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Estimation of nutritional content and isolation of amine forming bacterias of commercially important dry fish *Sardinella longiceps* collected from local market in Coimbatore, Tamil Nadu

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Abstract

Dry fish is the low cost dietary protein source in India, especially in Tamilnadu. Sometimes dry fishes are kept for a long period that is the key factor for the deterioration of nutritional value of dry fishes, as they absorb moisture from the surrounding air. The general purpose of this study was to determine the nutritional content and amine forming bacterias of dry fish (*Sardinella longiceps*) collected from local market in Coimbatore, Tamilnadu. The proximate composition such as protein, carbohydrate, lipid and ash contents were analysed in dry fish samples using standard procedures. In the present study 100gm of dry fish sample of *Sardinella longiceps* had the highest protein content (46.14 gm) and ash content (13.17gm), where as carbohydrate and lipid values were recorded as 2.05gm and 1.19gm respectively. In this present investigation the pH value of dry *Sardinella longiceps* was 6.89 and the recorded moisture content was 39.25gm. The dry sample had the lowest bacterial count of 0.7×10^5 cfu/g, which is below the permissible limit of 7×10^5 cfu/g. In addition to the proximate composition, amine forming bacterias were also isolated from the dry fish samples and the isolated bacterias were *E.coli* and *Salmonella typhimurium*. This study clearly indicates that the nutritional composition and microbial status obtained could be helpful in choosing fish based products on the nutritional values.

Keywords: *Sardinella longiceps*, physicochemical parameters, TVC, amine forming bacterias

1. Introduction

Dry fish is low cost dietary protein source and used as a substitute of fish at the scarcity of fresh fish. About 15% of fishes are cured for mass people consumption at the scarcity of fresh fishes. It is also a very favourite food item and has a good market demand besides fish and seafood products. Some marine fish species people do not like to consume as fresh fish but they like to eat dry fish of these species [1]. Moreover, dry fish has a storage life of several years and is a great source of protein, essential fatty acids, vitamins and many minerals. So it is consumed all over the world for its nutritional value, taste and aroma.

Dry fish are generally stored in dump warehouses; sometimes fishermen do not dry fishes properly due to loss of weight, as they want to make more profit. Therefore, during the monsoon period the dry fish absorb moisture rapidly and become suitable for infestation by beetles and mites. Therefore, it is assumed that the nutritional value and the physical properties of dry fish will be deteriorated with the increasing of storage period [2, 3]. Nutrition is an important influencing factor of fish product seafood consumption. 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.

The changes of nutritional value of marine dry fishes (*Harpodon nehereus*, *Johnius dussumieri* and *Lepturacanthus savala*) were investigated with the increasing of storage period (Siddique and Aktar, 2011) [4]. The effect of different processing methods on the nutritional quality and microbiological status of cat fish (*Clarias lazera*) was studied (Adenike, 2014) [5]. Studies on amines and amine forming bacterias in edible marine fish *Sardinella longiceps* and its product were conducted (Merline *et al.*, 2015) [6]. However least research works were carried out on dried fish nutritional composition while the area of amine forming bacterias in relation to dry fish were not undertaken so far in Coimbatore markets, hence the present study has been framed to determine the nutritional content and to isolate the amine forming bacterias in the

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dry fish samples of *Sardinella longiceps* collected from local retail shop in Coimbatore, Tamil Nadu with reference to the human health.

2. Materials and methods

Experimental fish

The experimental fish sardine (*Sardinella longiceps*) is a species of ray-finned fish in the genus *Sardinella*. It is one of the most important commercial marine fishes in India. The Indian Oil Sardine is one of the more regionally limited species of *Sardinella* and can be found in the northern regions of the Indian Ocean.

Samples collection

The dry fish samples of *Sardinella longiceps* were procured from local markets in Coimbatore, Tamilnadu, India.

Sample preparation

The dry fish samples were weighed accurately and samples were cleaned and crushed by mortar and peston. Then the powdered samples were tested for proximate composition and the presence of amine forming bacterias.

