E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(1): 157-161

www.fisheriesjournal.com
Received: 24-11-2016
Accepted: 25-12-2016

Shuvra Roy
CAS Marine Biology
Annamalai University
Parangipettai, Tamil Nadu, India

Correspondence
Shuvra Roy
CAS Marine Biology
Annamalai University
Parangipettai, Tamil Nadu, India

Occurrence of *Listeria monocytogenes* and biochemical composition in some Seafood, Kakdwip coast, West Bengal, India

Shuvra Roy

Abstract
Samples of marine fishes (Oil Sardine, Silver bellies, Indian mackerel, Tuna and Ribbonfishes) were collected from Kakdwip Coast of India to examine the occurrence of *Listeria monocytogenes* and to estimate the proximate composition (moisture, protein, carbohydrate and fat). The results of the present study revealed that *L. monocytogenes* were found enumerated, which varied between 0.012×102 and 0.15× 102 CFU/ml in Indian Mackerel and Oil Sardine respectively. The average moisture content was ranged from 64.79 to 74.33%. The protein content was noticed between 14.53 to 16.87% while the fat content (3.75 to 6.54 %,) and the Carbohydrate were varied widely from 3.5 to 9.58%. The details of the nutritive value of the fishes were presented.

Keywords: *Listeria monocytogenes,* proximate composition, marine fish, Kakdwip

1. Introduction
Human *Listeriosis* is caused by the pathogen *Listeria monocytogenes* which was perceived in the year 1927 by E.G.D. Murry and J. Pirie (Rocourt, 1999) [24]. Most people are routinely exposed to *Listeria* on health consequences. Determination of proximate composition as protein content, carbohydrates, lipids, moisture and ash percentage is often necessary to ensure that fish tissues have a good nutrition quality and that they meet the requirements of food regulations and commercial specifications (Surtharshiny and Sivashanthini, 2011) [26]. Tawfik, (2009)[27] studied the proximate composition and fatty acid profile in most commonly available fish species in Soudi market. According to Waterman (2000) [28], quantifying proximate composition is important in ensuring the requirements of food regulation and commercial specification. Protein content, which is an important component, tends to vary little in healthy fish (Weatherly and Gills, 1987) [29]. Pelagic fish species (e.g. Clupeidae, Osmeridae) are usually high in lipid content and energy levels (KJ/100g raw tissue), whereas demersal species (e.g. Synodontidae, Gobiidae) generally have lower lipid and energy values (Ball *et al*., 2007) [3]. There is a wealth of literature available on body composition of various fish species elsewhere (Berg *et al*., 2000; Dempson *et al*., 2004) [5, 8]. Proximate body composition is the analysis of water, fat, protein and ash contents of fish. Carbohydrates and non-protein compounds are present in negligible amount and are usually ignored for routine analysis (Cui and Wootton, 1988) [7]. The percentage of water is good indicator of its relative contents of energy, proteins and lipids. The lower the percentage of water the greater the lipids, protein contents and higher is the energy density of the fish (Dempson, *et al*., 2004) [7]. However, these values vary considerably within and between species, size, sexual condition, feeding season and physical activity. Protein content, which is important component, tends to vary little in healthy fish (Weatherly and Gills, 1987) [29]. Fish is one of the main food constituents in our diet as it contains essential fatty acids, amino acids and some of the principal vitamins and minerals in sufficient a mounts for healthy living (Borgstrom, 1961) [6].

Proteins compose over 50% of the dry weight of an average living cell and are very complex to macromolecules. In food, proteins are essential for growth and survival and very depending upon a person's age and physiology.

Lipids in food include the oil of such grains as corn, soybean, from animal fats, and are parts of many foods such as milk, cheese and meat. The unique nutritional benefits of marine oils

