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Quality analysis of shidal - a traditional fermented fish product of Assam, North-East India

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ABSTRACT

In the present study, proximate composition and bacteriological analyses of the fermented fish product, locally known as shidal, sampled from producers as well as retailers in eight different districts of Assam, were carried out. In proximate principle analysis, lower level of protein and fat content in the products obtained from retailers indicated the relative nutrient losses occurring at different stages of marketing chain. High total volatile base nitrogen (TVB-N), peroxide values and moisture along with promising microbial load in the retailer's samples reflected poor quality, whereas those obtained in producer's samples were within the acceptable limit. None of the samples revealed presence of the coliform bacteria, *Escherichia coli* and *Salmonella* sp. Yeast and fungal colonies were detected in the samples.

Keywords: Bacteriological analysis, Fermented product, Proximate composition, Shidal

Introduction

Traditional processing of fish such as fermentation, salting, drying and smoking are the principal methods of fish preservation in South-east Asia, (Cooke *et al*., 1993). In the north-eastern states of India**,** fermentation is one of the oldest and most economical methods to produce and preserve food. The fermentation process causes enrichment and improvement of food by way of enhancement of flavour and aroma, change in texture, preservation by producing organic acids, nutritional enrichment, reduction of endogenous toxins and reduction in the duration of cooking and thereby reduction in fuel requirement. In some cases, the pharmacological and nutritional values of the product are enhanced, including the digestibility (Crisan and Sands, 1975). Fermentation may make the product more enjoyable and safer. Probiotics, or "good bacteria" are also formed through the process of fermentation.

Indigenous fermented foods, such as *shidal,* contribute a large portion of daily food intake in north-eastern states of India. It is a salt-free, solid, semi-fermented fish product, prepared from small sized fish mainly *Puntius* sp. (Muzaddadi and Basu, 2003a). It has several local names like *seedal, seepa, hidal* and *shidal* in Assam, Tripura, Arunachal Pradesh, Nagaland and *ngari* in Manipur. The preparation involves several steps, including semi-drying of *Puntius* sp. (usually done in the sunlight), filling them in vats/earthen pots for fermentation for 4-6 months, following a standard procedure, during which the final product gets a semisolid appearance (Mansur, 2007).

Shidal is a delicacy for most of the tribal, Bengali and other people of the state for its characteristic taste and flavour. But its preparation continues to be through unhygienic traditional methods and preparation is confined to some particular groups of people. Further, its marketing condition is also poor. Literature available on the various aspects of preparation of this product is scanty (Muzaddadi and Basu, 2003a), leaving enough scope for research on the nutritional quality, biochemical and microbiological aspects of the product. There appears to be no information on the microbiology of the traditionally processed fish products of Assam and other north-eastern parts of India. Scientific information regarding its nutritional value and protein quality is necessary to make this food more popular among the people, so that it can contribute much in improving the protein deficiency, especially among the poor section of the state. Considering all these aspects, the present study was undertaken to assess the nutritional and food quality of traditional semi-fermented product *i.e.*, fish shidal, available in the domestic market in Assam.

Materials and methods

Collection of samples

The samples were collected periodically from the producers of shidal in 3 districts (Morigaon, Nagaon and

Karbi-Anglong) as well as from retailers in ifferent markets of Morigaon, Nagaon, Kamrup, Jorhat, Golaghat, Diphu, Lakhimpur and Karbi-Anglong districts of Assam. Three samples from each district were collected . After collecting the samples aseptically, they were packed tightly in separate pre-sterile polyethylene bags, kept in ice-box and were transported to the laboratory of the Department of Fish Processing Technology, Faculty of Fishery sciences, West Bengal University of Animal and Fishery sciences, Kolkata for analyses.

The semi-fermented fish shidal collected from producers and retailers were separately minced and finely homogenised with a pestle and mortar and used for subsequent analyses.

Proximate composition analyses

The proximate composition of the shidal samples were determined following standard methods (AOAC, 1995). The ash content of the samples were estimated as the inorganic residues such as oxides, sulphates, silicates and chlorides left behind, in the dry muscle sample heated to temperatures of 500-600 ºC in a muffle furnace. Total volatile base nitrogen (TVB-N, mgN 100 g^{-1}) was determined by the Conway micro diffusion method described by Beatty and Gibbon (1937). The peroxide value (PV) of the lipid was determined from the lipid extract using iodometric method as described by Jacobs (1958). The free fatty acid (FFA) content in sample was determined by the method recommended by Nambudiri (1985) and expressed as % FFA as oleic acid.

