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Post-Fermentation Preservation of *Shidal*- a Fermented Fish Product of Northeast India

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Abstract

Shidal is a popular traditional semi-fermented fish product exclusively prepared from *Puntius* spp. Being a salt free product, it has short storage life outside the *mutka* (earthen pot) and thus traditionally preserved either by dusting with common salt during retailing or by keeping it inside *mutka* until sold in the market. Present study was conducted to develop a low cost packing and preservation method for *shidal* outside *mutka* using different concentrations of salt in glass bottles. *Shidal* was packed in glass bottles after sprinkling with salt (2% and 5%) and stored at ambient temperature (18-34^{0}C) for 120 days. Parameters related to microbial, biochemical and sensory changes were analyzed at 15 day intervals from August to December 2010 and the quality was compared with the control (without salting) to see the periodic quality loss. The 2% salt treated *shidal* (T1) showed comparatively stable pH (4.6 to 4.8) with negligible variation throughout the storage period while in control and 5% salt treated *shidal* (T2), pH increased significantly ($P<0.05$) after 75 days of storage and reached 7.09 from an initial pH of 4.4. Moisture, ash, salt, protein and fat content of all the samples did not vary significantly ($P>0.05$) during the storage period upto 60 days indicating insignificant effects by the treatments on proximate composition. All the parameters increased significantly ($P<0.05$) during storage in all the samples indicating hydrolysis and degradation of protein. But the treated samples had slower rate of increments in the non-protein nitrogen, free á-amino nitrogen and total volatile base nitrogen compared to the control. Similarly, the peroxide value, free fatty acid and thiobarbituric acid showed slower rate of increments and insignificant changes in the treated samples than in the control. Total plate count did not change significantly ($P>0.05$) and remained near 7 Log cfu g^{-1} and Total fungal count was low in almost all samples. The sensory scores indicated longer shelf life of 90 days for T1 and T2 while the control had shelf life of 60 days in glass bottles.

Keywords: *Shidal*, fermented fish, *Puntius* spp., storage-characteristics, salt

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Introduction

Shidal is one of the fermented fishery products, highly preferred in North-East region of India. The product was developed traditionally by the local fishers in pre-British era (Muzaddadi & Basu, 2003a). This fish product is liked by fishers, plain tribes, non-tribes (Bangla speaking community), hill tribes and daily wage workers as a condiment with everyday meal. It is a sticky, solid, semi-fermented, salt free product prepared from small carps (*Puntius* spp.) normally in dried form by fermenting in *mutka* (earthen pot specifically designed and made for *shidal* preparation). Similar products have also been reported in Bangladesh as *Chepa Shutki* (Nayeem et al., 2010; Mansur et al., 2000; Khanum et al., 2001) and in other North eastern states of India *viz*., in Assam as *seedal* or *hidal* (Muzaddadi & Basu, 2003a; 2003b), in Manipur as *Hentak* and *Ngari* (Sarojnalini & Viswanath, 1994; Thapa et al., 2004) and as *Tungtap* in Meghalaya (Thapa et al., 2004). All the authors reported traditional methods of preparation for different fermented fish products, but none of them have reported on the aspects of post-fermentation preservation of the product.

Shidal needs to be taken out of commercial *mutka* (30-40 kg capacity), once its normal fermentation and maturation period (4-6 months) is over and it quickly loses its characteristic odour and flavour thereafter. However, it is instantly wrapped in old newspapers or polythene after dusting with approximately 5% common salt while retailing (post-fermentation or ex-situ preservation), or otherwise it is kept inside *mutka* until sold in the market (in-situ preservation). The practice of addition of salt is believed to be effective in preserving the characteristic flavor of *shidal*. Hence, there is a need of a scientific study to analyze the effects of salt in ex-situ preservation of *shidal* to retain its typical flavour for longer period of storage, since the in-situ preservation cannot be extended due to the risk of over-fermentation. The findings may help develop an effective ex-situ preservation technique for the product. Thus, in the present study, two concentrations of NaCl i.e., 2% and 5% were used and the effects were analyzed at ambient temperature (18-34^{0}C) during 120 days of storage.

