

## Extension of shelflife of the fermented fish product, *shidal* by packaging in glass bottle and low temperature storage

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### ABSTRACT

*Shidal* is a very popular, salt free, semi-fermented traditional fish product of north-east India prepared using minor carps (*Puntius* spp.) in specially designed earthen pots (*mutka*). The prime quality of *shidal* is given by its characteristic aroma and flavor which is lost very fast, once it is taken out of *mutka*. A study was conducted to preserve the quality of *shidal* outside *mutka*, by packing in glass bottles and storing under refrigerated temperature. Microbiological, biochemical and sensory changes during storage period of 120 days were analysed at 15 days interval. The total plate count (TPC) did not change significantly ( $p > 0.05$ ) and remained near  $7 \log \text{CFU g}^{-1}$  during storage. The total fungal count (TFC) was negligible. The pH of *shidal* was initially acidic ( $4.42 \pm 0.25$ ) which increased significantly ( $p < 0.05$ ) towards the end of storage period. The non-protein nitrogen (NPN), free  $\alpha$ -amino nitrogen (AAN) and total volatile base nitrogen (TVB-N) increased significantly ( $p < 0.05$ ) during storage indicating hydrolysis and degradation of protein. Similarly, showing high hydrolytic rancidity, the peroxide value (PV), free fatty acid (FFA) and thiobarbituric acid (TBA) number significantly increased throughout the storage period ( $p < 0.05$ ). The sensory scores showed significant differences ( $p < 0.05$ ) during the storage period. *Shidal* retained good quality up to 60 days at room temperature whereas 90 days at refrigerated temperature showing significantly high sensory scores in the treatment.

Keywords: Biochemical composition, Fermented fish, *Puntius* spp., *Shidal*, Storage characteristics

### Introduction

*Shidal* is a salt free, semi-fermented and very popular fish product of north-east India. With high rancid odour and prepared from small carps mainly *Puntius* spp. *shidal* is an unavoidable fish-based product as far as food security of the people of north-east is concerned. There are several similar products in different north-eastern states such as *seedal* and *hidial* in Assam, *sepaa* and *shidal* in Tripura, Nagaland and Arunachal Pradesh and *ngari* in Manipur (Muzaddadi, 2002; 2003a). The fish is semi dried and almost anaerobically fermented in earthen pots. The fermentation process takes around four to six months in anaerobic condition till the product gains a characteristic odour, texture and appearance. Neither food additives/preservatives nor starter culture is added during the processing steps (Muzaddadi and Basu, 2003b). This product is also popular in Bangladesh which is known as *chepa shutki* (Nayeem *et al.*, 2010). Sarojnalini and Viswanath (1998) reported about almost similar type of fermented products in Manipur *viz.*, *hentak* and *ngari*, which are prepared from sun-dried freshwater fish.

The traditional products are generally contaminated with dirt, filth, sand and dust during retail marketing. Bamboo basket, jute bags and earthen pots are used for storing the fermented fish. The materials are usually used under poor sanitation and hygiene and these products often

turn brown to dark brown in colour and are heavily infested with insects. *Shidal* has a very short storage life once it is taken out from the *mutka*. Therefore, *mutkas* are used as primary packaging and transporting vessels. Since glass is inert material having highest impermeability to gas and volatile substances, glass bottles might act as effective packaging material which is expected to retain the volatile flavour components of *shidal*. Though *shidal* is very common in every household of north-eastern parts of India, scientific information regarding storage are very scanty. Moreover, *shidal* has not been explored scientifically in India. The present study is expected to provide a comprehensive information on low cost packaging method for retailing and house hold storage of *shidal*. The study aimed to analyse the preservative action of low temperature, under packaged condition.

### Materials and methods

The study was carried out in the fish processing technology laboratory, College of Fisheries, Central Agricultural University, Lembucherra, Tripura west. Required amount of first grade commercial *shidal* prepared using four species of *Puntius* (*P. chola*, *P. sarana*, *P. sophore* and *P. ticto*) were collected aseptically in sterile polyethylene bags immediately after opening the *mutka* from the production centres in Agartala. For storage study, 2 kg *shidal* was packed in glass bottles (3l capacity,

19.5 cm X 13.5 cm height X, 10 cm outer mouth dia and 0.5 cm glass thickness, with cork and polythene lined stainless steel screw caps) and then stored at 4 °C (T) and also at ambient temperature separately which served as control (C) (Table 1).

