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MICROBIAL STUDY AND PROXIMATE COMPOSITION OF SIX MARINE FISH SPECIES IN MUDASALODAI COASTAL REGION

S. Sankar, P. Sujith and S. Jayalakshmi
C.A.S in Marine Biology, Faculty of Marine Science, Annamalai University, Tamil Nadu, India - 608502
*Corresponding Author Email: spsankar1983@gmail.com

ABSTRACT
The present study was aimed to analyse the proximate composition and endophytic microbial colonization in six marine fishes such as Nemipterus japonicas, Epinephelus areolatus, Lutjanusquin quelineatus, Scolopsis bimaculatus, Lutjanus decussate and Lethrinus lensus, were collected from Mudasalodai coastal region, Tamil Nadu, South east coast of India. Maximum protein recorted Lethrinus lensus 4.7×10^{5},minimum in Lutjanus decussate 2.9×10^{5},Micrococcus sp and Escherichia sp was recorded in all six fish species. In the case of fungi not found in all the six fish species. The total viable count was within the acceptable limit in all the fish samples. The proximate content results revealed that of the Lutjanusquin quelineatus was indicated high values obtained providing an update to food composition value. Among the three nutrient elements investigated the most abundant was Fe followed by P and Ca much amount in Lutjanus decussatus. This work was provided the important scientific information of the proximate composition and endophytic colonization of six marine fish collected from south east coast of India.

KEY WORDS
Mudasalodai coastal region, Micrococcus sp, Calcium

1. INTRODUCTION

On a global scale, fish and fish products are the most important source of protein in the human diet. This protein is relatively of high digestibility compared to other protein source. It comprises of all the ten essential amino acids in desirable quantity for human consumption. All these properties brings the fish flesh to be in the same class as chicken protein and are superior to milk, beef protein and egg albumin (Srivastava, 1999).

In 2002, world total fishery production (excluding aquatic plants) was reported to be 133.0 million tonnes, of which 41.9 million tonnes from aquaculture practices. World capture fisheries production amounted to 93.2 million tonnes, representing a slight increase of 0.4% compared to that of 2001 (Vannuccini, 2004).Proximate composition generally means percentage composition of basic constituents such as water, protein, lipids, carbohydrate and minerals. The energy yielding nutrients like protein, lipid and carbohydrate are considered as macronutrients are present in high level where as non energy yielding nutrients like vitamins and minerals are micronutrients and are present in small quantities (Ramakrishnan,1995). Fish has long been recognized as a valuable source of high quality protein in the human diet.

In recent years, fish lipids have also assumed great nutritional significance owing to their protective role against the development of cardiovascular disease and rheumatoid arthritis (Shahidi,1994). Hence, consumption of fish, both freshwater and marine, is therefore being encouraged. Six fishes commonly preferred and consumed by all the economic group of people and it is also considered low cost fishes. Hence, the present work was undertaken to study the proximate, amino acids, fatty acids and mineral composition of marine fishes of Mudasalodai coast. However, according to Osman et al. (2001), generally Malaysians simply consider fishes from different types are of the same nutritional value. The selection process is usually made based on the availability, freshness, favors and other physical factors. They do not pay attention on the variability of the nutrient composition of the different fish species. Therefore, in order to make the consumers more attentive on the nutritional content of fish, information on nutrient values of Indian fishes must be made available. In the present study, attempt was made to provide information on the microbial, proximate values of 6 marine fish species.

2. MATERIALS AND METHODS

Source of samples

All fishes were purchased from the fishing harbor at Mudasalodai, Tamil nadu, India. Six fish species were included in this study. These species are among the most commonly consumable in Tamil nadu, India. The fish species collected were immediately dipped in ice, kept and transported in sterile polystyrene boxes to sustain freshness. Then, samples convoyed to the laboratory for analysis.

Microbiological analysis

The muscles of the fishes were cut aseptically using sterile forceps and scalpels and five grams (5g) aseptically weighted into conical flask containing 45 ml of sterile deionised water. The contents were then transferred into a sterile mortar and homogenate into a watery paste all within 40 min. Serial dilutions of the samples were used for microbial enumeration with the following media: Standard Plate count Agar for Total Viable Counts (TVC), Fungi were isolated on the potatoes dextrose agar (PDA) with chloramphenicol media .