Materials and Methods

Required amount (300 -500 g) of good quality *shidal* with sensory score 4-5 (Table 1 & 2) were collected aseptically in sterile polyethylene bags immediately after opening the *mutka* from the production centers at Agartala, Tripura (India). After dusting with 2% and 5% salt, *shidal* was packed in glass bottles with screw caps (2 kg capacity) and stored at ambient temperature (18-34°C) for the storage study. Treatments were coded as *shidal* with 2% salt (T1), *shidal* with 5% salt (T2) and control, i.e. *shidal* without salt (C). Fortnightly sampling was done for ascertaining changes in different microbiological, biochemical and sensory parameters during storage. The study was continued for 120 days during Aug-Dec 2010.

Total plate count (TPC) and Total fungal count (TFC) were determined by spread plate techniques (APHA, 1995). Exactly 10g of *shidal* sample was introduced aseptically in a sterile stomacher bag (Seward stomach BA6141CPG standard bags) and blended for 2 min with 90 ml of sterile diluents (0.85% NaCl for TPC and 0.1% peptone water for TFC) using a stomacher (Seward stomacher 400 circulator, England). Serial decimal dilution of 10^{-2} was prepared using 9 ml sterile physiological saline and 1 ml homogenized sample, and it was well mixed in cyclomixer. For TPC, 0.1 ml portions of various dilutions were spread on the surface of preset surface-dried Soybean Casein Digest Agar/ Trypticase Soya Agar (TSA) (MU290, Himedia, India) plates and for TFC, same portions were spread on the surface of preset surface-dried Rose Bengal Chloramphenical agar (M640, HIMEDIA) plates using a sterile glass spreader. For TPC, plates were incubated at 35°C for 24-48 hours, while fungal plates were incubated at 25°C for 3-5 days. Representative samples of the product were inoculated directly (0.1 ml without dilution) onto replicate plates of brain heart infusion (BHI) agar (M211, HIMEDIA) with different concentrations of NaCl such as BHI + 5% NaCl, BHI + 10% NaCl, and BHI + 20% NaCl in order to see the growth of halophilic/halotolerant bacteria. These plates were incubated at 35°C for 24-48 hours. The plates containing 25-250 colonies were counted for TPC and plates containing 15-150 colonies were selected for TFC.

The samples were analyzed in triplicate for moisture, ash, salt pH, free fatty acid (FFA), lipid, protein and non-protein nitrogen (NPN) by standard methods of AOAC (2000); total volatile base nitrogen (TVB-N) content was determined according to Conway's micro-diffusion method (Conway, 1947); thiobarbituric acid (TBA) value by the method given by Tarladgis et al. (1960); peroxide value (PV) was estimated according to Jacob (1958); and the free á-amino nitrogen (AAN) was analyzed by method of Pope & Stevens (1939).

50g of *shidal* was used per package for sensory evaluation by an expert panel of 10 judges and they were requested to give scores in 5 point scale after seeing, feeling and smelling the product (Nayeem et al., 2010). The overall acceptability was calculated by taking arithmetic average from the scoresheet (Table 1).

All bacteriological counts were converted to $\log_{10}$ cfu g^{-1} for statistical analysis. Statistical analysis was done by performing one way ANOVA (Post Hoc, Duncan) and student's t-test to compare the means at 5% level using SPSS 15.0 software (SPSS for Windows. Release15.00. Chicago: IL: SPSS Inc, 2005).

