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Lactic Acid Fermentation of Minced Meat of *Leiognathus splendens* (Cuvier, 1829) using Different Bacterial Sources*

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Fermentation of minced meat of silverbelly *Leiognathus splendens* (Cuvier, 1829) using three different lactic acid bacterial sources viz. pure culture of *Lactobacillus plantarum,* fermented cabbage and curd revealed fermented cabbage as the ideal source of lactic acid bacteria for the preservation of fish meat through lactic acid fermentation. 48h fermented cabbage could bring down the pH of the minced meat mixture to 4.4 within 48 h of fermentation whereas the other two minced meat mixtures took nearly 84 h to reach the same pH. Fermented cabbage can also be easily prepared and maintained for fermenting fish meat on commercial scale. Free sugar, lactic acid content, NPN, TVBN and lactic acid bacterial count were found to be good indices of quality of the fermented product under storage.

Keywords : ***Leiognathus splendens, minced fish meat,*** **fermentation,** ***Lactobacillus plantarum,*** **curd, fermented cabbage**

During the last decade, the marine fish production of India has registered an increase from 2.28 million tonnes in 1988-89 to 2.83 million tonnes in 1998-1999 (Anon, 2001). Apart from the increase in the production of commercially important fishes, there has been considerable increase in the bycatch also *Leiognathids,* popularly called Silverbellies, are known for their abundance and occurrence through out the year in Indian coast and particularly in Tamil Nadu coast. Silverbellies are under-utilized owing mainly to their small size and high bone content, and a major portion of the catch is converted to fishmeal by traditional method of preparation. In countries like Norway and Sweden, fermentation of fish on commercial scale has been considered as an alternate way to effectively utilize the trash fish (Windsor & Barlow, 1981). However, research on this line is yet in infant stage in India.

Earlier studies on fermentation of minced fish meat have dealt with the use of two types of lactic acid bacterial sources viz (i) starter culture of homofermentative lactic acid bacteria and (ii) pre-fermented food material. However, in general, *Lactobacillus plantarum,* one of the highest acid producing bacteria (Steinkraus, 1983), has been widely used in the preparation of fermented fish meat by many investigators (Carl, 1953; Kreuzer, 1953; Kreuzer, 1954, James 1966; Brown & Summer; 1985).

Attempts have been made to use pre fermented food materials in preserving minced fish by fermentation (Wirahadikusumah, 1968; Wirahadikusumah *et al.* 1972; Wignall and Tattession; 1976.Durairaj *et al.*1982; Lingren and Pleje, 1983; Lone gram, 1986; Twiddy *et al.*, 1987). Very few studies have been made in India on the preparation of fermented fish products using different lactic acid bacterial sources (James *et al.*, 1977 and Durairaj *et al.*, 1982). The present study deals with the preparation of fermented *Leiognathus splendens*

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by lactic acid fermentation and the quality changes of the product during storage.

Materials and Methods

Leiognathus splendens that mainly constitutes the silver belly catch of Thoothukkudi coast was used for the study. Three types of lactic acid bacterial sources *viz,* 24h old starter culture of *Lactobacillus plantarum*, (LP), 12h old curd and 48h old fermented cabbage, (FC) were used as lactic acid bacterial sources to prepare fermented minced meat.

Curd was freshly prepared by adding sour buttermilk to the boiled and cooled milk. The 12h old curd contained the lactic acid bacterial count of 1.5 x 10^9 per gram. Freeze dried culture of *Lactobacillus plantarum* obtained from the National Dairy Research Institute, Karnal, India, was sub cultured in MRS broth twice and transferred to MRS slants. To prepare starter culture, the bacteria was transferred to MRS broth and incubated at 37°C, 24h prior to fermented minced meat preparation. The starter culture, thus prepared had the lactic acid bacterial count of 1.6 x 10^9 colonies per ml. Fresh cabbage was cut into small pieces, mixed with 4% of sodium chloride and stored in airtight container for 48h at room temperature. The fermented cabbage, thus prepared had a lactic acid bacterial count of 1.4 x 10^9 per gram.

