Chemical and microbial properties of *shidal*, a traditional fermented fish of Northeast India

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Abstract Like Southeast Asian countries fermented fish is a well known strategy of food preservation in the Northeast region of India. *Shidals* are mostly preferred salt-free fermented fish product amongst all. Chemical and microbial composition, antioxidative potential, fatty acid profile and proteins profile in gel electrophoresis of *shidals* were studied. pH and total titratable acidity (TTA) have been found as 5.86 ± 0.11 , 0.115 ± 0.01 and 6.62 ± 0.07 , 0.092 ± 0.01 in *punti* and *phasa shidal* respectively. DPPH[•] radical scavenging activity of *punti* and *phasa shidal* was determined as 80.15 ± 5.67 and 68.30 ± 3.22 respectively. Presence of eicosapentaenoic, docosahexaenoic, arachidonic, linolenic and linoleic acid indicate the nutritional significance of *shidal*. However, the result showed that *punti shidal* was rich in omega-3 but poor in omega-6 fatty acid, whereas, reverse was observed in respect of *phasa shidal*. Poly acrylamide gel electrophoretic study of protein revealed disappearance of myosin head chain (MHC) in the dry *punti* fish (raw material of *shidal*). Proteins or peptides with low molecular weight between 45 and 29 kDa and between 45 and 66 kDa were noticed in both the *shidals* and indicative of intensive protein degradation during fermentation. Therefore, fermented fish product, *shidal* could be used as a potential source of nutrients and natural antioxidants.

Keywords Shidal · Fermented fish · Northeast India · Antioxidants · SDS-PAGE · Fatty acid profile

Introduction

Fermentation offers a wealth of possibilities and play an important role in improvement of nutritional and functional properties of food. Cleavage of food proteins by microbial or indigenous proteases yield the bioactive peptides, leading to substantial increase in the biological properties of the food (Steinkraus 2002). The fermented foods have been found to possess therapeutic properties. The WHO food safety unit has given high priority for the research in food fermentation, as it will improve the food safety by controlling the growth and activity of pathogens in foods. Moreover, fermented food products are a good source of peptides and amino acids (Rajapakse et al. 2005; Sathivel et al. 2003). Amongst indigenous fermented products, fermented fishery products have been widely consumed in Southeast Asia as main dishes or condiments, due to their delicacy and high nutritional properties.

Shidal is a salt-free fermented fish product indigenous to the Northeast sector of India. It is very much popular due to its strong flavour. Dried salt-free *Puntius* spp. and *phasa* fish (*Setipinna phasa*) are usually used for preparation of *shidal*. The appearance of the product is solid, bilaterally compressed and pasty and shape of the fish remains almost unchanged except little disintegration near belly and caudal portion. The colour of best quality product is dull white that gradually becomes slight brownish to deep brownish on continuous exposure to air. The strong odour permeates the air in and around the storage and gives the area a characteristic smell of *shidal*. Traditional method of production of *shidal* is given in Fig. 1.

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Fig. 1 Traditional method of *shidal* preparation

Much attention has been focused on the use of antioxidants, especially natural antioxidants, to inhibit lipid peroxidation and to protect bio-molecules from damage by free radicals (Haliwell et al. 1995). Proteins and peptides from food have been found to be physiologically active or bioactive. Many peptides that are released in vitro or in vivo from animal or plant proteins have regulatory functions in the human body, apart from serving as important nutrients. Enzymatic fermentation of small fish and shrimp mediated by indigenous proteases yields short chain peptides and free amino acids, rendering the typical flavour and taste. Antioxidative activities associated with enzymatic hydrolysis or fermentation of marine animals has been reported. An antioxidative peptide with molecular weight (MW) of 962 kDa from fermented blue mussel exhibited strong scavenging effects towards superoxide, hydroxyl and DPPH radicals (Rajapakse et al. 2005). Je et al. (2007) reported antioxidant peptide (1519 Da), which had a capability of quenching free radicals, including superoxide, hydroxyl and DPPH radicals from tuna backbone proteins hydrolysed by pepsin. Hydrolysate from silver carp using alcalase or flavourzyme was reported to show hydroxyl radical-scavenging activity and metal chelating activity (Dong et al. 2008). Additionally, *Mungoong*, a paste prepared from the cephalothorax of white shrimp, contained peptides which acted as an effective antioxidant with high stability over a wide pH and temperature range (Binsan et al. 2008).