Results and Discussion

The TPC showed significant differences (P<0.05) during the storage period (Fig. 1). It showed initial decrease and subsequently increased after 45th day of storage in all three samples. Although the salted sample (T2) showed relatively lower counts compared to the control, salting at a rate of 2 and 5%

Table 1. Quality scores for the sensory evaluation of *shidal*

Quality attributes	Characteristics	Sensory scores	Quality
Appearance	Bright, moist and shining surface	5	Excellent
	Slight dullness with shining surface	4	Good
	Dull with soft surface	3	Fair
	Definite dullness with soft surface	2	Average
	Dry fish like, loss of weight, fungal growth	1	Poor
Colour	Dark brownish or slight yellowish colour	5	Excellent
	Gray yellowish or black colour	4	Good
	Pale brown or gray colour	3	Fair
	Colour become fade and off-white	2	Average
	Whitish colour	1	Poor
Odour	Strong characteristic *Shidal* odour	5	Excellent
	Light characteristic *Shidal* odour	4	Good
	Slight *Shidal* odour with no sour odour	3	Fair
	Faint sour odour	2	Average
	Strong sour odour, rancid, ammonia smell	1	Poor
Texture	Firm elastic muscle, sticky surface and muscle not detached from the backbone	5	Excellent
	Soft abdomen and fairly firm muscle	4	Good
	Muscle with no elasticity and firmness	3	Fair
	Limp and flaccid muscle	2	Average
	Melting abdomen, muscle easily bruised and broken	1	Poor

had no significant difference on growth of bacteria (*P>0.05*). However, fluctuation of counts was less in samples with 2% salt (T1) and the same sample also showed a longer shelf life. All three samples showed almost equal counts (7 log cfu g^{-1}) after 45 days of storage and soon after, the sensory scores also started decreasing to around 3. This perhaps indicated that the salt concentration at the level of 2% and low initial pH were enough to control spoilage bacteria of *shidal*, because the pH increased profoundly after 45 days of storage and so did the TPC. Though the count was not detected on BHI agar plates with 10% and 20% NaCl, TPC increased on BHI with 5% NaCl, indicating growth of halotolerant bacteria rather than halophilic bacteria in the product. Unlike the Southeast Asian fermented fish products, *shidal* is a salt free product and thus the harbouring halophilic bacteria might have altered the typical flavour and taste. The findings of Achinewhu and Oboh (2002) in fermented *Sardinella* sp. agree with the findings of the present study. Similar increasing trends in TPC were reported by Sarojnalini and Suchitra (2009) in fermented *Setipinna* sp. of Manipur and by Thapa et al. (2004) in *Ngari*, *Hentak* and *Tungtap* of North east. Muzaddadi & Baiju (2003b) also reported a higher bacterial count in *Seedal* prepared with 2% and 5% salt.

Fungal counts did not follow any trend, showing nil or less than 10 counts and in rare occasions, count was more than 15 per plate. Thus, TFC were erratic, inconsistent and undetectable in all the samples. Probably, this is the sign of fungi having negligible role in spoilage of *shidal*. Occurrence of fungi, yeasts and obligate anaerobic bacteria could not be found in four fermented fish sauces of Thailand (Crisan & Sands, 1975). Thapa et al. (2004) also found a similar trend of fungal growth in fermented fish of Northeast India.

Proximate composition of shidal and *Puntius* spp. showed significant differences for all the parameters except TFC, ash and acid insoluble ash (Fig. 2 & Table 2, 3). This may be due to the effects of processing and microbial breakdown of the product. Perhaps, the water-soaking step of *shidal* preparation (Flow-chart 1) increases the moisture level in fish affecting many vital parameters such microbial growth, enzymatic activity and texture. The fragmentation of macromolecules, protein and lipids results in smaller molecules like peptides, amino acids, free fatty acids, ketones and aldehydes during fermentation, providing the characteristic odour and flavor to the final product with higher digestibility as well as nutritive value (Steinkraus, 1996). Khanum et al. (2001) analyzed the volatile components of *Chapa shutki* of Bangladesh using GC-MS and they arrived at a similar conclusion about the characteristic flavor.

