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PROXIMATE CHEMICAL COMPOSITION AND OCCURRENCE OF SOME PATHOGENIC BACTERIA IN FROZEN FISH FROM UPPER EAST COAST OF INDIA

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Abstract

Some studies on bacteriology and proximate chemical composition were carried out on the frozen fish and shell fish samples brought from 27th cruise of FORV *Sagar Sampada*. Out of 21 samples screened for bacteriological studies, 17 samples have shown the presence of enterobacters, 12 samples contained *Pseudomonas* and *Proteus* was found in two samples. *Salmonella* and *Shigella* were absent in these Samples.

The edible muscle of frozen and thawed samples was analysed for moisture, protein, fat, ash and nutritive minerals like calcium, phosphorous and iron. The total protein content of the muscle of different fishes studied varied from 16.25 to 20.88 per cent on wet weight basis. The fat content is found to be more in deep sea fishes namely black ruff (6.074 per cent) and *Psenes* (3.796 per cent) than in other common varieties of inshore fishes analysed.

Introduction

Since long time, a fishery fleet of Exploratory Fisheries Project (1983) presently known as Fishery Survey of India, has been surveying the fisheries resources of the east coast in offshore as well as deep sea waters. The research vessels of National Institute of Oceanography also have been surveying for fish and other marine resources in the region of Bay of Bengal. Recently as a part of exploratory survey of Bay of Bengal along the east coast of India, the FORV *Sagar Sampada* of Department of Ocean Development, Government of India, is also conducting some fishing operations. Earlier, Imam Khasim *et al.* (1987) have done proximate composition analysis of some deep sea varieties of fishes caught in Bay of Bengal off Andhra Pradesh coast by FSI vessel. Kureishy *et al.* (1983) had analysed some trace metals in fishes and zooplankton from the Andaman Sea. As such, much information is not available on the bacteriology and biochemical composition of the fishes available in the waters of Bay of Bengal. During the 27th cruise of FORV *Sagar Sampada*, some fishing operations both pelagic and bottom trawling were conducted and from seven different stations about 17 varieties of fish and shell fish samples were collected and in this paper, the qualitative bacterial flora, biochemical proximate composition and some nutritive mineral elements viz., Ca, P and Fe of the fish samples are reported which may serve as a useful reference data for fish processing technologists and the consumers.

Materials and Methods

Seventeen varieties of some common and deep sea fish and shell fish (as per the list given in Table 1) were collected off upper east coast in the Bay of Bengal from seven stations lying in the area between latitudes 18°30' and 20° 30' and longitudes 85° 13' and 88° 50' during the 27th cruise of FORV *Sagar Sampada*. Immediately after the catch, the fish were packed in polythene bags and were frozen onboard the vessel in the cold storage at —20°C. These frozen samples were brought to the laboratory in an insulated box along with ice and were again stored in deep freezer at —20°C. For analysis, the fish samples were taken from the deep freezer and thawed in closed polythene bag. These samples were washed with water and edible muscle was taken for further chemical analysis.

Bacteriological methods : Twenty one samples of fish and shell fish were screened for pathogenic and spoilage bacteria. The edible muscle was taken in sterile condition for analysis. Since the samples were kept in frozen condition prior to testing, to facilitate injured organisms to recover, the samples were inoculated into lactose broth in 1 : 10 ratio which served as non-selective or pre-enrichment broth. (North, 1961). For testing Coliforms, U.S. Pharmacopoeia method was used (USFDA, 1984). USFDA (1972) method was followed for screening of *Salmonella* and *Shigella*.

Isolates were picked up from selective and non-selective media, purified and characterised. Pre-enrichment broth, selective broths and media used in this study were of Difco make. Proximate composition analysis for moisture, total protein, fat and ash contents were analysed following AOAC (1975) methods . Calcium was done from the ash content following AOAC (1965) methods. Phosphorous was determined from the ash content using Fiske and Subbarao method (1925). Iron estimation was also done from the ash content following AOAC (1970) method.