Apart from serving as a good source of proteins and bioactive compounds, fermented fish products might possess bioactivities, especially natural antioxidants, which provide

health benefits. The activity of proteolytic enzymes present in the raw material of fermented products is considered to be a primary factor of protein hydrolysis. The proteolytic products such as amino acids, peptides and their decomposition products are formed during fermentation process (Kiesvaara 1975). Fermented food products are, therefore, good sources of peptides and amino acids. Many peptides released from food proteins exhibit biological activities, such as antimicrobial properties, blood-pressure lowering effects, cholesterol-lowering ability, antithrombotic and antioxidative activities (Hartmann and Meisel 2007). Accordingly, these products could be marketed as health foods with high market value. Besides proximate composition and dominant microbes, no information regarding the lipid profile and bioactivity of fermented fishery products, i. e. *shidals* have been reported. Also, there was no report on detailed characterization of dominant microbes. Therefore, the objective of this study was to scale up nutrient profile of *shidals*, a fermented product from *Punti* or *Phasa* fish, produced exclusively in Northeast India.

Materials and methods

Chemicals

Trichloroacetic acid (TCA), sulphuric acid (H₂SO₄), hydrochloric acid (HCl) potassium ferric cyanide, ferric chloride and sodium hydroxide (NaOH) was procured from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India. 2, 2-diphenyl-1-picrylhydrazyl (DPPH), tris-HCl, thio-barbituric acid (TBA), acrylamide, bis-acrylamide, Luria Bertani broth (LB broth), nutrient agar, De Man Rogosa and Sharpe (MRS) agar and Mueller Hinton (MH) agar were procured from Himedia Laboratories, Mumbai, India.

Samples

Shidal samples produced from *punti* fish (*Puntius sophore*) and *phasa* fish (*Setipinna phasa*) were purchased from different producers in local markets of Agartala, Tripura, India. All the samples were packaged in sterile polypouches and aseptically brought to the laboratory for chemical and microbial analysis.

Preparation of sample extract from *shidal*

Punti and *phasa shidal* sample (5 g) was mixed with distilled water (25 ml) and the mixture was homogenised at a speed of 12,000 rpm for 1 min. The mixture was shaken at 110 rpm on an orbital shaker for 40 min at room temperature. The mixtures were then centrifuged at 7000 × g for 15 min at 4 °C to remove undesired debris. The collected supernatant was used

for analysis of ferric chloride reducing power assay and antimicrobial assay.

Biochemical analysis

Moisture, ash, crude protein, fat and total titratable acidity (TTA) contents of *shidal* were determined according to AOAC methods (1995). Total volatile basic nitrogen (TVBN) was determined by distillation method (AOAC 1995). Thiobarbituric acid (TBA) value was determined by using the method given by Buege and Aust (1978). Sample was homogenised with TBA-TCA-HCl solution followed by heating and subsequent cooling in tape water. The supernatant was collected and absorbance was taken at 532 nm. The pH of samples were determined by a pH meter (Sartorius, Goettingen, Germany), as described by Benjakul et al. (1997).

DPPH radical-scavenging activity.

Antioxidant activity of the *shidal* samples was determined according to the method of Kulkarni and Aradhya (2005) with slight modification. Sample (1 g) was mixed with 9 ml of 100 mM Tris-HCl buffer (pH 7.4). The mixture was properly mixed and centrifuged at $7000 \times g$ for 10 min at room temperature. A known volume of supernatant was taken and mixed with equal volume of DPPH solution (0.5 mM in ethanol). The mixture was left to stand for 30 min at dark. Absorbance was measured at 517 nm using a UV-spectrophotometer (Shimadzu Corporation, Japan).

Ferric chloride reducing power assay

The reducing powers of the sample from the dialyzed extracts were determined by the method of Oyaizu (1986) and absorbance was taken at different concentrations (25, 50, 75 and 100 μ l). The extract (at different concentrations) was mixed with 0.2 M phosphate buffer pH 6.6 (400 μ l) and 1 % potassium ferric cyanide (400 μ l). The mixture after incubation at 50 °C for 20 min and then mixed with 10 % TCA (400 μ l). The mixture was centrifuged at $3000 \times g$ for 10 min. The upper layer of solution (500 μ l) was collected and mixed with 1 % FeCl_3 (100 μ l). The absorbance was read at 700 nm by using distilled water as blank.