Identification of Microorganisms:

Morphological characteristics of the various bacterial isolates were noted in the agar plates and characterized using staining reactions and series of biochemical tests, individual microbial species were identified as described in Slaby et al, 1981.The total viable cell count on each petridish, colonies were inventoried before culturing. Pure cultures of the isolates were Gram-stained, while the cell morphology was examined by phase contrast microscopy. After incubation at 37°C, the Petri dishes were examined in the laboratory.

Determination of Moisture

Moisture content of fish fillets and shellfish were determined according to method described by AOAC 1990 with slight modifications by Tee et al. (1996). The samples were dried in moisture dish in an oven (UM400 Memmert, Germany) at 105˚C until constant weights were obtained.

Determination of Ash

Ash content of fish fillets and shellfish were determined according to method described by AOAC 1990 with slight modifications by Tee et al. (1996). Pre-dried samples obtained from moisture content analysis were ashes in furnace (Barnstead, Iowa, USA) at 550˚C overnight.

Determination of lipid

The flesh (5g) was extracted with chloroform (5ml) and methanol (10ml) using chloroform - methanol extraction method. The amount of lipid was calculated from the residue remaining after evaporating the chloroform layer.

Determination of crude protein

The edible flesh (2g) was digested with anhydrous potassium sulphate (15g), copper sulphate pentahydrate (0.5g) and concentrated sulphuric acid (25ml). Amount of protein in the digested sample was determined using Kjeldahl method**.**

Determination of carbohydrate

The edible flesh (2.5g) was stirred with distilled water (10ml) and 52% perchloric acid (13ml) for 20 min. The contents were diluted to 100m1, filtered into a 250ml volumetric flask and made upto the mark. The diluted filtrate (l.0ml) was heated with 1%w/v anthrone reagent in sulphuricacid for 20 min. and the absorbance at 630nm was measured in a Shimadzu UV- 160 spectrometer. The concentration of glucose in the sample was calculated using a standard curve

Determination of calcium

The ash was digested with 3M hydrochloric acid (15ml) and filtered. Emissions for calcium were measured at 620nm to using a Corning-410 flame photometer. (S. Wimalasena and M.N.S. Jayasuriya 1995)

Determination of phosphorus

The ash was digested with hydrochloric acid and diluted sample (10ml) was treated with molybdovanadate reagent (5ml). The amount of phosphorus was calculated from the absorbance at 400nm.

Determination of iron

The ash was digested with hydrochloric acid and diluted sample (10ml) was treated with 10% hydroxylamine hydrochloride (lml), acetate buffer of pH 4.5 and 0.1% orthophenanthroline solution (1 ml). The absorbance at 5l0nm was measured. The amount of acetate buffer that has to be added to adjust the pH to 3.5-4.5 was predetermined using the above solution (10ml) diluted to 25ml.

Determination of Energetic value

The energetic value was determined indirectly using Rubner's coeffcients for aquatic organisms: 9.5 kcal g-1 for lipids, 5.65 kcal g-1 for proteins (Winberg, 1971) and expressed in kJ g-1 wet mass as described by Eder and Lewis (2005)

Statistical Analyses

The results obtained were analyzed statistically by performing ANOVA and Tukey's tests where there were significant differences. Significance level was set to an alpha level of 0.05 (Sokal and Rolf, 1974). Statistical significance is indicated with appropriate letters on the data tables.

3. RESULTS

The microbiological status of the samples is summarized in **Table 1 &2**. This table shows that total viable count (TVC) of the six fish species varied from 2.9 x 10^5 to 4.7 x 10^5 CFU/g. In three species (*Lethrinus lensus*, *Scolopsis bimaculatus* and *Epinephelus areolatus*), microbial load were highly found then other three species as 3.5 x 10^5 CFU/g , *Lethrinus lensus* was 4.7 x 10^5 CFU/g for huge densities of microbes were present. On fungal specific media, no microbial growth was observed for the six samples. Table.2 shows the microbial isolates obtained from six fish species samples analyzed. *Micrococcus sp* and *Escherichia sp* were found on all fishes, *Pseudomonas sp* are absent in *Lutjanusquin quelineatus* and *Lethrinus lensus*, *Staphylococcus sp* are absent in *Lutjanus decussate and Nemipterus japonica, Flavobacterium sp* are absent in *Lutjanusquin quelineatus and Nemipterus japonicas*, *Bacillus sp* are present in *Scolopsis bimaculatus and Epinephelus areolatus*, six microorganism groups present fish species on both of *Epinephelus areolatus* and *Scolopsis bimaculatus*. (**Table 3**)