Results and Discussion

In the Table 1, the proximate composition showing moisture, total protein, fat, ash content and some of the nutrient elements like calcium, phosphorous and iron contents of the fishes analysed are given. The protein content of the fishes ranged from a minimum of 16.25% to a maximum of 20.80%. From the table it appears that the protein content is as same as or more both in quality table-fish and shell fish such as pomfret, mackerel, prawn as well as in low cost fishes such as *Psenes*, threadfin bream, silver bellies and others. The fat content was within 2% in almost all fishes but conspicuously in the deep sea varieties viz., *Psenes indicus* and *Centrolophus niger* (black ruff). The fat content was high, 3.79 and 6.074% respectively which is in full agreement with earlier analysis of these varieties by Imam Khasim *et al.*, (1987). Ash content was normal in all the fishes. Calcium and phosphorus were present in good required amounts in most of the fishes whereas the values of iron content were found to be low. This might be mainly due to the leaching of blood contents from the flesh, along with the driploss,during thawing of the frozen samples. It is to be remembered that the above analysis pertains to the edible muscle of fishes which were frozen and then thawed but not as such fresh i.e. raw fishes.

Bacteriological analysis

Based on morphological and biochemical characters, all the isolates were divided into three

Table 1. *Proximate chemical composition of fish/shell fish smples (on wet weight basis)*

Station No.	Fish %	Moisture %	Protein %	Fat %	Ash %	Calcium mg/100g	Iron mg/ 100g	Inorganic phosphate mg/100g
930	Black ruff (*Centrolopohus niger*)	75.00	17.25	6.074	1.3325	78.52	2.89	911.10
934	Mackerel (*Rastrelliger kanagurta*)	76.15	17.50	1.239	2.459	114.08	5.49	753.44
934	Indian drift fish (*Psenes indicus*)	74.60	18.80	2.694	2.219	276.37	1.295	614.22
934	Synagris (*Synagris* sp.)	77.60	17.375	0.5354	1.5321	39.27	4.10	177.68
935	Indian white prawn (*Penaeus indicus*)	76.80	17.50	0.58	1.4639	257.728	0.80	644.48
935	Japanese threadfin bream (*Nemipterus japonicus*)	74.90	18.125	0.7655	3.1299	195.37	1.566	1131.87
935	Banded barracuda (*Sphyraena jello*)	75.90	18.50	0.755	2.536	406.47	2.50	747.42
936	Indian drift fish (*Psenes indicus*)	72.00	20.275	3.796	2.002	128.40	1.713	936.95
939	Caranx (*Caranx* sp.)	76.40	17.625	1.173	2.834	160.10	5.20	812.75
944	Prawn (*Metapenaeus monoceros*)	77.40	20.00	0.739	2.109	279.42	2.03	639.56
944	Silver belly (*Leiognathus bindus*)	74.70	18.75	0.881	1.971	273.67	0.996	743.71
944	Coastal mud prawn (*Solenocera crassicornis*)	76.58	20.50	0.63	1.379	256.89	2.80	578.18
944	Ribbon fish (*Trichiurus haumela*)	78.10	18.00	0.567	1.401	169.64	1.143	650.80
944	Yellow goat fish (*Upeneus sulphureus*)	74.80	19.46	2.796	1.172	223.17	1.366	600.86
944	Ilisha (*Ilisha filigera*)	77.65	18.125	1.266	1.223	167.20	1.03	427.80
946	White pomfret (*Stromateus chinensis*)	78.10	16.25	0.596	1.513	199.60	2.006	625.48
946	Snappers (*Lutjanus* sp.)	73.00	20.88	1.405	1.872	152.48	0.691	1086.49
946	Japanese threadfin bream (*Nemipterus japonicus*)	75.50	19.125	2.425	2.376	186.34	1.60	659.99
946	Yellow goat fish (*Upeneus sulphureus*)	75.60	18.50	2.667	2.852	467.19	1.774	760.85

groups. The source for first group of isolates is Mac Conkey agar. The indole, methyl-red, Voges-proskauer and citrate utilisation results (IMViC), in sequence of two negatives and two positives, and other biochemical characters indicates the first group of isolates are typical *Enterobacter aerogenes* (Table 2) as described by Morris Fishbein *et al.* (Morris Fishbein *et al.*, 1976). There is need for identifying the source of enterobacters. The reason is that enterbacter genera are described as coliforms because of their certain common properties with *E. coli*. Besides, they are found in a variety of habitats. (Hayes, 1985).

