



Changes in Physico-Chemical and Microbial Profiles of Lactic Acid Bacteria Inoculated Fish Mince under Refrigeration Storage

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Abstract

The present study was undertaken to evaluate the effect of lactic acid bacteria (LAB) on storage stability of croaker (*Johnius* sp.) minced meat under refrigeration storage. Minced meat of croaker was inoculated with *Lactobacillus acidophilus* and stored at refrigeration temperature (4°C). A control (untreated) sample was also prepared and stored. The samples were analyzed at 0, 7, 14 and 21 days of storage for physico-chemical and microbial characteristics. Decrease in pH of inoculated sample from 6.09 to 5.47 with simultaneous increase in titrable acidity from 0.566 to 0.809 during entire storage indicated acid production by LAB. Microbial counts were lower in inoculated sample than in control. LAB count significantly increased in inoculated sample. The lowering of pH by acid production inhibited microbial growth. Physico-chemical parameters showed a significant ($p < 0.01$) correlation with the microbial counts. Inoculation of LAB in minced fish enhanced its shelf life by inhibiting microbial growth and decreasing the protein and fat degradation products.

Keywords : Lactic acid bacteria, microbial quality, minced fish, microbial inoculation, starter culture

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Introduction

Lactic acid bacteria (LAB) are known to alter the flavour, texture and appearance of foods, to retard spoilage and to reduce contamination (Hammes &

Tichaczek, 1994). Metabolic byproducts of LAB have been shown to inhibit the growth of several important pathogens and to increase product shelf life (Daeschel, 1989; Abee et al., 1995). It is well known that starter cultures produce a wide range of anti-microbial metabolites which include organic acids, diacetyl, acetone, hydrogen peroxide, antibiotics and bacteriocins (Holzapfel et al., 1995) that prevents growth of resident microbial species in food (Levin, 1994; Helander et al., 1997). LAB are mainly associated with fermented dairy products such as cheese, butter milk and yoghurt. LAB have a major potential for use in biopreservation because they are safe for human consumption (GRAS status) and are the prevalent microflora during storage in many foods (Castellano et al., 2008). It was reported that the shelf-life of meat could be extended at chill temperatures by a few days by treatment with lactics (Gilliland, 1985).

The present study has been designed with the aim to evaluate the likely changes of minced meat of croaker fish (*Johnius* sp.) inoculated with lactic acid bacterial starter culture on storage under refrigerated condition.

Materials and Methods

Fresh croakers (*Johnius* sp.) obtained from Digba fish landing center were packed in polyurethane boxes by icing (1:1) and brought to laboratory of Dept. of Fish Processing Technology, Faculty of Fishery Sciences, West Bengal within 7-8 h. Whole fish were then beheaded, skinned, eviscerated and deboned. Meat was picked using knife and minced using meat mincer (Stadler Corporation, Bombay). Minced fish was washed with chilled water to remove fat and water soluble proteins. LAB strain *Lactobacillus acidophilus* stock was anaerobically propagated in Mann Rogosa Sharpe (MRS) broth for 48 h at

37°C. The culture was then concentrated by centrifugation at 4°C and 4000 rpm for 20 min. The supernatant was discarded and cell pellets washed twice in sterile physiological saline (0.85%) and re-suspended at 10^8 cells ml^{-1} concentration. This was incorporated immediately into the minced fish at a concentration of 10% of fish meat with thorough mixing. One un-inoculated portion of minced fish served as control. The required number of packs were prepared for analysis and stored at 4°C in the refrigerator. Packs were withdrawn at 0, 7, 14 and 21 days of storage and analyzed for physico-chemical and microbial characteristics. For the treatment and control, three independent batches ($n=3$) were studied.

Physico-chemical analyses: Physico-chemical parameters used to assess the freshness of fish were: proximate composition of minced fish viz., moisture, ash, protein (Kjeldahl method) and fat (Soxhlet apparatus) following the methods of AOAC (2000) and results were calculated as g 100 g⁻¹ of minced fish. pH of the samples was determined by the method described by Suzuki (1981) using a digital pH meter (Model-MK VI Systronics). Total acidity was determined by the method of Konecko (1979). Total volatile base nitrogen (TVBN) was estimated by Conway's micro-diffusion unit (Conway, 1947). Thio barbituric acid (TBA) value was estimated following the method of Sinhuber & Yu (1958) to know the extent of fat rancidity and results were expressed as TBA number (mg malonaldehyde kg⁻¹ of minced fish). Extract release volume (ERV) was determined by the method of Jay (1964).

