Fishery Technology 55 (2018) : 262 - 269

Assessment of Potential Hazards in *Shidal*, an Ethnic Fermented Fish Product of North-East India

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Abstract

Fermented fish products are very popular in North-East region of India because of its unique taste and flavour. Two popular products, *puthi shidal* and *phasa shidal,* from Tripura were analyzed for biogenic amines, toxic heavy metals and foodborne micro-organisms of public health significance *viz., Salmonella*, *E. coli* and *Staphylococcus aureus*. Biogenic amines were detected by HPLC with UV-VIS detector using gradient elution method. Highest concentration of histamine, cadaverine and putrescine were detected in *puthi shidal* collected from Nutanbazar with a value of 118.18±1.78 ppm, 380.26±2.15 ppm and 141.87±1.20 ppm respectively. Spermidine and spermine were detected to be in the range of 21.38±0.88 - 41.89±0.83 ppm and 47.99±0.65 - 124.50±0.87 ppm respectively which was comparatively at lower concentration. Arsenic (As), cadmium (Cd) mercury (Hg) and lead (Pb) were determined using ICP-OES. The concentrations of heavy metals detected were within maximum permissible limit. Arsenic concentration of 2.65±0.02 ppm was recorded as maximum level in *puthi shidal* from Gulbazar (West Tripura district). *Shidal* samples are found to be free from pathogen like *Salmonella* and *E. coli*. The study indicates that the fermented fish products are free from heavy metal contaminations. Pathogen free fermented fish products are safe to consume. The study further revealed the need for standard production method in order to achieve uniform sensory attributes in the products including the control on histamine formation.

Keywords: *Shidal*, biogenic amines, heavy metals, pathogens, North-East India

Received 12 July 2018; Revised 04 October 2018; Accepted 04 October 2018

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Introduction

Fermented fish products are important ethnic food commodities which combat protein malnutrition by serving as a regular source of animal protein for the people of North-East India (NEI). *Shidal* is one of the most consumed fermented fish products in NEI being considered as a health food with numerous physiological benefits. There are approximately 5000 varieties of fermented foods prepared and consumed by billions of people belonging to different communities and ethnicities across the worldwide (Tamang & Kailasapathy, 2010). It is estimated that approximately 50 - 400 g per capita of fermented foods is consumed daily worldwide, representing 5 - 40% of the total daily food intake (Tamang & Kailasapathy, 2010). Fermented fish products are found to be rich source of nutrients, having balanced amino acid and fatty acid profile. Fermentation process enhances nutritive value, destroys undesirable components, imparts antioxidant property, helps to produce antimicrobial compounds and improves flavor of some foods (Paredes-Lopez & Harry, 1988). According to Shah (2001) fermented food imparts physiological benefit, helps to prevent disease and improves mental health by way of acting as functional ingredient. Some of the major fermented fish products in NEI are *ngari* & *hentak* in Manipur, *tungtap* in Meghalaya, *puthi shidal*, *phasa shidal* & *lona ilish* in Tripura, *nghaum*, *nghathu* & *dan pui thu* in Mizoram, *ngyii papi* in Arunachal Pradesh, *seedal* in Assam (Thapa et al. 2004; 2006; 2007).

Biogenic amines are chemical hazards with low molecular weight produced by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones (Askar & Treptow, 1986). Presence of harmful biogenic amines and heavy metals may deteriorate the health. Generally, *shidal* is nutritive and safe to consume but use of spoiled

raw material, unhygienic preparation, cross contamination and unfavorable storage environment may favour growth of foodborne microorganisms of public health significance and degradation of nutrient quality. Presence of high biogenic amine content is an indication of low quality products. There is a good probability that *shidal* may be encountered with a potential hazard due to any of the above reasons.