This study shows occurrence of enterobacters which are freeze resistant. Raj and Liston (1961) have reported the effect of freezing enterobacters and pointed out the variability of strain to strain. Survival of this bacteria sometimes for longer periods at freezing temperatures was reported by Lamprecht and Elliott (1971). The actual quality of fish landed at ports is dependent upon the time it has been held in ice and hygienic conditions onboard fishing vessels (Hayes, 1985). The initial reduction in numbers of bacteria immediately after freezing can range from only one or two per cent to ninety per cent (Shewan,1961; Simmonds and Lamprecht, 1980).

TABLE 2. *Characterisation of th isolates*

Morphology	I	II	III
	rods	rods	rods
Motility	+	+	+
Gram staining	-	-	-
Oxidase	-	+	-
Indole	-	-	+
Methyl red	-	-	+
Voges - Proskauer	+	-	-
Citrate utilization	+	+	+
Urea broth	-	-	R
Urea slant	-	-	R
On triple sugar iron			
1. Slant	A	NC	A
2. Butt	Ag	NC	Ag
3. H_2S production	-	-	+
On Friewer shaughnessy medium			
1. Motility	-	+	+
2. Fermentation	Ag	NC	NC
3. H_2S	-	-	+
Dextrose	Ag	NC	Ag
Lactose	Ag	NC	NC
Mannitol	Ag	NC	NC
Salicin	Ag	NC	Ag
Growth in K. C. N. broth	+	+	+

-	No reaction	NC	No change
+	Reaction positive	A	Acid
R	Red	Ag	Acid gas

The second group of isolates have their origin from Mac Conkey agar as well *Salmonella* and *Shigella* agar (S.S. agar) (Table 3). Out of 21 samples of fish and shell fish tested, 12 samples have shown the presence of this group of isolates. These isolates were further tested on pigment enhancing media. Five isolates have produced blue water soluble pigment on medium - A and the rest seven isolates have produced water soluble, fluorescent yellow pigment (Table 3). These pigment producing *Pseudomonas* isolates come under *Pseudomonas aeruginosa* (Pyocyaenea) (King *et al.*, 1954).

Presence of indigenous flora composed of *Pseudomonas* in fishing vessels was reported by Shewan (1961). *Pseudomonas* is absent in all shell fish samples. Enterobacters were isolated from all the samples excepting *Synagris* spp., prawns (*Metapenaeaus monoceros*), ribbon fish and white pomfrets (Table 3).

The third group of isolates has the source Bismuth Sulphite agar. Use of TSI agar has helped in identification of these isolates as *Proteus vulgaris* (AOAC, 1975). Presence of *Proteus* in frozen foods is unwanted as it can spoil the fish when they are at ambient temperatures (Hayes, 1985).

Presence of *Pseudomonas* and *Proteus* could be attributed to a degree of protection afforded by greater numbers in initial stages. Clumping of cells could be another possibility. The occurrence of *Pseudomonas* shows their ability to compete with coliforms.

The important points to be observed in the study are that the fish and shell fish samples were screened for pathogenic and spoilage bacteria immediately after bringing the samples to the laboratory. Although , the samples were brought in frozen condition they were not stored for longer time in the same condition. Assessment of these bacteria is qualitative in nature, but not quantitative. *Salmonella* and *Shigella* are absent in these samples.

There is need for further studies to know the qualitative nature of spoilage and pathogenic bacteria in fresh and processed sea-foods in this area.

TABLE 3. *Occurrence of spoilage bacteria in fish samples*

Stn.No.	Name of the fish	*Pseudomonas*	*Enterobacter aerogenes*	*Proteus vulgaris*
930	Black ruff	Blue pigment	+	-
934	Mackerel	Blue	+	+
934	Oil sardine	-	+	-
934	Indian drift fish	Yellow	+	-
934	*Synagris* sp.	Blue	-	-
935	Indian white prawn	-	-	-
935	Japanese threadfin bream	Yellow	+	-
935	Banded barracuda	Yellow	+	-
936	Black ruff	Yellow	+	-
936	Indian drift fish	Blue	+	-
939	*Caranx* sp.	-	+	-
944	Prawns (*Metapenaeus monoceros*)	-	-	-
944	Silver bellies	-	+	-
"	Coastal mud prawns	-	+	-
"	Ribbon fish	Blue	-	-
"	Yellow goat fish	-	+	-
"	Ilisha	-	+	-
946	White pomfrets	-	-	+
"	Sciaenids	Yellow	+	-
"	Japanese threadfin bream	Yellow	+	-
"	Yellow goat fish	Yellow	+	-

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