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Effect of Different Levels of Fermentable Carbohydrate on the Degree of Hydrolysis of Fish Silage

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Fermented silage production by Lactic Acid Bacteria (LAB) represents a low cost method for the preparation of food and feed products characterized by hygienic quality and improved shelf life. The present study aimed to compare the effect of different levels of fermentable carbohydrate on the microbial level and the degree of hydrolysis in fermented fish silage from whole fish and dressed fish. Fermented fish silage was prepared with different levels of jaggery (w/w) i.e., 5%, 10% and 15% viz., type I type II and type III respectively. The study indicated that pH values decreased in all the samples during the period of ensilation except in type I sample which showed a higher pH after four days. Since the type I silage was spoiled at an early stage it is evident that 5% jaggery is not sufficient for proper preservation through fermentation. But in type II and type III, the changes in pH and degree of hydrolysis do not show significant difference during ensilation. The bacteriological results also indicate that the fermentation patterns of both type II and type III are almost same. Hence it is evident that for successful Lactobacillus fermentation of tilapia 10%, jaggery is sufficient either in whole condition or in dressed condition.

Key words: Lactic Acid Bacteria, Fish Silage, Fermentable carbohydrate

Lactic acid fermentation represents a low cost method for the preparation of food and feed products characterized by hygienic quality and improved shelf life (Frazier & Westhoff, 1988; McDonald *et. al.*, 1991). Fermentation of fish silages depends on *in situ* production of lactic acid by Lactic Acid Bacteria (LAB) added to the fish with a fermentable carbohydrate source. According to Nilsson & Rydin, (1965) fermentation of manure, slaughterhouse wastes and fish byproducts low in LAB require the addition of a fermentation flora. The technology of fermented silage production relies on the production of lactic acid at a rapid rate in sufficient concentrations by fermentation, which suppress spoilage organisms and preserve the feed until it is needed (McDonald et. al., 1991).

Lactic acid bacteria produce acids, reduce the pH and are tolerant to low pH and this can be the key factor in the competition with spoilers. Of the various starters like *L. plantarum. L. acidophilus, Pediococcus halophilus and P. acidilacti; L. plantarum* appears to be the most effective starter culture (Bello *et.al.*, 1992). They can operate over a wide range and have an extremely important effect in modified product such as silage and to a lesser extend in fish sauces and pastes (Han-Ching *et al.*, 1994). The present study aims to compare the effect of different levels of fermentable carbohydrate on the microbial level and the degree of hydrolysis in fermented fish silage from whole fish and dressed fish.

Materials and methods

Lactobacillus plantarum culture was procured from MTCC 1425 IMTECH Chandigargh, revived, repeatedly sub cultured in MRS broth. All the chemicals used were of analytical grade supplied by Merck, SRL, Sigma and Oxoid. Tilapia (*Orechromis mossambicus*) collected in fresh condition from local market, Cochin was used for the study. Fermented fish silage was prepared with different levels of jaggery (w/w) i.e.,

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5%, 10% and 15% viz. type I type II and type III respectively.

To prepare silage from whole fish, the fish was washed with potable water, homogenized in Tecator, 1094 Homogeniser for 5-8 minutes and cooked for 30 minutes with 30% of water and jaggery in a beaker. It was cooled and inoculated with *Lactobacillus plantarum* culture at 5% (W/V) level containing 10^9CFU / ml. The whole mass was mixed thoroughly, and covered tightly with polythene paper.

Preparation of silage from dressed fish was done by washing with potable water, followed by removal of gills and all internal organs and a second washing. It was thoroughly homogenized for 5-8 minutes, cooked and packed as above. Aliquots of samples were drawn at intervals and analysed for chemical and microbiological parameters.

For quantitative methods of microbiological analysis, USFDA (2001) methodology was followed for Total plate Count, Lactic acid bacteria (LAB) *E. coli, Staphylococcus* and *Salmonella*. For qualitative estimation, the colonies were isolated from TGA and identified by the method of Surendran *et. al.* (2003).

pH of the samples were directly measured using a Cyberscan 510 pH meter. Nitrogen content and Non Protein Nitrogen content were determined by the method of AOAC (2000). Carbohydrate content was determined by the method of Cleg (1956).

Statistical analysis of the results was expressed as mean or mean log ± SD for biochemical & microbiological parameters. Statistical analysis between the means using ANOVA and Tukey's test. Statistical package used in the study was SPSS, 10.

