Chapter 5

Discussion

Napham is a traditional fermented fish prepared from a mixture of small fishes. This fermented fish is the flavor and taste enhancing delicacy of the Bodo tribe of Assam. It is also an inexpensive method of fish preservation to overcome the perishable nature of fish. At a community level, *napham* is prepared at home and not on a commercial scale like *shidol, matka shidol,* and *ngari,* the well-known fermented fish products of North East India. However, a few enterprising people have started to prepare *napham* for commercial purposes. The rich water reservoirs in Kokrajhar district provide a suitable ecosystem and are the natural breeding ground for many fishes. During the interview, the informants informed that they collected fish from nearby water sources like beel, small streams, etc for the preparation of *napham*. The local populace exploits the seasonal availability of fish and preserves it for a lean period through this technique.

Napham is a 'fermented fish paste' where fishes is reduced to a paste form. Some of the fermented fish pastes found across different cultures and countries are *bagoong, petis* & *balao-balao* of the Philippines, *ngapi* of Burma, *padaek* of Laos, *prahok* of Indonesia, *trassi* of Indonesia, *numsing*, *hentak* & *tungtap* of India.

Eighteen fishes belonging to 10 families and 13 genera were recorded during the documentation. The plant materials commonly used as reported by informants are the stem of *Colocassia esculanta* (L.) Schott, fruit of Ash gourd, (Loc name: khumbra; Scientific name: *Benincasa hispida* (Thunb.) Cogn., leaf of mwitha (Scientific name*: Hibiscus sabdariffa* L.) and papaya fruit. In Thai fermented fish processing, enzymes like papain and other animal enzymes accelerate the fermentation (Oshima & Giri, 2014). The purpose of using plant materials in *napham* may be to add moisture and enzymes to fasten the process of fermentation.

The chemical composition of fermented fish paste *bagoong,* prepared from *Stolephorus* sp., *Sardinella fimbriata*, and *Decapterus* sp., was reported by Sanchez, 1999. This fish paste (*bagoong*) has total Nitrogen-2.26%, Formol Nitrogen-1.31%, Ammonia nitrogen 0.31%, Salt (NaCl) 26.1%, pH 5.85% and lactic acid 0.84%. The chemical composition of the fermented fish paste *ngapi*, produced from different fish and shrimp was reported by Tyn (1993). It consist of moisture 7.6%,

crude protein 41.9 %, fat 8.4 %, NaCl 37.95 %, free ammonia 0.17 %, and Ash 3.45 %. *Hentak* consisted of moisture content of 20.9%, crude protein of 52.86%, crude fat 17.10 %, ash content of 8.10%, free fatty acid (as oleic acid) of 39.6%, and pH 6.6 (Sarojinalili and Vishwanath, 1995). In *trasi* (shrimp paste), moisture content was reported as 12.13 %, crude protein as 25.89 %, fat as 3.8 %, ash content as 32-46 %, pH at 6 (Putro,1993). In *ngari* Sarojinalili and Suchitra (2009) reported moisture content of 36.03%, total lipid 13.34 %, ash content 5.49 %, and pH 6.74 %.

In *namsing*, pH, ash content, protein, mineral, and microbial diversity was studied from day 1st of fermentation to the 28th day of fermentation by Chowdhury et al., 2019. The pH increased from 6.05 to 7.00 from the first day to the 28th day of fermentation. Ash content reduced from 13.05 to 2.24% during the 28th day of fermentation and the result showed an increase in mineral contents of phosphorous, calcium, manganese, zinc, and iron.

The proximate composition of *napham* consisted of moisture content 38.8%, crude protein of 30.3% and crude fat of 24.4%. The pH of *napham* was found in alkaline range of 7.03. The dynamics of pH, ash content, protein content and moisture content were studied in four samples of differnt months. The statistical analysis through Kruskell Wallis test shows a significant difference in moisture content, ash content and pH amongst the samples of four different fermentation times. According to statistical analysis, no significant difference was not observed in protein content from the given sample size of the experiment. This may indicate that static protein content is present in *napham* after the degradation that takes place between the preliminary stage and the first month of fermentation. The increase in the water content was found in the two months old sample and this may be because of the release of volatile molecules during the process of fermentation (Angoo et al., 2015). It was also reported by them that moisture content depended on the use of packaging material. In the research, the moisture content in samples prepared in hollow bamboo stems were found less as compared to those prepared in glass bottles. In raw sample, the moisture content was 25.5% which subsequently increased after the addition of Colocassia stem. Hence, it can be inferred that plant materials in *napham* are used to give the required moisture content and enzymes to facilitate the microbial activity in fermentation. The pH of *napham* increases in the older fermentation stage due to accumulation of ammonia produced by proteolytic activity of fermenting