Fermented fish products (fish sauce and fish paste) are known to contain high concentration of histamine (Fardiaz & Markakis, 1979) and a level of 430 ppm in *nampla* and 1380 ppm was detected in Korean anchovy sauce (Sanceda et al., 1996). While, 721 to 757 ppm was reported in anchovy fish sauce in Germany by Kirschbaum et al. (2000). The wholesomeness is affected by presence of undesirable harmful microbes or fungus. Uncontrolled growth of harmful microbes in fermented fishery products results in production of various undesirable chemicals affecting organoleptic acceptability and in turn compromises the safety of the product (Abraham et.al., 1993). High chance of microbial contamination exists during the process of fermented fish production.

Presence of heavy metals above the maximum permissible limits in a processed food is a potential chemical hazard. Arsenic content in the water from three districts (West Tripura, Dhalai and North Tripura) of Tripura was determined by Bhattacharjee & Goswami (2018). Arsenic concentration of 191 ppm in Jirania (West Tripura); 65-444 ppm in Salema, Halhali, Halhooli, Kamalpur and Joynagar (Dhalai district) and 122-283 ppm Sanitala, Rajbari and Dharmanagar (North Tripura) were reported. Use of water ladden with high concentration of heavy metals in the production of fermented fish is also a source of hazard.

Even though several scattered studies on *shidal* for proximate composition, nutritive value, effect of temperature, use of starter culture and microbial diversity exist, no research work has been undertaken on assessment of chemical hazards *viz.,* heavy metals and biogenic amines and biological hazards *viz.,* pathogenic bacteria in *shidal* from different markets of Tripura. Thus, the study was aimed at assessing the potential hazards in *shidal* from different dry fish markets of Tripura, India, and has direct relevance to public health.

Materials and Methods

Puthi shidal and *phasa shidal* were collected from five different dry fish markets of Tripura (Table 1). Collected samples were packed aseptically in polythene pouches and brought to the laboratory for determination of biogenic amines, heavy metals and pathogenic bacteria.

Sensory evaluation of *shidal* samples was carried out by 9-point hedonic scale (Jones et al., 1955). Biogenic amines were analyzed according to the method of O¨zogul et al. (2002) by using HPLC (Shimadzu prominence UV-VIS detector, Japan).

Heavy metals such as arsenic, cadmium, lead and mercury were analyzed using inductively coupled

Table 1. Source of s*hidal* samples analyzed in the study

Sl. No.	Market	Fermented fish product	No. of samples	Geographical coordinates
1.	Gulbazar	*i) Puthi shidal*	3	23.8315° N, 91.2868° E
		ii) Phasa shidal	3	
2.	Teliamura bazar	*i) Puthi shidal*	3	23.8411° N, 91.6277° E
		ii) Phasa shidal	3	
3.	Belonia bazar	*i) Puthi shidal*	3	23.2505° N, 91.4676° E
		ii) Phasa shidal	3	
4.	Udaipur bazar	*i) Puthi shidal*	3	23.5360° N, 91.4870° E
		ii) Phasa shidal	3	
5.	Nutanbazar	*i) Puthi shidal*	3	23.4231° N, 91.7563° E
		ii) Phasa shidal	3	
		Total no. of samples	30	

plasma (iCAP 6000 Series, ICP Spectrophotometer, England, ICP- Optical Emission Spectrometer) according to AOAC (2012). The software used for the analysis was iTEVA.

Aerobic plate count (APC), *Staphylococcus aureus* and *Salmonella* were determined using standard methods (FDA, 2001; Andrews et al., 2007). Coliforms & *E. coli*; and Yeast & mould (YM) count were determined by following the method described by Matner et al. (1990) and Beuchat et al. (1990) respectively.

Statistical analyses of data were carried out using statistical package for social science software (IBM SPSS Statistics 20.0, Chicago IL. USA). Analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) was carried out to test significant difference between different market samples. The level of significance was considered at 5%.