Fatty acid profile analysis.

Total lipid was extracted from muscle tissues of threadfin breams by Folch method and the fatty acid methyl ester (FAME) was prepared from the isolated lipids by heating with the methanolic NaOH first and then with BF_3 methanol for esterification. 5 ml n-heptane was added to recover the methyl esters in organic phase. The mixture was washed with saturated NaCl solution and two phases were separated using a separating funnel. The upper n-heptane phase was pipette out and stored in 10 ml all glass vials until further analysis.

Fatty acid methyl esters were separated by using a Shimadzu QP2010 quadruple Gas Chromatography Mass

Spectrometer (GCMS) Instrument equipped with a Carbowax (30 m $\times$ 0.25 mm ID; 0.25- μ m film thickness) capillary column (Cromlab S.A.). Helium was used as the carrier gas. Injector and detector temperatures were set at 250 °C. Injection was performed in split mode (1:15). The column temperature was programmed initially at 50 °C for 2 min and then increased at a rate of 10 °C per min to a final temperature of 230 °C. FAME was separated at constant pressure (23.1 kpa) and peaks were identified by comparing standard mass spectra with the relative abundances of m/z ranging from 40.00 to 550. The values of fatty acids are presented in area percentage of total identified fatty acids.

Protein profile of *shidal* by SDS-polyacrylamide gel electrophoresis

The protein profile of *punti* and *phasa shidal* were determined by SDS-PAGE method by using 10 % resolving and 5 % stacking gel, according to the method of Laemmli (1970). 3 g of *shidal* samples were solubilised in 27 ml of 5 % SDS (85 °C). The mixture was homogenised for 1 min at a speed of 13,000 rpm using a tissue homogeniser and incubated at 85 °C for 1 h to dissolve total proteins. The dissolved proteins were loaded onto the gel and subjected to electrophoresis at a constant current of 400 mA for 2 h by using a Mini-Pro-tean II unit (Bio-Rad Laboratories Pvt. Ltd., Kolkata, India). After that the separated proteins were stained with 2.5 g of Coomassie Brilliant blue R250 (HiMedia, Mumbai), 450 ml of methanol and 100 ml of acetic acid were mixed, filtered and volume was made to 1 l with distilled water. De-staining of the gel was done by using a de-staining solution (300 ml of methanol and 100 ml of acetic acid mixed with distilled water to 134 make up 1 l.). Photographs of the de-stained and clear gels were taken with gel documentation system (BioRad Laboratories, Kolkata, India).

Microbiological analysis

Shidal sample (10 g) was homogenized with 90 ml of sterile normal saline solution (0.85 % w/v, NaCl) in a stomacher (Seward, UK) and ten-fold dilutions of each sample were prepared, then 0.1 ml of each dilution was spread with a bent sterile glass rod on duplicate plates of pre-poured and dried standard Nutrient agar (Hi-Media, India). After 48-h incubation at 37 ± 1 °C, colonies were counted and results were expressed as $\log_{10}$ cfu/g of sausage sample. Enumeration of lactic acid bacteria (LAB) in the samples was conducted on MRS agar. For enumeration of Lactic acid bacteria the MRS plates were incubated in an anaerobic condition at room temperature for 48–72 h.

The dominant bacterial colonies were randomly selected by their morphological characters from different dilutions of TPC plates. After further purification and isolation the dominant

colonies were bio-chemically characterized. Bacterial genera were identified following the method described in Bergy's Manual (Vol. 2, Sneath 1986). Gram staining was performed from young culture of dominant bacterial groups isolated from *shidal* samples. Oxidation-Fermentation test (O/F-test) and Voges-Proskauer (VP) test was done following the method of Mac Faddin (1980). Catalase test was done by dipping young cultures of bacteria in 3 % v/v hydrogen peroxide solution and formation of bubbles was recorded as positive test. Production of indole from the amino acid tryptophan by the bacteria was tested by growing the bacteria in tryptophan broth for 48–72 h and adding few drops of Kovac's reagent. Oxidase test was done by using oxidase strips. Salt tolerance test was performed by using a basal medium of tryptone and beef extract with different concentration of salt (0, 2, 4, 6 and 8 %). For sugar fermentation test seven different types of sugars (1 % each) were dissolved in nutrient broth solution. After 24–36 h of incubation bromocresol purple was added in each tube and formation of pink colour indicated positive reaction.