Table 1. Sampling schedule of *shidal* along with ambient temperature recorded

Sampling	Date	Ambient Temperature (°C)
1	20 August 2010	30-34
2	5 September 2010	28-33
3	20 September 2010	28-33
4	5 October 2010	24-28
5	20 October 2010	24-28
6	5 November 2010	20-28
7	20 November 2010	20-28
8	5 December 2010	18-25
9	20 December 2011	18-25

The sampling was done aseptically in sterile petridishes, from each glass bottles for different microbiological, biochemical and sensory analysis. The container was closed immediately after sampling and the sampling was continued at 15 days' interval up to 120 days of storage.

Total plate count (TPC) and total fungal count (TFC) were done by spread plate technique (APHA, 1995). For this, 10 g of *shidal* sample was introduced aseptically in a sterile stomacher bag (Seward stomach BA6141CPG standard bags) and macerated for 2 min with 90 ml of sterile

diluent (0.85% NaCl) using a stomacher (Seward stomacher 400 circulator, England). Serial dilutions were made and plated onto Soybean Casein Digest Agar (SCDA, HIMEDIA) for TPC and onto Rose Bengal Chloramphenicol agar (RBCPA, HIMEDIA) plates for TFC.

The samples were analysed in triplicate for moisture, ash, pH and free fatty acids (FFA) following AOAC (2000), lipid content by the Soxhlet method (AOAC, 2000), protein and non-protein nitrogen (NPN) by the Kjeldahl method (AOAC, 2000); total volatile base nitrogen (TVB-N) content according to the Conway's micro-diffusion method (Conway, 1947); the thiobarbituric acid (TBA) value following Tarladgis *et al.* (1960); peroxide value (PV) as per Jacob (1958); and the free  $\alpha$ -amino nitrogen (AAN) by the method of Pope *et al.* (1939).

Sensory studies of *shidal* were carried out by an expert panel of 10 expert judges by 5-points Hedonic scale (Table 2). The overall acceptability was calculated by taking arithmetic average from score-sheet.

#### Statistical analysis

Statistical analysis was done by performing one way ANOVA (Post Hoc, Duncan) and student's t-test to compare the means using SPSS 15.0 (2005) at 5% confidence level. All bacteriological counts were converted to log<sub>10</sub> CFU g<sup>-1</sup> for statistical analysis.

## Results and discussion

Total plate count (TPC) was analysed during the study period to see the effect of temperature on the survival of

Table 2. 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 <i>Shidal</i> odour	5	Excellent
	Light characteristic <i>Shidal</i> odour	4	Good
	Slight <i>Shidal</i> 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

bacteria under refrigerated condition in comparison with ambient temperature storage of 120 days and expressed as log CFU g<sup>-1</sup>. The TPC of control (C) showed significant differences ( $p < 0.05$ ) with that of the treatment (T) (Fig. 1). Initial decrease in TPC was recorded in T, which may be due to the cold shock to the mesophilic bacteria at lower temperature and the psychrotrophic bacteria took time under chilled temperature for adapting themselves to cold environment and the growth was observed during further storage period. Similar observations were reported in fermented cassava fish wherein the mesophilic bacterial count decreased from 6.25 to 4.94 log CFU g<sup>-1</sup> during initial fermentation (Anihouvi *et al.*, 2007). It was observed that the counts in C and T were almost same during initial 15 days of storage and subsequently the count of T became higher than that of C. During the end of the storage period the count in T was significantly ( $p < 0.05$ ) different from that of C. The reason for this may be attributed to the overgrowth of bacteria in C at ambient temperature, which resulted in reaching the lag phase of growth during the end of the storage period. However, low temperature preservation retarded the growth of psychrotrophic and mesophilic bacteria which in turn, prolonged the log phase and hence the growth of bacteria continued till the end of the storage period. Sarojnalini and Suchitra (2009) reported a similar increasing trend of Gram positive bacteria in fermented *Setipinna* sp. of Manipur. The findings of Thapa and Tamang (2004) in *ngari*, *hentak* and *tungtap* of north-east India also agree with the present findings.

