BIOCHEMICAL AND MICROBIOLOGICAL ANALYSIS OF NGA-PI COLLECTED FROM DIFFERENT REGIONS OF BANGLADESH

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Roll No.: 0120/09

Registration No.: 882

Session: 2020-2021

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Fishing and Post-Harvest Technology

Department of Fishing and Post-Harvest Technology
Faculty of Fisheries
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Chattogram-4225, Bangladesh

DECEMBER 2022

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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DECEMBER 2022

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The Author

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LIST OF ABBREVIATIONS

Words	Abbreviation
AOAC	The Association of Official Analytical Chemists
APW	Alkaline Peptone Water
BPW	Buffer Peptone Water
Cm	Centimeter
CFU	Colony Forming Unit
DW	Distilled Water
E.	*Escherichia*
EMB	Eosin Methylene Blue Agar
et al.	Et alia (L) and other
etc.	Et cetera
Gm	Gram
HCL	Hydrochloric Acid
Hr	Hour
L	Liter
Mac	Macconkey Agar
Mg	Miligram
Min	Minute
NaOH	Sodium- hydroxide
R	Replication
SD	Standard Deviation
SE	Standard Error
TCBS	Thiosulfate-citrate-bile-salts-sucrose Agar
TSA	Trypticase Soy Agar
TSI	Triple Sugar Iron
XLD	Xylose Lysine Deoxycholate
% N	Percent Nitrogen
µg	Microgram

ABSTRACT

Nga-pi, fermented product traditionally produced at Cox's Bazar and Rangamati region is one of the popular food items among the ethnic people in Bangladesh.The present study was carried out to investigate the microbiological and chemical characteristic of Nga-pi. Samples were collected from the producers of Nga-pi in from different sampling areas (1) Cox's Bazar, (2) Mahesh khali, (3) Rangamati and (4) Teknaf. After analyzing the proximate composition of collected samples, it was found that Nga-pi from the Teknaf region has the highest protein value (55.03%), lipid (6.62%), ash content (18.49%) and moisture (51.19%) was higher in Mahesh khali samples. Among the samples collected from four regions, moisture content was found to be higher in the samples from Rangamati. The values of amino acids greatly varied in terms of every amino acid among the samples. The microbial load of Nga-pi was determined against *Vibrio cholera*, *Vibrio vulnificus, Vibrio parahaemolyticus, E. coli, Salmonella* and *Shigella. Vibrio vulnificus* and *Vibrio parahaemolyticus* were present on the samples collected from Rangamati, Cox's Bazar, Mahesh khali and Teknaf region. No *Vibrio cholera, E. coli, Salmonella* or *Shigella* was found in either of the samples collected from different regions. The marketing channel of Nga-pi was also analyzed during the study period. Rangamati, Khagrachari, Bandarban was found to be the major trade point of Nga-pi. The wholesalers of these region collect Nga-pi from Cox's Bazar region and sell in the retail market. Whereas, producers of Mahesh khali and Teknaf region make Nga-pi for their own consumption and sells the products in a smaller extend. Therefore, the price and purchase rate of Nga-pi varies in different areas based on their consumer's demand and purpose of production.

Keywords: Nga-pi, fermented product, microbiological, bio-chemical, bacteria.

CHAPTER-1: INTRODUCTION

A nation or its culture is native to traditional fish items (BOBP, 1985). The majority of the fish caught in Bangladesh is consumed domestically to make a variety of processed goods. Different kinds of traditional fish and fisheries goods can be found on the domestic market. The most crucial items among them are fish products that have been dried, salted, smoked, fermented, and semi-fermented (Shikha et al., 2019). These goods are crucial for a number of reasons, including the fact that they have endured for years in the developing countries, that they are essential for the nourishment of the underprivileged and poor, and that they typically entail low-cost processing techniques. For Bangladesh, where a significant number of people suffer from different forms of chronic malnutrition and the majority of babies are born underweight because of their mothers' chronic under nutrition, traditional fish and fisheries products are crucial (Islam, 1998). One of Bangladesh's most critical issues is protein deficiency (Hossain, 1991). The demand for fish and fishery products in Bangladesh is outpacing supply due to rapid population expansion. Due to their superior taste and lower price compared to larger economically significant species like table fish, people choose traditional items.

1.1 Significance of the study

Fermented fish products are distinctive from other types of traditional fish products. Because fermentation is the process of converting complex organic compounds into simpler ones with the aid of enzymes and microbes, it is one type of cured fisheries product. The ancient Chinese text Churai, which was composed in the third century BC, has three Chinese ideographs that describe fermented fish products. Hae, Ja, and Chi are those. Hae is fermented meat or fish that is aged in salt with the addition of wine, koji, and/or alcohol fermentation starters on occasion. Southeast Asian nations including Laos, Kampuchea, Vietnam, Thailand, Malaysia, Indonesia, and Myanmar have a long tradition of fermenting fish (Martin, 1994).

There are various fermented and semi-fermented foods that are popular in specific regions of Bangladesh, such Chepa shutki, Shidhal, and Nga-pi. Nga-pi is one of them, and it is mostly produced, sold, and consumed as a common food item in the South-East region of this nation by tribal Rakhaing and Chakma people. It is a type of fermented fish product that is either made of fish or shrimp and compresses the

original fish into a paste. *Acetes* species of little shrimp are used to make Nga-pi (Hajeb and Jinap, 2012). For various Nga-pi products, white fish fry, fingerlings, small fish, and other larger shrimp are occasionally also utilized. Depending on the varieties of shrimp that are offered in the nation, they may be blended with salt and sun-dried for two days. After being mashed into a fine paste, the dried shrimp are fermented for two days before being put into a jar to continue fermention (Wittanalai et al., 2011; Chuon et al., 2014).

The quality of seafood and fishery products is a key concern in the fish industry all over the world, according to Teklemariam et al. (2015). Essentially, the goal of fish and fish product assessment is to prevent consumers from consuming tainted food, to analyze the nutritional value of food by looking for physical, chemical, and biological dangers, and to ultimately ensure customer safety. Alam (2007) discussed many aspects of the processing of Nga-pi in the Bangladeshi Cox's Bazar region of Bangladesh. However, there is a dearth of information regarding the quality evaluation of Nga-pi manufactured traditionally and sold to customers, which is crucial to ensuring the product's safety.

In the current study, the team traveled to Cox's Bazar, Mahesh khali, and Rangamati, where they collected Nga-pi samples from various stakeholders and used those samples to determine the quality of the samples using appropriate techniques. They also gathered pertinent data to examine the Nga-pi marketing channel.

1.2 Objectives of the study

The following goals are the focus of this investigation -

- To assess the biochemical composition of collected samples from selective areas
- To determine the pathogenic bacterial load in Nga-pi samples
- To compare the quality of the products from different production areas

CHAPTER-2: REVIEW OF LITERATURE

Before beginning any research under a specific experimental procedure, it is critical to review previous research activities on related topics. The following is a review of literature relevant to the current research work

2.1. Fermented fishery products

Fermented fisheries products are the byproducts of freshwater and marine finfish, shel lfish, and crustaceans that are treated by the concurrent presence of fish enzymes and bacterial enzymes with salt to promote fermentation and so prevent putrefaction.
Enzymes cause a change in the consistency of the fish and contribute to the production of flavor, while bacteria are responsible for the development of fragrance and flavor throughout the process of fish fermentation (Beddows, 1979). The preservation of fish takes place in regions where there is an abundance of native fish during certain times of the year. The fermentation process was carried out on the many species of freshwater fish that are found naturally in the hydrological systems of the area. The fermenting technique first emerged among settled farmers and only in locations where salt could be supplied without much effort. The fermentation of fish has been perfected in a central region of the Asian continent, which includes both East Asia and Southeast Asia (Ruddle and Ishige, 2010). East Asia is responsible for the production of such a diverse array of fermented fish. They are categorized in this way according to the characteristics of the finished product as well as the manner of their preparation.

2.2. Nga-pi

Burmese cuisine often includes a paste called Nga-pi, which can be produced from either fish or shrimp and has a very strong flavor. Sun-dried fish or shrimp that has been fermented with salt and crushed spices is the traditional ingredient for Nga-pi. Similar to cheese, it can be identified by its primary constituent and its place of origin to set it apart. The several kinds of fish used to produce Nga-pi lend the dish its unique flavor. Nga-pi can be made from a variety of different seafood, including entire fish (Nga-pi kaung), little fish (hmyin Nga-pi), or prawns (seinza Ngapi). In Lower Burmese cuisine, Nga-pi plays an important role as a condiment or seasoning used to a wide variety of foods. Other than a few notable instances, raw Nga-pi is not meant to be consumed by mouth.

Since there are so many different kinds of fermented fish items throughout continental Southeast Asia, Nga-pi has a long history in Myanmar. A Mon stone inscription from the first century CE is the earliest known use of the term "Nga-pi" in writing. The names of Nga-pi artisans can also be found in Burmese inscriptions carved into stone and marble that date back to the 1100s and 1400s. By the 1400s, the Mon had developed a regular trade pattern for Nga-pi with the Bamars of Upper Myanmar, exchanging Nga-pi, salt, and rice for rubies, benzoin, and musk (Shorto, 1662). In the pre-colonial era, Nga-pi trade was a major contributor to the economy. Observers during British colonial control in Burma described the aroma of Nga-pi as "extremely self-offensive" and "offensive," but they yet acknowledged the dish's significance in Burmese cuisine (Steinkraus & Keith, 2004).

2.3. Uses of Nga-pi

Nga-pi is the most important component in the cuisine of Lower Burma, which is often prepared in the maritime coastal districts of the west and the south espite the fact that more accessible transportation in the modern age has contributed to Nga-rise pi's in popularity in Upper Myanmar, it is not the primary component in traditional Older Burmese (Burman, Shan, etc.) cuisines. It can be consumed in a variety of ways and utilized in a wide range of foods, including salads, pounded mixtures with chili, fried dishes like balachaung, and watery preparations like Nga-pi yay. In addition, it's utilized in both major dishes and soup bases.

2.4. Nga-pi in Bangladesh

The Bangladeshi districts of Cox's Bazar, Barguna, Patuakhali, and Chittagong are known for producing Nga-pi, a fermented fish product. This product is mostly made by the Rakhaings people from marine fish, including their fry and fingerlings, and shrimp like *Mysid* sp. and *Acetes* sp. Rakhaings, who number over 300,000 according to Sein et al. (2001), are widely dispersed throughout Bangladesh and have brought with them this traditional fishing product from Myanmar. Fish or shrimp are ground into a paste called Nga-pi (fermented fish paste), which is then partially dried in the sun for three to four days. The mixture is kept by packing it firmly into concrete vats or clay jars and letting it mature for three to six months. As a byproduct of the fermentation of Nga-pi, nganpyaye (fish sauce) is produced (Tyn, 1996, 2004). It is a

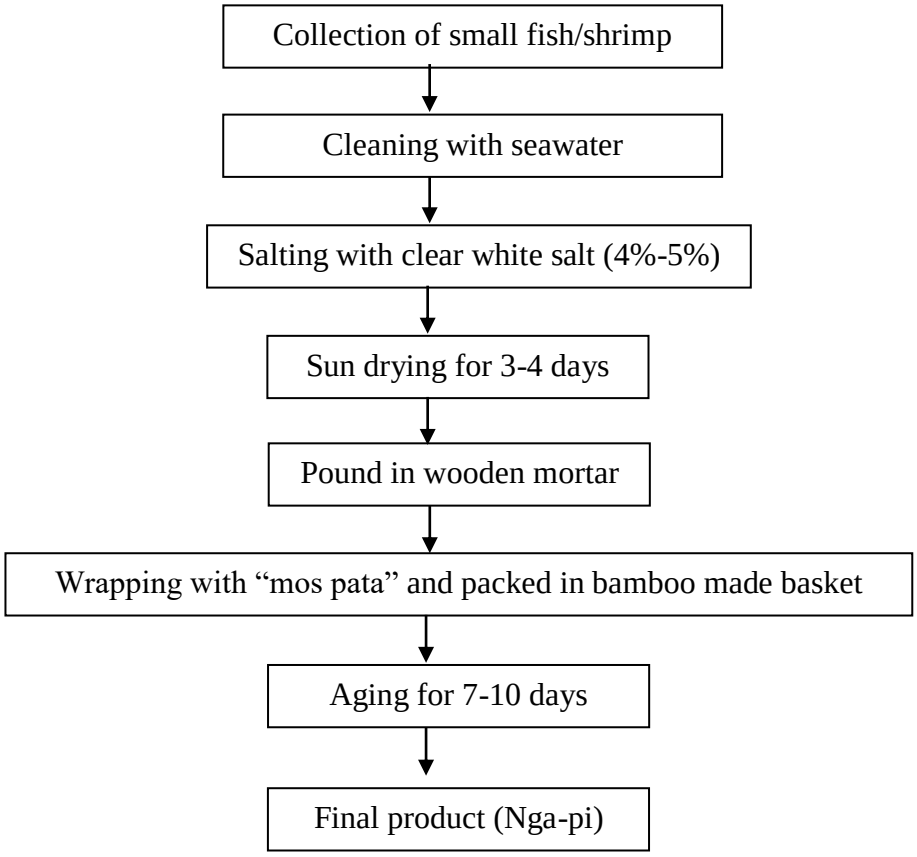
pounded dough that is blackish and has a strong, pungent flavor. It is typically eaten as a side dish with rice and curries.