Physico- chemical analysis

Measurement of pH

The dried sardine samples (10g) were homogenized in sterile blenders with 10ml of distilled water to make thick slurry. The pH of this slurry was then measured using a pH meter (Eco tester pH1), where three readings were recorded and the average was calculated Ronald and Ronald, 1991^[7].

Estimation of moisture: Jain and Singh, 2000^[8]

A known quantity of the sample is taken in a weighed fish and the moisture is removed by heating in a hot air oven. Finally it is cooled in desiccators and weighed. The difference between the weight of the sample before and after drying gives the moisture content and it is usually expressed as percentage (1%) of the weight of the sample.

Estimation of salt

The salt content in dry fish sample was determined according to the AOAC, 1995^[9] procedures by homogenizing 2 g of sample with 18 ml of distilled water. The homogenate was titrated with 0.1M AgNO₃ using 10% w/v. K₂Cro₄ solution used as an indicator.

Biochemical analysis

The total protein, carbohydrate and lipid contents were estimated in the dry fish samples by following the method of Lowry *et al.*, 1951^[10], Hedge and Hofreiter, 1962^[11] and Folch *et al.*, 1957^[12].

Total viable count

One gram (1g) of dry fish sample was dissolved in sterile deionized water and serially diluted. One millilitre (1ml) of appropriate dilutions was seeded on plate count agar using spread plate method, and then the medium was incubated at 37 °C for 24 hours. The plate count agar was examined and colonies present were counted and recorded after incubation at 37 °C for 24 hours to get the total colony count in cfu.

Isolation of microorganism

One gram (1g) of dry fish sample was serially diluted and 1 ml of an appropriate dilution was inoculated on nutrient agar

plates and the plates were incubated for 24 hours at 30 °C. After 24 hours, sterile wire loop was used to pick the isolate from the plate and was streaked on a freshly prepared sterile nutrient agar plates, then it was incubated for 24 hours at 30 °C in order to get pure cultures. The pure cultures were then stored in a refrigerator at 4 °C. The isolates were identified using their macroscopic, cultural and biochemical characteristics.

E. coli

Fish homogenate was transferred to lauryl sulphate tryptone broth (LSTB) tubes and incubated at 37 °C for 24 hours.

Salmonella typhimurium

Fish sample was homogenized and enriched in 225 ml lactose broth at 37 °C for 24 hours.

Staphylococcus aureus

Manitol salt agar was used to enumerate the number of *Staphylococcus aureus* colonies. The plates were incubated at 35 °C for 24hours and bright yellow colonies were counted.

Enterobacteriaceae aerogenes

The dilutions were pour-plated and overlaid with violet red bile glucose agar (VRBG; Oxoid CM0485). The plates were incubated at 37 °C for 24hours.

Shigella dysenteriae

Macconkey agar was used to enumerate the number of *Shigella* colonies. The plates were incubated at 35-37°C for 18 hours. *Shigella* colonies are appeared in pink colonies.

Pseudomonas aeruginosa

The strain was determined on centrimide agar by pour plating the plates were then incubated at 37 °C for 24 hours; strains of *Pseudomonas* produced green colour colonies.

Klebsiella pneumonia

Samples for detection of *Klebsiella* were plated out on Mac-Conkey Agar. The plates were incubated at 35 °C for 24hours. Mucoid colonies showed the presence of *Klebsiella pneumonia species*.

Proteus mirabilis

A loopfull from broth tube was streaked onto Urea agar base (Christensen) to isolate the *Proteus mirabilis*.

Streptococcus faecalis

A loopful from broth media was streaked onto the Luria agar to isolate the *Streptococcus faealis* and incubated at 35°C for 24 hours.

3. Results and Discussion

For the present research the selected dry fish *Sardinella longiceps* were collected from retail shop in Coimbatore, Tamilnadu. The samples were cleaned and powdered, then subjected to various analyses. The present results of physico chemical and biochemical parameters are shown in table 1and 2.

pH is an important factor that affects the microbial growth and spoilage of foods and may help to explain the observed differences in the effects of antimicrobial and antioxidant treatments. In this present investigation the pH value and the moisture content of dry *Sardinella longiceps* was 6.89 and

39.25gm respectively. Dry samples showed the highest protein content of 46.41 gm. It may be the result of dehydration of water molecules present between the proteins thereby, causing aggregation of protein and thus resulted in the increase in protein content of dried fishes. This result was in agreement with the findings of Sankar *et al.*, 2013 [13], who reported that dried fish had higher protein content. This result was also in agreement with the previous work of Ogbonnaya and Shaba, 2009^P [14] who observed that protein nitrogen was not lost during drying, so that protein content increased with the reduced moisture content in the fish samples.