Total fungal counts (TFC) recorded were  $< 2500$  CFU g<sup>-1</sup> in most of the samples, and in many samples TFC was undetectable. Similar trend was observed by Anihouvi *et al.* (2007) in fermented cassava fish.

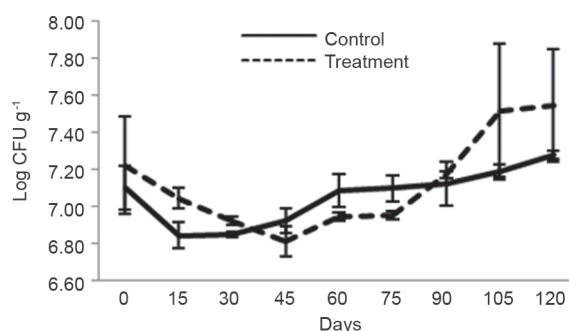


Fig. 1. Changes in TPC (log CFU g<sup>-1</sup>) of *shidal* during storage, values = mean  $\pm$  SE (error bars), n = 3

Proximate composition (Table 3) clearly showed that *shidal* is a highly nutritious food item. The pH showed significant differences ( $p < 0.05$ ) during the storage period (Fig. 2). The initial decrease in pH indicates fermentation process and formation of organic acids. The decrease in pH may also be due to the high buffering capacity of the fish flesh (Dakwa *et al.*, 2005). Nevertheless, fermentation

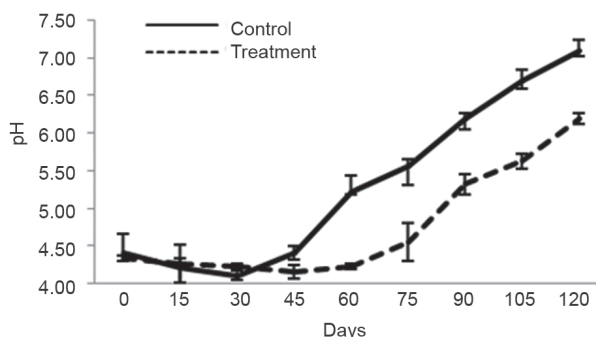


Fig. 2. Changes in pH of *shidal* during storage, values = mean  $\pm$  SE (error bars), n = 3

Table 3. Desirable microbial and biochemical quality parameters of *shidal*

Parameters	Values (mean $\pm$ SD)
Total plate count (log CFU g <sup>-1</sup> )	7.1 $\pm$ 0.12
Total fungal count (log CFU g <sup>-1</sup> )	ETFC $<$ 2500
pH	4.42 $\pm$ 0.43
Moisture (w/w %)	34.02 $\pm$ 1.55
Ash (w/w %)	13.80 $\pm$ 0.32
Protein (w/w %)	31.06 $\pm$ 0.05
Fat (w/w %)	18.87 $\pm$ 0.42
Acid-insoluble ash (w/w %)	0.53 $\pm$ 0.02
Non-protein nitrogen (w/w %)	5.49 $\pm$ 0.24
Free alpha amino acid (w/w mg %)	6.67 $\pm$ 4.67
Total volatile base nitrogen (mg %)	223.67 $\pm$ 3.28
Peroxide value (milliequivalent peroxide oxygen per 1000 g)	17.03 $\pm$ 0.32
Free fatty acid (% as oleic acid)	20.62 $\pm$ 0.17
Thiobarbituric acid number (mg malonaldehyde per 1000 g)	0.51 $\pm$ 0.01

could not continue for longer period due to the absence of fermenting bacteria in the later stages due to aerobic storage condition. Thus, in the later period of storage, fermentation stopped and protein was further degraded to form some volatile bases which led to increased pH. It may be attributed to putrefaction leading to formation of basic nitrogenous compounds (Kilinc *et al.*, 2006). Low temperature influenced the pH value which may be due to restriction in growth of fermenting bacteria. These findings agree with that of Sarojnalini and Suchitra (2009) in fermented fish of Manipur.

