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MICROBIOLOGICAL ASSESSMENT OF LOCALLY AVAILABLE RETAILED DRY FISHES

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ABSTRACT

The usage of dry fish usually obtained from the open shelf in most communities of the developing countries has raised some health related concerns. Curing is a traditional method for preservation of fish especially in rural areas. The quality of salted and sun dried fishes are adversely affected by the occurrence of microorganisms. The present study deals with the microbiological assessment of dry fishes such as Nethili, Panna, Thirrukkai, Valai, and Madavai were purchased from Uzhavar Sandhai, Mannargudi, Thiruvarur (Dt), Tamil Nadu, India. Thhe samples were then transported in clean polythene bags to the PG and Research Department of Microbiology laboratory for microbiological analysis. The bacterial and fungal species were isolated and identified from the five different types of the dry fishes. Nearly 6 bacterias, 8 fungal species are isolated and identified. The moisture and pH and level are monitored in the terms of three month of storage and also count the bacterial and fungal colonies using colony counter. The heavy metals of Fe, Cu, and Zn are also measured. All the bacterial and fungal colonies were analyzed by the mean values. Totally 6 bacteria and 8 fungi were isolated. *E.coli* and *Aspergillus niger* was predominant isolates from the all the dry fish samples. Microbial population frequently were high in Valai, followed by Madavai, Thirukkai, Panna and Nethili. The poor quality of dried fishes were mainly due to unhygienic processing, inadequate salting, unhygienic drying, use of spoilage fish for processing and lack of air tight packing of the dried fishes. Drying along with salting and smoking as a fish preservation technique had been practiced perhaps longer than any other food preservation technique.

KEYWORDS: Dry fish, pH, Heavy metals, Moisture.

INTRODUCTION

Fish is a highly nutritious food and an excellent source of proteins, vitamins, minerals and essential fatty acids. Interest in fish consumption has increased over the years, due to its health benefits it impart being a rich source of omega -3 fatty acids that reduces cholesterol levels and the incidence of heart disease and pre- term birth (Siscovick, 2000).[1] Dried fish are very important parts of the traditionally accepted diet for many in developing countries as well as a major source of protein. They are also often enjoyed for their characteristics flavor and are commonly used as a raw material for season's foods. Salting and drying are the oldest methods used in the preservation of fish. Although improvements in the techniques have evolved over the time, traditional methods of production continue to be practiced. Dried fish was better digested than beef or other types of protein. After processing the dried fishes were stored for particular period of time for local market and export, while the storage is a main problem of dried fish because of spoilage, it will lead to reduce the nutrient level, rotten odour, and unpalatable taste may constitute a public health hazard as well as many of economic losses (Hassan, 2007).[2] The main objective of this study to collect information regarding microbiological assessment of locally available dry fish in market.

MATERIALS AND METHODS

Sample collection

Samples of dry fishes such as Nethili, Panna, Thirrukkai, Valai, and Madavai were purchased from Uzhavar Sandhai, Mannargudi, Thiruvarur (Dt), Tamil Nadu, India. The samples were then transported in clean polythene bags to the PG and Research Department of Microbiology laboratory for microbiological analysis. Here all the samples were stored in room temperature for 6 months to examine the microbial population. Sampling was done for every month and all the samples were in duplicate. During analysis all the samples were surface sanitized using 70% ethanol and rinsed with sterile distilled water before grinding. Physical characteristics such as colour, odour, and texture of the traditionally dried fishes were examined by organoleptic test/sensory test on the basis of the method described by (Howgate, 1979).[3]

Moisture content

The moisture content of dried fish samples were carried out at the initial stage of collecting the dried fish and for three months storage using hot air oven (105˚ C).

