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STUDY ON MICROBIAL AND PHYSICAL CHANGES IN FISH SAUCE DURING FERMENTATION

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ABSTRACT

The production of fermented fish sauce was replicated in the laboratory of the Dept. of Fisheries Technology in order to study the microbiological and physical changes associated with the process. Fish sauce was produced by incubating mixtures of low valued small freshwater fish at different concentrations of sodium chloride (25% as Treatment-I, 30% as Treatment-II and 35% as Treatment-III) for 9 months of fermentation (March to December) and 15 days of aging. Changes in bacterial composition and physical condition were observed. Due to effect of salt the bacterial population gradually changed and finally salt tolerant bacteria survived and multiplied. The major bacterial groups identified in fish sauce were *Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*. Due to action of enzymes present in fish body and intestine the solid fish gradually converts in to liquid where salt acts a preservative to prevent the fish from spoilage. Finally the sensory analysis of fish sauce was done through panel test. Considering the values of different parameters (salt content, test, colour and flavour) fish sauce produced with 25% salt (Treatment-I) was found to be of best quality

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INTRODUCTION

Fermentation is one of the oldest techniques in food preservation as it not only extends the shelf-life but also enhances the flavor and nutritional quality of the product (Visessanguan et al., 2004). Fish sauce, a fermented product, is a brown, liquid seasoning commonly used in Asian countries. It has various names according to producing countries: patis in the Philippines, shotturu in Japan, budu in Malaysia, nam-pla in Thailand, nuoc-mam in Vietnam, ketjapikan or bakasang in Indonesia, yu lu in China and ngapi in Myanmar (Sanceda et al., 2003). It has been used in various prepared foods and sauce with the merit of its characteristic, favorable taste and nutritive value. It is basically produced from a mixture of fish and salt that has been allowed to ferment. During fermentation its degradation products, amino acids and peptides, have a considerable effect on the sensory characteristics of fish sauce. Enzymes from fish, bacteria or exogenous enzyme play a major role in fermentation process (Orejana, 1978; Beddows et al., 1979: Mclver et al., 1982). In Southeast Asia, fish sauce is not only popular as a condiment, but in some areas and certain social classes in the region, it is the main source of protein in the diet and has become a necessity in the household. This study, therefore, is aimed at investigating the microbiological and physical changes of fish sauce during its long period of fermentation.

MATERIALS AND METHODS

The present study was conducted in the laboratory of the Dept. of Fisheries Technology, Bangladesh Agricultural University, Mymensingh. The samples were collected from Kamal- Ranjit (KR) market of Bangladesh Agricultural University, Mymensingh in fresh condition. Fish species included were 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)*. All the fish samples were of small and non-commercial size.

Preparation of fish sauce

The collected fish samples were washed thoroughly with fresh water to remove all dirt, slime, and unnecessary 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 physical and microbial 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 (Figure 1).

Analytical methods

Microbiological analysis of the fish sauce was carried out weekly during the first month and at 15 days intervals thereafter. All the representative fish species were mixed together and ground in a sterile mechanical grinder to make a paste. This ground sample was used for bacteriological analysis. For culturing 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 rods followed by incubation at 37ºC for 48 hrs (FAO, 1979). The isolates from the fish sauce at different stages were examined for gram stain, cell shape, motility, oxidase, catalase etc. Isolation and identification of bacteria was done according to the method suggested in Cown and Steel's Manual for the Identification of Medical Bacteria (Barrow and Felthham, 1993). Physical changes during fermentation were continuously observed based on outer appearance, texture of fish and colour of exudates. Finally the sensory evaluation was done by panel test based on colour, aroma, test and texture. Taste panel were composed of 8 internal members (4 men and 4 women, having age 20 to 35) who had been trained to the standard level of proficiency on panelist on sensory evaluation.