Results and Discussion

The results of the sensory scores are presented in Fig. 1. The results showed higher sensory scores for *puthi shidal* indicating higher degree of acceptability of the products by the panelist, (Max. overall score of 8.55± 0.29, Teliamura bazar) except for the sample procured from Udaipur bazar. While *phasa shidal* got maximum overall score of 8.05±0.31 from Nutanbazar. In *puthi shidal*, the sensory parameters were found to have least differences in scores between the samples from different markets except Gulbazar. *Phasa shidal* from Gulbazar and Nutanbazar showed higher difference between the sensory parameters indicating inconsistency of the products. Both *puthi and phasa shidal* from Udaipur bazar got least overall scores, 6.55±0.32 and 6.65±0.24 respectively amongst the five markets indicating poor sensory attributes of the products. The reason behind the higher acceptability for *puthi shidal* as compared to *phasa shidal* might be due to special preference for *puthi* by the panels, because *phasa shidal* are treated as a substitute to *puthi shidal* by many consumers.

Biogenic amine content in *puthi shidal* and *phasa shidal* from different dry fish markets in Tripura is presented in Table 2. Representative profile of biogenic amines in *puthi shidal* and *phasa shidal* were illustrated in Fig. 2 and Fig. 3 respectively. Both the *shidal* samples showed high cadaverine content (380.26±2.15 ppm in *puthi shidal* and 346.53±1.98 ppm in *phasa shidal*) irrespective of the origin of production. However, *puthi shidal* samples showed higher content of all the amines detected during the study. Histamine content was detected in higher concentration in *puthi shidal* (118.80±1.78 ppm) compared to *phasa shidal* (98.53±0.98 ppm). This could be due to higher percentage of histidine (2.0±0.5 g 100 g^{-1} of protein) in fresh *Puntius* fish compared to fresh *Setipinna phasa* (1.5±0.2g/100g of protein) (Kakati & Goswami, 2017; 2018). It may also be due to presence of higher histamine formers in *puthi shidal*. There is also a possibility of formation of derivatives of amino acids such as amines and gluconeogenic substances during fermentation (Majumdar et al., 2015). However, spermidine and spermine were detected to be in the range of 21.38±0.88-41.89±0.83 ppm and 47.99±0.65-124.50±0.87 ppm respectively. Spermidine was observed to be the lowest amine in both the *shidal* samples. Mah et al. (2002) had reported with

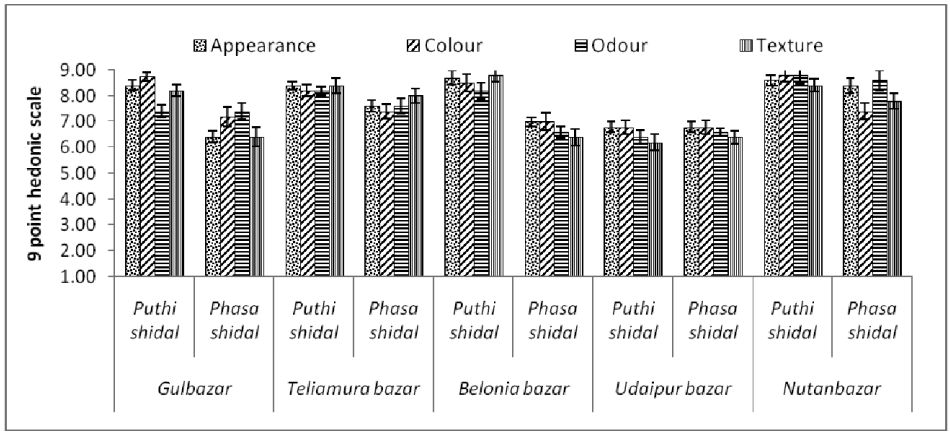

Fig. 1. Sensory scores of *puthi shidal* and *phasa shidal* from five different dry fish markets in Tripura

Table 2. Biogenic amines contents (ppm) in *puthi shidal* and *phasa shidal* from different dry fish markets in Tripura