Microbiological analyses: The Microbiological parameters such as aerobic plate count (APC), psychrotrophic plate count (PPC), coliforms, yeast & mould count and lactic acid bacterial counts were carried out as per APHA (2001). APC and psychrotrophs were counted in nutrient Agar plates. Enumeration of coliform was done in MacConkey broth. Staphylococcal count was determined on Baird Parker (BP) Agar and Lactic acid bacteria were counted on de-Man Rogosa Sharpe (MRS) Agar plates. The enumeration of yeasts and mould were carried out on potato dextrose agar. All microbiological counts were converted to log₁₀ cfu g⁻¹ for statistical analysis.

Statistical analysis: Observed data upto 21 days were analyzed statistically for one way Analysis of Variance (ANOVA) to find out significant difference

in changes of physico-chemical parameters during different storage days (Snedecor & Cochran, 1967). A simple correlation coefficient (r) was calculated to establish relation between microbial and physico-chemical parameters.

Results and Discussion

The protein content of control was 17.41 g% and *L. acidophilus* inoculated sample was 16.69 g%. Fat content in control was less than inoculated sample, the value of which was 2.37 g% and 2.91 g% respectively. Similarly, ash content was also less in control (1.02%) than in inoculated one (1.05%). The results of proximate composition reveal that starter culture of LAB has no significant effect on proximate composition of minced meat. Results obtained here are similar to the findings of Neethiselvan et al. (2002) in mince meat of silver belly.

There was an increase in total acidity (Fig. 1A) from 0.56 to 0.81 in *L. acidophilus* inoculated sample after 7 days of storage. The reduction in pH in the treatment indicated fermentation by LAB and subsequent lactic acid production which increases total acidity. The pH value of inoculated sample was lower with higher total acidity value than the control (Fig. 1B) during storage. However, the *L. acidophilus* inoculated sample showed a significant ($p < 0.05$) pH change from 6.09 to 5.47 during entire storage. In the present study, pH of the control sample was fairly stable ranging from 6.74 to 6.13 with little change from initial days during entire storage period, but not significant. Gilthead seabream and European sea bass under ice storage showed a reduction of pH from 6.2 to 6.1 and 6.39 to 6.35 respectively during initial days of storage (Abbas et al., 2008). This was explained as the glycogen in muscle might have metabolized to lactic acid which brought down the pH. A similar result was reported by Sakhare & Rao (2003) on minced meat & meat cubes of sheep fermented with mixed lactic acid cultures. No significant difference was observed in pH and total acidity value between the control and inoculated sample. However, pH and acidity of all the samples showed significant ($P < 0.01$) correlation with microbial counts.

TVBN content in the samples increased with storage period and ranged from 4 -11 mg% (Fig. 1C). The TVBN values of *L. acidophilus* inoculated meat sample increased from 4.36 to 7.74 whereas for control samples, it increased from 5.62 to 10.67