Twenty-four kilograms of silver bellies were washed well and minced as a whole in an electrical mincer. The minced meat was divided into six batches. To each batch of minced fish, molasses, water, sodium chloride and potassium sorbate were added at the level of 15, 30, 1 and 0.1 % (w/w) respectively. The resultant mixture was boiled for 30 minutes and allowed to cool to room temperature. To the mixtures in duplicate, broth containing starter culture of *Lactobacillus plantarum* at 2 % (LP), fermented cabbage at 5% (FC) and curd at 5% (Curd) levels were added to prepare 'LP', 'FC' and 'Curd' fermented minced meats respectively.

The inoculated minced meat mixtures were stored airtight in Polyethylene jars of 10 L capacity. Samples for recording drop in pH during initial phase of fermentation were drawn from each fermented minced meat mixture by keeping and opening the jars inside a laminar flow chamber to avoid external contamination during sampling. The drop in pH during the initial phase of fermentation was recorded up to 96 h at intervals of 12 h. The samples of fermented minced meat mixtures were analyzed for biochemical and microbial changes drawing samples on 5^{th} , 20^{th}, 45^{th},70^{th},100^{th} and 120^{th} day of storage. The proximate composition was determined as per AOAC (1985).

To determine the pH, 10 g of each fermented mixture was taken, mixed well with 40 mLof distilled water and pH was measured using a pH meter. Total free sugar was estimated as per the method of Caroll *et al.* (1956). To estimate the lactic acid content, 1 g of the fermented minced meat was diluted to 100 mL with distilled water and deproteinized using Metaphosphoric acid. From the protein free extract, 5mL was taken and used for the colorimetric estimation of lactic acid using Para hydroxy diphenyl reagent (Nani & Baldini,1964). The TCA-soluble nitrogen was estimated by Micro kjeldhal method (AOAC, 1985) and the Total Volatile Base Nitrogen (TVBN) was estimated by Conway's Microdiffusion method (Conway, 1947). For microbiological analysis, 10 g of the sample was transferred to 90 mL of 0.85 % sterile saline diluent and was used to prepare further serial dilutions for plating.

The Total plate count, Lactic acid bacterial count, Proteolytic bacterial count, Coliform, Aerobic spore formers and Yeast were estimated (APHA, 1976). Catalyze test was carried out by flooding the colonies on MRS plates with 10 % hydrogen peroxide solution. The catalase negative colonies were treated as lactic acid bacterial colonies.

To enumerate proteolytic bacteria, Nutrient Agar medium mixed with sterilized skimmed milk powder at 1% level was used. Violet Red Bile Agar was used to estimate coliform bacteria. To enumerate aerobic spore formers, the fermented minced meat was heated at 80° C for 20 minutes to kill the vegetative cells and Thioglycollate Agar medium was used for the culture. Selective medium for the estimation of yeast content in the fermented minced meat mixtures was prepared by bringing down the pH of Mycological agar to 4.5 prior to plating by adding 10% sterile lactic acid at a level of about 15 ml per litre of melted medium. Analysis of variance technique was employed to analyze the differences between the fermented minced meat mixtures with respect to various microbial and biochemical changes during storage. Simple correlation coefficient was worked out to understand the degree of relationship between sugar content and lactic acid production and between lactic acid bacterial count and lactic acid production.

Results and discussion

Proximate composition of different fermented mixtures are presented in Table 1. The protein contents of the fermented mixtures in general were comparatively higher than the values observed by Durairaj *et al.* (1982). The changes in pH during the initial phase of fermentation of minced meat of *Leiognathus splendens* are presented in Fig. 1. The minced meat added with 'FC,' 'Curd', and 'LP' prior to the on set of fermentation had different pH values viz. 6.35 6.8, and 7.0 respectively and the fermented mixtures also differed with respect to pH reduction both during initial phase of fermentation and during storage. The 'Curd' and 'LP' minced meat mixtures reached almost a stable pH of 4.4 in 84 h and 'FC-minced meat mixture reached the same pH by 48 h indicating faster fermentation in 'FC- minced meat mixture.