microorganisms. The decrease in ash content may be because of degradation of flesh and bones in *napham* which is also reported in other similar studies (Chowdhury et al., 2019). The difference in the physical and chemical composition in different time of fermentation is reported from the Thai fermented fish sauce and other fermented fish pastes (Oshima & Giri, 2014; Chaudhury et al., 2019). Chaudhury et al., 2019 reported this change in physical and chemical composition in fermented fish *namsing*. The fermentation process is unregulated and random in traditional fermented fish products, and since the products are frequently processed under local climatic conditions, sensory characteristics and consistency can vary (Oshima & Giri, 2014).

Minerals are inorganic substances that are essential for the normal life process. The plants absorb minerals from the soil. The animals get minerals from plants directly or from other animals indirectly. The body needs minerals called essential minerals. Essential minerals are sometimes classified as major minerals (macro-minerals) and trace minerals (micro-minerals). These two groups of minerals are equally important, but trace minerals are required in smaller amounts than major minerals (Soetan et al., 2010).

Macro minerals: - Sodium, Magnesium and Sulphur, Calcium, Phosphorus, and Chlorine

1. Micro minerals: - Iron, Zinc, Copper, Potassium, Iodine, Manganese, molybdenum, Fluorine, Chromium, and Selenium
2. Ultra-trace elements: - Boron, Silicon, Nickel, Vanadium, and Cobalt

In fermented fish *fessiekh* of Sudan, the mineral composition consisted of Phosphorous (34.6 µg/g), Manganese (0.162 µg/g), Barium-8.4%, Iron-4.4%, Zinc-3.8%, Alluminium-3.3%, Boron 1.5%, and Copper & Cadmium in trace amounts (Mohamed & Hamadani, 2013).

Smriga et al., 2009, reported that inorganic compounds in *garum* samples consisted of Sodium 16.50±1.19 mmol/100mg, Magnesium 34.33±3.68 mmol/100mg, Phosphorus 83.17±1.62 mmol/100mg, Calcium 213.33±6.08 mmol/100mg. Irianto& Irianto (1998) reported Calcium 174 mg, Phosphorus 316 mg, and Iron 3.1 mg in p*edah,* a fermented fish product of Indonesia.

The present study reports the mineral composition of fermented sample of three to four months. This report shows that *napham* is a good source of mineral compositions like calcium, sodium, iron, zinc, copper, potassium, manganese, molybdenum, chromium, nickel, and cobalt. It consists of macro, micro and trace

elements essential for human diet. In *tungtap* Ca 25.8 mg/100g, 0.9 mg/100g Fe, 1.6 mg/100g Mg, 0.8 mg/100g Mn and 2.4 mg/100g Zn were recorded. In *ngari* 41.7 mg/100g Ca, 0.9 mg/100g Fe, 0.8 mg/100g Mg, 0.6 mg/100g Mn and 3.1 mg/100g Zn were recorded (Tamang, 2009). Tamang (2009) determined the mineral contents of seven fermented fish of North East India and found that they are rich in calcium, zinc, magnesium, and manganese. In *suka-ko-macha* 38.7 mg/100g Ca, Fe 0.8mg/100g, Mn 1mg/100g and 5.2 mg/100g were found. In *gnuchi* 37 mg/100g Ca, 0.8mg/100g Fe, 5.0 mg/100g Mg and 1.0 mg/100g Mn were recorded.

