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An accelerated process for fermented fish (*seedal*) production in Northeast region of India

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ABSTRACT

Seedal is a traditional semi-fermented fish product of Northeast India. It is prepared with minor carp belonging to the genus *Puntius* which is endemic to the region. Fish is fermented in specially designed earthen pots normally for 5–6 months. This restricts the production to only 2 batches per year. Two predominant bacteria from fresh *seedal* and in-process study were isolated and identified as coagulase-negative *Staphylococcus aureus* and *Micrococcus varians*. A mixed starter culture of these 2 organisms was incorporated to the raw material during processing along with 2 food additives, i.e. common salt and sugar in different concentrations and combinations for accelerating the process. The accelerated method could be achieved by using 2% salt, 2% sugar and 100 ml bacterial inoculum (10^8cell/ml) of *S. aureus* and *M. varians* in traditional method and the fermentation period could be reduced to 45 days without losing the basic biochemical, microbiological and sensory characteristics of traditional best quality *seedal*. The quality of new *Seedal* was evaluated by 10 judges drawn from regular *seedal* eater. The scores given in 10 point hedonic scale were statistically analyzed. No significant difference was observed in comparison to the best quality traditional *seedal*.The storage characteristics of new *seeldal* and the traditional *seedal* were studied with different concentrations of salt in polythene bags at room temperature. The new *seedal* could be stored with 10% salt for 15 days keeping acceptable quality and the traditional *seedal* could be stored for 10 days at same conditions.

Key words: Fermented fish, *Micrococcus, Puntius, Seedal*, *Staphylococcus*

Seedal is a sticky-solid fermented product in which the shape of the fish (*Puntius* spp.) remains almost intact. The preparation methods of *Ngari* (a similar product of Manipur) and *hentak* (a paste fermented product of Manipur) were described by Sarojnalini and Vishwanath (1988). In Bangladesh, the use of freshwater minor carp *puti* (*Puntius* spp.) was reported to be used in the production of semi-fermented product locally called *Sheedal Shutki* or *Chapa shutki* (Mansur *et al.* 2000) and the volatile components of it was analyzed by Khanum *et al.* (2001). *Ngari* and *tungtap* (similar product of Meghalaya) were studied and lactic acid bacteria were isolated (Thapa *et al*. 2004, 2007). In the present study a starter culture with convenient food addtitives was aimed at to develop for reducing the total fermentation period of *seedal* production.

MATERIALS AND METHODS

The traditional *seedal* samples were collected aseptically in sterile polyethylene bags immediately after opening the *mutka* (earthen pot) from the *Seedal* production centers in Assam. *Seedal* was prepared in the laboratory following the traditional method (Fig. 1) and an in-process study was done at monthly intervals to record the bacterial activity and stages of fermentation (Table 1). A preliminary study for earmarking the best quality *seedal* in terms of resident bacterial flora, pH, titratable acidity, physical appearance, color and texture was done by following the traditional method (Table 2).

The objective was to prepare *seedal* with a shorter fermentation time with primary qualities similar to that of traditional S4 *seedal* (Table 1). For isolation of resident bacterial flora, standard method was followed (APHA 1998). Preliminary bacterial identification up to genus level was made following the identification scheme given by Le Chevallier *et al*. (1980). Isolates were identified and enumerated for *Micrococcus varians* by the method given by Kloos (1976) and for *Staphylococcus aureus* Baird-Parker (1969) method was adopted (Table 3). Species characterization was done as per Bergey's Manual (1986). The identified bacteria were stock-cultured in the TSA slants and sub-cultured every fortnight. These 2 bacteria were grown in trypticase soy broth, each in separate conical flasks and

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Table 1. Different stages of fermentation of traditional *Seedal* (mean±SD, n=10)