The pH of *shidal* showed significant differences (P<0.05) during storage with an initial decrease till 45 days of storage and subsequent increase. The treatments showed initial higher pH with less fluctuation and pH remained acidic till the end of the storage period (Fig. 2). The initial decrease indicated the formation of organic acids by fermenting bacteria leading to acidic pH. Similar pH changes were also reported by Subasinghe et al. (1990), Abbey et al. (1994), Achinewhu & Oboh (2002) and Anihouvi et al. (2007). Nevertheless, the absence of fermentation in the later period and degradation of protein resulted in the formation of some volatile bases leading to increased pH. The effects of salt on pH were apparent after 60 days of storage, showing constant increase in pH in control samples.

1. Sort the uniform sized dried *Puntius* spp. and dry under sun for 2-3 days

↓

2. Wash in river water taking in bamboo basket and dip in water for 5-15 minutes

↓

3. Spread on a shaded floor and dry overnight

↓

4. Smear fish and earthen pots (*mutka*) with vegetable oil/fish oil and dry under sun

↓

5. Repeat step 4 for 5-10 days until mutkas become saturated with oil

↓

6. Fix the *mutka* (keeping underground up to the belly) tightly in the ground keeping it upright

↓

7. Spread a thin layer of fish inside *mutka* and press fish with bare foot in standing position so that no air gaps remain between fish

↓

8. Repeat step 7 until *mutka* is packed up to the neck

↓

9. Fill mutka from the neck to rim with fish-paste made from broken pieces of fish after grinding them in a wooden mortar

↓

10. Cover mouth with a sheet of polythene and seal with clay

↓

11. Promptly mend the cracks on the seal as and when cracks appear on it

↓

12. Incubator undisturbed for 4-6 months depending on the texture of raw material

↓

13. Remove the clay-seal and fish-paste carefully and discard

↓

14. Take out ready *shidal*

Flow-chart 1. The traditional method of shidal preparation from dry Puntius spp in Tripura

Table 2. Microbial quality and pH of *shidal* (mean ± SD, n=3)

Parameters	*Shidal*	*Puntius* sp. (dry)
pH*	4.42±0.43	7.02±0.20
Total Plate Count (log cfu g^{-1})*	7.1±0.12	5.1±0.22
Total Fungal Count (cfu g^{-1})	<10	<10

*Significantly different (*P<0.05*)

Moisture content increased initially and gradually decreased with extended storage period. The initial low pH which neutralized the change on proteins, caused decrease in water holding capacity of fish meat (Akahane & Shimizu, 1989). The brief decrease in salt content in T1 may be due to the leaching of mineral and gradual decrease in moisture content in T1 and T2. However, change in moisture was almost stable in the control (Fig. 2). Similar trends are recorded in *lona ilish,* a salted and fermented Hilsa (*Tenualosa ilisha*) (Majumdar et al., 2006) and in *chepa shutki* of Bangladesh (Nayeem et al., 2010).

Protein and protein degradation products such as NPN, TVB-N and AAN increased significantly (*P*<0.05) with storage period (Fig. 3) indicating protein degradation by microbial activity. Reduction in total nitrogen content may have occurred due to the escaping of some volatile nitrogenous compounds and thus there is reduction in protein content (Nayeem et al., 2010). Similar observation was also reported by Taira et al. (2007) during fermentation of fish sauce. The gradual and significant increase in NPN may be due to the breakdown of protein by enzymatic action (*P*<0.05). The salt in combination with low pH might have restricted the growth of bacteria and also inactivated the hydrolyzing enzymes during initial storage period. It has long been known that activities of enzymes of animal and plant origin are strongly inhibited by salts (Warren et al., 1966; Lanyi, 1974). Since the TPC increased in salt treated samples in later stages, the growth of halo-tolerant bacteria is apparent which led to production of more bacterial enzymes. This interpretation is based on the fact that halophilic microorganisms produce proteases with high stability at saturated salt concentrations or organic solvents (Akolkar et al., 2008). These enzymes might have hydrolyzed the protein, producing peptides and amino acids resulting in high NPN.

The TVB-N content was high (>200 mg %) in all the treatments and differed significantly from the control (*P*<0.05). Autolytic enzymes might have caused de-aminaton of protein resulting in the formation of volatile bases (Shenouda, 1980; Karacam et al., 2002; Srikar et al., 1993; Hernandez-Herrero et al., 1999).

