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Full Length Research Paper

Effects of salt, sugar and starter culture on fermentation and sensory properties in *Shidal* (a fermented fish product)

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There are several traditional fermented fish products in Northeast India and *shidal* is one of them. It is made from small carps (*Puntius* spp.) with a maturation period of 4 to 6 months. The traditional method of *shidal* preparation varies from place to place. In this study traditional methods of Assam and Tripura were studied and standardized. The traditional *shidal* was collected in monthly intervals from the production centers and microbiological study was done. Two predominant bacteria from fresh traditional *shidal* were isolated and identified as coagulase negative *Staphylococcus aureus* and *Micrococcus* sp. A mixed starter culture of these two organisms was incorporated to the raw material during processing along with salt and sugar in different concentrations in order to hasten the fermentation process. In-process study was conducted with fortnightly sampling and *shidal* produced was evaluated by 10 judges in comparison to the best quality traditional *shidal*. The method could be shortened to 2 months with no significant differences ($p<0.05$) between the products. This modified method of *shidal* preparation can be used by the *shidal* producers to increase the production by two folds per year.

Key words: *Shidal*, fermented fish, starter culture, salt, sugar, traditional product.

INTRODUCTION

Shidal is a salt free, semi fermented and solid fish based product of Northeast India. The product is known for its characteristic odour and flavor. The characteristic color of good quality shidal is dark reddish brown (Table 1). It is prepared exclusively from small carps locally known as *puthi* (*Puntius* spp.) and consumed after elaborate cooking (Muzaddadi and Basu, 2003a). *Shidal*-chutney is a highly relished cuisine made from this product amongst many people of Northeast India (Figure 1). The product is prepared in earthen pots (called mutka) and stored in it till marketing. There are several similar products in different Northeastern states such as *shidal* and *hidal* in Assam, *sepaa* and *shidal* in Tripura and Nagaland and Arunachal Pradesh and *ngari* in Manipur. The semi dried fish are tightly packed in specially made earthen pots, locally called *mutka* (Plates 1 and 2) and sealed with fish-paste, plant leaves and fine clay for fermentation. The fermentation process takes around 4 to 6 months in semi-anaerobic condition till the product gains a characteristic odour, texture and appearance (Muzaddadi, 2002). Neither food additives/preservatives nor starter culture is added during the processing steps (Muzaddadi and Basu, 2003b). Similar products have also been reported in Bangladesh as *chepa shutki* (Mansur et al., 2000; Nayeem et al., 2010). The volatile compounds of Bangladeshi *chepa shutki* were analyzed by using gas chromato-

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Table 1. Determining characteristics of a normal good quality *Shidal.*

Attribute	Determining characteristic	Sensory Score#
Appearance	Moist with very soft surface, sticky on touch	9
Colour	Dark reddish brown	9
Texture	Moderately hard texture	9
Odour	Typical strong *Shidal* odour	9
pH	5.7 ± 0.8	-
Titratable acid level	1.78 ± 0.05 (%)	-
TPC	10^3 – 10^6 (CFU/g)	-

#10-Point Hedonic scale given by 10 judges, 1-very poor, 5-border line of acceptability, 9- very good and 10-excellent. Values are rounded off figure of the arithmetic average, where n=10.

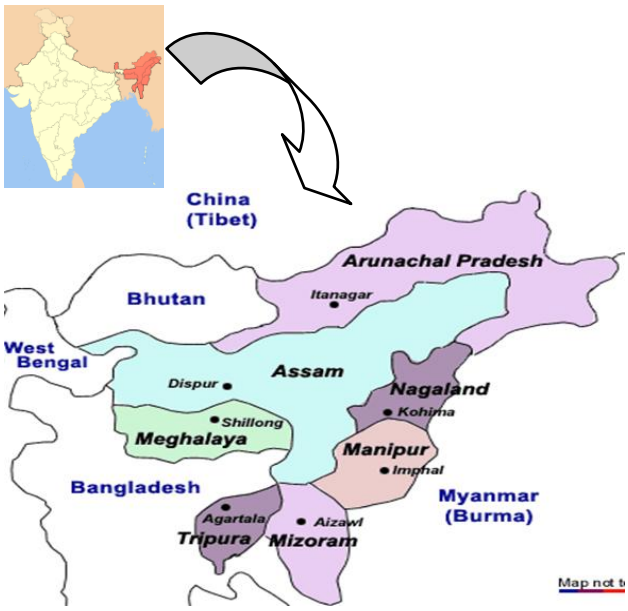

Figure 1. *Shidal* producing states of North East India.

Plate 1. Traditional oil processed *mutka.*

Plate 2. Traditional *Mutka* packed up to the rim.

graphy-mass spectrometry (GC-MS) (Khanum et al., 2001). *Hentak* and *ngari* are prepared from sun-dried fresh water fish in Manipur, India (Sarojnalini and Vishwanath, 1994). *Tungtap,* being another similar product of Meghalaya has been reported to be good source of protein (40.6%) (Murugkar and Subbulakshmi, 2006; Thapa et al., 2004).

In Nagaland, fermented fish products are prepared by keeping washed fish inside bamboo pipe and fermenting over the fireplace (Mao and Odyuo, 2007). Hastening of fish sauce fermentation process could be achieved by the use of proteolytic bacteria, that is, *Brevibacterium linens* and *Micrococcus* sp. (Putro, 1993). In the same line, present study aims at to improve and standardize the traditional method and develop starter culture for hastening the maturation period of *shidal* preparation. The improved method is expected to give an impetus to the *shidal* preparation industry by increasing production in two folds and above all, this cottage industry of the region which is facing a challenging competition from imported *shidal* from Bangladesh, would be rejuvenated.

