et al*. [13] Observed the absence of the spoilage organisms *Vibrio* sp. and *Salmonella* sp. in all the dry fish samples.

The results of the present study on the microbial and biochemical quality analysis of sun dried sea foods is an important warning signal for the consumers about the quality of the sun dried seafood. Therefore, it is important to train local fisher folk on proper processing and preservation to improve the quality of the sun dried sea foods for the benefits of the consumers.

ACKNOWLEDGEMENT

The authors are thankful to the Director of Suganthi Devadason Marine Research Institute, India for the facilities to carry out the work. Authors are also thankful to Dr. Paneerselvam for helping us in identifying the fungal species.

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