Antibiotic sensitivity test of isolated dominant groups of bacteria

Sensitivity was tested against 5 antibiotic discs namely Chloramphenicol (C) (30 mcg), Erythromycin (E) (15 mcg), Norfloxacin (NX) (10 mcg), Co.-Trimoxazole (COT) (25 mcg) and Ofloxacin (OF) (5 mcg). Young culture of bacteria from Luria Bertani broth (LB broth) was swabbed on Mueller Hinton Agar (MH agar) and a disc of particular antibiotic was placed at centre of the plate. The diameter of clear zone was measured after 24 h of incubation.

Statistical analysis

All data was analysed for analysis of variance (ANOVA) using SPSS software version 16.0 (SPSS, USA). Significance of means was determined using the Duncan's Multiple Range Test (DMRT) at a 95 % significant difference ($p < 0.05$).

Results and discussion

Bio-chemical and microbial quality of *punti* and *phasa shidal*

The mean values of proximate composition, biochemical and microbial quality of *shidals*, namely, *punti* and *phasa shidal* is given in Table 1. Low moisture content of '*Shidal*' was due to use of sun-dried fish (moisture content <10 %). *Puntius* belongs to the group of 'semi fatty' fish (fat content usually ranges from 2 to 5 % in breeding season). The variation of the lipid contents of the two *shidal* samples may be attributed

Table 1 Bio-chemical and microbial quality of *punti* and *phasa shidal* ($n = 5$)

Parameters	Punti shidal	Phasa shidal
pH	5.86 ± 0.11	6.62 ± 0.07
TTA (g %)	0.115 ± 0.01	0.092 ± 0.01
Moisture (%)	38.26 ± 0.89	43.48 ± 1.58
Crude protein (%)	36.84 ± 1.42	36.75 ± 1.75
Total lipid (%)	14.30 ± 2.24	7.85 ± 1.26
TVBN (mg %)	62.53 ± 1.61	120.27 ± 1.24
TBA (mg malonaldehyde/kg meat)	0.99 ± 0.06	1.10 ± 0.14
TPC (log cfu/g)	6.87 ± 0.11	6.36 ± 0.01
LAB (log cfu/g)	4.5 ± 0.08	4.8 ± 0.14

to the lipid content of the raw fish. Increased protein and fat content of the product is due to reduction of moisture content. Sarojnalinini and Vishwanath (1988) reported the proximate composition of fermented products of Manipur, i.e., '*Hentak*' and '*Ngari*' as ash (%) 11.43 and 5.49, moisture (%) 36.3 and 36.03, total nitrogen (%) 5.34 and 6.14, total lipid (%) 13.6 and 13.36 respectively.

The pH and total titratable acidity (TTA) have been found as 5.86 ± 0.11, 0.115 ± 0.01 and 6.62 ± 0.07, 0.092 ± 0.01 in *punti* and *phasa shidal* respectively. The pH seems to be slightly higher in respect of other fermented fish products, but this may be due to higher amounts of volatile nitrogenous compounds produced during fermentation that accumulate in the product. Similar pH values were also reported by several authors for different fermented fish products (Thapa and Pal 2007; Majumdar and Basu 2010; Kakati and Goswami 2013; Yatsunami and Takenaka 1996). Unlike salt fermented fish, there is no leaching out of nutrients from the '*Shidal*' product.

The TVBN content of the products was recorded to be 62.53 ± 1.61 and 120.27 ± 1.24 mg% in *punti* and *phasa shidal* respectively. However, such high concentration of TVBN usually does not manifest any ammonia-like odour in the product. This may probably be due to masking of ammonical odour by the characteristic strong odour of *shidal*. The content of TVBN of the product is the indication of high degree of fermentation. Other workers also reported higher TVBN value in fermented fish product samples (Anihouvi et al. 2006; Roy et al. 2014). Degree of lipid oxidation as measured by estimating thiobarbituric acid (TBA) value was found to be 0.99 ± 0.06 and 1.10 ± 0.14 mg malonaldehyde per kg meat. Absence of salt (a potential pro-oxidant) and metals (as the fermentation is carried out in earthen container) in the system may be attributed for such moderate values of TBA. Priyadarshini et al. (2014) reported a TBA value of 0.69 mg malonaldehyde/kg meat in *Tungtap*, a fermented fish product of Meghalaya. The TPC and LAB of both the *shidals* were estimated as 6.87 ± 0.11, 4.5 ± 0.08 log cfu/g and 6.36 ± 0.01, 4.8 ± 0.14 log cfu/g respectively.