The fermentation of fish may result in the breakdown of organic compounds into simple compounds like amino acids, peptides, and other nitrogenous compounds. Many amino acids are released during the fermentation process by the action of microbial enzymes (Feng, 2021). For the analysis of amino acids in traditionally fermented fish three stages were selected-one raw sample and two fermented samples. Significant difference was observed in the composition of amino acids in three different stages of fermentation. It was observe that there was an increase in amino acid composition in fermented samples as compared to the raw sample. The total amino acid in the raw material was 640.14pg/mg, whereas in fermented products F1 and F2 it was 1471pg/mg and 1027.72pg/mg, respectively. Koesoemawardani et al., (2018) also reported the increase in amino acids in fermented samples *rusip*. In anchovy fermented fish paste, 16 amino acids were reported by Angoo (2015). In *rusip,* a fermented fish from Indonesia*,* the dominant amino acids were glutamic and aspartic. Other amino acids found were alanine, arginine, glycine, histidine, methionine, serine, threonine, tyrosine, and valine (Koesoemawardani et al., 2018).

Essential and non- essential amino acids were detected in *napham*. In sample F1, the major amino acids detected were aspartic acid, asparagine, and serine, and in sample F2 it was aspartic acid, glutamic acid, glutamine, alanine, and lysine. Threonine, serine, valine, isoleucine, leucine, tryptophan and lysine were the essential amino acids detected during this study.

Koesoemawardani et al., (2018) also reported aspartic acid and glutamic acid as the major amino acid in fermented fish *rusip*. In fermented fish prepared from *Stolephorus* sp., the mojor essential amino acids present were L-Lysine HCL and Leucine, and the major non-essential amino acids were aspartic acid and glutamic acid (Anggo et al., 2014).

Jiang et al. (2007) reported that glutamic acid gave the flavor and aroma to *yu-lu*, a fermented fish product from China. Glutamic acid is a relatively stable amino acid in the absence of secondary decomposition during fermentation and gives a characteristic "umami" taste to fermented fish *yu-lu*. Glutamine was detected in both F1 and F2 samples and may be responsible to give the characteristic flavor to *napham*.

The fatty acids majorly found in *napham* were: Palmitic acid (16.01%), Linoleic-acid (10.41%), Alpha-Linoleic acid (9.53%), and DHA (8.03%). The study shows the presence of essential fatty acids like monounsaturated (MUFA), polyunsaturated fatty acids (PUFA), Docosahexaenoic acid (DHA), Omega 6 fatty acids, and Omega 3 fatty acids in *napham.* MUFAs are fatty acids consisting of a single bond, PUFA of two or more double bonds, and saturated fatty acids of double bonds (Schwingshackl & Hoffmann, 2012). The MUFAs present in *napham* were Oliec acid, Z-7-Hexadecenoic acid, 11-Hexadecenoic acid and 6-Hexadecenoic acid.

The PUFAs found in *napham* were Arachidonic acid, alpha-Linolenic acid, gamma-Linolenic acid, alpha-Linoleic acid, Linoleic acid, 5, 8, 11, 14, 17-Eicosapentaenoic acid and 5, 8, 11, 14- Eicosatetraenoic acid.

A significant difference is found in the composition of fatty acids of three different stages of fermentation. 4,7,10,13,16,19-Docosahexaenoic acid (DHA), Margaric acid, Palmitic acid,11-Hexadecenoic acid, Myristic acid, Linoleic-acid Alpha-Linoleic acid, Gamma-Linolenic acid and Stearic Acid increased significantly in fermented products. Fatty acids, 11-Eicosenoic acid Palmitic acid, Alpha-Linoleic acid and 11-Eicosenoic acid decreased in F2. Stearic Acid and 9-Octadecenoic acid were detected in higher percentage in raw sample. The fatty acids reported in *rusip* were Acid Palmitate (C16; 0), Acid Myristate (C14:0), cis-4, 7, 10, 13, 16 Acid, 19-Docosahexaenoate (C22:6n3), and stearic acid (C18:0). Docosahexaenoic acid gave *rusip* distinctive aroma (Koesoemawardani, 2018). Majumdar et al. (2015) reported the dominant fatty acids in *ngari* were Palmitic Acid (C16:0), Vaccenic Acid (C18:1n-7), and Oleic Acid. In *hentak,* they reported Stearic Acid (C18:0), Palmitoleic Acid (C16:1n-5), and Oleic Acid.

