

Microbiological quality of fermented fish silage

Madhusudana Rao B^{1*}, Jayaprakash Babu US², Imam Khasim D¹

¹Research Centre of Central Institute of Fisheries Technology, Visakhapatnam-530 003, India

²55-1-49, JR Nagar, HB Colony, Visakhapatnam-530 022, India

*Email:bmrcift@rediffmail.com

Three batches of fermented silage (FS) were made using silver bellies (*Leiognathus* sp), along with *Lactobacillus plantarum* (5%), sodium benzoate (0.5%) and molasses (6, 8 and 10%). The result indicated that 10% molasses with *Lb. plantarum* and sodium benzoate yielded a stable silage with desirable pH of <4.5, whereas molasses at 6 or 8% did not produce stable silage and had high levels of H₂S producing bacteria and total *Enterobacteriaceae* counts. The minimum pH attained was 4.0, 4.7 and 5.0 in 10, 8 and 6% molasses, respectively. Experiments were carried out to use material from previous FS as starter inoculum for converting fresh fish mince to silage using 1:1, 1:2, 1:4, 1:5 and 1:10 (inoculum: fresh fish mince) ratios. Stable FS (14 day old silage with LAB count of 7.4 log cfu/g and total plate count of 7.3 log cfu/g) was added to fresh fish mince, to which the required amount of molasses (10%) and sodium benzoate (0.5%) were added. The results suggested that 14-day old FS can be used as starter culture at 1:5 ratio effectively to produce stable FS.

Keywords: Fish silage, Fermented silage, *Lactobacillus plantarum*, Lactic acid bacteria, Hydrogen sulphide producing bacteria

Trawl bycatch, which includes juveniles of commercially important fish, slightly spoiled fish and other small fish, have low market value and less consumer appeal. High amount (66.6 to 94.2%) of economically low value bycatch of trawl fishery off Visakhapatnam constituted juveniles (Sujatha 1996). These under-utilized fish can be upgraded as a source of protein, safely and easily by ensilage with acid or by microbial fermentation methods. Fish ensilage is a potential source of protein for livestock nutrition and was tested as feed ingredient for fish and shell-fish in aquaculture (Raa and Gildberg 1982, Raghunath and Gopakumar 2002). Lactic acid bacteria (LAB) used for fish and shrimp silage preparation included, *Lb. plantarum* (Viète and Bello 1992, Ottati and Bello 1992, Fagbenro and Jauncey 1995, Lassen 1995), yoghurt bacteria like *Lb. bulgaricus* and *Streptococcus thermophilus* (Areche et al 1992, Yoon et al 1997), *Lb. dulbrecki* and *Strep. salivarius* (Martinez-Valdivieso et al 1996), *Lb. fermentum* (Mathew and Nair 2006) and natural inocula like sauerkraut (Dhatemwa 1989). *Lb. plantarum*, which is homofermentative, appears to be most effective starter culture (Bello et al 1992). As carbohydrate source, different workers have used molasses (10-15%) (Kompang et al 1980, Ottati and Bello 1990, Fagbenro and Jauncey 1994, 1995, Suchindra et al 1994, Ahmed and

Mahendrakar 1995, 1996), 20% malt meal and oat meal (Nilsson and Rydin 1963), tapioca meal, ragi meal (James et al 1977) and 5% dextrose (Lassen 1995). The present work was taken up to study the optimum level of molasses required for the conversion of small sized low value fish, silver belly (*Leiognathus* sp) into fermented silage (FS) by using *Lb. plantarum* and to determine the effect of using FS as an inoculum in place of lactic culture.

Fresh silver bellies (*Leiognathus* sp) brought from the fishing harbour, Visakhapatnam were minced thoroughly in mechanical mincer (BC60L, Stadler Corporation, Mumbai). Five kg of fish mince was used for each treatment. *Lb. plantarum* (NCIM 2083, National Chemical Laboratory, Pune) culture was grown in de Man Rogosa Sharpe (MRS) broth and incubated at 37°C for 24 h. The culture had LAB cell density of 9.3 log cfu/ml. The LAB culture in MRS broth was added at 5% (v/w) level to the fish mince which gave a concentration of 7.8 log cfu/g of fish mince. Molasses was used at 6, 8 and 10%. In fungal control, 0.5% (w/w) sodium benzoate was added to the raw fish mince (Babu et al 2005). Ensilation was done in airtight plastic containers kept at ambient temperature (28-34°C). The silages were stirred daily at 9.30 am and 4.30 pm and at the time of drawing the sample for analysis.