In this present study we investigated the length and weight relationship and edible portion of the 6 different marine edible fishes. The proximate compositions from edible tissues were determined and the results were tabulated. (**Table 4**) gives the data on length %Weight relationship and edible portion of the 6 different fishes, depicted the results of the mean percentage of moisture, ash, crude protein, fat, carbohydrate contents and energetic values of 6 fishes. Moisture contents were found high in *Epinephelus* areolatus and having significant differences in other five fish. The moisture content of this fishes were significantly lower in *Lutjanus decussatus*, while the fat content was significantly higher and it can be observed clearly in fish of *Lutjanusquin quelineatus and Scolopsis bimacula.* Regarding ash contents, two fishes Nemipterus *japonicus* and *Epinephelus areolatus* showed significant differences among the 6 fishes. All the fishes were observed to contain no carbohydrate except for *Nemipterus japonicus* with mean value of 0.25%. After all, the carbohydrate content in fish is generally very low and practically considered zero. Table shows the obvious difference of protein content of *Scolopsis bimaculatus* compared to other fish.

However, the protein content could be considered as significant instead, as the values were derived and estimated from the difference of other (Payne et al., 1999) Other than that, the energetic values for all pelagic fish were between the range of 060.21 and 120.57 (K cal/100g), for *Lutjanusquin quelineatus and Scolopsis bimaculatus*, with fairly higher values of 117.83 and 120.57 (K cal/100g).

Six fish samples were subjected to the treatment. The Calcium obtained after experiment were given in detail (**Table. 5**). The maximum Calcium was obtained in treatment with comparatively higher yield for *Lutjanus decussatus* 1888.10 ± 15.05), phosphorus contents were analyzed using spectrometry and were given in **Table 5**. Among the two orthogonal experiments, phosphorus found in higher levels

327.96 ± 19.1% for *Epinephelus areolatus* fish, this fish also have higher levels of iron 29.46 ± 0.73 % , it was inferred that the minerals were found such as calcium, phosphorus and iron in the six fish samples and the results were expressed in **Table 5**.

Table.1 - Densities of microbes isolated from the Fish sample

S.i. No	*Fish sample*	*Total ViableCounts Of bacteria Cfu/g*	*Total ViableCounts Of fungi Cfu/g*
1	*Nemipterus japonicas*	***3.4 x 10^5***	-
2	*Epinephelus areolatus*	***4.1 x 10^5***	-
3	*Lutjanusquin quelineatus*	***3.1 x 10^5***	-
4	*Scolopsis bimaculatus*	***3.5 x 10^5***	-
5	*Lutjanus decussate*	***2.9 x 10^5***	-
6	*Lethrinus lensus*	***4.7 x 10^5***	-

Table.2 Microorganisms isolated from six fish species

s.i.no	*Species*	*Pseudomonas sp*	*Micrococcus sp*	*Staphylococcus sp*	*Escherichia sp*	*Flavobacteri -um sp*	*Bacillus sp*
1	*Nemipterus japonicas*	+	+	-	+	-	-
2	*Epinephelus areolatus*	+	+	+	+	+	+
3	*Lutjanusquin quelineatus*	-	+	+	+	-	-
4	*Scolopsis bimaculatus*	+	+	+	+	+	+
5	*Lutjanus decussate*	+	+	-	+	+	-
6	*Lethrinus lensus*	-	+	+	+	+	-