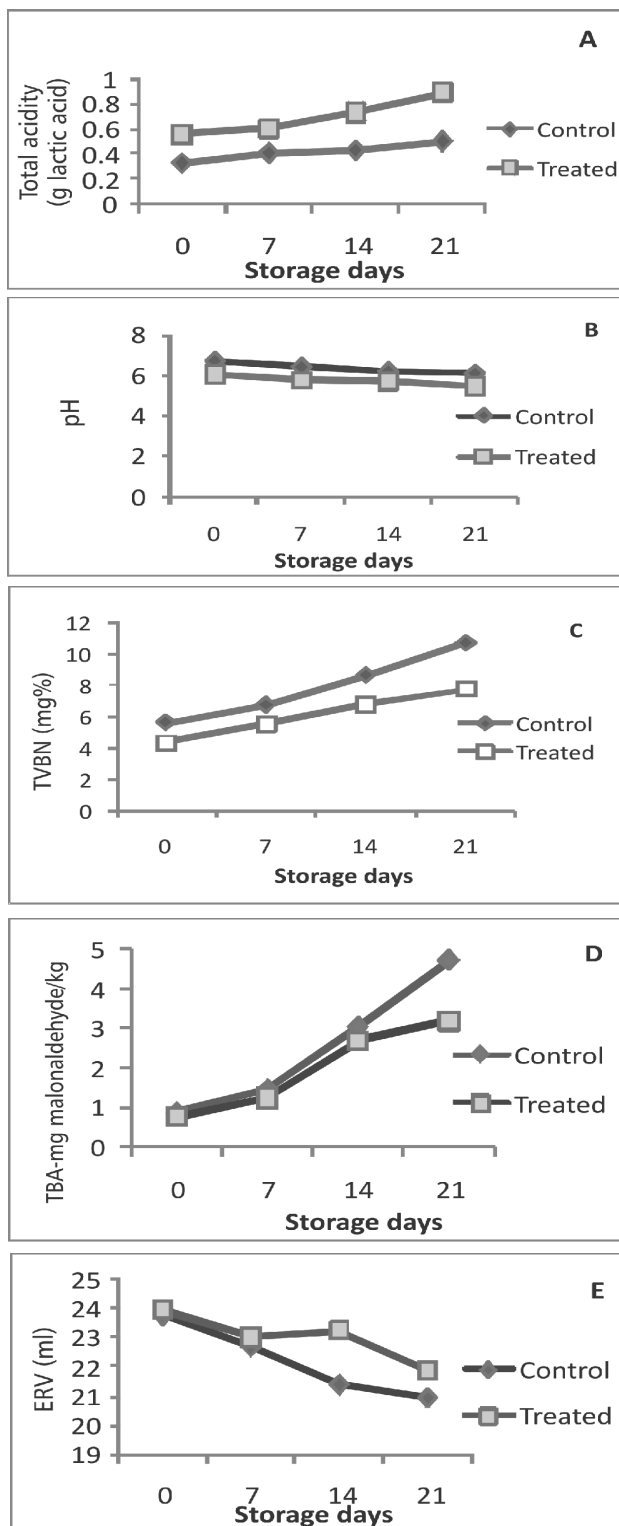


Fig. 1. Changes in physico-chemical profile of inoculated and control sample of minced *Johnius* sp. under refrigeration storage. A: Changes in total acidity; B: Changes in pH; C: Changes in TVBN; D: Changes in TBA; E: Changes in ERV

during entire storage period. This finding is supported by a similar study by Gelman et al. (2001) where TVBN values of LAB inoculated minced yellow fin tuna remained almost unchanged or slightly changed after 4 weeks of storage at 10°C. Their study revealed that TVBN content in minced fish samples inoculated with *L. plantarum* and *Pediococcus pentosaceus* changed from initial 24.2 (0 day) to 24.7 mg 100 g⁻¹ and 24.2 (0 day) to 27.1 mg 100 g⁻¹ respectively after 21 days of storage at 10°C. Similarly the control also showed a change from initial 24.2 (0 day) to 26.9 mg 100 g⁻¹ after 21 days of storage at the same temperature. ANOVA of the data revealed significant ($p < 0.05$) effect on storage days. pH and TVBN of the inoculated samples showed a highly significant ($p < 0.01$) correlation with storage. Changes in values for TBA in *L. acidophilus* inoculated and control samples were from 0.74 to 3.12 and 0.87 to 4.72 respectively. Highest value of 4.72 was observed in control on 21st day of storage which was unacceptable (Yu & Sinnhuber, 1957). A TBA value above 1-2 mg of malonaldehyde kg⁻¹ of fish is considered unacceptable (Laxmanan, 2002). However, consistently lower TBA (Fig. 1D) value in inoculated sample (0.741 to 3.168 mg kg⁻¹ fat) indicated the preservative action of LAB on lipid oxidation. A similar type of result was observed by Gelmen et al. (2001) who reported increased malonaldehyde level during first 4 weeks in LAB inoculated minced meat samples. Significant ($p < 0.05$) effect on storage days and significant ($p < 0.01$) correlation with all microbial counts were observed (Table 1).

ERV decreased during refrigerated storage both in control and inoculated sample (Fig. 1E). The ERV values for control ranged from 23.8 to 20.96 ml and in *L. acidophilus* inoculated sample from 23.96 to 21.86 ml. But, no significant differences were observed with storage days. However, a positive correlation ($p < 0.01$) of ERV with microbial parameters was found (Table 1).