Microbiological analyses

In order to assess the microbial load, 10 g of each sample was aseptically collected and macerated with 90 ml sterile saline. After making serial dilution in the same diluent, pour plating was done on nutrient agar plates. Total viable counts were recorded after 48 h of incubation at 37 ºC and was expressed as log cfu (colony forming units per gram). Lactic acid bacteria (LAB) were selectively isolated on MRS agar plates and incubated under anaerobic conditions in an anaerobic jar at 30 ^{0}C for 48 h. For selective enumeration of *Staphylococcus aureus*, Baird Parker medium, supplemented with egg yolk emulsion and potassium tellurite, was used. Samples were tested for enumeration of *Micrococcus* spp. and *Bacillus* spp. using selective fermentation medium for *Micrococcus* and *Bacillus* differentiation agar respectively. Yeasts (fungi) were isolated on fungal agar or mycological agar medium, supplemented with antibiotic penicillin @ 0.2 ml per 100 ml and streptomycin @ 0.3 ml per 100 ml to avoid growth of bacteria, and incubated aerobically at 28 ºC for 72-96 h. For enumeration of bacteria, plates having 30 to 300 colonies were considered and the results were expressed as log cfu per g wet weight of sample.

Statistical analyses

All the biochemical analyses and microbial counts were performed in three independent experiments for three samples each collected from different producers and retailers of the same district . The standard deviation and arithmetic mean of the triplicate samples were estimated . Data were analysed and compared by analysis of variance (two factorial ANOVA). Probability level was fixed at $p<0.05$. Correlation coefficient (r) was calculated for the different parameters to observe their acceptance level.

Results

Proximate composition

The proximate composition of fish shidal sampled from producers and retailers was analysed and the results are presented in Tables 1a and b and Fig. 1. Moisture content varied from 37.74±0.95 to 44.24±2.01%, with higher values recorded in the samples obtained from retailers and lower values for samples from producers. The average moisture content of producer and retailer samples were 39.34±1.70 and 43.20±0.99% respectively. Protein, content ranged from 34.66±1.68 to 35.25±0.58% in wet weight basis, with higher value obtained in the samples collected from producers. The average protein contents of producer and retailer samples were 35.20±0.23 and 34.73±0.06% respectively where as fat content varied from 17.25 ± 0.58 to 21.63 ± 0.89% with similar trend. The average fat content of producer and retailer samples were 21.00 ± 0.65 and 17.92 ± 0.58% respectively. Ash content varied from 2.22±0.94 to 2.41±0.08%, with higher value observed in the product from retailers. The average ash content of producer and retailer samples were 2.26±0.04 and 2.37±0.02% respectively. No significant difference

Table 1a. Proximate composition of shidal samples collected from producers in different districts of Assam

District	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Morigaon	37.74±0.95	35.25±0.58	21.63±0.89	2.30±0.45
Nagaon	39.16±0.74	35.40±1.25	21.05±1.23	2.22±0.94
Karbi-Anglong	41.14±1.02	34.95±0.82	20.32±1.76	2.28±0.57
Mean ± SD	39.34±1.70	35.20±0.23	21.00±0.65	2.26±0.04

Data are expressed as mean ± SD (n=3)

Table 1b. Proximate composition of shidal samples collected from retailers in different districts of Assam

District	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Morigaon	41.58±0.87	34.75±1.24	18.95±0.55	2.41±0.08
Nagaon	42.22±0.79	34.81±0.98	18.13±1.21	2.37±0.72
Kamrup	44.17±1.65	34.72±0.56	17.25±0.82	2.32±0.54
Jorhat	43.36±0.49	34.69±1.95	18.35±0.68	2.40±0.88
Golaghat	44.24±2.01	34.66±1.68	17.53±1.05	2.36±0.37
Diphu	43.18±1.55	34.70±0.72	17.82±0.37	2.39±1.04
Lakhimpur	42.70±0.82	34.85±0.45	18.13±0.92	2.35±0.66
Karbi-Anglong	44.21±0.68	34.68±1.75	17.25±0.58	2.38±0.46
Mean ± SD	43.20±0.99	34.73±0.06	7.92±0.58	2.37±0.02

Data are expressed as mean ± SD (n=3)