This study reports that *napham* consisted of essential fatty acids like MUFA, PUFA, DHA, omega-3- fatty acids, and omega-6- fatty acids in two different stages of spontenous fermentation. The decrease or increase in composition of fatty acids may

be due to the lipid oxidation that takes place during fermentation of fish products (Feng et al., 2021). Anggo et al., 2015 analyzed fatty acids and amino acids in fermented fish paste prepared from dried anchovy fish. He reported that on the 8th and 32nd day of fermentation Palmitic Acid, Oleic Acid, Stearic Acid, and Docosahexaenoic acid (DHA) were present in fish paste. He also reported that quantitative and qualitative parameters were different in both stages of fermentation and the change was not similar for all the fatty acids. According to his observation some of the fatty acids decreased in amount while some others increased.

Sixty-two volatile compounds were reported in Chinese traditional fermented shrimp, including alcohols, aldehydes, ketones, ethers, acids, esters, hydrocarbons, pyrazines, phenols, and other compounds (Fan et al., 2017). The study found that the pungent and rancid odor resulted from propanoic acid, butanoic acid, furans, and 2-hydroxy-3-pentanone in shrimp paste. The volatile compounds of fatty acids like ketones, aldehydes esters, and free fatty acids increased by the end of fermentation (Oshima & Giri, 2014). In the present study, twenty-five odd carbon number fatty acids and volatile compounds were detected in *napham*. These compounds may be the intermediate compounds during the fermentation process. Feng et al. (2021) reported that lipolysis and lipid oxidation generate flavor compounds in fermented fish product. Unsaturated fatty acids containing pentadiene like Linoleic acid and Arachidonic are the compounds that undergo enzymatic oxidation and transform into conjugated unsaturated fatty acids that forms volatile flavors in fermented fish (Feng et al., 2021).

In *napham,* the volatile compound Ritalin was detected. Ritalin is one of the most commonly known central nervous system (CNS) stimulants used in the treatment of attention deficit hyperactivity disorder (ADHD) and narcolepsy (Markowitz et al., 2003).

Microorganisms have a very significant role to play in the process of fermentation of fish. The fish provide a micro-environment to several micro-organisms. At different fermentation stages, the physical and chemical attributes in the micro-environment change, and, as a result, the dynamics, growth, and survival of micro-organisms also differ. An ecological interaction involving cell-to-cell communication occurs in the micro-environment of the raw fish substrate. Therefore, in any fermented fish product, an ecological approach is essential to understand the microbiological processes involved in the ripening of fish products (Giraffa, 2004).

In *namsing*, the mesophilic bacterial count increased from 3.44 log CFU/g on the first day to 8.2 logs CFU/g on the 7th day of fermentation and then further decreased to 3.36 log CFU/g on the 28th day. Yeasts and molds were not detected during the whole experimental stages of fermentation. Lactic acid bacteria appeared on the 21st day. Coliform bacteria were detected in the range of 2–2.8 log CFU/g from the first day to the end of the experiment (Chowdhury, 2019).

In the present study, the total live & aerobic bacteria, proteolytic bacteria, halophilic bacteria, and fungi were detected in the raw sample. These bacterial communities were present as natural microbial flora in the raw sample. The microbial load of total viable aerobic bacteria increased in first and second month but decreased in sixth and twefth month samples. The proteolytic bacteria increased in the early stages of fermentation and then gradually reduced in sixth and twefth month. In all the stages, the fungal count was low. The Lactic Acid Bacteria (LAB) was not detected in raw and the twelfth-month-old fermented samples. It was only detected in first, second, and sixth-month-old samples. Coliform bacteria were detected in the first-month and second-month-old samples. Their number slowly decreased to an undetectable level in the twelve-month-old sample.

A similar study was performed in *budu* where the total microbial load decreased gradually from 6.13 ± 0.01 to 3.45 ± 0.13 log CFU g–1 from the first month to the twelfth month of fermentation. A gradual reduction in the microbial count was observed in total plate count agar at the later fermentation stage in *budu*. The growth of halophilic bacteria decreased from the first month to the fifth month of fermentation and then increased slightly in the sixth month. The count of Streptococci and Lactococci was low in the early fermentation stages. It increased in the sixth month and twelfth-month samples in *budu* (Sims et al., 2015). .

The statistical analysis by Kruskell Wallis tests shows significant difference in the microbial load amongst different time of fermentation in *napham*. The bacterial count increases in the first and second months of fermentation, and get decreased in sixth-month and twelfth-month old samples. This trend of microbial dynamics in *napham* and other fermented fish like *budu* and *namsing* at different fermentation stages may be attributed to the difference in physical and chemical composition in the micro-environment.