MATERIALS AND METHODS

Basic study and identification of predominant bacteria

The traditional *shidal* samples were collected aseptically in sterile polyethylene bags immediately after opening the *mutka* (the earthen pots especially made for *shidal* preparation) from two dry fish markets of Tripura, that is, Lembucherra Bazar (LB) and Gol Bazar (GB). *Shidal* was also prepared in the laboratory following the traditional method (Figure 2) and an in-process study was done at monthly intervals. The market samples and laboratory samples were analyzed to record the bacterial activity, predominant bacteria and stages of fermentation for earmarking the best quality *shidal* in terms of resident bacterial flora, pH, titratable acidity, physical appearance, color and texture (Table 1). Depending on the texture, colour, appearance, odour and surface stickiness, the different fermentation stages were earmarked as R1, R2, R3, R4, R5 and R6. Stage R4 was found to be the ideal stage to open the *mutka* (Table 2).

Isolation and identification of resident bacteria

The bacteria showing comparatively higher number of colonies with similar colony characteristic on Soybean Casein Digest Agar Medium/Trypticase Soya Agar (TSA) (MU290, Himedia, India) plates were presumed to be predominant bacteria. The colonies were picked up and purified by streak plate method on Trypticase Soya Agar (TSA) plates. The purified cultures were preserved in TSA slants for further identification. Preliminary bacterial identification up to genus level was made following standard methods (Le Chavallier et al., 1980; Bain and Shewan, 1968).

Sample preparation

For microbiological study, 25 g fish was sampled aseptically and was introduced in a sterile stomach bag (Seward stomach BA6141CPG standard bags) aseptically and homogenized for 2 min with 225 ml of sterile diluents (0.85% physiological saline) using a stomacher blender (Seward stomacher 400 circulator, England) which made a muscle mixture of 10^{-1} dilution. For sensory evaluation and biochemical studies required amount of sample was collected in sterile Petri plates.

Sensory evaluation

Various sensory characteristics were evaluated after preparation and during storage at regular intervals by 10 experts using 10 point hedonic scale. The experts were selected from the regular *shidal* eaters who gave the scores for appearance, colour, texture and odour after doing physical observation of raw *shidal*. They were also requested to taste *shidal* in the form of *shidal* chutney with rice. The mean sensory score for each attribute with standard deviation was adopted. The scores for all products were recorded and subjected to statistical analysis.

Microbiological study

For Total Plate Count (TPC), serial decimal dilution of 10^{-2} was prepared using 9 ml sterile physiological saline and 1 ml homogenized sample (10^{-1}) and it was well mixed in cyclomixer. For TPC, 0.1 ml portions of various dilutions were spread on Trypticase Soya Agar (TSA) plates using a sterile glass spreader (APHA, 1995). The

Figure 2. The traditional method of *shidal* preparation from dry *Puntius* sp. in Tripura state.

plates were incubated at 35°C for 24 h for TPC and the plates containing 25 to 250 colonies were counted and reported. For Total Fungal Count (TFC) same portions were spread on the surface of preset surface-dried Rose Bengal Chloramphenical agar (M640, HIMEDIA) plates using a sterile glass spreader and were incubated at 25°C for 3 to 5 days. The plates containing 25 to 250 colonies were counted and reported. Differential counts for predominant bacteria (15 to 30% of TPC) were enumerated using differential media. The spread method was employed with 0.1 ml inoculum from different dilutions decided on the basis of the results of the pilot study.

Staphylococcus aureus counts

For staphylococcal count, 0.1 ml portion of various dilutions were spread on the surface of preset surface-dried Baird–Parker agar (M043, Himedia, India) plates supplemented with potassium tellurite and egg yolk emulsion. The plates were incubated at 37°C for 36 to 48 h. Convex, black and shiny colonies with narrow white margin surrounded by clear zone were regarded as *Staphylococcus aureus.* These colonies were confirmed by conducting Gram staining (colonial morphology), coagulase test, catalase test and anaerobic utilization of glucose and mannitol (USFDA, 2001). The colonies were purified onto Tryptose soya agar (TSA) plates and further subjected to coagulase test by slide technique for confirmation.

Micrococcus counts

Exactly 0.1 ml aliquots of various dilutions were spread on the surface of preset surface dried Vogel Johnson (MM023, Himedia, India) and Baird Parker agar plates for isolation and enumeration of *Micrococcus* spp. The plates were incubated at 37°C for 24 to 48 h. The yellow colonies on the plates were counted and further confirmed by Gram staining and the cells which were Gram-positive cocci with an arrangement in tetrads were considered. Micrococci were distinguished from staphylococci by a modified oxidase test (Baker, 1984; Faller and Schleifer, 1981).

Enterobacteriaceae and *E. coli* counts

Various members of the family Enterobacteriaceae were isolated and enumerated using MacConkey agar with crystal violet. Exactly 0.1 ml portions of various dilutions were spread on the surface of preset surface dried MacConkey agar (M007, Himedia, India) and Eosin Methylene Blue (EMB) agar (M022, Himedia, India) plates. Petri plates were incubated at 35°C for 20 h. The typical *Escherichia*

Table 2. Different stages of fermentation of traditional *Shidal*.