Aerobic Plate Count (APC) changed from 4.52 to 5.69 log cfu g⁻¹ in control and 4.18 to 2.21 log cfu g⁻¹ in inoculated sample (Fig. 2A). The inability of lactic acid bacteria (LAB) to grow well on nutrient agar has already been reported by some authors (Adams et al., 1987; Twiddy et al., 1987; Neethiselvan et al., 2002). Sreerekha et al. (2002) observed a reduction in APC in shark meat gel fermented with LAB for 2 months at 10°C and remained as low as 7.0×10 cfu g⁻¹. This finding is also similar to the

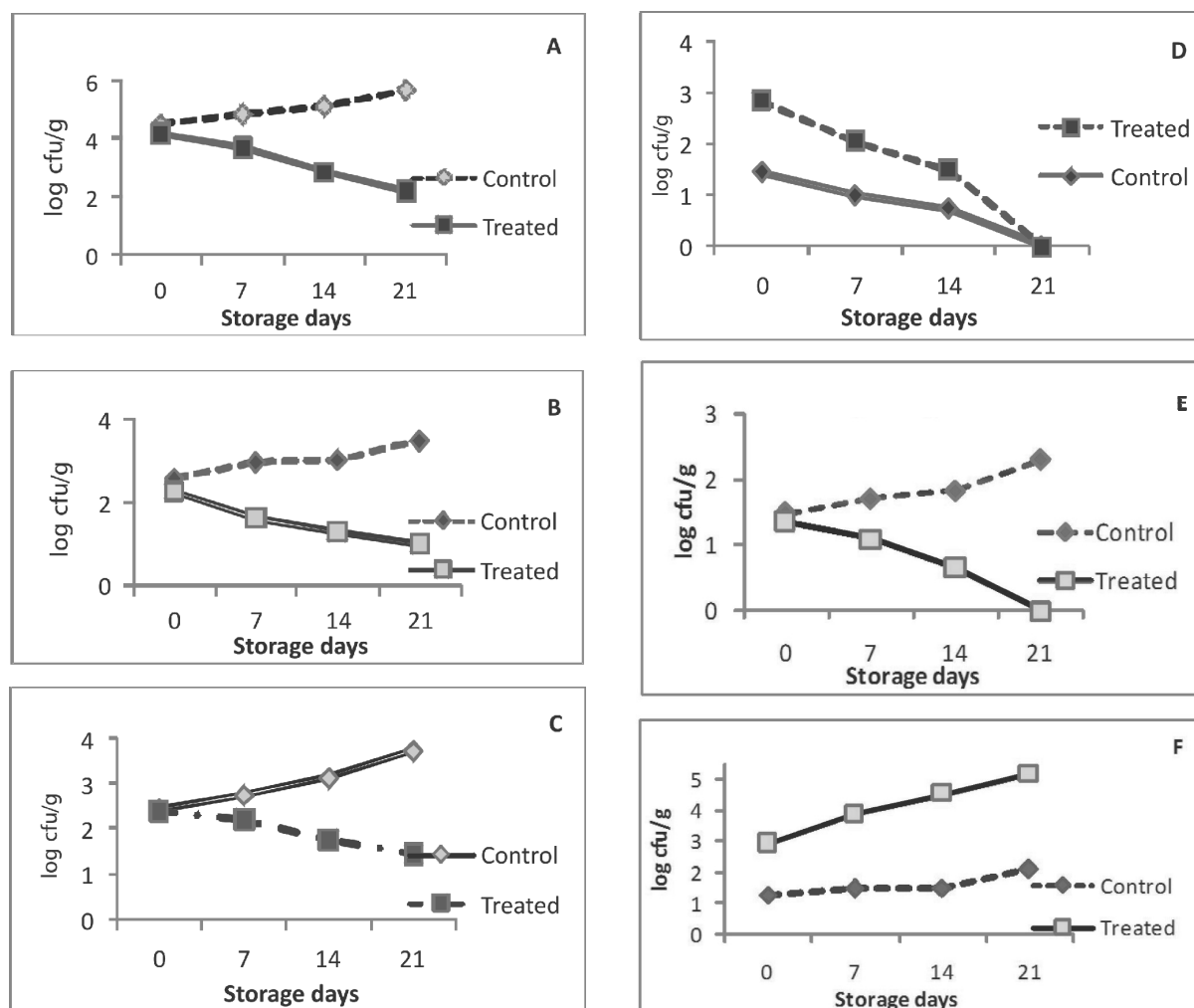
Table 1. Correlation co-efficient (r) computed between different physiological and microbial characteristics of LAB inoculated croaker (*Johnius* sp.) mince stored at refrigerated temperature

