

## PRODUCTION OF FISH SAUCE FROM LOW COST SMALL FRESHWATER FISH AND THEIR QUALITATIVE EVALUATION

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### ABSTRACT

The study was conducted to produce fish sauce from small freshwater fishes like Kholisa (*Colisa fasciatus*), Tit punti (*Puntius ticto*), Jat punti (*Puntius sophore*), Tengra (*Mystus vittatus*), Taki (*Channa punctatus*), Gutum (*Lepidocephalichthys guntea*), Guchi (*Macragnathus pancalus*), Veda (*Nandus nandus*), and Bujuri (*Mystus tengara*) by using different concentrations of salt (25% as Treatment-I, 30% as Treatment-II and 35% as Treatment-III). Fishes were fermented for 9 months by the combined action of microorganisms and enzymes present in fish tissue and viscera in presence of salt. During fermentation the initial protein content in fish was 15.17% which were nearly digested and liquefied at the end of fermentation. Crude protein content on the basis of total nitrogen were found to be 14.58, 13.88 and 12.63% in Treatment-I, II and III respectively while the highest ash content in fish sauce was 22.97% in Treatment-III. Initial Total Volatile Base Nitrogen in fish was 1.92 mg/100g, which reached 20.88, 20.52 and 20.31 mg/100g respectively in three treatments. Initial aerobic plate count (APC) in fish was  $5.99 \times 10^6$  cfu/g and after fermentation and 15 days of aging the APC in fish sauce was reduced to  $1.53 \times 10^4$  cfu/ml in Treatment-I,  $1.40 \times 10^4$  cfu/ml in Treatment-II and  $1.27 \times 10^4$  cfu/ml in treatment-III. The major bacterial groups identified in fish sauce were *Bacillus*, *Micrococcus*, *Lactobacillus* and *Pseudomonas*. Considering the values of different parameters and result of panel test, fish sauce produced with 25% salt (Treatment-I) was found to be of best quality.

**Key Words:** Fish Sauce, Salt treatment, Fermentation

### INTRODUCTION

Many fermented fish products are prepared in different parts of the world and the method of processing depends upon various factors, such as availability of raw materials, consumers' preference and climatic condition of the region (Al-Jedah *et al.*, 2000). Traditional fermentation in developing countries is one of the oldest processing methods which are used for preserving fish, where it is not only extend the shelf life but also enhanced a unique taste, flavor and nutritional quality of end products (Anihouvi *et al.*, 2006 and Nayeem *et al.*, 2010). There are a number of fish fermentation products such as fish

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sauce which are mainly prepared for flavor enhancers and condiments. Fish sauce is a product that can be made cheaply from various fish raw materials, which are not normally used for food fish. It is a brown, liquid seasoning commonly used in most parts of Southeast Asia. It is called by different names in different countries and it contains a mixture of amino acids and other protein degradation products (Gildberg, 2001; Fukami *et al.*, 2002 and Ichimura *et al.*, 2003). In Southeast Asia millions of liters of fish sauce are produced annually for daily consumption by at least 350 million people, which is prepared from both sea and freshwater fish. Almost every species of fish and even shellfish can be used as raw material (Beddows, 1979). All the fish sauce being sold in Bangladesh is imported and there is no report of producing fish sauce in Bangladesh. Many small fish species from both freshwater and marine water are underutilized or not utilized economically. Considering the above factors the present study was undertaken for the production of fish sauce using cheap local raw material and simple technology.

## MATERIALS AND METHODS

The present study was conducted in the laboratory of Fisheries Technology Department, Bangladesh Agricultural University (BAU), Mymensingh. The samples which included Kholisa (*Colisa fasciatus*), Tit punti (*Puntius ticto*), Jat punti (*Puntius sophore*), Tengra (*Mystus vittatus*), Taki (*Channa punctatus*), Gutum (*Lepidocephalichthys guntea*), Guchi (*Macrognathus pancalus*), Veda (*Nandus nandus*), and Bujuri (*Mystus tengara*) were collected from Kamal-Ranjit (KR) market of BAU, Mymensingh in fresh condition. All the fish samples were of small and non-commercial size.