Name of the Unit	Fermentation Period (month)	Average TPC (CFU/g)	Titratable acid level (%)	pH	Determining Characteristics/Stage	Code of Identity (Remarks)
A	0	1.1×10^2	ND	6.9 ± 0.2	Dry with hard surface, silvery colour	R (Raw material)
B	1	8.0×10^3	ND	6.9 ± 0.3	Moist with moderately soft surface, hard texture, almost no colour change, no characteristic *shidal* smell	R1 (initiation of fermentation)
C	2	3.3×10^4	0.21± 0.01	6.6 ± 0.2	Moist with moderately soft surface, silvery white colour disappears, moderately hard texture, mild putrefied smell	R2 (highest fermentation activity period)
D	3	2.7×10^6	0.51 ± 0.02	6.5 ± 0.2	Moist with soft and sticky surface, colour changes to pale brown, characteristic *shidal* smell moderately prominent, texture starts softening	R3 (Pre-ripened, low quality, under-fermented but acceptable)
E	4	1.6×10^4	1.78 ± 0.5	5.7 ± 0.8	Moist and sticky surface, dark reddish brown colour, characteristic strong *shidal* smell, moderately soft texture with shape of the fish intact	R4 (Ripened, Best Quality *shidal*)
F	5	7.5×10^3	2.04 ± 0.5	6.1 ± 0.2	Moist with very soft surface, colour dark brown to black, texture very soft, shape of the fish distorted, characteristic strong *shidal* smell prevails	R5 (Post-ripened, low quality, over-fermented but acceptable)
D	6	2.6×10^3	2.98 ± 0.5	6.0 ± 0.2	Moist with very soft surface, colour dark brown to black, the product almost turns to paste, characteristic strong *shidal* smell no longer prevails, deterioration starts	R6 (Degraded, highly over-fermented, poor quality, unacceptable)

ND = Not detected

coli colonies (brick red on MacConkey agar and dark centered and flat, with or without metallic sheen on EMB agar and colorless or slightly pink colonies on both the agar plates) were counted (USFDA, 2001). The colonies were purified onto Tryptose soya agar (TSA) plates and further subjected to Gram staining, Indole test, Methyl red (MR), Voges Proskauer (VP) tests and citrate utilization test.

Bacillus counts

Zero point one ml aliquots of various dilutions were spread on the surface of preset surface dried modified Mannitol-egg yolk-polymyxin (MYP) agar (M1139, Himedia, India) for isolation and enumeration of *Bacillus* spp. Plates were incubated 24 h at 30°C and the pink or red colonies, which becomes more intense after additional incubation were counted and further confirmed by Gram staining, microscopic observation, motility test, catalase test, VP reaction and acid production from mannitol (USFDA, 2001).

Enterococcus /*Streptococcus* counts

Enterococcus/*Streptococcus* counts were estimated by use of Blood Agar with Strepto Supplement (FD031, Himedia, India) and Slanetz and Bartley medium (M612, Himedia, India). Exactly 0.1 ml aliquots of various dilutions were spread on the surface of preset surface dried SB agar plates. Plates were incubated aerobically at 44°C for 24 to 48 h. The red or maroon colonies were counted. Isolates from primary culture were identified by colonial appearance, Gram stain, catalase test, Lancefield grouping (Group D) and optochin sensitivity (CDC, 2010).

Preparation of starter culture

From the basic study it was apparent that *Micrococcus* spp. and coagulase negative *S. aureus* were predominant in market samples as well as in the samples from in-process study (Table 3 and 4). Hence, these two bacterial cultures were further purified on TSA plates and were selected as starter cultures. These bacteria were grown in TSA broth, each in separate conical flasks and incubated at room temperature (35°C) for 48 to 72 h. The cells were collected by centrifuging at 8000 rpm at room temperature (35°C) for 10 min and then washed and diluted in sterile physiological saline (0.85% NaCl). The number of cells per ml saline (CFU ml^{-1}) was estimated by Total Plate Count (TPC) and the concentration was kept around 10^{10} ml^{-1}. The cultures of these bacteria were maintained by sub-culturing fortnightly on the TSA slants.

Modification of traditional method

The traditional *shidal* preparation method was standardized under laboratory conditions by trial and error (Figure 2) and further modified for laboratory preparation supplementing with starter culture to study the effects of starter during fermentation (Figure 3).

Table 3. TPC and identified isolates found in Samples from Lembucherra Bazar (LB) and Golbazar (GB) (mean ± SD, n=3).

Month	Log CFU g^{-1} of LB*	Predominant bacteria (LB)	Log CFU g^{-1} of GB*	Predominant bacteria (GB)
August	8.2 ± 0.2^{g}	A, B, E	7.5 ± 0.3^{f}	A, B, D, E
September	7.7 ± 0.1^{fg}	A, B, C, D, E	7.2 ± 0.4^{f}	A, B, D, E
October	7.1 ± 0.3^{de}	A, C, E	6.3 ± 0.3^{e}	A, B, C, E
Nov	6.3 ± 0.1^{c}	A, B, D, E	5.2 ± 0.2^{cd}	A, B, E
Dec	5.1 ± 0.4^{a}	A, B, D	4.1 ± 0.1^{a}	A, B, D
Jan	4.9 ± 0.3^{ab}	A, B, C, E	4.3 ± 0.1^{a}	A, D, E
Feb	5.7 ± 0.2^{bc}	A, B, C, E	4.9 ± 0.3^{b}	A, B, E
Mar	6.0 ± 0.1^{d}	A, B, D, C	5.9 ± 0.1^{bc}	A, B, C
April	6.8 ± 0.2^{ef}	A, B, E	6.7 ± 0.2^{de}	A, B, E
May	7.8 ± 0.1^{efg}	A, B, D	6.9 ± 0.2^{f}	A, B, C

*Means in a column with the same superscript letters are not significantly different (P>0.05). Predominant bacteria (15-30% of TPC): A. *S. aureus*, B. *Micrococcus* sp. C. *Bacillus* sp., D. *E. coli*, E. *Streptococcus*/*Enterococcus.*