There were significant differences in moisture and ash ( $p < 0.05$ ) (Fig. 3 and 4). The moisture content decreased gradually and the reason may be the initial low pH which electrically neutralised the proteins which might have caused decrease in water holding capacity of fish meat (Akahane and Shimizu, 1989). Itou *et al.* (2006) also reported a decreasing trend in moisture in *narezushi* (a Japanese fermented mackerel product) during processing. The ash content was too high which indicates sand adulteration as well as unhygienic conditions during

preparation in traditional production centers. Sarojnalini and Suchitra (2009) also reported high content of ash in fermented *Setipinna* sp. of Manipur. Majumdar *et al.* (2006) reported similar trends in ash content of *lona ilish* (salted and fermented *hilsa* (*Tenualosa ilisha*) from north-east India) during fermentation.

Being the major component of *shidal*, protein content differed significantly ( $p < 0.05$ ) during storage (Fig. 5) and gradually decreased at a constant rate during entire storage period. It may be due to hydrolysis of protein by intrinsic and microbial enzymatic action (Majumdar *et al.*, 2006). The initial decreases in protein percentage may be due to the increment in total weight by the increased moisture content. Nayeem *et al.* (2010) also reported similar decrease in protein content of *chepa shutki* collected from producer, whole-seller and retailer, with increasing storage period. Some volatile nitrogenous compounds might escape to the atmosphere due to which a reduction in total nitrogen content resulted and thus reduction in protein content. Similar observation was also reported by Taira *et al.* (2007) during fermentation of fish sauce.

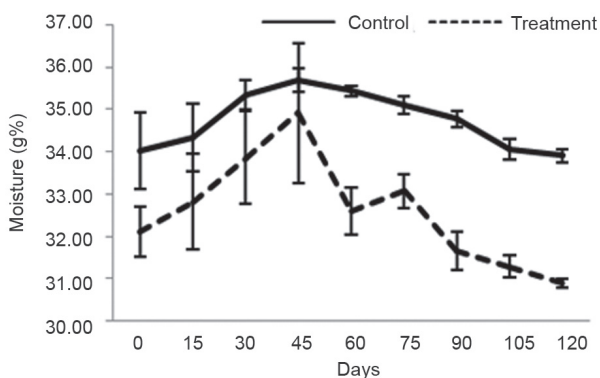


Fig. 3. Changes in moisture content of *shidal* during storage values = mean  $\pm$  SE (error bars), n = 3

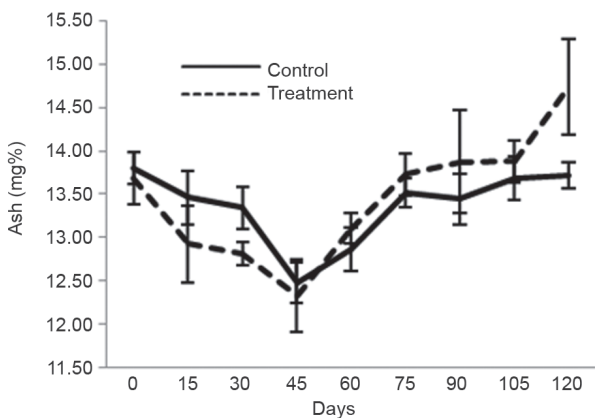


Fig. 4. Changes in ash content of *shidal* during storage values = mean  $\pm$  SE (error bars), n = 3