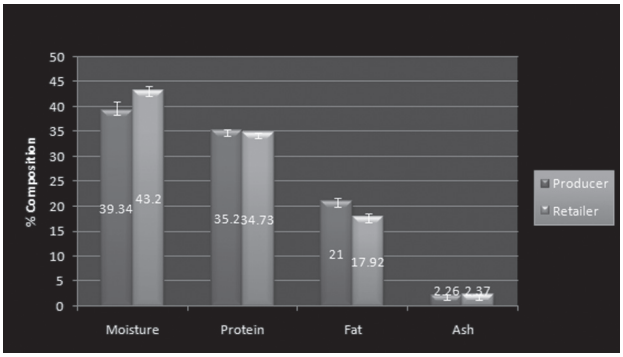

Fig. 1. Proximate composition (mean) of shidal collected from producers and retailers

(p>0.05) in proximate composition was observed among the samples collected either from producers or retailers inf different districts.

Biochemical parameters

The quality of the traditionally fermented fish product 'shidal' sampled from producers and retailers was evaluated by determining TVB-N, PV and FFA values and the results are presented in Tables 2a and b. The result showed that TVB-N value varied from 1.15±0.43 to 3.35±0.24 mg 100 g^{-1}, with higher value observed in products obtained from retailers (Fig. 2). The average TVB-N values of producer and retailer samples were, 1.34±0.20 and 2.96±0.35 mg 100 g^{-1} respectively. Peroxide value (PV) varied from 7.4±0.08 to 25.2±0.56 milliequivalent per kg with similar trend as that of TVB-N. The average peroxide values of producer and retailer samples were 9.5±2.95 and 17.52±4.63 milliequivalent per kg respectively (Fig. 3). Tables 2a and b also showed that the FFA content varied from 16.22±0.95 to 27.50±0.98%, with higher value in

Table 2a. TVB-N, PV and FFA values of shidal samples collected from producers in different districts of Assam

District	TVB-N (mg 100 g^{-1})	PV (milli equivalent kg^{-1})	FFA (%)
Morigaon	1.15±0.43	7.4±0.08	16.22±0.95
Nagaon	1.32±0.28	8.3±0.65	19.55±0.76
Karbi-Anglong	1.56±0.09	12.9±0.25	18.40±1.22
Mean ± SD	1.34±0.20	9.5±2.95	18.05±1.69

Data are expressed as mean ± SD (n=3)

Table 2b. TVB-N, PV and FFA values of shidal samples collected from retailers of different districts of Assam

District	TVB-N (mg 100 g^{-1})	PV (milli equivalent kg^{-1})	FFA (%)
Morigaon	2.23±0.56	10.5±0.98	19.74±0.72
Nagaon	2.71±0.05	12.8±0.46	23.15±0.08
Kamrup	3.35±0.24	17.6±1.12	25.32±0.65
Jorhat	3.15±0.76	15.2±0.60	24.25±0.71
Golaghat	2.98±0.92	19.5±0.37	27.50±0.98
Diphu	3.25±0.07	25.2±0.56	22.56±0.08
Lakhimpur	3.05±0.71	18.9±1.02	26.18±0.59
Karbi-Anglong	2.95±0.15	20.5±0.95	21.85±0.66
Mean ± SD	2.96±0.35	17.52±4.63	23.81±2.51

Data are expressed as mean ± SD (n=3)

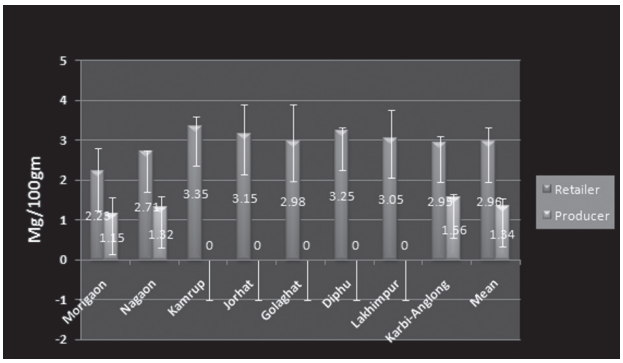

Fig. 2. TVB-N values of shidal collected from producers and retailers in different districts of Assam