The classical plate culture, enumeration, and isolation of pure culture in the study of food microbiomes are gradually shifting towards molecular-based culture-

independent methods. Several authors reported the application of molecular approaches to describe the complex interplay of microbial species during fish fermentation. The culture-dependent techniques have certain limitations, and this approach underestimates the large group of microbial populations that is not cultivable. The objective of using a metagenomic study was to fulfill the second objective of the study which is an analysis of microbial diversity in *napham*. The metagenomic study revealed the taxonomic composition and identification of microbial diversity in *napham*, the characterization of relative abundances of taxa, and understands the gene heterogeneity (Durazzi et al., 2021). To achieve this, two technique strategies WGS and 16S Metagenome sequencing were used. 16S Metagenome technique aims for the sequencing of 16SrRNA of the targeted gene in hypervariable regions (Scholz et al., 2012) and estimates the bacterial diversity of the sample. The output sequencing of 16S metagenomic consists of a set of clusters of nearly identical sequences called OTU which give significant information on the community diversity of bacteria, richness, and evenness (Choi et al., 2015). The scientific reports however state that sometimes biasness is seen as a result of the choice of primers used to amplify 16S rRNA, and the other challenging task for 16S metagenomics is to reveal the potential novel functional contribution of the community (Chistoserdova et al., 2009). WGS metagenomics addresses this issue, and long DNA sequences are sequenced in this method (Durazzi et al., 2021). WGS metagenomics aims to sequence all the genomes existing in a sample to analyse the biodiversity and the functional capabilities of community studied.

Investigation of bacterial and archeal communities by pyrosequencing was done in different types of *jeotgal*, a salted fermented fish of Korea (Roh et al., 2010). Depending on the type of *jeotgal* different bacterial and archeal communities were detected that consisted of both halophilic and mesophilic groups.

Halalkalicoccus and *Halorumbrum* were the halophilic Archaea found in most *jeotgals*. *Lactobacillus* and *Weissella* were also dominant bacterial groups in the majority of *jeotgals.* Gammaproteobacteria was dominant in *jeotgal* prepared from shellfish.

The microbial communities of *shikae* were also analyzed by pyrosequencing (Koo et al., 2016), and the investigation revealed *Lactobacillus sakei* was the dominant bacteriocin-producing bacteria in *shikae*. *Lactobacillus graminis*, *L. fructivorans*, *L. alimentarius*, and *Weissella thailandensis* were also observed *in*

shikae. The study showed that the addition of certain additives in *shikae* increased and decreased the population of *Lactobacillus* and *Weissella.*

The metagenomic studies show that the taxonomic assignment of *napham* samples was dominated by Phylum Firmicutes, Actinobacteria, and Proteobacteria. Similar metagenomic approach was used to investigate the microbiota of *hongeo*, a fermented skate (*Raja kenojei*) of Korea. The study showed that taxonomical assignment in *hongeo* samples was dominated by the Phylum Proteobacteria, Firmicutes, Fusobacteria, Bacteroidetes, and unclassified bacteria. The WGS metagenome study shows the presence of Ascomycota as this technique targets the sequencing of bacteria, fungi, and viruses.

Bacilli was the most dominant community in all the samples of *napham*. Other noteworthy communities of *napham* observed in all the samples were Actinobacteria, Gammaproteobacteria, Clostridia, Thermoleophilia, Betaproteobacteria, Deltaproteobacteria, and Negativicutes. In *hangeo*, the active bacterial community consisted mostly of Bacilli (39.10%), Gammaproteobacteria (35.3%), and Clostridia (10.1%) groups (Zhao & Eun, 2020).

Lactobacillales, Bacillales, Micrococcales, Enterobacterales, Corynebacteriales, Eurotiales, Clostridiales, Streptomycetales, Rhizobiales and Saccharomycetales were dominant communities found in S1 and S2. In S3 and S4, the dominant community orders detected were Lactobacillales, Bacillales, Clostridiales, Rhizobiales, Burkholderials, Rhodospirillales, and Micrococcales. Because of the alkaline fermentation of *hongeo* Lactobacillales and Clostridia were found in their microbiomes (Zhao & Eun, 2020). The microbiomes of *napham* also show the dominance of Lactobacilliales and Bacilliales. Clostridiales were also detected in all the samples.