Table.3. Physical parameters of marine fishes

Sl. No.	*Name of the Fish*	*Physical Parameters*	
		Length (in cm)	*Weight (in g)*
1.	*Nemipterus japonicus*	*20.20±0.17*	*152.67±22.57*
2.	*Epinephelus areolatus*	*22.70±0.71*	*226.00±07.07*
3.	*Lutjanusquin quelineatus*	*16.53±0.58*	*097.67±05.77*
4.	*Scolopsis bimaculatus*	*21.03±3.67*	*121.00±50.00*
5.	*Lutjanus decussatus*	*20.20±0.66*	*121.00±10.00*
6.	*Lethrinus lensus*	*18.78±0.93*	*018.33±13.66*

Table.4 Proximate composition of the marine fishes

Sl. No	*Name of the Fish*	*Proximate composition (Percentage Value)*					
		Moisture	*Protein*	*Carbohyd rate*	*Fat*	*Ash*	*Energy Value (K cal/100)*
1.	*Nemipterus japonicus*	75.72±0.75	13.24±0.08	0.26±0.0	1.78	3.97	075.98
2.	*Epinephelus areolatus*	79.99±0.23	13.84±0.35	0.06±0.0	0.95	3.89	060.21
3.	*Lutjanusquin quelineatus*	76.75±0.43	22.46±0.47	0.05±0.0	4.43	1.91	117.83
4.	*Scolopsis bimaculatus*	75.76±0.49	21.24±0.27	0.08±0.0	4.37	1.67	120.57
5.	*Lutjanus decussatus*	74.52±0.09	22.13±0.01	0.07±0.0	3.06	0.35	104.30
6.	*Lethrinus lensus*	78.63±0.74	16.19±2.26	0.10±0.0	0.51	1.88	066.81

Table 5: Calcium, phosphorous and Iron contents of Marine fishes

Sl. No	*Name of the Fish*	*Calcium*	*Phosphorous*	*Iron*
1.	*Nemipterus japonicus*	0799.63±10.04	256.16±19.89	01.16±0.06
2.	*Epinephelus areolatus*	1453.41±09.55	327.96±19.11	29.46±0.73
3.	*Lutjanusquin quelineatus*	0722.10±40.79	215.60±09.79	19.69±0.38
4.	*Scolopsis bimaculatus*	1176.00±17.68	267.54±21.33	01.42±0.03
5.	*Lutjanus decussatus*	1888.10±15.05	205.70±12.06	04.08±0.12
6.	*Lethrinus lensus*	0584.89±08.97	251.06±12.57	08.94±0.37

4. DISCUSSION

Microbiological count of the *Nemipterus japonicas*, *Lutjanusquin quelineatus* and *Lutjanus decussate* species were found to be lower in *Lethrinus lensus*, *Scolopsis bimaculatus* and *Epinephelus areolatus*.. Results recorded on the first three species indicated that the values nearer to the findings of Ouattara (1986) who observed 6.11 Log CFU/g. The higher values observed on marine fishes were similar to results reported by Kovacevic (1970). All the values obtained in microbiological analysis of the fish samples were higher than the acceptable International Commission on Microbiological Specification for Foods (ICMSF) and French Norms (FN) limits. In fact, ICMSF and FN have a limit of 4.70 Log CFU/g for frozen fishes. The analysis on specific medium showed that the predominant microorganisms *Micrococcus sp* and *Escherichia sp* were present in all samples and fungal species were not detected. The *Escherichia sp* were found in *C. auratus* and *P. peroteti* , Seydi et al. (1992) observed the same on fish sample.

Staphylococcus sp were present on four of the fish samples with values ranging from 1.20 to 3.43 Log CFU/g. These values were higher than findings from other authors (Bernadac et al., 1985; Ouattara, 1986; Ndiaye, 1998) and opposite to Bernadac et al. (1985) who reported that fish samples were free from staphylococci. The inventory of microorganisms found on the six samples reveals the presence of six bacterial species: *Micrococcus sp, Escherichia sp, Staphylococcus sp Pseudomonas sp*, *Flavobacteri-um sp* and *Bacillus sp* species. So, some strains of *S. aureus* are capable of producing a highly heat-stable protein toxin that causes illness in humans and *S.xylosus* is implicated in fish degradation (Jeantet et al., 2006) *Escherichia sp* belongs in the family Enterobacteraceae. They have been associated with nosocomial outbreaks, and are considered as opportunistic pathogens.