Parameters	pH		Total Acidity		TVBN		TBA		ERV	
	Control	Inoculated sample	Control	Inoculated sample	Control	Inoculated sample	Control	Inoculated sample	Control	Inoculated sample
APC	-0.943**	0.969**	0.980**	-0.993**	0.991**	-0.994**	0.985**	-0.989**	-0.944**	0.871*
PPC	-0.941**	0.976**	0.995**	-0.928**	0.956**	-0.985**	0.939**	-0.943**	-0.919**	0.884*
<i>Staphylococcus</i>	-0.941**	0.961**	0.971**	-0.996**	0.996**	-0.988**	0.992**	-0.990**	-0.948**	0.861*
Coliform	-0.985**	0.991**	0.973**	-0.945**	0.988**	-0.967**	0.974**	-0.925**	-0.990**	0.961*
Yeast & Mould	-0.893**	0.971**	0.963**	-0.978**	0.970**	-0.971**	0.969**	-0.956**	-0.890**	0.914*
Lactic acid bacteria	-0.917*	0.987**	0.938**	-0.956**	0.996**	-0.996**	0.999**	-0.962**	-0.939**	0.899*

ERV: Extract release volume

** 1% level of significance

* 5% level of significance

Fig. 2. Changes in microbial profile of inoculated and control sample of minced *Johnius* sp. under refrigeration storage. A: Changes in APC; B: Changes in PPC; C: Changes in staphylococcal count; D: Changes in coliform; E: Changes in yeast and mould; F: Changes in LAB count

results of Gelman et al. (2001) where decrease in APC was observed even after 5 weeks in LAB inoculated samples. APC was found to have a highly significant ($p < 0.01$) correlation (Table 1) with all the physico-chemical characteristics of inoculated mince. Psychrotrophic plate count (PPC) decreased in the inoculated sample from 2.27 to 1.0 log cfu g⁻¹ at the end of the storage period (Fig. 2B). This change is supported by the findings of other researchers (Russel, 1998; Bala, 2002). PPC showed highly significant ($p < 0.01$) correlation with all physico-chemical parameters (Table 1).

There was a highly significant ($p < 0.01$) effect of storage period on staphylococcal count. The count decreased from 2.40 to 1.46 log cfu g⁻¹ in the inoculated sample throughout the storage period (Fig. 2C) and the reduction was significant after 14th day of storage. *Staphylococcus* along with *Bacillus* and *Micrococcus* occur in fermented foods and are considered to produce metabolites that contribute to flavour and odour development (Crisan & Sands, 1975; Orejana, 1983; Adams, 1986; Saono et al., 1986). So, in the present study the staphylococcal count was considered to see the effect of lactic acid bacteria on the microbial profile of minced fish.

Coliform count reduced in inoculated samples during entire storage and no colonies were detected on 21st day of storage (Fig. 2D). This finding closely resembled with Neethiselvan (2002) where no coliform was found in fermented mixtures. Complete absence of coliforms may be attributed to the low pH which did not provide a conducive environment for the coliform to grow.

Inoculation of fish samples with LAB prevented the growth of yeast and mould (Fig. 2E) and no colonies were detected. The inhibition of yeast and mould count had already been described in fermented food stuffs (Leroi et al., 1996; Neethiselvan et al., 2002). Increase in LAB count from 2.94 to 5.2 log cfu g⁻¹ (Fig. 2F) in inoculated sample indicated the development of spontaneous lactic flora in the inoculated sample (Babji & Murthy, 2000). The antagonistic effect of LAB and their metabolic products were observed on microbial counts. Such antagonistic effect may be due to the production of organic acid, consequent lowering of pH and production of antibiotics and hydrogen peroxide (Bacus, 1984; Lucke, 1985; Jeppesen & Huss, 1993).

It is observed in the present study that inoculation of LAB to minced fish can enhance the shelf life of

fish meat by suppressing the growth of indigenous microbial species as well as potential food poisoning bacteria. There was no significant differences in control and inoculated sample with respect to chemical parameters such as TVBN, TBA and ERV. However, it was observed that microbial load reduced significantly throughout the study period. Growth of LAB may have been more prominent if samples were stored at higher temperature. Future studies may be carried out on microbial and sensory qualities of LAB inoculated fish meat at ambient temperature.

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