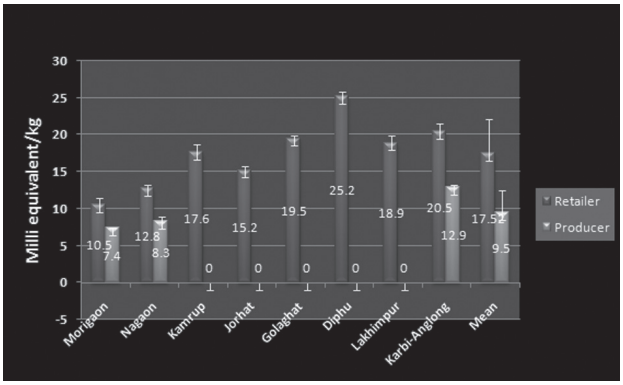

Fig. 3. Peroxide values of shidal collected from producers and retailers in different districts of Assam

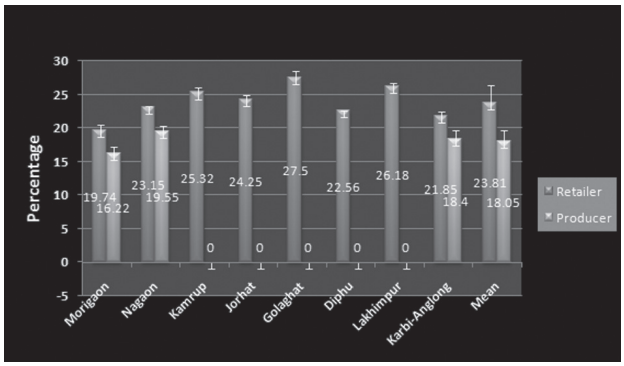

Fig. 4. FFA values of shidal collected from producers and retailers in different districts of Assam

product obtained from retailers. The average FFA values of producer and retailer samples were 18.05±1.69 and 23.81±2.51% respectively (Fig. 4).

Statistically no significant difference was recorded in the quality parameters among the samples collected from producers and retailers in different districts.

Microbiological characteristics

Total plate count (TPC) and individual microbial count (lactic acid bacteria, *Bacillus* spp., *Micrococcus* spp., *Staphylococcus* sp. and yeast) from shidal samples collected from producers and retailers of different districts of Assam are presented in Tables 3a and b as well as Fig. 5.

Table 3a. Microbial count of shidal samples collected from producers in different districts of Assam.

District	Log cfu g^{-1} sample					
	TPC	LAB	*Bacillus* sp.	*Micrococcus* sp.	*Staphylococcus* sp.	Yeast
Morigaon	6.03±0.46	3.99±0.44	2.06±0.39	1.67±0.17	2.37±0.47	2.45±0.48
Nagaon	6.43±0.43	4.46±0.46	2.12±0.42	2.02±0.44	2.75±0.19	2.50±0.46
Karbi-Anglong	6.68±0.12	4.63±0.13	2.06±0.49	2.10±0.45	2.89±0.15	2.42±0.44
Mean ± SD	6.38±0.32	4.36±0.33	2.08±0.03	1.93±0.22	2.67±0.26	2.45±0.04

Data are expressed as mean ± SD (n=3)

Table 3b. Microbial count of shidal samples collected from retailers in different districts of Assam

District	Log cfu g^{-1} sample					
T.P.C	LAB	*Bacillus* sp	*Micrococcus* sp.	*Staphylococcus* sp.	Yeast	
Morigaon	6.65±0.18	4.50±0.14	2.14±0.41	1.88±0.10	2.93±0.06	2.86±0.04
Nagaon	6.80±0.15	4.51±0.49	2.32±0.41	2.10±0.43	3.08±0.34	2.95±0.47
Kamrup	7.29±0.29	4.75±0.17	2.51±0.50	2.10±0.51	3.10±0.39	2.77±0.09
Jorhat	7.34±0.38	4.73±0.11	2.40±0.41	1.80±0.10	3.19±0.40	2.72±0.09
Golaghat	8.08±0.32	5.18±0.47	2.69±0.15	2.10±0.34	3.76±0.15	3.12±0.35
Diphu	7.46±0.18	4.53±0.47	2.17±0.39	1.88±0.06	3.10±0.36	2.75±0.13
Lakhimpur	7.02±0.33	4.74±0.05	2.37±0.45	1.82±0.12	3.37±0.36	2.42±0.38
Karbi-Anglong	7.41±0.39	4.94±0.40	2.42±0.38	2.10±0.31	3.32±0.33	2.73±0.17
Mean ± SD	7.25±0.44	4.73±0.23	2.37±0.17	1.97±0.13	3.23±0.25	2.79±0.20

Data are expressed as mean ± SD (n=3)