Name of the Unit	Fermentation period (Days)	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	30	7.7×10^5	1.21± 0.01	6.5 ± 0.3	Moist with moderately soft surface, hard texture, almost no colour change, no characteristic *seedal* smell	S1 (initiation of fermentation)
C	60	3.7×10^5	1.46± 0.01	6.2 ± 0.2	Moist with moderately soft surface, silvery white colour disappears, moderately hard texture, mild putrefied smell	S2 (highest fermentation activity period)
D	90	2.4×10^5	1.75 ±0.02	5.8 ± 0.2	Moist with soft and sticky surface, colour changes to pale brown, characteristic *seedal* smell moderately prominent, texture starts softening	S3 (Pre-ripened, low quality, under-fermented but acceptable)
E	120	2.7×10^4	1.78 ±0.05	5.7 ± 0.2	Moist and sticky surface, dark reddish brown colour, characteristic strong *seedal* smell, moderately soft texture with shape of the fish intact	S4 (Ripened, Best quality *seedal*)
F	150	7.5×10^3	1.84 ±0.04	5.4 ± 0.2	Moist with very soft surface, colour dark brown to black, texture very soft, shape of the fish distorted, characteristic strong *seedal* smell prevails	S5 (Post-ripened, low quality, over-fermented but acceptable)
D	180	2.6×10^3	1.98 ±0.05	5.2 ± 0.2	Moist with very soft surface, colour dark brown to black, the product almost turns to paste, characteristic strong *seedal* smell no longer prevails, deterioration starts	S6 (Degraded, highly over-fermented, poor quality, unacceptable)

incubated at room temperature (35°C) for 48–72 h. The cells were collected by centrifuging at 8000 rpm at room temperature for 10 minutes and then washed and diluted in sterile physiological saline (0.85% NaCl). The number of cells / ml saline (cfu/ml) was estimated by total plate count (TPC) (APHA 1998) and the concentration was kept as 10^8 cells/ml.

For accelerating the traditional method, 16 sets of *mutka,* each set consisting of 4 units of 3 kg capacity *mutka*s 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 e.g. *Seedal*. Traditional was denoted as S-T, *Seedal* with 2% salt as S-Sa2%, *Seedal* with 5% salt as S-Sa5% etc. (Tables 4, 5). The

Table 2. Determining characteristics of a normal good quality *Seedal*

Attributes	Characteristics
Appearance	Moist with very soft surface, sticky on touch
Colour	Dark reddish brown
Texture	Moderately hard texture
Odour	Typical strong *Seedal* odour
pH	5.7±0.2
Titratable acid level	1.78±0.05 (%)
TPC	$10^3 – 10^6$ (CFU/g)

Table 3. Occurrence of *Micrococcus varians* and *Staphylococcus aureus* in traditional *Seedal*

Fermentation Days	TPC[1] (CFU/g)	*M. varians*[2]		*S. aureus*[3]	
		Total Count	(%)	Total Count	(%)
0	1.1×10^2	UD	-	UD	-
30	7.7×10^5	2.1×10^2	0.0	1.4×10^3	0.0
60	3.7×10^5	1.1×10^5	29.7	2.4×10^5	64.8
90	2.4×10^5	4.2×10^4	17.5	1.6×10^5	66.6
120	2.7×10^4	2.2×10^2	0.0	1.4×10^4	51.8

UD – Undetectable by spread plate technique.[1-]TPC was done on Trypcase Soy Agar by spread plate technique.[2-] Circular, slightly convex, slightly yellow, smooth and glistening colonies with entire margin on Simmon's Citrate agar was counted (Kloos, 1976).[3-] Black or grey, convex colonies with >5 mm diameter with entire margin and clear zone on Baird Parker (BP) agar was counted (Baird-Parker, 1969)[1, 2,3] were done simultaneously from the same sample.

standardized traditional method was modified for experimental *seedal* preparation (Fig. 2) and all these sets were prepared by this method.

Sampling, in each *mutka*, was done in 3 strata such as top layer, center layer and bottom layer at 15 days interval. A *mutka* once opened was not used for further research to ensure equal and uniform anaerobic condition before opening. In-process changes in bacterial number were studied for S-SMS2%, S-SMS5% and S-SMS10%, since the fermentation period for these three products was promisingly less (Table 6).