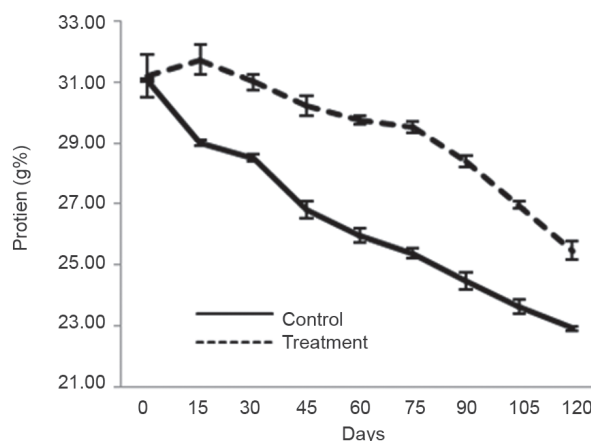


Fig. 5. Changes in protein content of *shidal* during storage values = mean  $\pm$  SE (error bars), n = 3

NPN, AAN and TVB-N showed significant differences ( $p < 0.05$ ) with increasing storage period. The significant increase in all these products may be the result of degradation of protein during storage (Fig. 6, 7 and 8). The TVB-N content was observed high ( $> 200$  mg%) in the treatment and differed significantly from control ( $p < 0.05$ ). The findings of Karthikeyan *et al.* (2007) and Majumdar and Basu (2010) agree with the present findings. TMA-N and TVB-N are products of bacterial spoilage and the content is often used as an index to assess the keeping quality and shelflife of seafood products (Vareltzis *et al.*, 1997). Karacam *et al.* (2002) reported similar increasing trend in TVB-N showing no temperature effect on brined

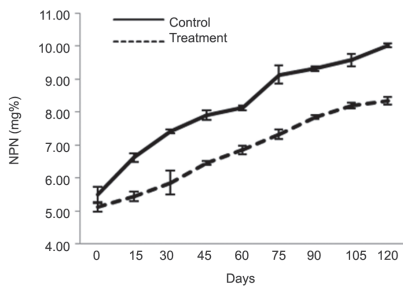


Fig. 6. Changes in NPN of *shidal* during storage, values = mean  $\pm$  SE (error bars), n = 3

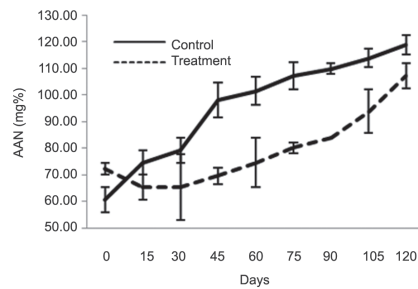


Fig. 7. Changes in AAN of *shidal* during storage, values = mean  $\pm$  SE (error bars), n = 3

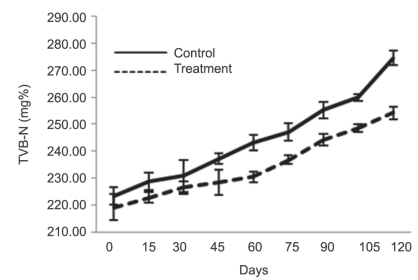


Fig. 8. Changes in TVBN content of *shidal* during storage, values = mean  $\pm$  SE (error bars), n = 3

anchovies. Ndaw *et al.* (2008) also reported increased TVB-N values in fermented Moroccan sardines (*Sardinella pilchardus*). TVB-N values recorded in the present study clearly indicate deterioration of proteins. The increase in AAN throughout the period may be because of the combined effect of autolysis and microbial degradation of the fish muscle (Voskresensky, 1965; Ijong and Ohta, 1996). This may be implicated in the activities of enzymes which

originated from fish gut, muscle and bacteria (Majumdar *et al.*, 2006). The contents of NPN and TVB-N of *Shidal* were indicative of high degree of fermentation.

The lipid content and different lipid degraded products like PV, FFA and TBA showed significant differences ( $p < 0.05$ ) during the storage period (Fig. 9 - 12). The fat content gradually decreased throughout the period, may