2.5. Processing technology of Nga-pi

Nga-pi is typically made from tiny *Acetes* and *Mysid* shrimps that have been ground into a paste and then salted to taste. The paste is then put through a series of alternate fermentation and sun-drying processes before maturing in an airtight container. The entire process is carried out traditionally in Rakhaing villages, where there are numerous opportunities for product and raw material contamination and degradation. The major steps involved in the processing of Nga-pi at Chowfaldandi in Cox's Bazar district are described here-

2.5.1. Collection of raw material

Nga-pi processors obtain raw materials from middlemen, landing areas, or fishermen. Processors buy the raw fish at relatively low costs during the height of the harvest. Nga-pi is prepared using little shrimp that are regularly gathered from the Mahesh khali waterway or from the shallow continental shelf close to the shore. The fish is neither salted nor iced on the boat. Typically, shrimp of the genera Acetes sp. and *Mysid* sp. are employed. However, other fish species are also used, such as different small marine fish, their fry, fingerlings, different shrimp, even sea snails and mollusks. While *Mysid* shrimps are very smaller in size with such a milky-white soft body and are known locally as "maishi," *Acetes* shrimp are reddish in color, somewhat larger in size, and have a tougher shell. The people claim that "ming" can yield the highest-quality Nga-pi. Crabs and fish can occasionally still be found in the combination. Prior to drying, however, the larger shrimp and fish larvae, small fish, other shrimp, sea snails, and other mollusks are not separated.



Flow chart-1: Preparation of Nga-pi

2.5.2. Salting and sun drying

Bamboo baskets are used to transport little, unsalted shrimp from the boats. The bamboo "chatai" is covered with a thin layer of salt, and the shrimp are arranged over the mats in thin layers. The amount of salt used is determined by the type and quality of crustaceans. Shrimp on the deck are typically left to dry in the sunshine for 3–4 days.

2.5.3. Pounded by wooden mortar

The following night, semi-dried shrimp is pounded with salt in a wooden mortar. For every 40 kg of shrimp, there is between 1 and 2 kilogram of salt (2.5%–5%). The salt-ground material is dried under the sun on the mat the following day for the entire day before being processed again at night. During the second grinding, salt is not added. Similar to that, the result is dried for a third day before being ground into a paste at night without any additional salt.

2.5.4. Wrapping with leaves

The finished paste seems to be deep gray to black in color. There isn't any additional color used. The finished paste is formed into blocks or balls and covered with the broad leaves of the "mos-pata" tree, a wild highland tree. Due to the salt action, the extremely thick leaves of these trees can absorb the water that is released from the product. Bamboo baskets are used to bundle the wrapped Nga-pi.

2.5.5. Aging

One bamboo basket contains 20 kg of dough, which is aged for 7–10 days. The product is typically sold in one week, though it may occasionally be kept for longer to fetch a higher price. The processors claim that Nga-pi made in this manner has a shelf life of six months.

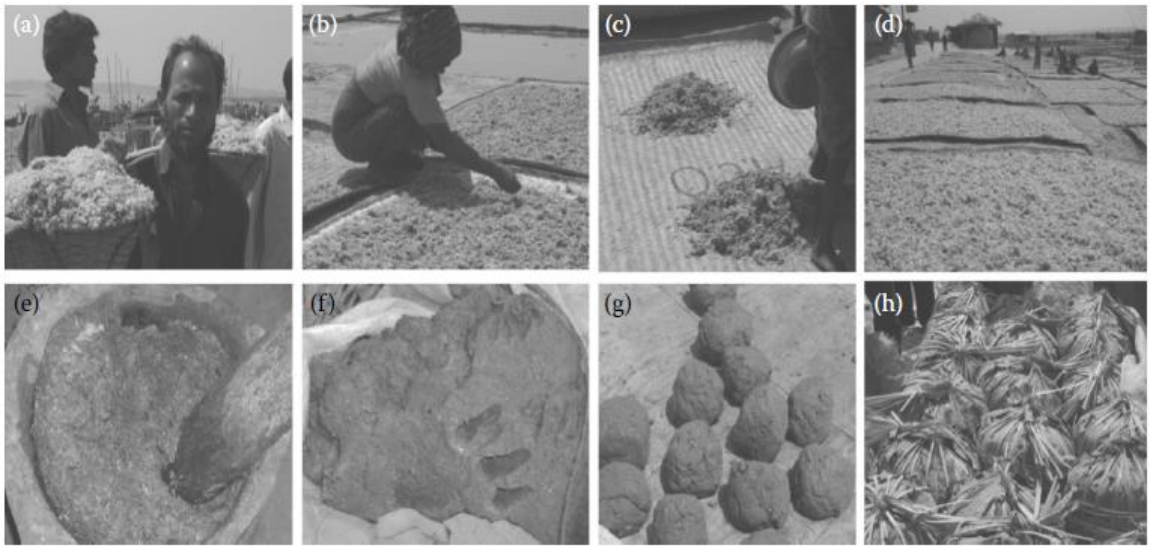


Plate-1: Processing technique of Nga-pi in Bangladesh. (a) collection of raw shrimp, (b) sorting of big shrimp and crab, (c) salting of raw materials, (d) drying on chatai, (e) pounding in wooden mortar, (f) pounded Nga-pi, (g) round shaped Nga-pi, (h) aging (Source: Rosma et al, 2015.)

2.6. Nutritional value of Nga-pi

Since fish, shrimp, or beans are used in its preparation, Nga-pi is a good source of protein. The Nga-pi created from marine fish and prawns also provides a supply of iodine (which is plentiful in all seafood). This may be advantageous for those inland customers whose diet may be weak in iodine and who do not have contact to iodized salt. In addition, iodine is prevalent in all seafood. Because of the substantial amount of salt that is used in its production, Nga-pi, just like any other dish that is high in salt

content, should only be ingested in small amounts by people who have salt-sensitive hypertension.

2.7. Microbiology of fermented fishery products

Marine fish have vegetation on their bodies that is specific to their natural habitat. These microorganisms generate putrefactive changes in fish tissues after death, along with the tissues' enzymes (Phithakpol, 1993). The microorganisms that are normally present in the salt used for salting the fish also have an impact on its degradative changes (Lertprakobkit, 2011). In essence, salt has been used to diminish the water activity of fish that have been exposed to fermentation and stop fish or shellfish from spoiling. Different types and batches of fermented fisheries products may contain between 10% and 30% (w/w) salt (Saisithi, 1994). This is probably going to have a big impact on how quickly bacteria multiply and ferment, which will then have an impact on the product's sensory quality and safety. Finding the ideal salt concentration that enhances the product's flavor and texture while not limiting the growth of the bacteria that fermented food is therefore important.

At water activity (Aw) levels under 0.60, all microbial development is suppressed. Halophiles do best in environments with high salt concentrations since they cannot develop in media without salt. Halophiles can counteract the environment's osmotic pressure and withstand salts' denaturing effects (Grant, 2001). According to Kushner (1993), microorganisms can be divided into five groups based on how much sodium chloride they require: extreme halophiles (2.5-5.2 M), moderate halophiles (0.5-2.5 M, -3-15% salt), non-halophiles (0.2 M, -1% salt), slight halophiles (0.2-0.5 M, -1-3% salt), borderline extreme halophiles (1.5-4.0 M, 9-23% salt). Halotolerant organisms can grow at quantities greater than saltwater, but do so optimally in the absence of considerable amounts of salt. Additionally, fisheries products like fish sauce, fermented fish pastes, and salted fish might include halophilic bacteria. Osmophiles may grow under high osmotic pressure, but xerophiles can grow under somewhat dry circumstances or below Aw of 0.85. (Grant, 2001). According to Namwong et al. (2007), the majority of food-borne bacterial pathogens cannot thrive in the Aw range of 0.98-0.9.

2.8. Microorganisms of fermented shrimp products

The use of microflora is crucial in making fermented shrimp pastes. It is the existence of microorganisms during fermentation that breaks down proteins and creates flavor and aroma. Yeast (*Candida*, *Saccharomycopsis*), homo fermentative and hetero

fermentative rods (*Lactobacillus*), endospore-forming rods (*Bacillus*), aerobic coccus (*Micrococcus*), and pathogenic contaminants (*B. cereus, S. aureus*, and *Enterobacteriaceae*) are just some of the microbes that can be found in fermented shrimp pastes (Sand and Crisan, 1974). *Bacillus spp*. was the other major bacterium found in the fermented shrimp paste. *Bacillus spp*. is widespread because they can adapt to their environment by producing endospores (Sand and Crisan, 1974). Several shrimp paste microbiological research has been completed in the past. To name a few, *Bacillus sp., Micrococcus sp.*, and *Moraxella sp.* are all microflora that have been found in the Filipino fish or shrimp paste, Bagoong (Beddows and Ardeshir, 1979). Microorganisms found in belacan include *Bacillus, Pediococcus, Lactobacillus, Corynebacteria, Micrococcus, Clostridium, Brevibacterium,* and *Flavobacterium.* Common microorganisms include lactic acid bacteria (LAB), Micrococcus, Bacillus species, and high salt-tolerant species (Sobhi et al., 2013).

Terasi, a fermented shrimp of Indonesia, is produced through the slow digestion or fermentation of salted shrimp, followed by the separation of the solid from the liquid portion of the hydrolysate. The resulting culture contains 11 species of *Bacillus*, 4 species of *Pseudomonas*, 3 species of *Micrococcus*, 1 species of Kurthia, and 1 species of *Sporolactobacillus* (Kobayashi et al., 2003). Protein hydrolysis was triggered by both intestinal and systemic enzymes, including those produced by the gut microbes *Bacillus subtilis* and *Bacillus* coagulans. Amino acids were mostly deaminated and decarboxylated by enzymes produced by bacteria. The flavor is created in part by fatty acids and amides with a lower MW. Throughout the fermenting process, *B. pumilus* predominates. Additional bacteria such as *B. coagulans, B. megaterium,* and *B. subtilis* contributed to the initial stages of fermentation, whereas *B. licheniformis* was the primary player in the later stages, the genus *Staphylococcus*, as well as the genera *Micrococcus* (Colpogenes), *Micrococcus* (Roseus), and *Micrococcus* (Varians) (Kobayashi et al., 2003). *Staphylococcus*, *Micrococcus*, and *Bacillus*, among other lactic acid bacteria, were found in Nga-pi, as was described by Chotwanawirach (1980). Protease-producing halophilic bacteria have also been found in Nga-pi. These microorganisms were *Lentibacillus kapialis, Salinicoccus siamensis, Virgibacillus halodenitrificans,* and *Oceanobacillus kapialis* (Pakdeeto et al., 2007a, b: Namwong et al., 2009).

Typically, the salt content of fermented shrimp pastes is between 25% and 30%. As a result, most of the bacteria discovered in the manufacturing process are categorized as halophiles. Bacteria have crucial roles in the production of both flavor and scent in fermented foods (Disarapong, 2005). Therefore, normal fragrance or volatile chemicals are not formed during aseptic production (Beddows and Ardeshir, 1979). There are two main types of bacteria that play a role in the fermentation process: (1) bacteria that create proteolytic enzymes, and (2) bacteria that participate in the creation of flavor and aroma.

CHAPTER-3: MATERIALS AND METHODS

3.1 Study Area

This study was conducted at the Nutrition and fish processing laboratory and Microbiology laboratory of Chattogram Veterinary and Animal Sciences University for different Nga-pi samples collected.