came from the effects of their long chain omega 3 polyunsaturated fatty acids, particularly EPA and DHA. Sea food is an ideal source of these nutrients (Potter and Hotchkiss, 1995) [23]. Fish oils contain omega 3 polyunsaturated fatty acids, which are essential for the diet. They help against coronary heart disease, high blood pressure and rheumatoid arthritis. Omega 3 polyunsaturated fatty acids, in particular DHA, may also be beneficial for infant brain and retina function and development. Other beneficial oils present in sea food include the omega-6 fatty acid M which is important for growth, and also seems to play a role in our general good health and well-being (Nichols *et al*., 1998) [21]. Carbohydrate, fat and enumeration of the *L. monocytogenes* were aimed to concentrate in some commercially important fishes from the landing centres of Kakdwip. Fish is considered as a major source of *Listeria* contamination. Fresh and marine water fish could be sources of human infection via eating raw or undercooked fish. Saurus and Sardine fish are a cheap fish sold as fresh in retail markets as well as imported as frozen fish. These fish could be contaminated by bacteria particularly Listeria from public health perspectives, Listeria contamination considered great public health significance. The Egyptian standards for food safety regulations tolerate zero limits for *L. monocytogenes* in frozen and fresh fish (EOS, 2005) [10].

2. Materials and Methods

The Kakdwip ((Station 1) and Diamond harbour (Station 2) region of Hooghly estuary (Lat. 21° 40' and long 87°47' E) is most diversified area of marine and brackish water fishes and so the fishing is one of the prime activity. Samples of marine fishes (Oil Sardine (*Sardinella longiceps*), Silver bellies (*Leiognathus equulus*) Indian mackerel (*Rastrelliger kanagurta*), Tuna (*Euthynnus affinis*) and Ribbonfish (*Trichiurus lepturus*) were collected to examine the occurrence of *Listeria monocytogenes* and to estimate the proximate composition (moisture, protein, carbohydrate and fat) from them. The specimens (12.5 - 16.5 cm TL) were properly cleaned for biochemical analysis, a portion of the muscle from the widest part of the body (devoid of bones) after removal of the skin was taken and dried at constant temperature (60^0C) for 24 hours in a hot air oven. Then the dried meat was powdered and the required quantity used for the determination of moisture, calorific value, protein, fat and carbohydrate. Each analysis was carried out in triplicates. Moisture content of fish fillets were determined according to method described by AOAC (1990) [2] with slight modifications by Tee *et al*. (1996) [27]. The calorific content was estimated by incinerating the pre-weighed test material (1g dry weight) in a Muffle furnace at 560 ^{0}C for a period of 5 hours and the residue was weighed and calculated as percentage. The Folin- Ciocalten Phenol method of Lowry *et al*., (1951) [19] was used for the determination of the total protein in the tissue. The total carbohydrate was estimated by Phenol- Sulphuric acid method of Dubois *et al.,* (1956) [9]. The lipid content was estimated by Folch *et al*., (1957) [12]. The bacterium *L. monocytogenes* was identified on the basis of morphological and biochemical characters (Balaswaminathan, 2001) [4].

3. Results

3.1 Biochemical composition of marine fish samples studied

The moisture, calorific value, protein carbohydrate and lipid of marine fish samples of five different species of commercial fishes were estimated in stations 1 and 2. The mean values (%) of biochemical composition of samples are given in Table 1 and Fig 1-5.

Table 1: Biochemical composition (%) of fishes estimated in station 1 and 2

Fishes	Moisture (%)		Calorific value (%)		Protein (%)		Carbohydrate (%)		Fat (%)	
	St.1	St.2	St.1	St.2	St.1	St.2	St.1	St.2	St.1	St.2
Oil sardine	70.37	69.28	3.73	3.8	16.72	16.87	4.31	5.31	3.75	3.67
	±1.98	± 1.31	±1.13	±1.06	±0.15	±0.22	±0.08	0.06	±1.48	±1.27
Indian Mackerel	74.24	67.72	3.57	5.62	12.35	15.48	5.78	3.42	3.56	4.17
	±2.39	±2.29	±0.16	±0.10	±0.13	±0.28	±0.11	±0.16	±0.10	±0.06
Silver Bellies	69.39	65.18	3.64	5.7	15.08	18.96	7.36	6.33	5.48	4.86
	±1.09	±3.02	±0.01	±0.20	±0.15	±0.45	±0.12	±0.07	±1.05	±1.19
Tuna	74.33	67.39	5.31	6.18	18	14.84	6.61	5.84	5.16	4.45
	±1.27	±1.27	±0.11	±0.06	±0.03	±0.20	±0.16	±0.13	±0.04	±0.13
Ribbonfish	71.88	64.79	5.45	4.43	10.45	14.53	8.66	9.58	6.54	5.56
	±2.12	±2.30	±0.26	±0.16	±0.1	±0.15	±0.08	±0.15	±0.12	±0.15

3.2 Moisture

The moisture content varied between 69.39 ± 1.09 to 74.33 ± 1.27% and 64.79 ± 2.30 to 69.28 ± 1.31% in St.1. However it was low in *L. equulus* and was very high in *Euthynnus affinis* in St.1 but in St.2. *S. longiceps* was highest and the Ribbonfish was lowest.