Determination of pH

2g of each of the dried fish samples were weighted in triplicates. Water was added and mixed thoroughly to make a fish slurry. The pH readings were taken by using digital pH meter equipped with a glass electrode was rinsed and immersed into the fish slurry. The pH readings were then recorded. (AOAC, 1990).[4]

Total bacterial count

The fish samples were surface sterilized separately in 3.5% sodium hypochlorite solution with constant agitation for 7 minutes, rinsed thoroughly with sterile distilled water until the traces of hypochlorite were removed and were then dried in an oven at 45˚C for 24 hours. The heads, muscles and the tails of the fish sample were pulverized separately using a blender (maker). Five milliliters were taken from each sample into a sterile bottle containing 450 ml of sterile peptone physiological saline to from a stock culture. The sample bottles were peptone placed on a rotatory shaker at 120 rpm for 1 hour. The colonies were counted to determine the total bacterial count. (Immaculate jeyasanta, 2015).[5]

Total fungal count

The results of the fungal counts in different dried sea foods are presented in Table-3. Visible fungal counties appeared quickly due to the moisture content of the fish samples and higher relative humidity of the atmosphere. The predominant fungal isolates namely of the atmosphere. The predominant fungal isolates namely *Aspergillus niger, A. fumigatus, A. flavus, A.versicolor, Fusarium oxysporum. solani, P.citrinum, Rhizopus orayzae* were isolated from all the five samples. No fungal contamination was seen in shrimps. The quality of salted and smoked fishes was adversely affected by the occurrence of fungi (Dhiva, 2017).[6] Also, the dominant fungi in salted and smoked sea food vary with the place. The commonly occurring fungi in the local market of Mannargudi, Thiruvarur, Tamil Nadu, India were *Aspergillus, Pencillium*.

Isolation of *Vibrio spp*

25g of sample was homogenized and enriched with 225ml of alkaline peptone water and inoculated into TSBS agar. (Thiosulphate 8.5g, citrate 10g, Bile salt 8.5g, Sucrose7.5g, Agar 15g, Distilled water 1000ml, pH 7.0) Plates for the enumeration of vibrio spp. Plates were incubation at 37˚C for 24-48 hours (2 days). (FDA, 1982).[7]

Isolation of *Salmonella spp*

25g of sample was homogenized and incubated into *Salmonella Shigella* agar (Peptic digest of animal tissue 5g, Protease peptone 5g, Beef extract 5g, Lactose10g, Bile salt mixture 10g, Sodium thiosulphate 8.5g, Ferric citrate 1g, Brilliant green 0.00033, Neutral red 0.025, Agar 15g, Distilled water 1000ml, pH 7.0) plates for the enumeration of *Salmonella spp*. The plates ere incubated at 37˚C for 24-48 hours (2 days). (Smith, 1970).[8]

Calculation of microbial load

The microbial load of dried fish product was calculated by using the following formula.

$$\text{No of cell/ ml} = \frac{\text{No of colonies}}{\text{Amount plated} \times \text{dilution}}$$

Detection of heavy metals

The collected dried fish samples were subjected to analysis for the detection of heavy metals namely Fe, Cu, and Zn. A known quantity of dried fish samples was weighed by an electric balance and 5ml of diacid mixture (5ml conc.$HNO_3$and $HC10_4$ or HNO_3 and $H_2SO_4$was added to each sample. The content was mixed for overnight. Samples were then digested initially at 80˚C temperature and later at 150˚ C for 2 hours. The completion of digestion was indicated by almost colourless condition of the material. The brown fumes also cease to exist at completion of digestion. The samples were separately filtered by using an ash less filter paper and volume made up to 25ml with 0.5% HNO_3 prepared for the determination of Fe, Cu, Zn (Tayor, 1958).[9] The samples were subjected to analysis by Atomic Absorption Spectrophotometer. ELICO- 618, Biominin Laboratory, S.T.E.T Women's College, Mannargudi.