For oil processing, the inner surface of the *mutka* was smeared with mustard oil and allowed to sun dry under. This process was continued till the *mutka* became fully saturated with oil. As the *mutka* absorbed the oil and became dry, another smear of oil was given in the same way and again dried. This oil-smearing and subsequent drying process was continued for about a week, until they become fully saturated with oil and unable to absorb any more oil even after a fresh drying. For packing, a *mutka* was fixed in a thermo-coal board tightly in vertical position. Now packing was done by spreading the fishes in a layer inside the *mutka* and uniform pressure was applied with hand after wearing gloves. Once the layer gets tightly packed, subsequent layers are put in a similar manner up to the mouth region.

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 *seedal* eaters who gave the scores for appearance, colour, texture and odour after doing physical observation of raw *seedal*. They were also requested to taste *seedal* in the form of *seedal bhorta* with rice. *Seedal bhorta* was prepared with precise recipe Ingredient (Table 7). The mean sensory score for each attribute with standard deviation was adopted.

The sensory scores for all 17 products were recorded and subjected to statistical analysis (Table 8). The SPSS (2000) statistical package was used for analysis of the experimental results. The data were analyzed as per analysis of variance to calculate the significant difference ($P<0.05$) (Snedecor and Cohran 1967).

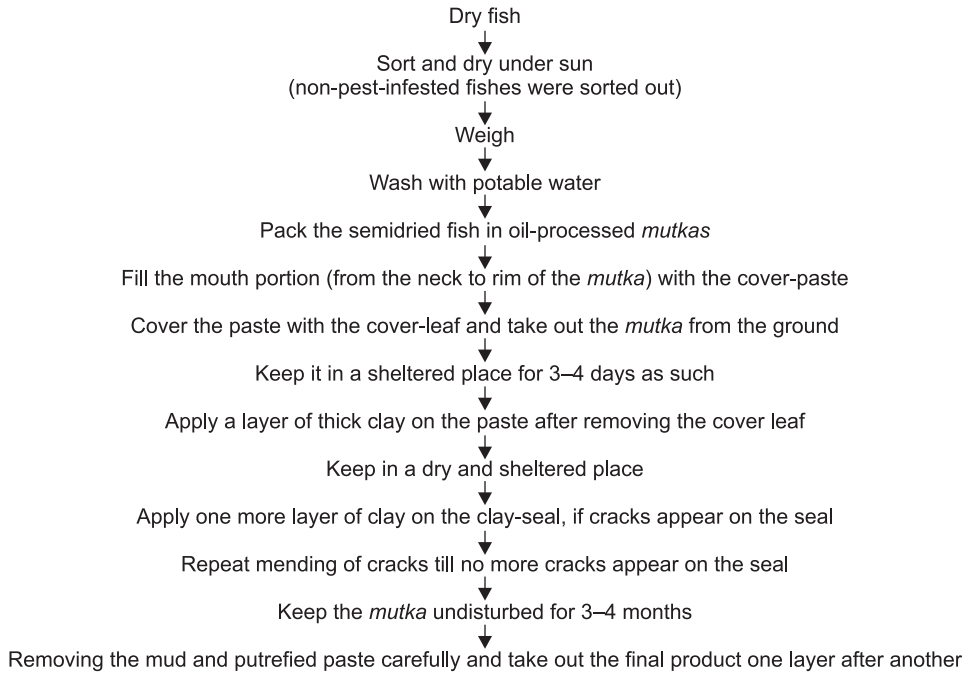

Fig 1. The standardized traditional method of *Seedal* preparation

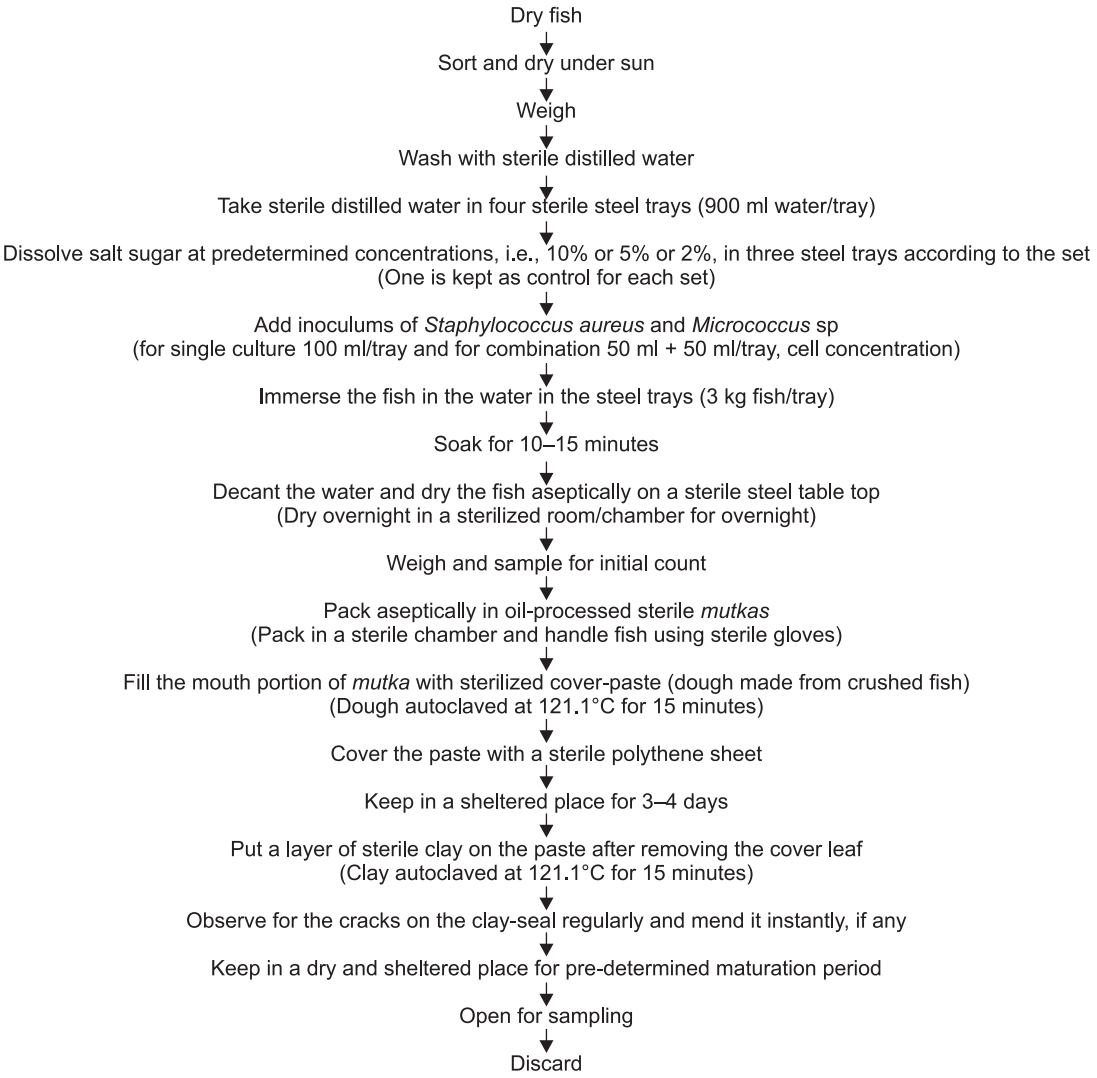

Fig 2. The method of *Seedal* preparation used in laboratory experiments

For storage study *seedal* was packed in polyethylene bags with salt or without salt and stored at room temperature. The quality of the product was examined at 5 days interval. Table salt was dusted on *seedal* at 10: 1 ratio before packing which is commonly done before retailing in the dry fish markets. The quality examination was based on physical appearance, smell and sensory score by the expert panel. The biochemical analysis such as pH, peroxide value, free fatty acid content, α-amino acid, titratable acid level, non protein nitrogen and TPC were carried out to see level of degradation (Table 9). Loss of characteristic *seedal* smell was considered to be the prime criterion for quality deterioration.

Moisture, protein, pH, free fatty acid, α-amino nitrogen, titratable acid level, non protein nitrogen (NPN), total volatile nitrogen (TVN) and peroxide value (PV) were estimated following AOAC (1995).