The common and dominant communities at the family level found in all the samples of *napham* were Staphylococaceae, Enterococaceae, Lactobacillaceae, Bacillaceae, Leuconostocaceae Carnobacteriaceae, and Streptococcaceae. Aspergillaceae was the fungal family detected in S1 and S2. S3 was mostly represented by Staphylococaceae and S4 by Enterobacteriaceae. A few families from Archaea were detected in 16S metagenomic data which indicates their relevant role in *napham* fermentation. In *jeotgal* also different bacterial and archeal communities were detected that consisted of both halophilic and mesophilic groups (Roh et al., 2010).

LAB genera including *Lactococcus*, *Streptococcus*, *Weissella*, and *Pediococcus* were not detected in all *hongeo* samples. In addition, some alkaliphilic marine LAB belonging to the genera *Marinilactibacillus* and *Jeotgalibaca* have been detected in hongeo samples (Zhao & Eun, 2020). There is far more diversity at the genus level in all the *napham* samples. Similar diversity at the genus level was observed in *hangeo* samples (Zhao & Eun, 2020). Common genera present in all the *napham* samples were *Bacillus*, *Enterococcus*, *Clostridium*, *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Pedicoccus*, *Pisciglobus*, *Staphylococcus*, *Streptococcus*, *Streptomyces*, *Weissella* and *Yaniella.* Roh et al. (2010) in their investigation of *jeotgal* found the use of different raw materials gave variations in microbial composition. The difference in the composition of dominant bacteria in all the samples may be due to raw materials used, method of processing and the fermentation environments.

The study revealed that *Staphylococcus* is dominant genus in all *napham* microbiomes. According to the scientific investigations, *Staphylococcus* was dominantly found in the fermented fish products of the world due to its lipolytic and proteolytic activity (Cachaldora et al., 2013). It was found that in a microbial succession study of fish sauce for up to 8 months, Staphylococcus was increased in the first four weeks (Fukui, 2012).

Staphylococcus was dominant the genus in the microbiomes of all samples S1, S2, S3, and S4. The community structure in the WGS study showed even distribution, but 16 S metagenomic revealed a biased trend with 93.16 % dominance of *Staphylococcus* in sample S3, and 96.58% dominance of *Enterococcus* in S4. This variation was also seen in *hangeo* samples (Zhao & Eun, 2020) even after use of similar techniques to detect microbial communities. Besides *Staphylococcus* the dominant genera found in S1 were *Oceanobacillus*, *Virgibacillus*, *Brevibacterium*, *Pediococcus*, *Enterococcus*, *Yaniella, Bacillus*, *Carnobacterium* and *Lactobacillus*. In S2, the dominant genera were *Enterococcus*, *Lactobacillus*, *Oceanobacillus*, *Aspergillus*, *Pediococcus*, *Lactococcus*, *Tetragenococcus*, *Weissella*, and *Vagococcus*. In S3, the dominant genera were *Enterococcus*, *Yaniella*, *Lentibacillus*, *Lactobacillus* and *Pseudogracilibacillus.* In S4, the other dominant genera were *Lactococcus*, *Staphylococcus*, *Weissella* and *Lactobacillus*. *Bacillus*, *Staphylococcus*, *Enterococcus*, *Pseudomonas*, *Clostridium*, *Oblitimonas*, and *Psychrobacter* showed high relative abundance in most of the studied samples of *hangeo*. Variations were

seen in the relative abundance of genera in the samples of *hangeo* (Zhao & Eun, 2020).

At the species level, diversity was observed in the microbiomes of all the samples. The variations may be because of the use of techniques used to analyze the microbiomes. The other factors may be the use of raw materials, the time of fermentation, the pH, and chemical and physical parameters in micro environments. However, some species are common in all the samples viz. *Lactobacillus pentosus*, *Enterococcus durans*, *Pediococcus pentosaceus*, *Lactococcus lactis*, *Staphylococcus lentus*, and *Lactobacillus brevis*.

Staphylococcus spp. showed the maximum diversity with a total of 19 species detected in all four microbiomes. S*taphylococcus xylosus* was the most abundant species in S1 followed by *S. lentus, S. nepalensis,* and *S. saprophyticus*. In S2, the most abundant Staphylococci were S*taphylococcus xylosus* followed by *Staphylococcus lentus, Staphylococcus nepalensis,* and *Staphylococcus saprophyticus.* In S3 and S4 *Staphylococcus lentus* was detected.