Statistical analysis

All the experiments were carried out in the mean values and standard deviation was calculated. Those data was presented as mean for each samples by using formula given by (Aneja, 1996).[10]

RESULTS

Isolation and identification of bacteria

The bacterial isolates were identified by using Bergey's manual of Determination bacteriology. Distinct developing on the culture plates were observed for their pigmentation, margin, elevation and opacity. Gram's staining was done using 24 hours pure culture. The

stained slide was then examined under the microscope with oil immersion objective lens (100x). The Gram's reaction, shape, arrangement and size of the cells were examined. Biochemical characteristics of the isolates were determination by employing the following tests on a fresh culture: Catalase test, Coagulase test, Citrate utilization test, Urease test, Indole formation test, motility test and Triple sugar ion test. The bacterial colonies were isolated from dry fishes and it was identified as *Bacillus subtilis, Streptococcus pyogens, E. coli, Staphylococcus aureus, Staphylococcus saprophiticus, Micrococcus* by Gram staining and biochemical characteristics. The identified bacteria were transferred to fresh nutrient agar plate and the pure culture was maintained for the isolated pathogens. Frequently *E.coli* was isolated in all the dry fish samples.

Isolation and identification of fungi

The fungi were identified by using standard manual, such as Manual of fungi (Ellis, 1971)[11], Dematiaceous Hyphomycetes (Ellis, 1976)[12], More Dematiaceous Hyphomycetes (Gillman, 1957)[13], Hyphomycetes (Subramanian, 1971).[14] Although total of 8 species were isolated namely *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Aspergillus versicolor, Fusarium solani, Fusarium oxysporum, Pencillium citrinum, Rhizopus oryzae. Aspergillus niger* was present in the all the dry fish samples. (Table-1).

Total bacterial colonies from the dry fish samples

In Valai 32, colonies were counted. Similarly Madavai 12, Thirukkai 16, Panna 12, Nethili 24 were counted using colony counter. Totally 96 colonies were counted. The highest number of colonies was counted in Valai, followed by Nethili, Thirukkai, Panna, and Madavai. (Table-1).

Total fungal colonies from the dry fish samples

Table- 4 showed that, 28 colonies were counted in Valai. Similarly Madavai 40, Thirukkai 84, Panna 76, Nethili 48 were counted using colony counter. Totally 276 colonies were counted. The highest number of colonies was counted in Thirukkai 84, followed by Panna, Nethili, Madavai and Valai. (Table-1).

Table 1: Total bacterial and fungal count of dried fishes during the storage period.

Dry Fish Samples	Dilution Factor for Bacteria	Ditution Factor for Fungi	No of Colonies		Population	
			Bacteria	Fungi	Bacteria	Fungi
Valai	10^{-5}	10^{-4}	32	28	3.2×10^{-4}	2.8×10^{-4}
Madavai	10^{-5}	10^{-4}	12	40	1.2×10^{-4}	4.0×10^{-4}
Thirukkai	10^{-5}	10^{-4}	16	84	1.6×10^{-4}	8.4×10^{-4}
Panna	10^{-5}	10^{-4}	12	76	1.2×10^{-4}	7.6×10^{-4}
Nethili	10^{-5}	10^{-4}	24	48	2.4×10^{-4}	4.8×10^{-4}

Total colonies of *Salmonella, Vibrio* in dry fish samples

The prevalence of *Salmonella* in Valai, 24 colonies was counted. Followed by Thirukkai 16, Nethili 32 from. *Salmonella* was totally absent in Panna, and Madavai. Similarly *Vibrio* count was also in the same way. Nearly 36, 28 colonies were isolated for Madavai, Thirukkai. In Panna, Nethili, *Vibrio* was absent. Totally 136 colonies of *Vibrio and Salmonella* were counted in using colony counter. The highest prevalence *Salmonella*, *Vibrio* was counted in Nethili, Madavai. (Table-2).

Table 2: Total *Salmonella* and *Vibrio* colony count for dry fish samples.

DRY FISH SAMPLES	DILUTION FACTOR	NO OF COLONIES		POPULATION	
		Salmonella	*Vibrio*	*Salmonella*	*Vibrio*
Valai	10^{-5}	24	-	2.4×10^{-4}	-
Madavai	10^{-5}	-	36	-	3.6×10^{-4}
Thirukkai	10^{-5}	16	28	1.6×10^{-4}	2.8×10^{-4}
Panna	10^{-5}	-	-	-	-
Nethili	10^{-5}	32	-	3.2×10^{-5}	-

Determination of pH content

The pH value was recorded in the three month of storage time. In the 1-3rd months, pH value of Valai dry fish are 6.35, 5.07, 5.11 respectively. Followed by Madavai, pH value are 7.00, 5.00 and 5.11, Thirukkai pH value are 5.65, 3.01and 6.17 in Panna pH value are 6.02, 7.00 and 5.35, Nethili pH value 7.10, 6.09 and 4.04 respectively.(Table- 3).