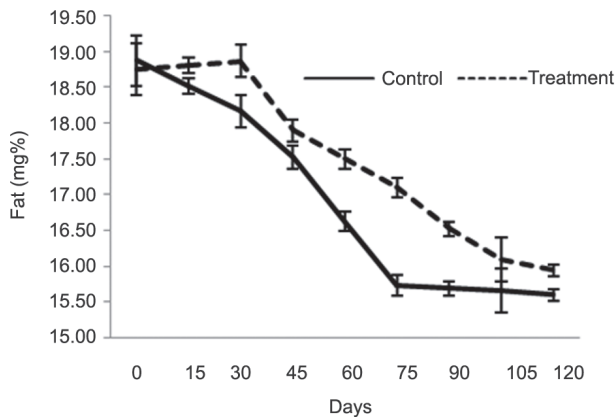


Fig. 9. Changes in fat content of *shidal* during storage values = mean  $\pm$  SE (error bars), n = 3

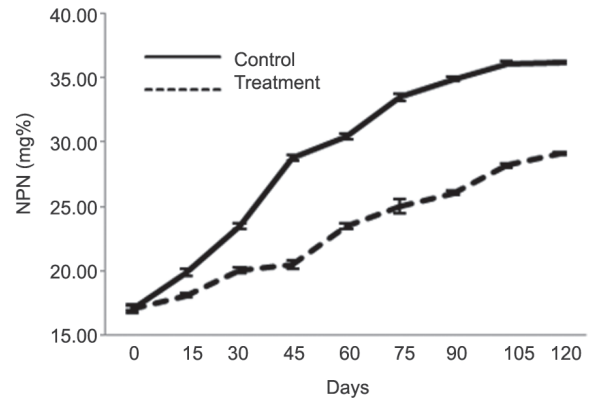


Fig. 10. Changes in peroxide value (PV) of *shidal* during storage values = mean  $\pm$  SE (error bars), n = 3

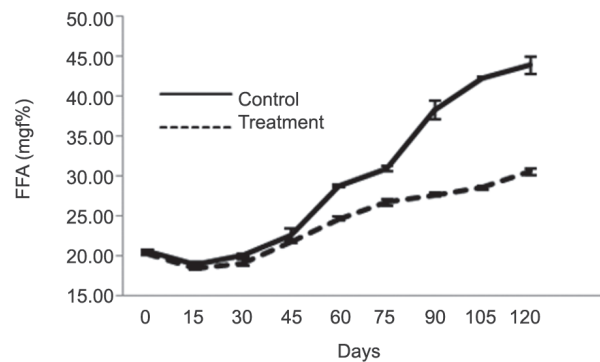


Fig. 11. Changes FFA content of *shidal* during storage values = mean  $\pm$  SE (error bars), n = 3

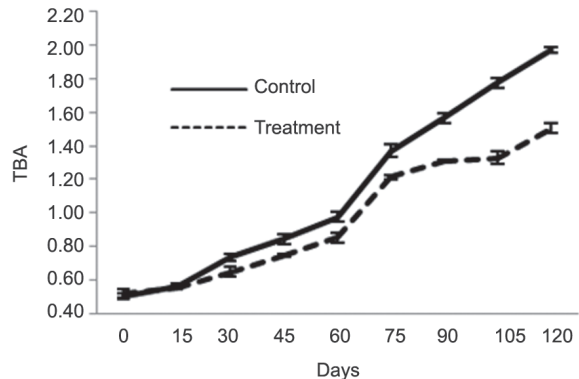


Fig. 12. Changes in TBA number of *shidal* during storage values = mean  $\pm$  SE (error bars), n = 3

be due to oxidative and hydrolytic rancidity of fat during storage. Considerable decomposition of triglyceride and phospholipids in the lipid may occur, accompanied by the production and accumulation of large amounts of free fatty acids throughout processing (Kilinc *et al.*, 2006). The high amount of fat may be due to additional fish oil which is normally used for smearing *mutka* and the raw material, and also *Puntius* spp. contain high amount of fat and they are caught during monsoon period (Sarojnalini and Vishwanath, 1994). The decreasing trend in fat content was also observed by Karthikeyan *et al.* (2007) in smoked

*Colisa fasciata* of Manipur, by Rahman *et al.* (1999) in salted *Hilsa* sp. and by Nayeem *et al.* (2010) in *chepa shutki* with increasing storage time. There are formal chemical definitions of oxidation, involving electron transfer and free radical reactions but, in the context of fish technology, it can be considered as the chemical reaction in which oxygen combines with a compound (Kilinc *et al.*, 2006). Among the different lipid degraded products, the primary oxidation indicator PV which is the index to assess the lipid oxidation of *shidal* significantly differed ( $p < 0.05$ ) in the treatment and the control (Fig. 10). The gradual increase in PV values