3.3 Calorific value

Total caloric content was calculated by adding the calorific contents of the protein, carbohydrate and lipid of the three tissues of five species of fishes. The highest total calorific content was observed in *T. lepturus* (5.45±0.26) and lowest in Indian mackerel in St.1. (3.57±0.16) and in St.2. it was varied from 3.8± 1.06 to 6.18± 0.06 in Oil sardine and Tuna fish respectively

3.4 Protein

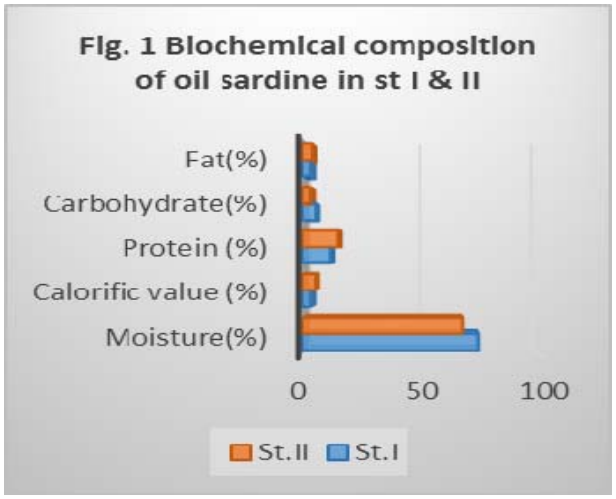
The protein content ranged from 10.45± 0.1 to 18±0.03% and 14.53±0.15 to 18.96±0.45% in the samples collected from the St.1.and St.2.respectively. However the protein content was very high in Tuna fish in St.1.while in St.2 it was high in Silver bellies. The lowest was found in *T. lepturus* from both the stations.

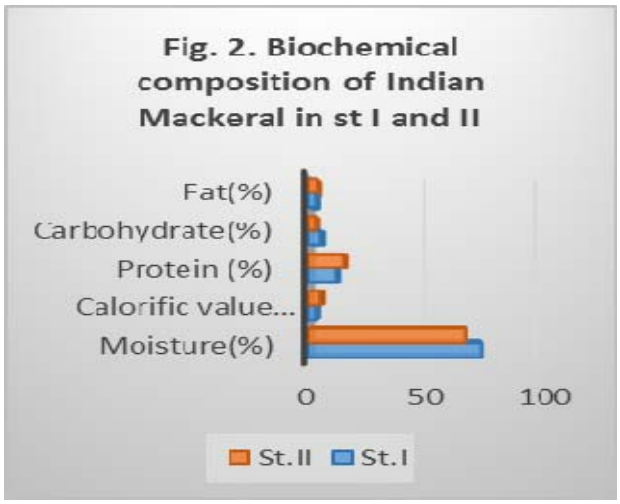
3.5 Carbohydrate

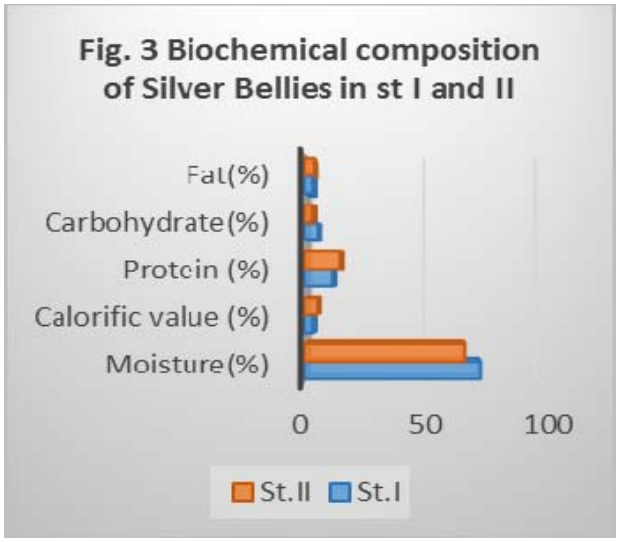
The carbohydrate content ranged from 4.31±0.08 to 8.66±0.08% and 3.42±0.16 to 9.58±0.15% in Kakdwip coast respectively. *T. lepturus* was conspicuous in processing high content of carbohydrate in both the stations. The lowest were found in and *S. longiceps* and *R. kanagurta* in both the St. respectively.