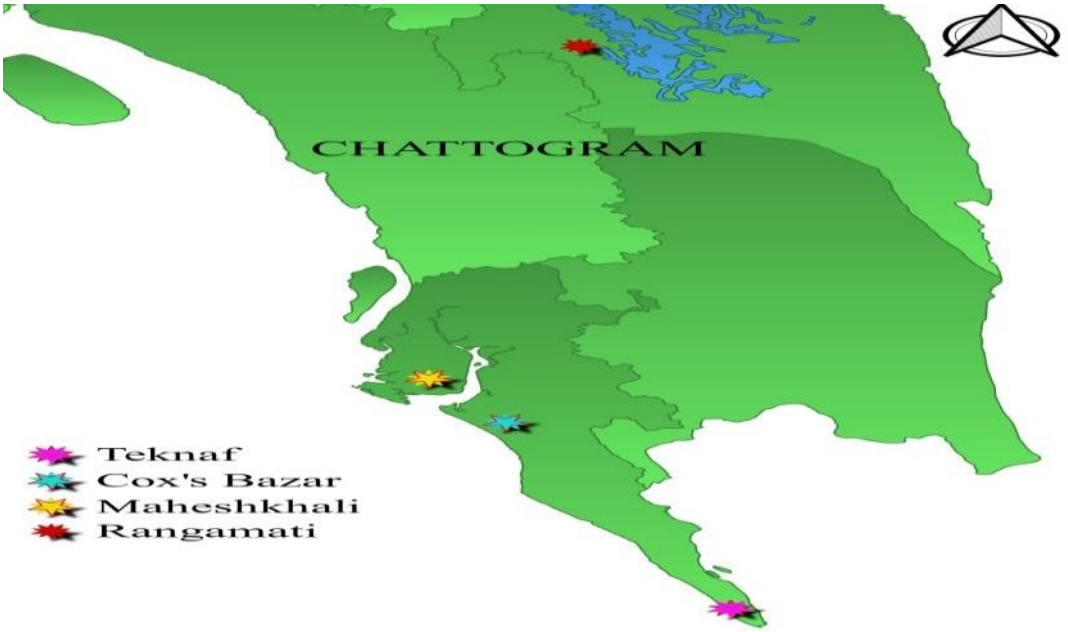


Figure-1: Map of sampling sites

3.2. Collection of samples

The samples were collected periodically from the producers of Nga-pi in four different sampling areas (1) Cox's Bazar, (2) Mahesh khali, (3) Rangamati and (4) Teknaf, where two stations were selected in Mahesh khali region and three stations in Rangamati region. Information on marketing price from retailers in different markets was also collected from those selected places.

3.3. Transportation and storage of the samples

Nga-pi samples were collected from previously selected different collection areas and immediately packed in zip-lock polythene packs at the collection sites to be transported to the Fish Processing Laboratory, Department of Fisheries Technology, Chattogram Veterinary and Animal Sciences University, Chattogram.

To track changes in biochemical parameters of Nga-pi packed in the airtight polythene condition, a predetermined number of samples (air-tight polythene packs; each pack containing roughly 250g Nga-pi sample taken from producers of different areas of Cox's Bazar, Teknaf, Mahesh khali, and Rangamati) were stored separately.

3.4. Proximate composition analysis

The proximate composition of the Nga-pi samples will determine following standard methods (AOAC, 2005). The ash content of the samples was estimated as the inorganic residues such as oxides, sulphates, silicates and chlorides left behind, in the dry muscle sample in a muffle furnace. Amino acid was determined by High Performance Liquid Chromatography (HPLC). Protein content was determined by Kjeldhal apparatus and moisture content are estimated by hot air oven. The lipid content of Nga-pi was determined by a soxhlet apparatus.

3.4.1. Protein determination

Protein content was determined through micro Kjeldhal Apparatus. Digestion compact system (DK20/26, VELP scientific) and Distillation system (Model: UDK 129, VELP scientific). After taking 0.3 g of sample in the digestion tube, 4 g catalyst and 5 ml concentrated H_2SO_4 was added. Then the digestion tube was placed in the digestion unit and digested for 30 minutes. Once digestion of the samples is completed, the tubes were taken out from the unit and kept in room temperature for 30 minutes for being cool. Then 25 ml of distilled water was added in the digestion tube. 10 ml of the mixed indicator was taken in the conical flask of the distillation unit. 25 ml NaOH and distilled water were placed below the pipe (white and black) of the distillation unit. Then the sample was titrated with 0.2 N HCl.

Formula:

$$\% \text{ of N} = [\text{ml of titrant} \times \text{strength of HCl (0.2 N)} \times \text{equivalent of nitrogen (0.014)}]/ (\text{weight of sample}) \times 100$$

$$\% \text{ protein} = \% \text{ N} \times 6.25 \text{ (animal origin)}$$


Plate-2: Protein determination

3.4.2. Lipid determination

The lipid content of Nga-pi was determined by a soxhlet apparatus (Model: RD 40, Food ALYT). At first, 2 g sample was taken in the thimble paper and then the thimble paper with the sample was placed under a magnetic holder by a magnetic ring and lifted up. Sterilized empty beaker was marked and measured to take the weight. Then 70 ml diethyl ether was taken into the marked beaker and the beaker with solvent was screwed under the condenser. Then the thimbles were lowered into the beaker, and extraction beaker was placed in the burner and boiled for 20 minutes at 100°C temperature. Following that, the thimbles were lifted up, the beakers were cooled for 20 minutes and let the solvent was evaporated for 10-15 min. Then the extraction beakers were kept in the hot air oven at 105°C for 30 minutes and cooled down in the desiccator. After that, the weight of the beaker was measured.

Formula:

$$\% \text{ of Lipid} = (\text{weight of lipid}) / (\text{weight of sample}) \times 100$$


Plate-3: Lipid determination

3.4.3. Determination of ash and moisture

3.4.3.1. Ash

Ash content was determined by Muffle furnace (Model: LHMF 100A, LABNICS Equipment). The weights of the sterilized empty crucibles were measured and then 3 g of samples were taken in each crucible. The crucibles containing samples were kept in the muffle furnace for 5 hours at 550°C temperature. Then they were kept in the desiccator for cooling and followed by taking weight of the crucibles.

Formula:

$$\% \text{ of Ash} = (\text{Ash weight} / \text{ sample weight}) \times 100$$

3.4.3.2. Moisture

Moisture content was determined by Drying oven (Model: BINDER, ED 115). 3 g of each sample were taken in the previously weighted empty crucible. Then the crucibles were kept in the chamber of a hot air oven for overnight (12 hours) at 105°C temperature. After that, the crucibles were kept in the desiccator for being cooled down and finally the weight of the crucibles was measured.

Formula:

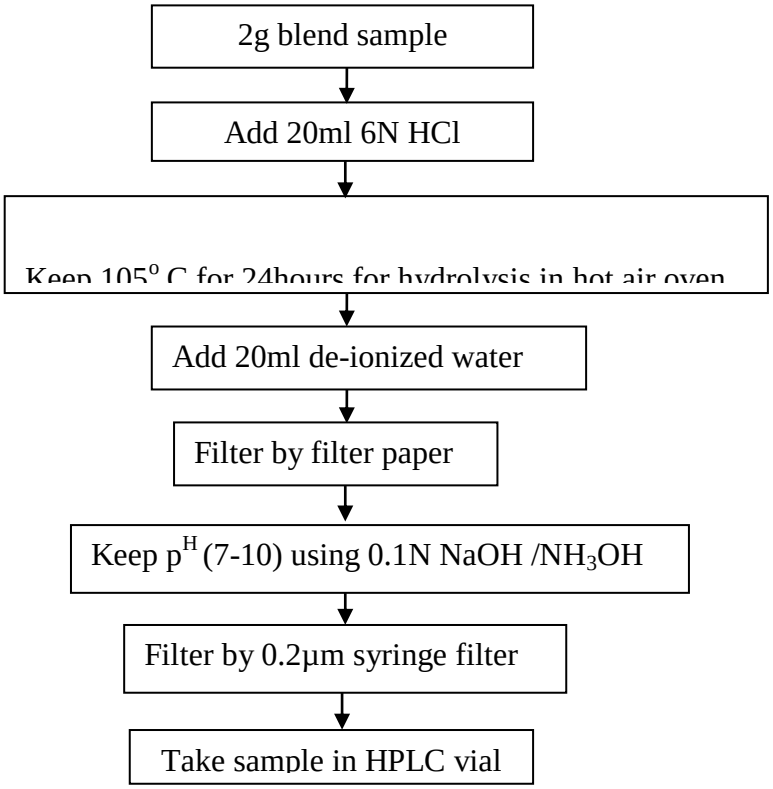
$$\% \text{ of Moisture content} = (\text{weight of wet materials} - \text{weight of dry materials}) / (\text{weight of wet materials}) \times 100$$

Plate-4: Determination of ash and moisture

3.5. Amino acid analysis by HPLC

Reliable and sensitive amino acid analyses are important steps in studies of protein structures. In this respect, high performance liquid chromatography (HPLC) (Waters e2695 separation Module) has greatly increased speed and sensitivity. Separation of underivatized amino acids by ion exchange HPLC and subsequent detection by post-column derivatization, as well as pre-column derivatization and subsequent separation of amino acid derivatives by reverse phase HPLC have been widely used. 6N HCl and 0.1 N NaOH or 0.1 N NH_3OH are used for sample preparation.

Sample preparation



Flowchart-2: sample preparation for amino acid analysis

Plate-5: HPLC (High Performance Liquid Chromatography) machine

3.6. Microbiological analysis of Nga-pi

To assess the microbial load, 10 g of each sample was aseptically taken in screw capped test tube and macerated with 90 ml sterile bacteriological peptone water (BPW) and the same for the Alkaline Peptone Water (APW). This is the first stock of serial dilution (10^{-1}). Then, serial dilution was continued up to10^{-8} times in the same diluent. Total viable counts were recorded after 48 h of incubation at 37ºC and expressed as log CFU (colony forming units per gram).

3.6.1. Total plate count

Total plate count was determined using the pour plate technique. Plate Count Agar (Oxoid, CM463) was used as medium. 19 ml of PCA (Plate Count Agar) was poured into sterile petri dish with 1 ml of 1 ml solution from first serial dilution and gently mixed together and then kept in the incubator. Total viable counts were recorded after 24 h of incubation at 37 ºC and express as log CFU (colony forming units per gram).

3.6.2 *E. coli* determination

To determine the presence of *E. coli*, streak plate method was followed. For *E coli* identification EMB (Eosin Methylene Blue) and MacConkey agar was prepared according to the instruction. After pouring the hot liquid agar on the agar plates, they were kept in room temperature for some time for solidifying. When the agar became solid enough, sample from BPW (Buffered peptone water) was taken with an inoculating loop (red heat incinerated). The loop was gently streaked on the agar in zigzag form for four times in each plate. Then, the plates were incubated in inverted positions at 37°C for 24-48h in the incubator.

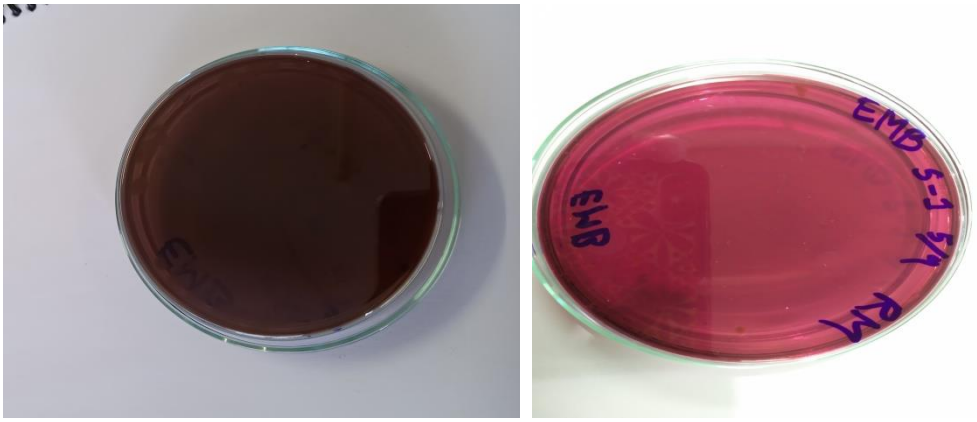
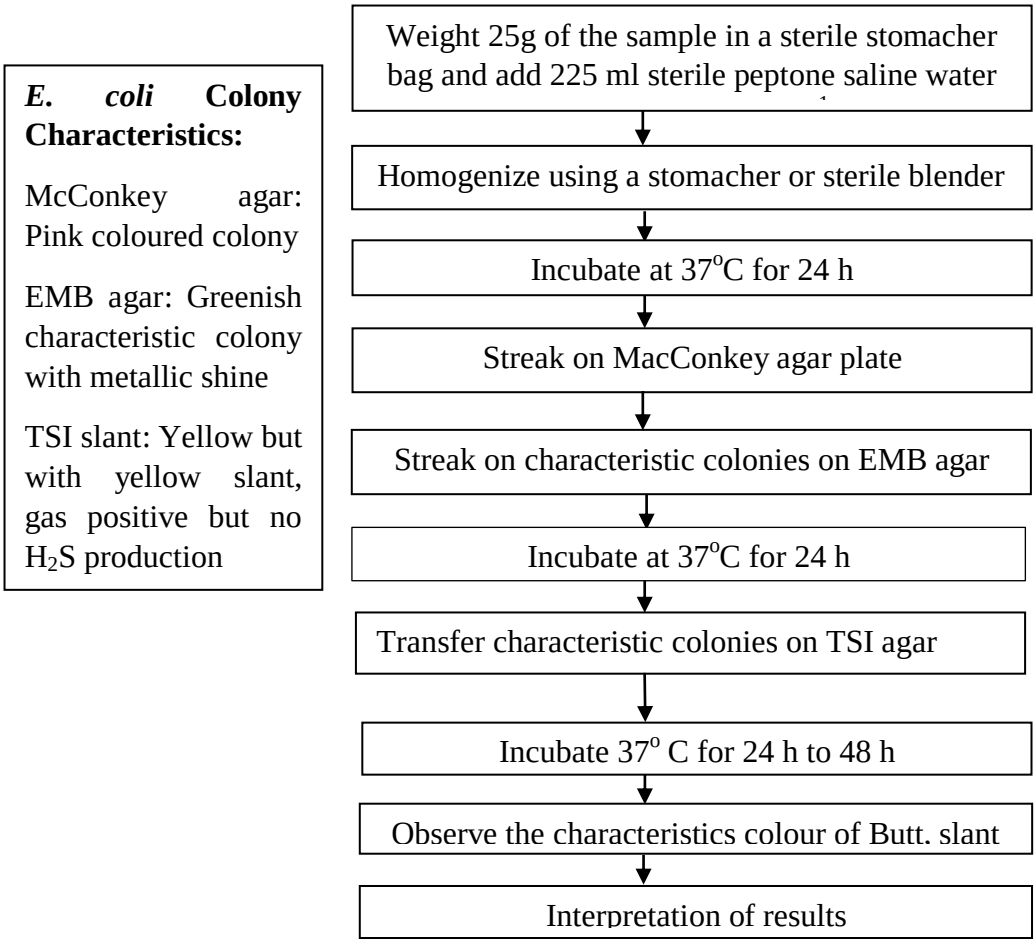