Biogenic amines (ppm)	Gulbazar		Teliamura bazar		Belonia bazar		Udaipur bazar		Nutanbazar	
	Puthi shidal	*Phasa shidal*	*Puthi shidal*	*Phasa shidal*	*Puthi shidal*	*Phasa shidal*	*Puthi shidal*	*Phasa shidal*	*Puthi shidal*	*Phasa shidal*
Putrescine	130.80 ±1.10	98.93 ±1.01	119.57 ±0.87	95.50 ±1.03	116.29 ±1.22	115.17 ±1.05	134.93 ±1.30	90.93 ±0.91	141.87 ±1.20	88.93 ±1.30
Cadaverine	357.00 ±1.87	346.53 ±1.98	385.24 ±2.8	294.37 ±2.11	340.88 ±1.65	287.92 ±1.96	377.26 ±2.15	329.74 ±2.64	380.26 ±2.15	312.55 ±2.01
Spermidine	41.89 ±0.83	29.49 ±0.77	30.07 ±0.86	28.02 ±0.92	36.06 ±0.91	28.79 ±0.98	32.46 ±0.58	24.22 ±0.92	29.22 ±0.92	21.38 ±0.88
Spermine	124.50 ±0.87	112.75 ±1.23	119.75 ±1.22	75.58 ±1.08	108.18 ±1.25	47.99 ±0.65	77.96 ±1.27	69.46 ±1.76	84.46 ±1.76	67.09 ±1.87
Histamine	115.48 ±1.27	95.67 ±1.54	105.08 ±1.06	98.53 ±0.98	110.29 ±1.06	95.97 ±1.86	105.25 ±2.01	98.50 ±1.78	118.80 ±1.78	98.18 ±1.22

(n=3, Mean±SD)

spermidine and spermine contents of 43 and 77 mg kg^{-1} and higher level of cadaverine content (665 mg kg^{-1}) in myeolchi-jeot (Korean fermented fish). The same author had reported histamine, tyramine and putrescine reaching to 155–579 mg kg^{-1}, 63–244 mg kg^{-1} and 92–241 mg kg^{-1}, respectively.

Ingestion of histamine with concentration ranging from 8 - 40 mg, 40 - 100 mg or more than 100 mg at a time may cause slight, intermediate and severe poisoning respectively. Histamine poisoning will occur in a healthy individual only when a dose of at least 50 mg histamine is ingested and it can happen only when the fish / fishery product is having a histamine level of more than 200 ppm (Parente et al., 2001). In the present study it was noticed that histamine level in both the *shidal* samples were below 200 ppm and within the acceptable limit set by various regulatory authorities including Food Safety and Standard Authority of India (FSSAI). Moreover, *shidal* were mostly consumed as condiment with little quantity at a time. This type of *shidal* consumption habit might have prevented the occurrence of histamine poisoning amongst the *shidal* consumers. Brilliantes & Samosorn (2001) reported histamine content in 549 commercial fish sauces of Thailand in the range of 200-600 ppm which were at higher level compared to the current

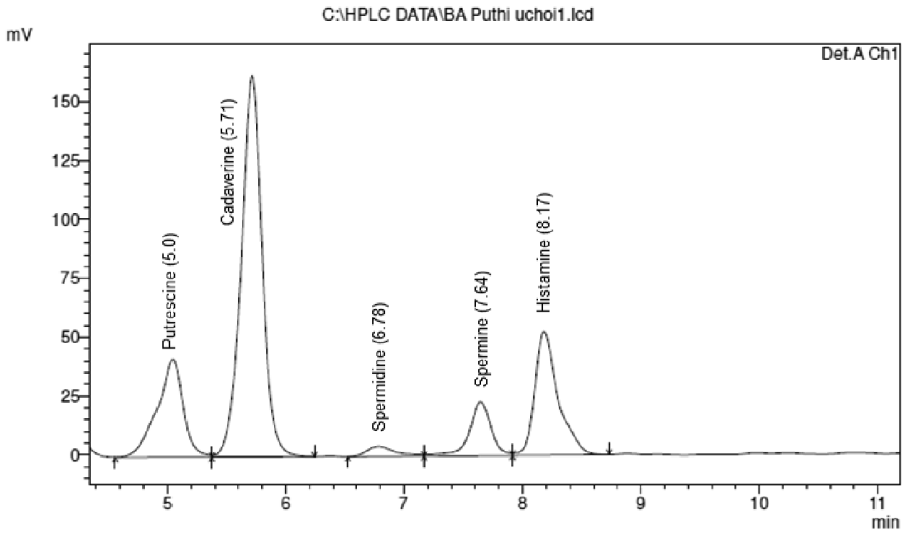

Fig. 2. HPLC chromatogram of biogenic amines in *puthi shidal*

study. However, the Canadian Fish Inspection Agency and US Food and Drug Administration had set up the maximum limit for histamine in fish sauce at 200 ppm and 500 ppm, respectively.