### *Preparation of fish sauce*

The collected fish samples were washed thoroughly with freshwater to remove all dirt, slime, and unwanted particles. Then the samples were weighed and divided into three parts (5 Kg each), then mixed properly with three different concentration (25%, 30% and 35%) of salt. A layer of salt was placed at the bottom of each plastic container, and then salt mixed fish were poured in it. Finally, a layer of salt was spread to cover the surface of the fishes. Plastic container with salted fish samples were covered with lids and kept in a dry place. After 7 days unsaturated brine solution was added to submerge the fish to prevent it from oxidation. Floating fishes on the surface of brine water were kept in submerged condition by placing a weight and allowed to ferment for 9 months. The chemical and bacteriological changes of the fish samples during fermentation were determined at 15 days intervals during this period. After 9 months the liquid part was extracted from the fermented fish samples, aged in the direct sunlight for 15 days, and filtered to separate the undigested portions. Thus the fish sauce became ready to use (Fig. 1).

### *Analytical procedures*

All the representative fish species (fresh) were mixed together and ground in a sterile mechanical grinder to make a paste. This ground sample was used for all biochemical and bacteriological analysis. The moisture, crude protein, total lipid and ash contents were determined using standard methods (AOAC, 1995), Total Volatile Base Nitrogen (TVB-N,

mg N/100g) was determined according to the methods given in Pearson (1976). For aerobic plate count (APC) of bacteria, 10 g of fish sample was transferred aseptically to 90 ml of sterile 0.2% (w/v) peptone water. Serial decimal dilutions in 0.2% peptone water were prepared and 0.1ml of appropriate dilutions was poured on selective agar plates and spread with sterile L shaped glass rod followed by incubation at 37°C for 48 hrs (FAO, 1979). Bacterial colonies developed were counted for the determination of standard plate count as cfu/g of sample. Isolation and identification of bacteria was done according to the method suggested in Cowan and Steel's Manual for the Identification of Medical Bacteria (Barrow and Feltham, 1993).