Table 4. Changes in Total Plate Count (TPC), Total Fungal Count (TFC) and pH in *Shidal* during fermentation (in-process) under laboratory condition by traditional method (mean ± SD, n=3)

Month	Fermentation Period (month)	TPC (Log CFU g^{-1})	TFC (Log CFU g^{-1})	pH	Predominant bacteria
August To January	0	6.5 ± 0.2^{a}	3.3 ± 0.1^{a}	6.9 ± 0.2^{a}	A, B, C, D, E
	1	2.9 ± 0.1^{c}	4.1 ± 0.2^{c}	5.9 ± 0.3^{b}	A, B, C, D, E
	2	3.7 ± 0.3^{c}	5.1 ± 0.4^{d}	4.8 ± 0.2^{ab}	A, B, C
	3	4.4 ± 0.2^{b}	6.8 ± 0.2^{cd}	4.6 ± 0.2^{ab}	A, B, C
	4	4.1 ± 0.2^{b}	4.2 ± 0.2^{c}	4.4 ± 0.2^{c}	A, B
	5	**5.5** $\pm 0.2^{a}$	**2.6** $\pm 0.3^{b}$	**5.5 ± 0.2**^{b}	**A, B**
	6	4.2 ± 0.2^{ab}	2.4 ± 0.2^{b}	5.0 ± 0.3^{ab}	A, B
February to July	0	6.0 ± 0.2^{a}	3.6 ± 0.2^{a}	7.0 ± 0.2^{a}	A, B, C, D, E
	1	2.9 ± 0.2^{c}	4.6 ± 0.2^{c}	5.8 ± 0.2^{abc}	A, B, C, D
	2	7.0 ± 0.1^{d}	5.7 ± 0.2^{d}	4.7 ± 0.2^{ab}	A, B, C
	3	9.8 ± 0.2^{e}	6.5 ± 0.1^{cd}	4.5 ± 0.3^{ab}	A, B
	4	**5.8** $\pm 0.2^{a}$	**2.1** $\pm 0.2^{b}$	**5.7 ±0.2**^{abc}	**A, B**
	5	4.6 ± 0.3^{b}	2.8 ± 0.2^{b}	5.6 ± 0.2^{abc}	A, B
	6	4.6 ± 0.2^{ab}	2.0 ± 0.1^{b}	5.3 ± 0.2^{abc}	A, B

*Means in a column with the same superscript letters are not significantly different (P>0.05)
Predominant bacteria (15-30% of TPC): A. *S. aureus*, B. *Micrococcus* sp. C. *Bacillus* sp., D. *E. coli*, E. *Streptococcus*/*Enterococcus*
Bold row indicates "*Shidal* attained its final stage"

Sampling plan

For hastening the traditional lengthy fermentation process, 16 sets of *mutka,* each set consisting of 4 units of 6 kg capacity *mutka*s (Plate 3) were prepared with different combinations of food additives and/or starter. The *mutkas* in each set were encoded denoting initials of additives and organisms used as shown in Table 5 and 6. For preparation of each set of *mutka*, four sterile steel trays with 900 ml sterile distilled water were taken (Plate 4). Salt and sugar were dissolved separately in three trays in predetermined concentrations, that is, 10, 5 or 2% or in combinations with or without the starter culture according to the designation of the set. One *mutka* per set was kept as control. 100 ml inoculum with concentration of 10^{10} CFU ml^{-1} was used separately for *Micrococcus* and *S. aureus* as starter culture and 50 ml inoculum of each bacterium was used when these cultures were used in combination. *Shidal* was prepared by following the modified method (Figure 3). Thick sterilized heavy gloves (Plate 5) were used for packing and every operation was done using sterilized gloves (Plate 6).

Biochemical study

Moisture, Protein, Ash, Acid insoluble ash, total fat content, pH, Free Fatty Acid, titratable acid level and Non Protein Nitrogen (NPN) were estimated following standard AOAC methods (AOAC, 2001). α-amino nitrogen was determined by the method given by Pope and Stevens (Pope and Stevens, 1939). Total Volatile Base Nitrogen (TVB-N) was determined by Conway's micro-diffusion method (Conway, 1947), Peroxide Value (PV) by the method given by Jacob (1958) and Thiobarbituric Acid (TBA) number by method given by Tarladgis et al. (1960).