The lipid content and different lipid degraded products like peroxide, free fatty acid and malonaldehyde showed significant changes (*P*<0.05) in all the treatments including control during the

Table 3. Biochemical quality parameters of *shidal* (mean ± SD, n=3).

Parameters	*Shidal*	*Puntius* sp. (dry)
Moisture (w/w %) *	34.02 ± 1.55	16.82 ± 2.25
Ash (w/w %)	13.80 ± 0.32	12.90 ± 1.02
Protein (w/w %)*	31.0 6± 0.05	62.06 ± 0.65
Fat (w/w %)*	18.87 ± 0.42	12.31 ± 0.50
Acid-insoluble Ash (w/w %)	0.53 ± 0.02	0.83 ± 0.22
Non-protein Nitrogen (w/w %)*	5.49 ± 0.24	3.55 ± 0.32
Free Alpha Amino Acid (w/w mg %)*	60.67 ± 4.67	32.14 ± 3.77
Total Volatile Base Nitrogen (mg %)*	223.67 ± 3.28	55.35 ± 2.00
Peroxide Value (meq peroxide oxygen 1000 g^{-1})*	17.03 ± 0.32	12.25 ± 0.30
Free Fatty Acid (% as oleic acid)*	20.62 ± 0.17	11.22 ± 0.47
Thiobarbituric Acid Number (mg malonaldehyde 1000 g^{-1})*	0.51 ± 0.01	0.23 ± 0.11

*Significantly different (*P<0.05*)

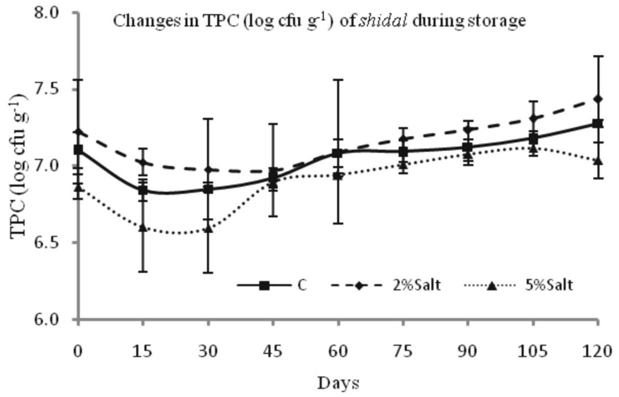

Fig. 1. Changes in Total Plate Count (TPC) of *shidal* during storage

storage period (Fig. 2 & 3). The high amount of fat may be due to the raw material i.e., *Puntius spp.* and additional fish oil which is normally used for smearing *mutka* and fish (Muzaddadi & Basu, 2003b; Sarojnalini & Vishwanath, 1994). Rahman et al. (1999) also reported a decreasing trend of lipid content in dry salted Hilsa.

Peroxide value increased in the control, but it decreased initially in the treatments primarily due to the effects of salt. Similar observations were recorded by Srikar et al. (1993).

TBA values in the present study are in the acceptable range and similar to the findings of Karacam & Boran (1996). The marginal increment in the treated