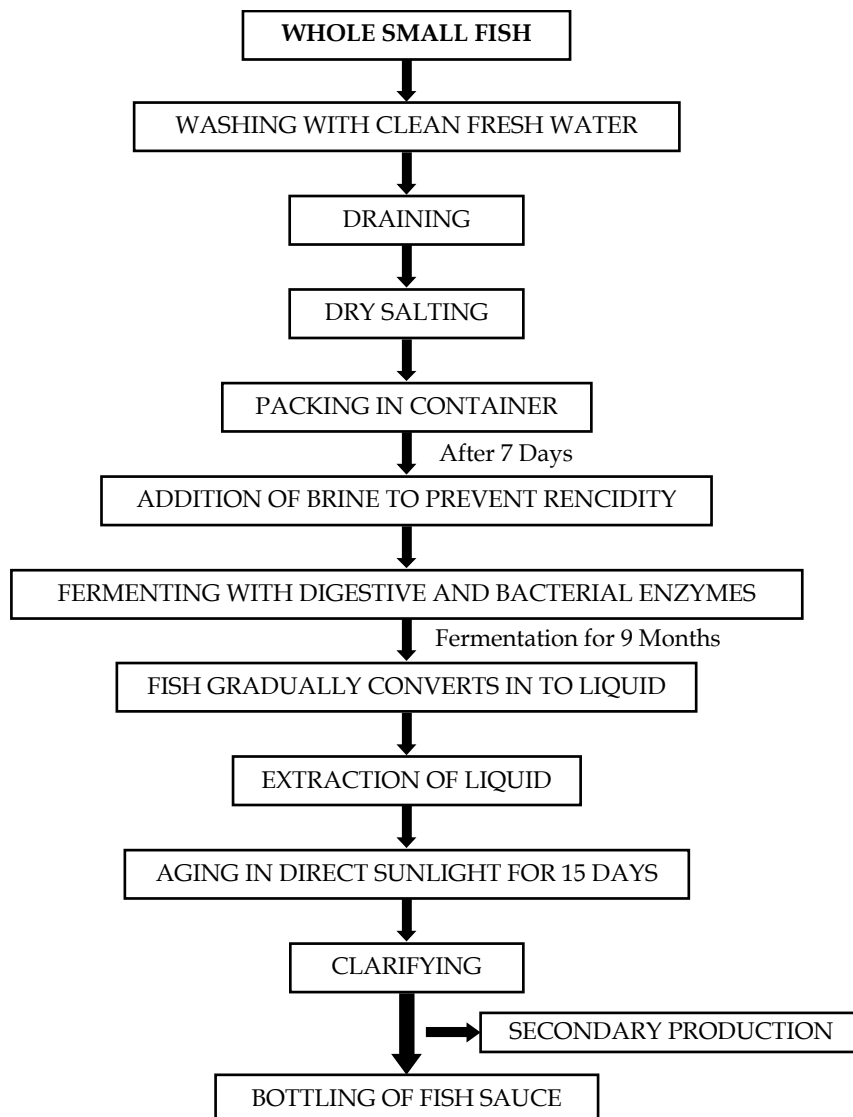


Fig. 1. Flow diagram of production of fish sauce

## RESULTS AND DISCUSSION

### *Chemical and microbial analysis of fresh fish*

Proximate composition and bacterial load of fresh raw materials were determined and it was found that initial average moisture, total nitrogen content, crude protein, lipid and TVB-N contents in fish were 75.61%, 2.42%, 15.17%, 2.94% 1.918 mg/100g respectively while initial APC was  $5.99 \times 10^6$  cfu/g. Major bacterial genera that identified from the fresh fish were *Bacillus*, *Micrococcus*, *E. coli*, *Mycobacterium*, *Pseudomonas* and *Staphylococcus*, among them *Bacillus* was dominant.

### *Chemical and microbial changes during fermentation*

Salted fish samples were allowed to ferment for a period of 9 months. During fermentation process fish samples in 3 treatments became liquefied gradually due to combined action of salt and enzymes from fish and microorganisms. Changes in different parameters during fermentation process have been shown in Fig. 2 to 5.

Fig. 2 showing that initially total nitrogen content in fish was 2.43%, after 24hrs of addition of salt the total nitrogen content was dramatically increased and reached to 4.10%, 4.19% and 4.30% in treatment-I, treatment-II and treatment-III respectively due to reduction of moisture content and then gradually decreased with the progress of fermentation. At the same time the total nitrogen in brine increased gradually. The total nitrogen content in fish body was always lower in treatment-I than treatment-II and treatment-III. Similar results have been reported by Hjalmarsson *et al.* (2007) and Xu-Wei *et al.* (2008).