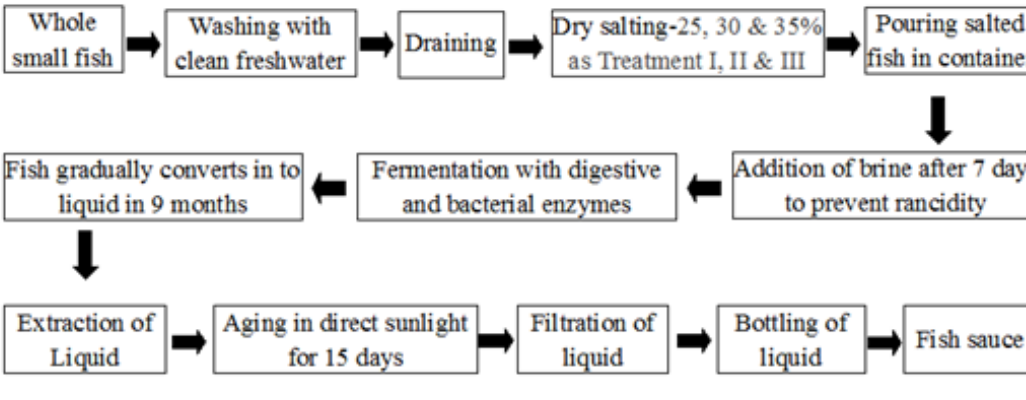

Figure 1. Flow diagram of preparation of fish sauce

RESULTS AND DISCUSSION

Generic distribution of bacteria in different treatments during fermentation

The generic distribution of bacteria in different treatments during fermentation has been represented in Table 1. *Bacillus* was dominant during the whole period of fermentation, because of its spore forming and salt tolerant ability. At the initial stage *Micrococcus, Lactobacillus, Corynebacterium, Escherichia .coli, Streptococcus* and *Pseudomonas* were also present. As the fermentation progressed due to higher concentration of salt *Corynebacterium, E.coli and Streptococcus* failed to survive and removed. At the end of 9 months fermentation and 15 days aging in sunlight *Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas* were the dominant species.

The predominance of *Bacillus* species in the microflora of fermented fish sauce has been reported by Saisithi et al. (1966), Crisan and Sands (1975) and Beddows (1985). However, Thongthai and Siriwongpairat (1990) demonstrated the presence of extremely halophilic bacteria identified as Halobacteria in Thai fish sauce. Sanchez (2001) stated that the microorganisms responsible for the fermentation and preservation are *Lactobacillus, Streptococcus, Leuconostoc, Pediococcus, Bacillus, Debaryomyces, Pseudomonas, Alcaligenes, Rhizopus, Acetobacter, Saccharomyces, Candida, and Torulopsis spp.* According to Lopetcharat and Park (2002) *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 present study of fish sauce also agrees with the results of the above reports. According to the result of the present study there was only a little difference among the generic distribution of bacteria in three treatments.

Physical changes in fish sauce during fermentation

The physical changes in 3 (three) treatments during fermentation has been represented in Table 2. Before addition of salt fish sample had shiny appearance and elastic texture of fresh fish. After the addition of salt within few days fishes loss shininess and gradually lost the scales, skin, flesh exposed, belly disappeared and finally skeleton become exposed. Elastic texture of fish become soft and gradually muscles become digested due to action of enzymes. Similarity, at the beginning colours of the exudates (brine) was light yellowish and gradually it became light red, dark red, dark red and turbid and finally highly turbid. Infact those physical changes are common phenomenon for the fish fermentation process. Enzymes from fish, bacteria or exogenous enzyme play a major role in fermentation process (Orejana 1978, Mclver et. al., 1982). After 9 months of fermentation fishes in 3 (three) different treatments are fully digested and liquefied. The liquid from three different treatments was collected and poured in the conical flasks separately. The mouth of each conical flask was properly sealed with aluminum foil to prevent cross contamination.

The conical flasks were kept in the direct sunlight for 15 days for aging the liquid. When aging was completed a deep brown colour liquid aggregated at the bottom of the flasks. This deep brown colour liquid was filtered and separated by using fine meshed filters and siphoning process from the undigested parts. The filtered liquid was transferred separately in to other clean conical flasks and marked properly according to their treatments. According to Saisithi (1967) fish sauce colour varies from straw colour, amber or reddish brown. The similar result was found in this study also.

Table 1. Generic Distribution of Bacteria in 3 (three) treatments during fermentation