Plate-6: *E. coli* determination

Identification of *E. coli*



Flowchart-3: *E. coli* determination

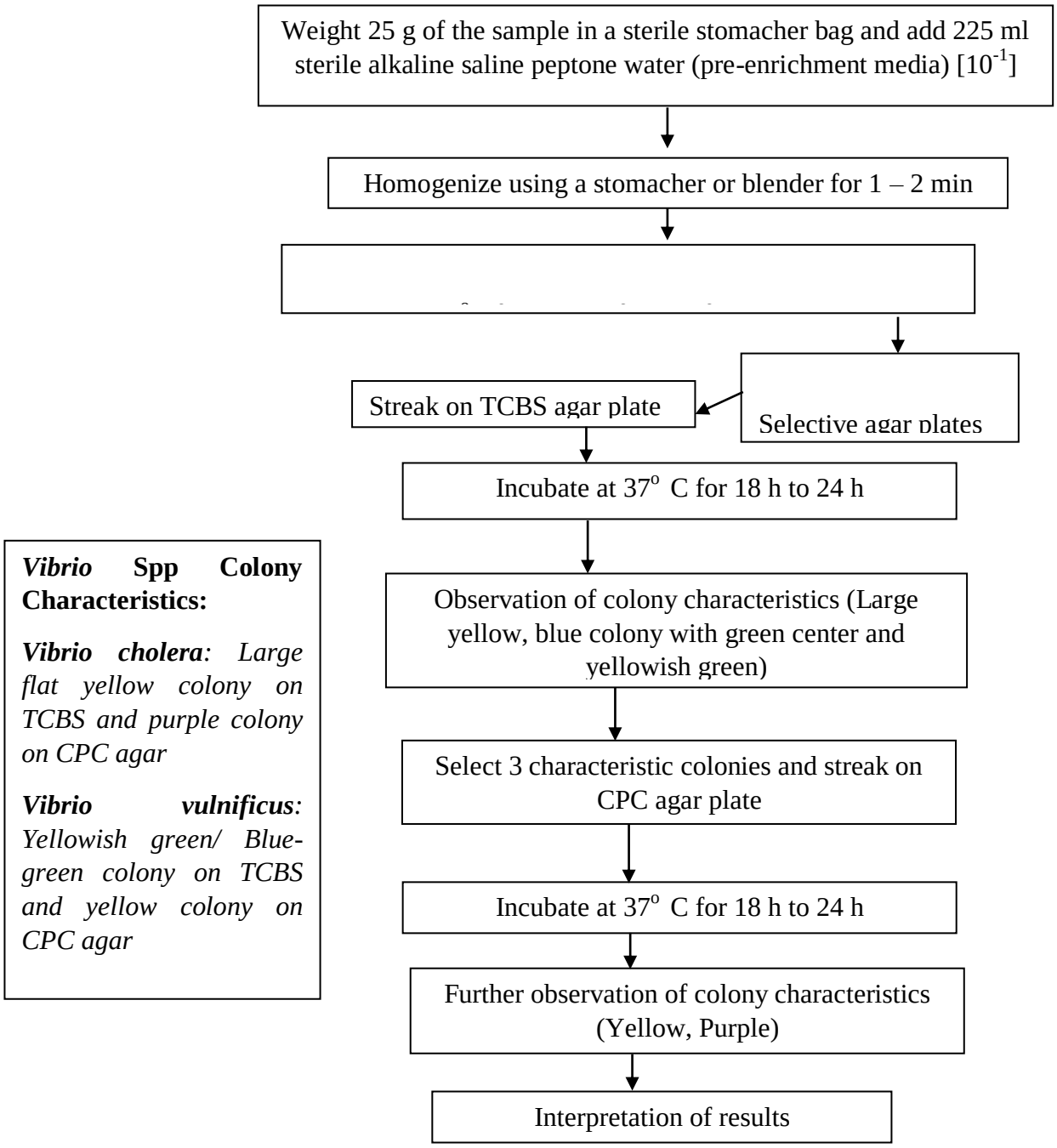
3.6.3. *Vibrio cholera* identification

APW (Alkaline peptone water) is recommended as an enrichment broth as it can enhance the isolation of *V. cholera* when few organisms are present. At 6 to 8 hours was present in greater numbers than non-*Vibrio* organisms. Sample with APW was incubated in the tube with the cap loosened at (35° to 37°C) for (6 to 8 hours) in the incubator.

After incubation, subculture on TCBS (thiosulfate citrate bile salts sucrose agar) is the selective agar medium of choice and on MacConkey agar. One to two loopfuls of APW were taken with the help of red heat incinerated inoculating loop from the surface and topmost portion of the broth, since *Vibrio's* preferentially grow in this area. The samples were placed in the agar plate by streaking method. Then, the plates were incubated in inverted positions at 37°C for 24h in the incubator. *Vibrio* strains

grow as pale, non-lactose-fermenting colonies on MacConkey agar. On TCBS agar *V. cholera* grows as medium-sized convex, smooth, yellow colonies.

Detection of *Vibrio cholera*



Flowchart-4: *Vibrio cholera* identification

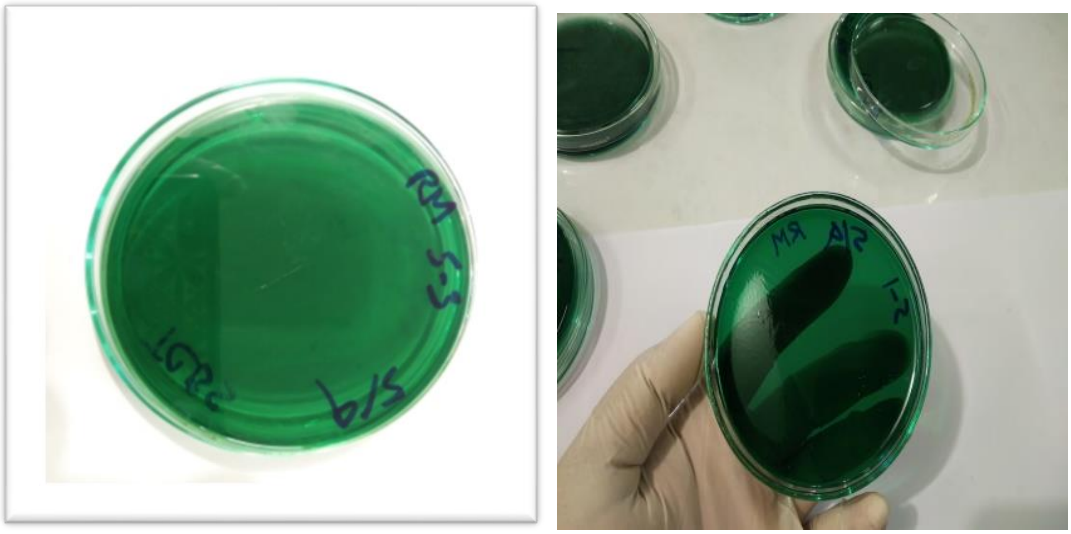
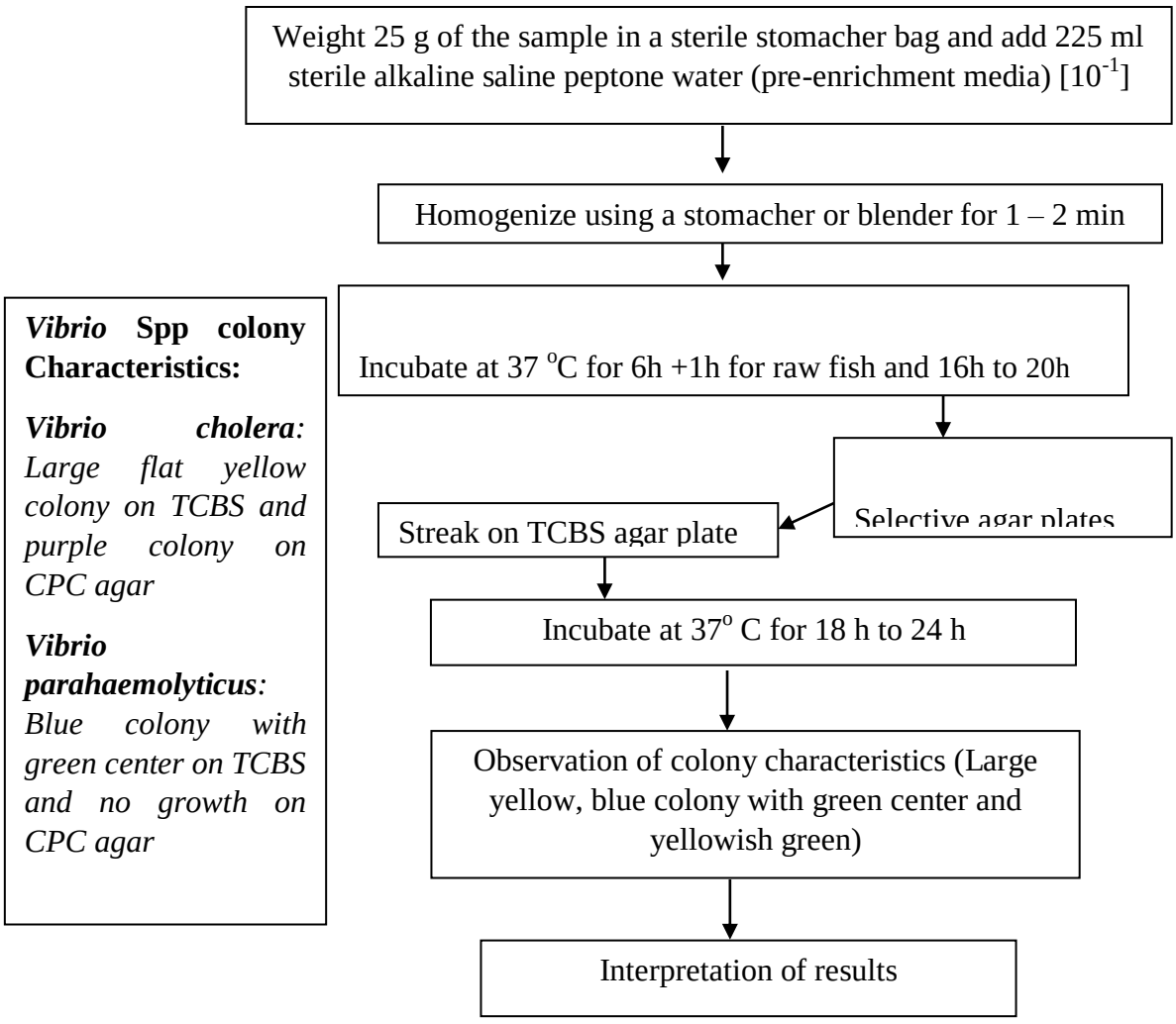


Plate-7: Detection of *Vibrio cholera*

3.6.4. Detection of *Vibrio parahaemolyticus*



Flowchart-5: *Vibrio parahaemolyticus* identification

3.6.5. Identification of *Salmonella* and *Shigella*

Non-selective enrichment of the collected sample was done in BPW (Bacteriological peptone water). The broth was kept in the incubator for 20-24h at 37°C temperature for bacterial growth. RV (Rappaport vassiliadis soya peptone) broth was used as selective medium for *Salmonella* identification. Sample was added to the RV broth following 1:9 ratios and incubated for 24 to 48h at 37°C temperature. XLD (Xylose Lysine Deoxycholate) and BGA (Brilliant Green Agar) was prepared according to the instruction and poured in the agar plate for solidifying. Also, TSI (Triple Sugar Iron) slant was prepared in the sterile test tubes. Afterwards, sample from broth was taken with an inoculating loop (red heat incinerated) and streaked in the solidified agar plates (XLD and BGA) and stabbed in the agar slants (TSI). Then the plates were incubated for 24 to 48h at 37°C and slants were kept in the incubator for 24h in 37°C temperature.