The carbohydrate content was relatively low when compared to protein content of the samples. From the results recorded carbohydrate content in dry Sardines samples was 2.05 gm. Lipid content of the dry fish *Sardinella longiceps* was 1.19 gm. According to Ackman, 1989 [15], generally fish can be grouped into four categories according to their fat content lean fish, low fat (2-4%), medium fat (4-8%) and high fat. In the present study the selected species of *Sardinella longiceps* were having low fat content (2-4%). The ash content in dry fish sample was 11.37 gm (Table 2). The value of ash in the processed fishes may be attributed to analysis which was carried out on the edible portion of the fish not incisive of the bone.

The total viable counts of dry sample are shown in table (2). The total viable count expressed as colony forming unit in one gram of sample (cfu/g) of the representative sample was determined by standard plat count method on plate count agar media. Total viable count of the dry sample was recorded as 0.7 cfu/g, which is below the acceptable level (7×10^5 cfu/g). The present study agreed with the findings of Oladipo and Bankole, 2013 [16], on microbial quality of dried *Clarias gariepinus* and *Oreochromis niloticus*.

The distribution of the bacteria species present (table 3) in the dry sample of *Sardinella longiceps* were *Echerichia coli*, *Proteus mirabilis*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Enterobacter aerogens*. The isolated amine forming bacteria from the selected dry sample were *E.coli* and *Staphylococcus aureus*. *Staphylococcus aureus* has been detected during the process of drying and subsequent smoking of eels in Alaska in 1993 (Eklund *et al.*, 2004) [17].

E.coli and *Salmonella* can survive for very long periods and that once introduced, they may become indigenous to the treatment (Fujioka *et al.*, 1989) [18]. *Escherichia coli* belong to the family Enterobacteraceae and they have been associated with nosocomial outbreaks, and are considered as opportunistic pathogens. The importance of *Salmonella typhimurium* as a cause of food borne illness is well recognized. The occurrence of the organism in fish is normally associated with the faecal contamination of the environment from which they are harvested.

4. Conclusion

Drying of marine fish is very common in the entire coastal areas of Tamilnadu and these dried fishes have demand both in domestic and international market. In this present study the collected dry samples of *Sardinella longiceps* were very rich in protein and lipid content. However, the nutritional value of dry fishes are greatly deteriorates due to the longer storage. Therefore, the dry fish industries should be kept more precautionary steps during storage of dry fish in the warehouse and in the sales center.

Table 1: Physico chemical parameters of dry sample of *Sardinella longiceps*

S.No	Parameters	Dry sample
1	pH	6.89
2	Moisture (gm)	39.25
3	Salt (gm)	0.20
4	Total viable count @ 37 °C	0.7×10^5 cfu/g

Values are expressed as mean

Table 2: Biochemical parameters of dry sample of *Sardinella longiceps*

S. No	Parameters	Dry sample
1	Protein (gm)	46.14
2	Carbohydrate (gm)	2.05
3	Lipid (gm)	1.19
4	Ash (gm)	11.37

Values are expressed as mean

Table 3: List of amine forming bacteria isolated from dry sample of *Sardinella longiceps*

S. No	Microorganisms	Dry sample
1	<i>Echerichia coli</i>	+
2	<i>Proteus mirabilis</i>	-
3	<i>Shigella dysenteriae</i>	-
4	<i>Staphylococcus aureus</i>	+
5	<i>Streptococcus faecalis</i>	-
6	<i>Salmonella typhimurium</i>	-
7	<i>Pseudomonas aeruginosa</i>	-
8	<i>Klebsiella pneumonia</i>	-
9	<i>Enterobacter aerogens</i>	-

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