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EFFECT OF TEMPERATURE AND USE OF FERMENTED GREEN GRAM AS STARTER CULTURE ON THE PREPARATION OF FERMENTED FISH SILAGE

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Fermented fish silage was prepared at different controlled temperatures: 10°, 20°, 30° and 40°C. Samples were taken out at 0, 3, 7, 14 and 21 days for determining changes in pH value, non protein or soluble nitrogen, peptides, ammonia, amino acid nitrogen and lactic acid bacterial (LAB) count. According to the findings of this study, the temperature has a profound effect on the quality and the duration of fermentation of fish silage. Good quality silage can be prepared within 3 days at 40°, 7 days at 30°, 14 days at 20° and 21 days at 10°C. Merits of fermented green gram as starter culture were also evaluated at these temperatures.

Key words: Temperature, Fermentation, Lactic acid bacteria, Green gram, Starter, Fish silage.

Introduction

A previous communication [1] reported the preparation of fermented fish silage. The process may either be utilized by local fishermen to prepare feed component for poultry and other animals or may form the basis of a rural cottage industry.

The high ambient temperatures in tropical and subtropical countries emphasize the need to obtain information on the speed and efficiency of traditional fermentation, at a range of various temperatures. In Karachi, the temperature varies between 10°- 40°C during the year. A number of studies have shown that variation in temperature used for the preparation of fish silage has a profound effect on its quality [2-4].

In preparing fish silage, particularly, for use as ruminant feed, it is recommended to check the amount of soluble nitrogen in the feed [5]. The protein liquefaction is almost entirely due to autolysis which is temperature dependent. Knowledge of the extent of protein hydrolysis during the liquefaction from the view point of nutritional quality of silage becomes obvious.

Several studies are available on the microbiology, biochemistry and nutritional quality of vegetative and acid fish silage [5 - 7]. Not much information is available on the effect of storage temperature on the quality of liquid fermented silage.

The present study reports the effect of incubation temperature on the quality of fermented fish silage and the merits of fermented green gram *(Phaseolus aureus)* pulse as starter at different temperatures.

Materials and Methods

Methods for removal of samples, assessment of microbial load and analytical methods were reported previously [1]. For the preparation of silage, 2 kg of fish were minced once in an electric mincer having 7.5 cm diameter, plate (with 105, 4.5 mm holes/plate), 5% w/w each of commercial molasses and sucrose added and mixed thoroughly using stainless steel rod (1 cm diameter) for 5-10 mins. To one half of this mixture was added 10% v/w of active culture of *Lactobacillus plantarum* grown in de Man, Rogosa and Sharpe (MRS) broth (Oxoid), mixed again using the same rod. To the other half of the mixture was added 10% (w/w) fermented green gram *(Phaseolus aureus)*. Each of the inoculated fish carbohydrate mixture was divided in four plastic containers, one container from each of these two different inocula was incubated at 10°, 20°, 30° and 40°C for 21 days. The samples were drawn at 0,3,7,14, and 21 days for determining changes in pH, total soluble nitrogen in terms of peptide, ammonia and amino acids nitrogen (AAN) during ensilation. Six such experiments were carried out and results were expressed as means of six values.

The increase in autolysis in microbial silage in relation to time and temperature was estimated by measuring increases in the non protein or soluble nitrogen content of the samples during storage. Further information of soluble nitrogen was taken in terms of peptides, ammonia and amino acids during ensilation.

Peptide nitrogen was determined by substracting free amino acids (FAA) and ammonia nitrogen from the trichloro acetic acid (TCA) soluble non protein nitrogen (NPN).

Preparation of fermented gram pulse. 200 grams of pulse namely green gram *(Phaseolus aureus)* was soaked in tap water with a ratio of 1 : 3. The starter culture may be prepared using the whole seed or paste of soaked pules containing 2.5% (w/w) NaCl and kept in glass or plastic container at 28°- 30°C for 4 - 8 days. Usually 10^8 cfu/g of lactic acid bacteria were produced by natural fermentation and the pH value was observed to fall from 6 - 4. To prevent mould and yeast growth,

walls of the container and surfaces were sprayed with propionic acid or 1% potassium sorbate after 24 hr of fermentation [8].

Lactobacillus plantarum culture was the same used in the previous studies [1].

Results and Discussion

Figure 1 and 2 present the changes in pH and lactic acid bacteria (LAB) count in silage prepared at four different temperatures (10°, 20°, 30° and 40°C) in two batches using *L. plantarum* and fermented green gram as starter culture, respectively. The changes in pH values during storage, irrespective of the type of inoculum were of the same order. The pH dropped gradually in both batches of silage.