Days	Treatment-I*	Treatment-II*	Treatment-III*
0	*Bacillus, Micrococcus, Lactobacillus, Corynebacterium, E. coli, Streptococcus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus, Corynebacterium, E. coli, Streptococcus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus, Corynebacterium, E. coli, Streptococcus* and *Pseudomonas*
1	*Bacillus, Micrococcus, Lactobacillus, Pseudomonas, Corynebacterium* and *E. coli*	*Bacillus, Micrococcus, Lactobacillus, Corynebacterium* and *E. coli*	*Bacillus, Micrococcus, Corynebacterium* and *Pseudomonas*
8	*Bacillus, Micrococcus, Lactobacillus E. coli* and *Corynebacterium*	*Bacillus, Micrococcus, Lactobacillus* and *Corynebacterium*	*Bacillus, Micrococcus, Lactobacillus, Pseudomonas* and *Corynebacterium*
15	*Bacillus, Micrococcus, Pseudomonas, Lactobacillus* and *Corynebacterium*	*Bacillus, Micrococcus, Lactobacillus, Pseudomonas* and *Corynebacterium*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*
22	*Bacillus, Micrococcus, Lactobacillus* and *Corynebacterium*	*Bacillus, Micrococcus, Lactobacillus* and *Corynebacterium*	*Bacillus, Micrococcus* and *Lactobacillus*
30	*Bacillus, Micrococcus, Lactobacillus* and *Corynebacterium*	*Bacillus, Micrococcus* and *Lactobacillus*	*Bacillus, Lactobacillus* and *Micrococcus*
45	*Bacillus, Micrococcus* and *Lactobacillus*	*Bacillus, Micrococcus* and *Lactobacillus*	*Bacillus, Micrococcus* and *Lactobacillus*
60	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus* and *Lactobacillus*
75	*Bacillus, Micrococcus,* and *Pseudomonas*	*Bacillus, Micrococcus,* and *Pseudomonas*	*Bacillus, Micrococcus* and *Lactobacillus*
90	*Bacillus, Micrococcus* and *Lactobacillus*	*Bacillus, Micrococcus* and *Lactobacillus*	*Bacillus, Micrococcus* and *Lactobacillus*
105	*Bacillus, Micrococcus, Pseudomonas* and *Lactobacillus*	*Bacillus, Micrococcus* and *Lactobacillus*	*Bacillus, Micrococcus* and *Lactobacillus*

Table 1. Generic Distribution of Bacteria in 3 (three) treatments during fermentation (Contd.)

120	*Bacillus, Micrococcus, Pseudomonas* and *Lactobacillus*	*Bacillus, Micrococcus, Pseudomonas* and *Lactobacillus*	*Bacillus, Micrococcus* and *Lactobacillus*
135	*Bacillus, Micrococcus* and *Lactobacillus*	*Bacillus, Micrococcus* and *Lactobacillus*	*Bacillus, Micrococcus* and *Lactobacillus*
150	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*
165	*Bacillus, Lactobacillus,* and *Pseudomonas*	*Bacillus, Micrococcus, Streptococcus* and *Pseudomonas*	*Bacillus, Micrococcus, Streptococcus* and *Pseudomonas*
180	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*
195	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*
210	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*
240	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*
270	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*
Final Fish Sauce (After 15 days of aging and filtration)	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*	*Bacillus, Micrococcus, Lactobacillus* and *Pseudomonas*

*Treatment-I- 25% salted fish, Treatment-II- 30% salted fish, Treatment-III- 35% salted fish

Panel test of fish sauce

After aging and filtration the physical quality of final fish sauce in 3 (three) different treatments was judged through panel test and the result has been represented in Table 3. Colour of the fish sauce in treatment-I was reddish brown, whereas treatment-II and treatment-III had deep red and blackish red colours respectively. The aroma of treatment-I was light fishy sweet flavor, but the rest two treatments had strong fishy flavor. Fish sauce of treatment-I was more acceptable than other 2 (two) treatments due to its less saltiness and also the overall appearance was perfectly matched with the imported fish sauce available in the market. Taniguchi and Takano (2007) stated that 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. This result matched with the present study.