Moisture of a given sample simply refers to the water content of that sample. Results obtained from the proximate analysis of the various fish species showed that of all the fish samples, *Epinephelus areolatus* which was marine harvested has the highest percentage of moisture, 79.99 ± 0.23 % while *Lutjanus decussatus* has the lowest moisture content, 74.52±0.09 %. The fishes had moisture ranging from 79.99 to 74.52% indicating that the percentage moisture in fish muscles and it was within the acceptable level (70-80 %) in all the samples which could be due to the stable water levels in the environmental location where the fishes collected. The percentage of water is also a good indicator of its relative content of energy, protein and lipid. There was high significant difference between two fishes *Lutjanusquin quelineatus , Scolopsis bimaculatus*.

The results in (**Table 1**) showed that all the fish species were good sources of protein of those in *Lutjanusquin quelineatus* having the highest protein content (22.46±0.47 %) and *Nemipterus japonicus* with a significantly lower protein content (13.24±0.08 %). The protein content of the fish samples ranged from 13.24 to 22.46 % and it was relatively high to moderate percentage of crude protein and it may be attributed to the fact that fishes are good source of pure protein, but the differences observed in values obtained could also be as a result of fish consumption or absorption capability and conversion potentials of essential nutrients from their diets or their local environment into such biochemical attributes needed by the organisms body [Burgress GH,1975 and Adewoye et.al,1997].

Generally, lipids are soluble in ether hence they are ether extractable. They serve as source of energy during starvation and fasting. The mean oil content (3.13 ± 0.06 %) of *Lethrinus lensus* was relatively low compared to the oil content of the other five fishes. *Lutjanusquin quelineatus* had the highest mean oil content (4.43%). According to Ackman (1989), generally fish can be grouped into four categories according to their fat content lean fish, low fat (2 to 4 %), medium fat (4 to 8%), and high fat. The marine fishes (*Lutjanusquin quelineatus* and *Scolopsis bimaculatus*) had higher lipid content than other fishes; hence they were classified as high fat group. This indicates that the marine fishes are better sources of lipid in the body when consumed.

The low concentrations of lipid in the muscles of the fishes species could be due to poor storage moisture content. There is a disadvantage in that it increases the fishes' susceptibility to microbial spoilage, oxidative degradation of polyunsaturated fatty acids and consequently decreases in the quality of the fishes for longer preservation time [Omolara,2008] mechanism and the use of fat reserves during spawning activities [Osibona et.al,2009].

The ash content in marine fish species were generally higher (*Nemipterus japonicus* 3.97%; *Epinephelus areolatus* 3.89 %) than that of the other samples. There was no significant difference between the values. The observed range of ash content in the fishes indicated that the species is a good source of minerals such as calcium, potassium, and iron. Ash is a measure of the mineral content of food item. It is the inorganic residue that remains after the organic matter has been burnt off. A good source of instant energy that comes to the mind is carbohydrate. It also helps in the body's development and growth. The carbohydrate content in fish is generally very low and practically considered zero [Payne, 1999]. The result in **Table.1** showed that the various fish species having poor sources of carbohydrate. *Nemipterus japonicus* has a high mean carbohydrate content of 0.26±0.00 % while other fishes had a low mean carbohydrate content of 0.05±0.00 % respectively. There was no significant difference between the values. The relatively low values of carbohydrate could be due to higher values of moisture and a relatively high value of protein content. This study showed that the preferential fish species consumed in *Lutjanusquin quelineatus* presented a good nutritional composition. The high protein content confirmed that these species were good animal proteins sources; they are particularly consigned for the children growing stages. The caloric value confirmed their energetic plus value. However, microbial results showed low count; these microbial qualities are acceptable.

CONCLUSION

This study clearly indicates that the microbial and proximate values obtained would be useful to help the consumers in choosing fish based on their nutritional values besides providing an update to food composition value.

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***Corresponding Author:**
S. Sankar
Research Scholar
CAS in Marine Biology,
Faculty of Marine Sciences,
Annamalai University,
Parangipettai, Cuddalore (Dt)
Tamil nadu, india.