Identifying color of the colony-

- XLD- Black colony (*Salmonella* or *Shigella*)
- BGA- Whitish pink colony (*Salmonella*)
- TSI- Yellow media turns into black with gas at bottom (*Salmonella*)
- TSI- No color change in media (*Shigella*)

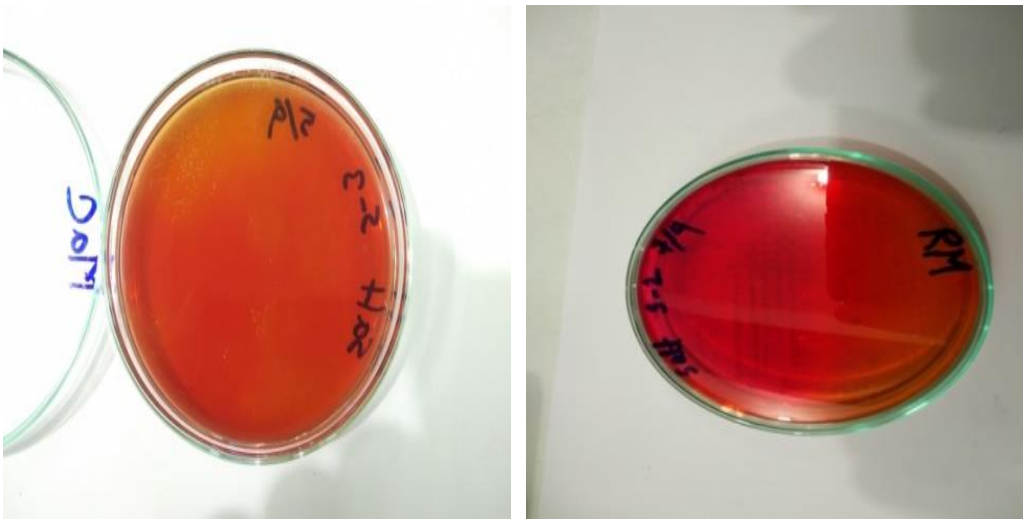
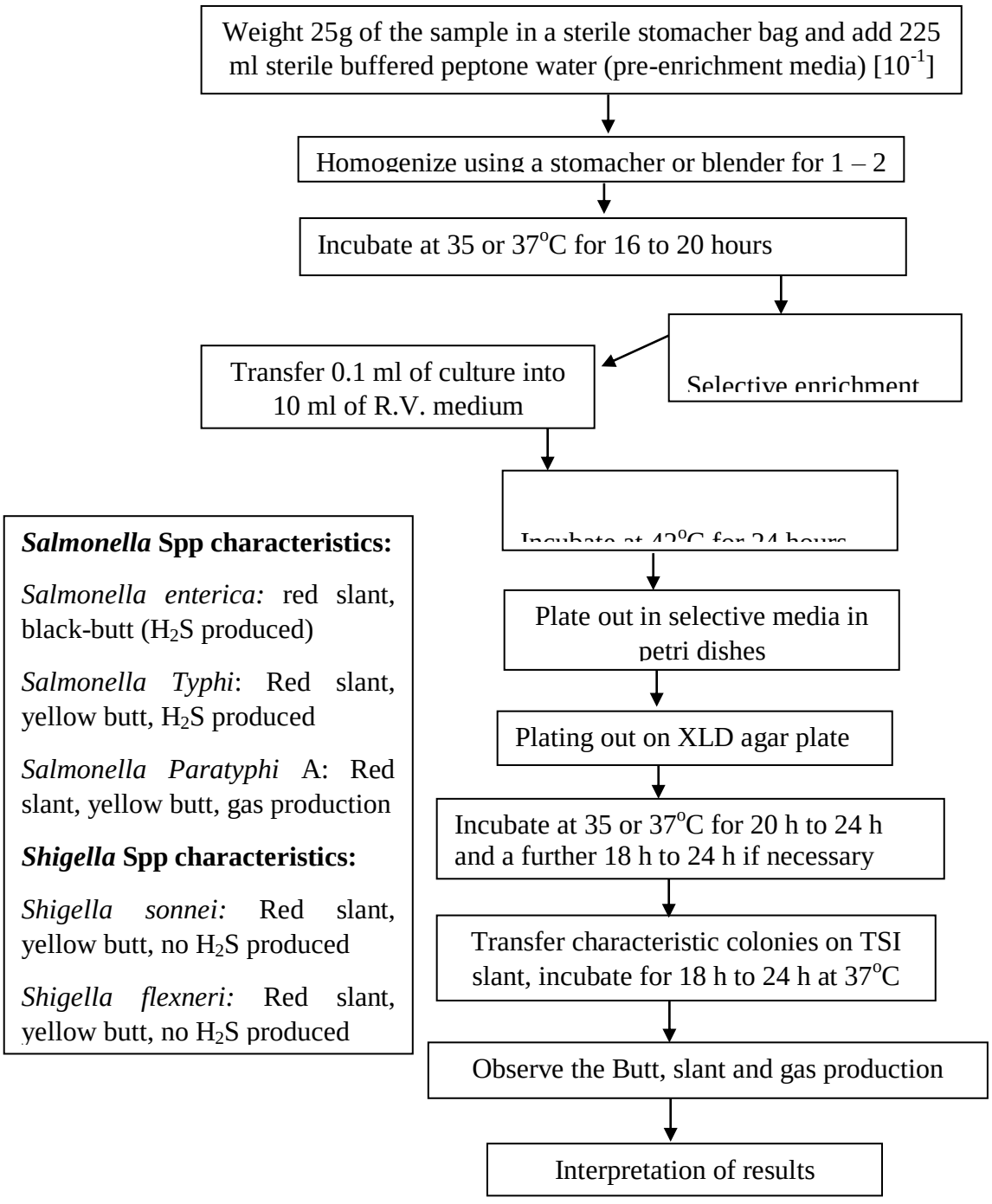


Plate-8: Identification of *Salmonella and Shigella* spp.

Detection of *Salmonella and Shigella* spp



Flowchart-6: Detection of *Salmonella* and *Shigella* spp.

3.7. Statistical analysis

The data on proximate composition and amino acids were analyzed by using MS Excel software. The mean and standard error of the mean of all the data were calculated in MS Excel and reported throughout the text as means and standard error.

CHAPTER-4: RESULTS

4.1. Proximate composition of Nga-pi

The data illustration in Figure 2 showed that Nga-pi from the Teknaf region has the highest protein value (55.03%) and Cox's bazar region has the lowest value (35.54%). On the other hand, the lipid content was higher in Nga-pi collected from the Mahesh khali station 1 and station 2. The lowest lipid content was found in the sample from the Rangamati station 3 regions (3.92%). The ash content was also found to be higher (18.49%) in the Mahesh khali region (station 2) but lower in Teknaf (13.10%) at figure 3. The results also indicated that the value of moisture content was very lower (12.89%) in the Nga-pi sample from Teknaf region and higher in the Rangamati region at figure 4. Among the samples collected from 3 stations of Rangamati, station 3 (51.19%) contains more moisture than station 1 (43.79%) at figure 5.

Proximate composition (protein, lipid, ash and moisture) of Nga-pi samples from different regions of Bangladesh (Cox's-Cox's bazar; Tek-Teknaf; Mhs (1) - Mahesh khali station 1; Mhs (2) - Mahesh khali station 2; Rang (1)- Rangamati station 1; Rang (2)- Rangamati station 2; Rang (3)- Rangamati station 3

a)

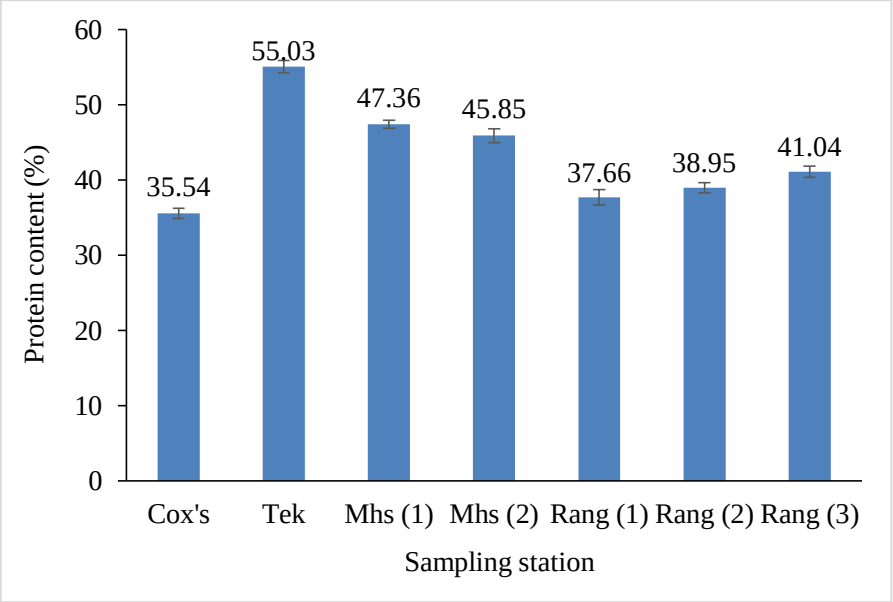


Figure-2: Protein percentage of Nga-pi samples from different regions of Bangladesh

b)

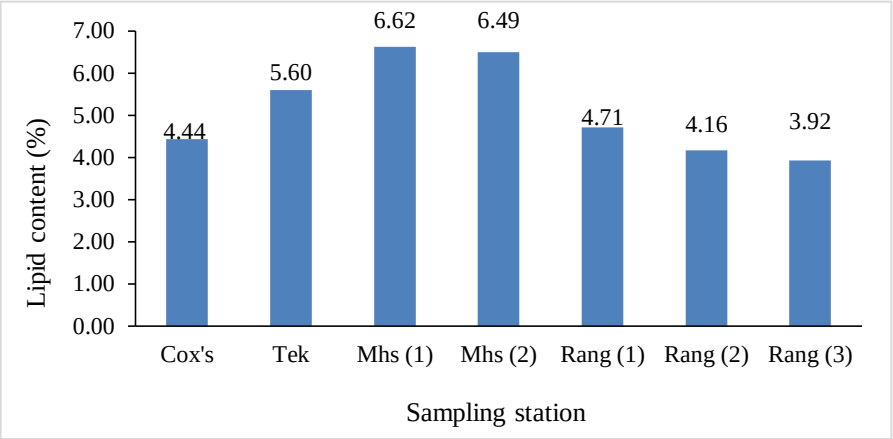


Figure-3: Proximate composition (lipid) of Nga-pi samples from different regions of Bangladesh

c)

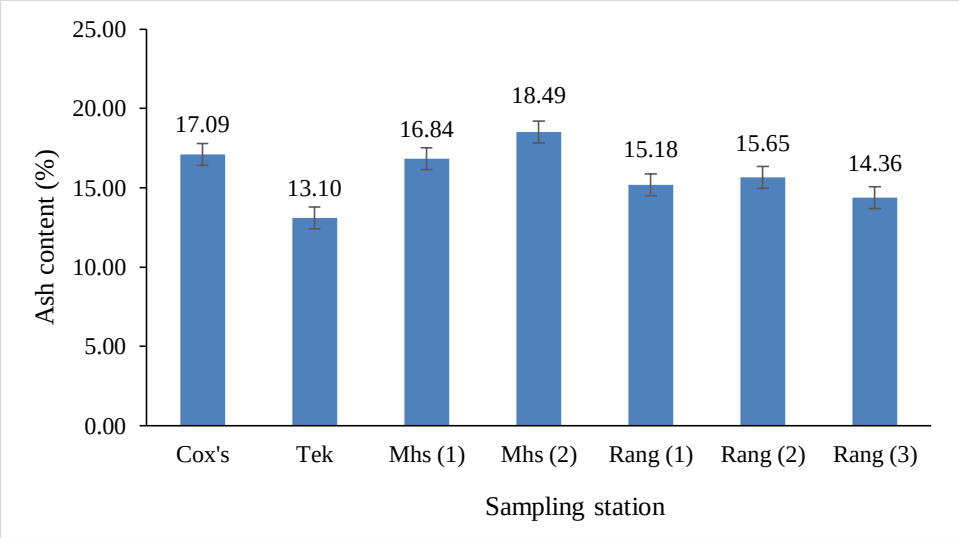


Figure-4: Proximate composition (ash) of Nga-pi samples from different regions of Bangladesh

d)

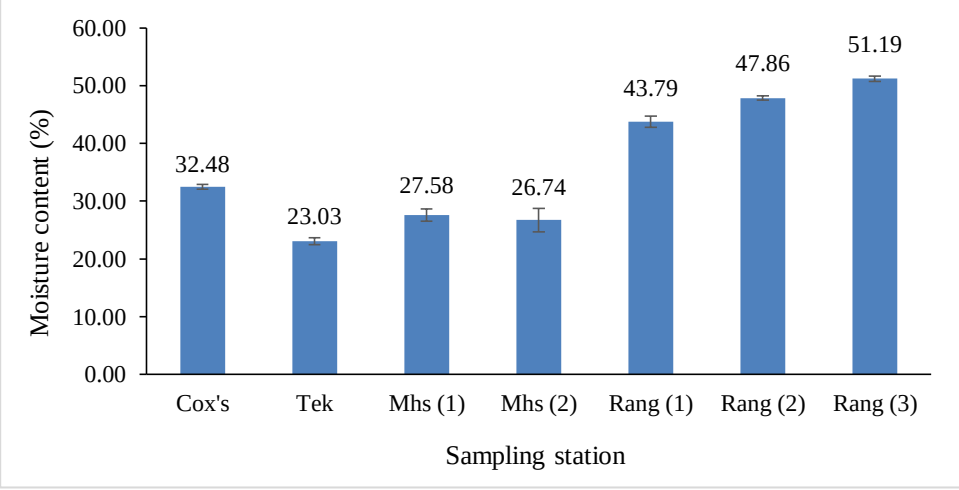


Figure-5: Proximate composition (moisture) of Nga-pi samples from different regions of Bangladesh

4.2. Amino acid profile

The results of amino acid determination have been shown in Table 1 there are 17 amino acid contents have been determined for each sample. The values greatly varied in terms of every amino acid among the samples.