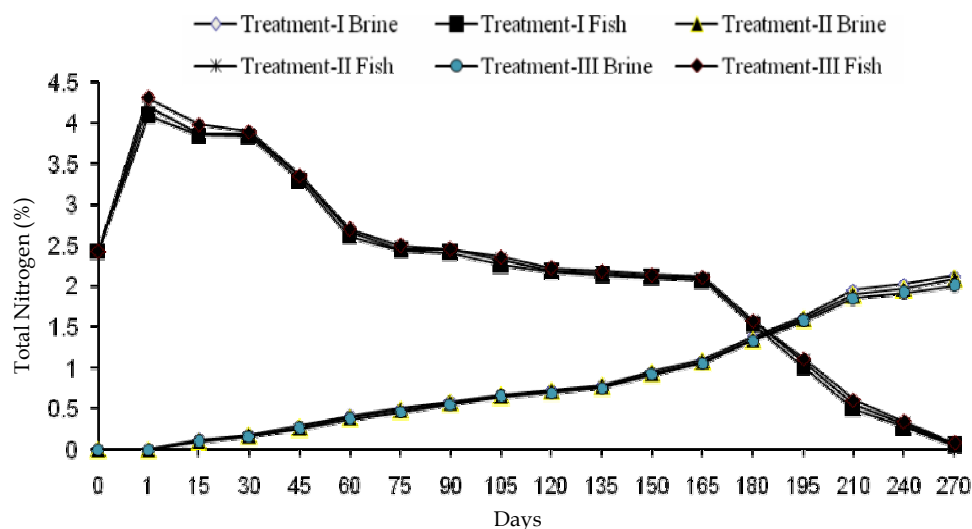


Fig. 2. Changes of total nitrogen content in salted fish and brine during fermentation

Changes in TVB-N in fish during fermentation have been shown in Fig. 3. TVB-N in fish body was initially 1.92 mg/100g. After addition of three different concentration of salt (25%, 30% and 35%) the quick increase of TVB-N of fish body was retarded due to action of salt.

With the progress of fermentation process protein in fish body gradually broke down and the amount of total volatile nitrogen increased and after 270 days the TVB-N reached the maximum level 19.93 mg/100g in treatment-I, 19.69 mg/100g in treatment-II and 19.07 mg/100g in treatment-III. Among the three different concentrations of salt, TVB-N was higher in treatment-I due to maximum break down of protein into nitrogenous compounds as because the enzymes in fish intestine and the microorganisms can tolerate comparatively lower concentration of salt. Similar result was found by Xu. Wei *et al.* (2008). Fish sauce was made from kilka fishes of the Caspian Sea (Iran) from 3 different preparations (whole kilka, cooked whole kilka and dressed kilka) the total volatile nitrogen was 25 mg/100g after 6 months of fermentation (Moini and Koochekian, 2003).

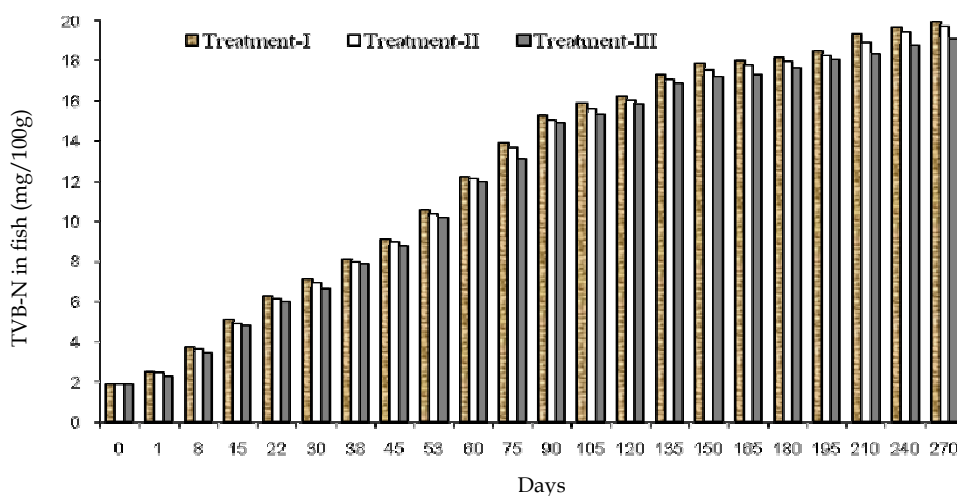


Fig. 3. Changes in Total Volatile Base Nitrogen (TVB-N) during fermentation

Initial moisture content of fish was 75.61% before addition of salt. After addition of salt the moisture content dramatically decreased due to entrance of salt in fish muscle. With the progress of fermentation process moisture level again started to increase and after 270 days the moisture content reached the maximum level, 73.29%, 72.04% and 69.98% for treatment-I, II and III respectively. These gradual increases in moisture level were happened in fish due to entrance of moisture in muscle and liquefaction of proteins. Kim *et al.* (1999) stated that the moisture content of anchovies and other small fishes varies from 46 to 77%, during fermentation period. Result of the present study fall within this range.