Majumdar & Gupta (2020) isolated seven *Staphylococci* spp. from *Sheedal* of Northeast India namely *S. arlettae*, *S. condimenti*, *S. hominis S. nepalensis*, *S. sciuri*, *S. piscifermentans* and *S. warneri*.

In Korean fermented fish, *Staphylococcus xylosus, S. warneri, S. epidermidis, S. cohnii, S. hominis, S. saprophyticus, S. haemolyticus*, and *S. aureus* were isolated (Um & Lee, 1996).

Stahnke (1994) reported that fermented sausage contained several aromatic esters when fermented with *Staphylococcus xylolus* as a starter culture, which were not present in controlled products. In microbiomes of S1 and S2 *Staphylococcus xylolus* is the dominant species and may play a significant role in producing the typical aroma of fermented fish *napham*. Mah & Hwang (2009) reported that *Staphylococcus xylolus* No. 0538 inhibited biogenic amine formation in a salted and fermented anchovy m*yeolchi-jeot. Staphylococcus xylolus* No. 0538 produced an inhibitory bacteriocin like substance that inhibited the growth of biogenic producing *Bacillus lichenoformis*. *Staphylococcus lentus,* another dominant staphylococcus of *napham*, is a member of the *Staphylococcus sciuri* group, and they appeared to be isolated from various animal-derived fermented food products (Anihouvi et al., 2007).

In S2, there were three fungal species: one species of *Aspergillus* and two species of *Candida*, whereas in S1, a single fungal species *Aspergillus taichugensis* was observed. *Aspergillus taichugensis* is a filamentous fungus belonging to *Aspegillus* section *Caddidi*. It is moderately xerophilic, often found in stored grains, is capable of raising the moisture content, and temperature of the substrate up to 55°C (Lacey & Magan, 1991). They are used in the meat industry for spontaneous sausage ripening (Sunesen & Stahnke, 2003). Five dominant Aspergillus species were isolated and identified from the fermented food karebushi: *A. amstelodami*, *A. chevalieri*, *A. pseudoglaucus*, *A. ruber*, and *A. sydowii* (Takenaka et al., 2020). So, it can be inferred that fungi may also take active role in fermentation of fish.

Brevibacterium is another dominant genus in S1 and S2 microbiomes. Brevibaterium belongs to Class Actinobacteria. This grows in association with salt-tolerant yeast on the surface of smear-ripened cheese and produces several potent antimicrobial compounds, bacteriocins capable to inhibit the spores of germination of *Clostridium botulinum* (Grecz et al., 1959). *Brevibacterium linens* was the most dominant Bravibacterium in both S1 and S2, with 4.39% and 0.74% abundance, respectively. *Brevibacterium* bacterium is a major surface micro-organism in a variety of smears surface-ripened cheeses, such as Limburger and its growth on the surface gives the characteristic color, flavor, and aroma to the cheeses (El Soda & Awad, 2014). This bacterium is extensively studied, has proteinase enzymes, and esterase enzymes that are prerequisites for the development of flavor and texture in the cheese, and is required commercially in the dairy industry to produce smear surface-ripened cheeses (Ades et al., 1969; Foissy, 1974; Rattray & Fox, 1999). Tokita & Hosono (1968) studied volatile sulfur compounds produced by *B. linens* in the culture medium.

Oceanobacillus is detected in microbiomes of S1 and S2. *Oceanobacillus sojae* formed 12.45% of the total population in the S1, and *Oceanobacillus oncorhynchi* formed 6.01% in S2. *Oceanobacillus sojae* is a Gram-positive, spore-forming, motile, rod-shaped bacterium isolated from the bottom of a mold fermenter during the process of soy sauce production (Tominaga et al., 2009). *Oceanobacillus oncorhynchi* was first isolated from rainbow trout (*Oncorhynchus mykiss*a) skin and are a halotolerant, obligately alkaliphilic bacterium (Yumoto et al., 2005). Report of presence of *Oceanobacillus* was reported from fermented fish hakral (Osimani et al., 2018)

Kim et al., 2011 reported the isolation of *Virgibacillus alimentarius* from traditional salt-fermented seafood made of gizzard shad in Korea. *Virgibacillus alimentarius* formed 5.37% of the total population in S2.