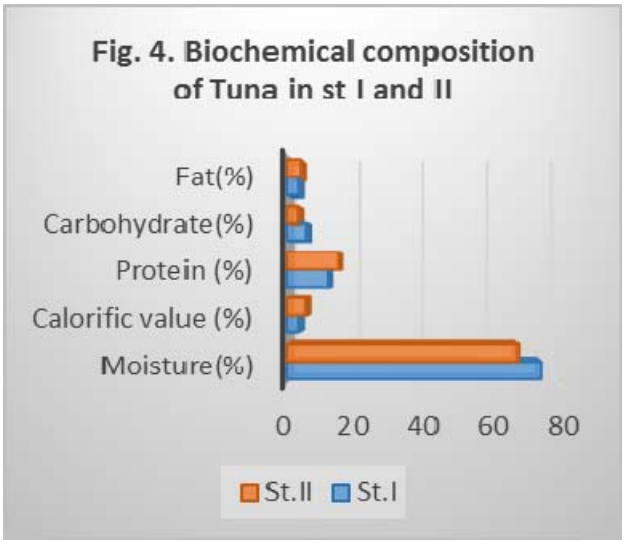
3.6 Lipid

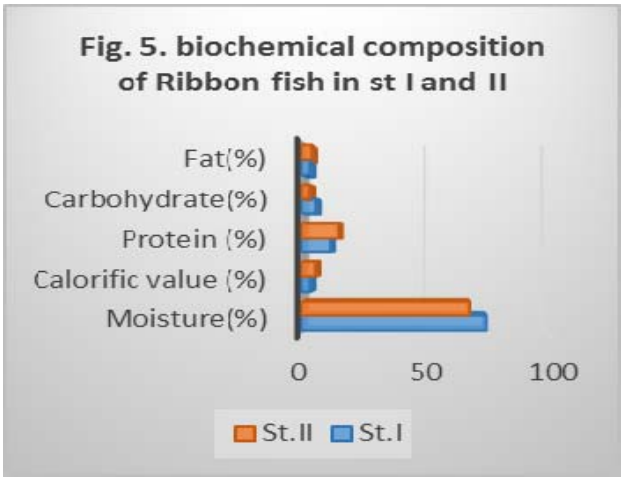
The lipid content varied between 3.56±0.01-6.54 and ±0.12% and 3.67± 1.27 and 5.56±0.15% in St.1. and St.2. respectively. However *S. longiceps* was low and was very high lipid content when compared to other species in both the species.











3.7 Isolation and Identification of *L. monocytogenes*

L. monocytogenes population was determined in different fish samples collected from different sites. In some of fish species the population of *Listeria* was not observed. But in some species it occurred and the population varied significantly. The differences in their population are presented in Table 2.

Table 2: Distribution of *L. monocytogenes* in station 1 and 2

Fishes analyzed	Station 1 (CFU/ml)	Station 2 (CFU/ml)
Oil sardine	0.15×10^2	0.003×10^2
Indian Mackerel	0.012×10^2	0.001×10^2
Silver Bellies	0.014×10^2	0.001×10^2
Tuna	0.054×10^2	0.002×10^2
Ribbon fish	0.013×10^2	0.001×10^2

The station 1 it was varied between 0.012×102 and 0.15× 102 CFU/ml in Indian Mackerel and Oil Sardine respectively, whereas 0.001×102 and 0.003×102 CFU/ml of *L. monocytogenes* were found enumerated in Indian Mackerel and Oil Sardine respectively in station 2.