Results and Discussion

Changes in pH during ensilation of whole tilapia with different levels of carbohydrate are given in Table 1. During ensilation the carbohydrate added was fermented by the bacteria, which resulted in the production of lactic acid. As a result the pH of the samples decreased. The rate of decrease was faster during first 2 days followed by a slow decrease. The rapid growth of lactic acid bacteria during the early stages of fermentation resulted in the production of lactic acid with a concomitant reduction in pH. Ahmed & Mahendrakar (1996) also reported almost the same results during the fermentation of fresh water fish viscera. In type I (with 5% jaggery) the pH decreased to 4.7 on fourth day and increased thereafter. This could be due to the depletion of sugar and consequently the pH increased and the samples were spoiled. The fermented fish silage prepared by Kompiang *et. al* (1979) with 5% molasses had a pH of 5.0 by three days and got spoiled by 10 days. The pH of fermented fish silage prepared using fermented cabbage and curd as the inoculum reached 4.6 and 4.9 respectively (Neethiselvan *et. al.* 2002). In type II, the pH decreased to 3.9 on 10^{th} day and then started to increase probably due to the neutralization of lactic acid with the nitrogenous compounds produced. But in the case of type III, the pH showed the decreasing trend throughout the experiment due the presence of excess sugar. It is observed that the addition of sugar at higher concentrations does not have significance on the pH change. Durairaj *et. al.* (1985) reported that the pH of the bacterial silage prepared from silver belly stored in jerry cans was stable at 4.0 through out the storage period of 12 months and even beyond.

Table 1. Changes in pH during ensilation of whole tilapia at different levels of carbohydrate

Days	5%	10%	15%
1	6.97 ± .06 ^{a}	6.9 ± 0.03 ^{a}	7.0 ± 0.15 ^{a}
2	5.26 ± 0.1 ^{a}	5.23 ± 0.04 ^{a}	5.1 ± 0.1 ^{a}
4	4.7 ± 0.1 ^{a}	4.21 ± 0.03 ^{b}	4.2 ± 0.1 ^{b}
6	4.9 ± 0.05 ^{b}	4.02 ± 0.04 ^{a}	4.1 ± 0.05 ^{a}
10	-	3.97 ± 0.06 ^{a}	4.06 ± 0.05 ^{a}
14	-	4.12 ± 0.01 ^{b}	4.06 ± 0.15 ^{a}

Results are presented as mean ± standard deviation (SD) of 3 replications .^{a,b,c,d,e}, mean in a row with the same superscript values are not significantly different ($P<0.05$).

Changes in pH during ensilation of dressed tilapia at different levels of carbohydrate is given in Table 2. As in the case of whole fish silage, the pH values decreased in all the samples during the period of ensilation except in type I sample which showed a higher pH after four days. This could be due to depletion of carbohydrate as in the case of whole tilapia. The decrease in the case of whole fish was marginally higher compared to the dressed fish with the same level of sugar. This could be due to the action of intestinal cathepsins which could result in partial hydrolysis of the muscle during initial cooking and a consequent enhanced activity of the added bacteria. According to Babu *et. al.*, (2005), a concentration of bacteria of 10^8 cells/g caused a decrease of the pH to 4.5 by the end of first day. Lindgren (1992) observed that *L. plantarum* produced about 4.5% lactic acid in fish silage.

Table 2. Changes in pH during ensilation of dressed tilapia at different levels of carbohydrate

Days	5%	10%	15%
1	6.94 ± .06^a	6.96 ± .05^a	7.13 ± 0.11^b
2	5.40 ± 0.1^a	5.6 ± .05^a	5.30 ±0.10^a
4	4.09 ±.09^a	4.31 ± .01^b	4.38 ± 0.32^b
6	5.20 ± 0.1^b	4.22 ± .005^a	4.14 ± 0.04^a
10	5.60 ± 0.26^b	4.07 ± .01^a	4.12 ± 0.005^a
14		4.03 ± 0.11^b	4.03 ± 0.05^b

Results are presented as mean ± standard deviation (SD) of 3 replications .^{a,b,c,d,e}, mean in a row with the same superscript values are not significantly different ($P<0.05$).

Changes in LAB count during ensilation of whole tilapia at different levels of jaggerry are given in Fig 1. The pH of this set showed the highest value of 5.26 on 2nd day. But on 4th day of inoculation, the counts increased to log 8.3cfu/ml and remained more or less same upto 6th day. Due to spoilage, no further observation was possible for this lot. But in type II and type III samples LAB count increased initially upto 6th day of fermentation and thereafter showed a declining trend indicating the depletion of carbohydrate source. As a consequence during the next observation on 9th day and 14th day the LAB count was reduced to log 6.3 cfu/ml in type II and log 7.4 cfu/ml in type III respectively. Fadda *et. al* (2002) observed that in a model sausage system, the LAB count remained at 10^8 cfu/g through out the course of fermentation whereas, according to Neethiselavan et. al (2002) the LAB count increased from the initial 1.6 X 10^6 cfu/ g on 5th day and further increased to reach a maximum count of 1.9 X10^7 /g on 7th day.