Table 2 Antioxidant activity of *punti* and *phasa shidal*

Product	DPPH radical scavenging activity (%)	Ferric chloride (FeCl ₃) reducing power assay (Absorbance at 700 nm)			
		25 µl	50 µl	75 µl	100 µl
<i>Punti shidal</i>	80.15 ± 5.67	0.133	0.178	0.225	0.294
<i>Phasa shidal</i>	68.30 ± 3.22	0.106	0.150	0.195	0.242

Antioxidant activity of *shidal*

DPPH[•] radical scavenging activity of *punti* and *phasa shidal* was determined as 80.15 ± 5.67 and 68.30 ± 3.22 respectively (Table 2). Radical scavenging against DPPH[•] radical in respect of different fermented fish products has been reported. Thai traditional fermented shrimp and krill products including *Jaloo*, *Koong-Som* and *Kapi* had shown antioxidative activity against DPPH[•] radicals (Faithong et al. 2010), and the water-soluble fraction from *Kapi* showed the strongest antioxidative activity amongst soluble fractions from all other products. Philippine's salt-fermented shrimp pastes showed DPPH[•] scavenging activity in the range of 24.3–61.5 % in 80 % ethanolic extract (Peralta et al. 2008). Squid *miso* prepared with *Aspergillus oryzae*-inoculated *koji* exhibited almost 90 % DPPH[•] scavenging activity and showed rapid development of reducing property at an early stage of fermentation (Giri et al. 2011). Usually peptides from fermented fish products have been reported to act as antioxidants. The presence of

organosulfur compounds of garlic inhibit the peroxidation of lipids and possess antioxidant and free radical-scavenging activity (Karovicova and Kohajdova 2005). Food-derived peptides exhibit antimicrobial properties, blood-pressure lowering effects, cholesterol-lowering ability, antithrombotic and antioxidant activities (Hartmann and Meisel 2007). Ferric chloride reducing power of *punti* and *phasa shidal* is given in Table 2. From the observed DPPH[•] radical scavenging activity and ferric reducing power, *shidal* may be considered as a health-food. It is well established that bioactive compounds from our diet can promote protection, prevention or reduction of the effects caused by oxidative stress (Huang et al. 2005; Pietta 2000).

Fatty acid profile

Fatty acid profiles of *punti* and *phasa shidal* are presented in Tables 3 and 4 respectively. Amongst saturated fatty acids, palmitic acid (C16:0) was found to be dominant in both the

Table 3 Fatty acid profile of *punti shidal*

Fatty acids	Name	Symbol	Percent	Retention time (min)
Saturated	Lauric acid	C12:0	1.10 ± 0.12	14.340
	Tridecanoic acid	C13:0	0.19 ± 0.04	15.132
	Myristic acid	C14:0	3.87 ± 0.1	16.063
	Pentadecylic acid	C15:0	1.69 ± 0.07	16.789
	Palmitic acid	C16:0	10.25 ± 0.07	17.868
	Margaric acid	C17:0	0.57 ± 0.06	19.612
	Stearic acid	C18:0	0.62 ± 0.19	22.167
	Nonadecylic acid	C19:0	1.23 ± 0.11	28.310
	Arachidic acid	C20:0	0.87 ± 0.16	28.310
Monoenoic	NI [#]	C16:1(n-5)	34.57 ± 1.55	18.302
	Oleic acid	C18:1(n-9)	21.71 ± 0.39	23.123
	Eicosanoic acid	C20:1(n-9)	0.78 ± 0.21	28.698
Polyenoic	Linoleic acid	C18:2(n-6)	2.08 ± 0.17	23.775
	Linolenic acid	C18:3(n-3)	0.34 ± 0.05	27.417
	NI [#]	C20:2(n-9)	0.48 ± 0.14	28.981
	Arachidonic acid	C20:4(n-6)	2.58 ± 0.13	30.057
	EPA	C20:5(n-3)	1.77 ± 0.17	31.208
	DHA	C22:6(n-3)	15.29 ± 0.75	37.090