RESULT AND DISCUSSION

Seedal prepared by the traditional method (Fig.1) was perfectly ripe in S4 stage which was achieved after 120 days of fermentation (Table 1). S4 *seedal* was kept as target *seedal* in further studies and quality of experimentally prepared *seedal* was compared with it by an expert panel (Table 2).

Microbiological studies were carried out at every stage from R to S6 (Table 1) and most of the isolates were found to be *M. varians* and *S. aureus* (coagulase-negative). After second month of fermentation, *M. varians* occurred 29.7% of the total count and *S. aureus* occurred 64.8%, whereas the count decreased to 17.5% and increased slightly to 66.6% for these two bacteria respectively after third month of fermentation (Table 3). The findings of Thapa *et al*. (2004)

Table 4. Sampling schedule for in process studies of different types of *Seedal*

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

ND, Not detectable by spread plate technique.

supports this findings which showed the presence of *S. aureus* and *Micrococcus* spp. in traditional fermented fish products of North-east India. Maximum fermentation occurred in initial 3 months of fermentation in terms of highest microbial activity combined with desirable pH and titratable acidity change and organoleptic changes (Table 1). Thus, it was obvious that these 2 bacteria have direct or indirect role in fermentation. The results are in lined with the findings of Anihouvi *et al.* (2007) which showed that the predominant organisms isolated from naturally fermented cassava fish were gram positive *Micrococcus* spp. *Bacillus* spp. and *Staphylococcus* spp. Since these 2 bacteria can ferment glucose and have a better growth in saline condition (Kloos 1976), it was assumed that an external supply of sugar and salt in certain concentrations would lead to instant proliferation and uniform growth of these two bacteria in all the strata inside the *mutka* just after packing. Simultaneously, the introduction of starter mixture of *M. varians* and *S. aureus* indeed would initiate the fermentation process instantly (Mabesa *et al*. 1989). In traditional method, eventually the number of resident fermenting bacteria was insufficient soon after packing due to vigorous sun-drying and would require a certain amount of time to grow sufficiently for initiating the fermentation process. These 2 bacteria in the raw material were undetectable by spread plate technique (Table 3).

To reach S3 stage, the traditional method took more than 90 days, and when *seedal* was prepared with the inoculum of single starter i.e. either *S. aureus* or *M. varians* and without any additive, S3 could be attained after 60 days for *S. aureus* and after 45 days for *M. varians* and advanced S3 stage could be attained after 45 days of fermentation when both the cultures were used in equal proportions (Table 5). In a separate experiment with *Lactobacillus* (isolated from curd) inoculum, S3 could be attained after 15 days of fermentation, but its quality was poor having significantly different overall acceptability with traditional *seedal* ($P<0.05$) (Tables 5, 8)). Saithong *et al*. (2010) could accelerate the fermentation process with *Lactobacillus* starter in Thai plaa-som which supports the present findings. Gelman *et al.* (2001) evaluated the efficiency of lactic acid bacteria as starter culture in fermented fish products and found similar results. 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 *seedal* altering its traditional identity in terms of texture, flavour, colour and smell.

The stage-S3 could be attained after a maturation period of 15 days only, when bacterial starter of *S. aureus* and *M. varians* along with 2% salt and 2% sugar were added to the raw material. The initial count in the raw material remained almost same in the top layer after 30 days and gradually decreased in the layers beneath and bottom layer respectively. The bacterial counts gradually decreased with the increasing time of fermentation in all strata, while the top layer always had the highest count (Table 6). All the other criteria matched with S3 stage except the TPC. 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

Table 5. Total fermentation time required for each product to attain pre-ripened stage (S3) and respective end-product microbiological and chemical quality