Histamine is the most extensively studied amine in fishery products, and is the only amine with the established permissible limits for the human consumption (Prester, 2011). FSSAI has set a limit for histamine content in dried, cured and fermented fishery products as 200 mg kg^{-1}. Histamine levels above 500 mg kg^{-1} are considered toxic and dangerous for human health (Ten Brink et al., 1990). Biogenic amines at considerably lower level in food may pose insignificant hazard as the amine oxidase (mono amine oxidase and diamine oxidase) in human gut can detoxify these amine. (Biji et al., 2016). Presence of histamine and other biogenic amines in fermented fish products at higher concentration could also indicate over fermentation or advanced stage of microbial contamination. Askar & Treptow (1993) observed that, histamine, spermine, spermidine, putrescine and cadaverine content in the myeolchi-jeot increased during storage. Karmas (1981) investigated fish sauces for biogenic amines which showed high amounts of tyramine,

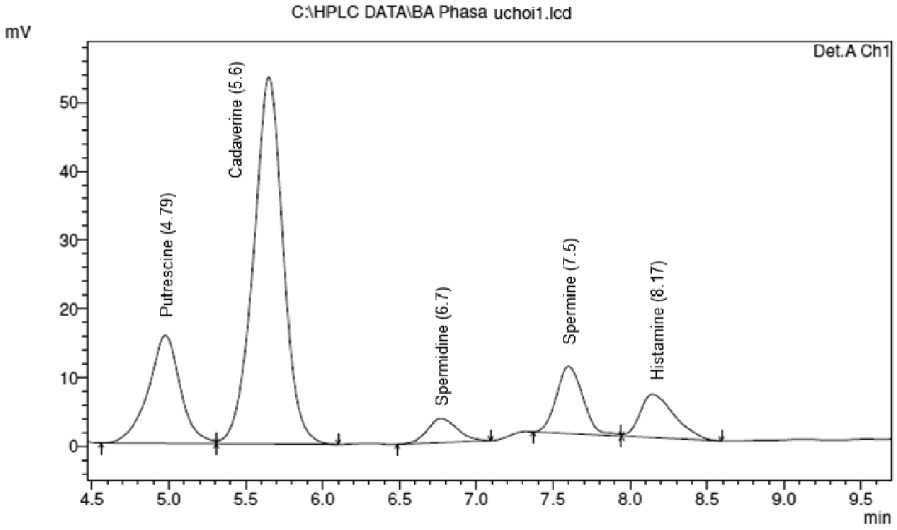

Fig. 3. HPLC chromatogram of biogenic amines in *phasa shidal*

Table 3. Mineral content (ppm) in *puthi shidal* and *phasa shidal* from different dry fish markets in Tripura

