Int J Pharm Bio Sci 2015 Oct; 6(4): (B) 818 - 823

Research Article **Allied science**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

MICROBIAL SCREENING ON RAW AND DRY SELECTED MARINE SPECIES FROM NAGAPATTINAM DISTRICT, TAMIL NADU, INDIA

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ABSTRACT

The present study was conducted for microbiological assessment of six selected marine species from Nagapattinam district. Total viable colony count, E.coli, Vibrio species, Salmonella species, total yeast count and total mould count were determined in *Stolephorus commorsonnii*, *Hilsa ilisha*, *Otolithes ruber*, *Sardinella longiceps*, *Ilisha elongata* and *Penaeus indicus*. The total viable colony count of the raw fishes and prawn varied from 0.10- 8.30% and the dry fishes and prawn varied from 0.10-1.20%. The *E.coli* of the raw fishes and prawn varied from 0.20-0.80% and the dry fishes and prawn varied from 0.08 to 0.20%. The vibrio species of raw fishes and prawn varied from 1-4% and the dry fishes and prawn varied from 0.2 – 0.20%.In the present study salmonella, yeast and mold were not detected in both fresh and dried samples. The total viable colony count, *E.coli* and vibrio species were reduced in the dry fishes while *E.coli* was completely absent in *Stolephorus commersonnii*, *Sardinella longiceps* and *Ilisha elongata*.

KEYWORDS: **Fresh samples, dried samples, microbial analysis.**

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INTRODUCTION

Fish, an important source of protein to the large teaming population of developing countries like India provides 40% of the dietary intake of animal protein along with vitamins and minerals necessary for the maintenance of a healthy body. Among the fishes, sea fishes are known to provide high contents of important constituents for the human diet such as nutritional proteins, digestive proteins, soluble vitamins and microelements, highly unsaturated fatty acid and lipids which plays a positive role in preventing certain human diseases. But inspite of it, only little attention has been paid to the commercial use of these fish and fishery byproducts which are produced in large amount annually in the world. About 50% of fish produced in the remote coastal settlements perish before reaching the consumers as a result of poor post harvesting technology like handling, preservation and processing practice adopted by fishermen's, fish farmer and fish entrepreneurs. Since fish is a risky product it needs proper handling and processing in order to preserve its nutrients, functional components and wholesomeness that promote good health. Because of the presence of wide fish varieties and post harvesting problems, the public has always required continuous reassurance about its quality and are often unsure of particular species product made from them are good to eat or not. The major quality control issues encountered in dried salted fish are the variable but often low quality final product, its high salt content, insect infestation and microbial contamination which induce a rapid rate of deterioration during transport, distribution and storage[5]. Moreover dry fish production plants in most countries are located in very remote rural locations, characterized by lack of infrastructure facilities and poor sanitary conditions. Thus from several points of view fish quality has become very important in the world. This is because consumers now are more aware of possible food hazards and malpractices which will affect the quality as a result of bad handling, providing a suitable environment for microorganisms and processing procedures. Therefore, consumers individually or collectively become more demand in respect of freshness, naturalness, microbial safety, free from pollutants and protect from damage. Considering the popularity of fresh and dried marine species, nutrition, an important influencing factor of fish product/seafood consumption among the South Indians it was envisaged to prepare good quality fresh and dried fish products. It was expected that such type of marine products knowledge, chemical composition of any edible organisms was extremely important since the nutritive value reflects on its biochemical contents. Fish species should be recommended for human consumption only after assessing the nutritive value of the species with regards to its nutritional merits. But still our knowledge on its nutritive value is fragmentary. In this respect the present study was carried out to evaluate microbiological assay which play a major role in recent years in estimating the nutritional status of selected marine raw and dried fish.

MATERIALS AND METHODS

Collection of samples

Fresh and dry fish samples and prawn were procured from Nagappatinam district, Pushpavanam town fish market and were identified, labeled and packed in air tight polythene bags and brought to the laboratory in aseptic condition.

Sample preparation

Selected freshly harvested fishes and prawn were washed with tap water to remove the ice, and then rinsed with double distilled water and cleaned thoroughly. The fresh edible species were eviscerated beheaded and the muscle tissues were collected for further experimental work. Dry samples were cleaned to remove the external dirt and debris and powdered for microbial analysis.