Products	Total fermentation time required (Days)	TPC (cfu/g)	Titratable acid level (%)	pH	Remark
S-Traditional	90+	2.4×10^5	1.75±0.02	5.8±0.2	(S4)
S+Salt2%	75+	8.6×10^4	1.58±0.02	6.7±0.2	(S3)
S+Salt5%	75+	4.7×10^4	1.58±0.02	6.7±0.2	(S3)
S+Salt10%	75+	1.0×10^5	1.59±0.02	6.4±0.2	(S3)
S+Sugar2%	75+	1.8×10^5	1.65±0.02	6.3±0.2	(S3)
S+Sugar5%	75+	4.7×10^5	1.64±0.02	6.3±0.2	(S3)
S+Sugar10%	75+	2.8×10^5	1.67±0.02	6.3±0.2	(S3)
S+Salt 2% +sugar 2%	60+	2.7×10^5	1.48±0.02	6.5±0.2	(S3)
S+Salt5% +sugar 5%	60+	4.7×10^4	1.54±0.02	6.5±0.2	(S3)
S+salt 10% +sugar10%	60+	2.1×10^5	1.68±0.02	6.3±0.2	(S3)
S+Micrococcus	45+	2.6×10^6	1.18±0.02	6.8±0.2	(S3)
S+Staph.	60+	3.5×10^6	1.22±0.02	6.8±0.2	(S3)
S+Staph.+Micro.	45+	3.6×10^6	1.30±0.02	6.7±0.2	(S3)
S+Lactobacillus	15+	3.7×10^5	1.81±0.02	5.5±0.2	*
S+Salt 2% +sugar 2%+ Staph.+Micro.	15+	1.6×10^8	1.78±0.02	5.6±0.2	(S4)
S+Salt 5% +sugar 5%+ Staph.+Micro.	60+	2.5×10^8	1.66±0.02	5.9±0.2	(S3)
S+salt 10% +sugar 10%+ Staph.+Micro.	45+	1.3×10^8	1.78±0.02	5.7±0.2	(S3)

*Sensory score below 5.

Table 6. The in-process bacterial profile for the SMS2%, SMS5%, SMS10%

Set	Fermentation period (days)	TPC (CFU/g)				Remark
		Top	Centre	Bottom	Average	
S-SMS2%	0				4.6×10^8	(R)
	15	1.1×10^7	1.0×10^6	1.8×10^6	4.6×10^6	(S3)
	30	4.1×10^8	7.1×10^7	8.9×10^6	1.6×10^8	(S3)
	45	3.2×10^8	1.9×10^7	5.9×10^7	1.3×10^8	(S4)
	60	5.5×10^7	6.7×10^6	4.2×10^6	2.2×10^7	(S4)
	75	7.8×10^6	5.0×10^6	2.9×10^6	5.2×10^6	(S5)
S-SMS5%	0				1.1×10^7	(R)
	30	6.8×10^8	6.5×10^7	5.9×10^6	2.5×10^8	(S2)
	45	3.9×10^8	7.9×10^7	4.5×10^7	1.7×10^8	(S3)
	60	2.3×10^7	3.7×10^6	6.4×10^6	1.1×10^7	(S3)
	75	9.2×10^6	4.4×10^5	7.2×10^5	3.5×10^6	(S4)
S-SMS10%	0				2.3×10^7	(R)
	30	3.6×10^8	4.6×10^7	9.9×10^5	1.4×10^8	(S3)
	45	4.5×10^7	7.8×10^6	3.8×10^5	1.8×10^7	(S3)
	60	3.8×10^6	3.7×10^6	7.0×10^4	2.5×10^6	(S4)
	75	8.2×10^5	7.3×10^5	6.1×10^4	5.4×10^5	(S4)

S-SMS 2%, *Seedal* with 2% salt, 2% sugar, *Micrococcus* and *Staphylococcus;*S-SMS 5%, *Seedal* with 5% salt, 5% sugar, *Micrococcus* and *Staphylococcus*; S-SMS 10%, *Seedal* with 10% salt, 10% sugar, *Micrococcus* and *Staphylococcus*; bold row, *Seedal* has attained its final stage.

fermentation period further increased, more degradation of quality was recorded. This findings match with the findings of Thapa *et al.* (2007) in which a increased number of bacteria was found in the end products of several fermented products from North-east India.