Fig. 4 shows the total bacterial load in fish during fermentation. APC value was initially  $5.99 \times 10^6$  cfu/g but after 24 hrs of addition of salt, the total bacterial load decreased to  $3.38 \times 10^5$ ,  $2.47 \times 10^5$  and  $2.75 \times 10^5$  cfu/g in three treatments respectively. The total bacterial load gradually decreased, but on 8<sup>th</sup> day the total bacterial load increased due to addition of brine solution to prevent rancidity. After 15 days the total bacterial load reached the lowest level and APC was  $1.21 \times 10^5$ ,  $8.01 \times 10^4$  and  $5.12 \times 10^4$  cfu/g in treatment- I, II and III respectively. As the time passed the protein in fish body gradually broken-down into amino acids and other nutritive compounds, as a result the bacterial population got more nutrients

and started to multiply rapidly. After 270 days the total bacterial load was  $1.41 \times 10^7$ ,  $1.34 \times 10^7$  and  $1.25 \times 10^7$  cfu/g in treatment- I, II and III respectively.

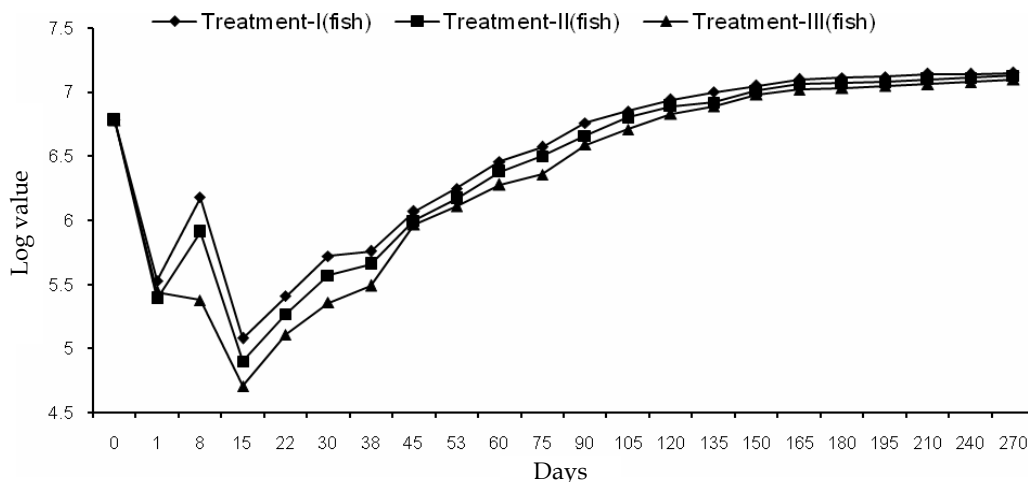


Fig. 4. Changes in bacterial load in fish during fermentation

Fig. 5 shows the changes in bacterial load in brine. On 7<sup>th</sup> day brine was added to prevent oxidation of fish, after 24hrs of initial load of bacteria in brine solution counted  $3.61 \times 10^6$  cfu/ml in treatment-I,  $5.55 \times 10^5$  cfu/ml in treatment-II and  $3.04 \times 10^5$  cfu/ml in treatment-III. The total bacterial load in brine quickly reduced and reached to the lowest level after 15 days. At 15<sup>th</sup> day the bacterial load in brine sample was  $6.29 \times 10^4$ ,  $3.20 \times 10^4$  and  $1.29 \times 10^4$  cfu/ml in three treatments respectively. After 270 days the total bacterial load reached the maximum levels that were  $1.33 \times 10^7$ ,  $1.24 \times 10^7$  and  $1.17 \times 10^7$  cfu/ml in treatment-I, II and III. Among three different concentrations of brine bacterial load was always higher in brine of treatment-I and lowest at the brine of treatment-III due to less survival rate and less enzyme activity at high concentration of salt.