Table 2. Physical changes in 3 (Three) Treatments during fermentation

Days	Outer Appearance			Texture of fish body			Colour of Exudates		
	Treatment-I*	**Treatment-II***	**Treatment-III***	**Treatment-I***	**Treatment-II***	**Treatment-III***	**Treatment-I***	**Treatment-II***	**Treatment-III***
0	Fresh fish	Fresh fish	Fresh fish	Hard	Hard	Hard	No Exudates	No Exudates	No Exudates
1	Fresh like appearance	Fresh like appearance	Fresh like appearance	Hard	Hard	Hard	Light yellowish	Light yellowish	Light yellowish
15	Less shiny	Less shiny	Less shiny	Hard	Hard	Hard	Yellowish	Yellowish	Yellowish
30	Loss of shine	Loss of shine	Loss of shine	Slight soft	Slight soft	Slight soft	Light reddish	Light reddish	Light reddish
45	Complete loss of shine	Complete loss of shine	Complete loss of shine	Slight soft	Slight soft	Slight soft	Reddish	Reddish	Reddish
60	Complete loss of shine	Complete loss of shine	Complete loss of shine	Belly portion more soft	Belly portion more soft	Belly portion more soft	Dark red	Dark red	Dark red
75	Few scale start to loss	Scale intact	Scale intact	Belly portion more soft	Belly portion more soft	Belly portion more soft	Red and slight turbid	Red and slight turbid	Dark red
90	More scale loss	Few scale start to loss	Few scale start to loss	Belly flaps start to digest	Belly flaps start to digest	Belly portion more soft	Turbid red	Turbid red	Red and slight turbid
105	30% scale loss	15% scale loss	10% scale loss	Stomach digested	Stomach digested	Belly flaps start to digest	Turbid and deep red	Turbid and deep red	Turbid red
120	45% scale loss	30% scale loss	25% scale loss	Stomach digested	Stomach digested	Belly flaps start to digest	Turbid and deep red	Turbid and deep red	Turbid red
135	55% scale loss	42% scale loss	35% scale loss	Belly flaps digested	Belly flaps digested	Stomach digested	Deep turbid red	Deep turbid red	Deep turbid red
150	72% scale loss	65% scale loss	50% scale loss	Belly flaps digested	Belly flaps digested	Stomach digested	Deep turbid red	Deep turbid red	Deep turbid red
165	85% scale loss	75% scale loss	60% scale loss	Muscle near the belly become more soft	Muscle near the belly become more soft	Belly flaps digested	Deep turbid red	Deep turbid red	Deep turbid red
180	Scale completely loss	90% scale loss	80% scale loss	Complete body become more soft	Complete body become more soft	Muscle near the belly digested	Deep turbid red	Deep turbid red	Deep turbid red

Table 2. Physical changes in 3 (Three) Treatments during fermentation (Contd.)

195	Skin start to loss	Scale completely loss	95% scale loss	Muscle of other portion start to digest	Muscle of other portion start to digest	Complete body become more soft	Highly turbid red	Highly turbid red	Highly turbid red
210	Most of skin part loss	Skin start to loss	Scale completely loss	About 30% muscle digested	About 20% muscle digested	Muscle of other portion start to digest	Highly turbid red	Highly turbid red	Highly turbid red
225	Flesh exposed	Most of skin part loss	Skin start to loss	About 65% muscle digested	About 55% muscle digested	About 35% muscle digested	Highly turbid red with flesh parts	Highly turbid red with flesh parts	Highly turbid red with flesh parts
240	Bones exposed	Bones exposed	Bones exposed	About 90% muscle digested	About 82% muscle digested	About 63% muscle digested	Highly turbid with flesh parts	Highly turbid red with flesh parts	Highly turbid red with flesh parts
255	Bones fully exposed	Bones exposed	Bones exposed	Muscle fully digested	About 95% muscle digested	About 85% muscle digested	Highly turbid with dissolved flesh	Highly turbid red with dissolved flesh	Highly turbid dark red with flesh parts
270	Bones fully exposed	Bones fully exposed	Bones fully exposed	Muscle fully digested	Muscle fully digested	About 98% muscle digested	Highly turbid red with dissolved flesh	Highly turbid dark red with dissolved flesh	Highly turbid dark red with dissolved flesh

*Treatment-I- 25% salted fish, Treatment-II- 30% salted fish, Treatment-III- 35% salted fish

Table 3. Panel test report of fish sauce

Parameters	Treatment-I*	Treatment-II*	Treatment-III*
Colour	Reddish brown	Deep Red	Blackish red colour
Flavour	Light fishy sweet flavour	Strong fishy flavour	Strong fishy flavor
Test	Salty	More salty	Highly salty
Clearness	Clear	Slight cloudy	More cloudy
Comparison with imported fish sauce available in the market	More similar	Less similar	Less similar
Acceptability	Highly acceptable	Acceptable	Less acceptable

*Treatment-I- 25% salted fish, Treatment-II- 30% salted fish, Treatment-III- 35% salted fish

CONCLUSION

Based on above discussion it can be concluded that fish sauce was prepared due to combined action of enzymes present in fish body and microorganisms, where salt worked as a preservative. Moreover only the salt tolerant bacteria can survive in this long period of fermentation. Considering the result of physical parameters of fish sauce it can be declared that fish sauce prepared with 25% salt (Treatment-I) was found to be the best quality.

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