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 S3 stage by 15 days of fermentation (Table 5). Interestingly, the product quality neither improved much, nor deteriorated as the fermentation time increased. Moreover, the product had maximum resembling characteristics of traditional *Seedal* in terms of appearance, colour, taste, texture and odour and had no significant difference ($P>0.05$) with the traditional *seedal* (Table 8). The reasons for these

Table 7. Ingredients for recipe of *seedal bhorta*

Spices	Amount (g)
Seedal	250.0
Red/green pepper	10.0
Onion	150.0
Garlic	25.0
Ginger	25.0
Salt	5.0
Oil	50.0

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. In support of this results, the work of Whiting *et al.* (1984) showed that reducing the level of salt by 60% to 1.5% resulted in a more rapid growth in natural flora of frankfurters. Reducing the salt level by 50% to 1.25% in ground pork resulted in slight increases in the growth of *Lactobacillus* spp. (Terrell 1983). Thus, the large population of *S. aureus* and *M. varians* might have not allowed other undesirable bacteria to proliferate sufficiently to slow down fermentation and deteriorate the quality. In the same line Mah and Hwang (2009a) used *S. xylosus* as starter culture to inhibit formation of biogenic amines by other groups of bacteria in salted fermented anchovy.

The total fermentation time was 30 days for reaching S3-stage when *Seedal* was prepared with *S. aureus* and *M. varians* inoculum along with 5% salt and 5% sugar. Though higher count of bacteria was encountered in all the strata, the quality of the product was inferior to S-SMS2% ($P<0.05$) (Tables 6, 8). 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 *seedal* (Table 8). The possibility of over fermentation and undesirable chemical activity in presence of higher concentration of food additives may be the reason for this. Similar findings were recorded by Mah and Hwang (2009b) that food additives could inhibit formation of biogenic amines in salted fermented anchovy and thus controlled the growth of bacteria. The experiment with 10% salt and sugar and with starter-mix could reduce the fermentation period to 30 days (Table 5). However, the product quality here was remarkably inferior to S-SMS2% ($P<0.05$) (Table 8). One explanation of this can be the high concentration of food additives like sugar, promoted a different type of fermentation, which entirely changed the kinetics of fermenting bacteria in *seedal* fermentation.

Among all, S-SMS2% showed the best results and the overall acceptability of this product did not differ significantly ($P>0.05$) with the best quality traditional *seedal* (Table 8).

When stored without salt, the traditional *seedal* (S-T) was good for consumption up to 5 days of storage and and after storage of 10 days its quality deteriorated to an unacceptable level. When stored with 10% salt, its shelf life increased to 10 days. Salt solubilizes the functional myofibrilar proteins in meat and activates the proteins to increase hydration and water-binding capacity, ultimately increasing the binding properties of proteins to improve texture (Desmond 2006).

Seedal S-SMS2%, when stored in polyphone bags with

Table 8. Sensory scores of *Seedal* on 10 point hedonic scale (mean±SD, n=10) with 45 days fermentation period

Attributes / Products	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+salt 2%	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+salt 5%	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+salt 10%	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+sugar 2%	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+sugar 5%	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+sugar 10%	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 10-point scale (1, least and 10 being the most). Means in a column with the same superscript letters are not significantly different ($P>0.05$).