Above results indicated that the addition of salt destroyed many types of bacteria except probably the halophilic and halotolerant ones. The remaining bacteria survived and gradually adjusted with the condition to multiply. Multiplication was accelerated due to breakdown of macromolecular compounds that released the low molecular weight compounds. These compounds served as nutrient source of the bacterial population. Al-Jedah *et al.* (2000) stated that the APC during preparation of fish sauce reached  $1.8 \times 10^7$  cfu/ml after 240 days of fermentation. Fish sauce was made from kilka fishes of the Caspian Sea (Iran) from 3 different preparations (whole kilka, cooked whole kilka and dressed kilka) the total bacterial count was 2.1 to 6.15 log cfu/ml after 6 months of fermentation (Moini and Koochekian, 2003). The present study also agrees with the above reports.

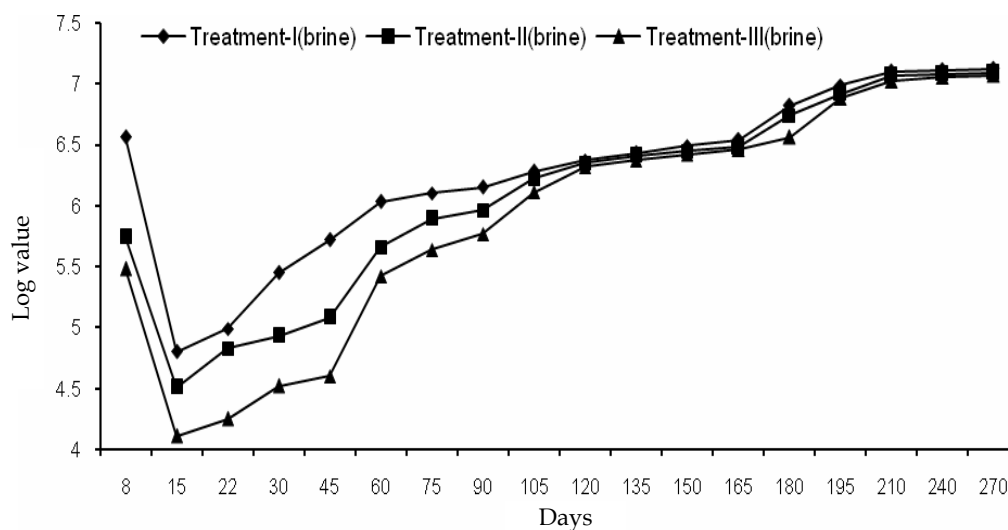


Fig. 5. Changes in Bacterial load in brine during fermentation

***Physical, chemical and microbial composition in final fish sauce (after aging in direct sunlight)***

After 9 months fishes in 3 treatments were fermented and liquefied completely, the liquid were collected and poured in the conical flask. The mouths of conical flasks were properly sealed with aluminum foil to prevent any kind of contamination. Those were kept in the direct sunlight for 15 days for aging. When aging was completed the upper layer of sauce became transparent but deep brown coloured and a layer of undigested fish body parts particularly scales and bones aggregated at the bottom of the flasks. The brown coloured liquid was separated from the undigested body parts of fish by using fine meshed filters. The filtered liquid was transferred in to other clean conical flasks and marked properly according to their treatments. In Table 1 physical properties of fish sauce in three different treatments is shown. Comparison of physical properties of fish sauce among the three treatments has shown that colour of sauce in treatment-I is comparatively darker than treatments II and III and that was judged as of best quality containing less salty than other two treatments. Table 2 shows the chemical and microbial properties sauce in three treatments. In Treatment-I protein and lipid content were higher than other two treatments. After 9 months of fermentation the bacterial load in three treatments were comparatively higher ( $1.33 \times 10^7$ ,  $1.24 \times 10^7$  and  $1.17 \times 10^7$  cfu/ml in treatment-I, II and III respectively) but after 15 days of aging it dramatically reduced due to effect of UV ray of sunlight. Bacterial load in treatment-I was slightly higher ( $1.53 \times 10^4$  cfu/ml) than treatment-II and III. Bacterial species identified were *Bacillus*, *Micrococcus*, *Lactobacillus* and *Pseudomonas* apparently no pathogen was present and hence safe for human consumption.