Table 1. Proximate composition of 10 days old fermented mixtures

Types of fermented mixture	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Curd	71.9±0.52	17.20±1.25	4.64±0.32	4.39±0.06
LP	74.89±0.13	16.94±0.21	3.45±0.26	3.37±0.27
FC	72.34±0.11	16.10±0.15	2.84±0.21	3.35±0.18

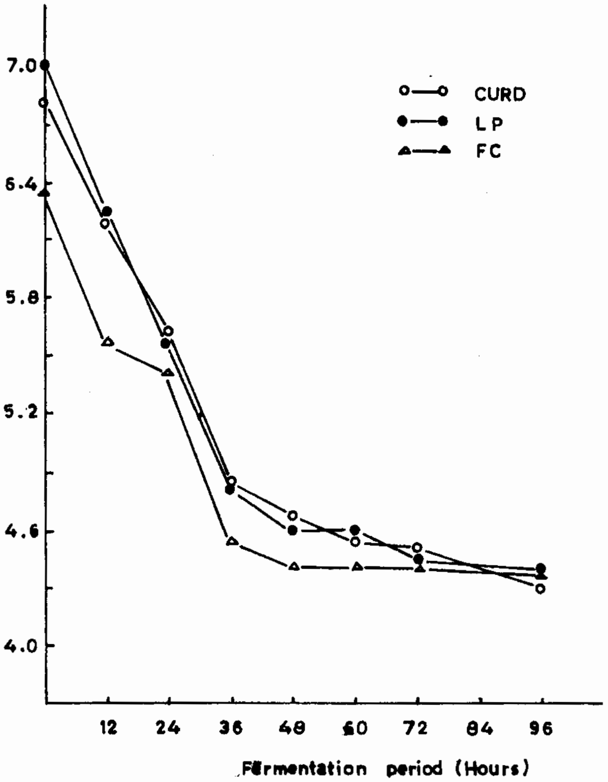

Fig. 1. Changes in pH during initial fermentation period

In the present study, a steady reduction of free sugar in all fermented mixtures on storage up to 45th day could be observed and it could not be detected in the 70th day samples (Table 2). A clear inverse relationship could be observed between free sugar content and lactic acid production in all the three types of fermented mixtures during storage. The correlation co-efficients explaining the relation between sugar content and

Table 2. Changes in percentage of free sugar content of fermented mixtures during storage

Storage period (in days)	Types of fermented minced meat Curd	LP	FC
5	1.03	0.51	2.35
20	0.35	0.42	0.33
45	0.02	0.05	0.03
70	0	0	0
100	0	0	0
120	0	0	0

lactic acid production were,-0.86, -0.91 and –0.94 in 'Curd', 'LP' and 'FC' fermented mixtures respectively. Paul & Southgate (1978) reported that fresh cabbage contains about 3.5% of carbohydrate which may be the reason for the relatively higher amount of free sugar recorded in 'FC' fermented mixtures on 5th day of storage (Table 2). The initial free sugar content was higher in 'FC' (2.35%) and 'Curd' (1.03%) fermented mixtures than in 'LP' fermented mixture (0.51%) and the rate of reduction of free sugar was also higher in 'FC' and 'Curd' fermented mixtures than in 'LP' fermented mixture (Table 2). The faster rate of sugar utilization in 'FC' and 'Curd' fermented mixtures than in 'LP' fermented mixture may be attributed to higher lactic acid bacterial count in 'FC' and 'Curd' fermented mixtures (Fig. 5).

A clear negative relation between the lactic acid formed and sugar utilization could be observed in all fermented mixtures (Table 2 and Fig.2). The free sugars in fermented minced meats were found fully utilized within 45 to 50 days of storage. The formation of lactic acid in 'LP' fermented mixture on par with 'FC' and 'Curd' fermented mixtures may be attributed to the effective production of lactic acid by pure