Heavy metals (ppm)	Gulbazar		Teliamura bazar		Belonia bazar		Udaipur bazar		Nutanbazar	
	Puthi shidal	*Phasa shidal*	*Puthi shidal*	*Phasa shidal*	*Puthi shidal*	*Phasa shidal*	*Puthi shidal*	*Phasa shidal*	*Puthi shidal*	*Phasa shidal*
Arsenic (As)	2.65 ±0.02^{E}	1.98 ±0.02^{D}	2.20 ±0.01^{A}	2.51 ±0.03^{E}	0.52 ±0.01^{A}	1.53 ±0.05^{C}	0.53 ±0.01^{A}	0.53 ±0.02^{A}	0.54 ±0.02^{A}	0.79 ±0.02^{B}
Cadmium (Cd)	0.05 ±0.00^{A}	0.05 ±0.00^{A}	0.05 ±0.00^{A}	0.09 ±0.00^{C}	0.05 ±0.00^{A}	0.12 ±0.00^{D}	0.06 ±0.00^{AB}	0.05 ±0.01^{A}	0.05 ±0.00^{AB}	0.07 ±0.00
Lead (Pb)	0.04 ±0.01^{C}	0.02 ±0.01^{A}	0.05 ±0.01^{C}	0.01 ±0.00^{A}	0.03 ±0.02^{B}	0.03 ±0.00^{B}	0.03 ±0.02^{B}	0.03 ±0.01^{B}	0.03 ±0.00^{B}	0.04 ±0.02^{C}
Mercury (Hg)	0.11 ±0.04^{A}	0.16 ±0.04^{B}	0.13 ±0.05^{A}	0.19 ±0.05^{B}	0.12 ±0.04^{A}	0.13 ±0.06^{A}	0.18 ±0.04^{B}	0.17 ±0.04^{B}	0.10 ±0.05^{A}	0.10 ±0.04^{A}

$^{A-E}$ Different capital letters with mean value indicates difference between different fermented fish products (Rows). Values in parentheses indicate the standard error. Means in a row with the different superscript letters are significantly different ($p<0.05$), (n=3, Mean±SD)

Table 4. Aerobic plate count (APC) and health significance micro-organisms load in *puthi shidal* and *phasa shidal* from different dry fish markets in Tripura

Parameters	Gulbazar		Teliamura bazar		Belonia bazar		Udaipur bazar		Nutanbazar	
	Puthi shidal	*Phasa shidal*	*Puthi shidal*	*Phasa shidal*	*Puthi shidal*	*Phasa shidal*	*Puthi shidal*	*Phasa shidal*	*Puthi shidal*	*Phasa shidal*
APC (log cfu/g)	6.94 ±0.23	6.91 ±0.12	6.46 ±0.84	6.63 ±0.88	6.47 ±0.06	6.38 ±0.15	7.23 ±0.17	7.75 ±0.13	6.18 ±0.17	6.14 ±0.09
Coliforms & *E. coli*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S. aureus (log cfu/g)	1.8±0.17	1.6±0.23	1.8±0.28	2.01±0.38	1.5±0.21	1.42±0.47	1.68±0.55	2.0±0.74	2.01±0.76	2.20±0.82
Salmonella	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND\
YM	1.12±0.24	1.26±0.21	1.35±0.32	1.20±0.08	1.33±0.09	1.45±0.23	1.25±0.44	1.04±0.47	1.42±0.36	1.48±0.74

APC- Aerobic plate count; YM- yeast & mold; ND- Not Detected; (n=3, Mean±SD)

cadaverine, putrescine and histamine (BAs all exceeding 100 mg L^{-1}). The high amounts of putrescine, cadaverine and tyramine are indicators for very long storage or spoilage of the products (Karmas, 1981). Generally, people of North-East India consume fermented fish products on daily basis and there is very little chance for longer storage period.

Mineral contents of *puthi shidal* and *phasa shidal* are presented in Table 3. The samples were analyzed for arsenic, cadmium, lead and mercury content. Minerals such as iron, copper, cobalt and zinc were essential nutrients and were harmless but the former four heavy metals were poisonous and harmful. Hence, the focus was kept on these four elements as potential hazards. Arsenic concentration of 2.65±0.02 ppm in *puthi shidal* sample from Gulbazar was recorded as maximum level amongst the entire sample analyzed during the study. And the sample belonged to *puthi shidal* from Gulbazar (West Tripura district). Bhattacharjee & Goswami (2018) reported three districts (West Tripura, Dhalai and North Tripura) of Tripura with high arsenic contamination. But the values found in the samples were much below the level of maximum limit (76 ppm) set by FSSAI (2011). Therefore, the results suggested that the samples from the selected markets were comparably nil in arsenic. Cadmium, lead and mercury content of the samples were detected less than 0.13 ppm, 0.06 ppm and 0.2 ppm respectively; which were within the standard set by FSSAI for fish which were 0.3 ppm, 0.3 and 0.5 ppm for Cd, Pb and Hg [FSSAI, Article no. 2.1.1 (1), 2011] respectively.