In the *L. plantarum* inoculated batch, a sharp decrease was noticed at 30° after 3 days and 20° after 7 days. From an initial pH value around 6, the pH ranged between 4.5 - 5.0 after 21 days. The sharp decrease in pH value at 30°C after 3 days of ensilation may be attributed to the optimum growth temperature of *L. plantarum.*

In fermented green gram batch, with the exception at 10°C, there was a sharp drop in pH at 20°, 30° and 40°C after 3 days followed by slow decrease throughout the storage. At 10°C a slow and gradual drop was obtianed. After 21 days storage the pH value ranged between 4.7 - 5.1 and no unacceptable off odours were obtained. The pH pattern showed that at 10°C there was slow production of acid from carbohydrate in both batches of silage (Fig 1, 2), however, fish may safely be preserved as good quality silage within 21 days. It has been established that fish and shelfish spoiled at 10°C within 2 - 4 days [9,10].

The LAB count increased during 3 days storage at each temperature in silage prepared using *L. plantarum.* After 3 days, a decrease in count was slower at 10° and 30°C. A sharp decrease was noticed at 40°C (Fig. 1).

Slightly different pattern of LAB count was observed in silage prepared using fermented green gram as starter culture (Fig 2). The counts increased at 20°, 30° and 40°C and decreased at 10°C during 3 days of storage. This may be attributed to the heterogeneous nature of LAB in fermented green gram. At 10°C the counts continued to increase during the remaining storage. Whereas at 20° and 30°C, the counts decreased throughout the remaining storage. A sharp decrease was noticed at 40°C after 3 days, followed by a gradual decrease during the storage.

As regard the microbial pattern, *L. plantarum* growth was observed at 10°, 20°, 30° and 40°C in silage prepared by adding pure *Lactobacillus* culture. However, a different pattern was observed in the silage batch prepared by adding fermented green gram as starter culture.

After 3 days storage at 10° and 20°C the dominant organisms on MRS agar were of *Leuconostoc* sp. which remained dominant only at 10°C up to 14 days. At 20°C mixed flora of *Leuconostoc* and *Lactobacillus* sp. appeared after 7 days of storage. As the storage progressed, *Lactobacillus* sp. became the dominant organisms at 10° and 20°C after 21 days.

At 30° and 40°C *Lactobacillus* sp. were the dominant organisms throughout the storage of 21 days. The observed

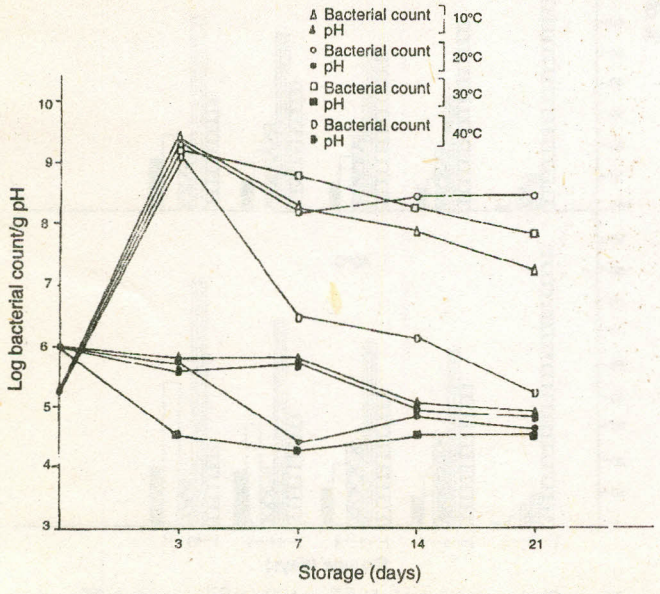

Fig. 1. Changes in log bacterial count and pH in fish silage prepared using *Lactobacillus plantarum* as starter during storage at 10°,20°, 30° and 40°C.

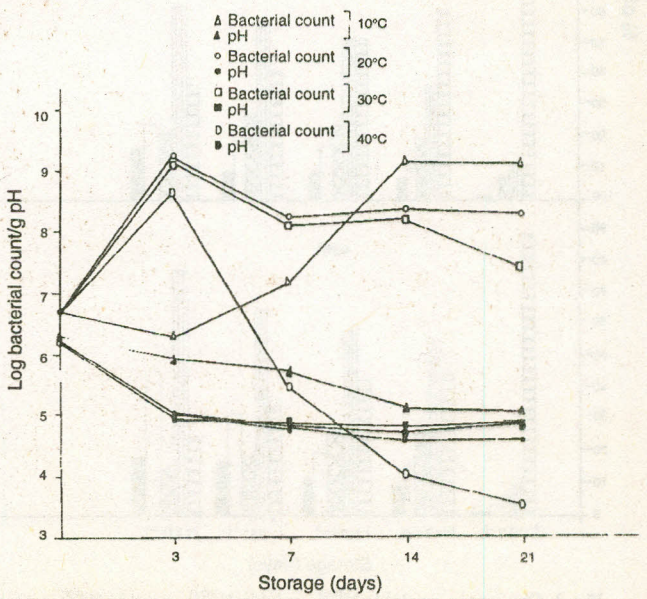

Fig. 2. Changes in log bacterial count and pH in fish silage prepared using fermented green gram as starter during storage at 10°, 20°, 30° and 40°C.