* mean of 3 determinations ± SD, # not identified

Table 4 Fatty acid profile of *phasa shidal*

Fatty acids	Name	Symbol	Percent	Retention time (min)
Saturated	Lauric acid	C12:0	0.74 ± 0.06	14.340
	Tridecanoic acid	C13:0	0.17 ± 0.01	15.333
	Myristic acid	C14:0	1.37 ± 0.09	16.083
	Pentadecylic acid	C15:0	1.62 ± 0.13	16.962
	Palmitic acid	C16:0	10.76 ± 0.36	18.010
	Margaric acid	C17:0	2.21 ± 0.25	19.584
	Stearic acid	C18:0	0.29 ± 0.05	22.092
	Nonadecylic acid	C19:0	0.50 ± 0.11	27.148
	Arachidic acid	C20:0	0.18 ± 0.03	28.517
	Heneicosylic acid	C21:0	0.19 ± 0.08	31.168
Monoenoic	Palmitoleic acid	C16:1(<i>n</i> -7)	21.75 ± 0.85	18.397
	Oleic acid	C18:1(<i>n</i> -9)	44.96 ± 1.65	23.115
	Eicosanoic acid	C20:1(<i>n</i> -9)	1.24 ± 0.09	28.801
Polyenoic	Linoleic acid	C18:2(<i>n</i> -6)	9.71 ± 0.55	24.212
	NI [#]	C20:2(<i>n</i> -9)	0.18 ± 0.05	29.176
	NI [#]	C20:3(<i>n</i> -9)	0.11 ± 0.05	29.470
	EPA	C20:5(<i>n</i> -3)	2.77 ± 0.13	30.441
	DHA	C22:6(<i>n</i> -3)	1.26 ± 0.13	37.575

* mean of 3 determinations ± SD, [#] not identified

fermented fish and contributed about 10 % of the total fatty acids. Amongst the monoenoic fatty acids, one unknown with symbol C16:1(*n*-5) contributed about one third (34.57 %) of the total fatty acids followed by oleic acid (21.71 %) in *punti shidal*. In case of *phasa shidal*, monoenoic fatty acids were dominated by oleic acid (44.96 %) followed by palmitoleic acid (21.75 %). The *n*-6 PUFA in *punti shidal* was composed only of arachidonic acid (2.58 %) and linoleic acid (2.08 %), whereas, in *phasa shidal*, it was only linoleic acid (9.71 %). Contribution of EPA and DHA in both the *shidals* was 1.77 %, 15.29 % and 2.77 %, 1.26 % in *punti* and *phasa shidal* respectively. Presence of EPA, DHA, arachidonic, linolenic and linoleic acid indicate the nutritional significance of *shidal*. However, the result showed that *punti shidal* was rich in omega-3 but poor in omega-6 fatty acid, whereas, reverse was observed with respect to *phasa shidal*.

The *n*-3/*n*-6 ratio was found 3.73 and 0.41 in *punti* and *phasa shidal* respectively, which further emphasize the nutritional richness of *punti shidal*. Majumdar (2005) reported the *n*-3/*n*-6 ratio of salt fermented *hilsa* as 0.39. The percentage of oil in fish and the percentage of fatty acids in fish oil, including *n*-3 fatty acids, vary widely with the species, the geographic location, the food available to the fish and the season (Sikorski and Kolakowska 1990). During fermentation or salting, significant loss occurs in some *n*-3 fatty acids (Pigott and Tucker 1990). Hanafiah and Pigott (1987) reported a considerable loss of *n*-3 fatty acids during fermentation of Indian mackerel and preparation

of fermented '*pedah*'. Further, Yankah et al. (1996) reported a loss in docosahexaenoic and eicosapentaenoic acids during processing and storage of Ghanaian fermented fish product '*momoni*'.