Table 1: Amino acids profile of Nga-pi collected from different regions of Bangladesh (unit PPM)

	Sampling Area		
Amino acid	Cox's Bazar	Mahesh khali	Rangamati
Alanine	24260.30	12157.86	13967.44
Arginine	115774.45	47938.61	60018.41
Aspartate	272.96	177.14	162.44
Cystine	21.41	1085.55	1.10
Glutamine	324.78	315.74	226.75
Glycine	100.01	72.04	55.82
Histidine	591.24	23.00	14.22
Isoleucine	14385.74	63805.56	35624.54
Leusine	151.53	176.59	179.07
Lysine	108.49	70.44	164.26
Metathione	247.38	228.60	175.40
Phenylalanine	284.70	215.87	260.14
Proline	127.61	213.65	90.68
Serine	180.29	199.37	197.08
Threonine	149.43	280.11	163.39
Tyrosine	138.13	31.02	51.76
Valine	2.36	85.95	79.93

4.3. Microbiological analysis

The results of the microbiological analysis are given in Table. The microflora of salted-naturally fermented fish consisted of various species of micro-organisms such as aerobic, halophile, and staphylococcal bacteria, yeasts and molds. The microbial load of Nga-pi was determined against *Vibrio cholera*, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *E. coli*, *Salmonella* and *Shigella*. *Vibrio vulnificus* and *Vibrio parahaemolyticus* were present on the samples collected from Rangamati, Cox's Bazar, Mahesh khali and Teknaf region. No *Vibrio cholera*, *E. coli*, *Salmonella* or *Shigella* was found in either of the samples collected from different regions.

4.3.1 Total plate count of bacteria

From the first sampling, Nga-pi samples were collected from previously selected sampling area. Numbers of bacterial colony found in the sample were shown in Figure-6. Highest bacterial load was found in Rangamati(2) sample (16.33×10^3 CFU/g). Whereas, the lowest number of bacterial colony was found in Teknaf sample (0.87×10^3 CFU/g). Comparatively lower number of bacterial colonies were determined in the samples from Mahesh khali(1), Mahesh khali(2), Cox's Bazar, respectively (1.33×10^3, 2.17×10^3, 4.81×10^3). 13.00×10^3 CFU/g and 15.00×10^3 CFU/g bacterial colony were recorded from Rangamati(1) and Rangamati(3)'s sample respectively.

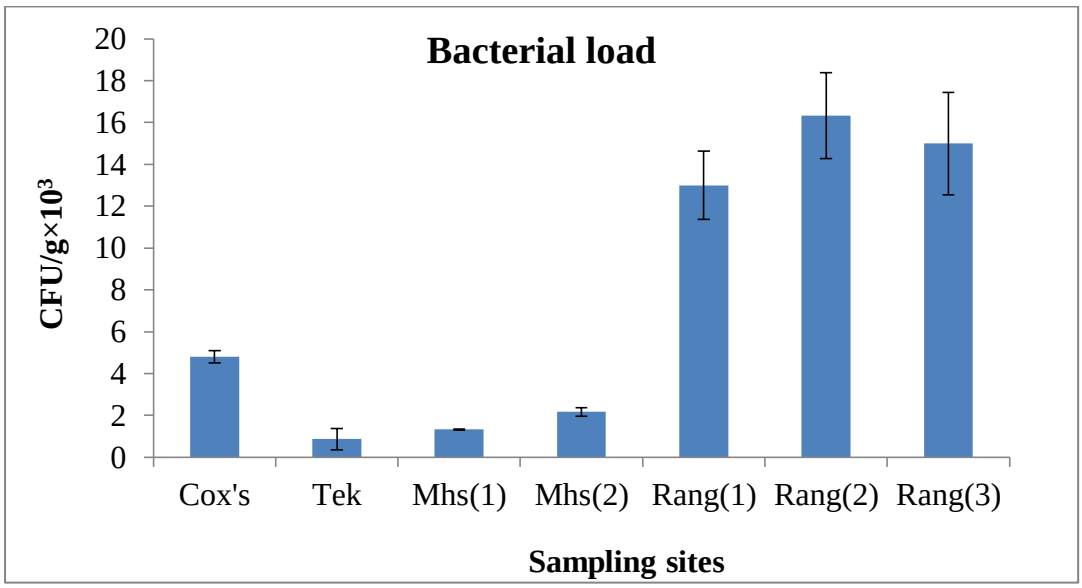


Figure-6: Bacterial load of Nga-pi samples collected from different regions of Bangladesh

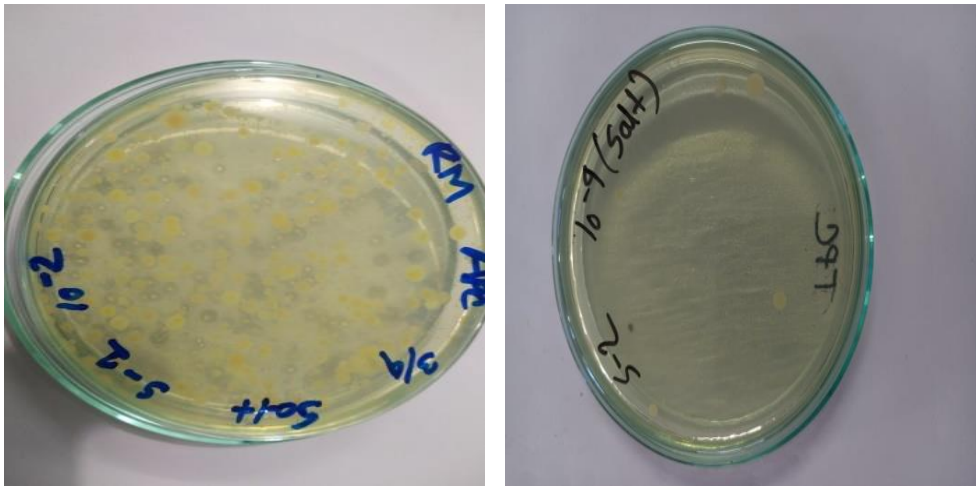


Plate-9: Total plate count bacteria

4.4.2 Pathogenic bacteria

Nga-pi samples were collected from previously selected sampling area. In all of the samples there were no colonies of *Salmonella* spp., *Shigella* spp., *Vibrio cholera*, *E.coli* bacteria. But there are some blue green colonies in TCBS media. For this the blue green colony were anticipated as *Vibrio parahaemolyticus*. The results of different media are shown in Table 2.

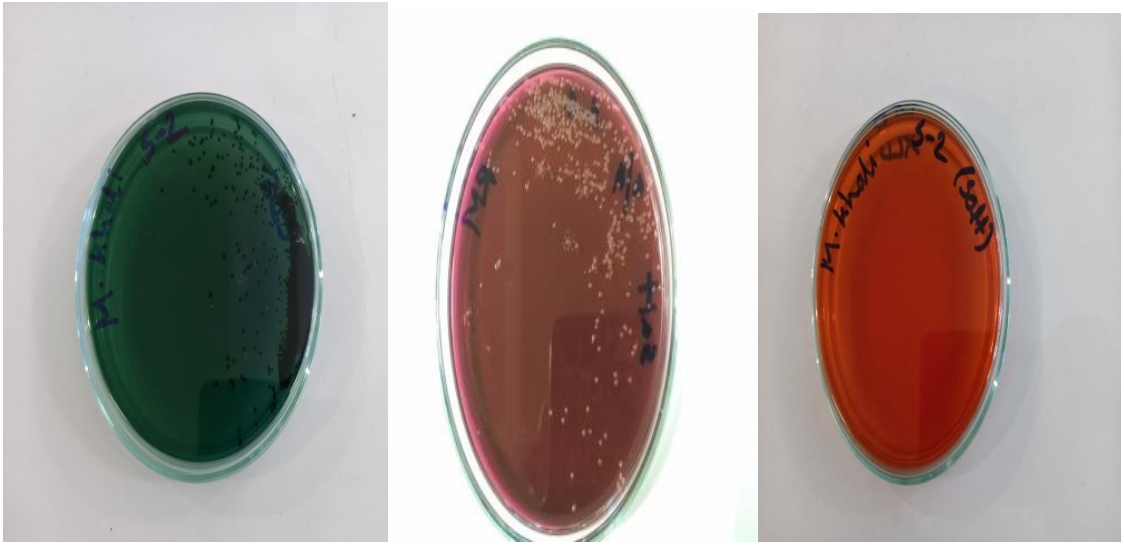


Plate-10: Pathogenic bacteria identification

Table 2: Microbial load in Nga-pi sample collected from different stations where "R", "S", "W. salt", "C.B", "Mhs", "Tek" represents the replication, Sample, without salt, Cox's Bazar, Mahesh khali and Teknaf (-ve means negative, +ve means positive)

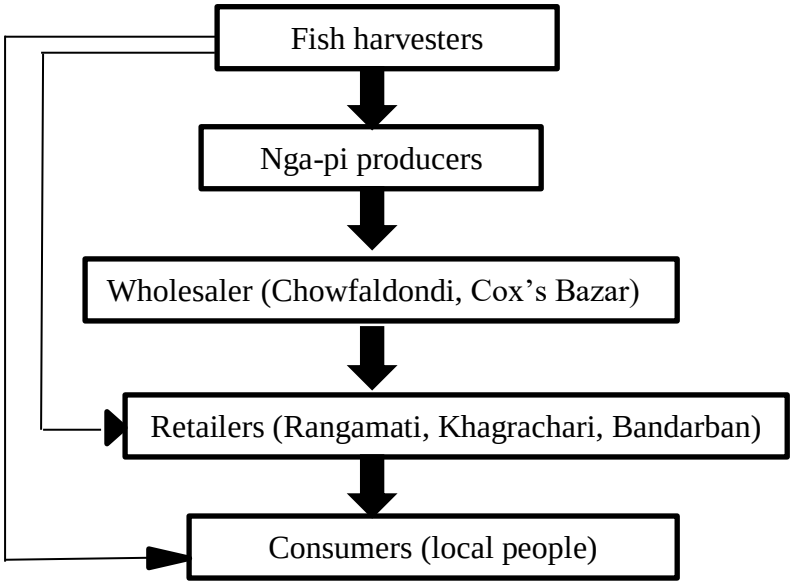
Sample	**R**	***Salmonella* (XLD)**	***Vibrio cholera* (TCBS)**	***E. coli* (EMB/ MacConkey)**	***Shigella* (XLD)**	***Vibrio parahaemolyticus* (TCBS)**
Rangamati						
S-1 (salt)	R-1	-ve	-ve	-ve	-ve	+ve
	R-2	-ve	-ve	-ve	-ve	+ve
	R-3	-ve	-ve	-ve	-ve	+ve
S-1 (w. salt)	R-1	-ve	-ve	-ve	-ve	-ve
	R-2	-ve	-ve	-ve	-ve	+ve
	R-3	-ve	-ve	-ve	-ve	-ve
S-2 (salt)	R-1	-ve	-ve	-ve	-ve	+ve
	R-2	-ve	-ve	-ve	-ve	+ve
	R-3	-ve	-ve	-ve	-ve	+ve
S-2 (w. salt)	R-1	-ve	-ve	-ve	-ve	-ve
	R-2	-ve	-ve	-ve	-ve	+ve
	R-3	-ve	-ve	-ve	-ve	+ve
S-3 (salt)	R-1	-ve	-ve	-ve	-ve	+ve
	R-2	-ve	-ve	-ve	-ve	-ve
	R-3	-ve	-ve	-ve	-ve	+ve
S-3 (w. salt)	R-1	-ve	-ve	-ve	-ve	-ve
	R-2	-ve	-ve	-ve	-ve	+ve