4. Discussion

The commercial value of fish and fishery products are often related to their biochemical composition, as they contribute to their nutritive value. The biochemical composition of fish and fishery products are determined based on the estimation of proximate composition such as moisture, protein, fat,

carbohydrate and mineral substances. In the present study Oil sardine, Indian mackerel, Ribbonfish, Tuna and Silver bellies were carried out along with *L. monocytogenes* in St1 and St.2. The moisture content varied between 69.39 ± 1.09 to 74.33 ± 1.27% and 64.79 ± 2.30 to 69.28 ± 1.31% in St.1 and St.2 respectively. Fish has very high water content and hence spoils easily due to the action intrinsic enzyme and microorganisms (Indira Jasmine and Jeya Shakila, 2004) [15]. The highest total calorific content was observed in *T. lepturus* (5.45±0.26) and lowest in *S. longiceps* in St.1 (2.73±1.13) and in St.2 it varied from 3.8± 1.06 to 6.18± 0.06 in Oil sardine and Tuna fish respectively. Besides minerals such as Ca, Na, K which normally from the major constituents of ash, the fish may also contain Cu, Fe, Mg etc as minor constituents. They help to build tissue regulate body fluids or assist in various body functions. Proteins provide essential amino acids which are not synthesized in the body. The diet contains a variety of different animal and plant proteins (Achuthankutty and Parulekar, 1984; Snehalata Das and Sahoo2001) [1, 25]. The nutritional value or quality of a given protein depends upon two factors (1) it content essential amino acids and (2) its digestibility. The protein content ranged from 10.45± 0.1 to 18±0.03% and 14.53±0.15 to 18.96±0.45% in the samples collected from St.1.and St.2.respectively. Fish is a rich source of all essential amino acids especially of Methionine, Thrionine, Lysine and Isolysine (Indira Jasmine and Jeye Shakila, 2004.) [12] The carbohydrate content ranged from 4.31±0.08 to 8.66±0.08% and 3.42±0.16 to 9.58±0.15% in St.1. respectively. Body's energy is derived from carbohydrates and fats and are set to be proteins spareres (Guyton and Hall, 1998; Snehalata Das and Sahoo, 2001) [13, 25]. The low value of carbohydrates recorded in the present study, which could be due to the fact that glycogen, in many marine animals do not contribute much to the reserves in the body (Jayasree *et al*. 1994) [16].

The lipid content varied between 3.56±0.01-6.54 and ±0.12% and 3.67± 1.27 and 5.56±0.15% in St.1.and St.2 respectively. Fat is a concentrated source of energy and it supplies per unit weight more than twice energy furnished either protein or carbohydrate. Fat also provides calories and essential fatty acids. Triglycerides constitute about 98% of total dilatory lipids, the remaining 2% consist of phospholipids and cholesterol and its esters. Fishes require lipids in the muscle for energy during starvation and reproduction (Love, 1980) [18].

L. monocytogene is responsible for nearly ¼ of all estimated food borne disease related death which highlights its significance as a health concern (Mead *et al*, 1999) [20]. The majority of human listeriosis occurs in pregnant women, immune suppressed individuals and elderly (Farber and Peterkin, 1991) [11]. As growing segments of human population falls include high risk group improved methods for reducing the levels of *L. monocytogenes* in foods are essential. While *L. monocytogenes* causes relatively few human disease of particularly compare to many other food borne pathogens (Mead *et al*, 1999) [20]. Jinneman *et al* (1999) [17] isolated *L. monocytogenes* from domestic and fresh, frozen and processed sea food products, including Crustaceans molluscs and fin fishes. In the present study *L. monocytogenes* was varied between 0.2×10^2 and 0.15×10^2 CFU/ml and 0.001×10^2 and 0.003×10^2 CFU/ml inSt.1.and St.2. Norton *et al* (2001) [22]. Noticed and isolated *L. monocytogenes* representing the 3 of the 18 ribotypes found among isolated from the smoked fish industries. Oil sardine is the dominant marine fish catches in India and *L. monocytogenes* distribution was enumerated in higher number in both stations. This could be attributed due to handling improper processing etc. Huss *et al* (2000) [14] have classified the sea food as potential high risk food for Listeriosis. Molluscs, including fresh and frozen mussels, clams and oysters in shell or shecked, raw fish, lightly processed fish products, including salted, marinated, fermented, cold smoked and graded fish, and mildly heat accessed fish products and crustaceans.

5. Acknowledgements

The author is grateful to the Dean and Director, CAS in Marine Biology and the authorities of Annamalai University for the facilities provided.

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