Moreover, some fatty acids were found but could not be identified such as C16:1(*n*-5) [in *punti shidal*], C20:2(*n*-9) [in both] and C20:3(*n*-9) [in *phasa shidal*]. Fatty acids with odd number carbon were also detected such as tridecanoic acid (C13:0), pentadecylic acid (C15:0), margaric acid (C17:0) and nonadecylic acid (C19:0) in both *punti* and *phasa shidal* and heneicosylic acid (C21:0) only in *phasa shidal*. There is

Fig. 2 Protein profile of raw material (dry phasa and punti fish) and *shidal* in PAGE

Table 5 Bio-chemical characteristics of dominant bacterial groups isolated from puti and phasa *shidal*

Products	Colony colour	Cell morphology	Gram stain	Catalase	Oxidase	pH	O/F test			MR/VP	Citrate test	Indole test	Amylase test	Lipolysation test	De-aminase test	Salt tolerance test (%)					Identified bacterial genus
							4.0	7.0	9.0							0	2	4	6	8	
Puti <i>shidal</i>	Yellow	Coccus	+	+	–	–	–	–	–	+/–	–	–	–	–	–	+	+	+	–	–	<i>Staphylococcus</i>
	White	Coccus	+	+	–	+	–	+	+	+/–	–	–	–	–	–	+	+	+	–	–	<i>Staphylococcus</i>
Phasa <i>shidal</i>	Yellow	Coccus	+	+	+	–	–	–	–	+/–	–	–	+	–	–	+	+	+	–	–	<i>Micrococcus</i>
	White	Rod	+	+	–	–	–	+	–	+/–	+	–	–	+	–	+	+	+	+	–	<i>Bacillus</i>

possibility that such odd carbon chain fatty acids might be from the microbes involved in fermentation. Although, not much work on the fatty acid profile of such salt-free fermented fish products has been reported but possibilities of loss of PUFAs during fermentation as well as post fermentation exposure of the product out of fermenting containers cannot be ignored. However, to increase the PUFAs in the product by modifying the fermentation process needs further study.

Proteins pattern in electrophoretic study

Poly acrylamide gel electrophoretic (PAGE) study to know the protein patterns in raw material (dry *phasa* and *punti* fish) as well as in *shidal* has been done and presented in Fig. 2. In the raw materials (dry *phasa* and *puntius* fish) of both the fermented products *shidal*, disappearance of myosin head chain (MHC) was noticed and this may be attributed to the proteolytic degradation by indigenous proteases during drying. Moreover, low pH of fish before drying might favour the hydrolysis of myofibrillar proteins (Visessanguan et al. 2004). That may be the reason of absence of MHC in *shidals*. But actin was present in all except in dry *phasa* fish where the band was not distinct. MHC was more susceptible to hydrolysis, compared with actin (Riebroy et al. 2008).

From the results, proteins or peptides with low molecular weight between 45 and 29 kDa and between 45 and 66 kDa were noticed in both the *shidals* and indicative of intensive protein degradation during fermentation. The results of the present study supports the observation of Faithong et al. (2010) who reported that proteins or peptides with low molecular weight including 50–54, 30–35 and 18–22 kDa were found in *Jaloo* and *Koong-Som* (salt-fermented shrimp product of Thailand), with a longer period of fermentation of 3–6 months. Zeng et al. (2013) observed low intensity of MHC and actin chain bands due to degradation of protein during ripening condition in *Suan yu*- a traditional low salt fermented whole fish product of China.

Microbiology of *shidals*

The total plate count was found to be 6.87 ± 0.11 and 6.36 ± 0.01 log cfu/g in *punti* and *phasa shidal* respectively (Table 1). Muzaddadi (2002) also reported a higher bacterial count of 6.5 to 8.0 log cfu/g in *shidal* prepared with 2 % and 5 % salt. Aerobic mesophilic counts from three fermented fish products such as 'Ngari', 'Hentak' and 'Tungtup' from NE India was reported to be in the range of log 4.3–7.3 log cfu/g (Thapa et al. 2004). Similar TPC value also reported for *Lanhounin*, a traditional fermented fish product in the Republic of Benin (Anihouvi et al. 2006).

The dominant bacterial colonies were randomly selected by their morphological characters from different dilutions of TPC plates. It was found that in every dilution plates yellow and

Table 6 Sugar fermentation characteristics of dominant bacterial groups isolated from puti and phasa *shidal* samples