Analysis

The selected fresh and dried seafood samples were analyzed for total bacterial count (TBC),

total fungal count (TFC), *E.coli*, *Salmonella* species and *Vibrio* species. All microbial analysis was carried out in selective medias which were prepared according to the manufacture's specification. These selected media were sterilized in an autoclave at 121˚C for 15 minutes. Petri dishes, homogenizers, pipettes were sterilized using dry heat at 180˚C for 1 hour. Standard chemicals of analytical grade were obtained from Merck limited (Mumbai, India) and used for the present lab analysis .

Isolation and Enumeration of (i) Bacterial count

Enumeration of bacterial load was done using plate count agar by spread plate technique. 10g of the sample was mixed with 90ml saline water. Approximate dilutions of fish homogenate were spread on plate count agar and incubated at 37°C for 24 – 48 hrs and the colonies were counted for total plate count and the count was expressed as cfu/g[3].

(ii) Fungal count

Isolation and enumeration of fungi was carried out in Sabouraud's dextrose agar to which penicillin and streptomycin had been added and identified according to[4&10]. The samples were spread on the surface of the medium and incubated at room temperature (28 ± 7°C) for 3-5 days and the colonies were counted for total fungal count and the count was expressed as cfu/g.

(iii) *E.coli*

The total coliform number was determined by MPN (maximum probable number) technique[2]. Fish homogenate was transferred to lauryl sulphatetryptone broth (LSTB) tubes and incubated at 37°C for 24 hrs and observed for growth and gas production. Samples from positive LSTB tubes were transferred to EC broth tubes and incubated at 37°C for 24 – 48 hrs. Samples from positive EC broth were streaked on to Eosine methylene blue agar plate to confirm the E.coli.

(iv) Salmonella

For the isolation of salmonella 25g of the sample was homogenized and enriched in 225 ml lactose broth at 37°C for 24 hrs. Selective enrichment of salmonella was carried out in tetrathionate (TT) broth and Rappa port vassilidis medium in thermo statically controlled water both. Each of this enriched culture was streaked in XLD (Xylose lysine deoxycholate) agar. Typical salmonella exhibits pink colonies with or without black centers.

(v) Vibrio

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

Table 1
Microbial quality and quantity of selected marine raw fishes from Nagappatinam district, Tamilnadu

Fishes	Microbial quality and quantity of selected marine raw samples				
	TVC	E.coli	Vibrio Sp.	Yeast & mould	Salmonella Sp.
Stolephorus commersonnii	1.30	0.20	1	AB	AB
Hilsa ilisha	8.30	0.60	3	AB	AB
Otolithes ruber	3.0	0.80	4	AB	AB
Sardinella longiceps	7.20	0.40	2	AB	AB
Ilisha elongate	1.0	0.30	2	AB	AB
Penaeus indicus	0.10	0.40	4	AB	AB

**** AB- absent; E.coli- cfux10[5]; Vibrio- cfu/g[3]; TVC- cfu/g[3]***

Table 2
Microbial quality and quantity of selected marine dry fishes from Nagappatinam district, Tamilnadu

Fishes	Microbial quality and quantity of selected marine dry samples				
	TVC	E.coli	Vibrio Sp.	Yeast & mould	Salmonella Sp.
Stolephorus commersonnii	0.30	AB	0.20	AB	AB
Hilsa ilisha	0.40	0.10	0.3	AB	AB
Otolithes ruber	1.20	0.20	1.0	AB	AB
Sardinella longiceps	0.20	AB	0.2	AB	AB
Ilisha elongata	0.10	AB	0.2	AB	AB
Penaeus indicus	1.0	0.08	0.8	AB	AB