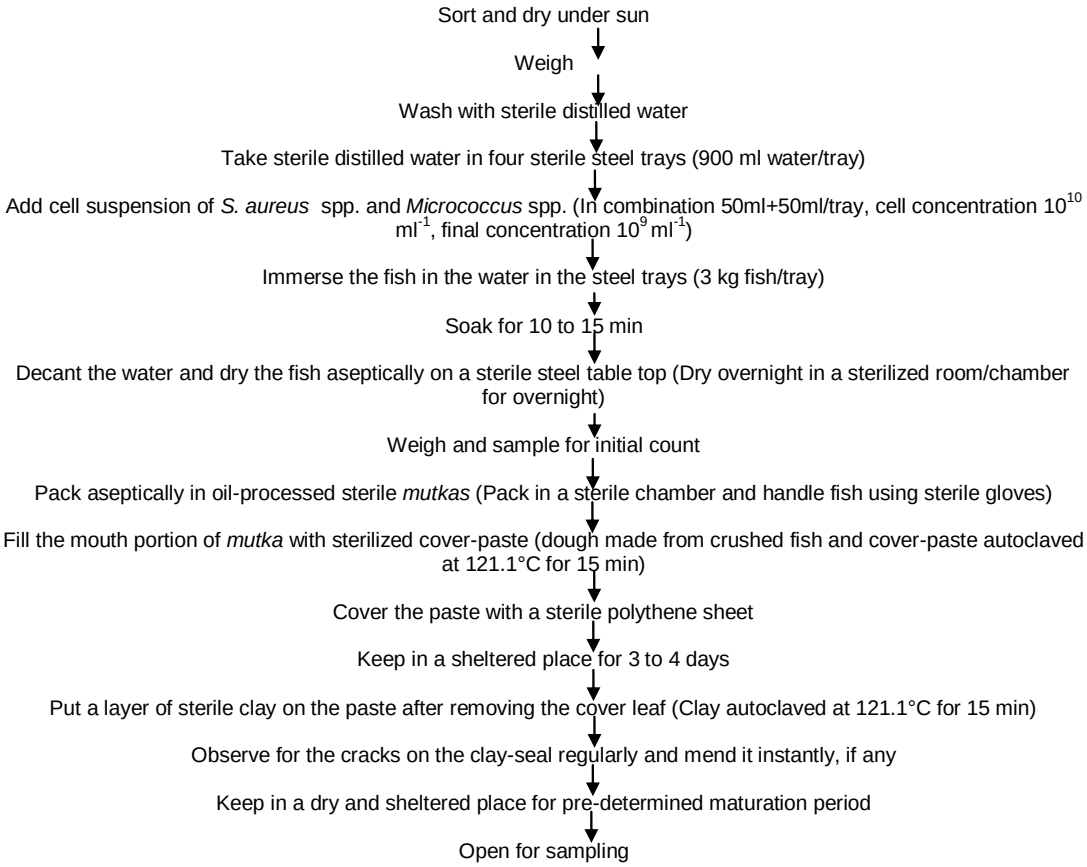

Figure 3. The modified method of *Shidal* preparation used in laboratory experiments.

Plate 3. Oil processed *mutka* used in modified method.

Determination of final stage

After each sampling the parameters such as the no. of resident bacterial flora (TPC), pH, titratable acidity, proximate composition, AAN, FFA, NPN, PV, TBA and TVB-N were compared with the parameters of standard good quality *shidal* (Tables 1 and 2). For determining the final stage, these findings were further co-related with the sensory scores given by 10 expert judges. The final stage (R4) was determined with the determination of pre-maturation stage (R3). The duration of fermentation after which the product attained the

Table 5. Sampling Schedule for in process studies of different types of *Shidal.*

Name of the set	Salt sugar used (%)	Name of the unit	Fermentation period (day)	Number of *Micrococcus* and *Staphylococcus* in the raw material	Bacteria used in Starter
S-T (Control)	0	S-T30	30	ND	None
		S-T45	45		
		S-T60	60		
		S-T75	75		
		S-T90	90		
S-SMS2% (*Shidal* with *S. aureus* and *Micrococcus* and 2% salt and 2% sugar)	2+2	S-SMS2%15	15	ND	*Micrococcus* and *S. aureus*
		S-SMS2%30	30		
		S-SMS2%45	45		
		S-SMS2%60	60		
		S-SMS2%75	75		
S-SMS5% (*Shidal* with *S. aureus* and *Micrococcus* and 5% salt & 5% sugar)	5+5	S-SMS5%30	30	ND	
		S-SMS5%45	45		
		S-SMS5%60	60		
		S-SMS5%75	75		
S-SMS10% (*Shidal* with *S. aureus* and *Micrococcus* and 10% salt and 10% sugar)	10+10	S-SMS10%30	30	ND	
		S-SMS10%45	45		
		S-SMS10%60	60		
		S-SMS10%75	75		

ND= Not detectable by spread plate technique.

Plate 4. Starter culture treated raw material.

Plate 5. Fish packed aseptically with a sterilized heavy glove.

final stage has been indicated by a bold line in all the related tables.

Sensory evaluation

Sensory studies of *shidal* were carried out by expert panel of 10 judges. The sensory characteristics included General Appearance, Texture, Odour and Colour. The judges were requested to give the scores in 10 points scale after seeing, feeling by touching and smelling the product. The overall acceptability was calculated by taking arithmetic average from the score-sheet.

Data analysis

All bacteriological counts were converted to $\log_{10}$ $CFUg^{-1}$ for statistical analysis. Statistical analysis was done by performing one

Table 6. Total fermentation time required for each product to attain pre-ripened stage (R3) and their respective end-product quality.

Product	Total fermentation time required (Day)	TPC (CFU/g)	Titratable acid level (%)	pH	Remark
S-Traditional	90+	6.4	1.75 ± 0.02	6.5 ± 0.2	(R3)
S+Salt2%	75+	4.9	1.58 ± 0.02	6.7 ± 0.2	(R3)
S+Salt5%	75+	4.7	1.58 ± 0.02	6.7 ± 0.2	(R3)
S+Salt10%	75+	5.0	1.59 ± 0.02	6.4 ± 0.2	(R3)
S+Sugar2%	75+	5.3	1.65 ± 0.02	6.3 ± 0.2	(R3)
S+Sugar5%	75+	5.7	1.64 ± 0.02	6.3 ± 0.2	(R3)
S+Sugar10%	75+	5.4	1.67 ± 0.02	6.3 ± 0.2	(R3)
S+Salt 2% +Sugar 2%	60+	5.4	1.48 ± 0.02	6.5 ± 0.2	(R3)
S+Salt5% +Sugar5%	60+	4.7	1.54 ± 0.02	6.5 ± 0.2	(R3)
S+Salt 10% +Sugar10%	60+	5.3	1.68 ± 0.02	6.3 ± 0.2	(R3)
S+*Micrococcus*	45+	6.4	1.18 ± 0.02	6.8 ± 0.2	(R3)
S+*Staph.*	60+	6.5	1.22 ± 0.02	6.8 ± 0.2	(R3)
S+*Staph.*+*Micro.*	45+	6.6	1.30 ± 0.02	6.7 ± 0.2	(R3)
S+*Lactobacillus*	15+	5.6	1.81 ± 0.02	5.5 ± 0.2	*
S+Salt 2% +Sugar 2%+ *Staph.*+*Micro.*	**15+**	**8.2**	**1.58 ± 0.02**	**6.0 ± 0.2**	**(R3)**
S+Salt 5% +Sugar 5%+ *Staph.*+*Micro.*	60+	8.4	1.66 ± 0.02	6.2 ± 0.2	(R3)
S+Salt 10% +Sugar 10%+ *Staph.*+*Micro.*	45+	8.1	1.78 ± 0.02	6.2 ± 0.2	(R3)