Table 1. Physical properties of fish sauce

Parameters	Treatment-I 25% salt	Treatment-II 30% salt	Treatment-III 35% salt
Colour	Light brown coloured liquid similar as world class fish sauce	Comparatively deep brown coloured than treatment-I	Deep brown coloured than treatment-II
Flavour	Light fishy flavour	Light fishy flavour	Light fishy flavour
Taste	Less Salty	More salty	Highly salty
Clearness	Clear	Clear	Clear
Liquidity	Liquid like water	Liquid like water	Liquid like water
Acceptability	Highly acceptable	Acceptable	Less acceptable

Table 2. Chemical composition and microbial analysis of fish sauce

Parameters	Treatment-I (25% Salt)	Treatment-II (30% salt)	Treatment-III (35% salt)
Protein (%)	12.28	11.88	11.63
Total Nitrogen (%)	1.97	1.90	1.86
Ash (%)	17.32	20.55	22.97
Moisture (%)	67.44	64.79	62.70
Lipid (%)	2.81	2.74	2.68
Salt content (%)	23.89	28.94	34.63
TVB-N (mg/100g)	19.88	19.30	18.67
Bacterial load in salted agar medium (cfu/ml)	$1.53 \times 10^4$	$1.40 \times 10^4$	$1.27 \times 10^4$
Major bacterial species	<i>Bacillus</i> , <i>Micrococcus</i> , <i>Lactobacillus</i> and <i>Pseudomonas</i>	<i>Bacillus</i> , <i>Micrococcus</i> , <i>Lactobacillus</i> and <i>Pseudomonas</i>	<i>Bacillus</i> , <i>Micrococcus</i> , <i>Lactobacillus</i> and <i>Pseudomonas</i>

According to Hjalmarsson *et al.* (2007) total nitrogen content in the fish sauce from summer capelin was 2.03% after 250 days of fermentation. Kim *et al.* (2004) stated the moisture percentage of anchovies and other small fishes varies from 46 to 77%, during fermentation period. Lopetcharat *et al.* (2002) mentioned that the ash content of Pacific whiting during preparation of fish sauce reached up to 25%. Low salt fish sauce was prepared experimentally from squid processing by-products in three different manufacturing techniques and total protein in three fish sauce were 13.344%, 12.238% and 12.738%, respectively (Xu-Wei *et al.*, 2008). The ranges of protein content of these results during fermentation are quite similar with this study. Moini and Koochekian (2003) stated that the total volatile nitrogen was 25 mg/100g after 6 months of fermentation of fish sauce, made from kilka fishes of the Caspian Sea (Iran). High-quality salmon sauce was developed with mature chum salmon (*Oncorhynchus keta*) samples, were eviscerated, deboned, washed, cut in small pieces, added 25% salt and internal organs (Taniguchi and Takano, 2007). Fish



sauce was made from kilka fishes of the Caspian Sea the total bacterial count was 2.1 to 6.15 log cfu/ml after 6 months of fermentation (Moini and Koochekian, 2003). Lopetcharat and Park (2002) stated that *Staphylococcus*, *Bacillus* and *Micrococcus* were found as predominant microorganisms during fermentation of fish sauce. Zaman *et al.* (2009) reported the presence of several microorganisms such as Enterobacteriaceae, Micrococci and Lactobacilli in fish sauce. The above discussion suggests that fish sauce prepared in this study was quite similar in qualitative aspects with fish sauce prepared elsewhere in the world. It is concluded that by simple salt treatment fermentation high quality fish sauce could be prepared from low priced non commercial fresh water fishes of Bangladesh.

## CONCLUSION

Based on above discussion and considering the result of different parameters it can be concluded that fish sauce produced with 25% salt (Treatment-I) was found to be of best quality.

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