Stable fermented silver bellies silage

of 14 day old FS with 10% molasses with LAB count of 7.4 log cfu/g and total plate count (TPC) of 7.3 log cfu/g was added as inoculum to fresh fish mince prepared from underutilized fishes at different ratios viz., 1:1, 1:2, 1:4, 1:5 and 1:10 (inoculum: fresh fish mince). Molasses and sodium benzoate were added to the fish mince at 10 and 0.5% levels, respectively to all batches.

Moisture, protein, fat and ash were determined as per AOAC (1990). pH was determined using pH meter (Griph D pH meter 327, Systronics, Mumbai) by directly dipping the electrode into the ensilage. TPC, *Enterobacteriaceae* count on violet red bile glucose agar (Speck 1978), total yeast and mould count (YMC), coliforms, faecal coliforms, *Escherichia coli*, *Salmonella*, *Vibrio cholerae*, coagulase positive *Staphylococci* (Anon 1995), LAB count on MRS agar (de Man et al 1972) and H₂S producing bacterial counts (H₂SPB) on peptone iron agar (Gram et al 1987) were determined. Samples for analysis were drawn on 1, 3, 5, 7, 14, 21 and 28 days of fermentation.

Silver bellies fish mince contained 75.7% moisture, 17.2% crude protein, 1.7% crude fat and 4.9% ash. TPC, H₂SPB count, *Enterobacteriaceae* count, YMC and coagulase positive *Staphylococci* count of silver bellies fish mince were 4.4, 2.7, 2.8, 2.3 and 1.6 log cfu/g, respectively. The levels of coliforms,

faecal coliforms and *E. coli* in silver bellies fish mince were >1100, 16 and 16 MPN/g, respectively. The silver bellies fish mince had a LAB count of only 1.5 log cfu/g. *Lb. plantarum* grown in MRS broth and incubated at 37°C for 24 h had a count of 9.3 log cfu/ml, which appeared turbid with white precipitate and when inoculated to fresh fish mince at 5% (v/w) level as starter culture, the LAB concentration of mix was 8.0 log cfu/g.

The results are presented in Fig. 1. The pH of desirable and stable silage is less than 4.5 (Fagbenro and Bello-Olusoji 1997). pH dropped to <4.5 at the end of 1st day in FS with 10% molasses indicating successful lactic acid fermentation by *Lb. plantarum* culture (5%). The pH stabilised at 4.0 at the end of 3rd day. However, in FS with 6 or 8% molasses, pH <4.5 was not attained. Relatively intense off odour was noticed in silages after 4th day in FS with 6% molasses and after 15 days in FS with 8% molasses when pH reached 5.5. The major metabolite of LAB is lactic acid which is responsible for significant pH reduction sufficient to antagonise many micro-organisms (Earnshaw 1992). TPC of FS (10% molasses) increased during the first 3 days of ensilation and thereafter it showed a decreasing trend reaching 5.8 log cfu/g on 28th day. LAB counts were high in all the fermented silages on the first day with LAB counts of 9.5 log

cfu/g in FS with 10% molasses, 8.8 log cfu/g in FS with 8% molasses and 8.6 log cfu/g in FS with 6% molasses. Thereafter there was decrease in LAB counts in FS. The LAB counts reached 5 log cfu/g level on 2nd week in FS with 6% molasses and on 4th week in FS with 8% molasses. In stable FS with 10% molasses the count was 6 log cfu/g on 4th week. H₂SPB are associated with the spoilage of fish. H₂SPB count of silver bellies fish mince was 2.7 log cfu/g. H₂SPB count in FS with 10% molasses reduced to nil on 3rd day. In FS with 6% molasses the H₂SPB count increased to 5.3 log cfu/g on 2nd week of storage whereas in FS with 8% molasses the increase was slower and reached 4.8 log cfu/g on 4th week of fermentation. The increase in H₂SPB count was associated with an increase in the intensity of off-odour which led to discarding of FS with 6% molasses after 2 weeks and FS with 8% molasses after 4 weeks of fermentation. The *Enterobacteriaceae* count of fresh fish mince was 2.8 log cfu/g. In FS with 10% molasses, *Enterobacteriaceae* count was nil on 7th day. *Enterobacteriaceae* count increased with lower levels of molasses.

FS with 10% molasses contained 70.5% moisture, 19.2% crude protein, 1% crude fat and 5.2% ash. LAB was the predominant bacteria in FS with 10% molasses. Complete reduction in YMC,

coliforms, faecal coliforms and *E. coli* counts was attained within 3 days in FS with 10% molasses. The microbiological quality of FS with 10% molasses was good as indicated by the absence of *Salmonella*, *Vibrio cholerae* and coagulase positive *Staphylococci*.