*Sensory score below 5.

Table 7. Sensory scores of *Shidal* on 10 point hedonic scale (mean ± SD, n = 10) with 60 days fermentation period.

Treatment	Appearance*	Colour*	Taste*	Texture*	Odour*	Overall Acceptability*
S-Traditional	**9.5±0.2** ^{a}	**9.4±0.2**^{a}	**9.5±0.2** ^{a}	**9.2±0.2** ^{a}	**9.0±0.2**^{a}	**9.3±0.2**^{a}
S+Salt2%	7.4±0.4^{b}	6.5±0.4^{b}	6.0±0.4^{b}	5.5±0.4^{b}	6.1±0.4^{b}	6.3±0.4^{b}
S+Salt5%	7.5±0.4^{b}	6.3±0.4^{b}	5.8±0.4^{b}	5.0±0.4^{c}	5.9±0.4^{b}	6.1±0.4^{b}
S+Salt10%	7.0±0.5^{c}	5.5±0.5^{c}	4.0±0.5^{c}	4.2±0.5^{c}	5.3±0.5^{c}	5.2±0.5^{c}
S+Sugar2%	7.6±0.5^{b}	6.2±0.5^{b}	6.0±0.5^{b}	6.5±0.5^{b}	6.7±0.5^{b}	6.6±0.5^{b}
S+Sugar5%	7.3±0.5^{b}	6.6±0.5^{b}	6.2±0.5^{b}	4.0±0.5 ^{c}	6.4±0.5^{b}	6.1±0.5^{b}
S+Sugar10%	6.9±0.5 ^{c}	6.4±0.5^{b}	5.7±0.5^{c}	3.6±0.5 ^{d}	5.8±0.5^{b}	5.7±0.5^{c}
S+Salt 2% +Sugar 2%	**7.8±0.2**^{ab}	**7.0±0.2**^{b}	**6.5±0.2**^{ab}	**6.8±0.2**^{b}	**7.1±0.2**^{ab}	**7.0±0.2** ^{ab}
S+Salt5% +Sugar5%	7.2±0.3^{b}	6.1±0.3^{b}	5.4±0.3^{b}	6.0±0.3^{b}	6.6±0.3^{b}	6.3±0.3^{b}
S+Salt 10% +Sugar10%	6.4±0.3 ^{bc}	5.5±0.3^{c}	5.0±0.3^{b}	5.3±0.3^{b}	6.4±0.3^{b}	5.7±0.3^{c}
S+*Micrococcus*	7.5±0.4^{b}	7.1±0.4^{b}	7.2±0.4^{ab}	7.8±0.4^{ab}	7.1±0.4^{ab}	7.3±0.4^{ab}
S+*Staph.*	7.0±0.7^{c}	6.9±0.7^{b}	6.7±0.7^{ab}	7.1±0.7^{ab}	6.8±0.7^{ab}	6.9±0.7^{ab}
S+*Staph.*+*Micro.*	**7.9±0.4**^{ab}	**8.0±0.4**^{ab}	**8.5±0.4**^{bc}	**8.6±0.4**^{ab}	**7.3±0.4**^{ab}	**8.1±0.4**^{ab}
S+*Lactobacillus*	3.0±0.5^{bc}	4.0±0.5^{c}	2.5±0.5^{d}	2.0±0.5^{d}	3.5±0.5^{d}	3.0±0.5^{d}
S+Salt 2% +Sugar 2% + *Staph.* + *Micro.*	**9.5±0.2**^{a}	**9.8±0.2**^{a}	**9.4±0.2**^{a}	**9.8±0.2**^{a}	**8.8±0.2**^{a}	**9.4±0.2**^{a}
S+Salt 5% +Sugar 5%+ *Staph.*+*Micro.*	7.7±0.2^{ab}	7.4±0.2^{ab}	8.2±0.2^{ab}	8.0±0.2^{ab}	7.2±0.2^{ab}	7.7±0.2^{ab}
S+Salt 10% +Sugar 10%+ *Staph.*+*Micro.*	6.9±0.2^{c}	7.3±0.4^{ab}	7.0±0.4^{ab}	6.1±0.4^{b}	6.2±0.4^{b}	6.7±0.4^{ab}

*All traits measured on ten-point scale with 1 being least and 10 being the most. The products having means in a column with the same superscript letters are not significantly different (p<0.05).

way ANOVA (Post Hoc, Duncan) and students' t-test to compare the means using SPSS-15.0 (2005).