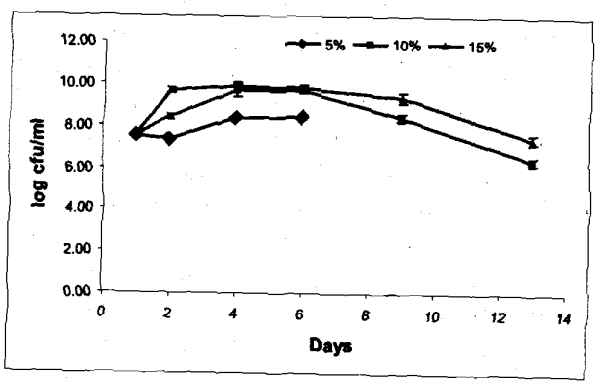

Fig. 1. Changes in Lactobacillus count during ensilation of whole tilapia at different levels of carbohydrate

Changes in LAB count during ensilation of dressed tilapia at different levels of carbohydrate are given in Fig. 2. The LAB count of all the three samples increased initially indicating the utilization of carbohydrate by the starter culture. In type I, the count increased up to four days and showed decreasing trend. The LAB count in dressed fish silage was found to be higher. This

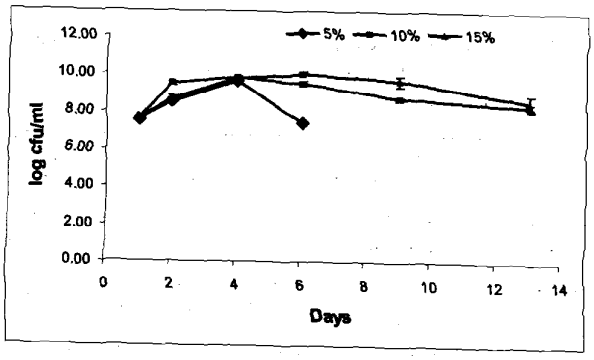

Fig. 2. Changes in LAB count during ensilation of dressed tilapia at different levels of carbohydrate

value declined in case of type II and type III after 6 days. But the rate of reduction was very low which could be due to the presence of sufficient quantity of sugar in the substrate. Through out the period of fermentation, the count of LAB remained in the range of 7.4 log and 10.0 log CFU/ ml indicating satisfactory conditions for fermentation.

Table 3 depicts the changes in Total Plate Count (log cfu/ml) of whole tilapia during ensilation at different levels of carbohydrate. The TPC levels during ensilation showed an inverse relation as that of LAB count. For the initial 4 days, no TPC was detected indicating the inhibition of spoilers by *Lactobacillus plantarum*. In type I, the total plate count observed on 6th day was 2.4 CFU/ ml and since sample got spoiled no further observation was made. Depletion of carbohydrate (Fig.3) and consequent decrease in LAB count and increasing pH caused the growth of spore forming spoilage bacteria which might have caused the spoilage of samples. In type II and type III samples, TPC was not detected till 9th day. This could be due to the inhibition of normal bacterial population by *L. plantarum*. According to Faid *et.al.* (1997) the lactic acid fermentation is usually accompanied by some metabolites (Bacteriocins), which may help the preservation of fermented foods by way of suppressing the spoilage organisms Kannappan & Manja (2004). Since the carbohydrate supplied is limited by 12 days, and due to release of other volatile compounds, the pH started

Table 3. Total Plate Count (log cfu/ml) of Whole Tilapia During Ensilation at different levels of carbohydrate

	5%	10%	15%
2 day	Not detected	Not detected	Not detected
4 day	Not detected	Not detected	Not detected
6 day	2.49 ± 0.14	Not detected	Not detected
9 day	Not done	2.72 ± 0.21	2.66 ± 0.19
13 day	Not done	5.68 ± 0.20	5.19 ± 0.06

Results are presented as mean ± standard deviation (SD) of 3 replications.

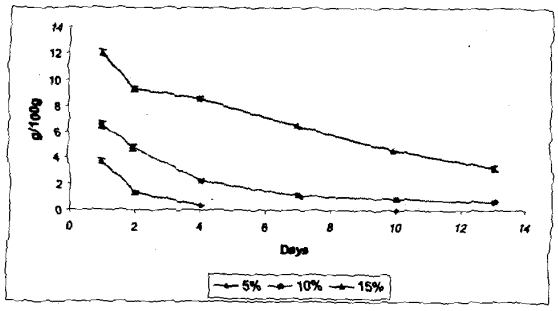

Fig. 3. Changes in the levels of carbohydrate during ensilation of whole tilapia

increasing which consequently resulted in the growth of other spoilage organisms as evidenced by the increase in total plate count. All the colonies were of same physical appearance. Typical colonies were selected and the isolated colonies were identified as *Bacillus* sps. (Surendran *et. al.* 2003). *E. coli, Staphylococcus and* Salmonella were not detected in the samples.