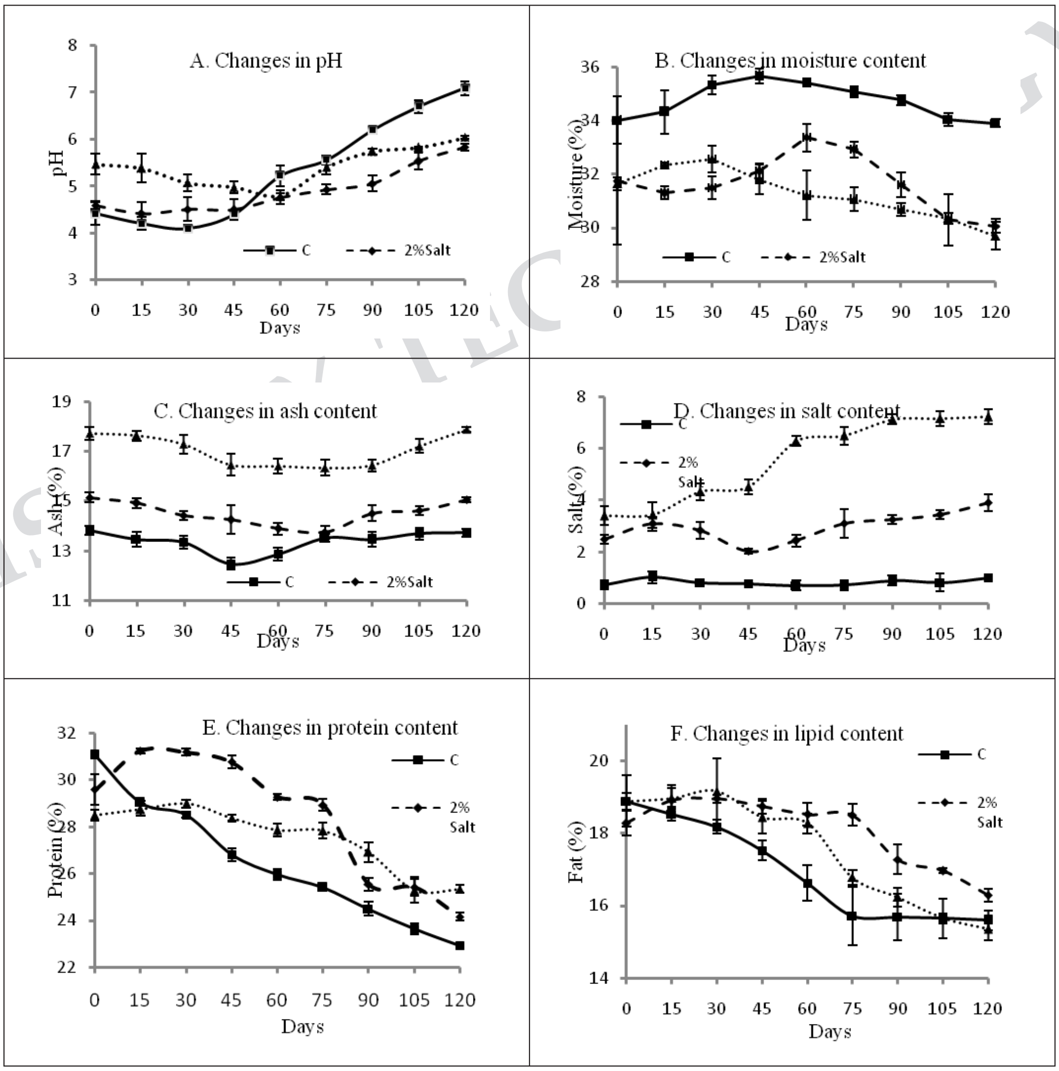

Fig. 2. Changes in proximate composition and pH of *shidal* during storage

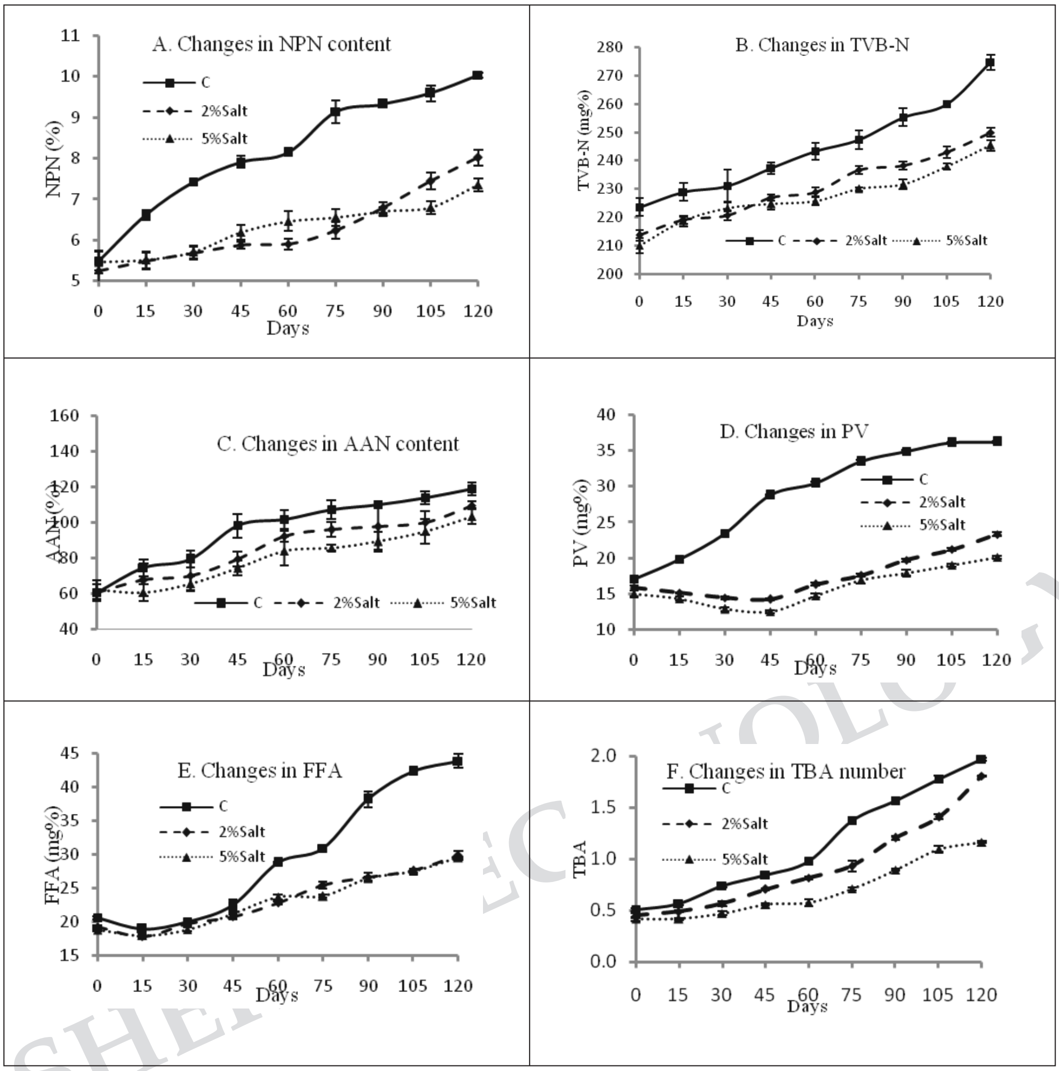

Fig. 3. Changes in NPN, TBV-N, AAN, PV, FFA and TBA of *shidal* during storage

samples may be incriminated to the effect of salt. Many authors support such effects of salt in escalating TBA values (Aubourg, 1998; Koral et al., 2010).

Since *shidal* retains the original morphological structure of the raw material (*Puntius* spp.), the sensory characteristics (Table 1) were decided on the basis of physical observation of the product along with sensory evaluation by a panel of judges. The sensory attributes such as appearance, color, texture, odor and the overall acceptability of *shidal* significantly differed ($P<0.05$) during the storage period in all the treatments including control (Fig. 4). Scores were fairly good for control (C) till 75 days and for treatments till 90 days of storage. The decreasing trends in sensory scores in different salted fish were reported by many authors (Achinewhu & Oboh, 2002; Karacam et al. 2002; Koral et al., 2010; Ozden & Erkan, 2006).

From the findings of the study, T2 (with 2% salt) is recommended as a cheapest and easy method to preserve *shidal*. Hence, post-fermentation preservation of *shidal* may be achieved by using 2% salt in glass bottles or jars in view of health concerns and for retention of characteristic *shidal* odour and texture. Further studies are necessary to determine the specific microbial succession that occurs during fermentation and storage.