RESULTS AND DISCUSSION

The samples collected from Lembucherra Bazar and Gol Bazar showed moderately high values of total plate count during the summer months and early winter (Tables 3 and 4). In the summer months (August to October) the counts were 10^{6} to10^{8} CFU g^{-1}. Contrastingly, the TPC was comparatively lower (10^{4} to 10^{7} CFU g^{-1}) during the winter months and early summer (November to May). Slight

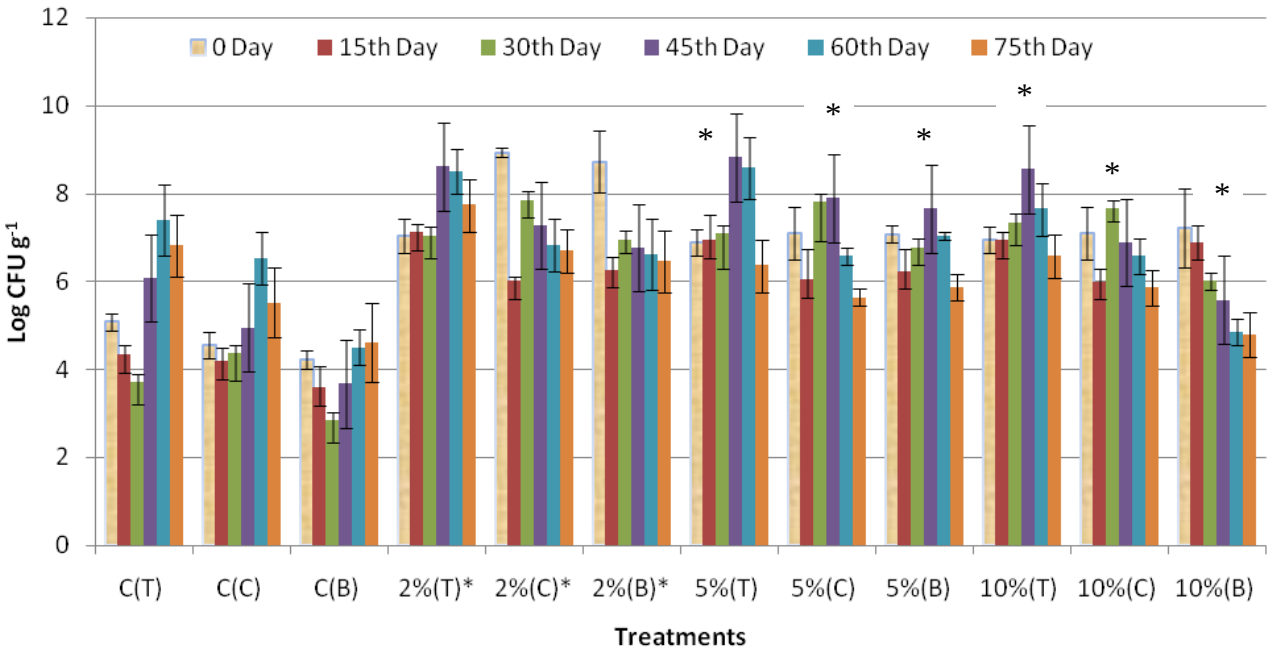

Figure 4. Process bacteria dynamics during fermentation in different layers of the fermenting pot (mutka) at 15 days interval. Top layer is denoted by (T), Center by (C) and Bottom by (T). The Control is denoted by C and Treatments as 2, 5 and 10%. Values are mean ± SD (error bars), n=6.

Noted: Treatments, 2%= *Shidal* with 2% Salt, 2% Sugar, *Micrococcus* and *S. aureus;* 5%= *Shidal* with 5% Salt, 5% Sugar, *Micrococcus* and *S. aureus*; 10%= *Shidal* with 10% Salt, 10% Sugar, *Micrococcus* and *S. aureus.*
Treatments with asterisks denote achievement of maturity within 60th day of fermentation. Bars with asterisks denote significant difference (P<0.05) with best quality *shidal.*

variation was observed in some of the samples which may be attributed to external contamination. The bacterial count was high during the summer months, perhaps due to the ambient temperatures (25 to 35°C) which lie in favorable range of temperature for most of the mesophilic bacteria. During the winter months, due to the low ambient temperature (15 to 25°C) the general flora of bacteria might have showed limited growth. However, the counts did not differ significantly (P>0.05) throughout the year. Similar increasing trends in TPC were reported by Sarojnalini and Suchitra (2009) in fermented *Setipinna* sp. of Manipur and by Thapa et al. (2004) [9] in *Ngari*, *Hentak* and *Tungtap* of Northeast.

In all the samples the predominant bacteria were *S. aureus* and *Micrococcus* sp. regardless market samples or laboratory samples (Table 3 and 4). Hence, both of these bacteria were chosen for preparation of starter for hastening the process. The findings of Tanasupawat et al. (1991) who isolated *Staphylococcus* spp. from fermented fish supports the present observation.

In all the processes the fungal count was found to be 10^2 to 10^3 CFU g^{-1} and insignificant in number (Table 4). Fungal counts were very erratic and inconsistent the market samples and not shown in the tables. Near anaerobic condition inside the *mutka* may be one of the reasons for low fungal growth.

The assumption was to supply readily available nutrients, that is, sugar and salt externally, in certain concentrations which would help instant proliferation and uniform growth of bacteria in all the strata inside the *mutka* and also to introduce starter mixture of *Micrococcus* and *S. aureus* to make fermentation process set in early. In traditional method the number of resident fermenting bacteria was insufficient soon after packing and would require a certain amount of time to grow sufficiently for initiating the fermentation process. The resident fermenting bacteria in the raw material was undetectable by spread plate technique (Table 5). The reason may be the vigorous sun - drying, which might reduce the number to an undetectable level. Among the Gram negative bacteria, *E. coli* was found in negligible level (<10^2 g^{-1}) in the initial stage, which may be due to incidental pre-process contamination and was absent in the later stages of fermentation.