**** AB- absent; E.coli- cfux10^5; Vibrio- cfu/g^3; TVC- cfu/g^3***

RESULTS AND DISCUSSION

The individual results of microbiological analysis of selected fresh and dry samples are presented in Table 1and 2.The total viable colony count of the raw fishes and prawn varied from 0.10- 8.30 cfu/g^3 and the dry fishes and prawn varied from 0.10-1.20 cfu/g^3. The yeast and mould which are examples of fungi, responsible for food spoilage were absent in the raw and dried fishes and prawn samples. The *E.coli* of the raw fishes and prawn varied from 0.20-0.80 cfu/g^3. The highest value (0.80 cfu/g^3) was recorded in *Otolithes ruber*. The lowest value (0.20 cfu/g^3) was recorded in *Stolephorus commersonnii*. The *E.coli* of the dried fishes and prawn such as *Stolephorus commersonnii*, *Sardinella longiceps* and *Ilisha elongata* were absent which may be attributed to use of clean potable water for washing of fish and maintaining a totally hygienic conditions during drying operation. On the Other hand three other study species such as *Hilsa ilisha, Otolithes ruber* and *Penaeus indicus* showed the presence of *E.coli* which varied from 0.08 to 0.20 cfu/g^3 may due to use of near shore water for washing and unhygienic drying conditions [8] observed that market sample of dry fish in Sri Lanka showed the presence of *E. coli* because the beaches in Sri Lanka were subjected to defaecation by people and [6] isolated *E. coil* from some of the dry fish samples in the market of Kakinada living nearby. In the present study salmonella was not recorded in all the raw and dried fishes and prawn which was in accordance to [11] who recommended that the dried fish should be totally free from *Salmonella*. Similar results was obtained in the present study which may be attributed to adoption of good manufacturing practices during higher count of indicator organisms in the worker's hand, soil, water and curing tanks. The *vibrio* species of raw fishes and prawn varied from 1-4 cfu/g^3. The highest value (4 cfu/g^3) was recorded in two species *Otolithes ruber* and *Penaeus indicus* and the lowest value (1 cfu/g^3) was recorded in *Stolephorus commersonnii*. The various species of dried fishes and prawn varied from 0.2 – 0.20 cfu/g^3 and the highest value (0.20%) was recorded in *Stolephorus commersonnii*. The lowest value (0.2 cfu/g^3) was recorded in two species such as *Sardinella longiceps* and *Ilisha elongata*. Microbial contamination was observed in fresh and naturally sun dried samples.[7] studied the microbial quality of commercially available dried mackerel and reported the presence of microbes. All the raw samples collected from the selected site were positive for coliform and *vibrio* species and their levels were 0.20- 0.8 cfu×10^5 and 1- 4 cfu×10^5. Comparison of the total bacterial count of the fresh and dried fish and prawn samples showed that fresh samples showed highest colony count and dried samples had the lowest colony count, which indicates that drying a reduced microbial load of the samples. Present results revealed that direct relationship between the microbial count and the moisture content of dried fish and prawn sample. Similar work was carried out in dried fishes in Tuticorin fish market[1&9]. Moisture content of fish plays an important role in spoilage, lowering of moisture retards the spoilage. Present results revealed that *E.coli* count was 0.2 to 0.8cfu×10^5 in the fresh marine fishes and

prawn. *E.coli,* a commensal bacterium which colonies in the intestinal tract of humans are pathogenic causing diarrhea in human are termed as enteropathogenic bacteria. Washing the catches in polluted coastal water definitely adds the faecal indicator bacteria and faecal contamination near the landing center is also responsible for this. Few of the selected dried samples also showed low numbers of *E.coli* and this may be due to the unhygienic way of drying in selected site.

CONCLUSION

In the present work, it was concluded the fish sold in the retail markets are not standard to consume since the observed microbial levels are always higher than the recommended levels. So to overcome this situation proper hygienic code of practice should be maintained at every step from catching, landing, icing, post-harvesting procedures, storage including depuration, transportation, processing till marketing because freshness, healthiness and safety are increasingly important to Indian consumers as they have become more health conscious increasingly demanding low-calorie, low-cholesterol, low-fat foods and products rich in vitamins, minerals and fiber and try to avoid foods which contain synthetic or chemical additives. At present, people are aware about health and nutritional issues and they concern about the nutritional value of the food items when they buy food items for their household. So the present work was to contribute data regarding microbial quality and quantity as a tool for safe consumption of fresh and dried fish product which is highly desirable to the health conscious people in the country and to achieve this scientific and improved drying method should be practiced throughout the country during period of storage for better quality and to enhance their income.

ACKNOWLEDGEMENT

The authors are thankful to the Post graduate and Research Department of Zoology, Nirmala College for Women, Coimbatore for providing necessary laboratory and library facilities for this study

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