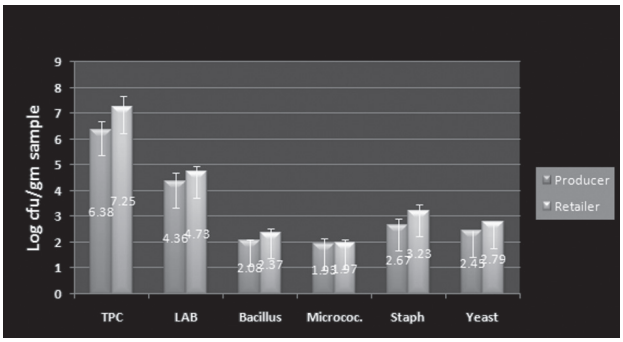

Fig. 5. Microbial count (mean) of shidal collected from producers and retailers

Microbial load (total plate count, TPC) in shidal samples collected from the producers and retailers ranged from 6.03±0.46 to 8.08±0.32 log cfu g^{-1} with comparatively higher load observed in samples collected from retailers. The average TPC from producer and retailer samples were 6.38±0.32 and 7.25±0.44 log cfu g^{1} respectively.

The lactic acid bacterial load ranged from 3.99±0.44 to 5.18±0.47 log cfu g^{1} with average of 4.36±0.33 and 4.73±0.23 log cfu g^{1} for producer and retailer samples respectively. *Staphylococcus* spp. ranged from 2.37±0.47 to 3.76±0.15 log cfu g^{1} with average of 2.67±0.26 and 3.23±0.25 log cfu g^{1} respectively for producer and retailer samples. The average *Bacillus* spp. count recorded were 2.08±0.03 and 2.37±0.17 log cfu g^{1} in producer and retailer samples respectively. Average *Micrococcus* spp. count recorded in producer and retailer samples were 1.93±0.22 and 1.97±0.13 log cfu g^{1} respectively. The average yeast count was 2.45±0.04 and 2.79±0.20 log cfu g^{-1} for producer and retailer samples respectively (Table 3a and b). None of the samples in the present study revealed the presence of coliform bacteria, *E. coli* and *Salmonella* sp.

Discussion

Proximate composition

From the Tables 1a and b, it is evident that moisture content was comparatively higher in samples from retailers than from producers. The higher percentage of moisture content observed in the retailer's sample might be due to the absorption of moisture from the environment during selling and storage, since in most of the places shidal are stored in bamboo basket or plastic bucket under open air where there are chances of absorbing moisture (Mansur, 2007). There is also possibility of adding moisture intentionally or unintentionally by the retailer in order to increase the weight of the product, which can not be denied.

Protein, the most important proximate principle among the chemical composition as well as fat recorded higher values in the samples collected from producers as compared to samples from retailers. Lower level of protein and fat content in the products obtained from retailers could be probably due to the loss of quality at different stages of marketing chain. This result was in agreement with the work reported by Nayeem *et al.* (2010).

Ash content was recorded higher in retailer samples compared to producer samples. The higher ash content noticed in the products sampled from retailers are probably associated with contamination with filth, sand, dust *etc.* which might occur during handling, transportation and preservation in the marketing chain. This observation from the present study are more or less in agreement with the opinion of Khanum *et al.* (1999).

Biochemical parameters

The food qualities of shidal evaluated by determining TVB-N, PV and FFA, indicated higher values in product from retailers as compared to that of producer samples. Similar trend of TVB-N value was also observed by Nayeem *et al.* (2010). The high PV in retailer's sample is due to the oxidation of unsaturated fatty acids of lipid (Achinewhu *et al.*, 2002). Sodium chloride added to the product by retailers in order to repel flies, has been reported to act as a pro-oxidant (Kanner and Kinsella 1983; Connell 1995; Achinewhu *et al.*, 2002). The high value of FFA in retailer's samples might be due to the lipid hydrolysis during storage. Majumdar *et al.* (2006) also observed similar kind of results in salt fermented Indian shad. Although the values of TVB-N, PV and FFA were observed to be higher in retailer's samples which indicate poor quality, the values recorded were within the acceptable limits, as suggested for fishery products (Connell, 1995).

Microbiological characteristics

Comparatively higher microbial load was recorded in samples collected from retailers. The average TPC values recorded in producer and retailer samples were 6.38±0.32 and 7.25±0.44 log cfu g^{-1} respectively. Sarojnalini and Vishwanath (1988) recorded total viable bacterial count of 7.69 log cfu g^{-1} in the fermented product, ngari collected from Imphal market in Manipur. Similar result was also observed by Muzaddadi and Basu (2003b) after 4 months maturation in shidal.