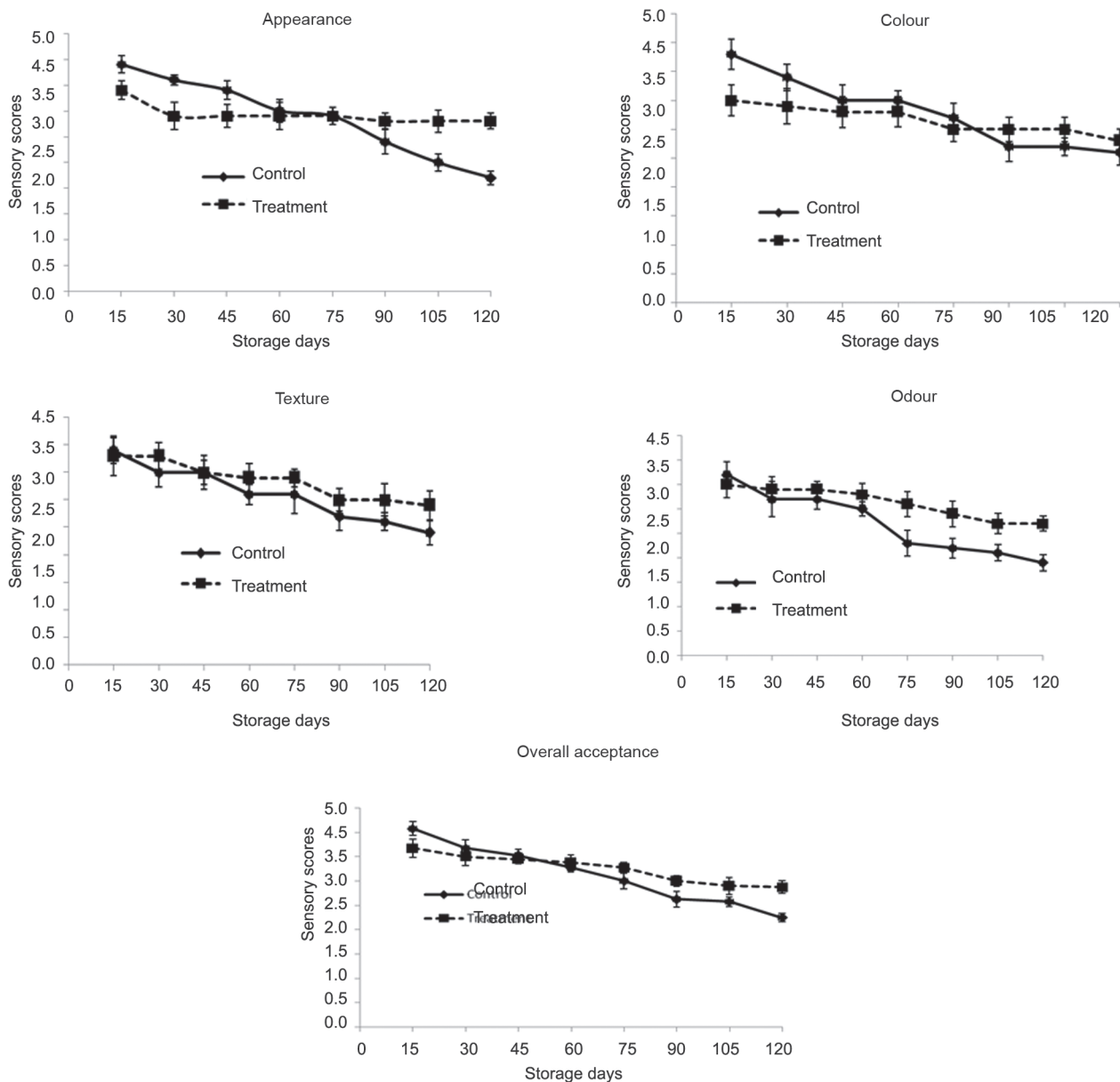


Fig. 13. Changes in sensory scores for different sensory parameters (appearance, colour, texture, odour and overall acceptability) of *shidal* during 120 days of storage, values = mean  $\pm$  SE (error bars), n = 10

may be due to more amount of initial fat oxidation and it also indicates the formation of peroxide or hydroperoxide (Vernam and Sutherland, 1995).