To reach R3 stage, the traditional method took more than 90 days, while the *shidal* prepared with different concentration of salt and sugar alone could reduce this period by 15 days (Table 5). In the experiments with salt and sugar mixture with equal proportions and with different concentrations, R3 stage was achieved after 60 days (Table 6). On the other hand, when *shidal* was prepared with single starter, that is, the inoculum of either *S. aureus* or *Micrococcus* without any additive, R3 could be attained

Plate 7. Traditional good quality *shida.*

Plate 8. *Shidal* after maturation by modified method.

after 60 days for *S. aureus* and after 45 days for *Micrococcus*, whereas advanced R3 stage could be attained after 45 days of fermentation when both the cultures were used in equal proportions (Table 6). In a separate experiment with *Lactobacillus* (isolated from curd) inoculum, R3 could be attained after 15 days of fermentation, but there was a complete loss of *shidal* flavour and the overall acceptability was significantly different with traditional *shidal* ($p>0.05$) (Table 7).

Further experiments were done with low doses of food additives and the starter, as the experimental results so far clearly indicated that the treatment with a high dose would induce the development of undesirable qualities in *shidal* altering its traditional identity in terms of texture, flavour, colour and smell.

The stage R3 could be attained after a maturation period of 15 days only, when bacterial starter of *S. aureus* and *Micrococcus* (starter-mix) along with 2% salt and 2% sugar were added to the raw material. The bacterial counts gradually decreased with the increasing time of fermentation in all the strata significantly ($P<0.05$) from 10^8 CFU/g to 10^6 CFU/g, while the top layer always had the highest count (Figure 4). All the other criteria matched with R3 stage except that its count was more. It indicates that the overpopulation of bacteria through artificial inoculation cannot accelerate the fermentation process proportionately. Despite of their existence in the system in high numbers, all of the active bacteria might not take part in the fermentation process due to overcrowding. Nevertheless, once the fermentation period further increased, more degradation of quality was recorded.

A promising combined effect of food additives and culture-mix was clearly visible, when 2% salt+ 2% sugar was added along with a starter-mix population of 10^8 CFU/g, the product had attained the final stage just after 45 days crossing the R3 stage by 15 days of fermentation. Though the bacterial count in the top layer was higher than in other two strata, invariably all the strata had a high bacteria count. The growth of bacteria was sustained in all three layers till 75 days of fermentation (Figure 4). Interestingly, the product quality neither improved much nor deteriorated as the fermentation time increased. Moreover, the product had maximum resembling characteristics of traditional *Shidal* in terms of appearance, colour, taste, texture and odour and there was no significant difference ($p<0.05$) with the traditional *shidal* (Table 7). The reasons for these desirable results can be interpreted as, due to external supply of active bacterial culture and basic nutrients in form of additives, an instant and uniform proliferation of bacteria occurred in all parts of *mutka* which, in turn, triggered an early start of fermentation and thus reducing the total fermentation time. Large population of *S. aureus* and *Micrococcus* sp. might have not allowed other undesirable bacteria to proliferate sufficiently to slow down fermentation and deteriorate the quality.

The total fermentation time was 30 days for reaching R3 stage when *Shidal* was prepared with *S. aureus* and *Micrococcus* inoculum along with 5% salt and 5% sugar. The bacterial count steadily decreased with increased time of fermentation time. Though higher count of bacteria was encountered in all the strata, the quality of the product was inferior to S-SMS2% ($p>0.05$) (Table 7). All the strata had a sustained bacterial count till 75 days of fermentation (Figure 4). The product quality here was not that good and the quality slightly deteriorated as the fermentation time increased. The overall acceptability of this product differed significantly ($p>0.05$) with traditional *shidal* (Table 7). The possibility of over fermentation and undesirable chemical activity in presence of higher concentration of food additives may be the reason for this.

The experiment with 10% salt and sugar was in the same line to the previous one, where the concentration of salt and sugar was raised to 10% and starter-mix was added. The dynamics of bacterial proliferation was found to be almost same to that of S-SMS5% and the total fermentation period for attaining the final stage remained

same, that is, 30 days (Figure 4). The product quality here was remarkably inferior to S-SMS2% ($p>0.05$) (Table 7). The explanation of this can be stated as the high concentration of food additives like sugar, promoted a different type of fermentation, which entirely changed the concept of *Shidal* fermentation. Among all, S-SMS2% showed the best results, having no significant difference with the traditional *shidal* and it was given even higher sensory scores than many traditional products collected from dry fish market. The overall acceptability of this product did not differ significantly ($p<0.05$) with the best quality traditional *shidal* (Table 7).

The biochemical properties of traditional *shidal* (S-T) (Plate 7) and newly prepared *shidal* (S-SMS2%) (Plate 8) have been showed in Table 8. The properties do not differ significantly ($P>0.05$) except acid insoluble and TBV-N content. Probably the hygienic preparation method practised in laboratory ensured less inclusion of sand particles and foreign materials resulting in less acid insoluble ash in modified *shidal*. Frequently handling for sampling might be one of the reasons for increased TVB-N in the new product. Hence, the biochemical properties have also been maintained in the new product.

Conclusion

A method with a maturation period of only two months could be obtained. This method, if commercialized, can contribute immensely to the annual production of *shidal* in the northeastern region of the country. At present, commercial and traditional *shidal* producers can produce only two batches per year due to the long maturation period which is about 4 to 6 months. As a result there is short supply of *shidal* in the local markets leading to the high price and sometimes it escalates to more than INR 600.00 kg^{-1}. We are still engaged in further refinement of the technology with starter culture with different combination and food additives.

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