Among the different microorganism isolated from shidal, lactic acid bacteria was found to be predominant in all the samples. LAB species were also reported from other Asian fermented fish products such as nam-plaa and kapi, fermented fish products of Thailand (Tanasupawat *et al.*, 1992).

Population of *Staphylococcus aureus* was less than 4 log cfu g^{-1} in all the samples of shidal, which would be the impact of competition and/or antagonistic reaction of

predominant lactic acid bacteria that have prevented the proliferation (Adams and Nicolaides, 1997). However, the presence of *S. aureus* in fermented fish products could be attributed to contamination during processing. *S. aureus* is regarded as a poor competitor and its growth in fermented foods is generally associated with failure of the normal microflora (Nychas and Arkoudelos, 1990). Since the *S. aureus* count was less than 4 log cfu g^{-1} , the possibility of *S. aureus* food poisoning resulting from consumption of this product is ruled out (Garbutt, 1997). According to Bergdoll (1979), *Staphylococcus* count exceeding to 6.0 log cfu g^{-1} is considered to be hazardous. However, in the present investigation, the *Staphylococcus* count was far below the above value. Thapa *et al*. (2004) also observed *S. aureus* count in the range, 2.5 - 3.5 log cfu g^{-1} in Ngari from Manipur. Though there has been no reported case of toxicity or illness due to consumption of fermented fish products in Assam, gross contamination of the products by *S. aureus* in the region suggests that the products need to be investigated clinically.

Though the load of *Bacillus* spp. was around 10^2 cfu g^{-1} in retailer and producer samples, their presence shows the dominance next to LAB and *S. aureus* in fish products. *Bacillus* species were found in the fish products, due to their ability for endospore formation to survive under the prevailing conditions (Crisan and Sands, 1975). Similar load of *Bacillus* spp. was also observed by Thapa *et al*. (2004) in ngari sampled from Manipur.

As recorded in shidal in the present study, *Micrococcus* sp. were also reported from some fermented fish products of Thailand (Phithakpol, 1993) and Japan (Wu *et al*., 2000). The presence of *Bacillus* and *Micrococcus* species suggested that spore forming bacilli as well as non-spore forming cocci might play an active role during fermentation (Sarojnalini and Suchitra, 2009). Perez-Villarreal and Pozo (1992) also observed that during ripening of salted anchovy, the microflora was dominated by halophilic and halotolerent bacteria like micrococcaceae, lactic acid bacteria and some moulds and yeasts.

As observed in the present study, yeast (*Candida* sp.) was also reported from nam-plaa and kapi (Watanaputi *et al.,* 1983). Thapa *et al.* (2004) also observed yeast count in the range 2.8-3.3 log cfu g^{-1} sample in ngari in Manipur comprising *Candida* sp. and *Saccharomycopsis* sp.. Sarojnalini and Suchitra (2009) recorded total plate count of 3.28 log cfu g^{-1} of molds in fermented *Setipinna* sp.

The microbial count revealed comparatively higher load in products from retailers. From the Tables 3a and b, it was also observed that the *Staphylococcus* sp. count is atleast one log cycle lower than the LAB count in all the samples.. The load of LAB was higher than that of other microorganisms in all the producers as well as retailer's samples. Significant difference ($p<0.01$) was noticed in the microbial count among the samples collected from producers of different districts. The method of preparation, handling and environmental factors might have been responsible for the observed difference in microbial load.

From the experimental study, it was also observed that none of the samples revealed the presence of coliform bacteria, *E. coli*, and *Salmonella* sp., which indicate that the products are safe for consumption. Sarojnalini *et al.* (2009) also did not detect the presence of coliforms, *E. coli*, and *Salmonella* sp. in the fermented *Setipinna* sp.

From the preceding discussion, it can be concluded that quality of shidal was in acceptable condition in producer's sample and the quality was comparatively poor in the retailer's sample, which is presumably due to lack of adequate knowledge about the quality aspect among the retailers, lack of proper packaging system and poor marketing conditions. The hygienic conditions are far from satisfactory with retailers as well as in manufacturing units. The product is often sold prior to proper maturation. Moreover, the retailer's samples were of unknown history and could have been improperly handled during marketing. There should be emphasis on the proper packing of the products and on quality awareness among the retailers and producers. Nevertheless, an urgent scientific as well as feasibility study for these products is the need of the hour.

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