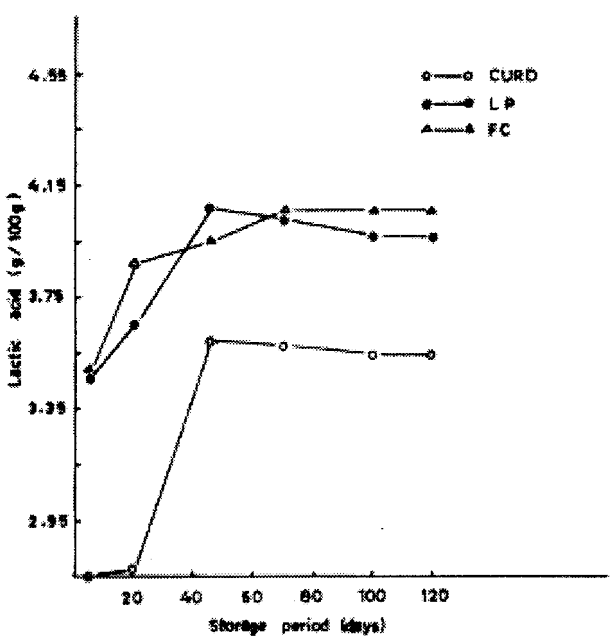

Fig. 2. Changes in lactic acid content during storage

culture of homofermentative lactic acid bacteria *Lactobacillus plantarum* utilizing limited source of sugar. The 'FC' fermented mixture meat showed the maximum lactic acid content of 4% on 70th day while the 'Curd' and 'LP' fermented minced mixture showed their maximum values of 4 and 3.5% respectively on 45th day of storage itself. This might be due to the formation of lactic acid from the bound sugars of cabbage. The levels of lactic acid observed in the fermented minced mixture of the present study were comparable with the levels recorded by James, (1966), Durairaj *et al.* (1982) and Lindgren & Pleje (1983).

Hall *et al.* (1985) reported that limited autolysis in fermented fish ensilages may be beneficial in restricting the release of free amino acids which are capable of reacting with lipid oxidation products resulting in the reduction of nutritional value of fermented minced meat based diets. Raa *et al.* (1983) have also reported that high amount of free amino acids and peptides released by protein breakdown in fermented minced meat might cause excessive deamination by ruminant microbes when fermented minced meat based diets are fed to them. Among fermented mixtures of the present study, the 'LP' fermented mixture had high level of TCA soluble Nitrogen throughout the study period (686mg% at 120th day of storage) followed by 'Curd' and 'FC' mixtures (651mg% and 560mg% respectively) indicating a relatively higher protein breakdown in 'LP' fermented mixture (Fig.3). The relatively low amount of TCA soluble Nitrogen observed in 'FC' fermented mixture indicated that it might serve as a good source of protein in the diets of ruminants than 'LP' and 'Curd' fermented mixtures. However, further studies are required to arrive at solid conclusion.

The Total Volatile Base Nitrogen (TVBN) content of a product is a measure of all volatile amines and ammonia which might have resulted from nucleotide breakdown or by the action of Proteolytic enzymes (Jones

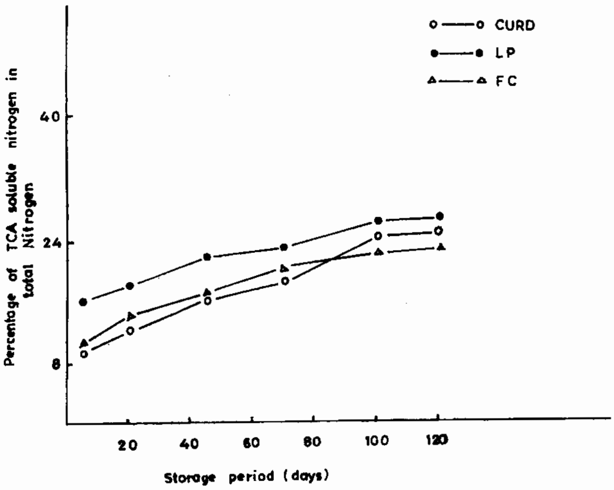

Fig. 3. Changes in percentage of TCA soluble nitrogen during storage

& Murray, 1962). Lindgren & Pleje (1983) defined TVBN as the measure of total amount of amines and ammonia derived from amino acids. The TVBN values increased with the increase in storage period and ranged between 35 - 155 mg%, 28 - 98mg% and 30 - 70 mg% in 'Curd', 'LP' and FC fermented mixtures respectively during storage. The changes in percentage of TVBN to total Nitrogen of the fermented mixtures during storage are presented in Fig.4. Irrespective of the type of fermented mixtures, the TVBN steadily increased during storage. The TVBN content of the fermented mixtures recorded in the present study during the initial period of storage are in concurrence with the results reported by Durairaj *et al.* (1982). Among three types of fermented mixtures, the fermented mixture prepared with 'Curd' was found to contain relatively a higher amount of TVBN throughout the study period (Fig 4). The steady increase in TVBN indicated the existence of certain spoilage process in the 'Curd' fermented mixture.