changes in the microbial pattern are quite understandable. Naturally fermented green gram under controlled conditions produce heterogeneous species of lactic acid bacteria. The organisms may grow at the respective suitable growth temperatures during storage at 10°, 30° and 40°C. At lower temperatures (10° and 20°C), initially *Leuconostoc* sp. dominated due to their psychrotrophic nature. The *Lactobacillus* sp. usually dominated at 30° and 40°C.

Figures 3 and 4 present the changes in soluble and insoluble nitrogen peptides, amino acids and ammonia during storage of silage prepared at different temperatuers using *L. plantarum* and fermented green gram as starter culture, respectively.

The ensiling mixture had the following composition : TCA insoluble N around 90%, TCA soluble nitrogen (AAN) 4.2% and traces of ammonia. Temperature was found to be the

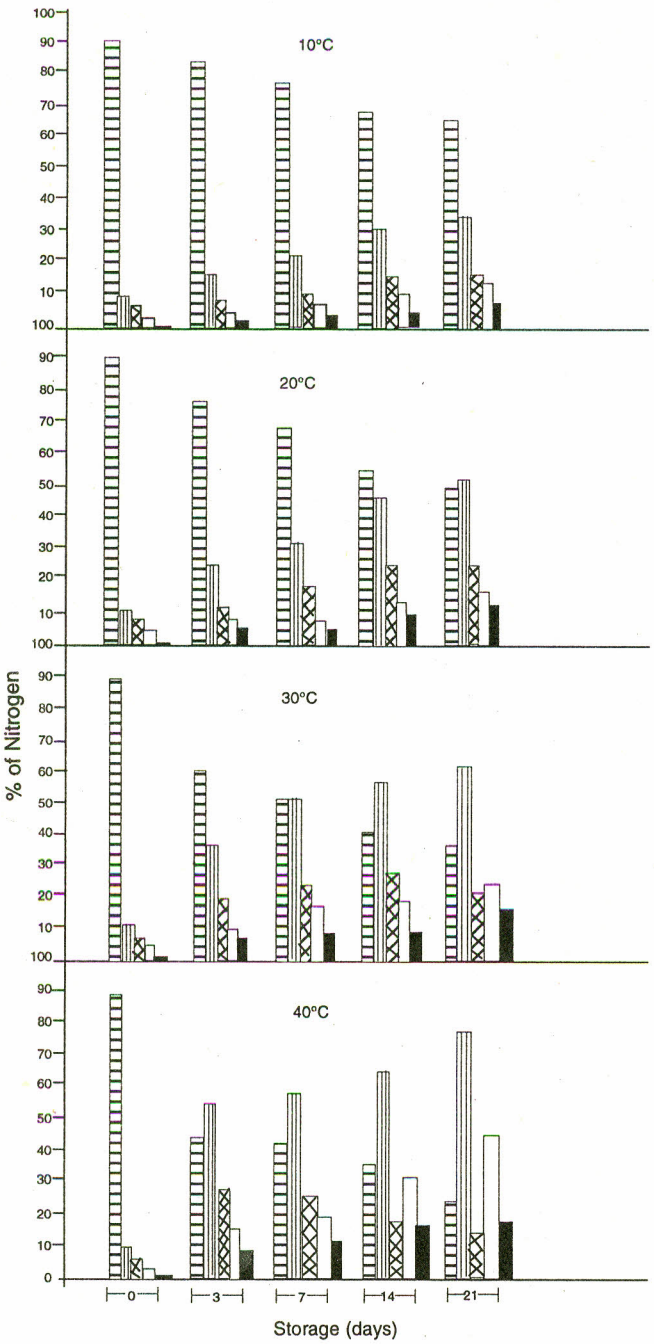

Fig. 3. Changes in insoluble N ▤, soluble N ▥, peptide N ▩, amino acid N □ and ammonia N ■, percentage in fish silage prepared using *Lactobacillus plantarum* as starter during storage at 10°, 20°, 30° and 40°C.