The FFA content increased throughout the storage period (Fig. 11). FFA showed less values in refrigerated samples indicating that low temperature storage could reduce lipid hydrolysis. These findings agree with the findings of Srikar *et al.* (1993) who reported lower PV and lower content of FFA in salted mackerel and pink perch when stored at 2.5 °C after 35 days of storage. The high TBA values represent the degree of rancidity in the products and the values above 3-4 indicate quality loss (Karacam and Boran, 1996). It was observed that in the present study, the values are in the acceptable range.

Primary and secondary lipid oxidation products are the biological amino compounds, protein, peptides, free amino acids and phospholipids; they react to produce interaction compounds and this make the colour of the product brown, causes a change in flavour and loss in aromatic nutrient elements (Aubourg, 1998).

The sensory score for all sensory characters significantly differed ( $p < 0.05$ ). The scores for appearance, colour, texture, odour and overall acceptability (Fig. 13) of *shidal* significantly differed ( $p < 0.05$ ) and gradually decreased at a higher rate.

It was observed that the low temperature storage of *shidal* had positive effects on extending shelflife of *shidal*. Low temperature stored *shidal* showed less fluctuations in the scores of all the sensory parameters which denotes a stable quality of *shidal* during the storage period (Fig. 13). The product remained acceptable up to 90 days of storage with an overall acceptability score of  $> 3.0$ . *Shidal* after 90 days storage under refrigerated condition is shown in Fig. 14. This agrees with the findings of Akande *et al.* (1991) in spiced minced fish cake and Koral *et al.* (2010) in hot

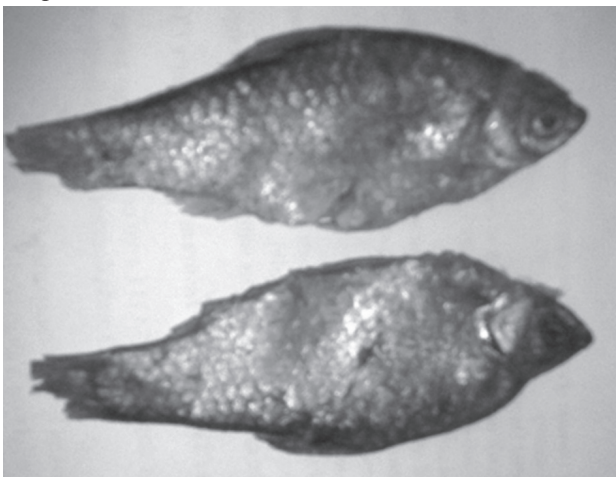


Fig. 14. Good quality *Shidal* after 90 days of storage under refrigerated condition

smoked Atlantic bonito (*Sarda sarda*) packed in aluminium foil during storage. Ozden and Erkan (2006) also reported that sensory evaluation has an important role in determining the quality of fish and seafood. After analysing and observing all the parameters it was inferred that *shidal* may be kept at low temperature up to 90 days without losing its characteristic odour and biochemical quality. It became unacceptable after 120 days of storage under both conditions (Fig. 15).



Fig. 15. *Shidal* after 120 days of storage

Though *shidal* is one of the important fermented products available in north-eastern part of India, it does not have proper packaging and preservation methods and it loses its typical flavour within a short period after taking out from the *mutka*. The present study expected to provide basic scientific information to develop a suitable packaging and preservation technology for *shidal*. Limited research work has been done on all aspects of fermented fish products of north-east India, and there is a need for future research especially in developing better packaging methods utilising polythene, polyethylene terephthalate (PET) or modified atmospheric packaging (MAP).

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