Table 3. Analysis of variance of biochemical and microbial characteristics of different fermented mixtures during storage

Sl. No.	Quality characteristics	Sources of variation	Degrees of freedom	SSQ	MSSQ	'F' ratio
1.	PH	Between fermented mixtures	3	0.04	0.0130	1.40*
		Between periods	5	1.10	0.2200	23.66
		Error	15	0.14	0.0093	
2.	Free sugar (%)	Between fermented mixtures	2	540882	270441	0.867*
		Between periods	2	2574152	1287076	4.125*
		Error	4	1248140	312035	
3.	Lactic acid (%)	Between fermented mixtures	2	1.42	0.710	29.58*
		Between periods	5	1.15	0.230	9.58*
		Error	10	0.24	0.024	
4.	TCA-soluble nitrogen (mg %)	Between fermented mixtures	3	3854851	1284950.30	94.96*
		Between periods	5	607029	121405.80	8.97*
		Error	15	202970	13531.30	
5.	TVBN (mg %)	Between fermented mixtures	3	10292.0	3430.67	17.78*
		Between periods	5	18998.8	3799.76	19.69*
		Error	15	894.2	192.95	
6.	Lactic acid bacterial count	Between fermented mixtures	2	19.48	9.740	263.24*
		Between periods	5	1.12	0.224	6.05*
		Error	10	0.37	0.037	

* Significant at 1% level

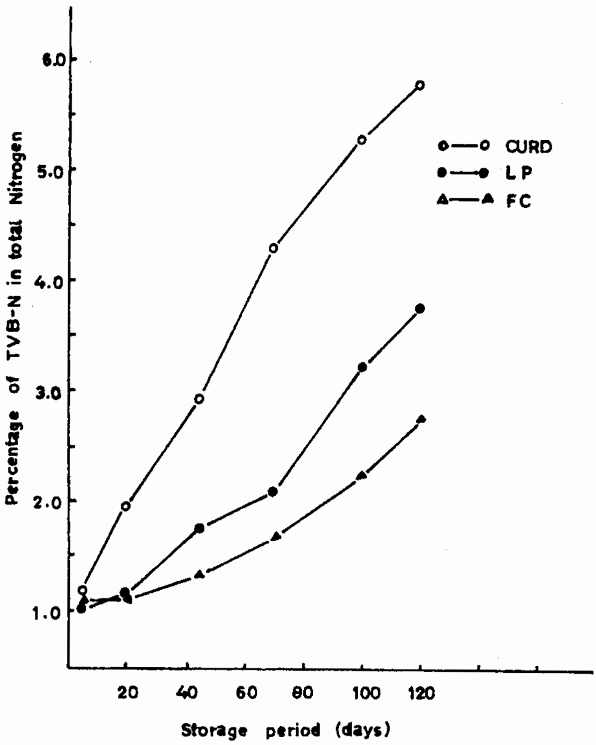

Fig. 4. Changes in TVBN during storage.

In the present study, TPC was generally lower than the lactic acid bacterial count indicating the inability of lactic acid bacteria to grow well on Nutrient Agar Medium. Durairaj *et al.*, (1982) in his studies, treated lactic acid bacterial count as TPC. However, Adams *et al.*, (1987) and Twiddy *et al.*,(1987) have treated these two counts separately as significant difference could be observed between the counts of them. The result of the present study supports the view of Adams *et al.* (1987) and Twiddy *et al.* (1987).

The changes in lactic acid bacterial count of fermented mixtures during storage are presented in Fig. 5. Though the changes were not significant, the lactic acid bacterial count of the fermented mixtures increased steadily to reach the maximum followed by a slight decrease on storage. Among fermented mixtures, 'FC' fermented mixture showed the highest lactic acid bacterial count of 1.60 x 10^6 per gram on 5th day and it increased to reach a maximum count of 1.9x10^7 per gram on 70th day. Though the lactic acid bacterial count of 'LP' fermented mixture was low and ranged between 7.20 x 10^3 and 4.6 x 10^4 per gram, the amount of lactic acid produced in this fermented mixture was notably high indicating the higher efficiency of *L.plantarum* in producing lactic acid compared to the mixed species of lactic acid bacteria of 'FC' and 'Curd' fermented mixtures (Fig. 2 & 5). Good correlation between lactic acid formation and lactic acid bacterial count could be observed in all fermented mixtures as evident from the correlation co-efficients of 0.98, 0.90 and 0.73 for 'Curd' 'LP' and 'FC' fermented mixtures respectively.