Table-3: Determination of pH in the dried fishes for three months of storage.

Dry fish sample	December	January	February
Valai	6.35	4.95	6.00
Madavai	7.00	5.11	6.95
Thirukkai	5.65	6.17	4.02
Panna	6.02	7.00	5.35
Nethili	7.10	4.04	6.95

Determination of moisture level in dry fish samples

The moisture level was also monitored in terms of dry weight basis for the three months storage. From the 1-3rd months, moisture level of Valai are 50, 46, 45.05 (mg). Similarly in Madavai 50, 47.05 and 47 (mg), Thirukkai 50, 46 and 45.08 (mg), Panna 50, 48 and 46.05(mg), Nethili 50, 48 and 47.05 (mg) respectively. The moisture level was showed slight variations in all the dry fish samples. (Table-4).

Table-4: Determination of moisture in the dried fishes for three months storage

Dry fish sample	MOISTURE CONTENT (mg)		
	December	January	February
Valai	50.00	45.05	44.00
Madavai	50.00	47.00	46.05
Thirukkai	50.00	45.08	40.00
Panna	50.00	46.05	42.05
Nethili	50.00	47.05	45.05

Determination of heavy metals

The results of heavy metal analysis of the dried fish samples, showed that Valai, had Fe, Cu, Zn content was 0.007, 0.097, 0.87 (ppm), next, Madavai, 0.001, 0,043, 0.025(ppm), Thirukkai, 0.002, 0.079, 0.078 (ppm), Panna, 0.004, 0.28, 0.053(ppm), Nethili, 0.005, 0.067, 0.072 (ppm) respectively (Table-5).

Taple-5: Detection of heavy metal from the dry fish samples.

Name of the samples	Fe (ppm)	Cu (ppm)	Zn (ppm)
Valai	0.005	0.079	0.045
Madavai	0.001	0.043	0.025
Thirukkai	0.002	0.097	0.078
Panna	0.004	0.082	0.053
Nethili	0.005	0.067	0.072

Determine the mean values of bacterial and fungal colonies from the dry fish samples

The range of mean values of bacteria for Valai 6.2, Madavai 2.2, Thirukkai 3.1, Panna 2.2, and Nethili 5.4. Similarly fungal colonies mean values are Valai 5.3, Madavai 8.0, Thirukkai 16.4, Panna 15.1, and Nethili 9.3 respectively. (Table-6).

Table-6: Mean values of bacterial and fungal colonies from the dry fish samples.

Name of the samples	Bacterial colonies	Fungal colonies	Mean values of bacterial colony	Mean values of fungal colony(cfu×10^5)
Valai	32	28	6.2	5.3
Madavai	12	40	2.2	8.0
Thirukkai	16	84	3.1	16.4
Panna	12	76	2.2	15.1
Nethili	24	48	5.4	9.3

Note: Values are representing as Mean

SUMMARY AND CONCLUSIONS

The present study was conducted to isolate and identify the microorganisms in the locally available retailed dry fishes in the market. Although 6 bacterial isolated are namely, *Bacillus subtilis, Streptococcus pyogens, E. coli, Staphylococcus aureus, Staphylococcus saprophiticus, Micrococcus*. Totally 8 fungi was isolated namely, *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Aspergillus versicolor, Fusarium solani, Fusarium oxysporum, Pencillium citrinum, Rhizopus oryzae.* The *E.coli* and *Aspergillus niger* was the predominant microorganism present in all the dry fish samples. Similarly the *Salmonella* and *Vibrio* were present only Valai, Madavai, Nethili. Moisture content was monitored in three month of storage period. The dry fishes had both acidic and alkaline nature of pH. The heavy metal content was also analyzed in all the dry fish samples. 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 product up to marketing of the produces, in order to avoid the infections and intoxication by the pathogens.

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