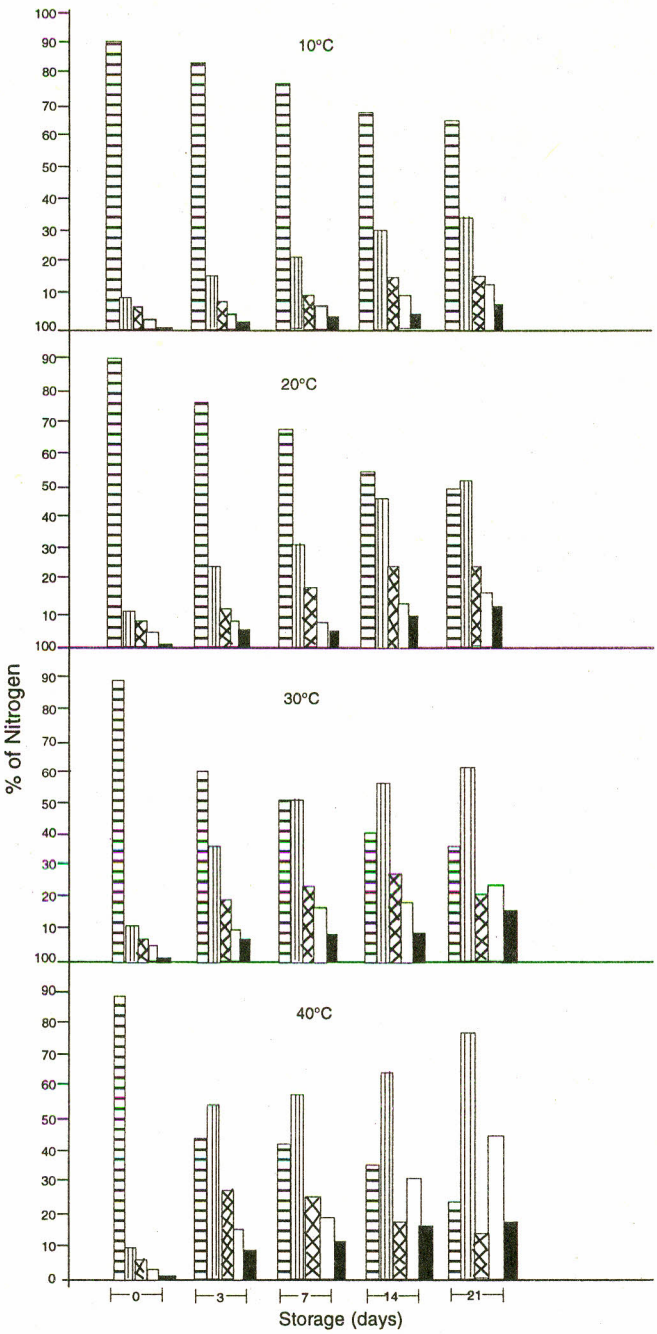

Fig. 4. Changes in insoluble N ▤, soluble N ▥, peptide N ▩, amino acid N □, and ammonia N ■, percentage in fish silage prepared using fermented green gram as starter during storage at 10°, 20°, 30° and 40°C.

determinative factor in the production of NPN during storage, the starter culture i.e. *L. plantarum* or fermented green gram played little role. The higher the temperature of incubation more the solubilization of N, peptide N, AAN and ammonia increased. A more rapid increase was noted at 30° and 40°C compared to 20° and 10°C.

Disney *et al.* [3] pointed out that the maintenance of a starter culture at village level in tropical countries is a serious handicap in the preparation of fermented fish silage. In a previous communication the successful use of sauerkraut as starter has been reported in detail [1]. Twiddy *et al.* [11] also reported that prefermented cassava (20% (w/w) of fish) gave rapid reproducible fermentation, the pH decreased to less than 4.5 and the LAB : spoiler ratio exceeded to 4 log cycles within 2 days. The handling and maintenance of pulses starter is easy for semi skilled worker to maintain. Also among pulses, use of green gram as starter is better than others, due to its better fermentation.

It has been reported by many workers that nutritional quality of fish silage can be improved by limiting the extent of protein hydrolysis into polypeptides and amino acids. Termination of the ensiling process after 3-7 days has been reported to improve the quality of silage resulting in more weight gain, protein efficiency ratios (PER), biological value (BV) and net protein utilisation when fed to mink, rats, rainbow trout and sheep [5, 12-14].

In has been recommended that good quality silage should contain an abundance of relative short peptides and less amino acids and ammonia [7]. If the fish silage fermentation is allowed to continue, more amino acids and ammonia are produced. Amino acids are produced due to autolysis which is temperature dependent [4 - 7]. The high ammonia-N production might be due to de-amination of amino acids by lactic acid bacteria [1]. Therefore, it is desirable to discontinue the process of silage fermentation by heating after 7 days at 30°C, or 3 days at 40°C, at a pH value close to 4.5.

From the chemical changes observed at different temperatures, it may be concluded that good quality fish silage can be prepared within 3 days at 40°C, 7 days at 30°C, 14 days at 20°C and 21 days at 10°C using green gram as a starter. Further studies and data are required on the nutritional aspects of silage as poultry and other animal feeds.

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