The 'Curd' and 'FC' fermented mixtures were found to contain certain aerobic spore formers to the tune of 3x10^3 and 1x10^4 /gram respectively on 5th day of storage. However this count decreased drastically on storage and reached a level of 4x10^1 and 3 x 10^2/ g respectively on 120th day of storage. The 'LP' fermented mixture was devoid of aerobic spore formers throughout the study period. The presence of aerobic spore forming bacteria in fermented mixtures prepared with pre-fermented food materials like curd and fermented cabbage revealed

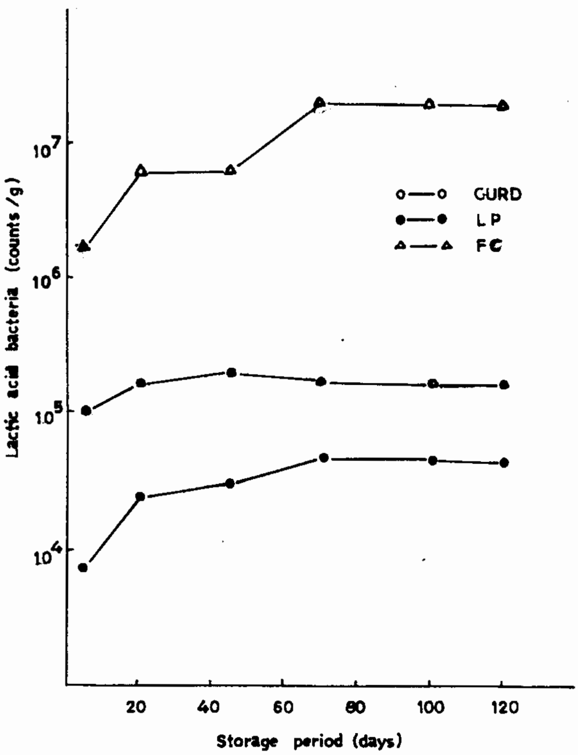

Fig. 5. Changes in lactic acid bacterial count during storage

that these two materials might have served as inoculums for spore forming bacteria in the respective fermented mixtures. All fermented mixtures of the present study were free from yeast, Coliforms and proteolytic bacteria during the entire period of study. The reason for the complete absence of yeast in all the fermented mixtures may be attributed to the incorporation of the yeast inhibitor, potassium sorbate in the preparation. Lindgren & Pleje (1983) have made similar observation in fermented fish meat incorporated with sorbic acid. The complete absence of Coliform in all the three fermented mixtures of the present study may be attributed to the low pH, which has not provided a conducive environment to Coliforms to grow. Wirahadikusumah (1968) observed the complete destruction of Coliform and enterococci in fermented fish mince at a pH of 4.5. The complete absence of proteolytic bacteria in all the three fermented products during storage reveals that the proteolysis of the fermented minced meat was due to protease enzymes of the fish and not due to the activity of proteolytic bacteria. This is in concordance with the findings of Lindgren & Pleje (1983) who reported that the proteolytic activity in fermented mixtures is mainly by tissue proteases and not due to microbial activity.

The results of the analysis of variance (Table 3) revealed significant differences between fermented mixtures with respect to most of the biochemical and microbiological changes studied during storage ($P < 0.01$). However the differences were insignificant ($P > 0.01$) with respect to pH, and total plate count. The significant differences between various fermented mixtures with respect to certain biochemical and microbiological characteristics may be due to the differences in the types of lactic acid bacterial sources used. The pH and total plate count of the products did not show significant differences on storage but parameters such as free sugar, lactic acid content, NPN, TVBN and lactic acid bacterial count recorded significant variations. Therefore, the latter parameters may be considered as indices of quality of fermented minced meat of fish under storage.

All the three lactic acid bacterial sources used in the present study could effectively produce lactic acid and preserve the fermented minced meat mixtures of *Leiognathus splendens* without any notable spoilage through out the study period. However, compared to the cost of maintenance of pure culture of *L.plantarum* in laboratory and preparation of curd, the fermented cabbage is cheap and easy to maintain without any sophisticated laboratory and hence fermented cabbage may be used as the lactic acid bacterial source for the preservation of minced meat of underutilized fishes on commercial scale.

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