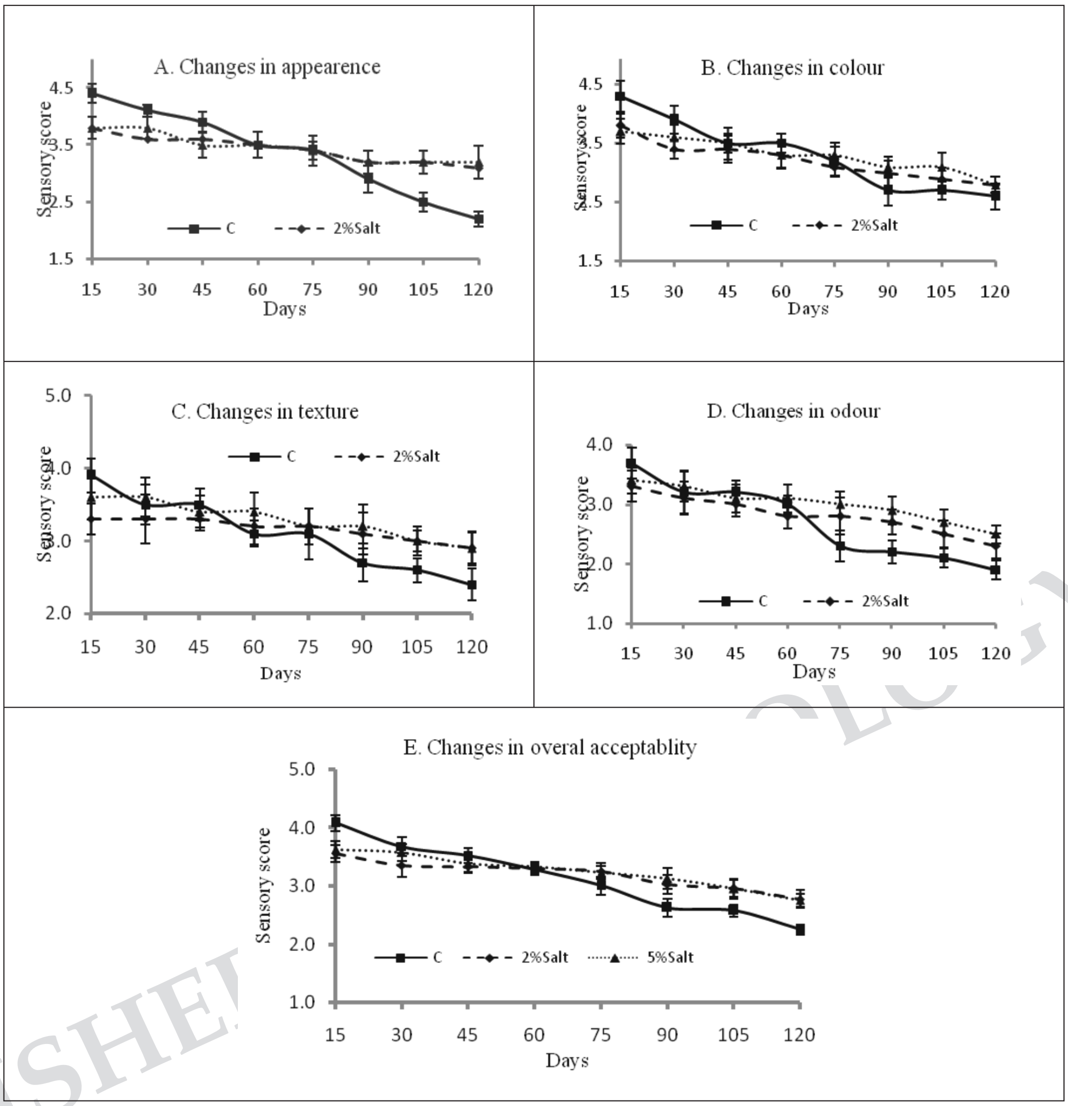

Fig. 4. Changes in sensory score (5 point scale) for different sensory characteristics of *shidal* during storage

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