Table 9. Storage characteristics of S-T and S-SMS2%

Product	Salt conc. (%)	Storage period (days)	Overall acceptability*	pH#	Moisture# (%)	Peroxide value#(milli eq./kg of oil)	Free fatty acid# (% of oleic acid)	α-amino Ntrogen# (%)	Titratable acid level# (%)	NPN# (%)	TVN# (mg%)	TPC# (cfu/g)
S-T	0	0	9.3±0.2[a]	6.3	15.0±1.5	9.35±1.2	6.48±0.33	16.75±0.11	1.75±0.02	8.62±0.5	560	2.7×10^6
		5	7.5±0.2[b]	6.2	14.0±1.5	15.45±1.2	17.47±0.33	27.75±0.11	1.80±0.02	9.62±0.5	570	1.5×10^3
		10	5.5±0.2[c]	6.2	12.0±1.5	18.45±1.2	38.57±0.33	49.75±0.11	1.95±0.02	11.72±0.5	670	1.8×10^4
		15	3.5±0.2[d]	ND	ND	ND	ND	ND	ND	ND	ND	ND
		0	9.3±0.2[a]	6.5	15.0±1.5	7.02±1.2	5.31±0.33	17.02±0.11	1.75±0.02	8.62±0.5	560.00	1.3×10^2
		5	8.4±0.2[ab]	6.7	14.3±1.5	10.11±1.2	22.32±0.33	29.21±0.11	0.68±0.02	7.12±0.5	510.00	4.2×10^2
	10	10	7.8±0.2[b]	6.7	13.0±1.5	20.71±1.2	35.48±0.33	44.66±0.11	0.67±0.02	9.12±0.5	570.00	1.1×10^3
		15	5.8±0.2	ND	ND	ND	ND	ND	ND	ND	ND	ND
S-SMS2%	0	0	9.4±0.2[a]	6.0	19.42±1.5	18.51±1.2	6.28±0.33	15.85±0.11	1.58±0.02	9.42±0.5	540.20	1.6×10^8
		5	8.5±0.2[ab]	6.2	18.50±1.5	25.15±1.2	26.77±0.33	16.85±0.11	1.45±0.5	9.82±0.5	570.00	2.5×10^3
		10	7.4±0.2[b]	6.5	17.67±1.5	19.15±1.2	37.27±0.33	17.75±0.11	1.55±0.5	10.72±0.5	680.00	3.2×10^5
		15	5.4±0.2[c]	ND	ND	ND	ND	ND	ND	ND	ND	ND
	10	0	9.4±0.2[a]	6.8	19.42±1.5	8.21±1.2	6.28±0.33	15.85±0.11	1.58±0.02	9.42±0.5	540.20	1.1×10^2
		5	9.1±0.2[a]	6.3	18.12±1.5	12.70±1.2	24.17±0.33	24.85±0.11	1.05±0.5	8.82±0.5	470.00	6.5×10^2
		10	8.5±0.2[ab]	6.3	17.67±1.5	17.15±1.2	34.20±0.33	34.75±0.11	1.58±0.5	7.72±0.5	480.00	7.2×10^2
		15	7.5±0.2[b]	6.1	16.11±1.5	28.15±1.2	54.57±0.33	64.75±0.11	3.55±0.5	7.95±0.5	496.00	3.2×10^4

*All traits measured in 10 point scale (1, least; 10 being the most keeping typical *seedal* smell as prime concern) mean±SD, n=10. Means in the column with the same superscript letters are not significantly different (P>0.05).

#mean±SD , n,3; ND, not detected.

10% salt, it remained acceptable with a sensory score more than 6 up to 15 days of storage and biochemical parameters showed dehydration, fat oxidation and protein degradation due to microbial activity and exposure to atmospheric oxygen. When S-SMS2% was stored without salt, it was good for consumption up to 5 days of storage (Table 9). This findings agree with the findings of Terrell (1983) that salt activates proteins to increase hydration and water-binding capacity to improve texture and it is essentially increases flavour and being bacteriostatic agent it increases shelf life of meat and meat products.

Thus, after opening the *mutka* storage may not be recommended for more than 10 days in ordinary polyethylene packaging system even if salt is dusted on the surface. This degrades the quality of *seedal* by reducing the characteristic *seedal* flavour due to increased rate of fat oxidation, loss of volatile substances, putrefying activity of microorganisms etc.

In conclusion, an accelerated method (Fig. 2) with a fermentation period of only one and half month could be obtained. This technique, if transferred to commercial *seedal* producers, can contribute immensely to the annual production of *seedal* in the northeastern region of the country. The traditional method restricts the production to only one batch per year due to the long fermentation period i.e. 6 months. As a result supply of *seedal* is less than the demand in the markets of northeast region of the country which leads to the high price of *seedal* approximately ₹ 400–450/kg. The present invention probably has the ability to bridge up this gap reducing the exorbitant price of *seedal*. It may also promote new entrepreneurship amongst the unemployed youths, women and weaker sections.

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