Microbial analysis of *puthi shidal* and *phasa shidal* from different dry fish markets are furnished in Table 4. *Phasa shidal* sample from Udaipur bazar with a value of 7.75±0.13 log cfu g^{-1} was recorded as the highest APC followed by *puthi shidal* from same market with a value of 7.23±0.17 log cfu g^{-1}. The remaining samples were in the range of 6.14±0.09 to 6.94±0.23 log cfu g^{-1}. The higher microbial loads in the products were reasonably due to prolong subjecting of the products under exposed condition to open air and unhygienic handling during the process of retailing. Comparable results of microbial load for different fermented fish products were reported by Anihouvi et al. (2006) and Roy et al. (2014).

Staphylococcus aureus count was highest in *phasa shidal* of Nutanbazar with a value of 2.20±0.82 log cfu g^{-1}. Majumdar et al. (2015) and Rapsang & Joshi (2012) reported presence of *Staphylococcus aureus* in *ngari, hentak* and *tungtap*. Muzaddadi & Basu (2003) reported presence of *S. aureus*, *Micrococcus* spp., *Bacillus* spp. and *Escherichia coli* in *shidal*. In *puthi seedal* of Assam, Kakati et al., (2018) reported *S. aureus*, *Streptococcus* spp. and *E. coli* count as 2.4 log cfu g^{-1}, 1.10 log cfu g^{-1} and less than 1.0 log cfu g^{-1} respectively; he further reported absence of *Salmonella* in the samples analysed. The presence of *S. aureus, Streptococcus* spp. and *E. coli* in fermented fish products might be attributed to poor handling practices and faecal contamination during processing and storage (ICMSF, 1996). The growth of *Staphylococcus* in the product is a potential health hazard since many strains can produce enterotoxins, which cause food poisoning if ingested. Barber

& Deibel (1972) reported the incidence of *Staphylococcus* food poisoning associated with fermented food causing gastroenteritis in humans. But in the present study *Salmonella* and *E. coli* were not detected. From this result, it can be concluded that the products were free from faecal contamination or they were handled with poor hygiene.

Yeast and mold count were found to be low, accounting for less than 1.5 log cfu g^{-1} in the entire samples analyzed. Similar result was also obtained in *puthi seedal* of Assam by Kakati et al. (2018). The contamination of *shidal* with pathogenic bacteria can be minimized by application of Good Manufacturing Practice (GMP) and Sanitation Standard Operating Procedure (SSOP) in the production of *shidal*.

In conclusion, the current study enabled us to identify the harmful potential hazards associated in *shidal* of which histamine is the most concern. High level of cadaverine content might accelerate the toxicity effect of histamine when consumed by histamine intolerant persons. In Indian scenario, most people do not report incidence of food poisoning due to lack of knowledge about the symptoms or failure to recall the identity of food consumed. *Shidals* are usually consumed in small amounts as side dish prepared with many vegetables, so 100-120 ppm concentrations of histamine could possibly be tolerated by the consumers without significant negative effect on their health. Toxic heavy metals were detected at much lower level than the maximum permissible limit recommended by FSSAI. All the samples analyzed were found to be free from pathogens like *Salmonella* and *E. coli* except for *S. aureus*, which is a poor hygiene indicator. Further investigation of potential hazards in other popular fermented fish products are required for quality assurance in ethnic fermented fish products of North-East India.

Acknowledgements

The authors are grateful to The Director, ICAR-Central Institute of Fisheries Technology, Cochin, for providing the facilities to carry out the research work. The authors are also very grateful to The Director, ICAR-Central Institute of Fisheries Education, Mumbai, for permitting to carry out work at ICAR-CIFT, Cochin.

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