	R-3	-ve	-ve	-ve	-ve	-ve
Cox's Bazar						
C.B (salt)	R-1	-ve	-ve	-ve	-ve	+ve
	R-2	-ve	-ve	-ve	-ve	-ve
	R-3	-ve	-ve	-ve	-ve	+ve
C.B (w. salt)	R-1	-ve	-ve	-ve	-ve	+ve
	R-2	-ve	-ve	-ve	-ve	-ve
	R-3	-ve	-ve	-ve	-ve	-ve
Teknaf						
Tek (salt)	R-1	-ve	-ve	-ve	-ve	-ve
	R-2	-ve	-ve	-ve	-ve	+ve
	R-3	-ve	-ve	-ve	-ve	-ve
Tek (w. salt)	R-1	-ve	-ve	-ve	-ve	-ve
	R-2	-ve	-ve	-ve	-ve	-ve
	R-3	-ve	-ve	-ve	-ve	-ve
Mahesh khali						
Mhs-1 (salt)	R-1	-ve	-ve	-ve	-ve	+ve
	R-2	-ve	-ve	-ve	-ve	-ve
	R-3	-ve	-ve	-ve	-ve	+ve
Mhs-1 (w. salt)	R-1	-ve	-ve	-ve	-ve	-ve
	R-2	-ve	-ve	-ve	-ve	-ve
	R-3	-ve	-ve	-ve	-ve	-ve
Mhs-2	R-1	-ve	-ve	-ve	-ve	+ve

(Salt)	R-2	-ve	-ve	-ve	-ve	-ve
	R-3	-ve	-ve	-ve	-ve	+ve
Mhs-2 (w. salt)	R-1	-ve	-ve	-ve	-ve	+ve
	R-2	-ve	-ve	-ve	-ve	-ve
	R-3	-ve	-ve	-ve	-ve	-ve

4.4. Marketing channel of Nga-pi

Commercially Nga-pi has been produced in Chowfaldandi, Cox's Bazar region, where the production rate is comparatively higher than the other areas. Most of the Nga-pi products are distributed from this area throughout the country. Major trade point of Nga-pi is Rangamati, Bandarban, Khagrachari. The wholesalers of these regions collect Nga-pi from Cox's Bazar region and sell in the retail market. Producers of Mahesh khali and Teknaf region basically make Nga-pi for their own consumption and sells the products in a smaller extend. Therefore, the price and purchase rate of Nga-pi varies in different areas based on their consumer's demand and purpose of production.



Flowchart-7: The marketing channel of Nga-pi

CHAPTER-5: DISCUSSIONS

Since the beginning of human evolution, gathering food has become essential to our survival and the preservation of our physiological processes. Along with the advancement of human civilization, perishable food ingredients from plant and animal sources have been preserved, their products have been diversified, and innovations in production of food and culinary techniques have also been made. Every community has its own unique fermented foods that are produced naturally (by local microorganisms) or artificially (using starter cultures). Fermented foods and alcoholic drinks are consumed all over the world. According to Campbell-Platt (1994), about one-third of the food we eat is fermented. Around 20% of all food consumed worldwide is fermented. Data on the frequency and consumption of fermented foods are scarce and not very reliable. This research was done to ascertain the nutritive benefits of "Nga-pi" that was gathered from various parts of Bangladesh.

5.1. Proximate composition of Nga-pi

Proximate composition for raw shrimps (traditionally used for Nga-pi production), traditionally produced Nga-pi samples collected from different stakeholders of four different regions are presented in Figure 2-5. The data illustration showed that Nga-pi from the Teknaf region has the highest protein value (55.03%) and Cox's bazar region has the lowest value (35.54%). On the other hand, the lipid content was higher in Nga-pi collected from the Mahesh khali station 1 and station 2. The lowest lipid content was found in the sample from the Rangamati station 3 region (3.92%). The ash content was also found to be higher (18.49%) in Mahesh khali region (station 2) but lower in Teknaf (13.10%). The results also indicated that the value of moisture content was very lower (12.89%) in the Nga-pi sample from Teknaf region and higher in the Rangamati region. Among the samples collected from 3 stations of Rangamati, station 3 (51.19%) contains more moisture than station 1 (43.79%).

Fermented fish products are generally high in protein and amino compounds. Shikha et al. (2019) reported that, the protein content varied between25.88% to 26.89% for different Nga-pi samples collected from Chowfaldandi and 26.10% to 27.12% Nga-pi samples collected from Nazirartak.

In this study, highest water content (moisture) was found in the shrimp paste samples collected from the Rangamati, which were 47.86% and 51.19% where the lowest value was found in the samples collected from Teknaf region (23.03%). There are many variations in the moisture content of different regional products. Because local products were handcrafted or produced in small quantities, some of the ingredients varied between samples. The amount of moisture in the finished shrimp paste depends on how long the sample was sun-dried (Pongsetkul et al., 2014). With an Aw of roughly 0.7, the shrimp paste falls into the category of food with intermediate moisture (Fennema, 1996). At room temperature, the low Aw of shrimp paste products may extend their shelf lives and protect them from microbial spoilage (Goulas and Kontominas, 2005; Prapasuwannakul and Suwannahong, 2015). Additionally, the low Aw would prevent product rancidity and restrict the development of food pathogens (Hajeb and Jinap, 2012). The salt that is required for preservation was present in thirteen samples of shrimp paste. The range of the salt content was 7.00 to 10.85%. Additionally, the salt content would lengthen the shelf life and improve the flavor of products made with shrimp paste. The percent moisture content of raw shrimp samples and traditionally produced Nga-pi samples collected from the Nazirartak were found to be higher than the values found in this study in a previous investigation report. The range of traditionally produced Nga-pi samples there was between 58.29% and 60.70%.

Raw shrimp samples from Nazirartak were found to have a percent lipid content of 4.36 (better grade) and 3.28 (lower grade), which is traditionally used in the production of Nga-pi. While the range of percent lipid content in various Nga-pi samples found from 4.81 to 5.12 collected from Chowfaldandi, the lipid content of various Nga-pi samples collected from the same area varied between 4.18% and 4.93%. Although raw shrimp samples' percent ash content ranged from 0.99 to 1.98, when Nga-pi was produced, the ash content ranged from 7.33 to 8.40 when gathered from Chowfaldandi and 7.30 to 8.29 when gathered from Nazirartak. An analysis of the quality of a traditional semi-fermented fishery product (Chepa Shutki) from Bangladesh that was gathered from the value chain was done by Nayeem et al. (2010). They looked at the near composition of Chepa shutki, a type of semi-fermented fish product that was purchased from a producer, wholesaler, and retailer. According to the findings of their investigation, moisture content ranged from 39.62 to 46.89%,

with the retailer-purchased products having the greatest value and the producer-purchased products having the lowest. Protein, the most significant chemical component, ranged from 32.46 to 33.83%, with the highest value found in products purchased directly from producers and the lowest value in those purchased directly from retailers. On the other hand, the lipid content ranged from 19.25 to 24.97%, with the producer's products having the highest value and the retailer's products having the lowest value. When the values of protein and lipid were recalculated on a dry weight basis, a similar trend was also seen. Ash content ranged from 0.81 to 1.01%, with products bought via wholesalers and merchants having the highest value and products bought directly from producers having the lowest value. Lower protein and fat concentrations in the goods purchased from retailers and wholesalers were likely caused by losses that occurred during various phases of the marketing chain, including handling, transit, and preservation. Another investigation of the nutritional and microbial value of Chepa shutki from Bangladesh's haor regions was conducted by Sabikon et al. (2017). Lower levels of protein, fat, fiber, and nitrogen-free extract content in the product purchased from the retailers were found in their investigation. These findings are likely attributable to losses that occurred during handling, transit, and preservation at various points along the marketing chain.

The quality of fish pickles made with Thai Pangus (*Pangasianodon hypophthalmus)* was studied by Rahman et al. (2019) at refrigeration (5°C to 8°C) and freezing (-20°C to -18°C) temperatures in a kitchen refrigerator. After a year of storage, the moisture content (%) in fish pickle decreased from 58.20 0.194 to 48.53 0.345 and from 58.67 0.180 to 43.90 0.245 at refrigeration and freezing storage, respectively. Similarly, over the course of storage, the protein content (%) decreased from 22.35 0.385 to 18.85 0.097 and from 22.70 0.141 to 14.69 0.137, respectively. Lipid content (%) increased for the first five months of storage before gradually declining at refrigeration temperature, whereas it gradually increased during the entire storage period at frozen temperature. In the refrigerator and freezer compartments, the ash content (%) increased from 4.08 0.043 to 7.38 0.081 and 4.83 0.130 to 9.18 0.085, respectively.

5.2. Amino acid profile

The shrimp paste obtained from the three distinct sampling regions had varying levels of amino acids (Table 2). The amino acids alanine, arginine, aspartate, cystine, glutamine, glycine, histidine, isoleucine, leusine, lysine, metathione, phenylalanine, proline, serine, threonine, tyrosine, and valine were the most prevalent in three different shrimp paste preparations. The three shrimp paste products have alanine (24260.30-12157.86 g/g), arginine (115774.45-47938.61 g/g), and isoleucine (63805.56-14385.74 g/g) as their main amino acids. Major amino acids such as glutamic acid (324.78-226.759 g/g), lysine (164.26-70.449 g/g), and leucine (179.07-151.539 g/g) (Table 2) varied in comparison to the glutamic acid content in shrimp paste and fish sauce, which ranged from 3.1-7.0% (w/w) and 0.5-1.5% (w/v), respectively (Kim et al., (2014). According to our investigation, SP4 has the highest levels of glutamic acid, followed by KS1 and SP2. High levels of glutamic acid were a sign of a robust umami flavor.

Arginine, aspartate, isoleucine, lysine, proline, serine, threonine, and valine were found to be associated to taste and flavor Kim and Rhee (1990). While arginine, histidine, isoleucine, leucine, methionine, phenylalanine, and tryptophan are connected to bitter taste, certain amino acids, such as alanine, glycine, serine, threonine, and valine, were related to sweet flavor. Furthermore, -aminobutyric acid (GABA) was found in all samples (0.7-2.6 g/g) of the sample. Many different types of fermented goods have been shown to have health benefits when GABA is present in the dish (Dhakal et al., 2012).

5.3. Microbiological analysis

Table 2 provides the microbiological analysis' findings. Fish that had been salted and spontaneously fermented included a variety of microbes, including yeasts, molds, halophile, aerobic, and staphylococcal bacteria. *Vibrio cholera, Vibrio vulnificus, Vibrio parahaemolyticus, E. coli, Salmonella,* and *Shigella* were tested against the microbiological load of Nga-pi. *Vibrio vulnificus* and *Vibrio parahaemolyticus* were found on the samples taken from the Teknaf, Mahesh khali, Cox's Bazar, and Rangamati regions. No *Vibrio cholera, E. coli, Salmonella, Shigella*, or other pathogens were discovered in any of the samples that were taken from various locations. Results from this study differ from several earlier reports that had been

looked into. According to Thapa et al. (2004), Staphylococci and Bacilli were the two most common bacterial species found in North-East Indian ngari, hentak, and Tungtag fermented fish products. Similar findings were made by Anihouvi et al. (2006, 2007), who noted that Staphylococci and Bacilli were the predominant bacterial groups recovered from the traditionally processed fermented fish product known as lanhouin and cassava fish in the Republic of Benin. On the other hand, according to Koffi-Nevry et al. (2011), lactic acid bacteria predominated in the fermentation of agjuevan, a fermented fish from the Ivory Coast.

CHAPTER-6: CONCLUSIONS

The components of the several shrimp paste samples used in this experiment varied. The proximate composition characteristics, value of amino acids, and SPC of bacteria have been examined in Nga-pi samples that have been generated locally. The metrics differed among the samples of traditionally manufactured Nga-pi from Teknaf, Cox's Bazar, Mahesh khali, and Rangamati region that were gathered from various stakeholders. They had a significant amount of protein, which suggested that they were good suppliers of proteins and amino acids. Additionally, these goods would remain stable for a long time because to the low Aw and low histamine concentration but high salt content. *Vibrio vulnificus* and *Vibrio parahaemolyticus* spp. were the main pathogens. There were no harmful microorganisms found. The current study draws the conclusion that the nutritional value of fermented food products (Nga-pi) differs in different regions depending on the raw materials used and the method of preparation based on the data collected. The study also demonstrated that Nga-pi made in Bangladesh's south-east has no pathogenic germs, making it safe for commerce and consumption. Both the future of production and the fundamental scientific information about the product will be established by this investigation.