Total Plate Count of dressed tilapia during ensilation at different levels of carbohydrate is given in Table 4. During the initial 4 days TPC was not detected except in sample type I which increased on 6th day, to the tune of 6 log CFU/ ml. Aerobic plate count was not detected in other two samples on the same day, which could be due to the low pH and higher lactobacillus count. Colonies were detected in other two groups on 9th day of fermentation and a slow increase in count was observed which might be due to the germination of spores that survived the initial heat treatment and its multiplication.

Table 4. Total Plate Count (log cfu/ml) of Dressed Tilapia During Ensilation at Different Levels of Carbohydrate

	5%	10%	15%
2 day	Not detected	Not detected	Not detected
4 day	2.67±0.16	Not detected	Not detected
6 day	6.63±0.17	Not detected	Not detected
9 day	Not done	4.23±0.09	3.68±0.12
13 day	Not done	4.99±0.12	5.12±0.21

Results are presented as mean ± standard deviation (SD) of 3 replications

The degree of hydrolysis (DH) during ensilation of whole tilapia is given in Fig 3. Even though in type I the degree of hydrolysis was progressing initially, due to depletion of sugar, the sample was spoiled. Lactic acid bacteria ferment the sugar present to organic acid, thus lowering the pH. If the pH falls sufficiently low (4.5) growth of putrefactive organisms and pathogens are inhibited. But in type II, the DH progressed at a faster rate initially and slowed during the end of ensilation, which could be due to the decreased activity of microbes due to depletion of sugar. In type III, the degree of hydrolysis was similar to that in type II. Increase of non protein nitrogen was observed when pilchard waste was fermented with 25% molasses throughout for the period of 11 days by Faid et. al (1997). In the present study, about 60% hydrolysis of the protein was observed in all the samples. A similar observation was noted in blue whiting ensilage by Maria et. al (1998). Since the DH of the three sets of samples was almost same, the levels of sugar could be limited to 10%.

Degree of hydrolysis (ratio of total nitrogen to non protein nitrogen) during ensilation of dressed Tilapia at different levels of carbohydrate is given in Fig 4. In case of dressed fish also, the DH was found to be increasing during the course of fermentation. After 7 days, the rate of hydrolysis was less up to 13 days, which could be due to the presence of complex proteins difficult to break. But compared to whole fish silage, the DH was less in all sets of samples. The final observation of less DH could be due to utilisation of hydrolyzed protein by the microbes on depletion of sugar. Backhof (1976) has reported a greater value of NPN (83.5%) in cod viscera compared to fish flesh.

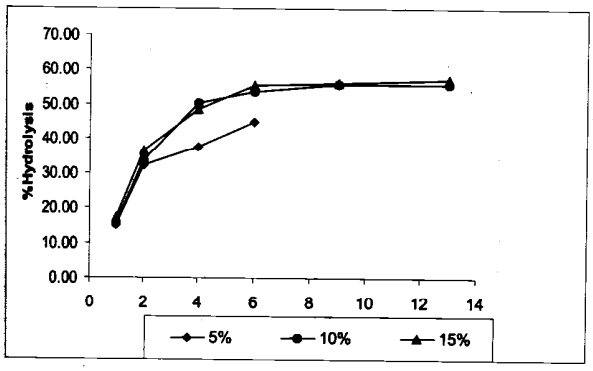

Fig. 4. Degree of hydrolysis during ensilation of whole Tilapia at different levels of carbohydrate

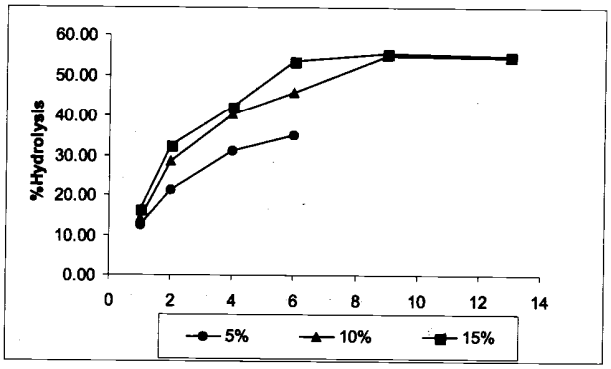

Fig. 5. Degree of hydrolysis during ensilation of dressed Tilapia at different levels of carbohydrate

Since the type I silage was spoiled at an early stage, it is evident that 5% jaggery was not sufficient for proper preservation through fermentation. But in type II and type III, the changes in pH and degree of hydrolysis do not show significant difference during ensilation. The bacteriological results also indicate that the fermentation patterns of both type II and type III are almost same. Hence it is evident that for successful Lactobacillus fermentation of tilapia 10%, jaggery is sufficient either in whole condition or in dressed condition.

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