One of the factors that increased production cost of fermented fish silage is the cost of MRS broth (Rs 688/l of MRS broth-imported brand, Rs 194/l of MRS broth-Indian brand, 2008). One litre of MRS broth culture is necessary for preparation of 20 kg of FS. In order to lower the expenditure, trials were carried out to use FS as starter inoculum. The pH dropped to less than 4.5 in 1:1, 1:2, 1:4 and 1:5 (inoculum : fresh fish mince) at the end of 3rd day. The pH drop was rapid in 1:1, 1:2 1:4 and 1:5. The inoculum of 14 days old FS with 10% molasses when used at 1:5 level gave a LAB concentration of 6.6 log cfu/g of fish mince. The LAB counts in 1:5 silage increased to 8.4 log cfu/g on third day and the pH of FS dropped to <4.5. Thereafter the LAB counts decreased slowly reaching 7.8 log cfu/g and 7.0 log cfu/g on 7th and 14th day, respectively.

Preparation of fermented fish silage was a simple process that can be done in containers at room temperature (28-34°C) from underutilized fish by fermentation using *Lb. plantarum*, 0.5% sodium benzoate and 10% molasses. Incorporation of molasses at either 6 or 8% did not result in stable silage. Stable FS with 10% molasses of 14 days could be used as starter culture possibly to minimise the cost of production of FS. Fish silage is an ideal alternative to fish meal as it is ecofriendly and has added advantages such as easy to prepare, can be done even in rainy season and produces

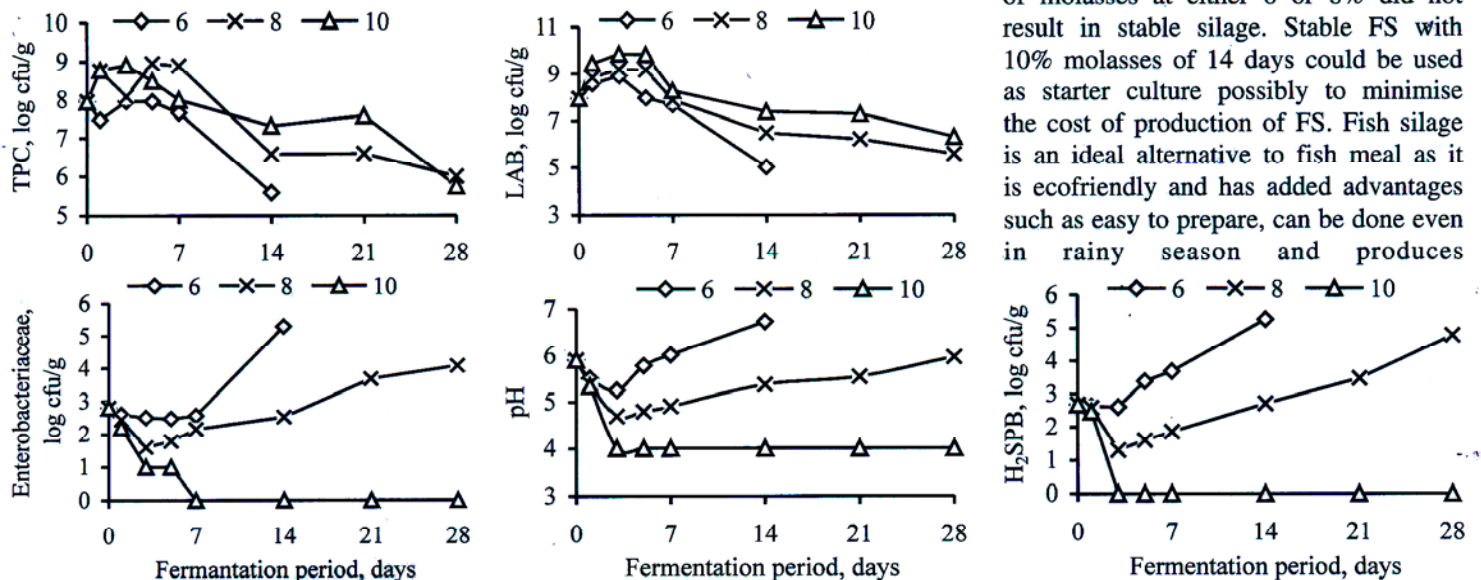


Fig. 1. Changes in microbiological quality and pH of silver bellies fish during fermentation at ambient temperature (28-34°C) with different levels of molasses (6, 8, 10%) and 0.5% sodium benzoate (n=3)

TPC: Total plate count, LAB: Lactic acid bacteria, H₂SPB: H₂S producing bacteria

microbiologically safe product. Fermented fish silage finds use as protein source in the diets of pigs, poultry and in aqua feed.

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