Products	Identified bacterial genus	Sugars						
		Maltose	Lactose	Mannitol	Sucrose	Sorbitol	Glucose	Dextrose
Puti <i>shidal</i>	<i>Staphylococcus</i>	–	+	–	–	–	+	+
	<i>Staphylococcus</i>	+	+	+	+	+	–	+
Phasa <i>shidal</i>	<i>Micrococcus</i>	+	+	+	+	+	+	+
	<i>Bacillus</i>	–	+	–	–	–	+	+

white colour colonies formed the dominant groups of bacteria in both the *shidals*. Isolation, purification and identification of dominant bacterial colonies were done in accordance with Bergey's Manual of Systematic Bacteriology (Vol. 2, Sneath 1986). On the basis of four major confirmatory characteristics, viz., colony morphology, gram staining, catalase and oxidase tests, *Staphylococcus* was found to be the dominant genus in *punti shidal*, whereas the dominant genera in *phasa shidal* was found to be *Micrococcus* and *Bacillus*. Colony as well as biochemical characteristics of the dominant bacterial genera are given in Table 5. In *punti shidal*, both yellow and white coloured colonies of *Staphylococcus* were found. All other biochemical tests conducted for all the dominant genera confirmed the description given for bacterial genus in Bergey's Manual (Vol. 2, Sneath 1986) except minor deviation in respect of Voges Proskauer (VP) test and amylase producing capacity. This deviation in characteristics indicates host specific variation in metabolic activity since *shidals* lack long chain carbohydrates such as starch.

Microflora plays an important role in fermentation of fish. The presence of microorganisms during fermentation contributes to the degradation of proteins and development of flavour and aroma (Salampessy et al. 2010). Dominant bacteria of few fermented fish products have been reported. The microflora of *hentak* and *tungtap* has been studied by Thapa et al. (2004) and predominant microorganisms were reported to be LAB and *Bacillus* spp. The presence of *Bacillus* spp. in LAB dominant products are due to their ability to form endospores to survive under prevailing conditions (Crisan and Sands 1975). *Bacillus* spp. represented the dominant flora in *ngari* (ethnic fermented fish product of Manipur in India) next to LAB

(Thapa et al. 2004). The microflora of fish or shrimp paste *bagoong* in the Philippines includes *Bacillus* sp., *Micrococcus* sp., and *Moraxella* sp. (Beddows 1985). Mabesa and Babaan (1993) reported that in *balao balao*, a popular fermented fish of Philippines, the microflora changes during 10 days of fermentation and Gram-positive cocci predominate the first 5 days of fermentation, while acid-producing rods predominate the rest of the fermentation period. Sanni et al. (2002) reported *Bacillus* and *Staphylococcus* as dominant bacterial group in *Momoni*, a fermented fish condiment of Ghana. Achinewhu et al. (2004) reported *Staphylococcus* and *Bacillus* species as the predominant bacteria in fermented *Sardinella* sp. Majumdar and Basu (2010) reported *Bacillus* and *Micrococcus* as the dominant bacterial group in *lona ilish*, a salt fermented fish product from *hilsa*. The sugar fermentation characteristics of the dominant bacterial groups isolated from both the *shidals* are given in Table 6. It was found that the bacterial groups can ferment lactose and dextrose whereas; only one bacterial group (white coloured colony of *Staphylococcus* from *Punti shidal*) did not ferment glucose. In case of antibiotic sensitivity test the diameter of clear zone for the dominant bacterial groups against the selected antibiotic discs ranged from 2.2–3.6 cm (Table 7).

Conclusions

Fermented fish product, *shidal* plays a very important role in the nutrition of people of Northeast sector of India and no scientific intervention regarding the nutrition-related qualities has been made so far. The study revealed that the product is

Table 7 Antibiotic sensitivity test of dominant bacterial groups isolated from punti and phasa *shidal* samples

Products	Identified bacterial genus	Diameter of clear zone (cm)				
		Chloramphenicol (SD006-5CT) (30 mcg)	Erythromycin (SD013-5CT) (15 mcg)	Norfloxacin (SD057-5CT) (10 mcg)	Co-Trimoxazole (SD010-5CT) (25 mcg)	Ofloxacin (SD087-5CT) (5 mcg)
Puti <i>shidal</i>	<i>Staphylococcus</i>	3.6	3.2	2.5	3.1	2.2
	<i>Staphylococcus</i>	3.0	2.6	2.7	2.5	2.6
Phasa <i>shidal</i>	<i>Micrococcus</i>	2.8	3.0	3.3	2.9	2.7
	<i>Bacillus</i>	3.4	3.1	3.6	3.3	2.4

having immense nutritional value in respect of essential fatty acids and antioxidative potential and short chain proteins or peptides.

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