CHAPTER 7: RECOMMENDATIONS AND FUTURE PERSPECTIVES

In the world, billions of people from various cultures and ethnicities create and consume about 5000 different major and minor unlisted forms of fermented foods and beverages. However, consumption of some lesser-known and uncommon ethnic, fermented foods is declining as a result of lifestyle modifications, a shift away from traditional foods in favor of fast food and commercial foods, as well as some locations' changing climates, which have a significant impact on traditional culinary practices.

Many individuals in Bangladesh live in poverty and experience chronic malnutrition because they cannot buy a sufficient supply of food (Islam, 1998). One of Bangladesh's most critical issues is protein deficiency (Hossain, 1991). People rely on low-cost techniques of processing fishing goods as a result. The taste and affordability of traditional goods make them more popular with consumers than larger, more valuable commercial fish used as table fish.

Therefore, regarding this area, some future research attempts may include the followings:

i. As Nga-pi was not showed effective result in enteric bacteria and others salt intolerant bacteria, further research might be observed for salt tolerant bacteria.
ii. Research can also be carried out to see the presence of heavy metals in Nga-pi.

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PHOTO GALLERY

Plate-11: Nga-pi preparation

Plate-12: Media preparation

Plate-13: Bacteria culture

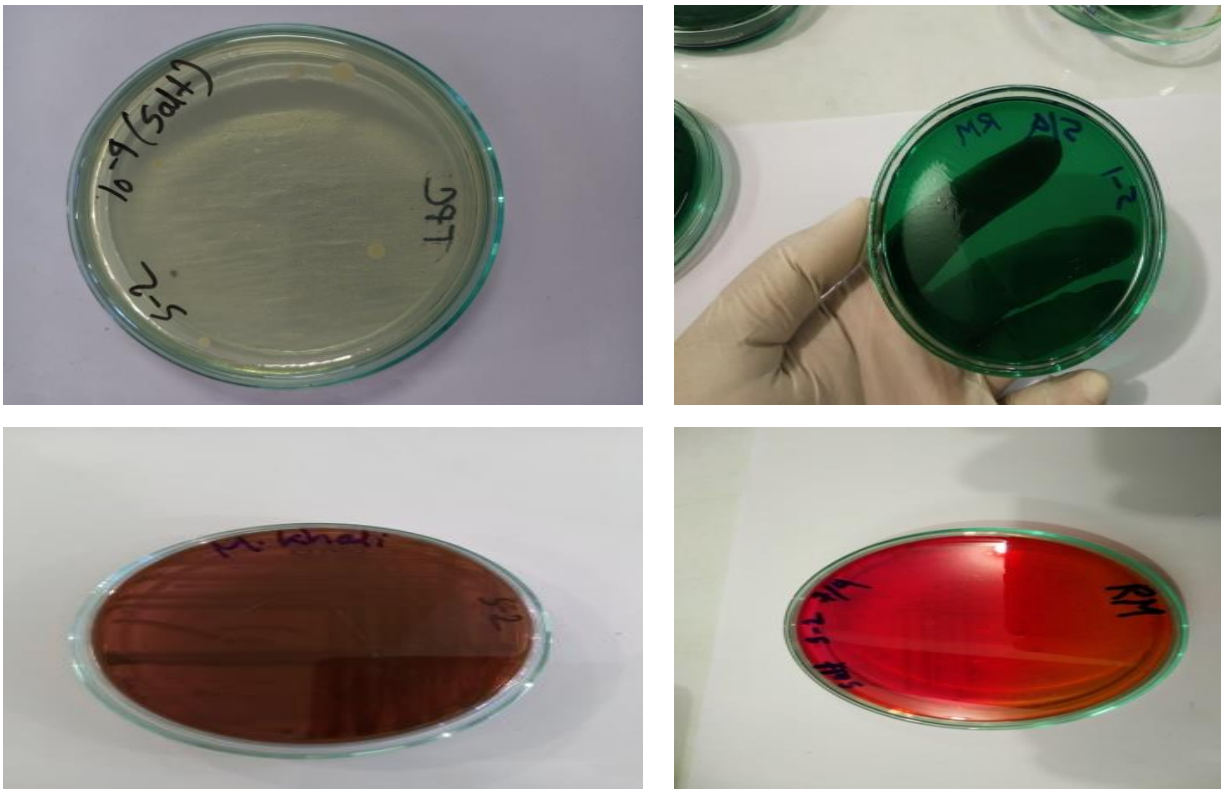

Plate-14: Identification of bacteria

Plate-15: Protein and Lipid determination

Plate-16: Ash and Moisture determination

APPENDICS

Appendix 1: Bacterial load of Nga-pi samples collected from different regions of Bangladesh ("Cox's", "Mhs", "Tek" represents the Cox's Bazar, Mahesh khali and Teknaf)

Samples	Bacterial load (CFU/g)			Bacterial load	Standard deviation
	Replication-1	Replication-2	Replication-3		
Cox's	4.92×10^3	5.1×10^3	4.4×10^3	4.81×10^3	0.30×10^3
Tek	1.585×10^3	0.49×10^3	0.534×10^3	0.87×10^3	0.51×10^3
Mhs(1)	1.32×10^3	1.37×10^3	1.31×10^3	1.33×10^3	0.03×10^3
Mhs(2)	2.4×10^3	1.9×10^3	2.2×10^3	2.17×10^3	0.21×10^3
Rang(1)	11×10^3	13×10^3	15×10^3	13.00×10^3	1.63×10^3
Rang(2)	19×10^3	14×10^3	16×10^3	16.33×10^3	2.05×10^3
Rang(3)	18×10^3	15×10^3	12×10^3	15.00×10^3	2.45×10^3

Appendix 2: Proximate composition (protein) of Nga-pi samples from different regions of Bangladesh ("Cox's", "Mhs", "Tek", "R", "SD", "SE" represents the Cox's Bazar, Mahesh khali and Teknaf, Replication, Standard Deviation, Standard Error)

Treatment	R	Final	Initial	Difference	Sample Weight	% of N	% protein	Average	SD	SE
Cox's	R-1	39.7	33.2	6.5	0.33	5.5	34.47	35.5	0.93	0.66
	R-2	47	39.7	7.3	0.35	5.7	36.09			
	R-3	9.3	2.6	6.7	0.33	5.7	36.08			

Tek	R-1	11.4	2.1	9.3	0.34	7.5	47.31	48.4	1.2	0.82
	R-2	24.1	11.4	12.7	0.33	10.9	48.17			
	R-3	33.2	24.1	9.1	0.32	7.9	49.61			
Mhs (1)	R-1	17.5	9.3	8.2	0.31	7.5	46.89	47.36	0.78	0.55
	R-2	25.6	17.5	8.1	0.30	7.5	46.94			
	R-3	33.9	25.6	8.3	0.30	7.7	48.26			
Mhs (2)	R-1	42.9	33.9	9	0.36	7.1	44.37	45.86	1.34	0.94
	R-2	10.5	1.7	8.8	0.33	7.4	46.25			
	R-3	19.3	10.5	8.8	0.33	7.5	46.95			
Rang (1)	R-1	9.5	2.3	7.2	0.35	5.8	36	37.66	1.46	1.03
	R-2	8.5	1.2	7.3	0.33	6.2	38.71			
	R-3	15.5	8.5	7	0.32	6.1	38.28			
Rang (2)	R-1	22.6	15.5	7.1	0.32	6.2	38.83	38.95	0.92	0.65
	R	30	22.6	7.4	0.34	6.1	38.03			

	-2									
	R -3	37.3	30	7.3	0.32	6.4	39.92			
Rang (3)	R -1	44.5	37.3	7.2	0.3	6.7	42	41.04	1.0 5	0.7 4
	R -2	51.8	44.5	7.3	0.31	6.5	41.21			
	R -3	41.3	34	7.3	0.32	6.4	39.92			

Appendix 3: Proximate composition (lipid) of Nga-pi samples from different regions of Bangladesh ("Cox's", "Mhs", "Tek", "R", "SD", "SE" represents the Cox's Bazar, Mahesh khali and Teknaf, Replication, Standard Deviation, Standard Error)

Treatment	R	Initial	Final	Sample weight	Net	Average
Cox's	R-1	92.48	92.56	2.01	4.27	4.44
Cox's	R-2	92.66	92.75	2.02	4.60	
Tek	R-1	91.58	91.68	2.03	5.24	5.60
Tek	R-2	92.42	92.54	2.03	5.96	
Mhs (1)	R-1	92.48	92.65	2.01	8.53	6.63
Mhs (1)	R-2	92.66	92.75	2.01	4.72	
Mhs (2)	R-1	91.58	91.67	1.98	4.52	6.49
Mhs (2)	R-2	92.42	92.60	2.10	8.46	
Rang (1)	R-1	92.48	92.57	2.07	4.51	4.71

Rang (1)	R-2	92.66	92.76	2.04	4.92	
Rang (2)	R-1	91.58	91.66	2	4.17	4.17
Rang (3)	R-1	92.42	92.5	2.04	3.92	3.92

Appendix 4: Proximate composition (moisture) of Nga-pi samples from different regions of Bangladesh ("Cox's", "Mhs", "Tek", "R", "SD", "SE" represents the Cox's Bazar, Mahesh khali and Teknaf, Replication, Standard Deviation, Standard Error)

Treatment	R	Final	Initial	Sample weight	Net	Average	SD	SE
Cox	R-1	24.93	23.98	3.1	30.52	32.48	1.98	1.40
	R-2	25.12	24.05	3.1	34.48			
	R-3	25.22	24.21	3.11	32.44			
Tek	R-1	25.39	24.71	2.98	22.75	23.03	2.26	1.60
	R-2	25.35	24.57	3.08	25.42			
	R-3	25.02	24.36	3.15	20.92			
Mhs (2)	R-1	25.597	24.85	3.08	24.25	27.58	2.90	2.05
	R-2	25.00	24.08	3.13	29.49			
	R-3	28.87	27.96	3.14	29.01			
Mhs (1)	R-1	24.86	23.92	3.14	30.06	26.74	2.88	2.04
	R-2	26.05	25.26	3.13	25.21			

	R-3	27.21	26.45	3.03	24.95			
Rang (1)	R-1	25.03	23.87	2.98	38.97	43.80	4.20	2.97
	R-2	26.50	25.00	3.21	46.63			
	R-3	20.30	18.95	2.95	45.78			
Rang (2)	R-1	24.73	23.04	3.52	48.08	47.86	0.57	0.41
	R-2	24.86	23.43	2.97	48.28			
	R-3	25.74	24.28	3.08	47.21			
Rang(3)	R-1	24.35	22.84	2.98	50.57	51.20	0.71	0.50
	R-2	24.66	23.07	3.07	51.96			
	R-3	26.78	25.17	3.15	51.05			

Appendix 5: Proximate composition (ash) of Nga-pi samples from different regions of Bangladesh ("Cox's", "Mhs", "Tek", "R", "SD", "SE" represents the Cox's Bazar, Mahesh khali and Teknaf, Replication, Standard Deviation, Standard Error)

	Treatment	Initial	Final	Sample Weight	Net	Average	SD	SE
Cox	R-1	23.03	23.55	2.98	17.38	17.09	0.68	0.48
	R-2	28.46	28.96	3.09	16.31			
	R-3	22.79	23.34	3.13	17.57			
Tek	R-1	31.47	31.89	3.22	13.17	13.10	0.06	0.04
	R-2	27.65	28.07	3.21	13.05			

	R-3	23.26	23.66	3.08	13.08			
Mhs (1)	R-1	28.31	28.81	3.04	16.48	16.84	0.68	0.48
	R-2	25.16	25.68	3.15	16.41			
	R-3	24.01	24.55	3.06	17.61			
Mhs (2)	R-1	25.04	25.62	3.00	19.23	18.50	0.81	0.57
	R-2	27.90	28.44	3.08	17.63			
	R-3	25.15	25.72	3.08	18.64			
Rang (1)	R-1	25.22	25.71	3.20	15.18	15.18	0.02	0.01
	R-2	23.73	24.19	3.01	15.21			
	R-3	23.86	24.32	3.01	15.17			
Rang (2)	R-1	23.24	23.71	3.04	15.58	15.65	0.07	0.05
	R-2	23.77	24.25	3.00	15.72			
	R-3	23.39	23.86	3.05	15.64			
Rang(3)	R-1	24.69	25.13	3.02	14.49	14.36	0.13	0.09
	R-2	23.31	23.72	2.94	14.24			
	R-3	23.74	24.18	3.07	14.36			

BIOGRAPHY

This is Hasnain Mostari Sahi, daughter of Aminul Hoq and Roksana Begum, Mahesh khali, Cox's Bazar, Bangladesh. She passed the Secondary School Certificate Examination in 2012 from Yunus khali Nasir Uddin High School, followed by the Higher Secondary Certificate Examination in 2014 from Hazera Taju University College. She graduated in 2019 with a B. Sc. in Fisheries (Hons.) degree from the Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh. She is now a candidate for the degree of MS under the Department of Fishing and Post-Harvest Technology, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.