Several investigations have reported the dominance of LAB like *Lactobacillus*, *Leuconostoc, Streptococcus, Pediococcus, Lactococcus, Weissella, Tetragenococcus, Vagococcus* in many fermented fish products of the world (Paludan-Müller et al., 2002; Thapa et al.,2004; Kuda et al.,2014; Gelman et al.,2000; Hwanhlem et al.,2011; Siddegowda et al.,2017; Udomsil et al.,2016; Dai et al.,2013).

The present study showed that there is a good diversity of LAB species in all the samples of *napham* microbiome. The LAB community in S1 was comprised of *Enterococcus faecalis, E. faecium, Lactococcus garvieae, Lactococcus lactis, Lactobacillus brevis, Lactobacillus plantarum, Pediococcus acidilactici, Pediococcus pentosaceus*, *and Weissella paramesenteroides*.

In S2 the LAB community consisted of *Enterococcus faecalis*, *Enterococcus faecium*, *Lactobacillus brevis*, *Lactobacillus pentosus*, *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Pediococcus acidilactici, Lactocillus garvieae*, *Lactcaccus lactis*, *Lentibacillus jeotgali, Tetragenococcus halophilus*, *T. muriaticus, T. solitaries, Weisella hellenica, Weissella jogaejeotgali and Weissella*.

In S3 and S4 the LAB species detected were *Enterococcus durans*, *Lactobacillus brevis*, *Lactococcus garvieae*, *Lactobacillus pentosus*, *Pediococcus pentosaceus* and *Lactococcus lactis*.

Bacterial species such as *Lactobacillus plantarum*, *Pediococcus pentosaceus* and *P. acidilactici* together with *Micrococcus* and *Staphylococcus* release flavour compounds during fermentation to provide products with specific tastes (Boyacioglu et al., 2010). The pediococci are facultatively anaerobic to microaerophilic bacteria belonging to the order Lactobacillales. *Pediococcus acidilactici* and *Pediococcus pentosaceus* have proteolytic enzymes, such as protease, di-peptidase, dipeptidyl aminopeptidase, amino-peptidase, which shows strong leucine and valine arylamidase activities (Raccach, 2014). *Pediococcus acidilactici* has application in meat fermentation and as probiotic and bio-protection. *Enterococcus faecium* CN-25 was isolated from Thai fermented fish *kai-pla* that produced enterocin and inhibited *Listeria monocytogenes* (Sonsa et al., 2015*). Enterococci faecalis* was isolated from traditional fermented tungtap of NE, India, (Rapsang & Joshi, 2012) and plasom of Thailand (Hwanhlem et al., 2011). But the study of Biswas et al. (2019)

raised a concern that Enterococci may be a reservoir in traditionally processed products for antimicrobial resistance and virulence genes enabling the propagation of these genes to the human microbiota through the food chain. Kopermsub & Yunchalard (2010) reported the presence of *Lactococcus garvieae*, *Streptococcus bovis, Weissella cibaria*, *Pedococcus pentosaceus*, *Lactobacillus plantarum* and *Lactobacillus fermentum* in *plaa-som*.

Three species of *Tetragenococcus* were present in S1 and absent in S2 which were *Tetragenococcus halophilus*, *Tetragenococcus muriaticus*, and *Tetragenococcus solitaries*. *Vagococcus fluvialis*, *Virgibacillus alimentarius*, *Virgibacillus siamensis*, *Weissella hellenica* and *Weissella jogaejeotgali* were also peresent in S2 and absent in S1. *Weissella hellenica* was reported to have probiotic and bacteriocinogenic properties (Panthee et al., 2019). *Tetragenococcus* was isolated and studied in many fermented fish like *ngari*. *Tetragenococcus halophilus* was isolated in the Japanese fermented puffer-fish (Kobayashi et al., 2000).

The pathogenic species in S1 consisted of *Staphylococcus aureus* with 1.06 % abundance and *Listeria monocytogenes* with a merge abundance of 0.09%. In S2, *Staphylococcus aureus* was present but *Listeria monocytogenes* was absent. In another study, it was reported that *Staphylococcus aureus* was important for the formation of colour, flavour and texture in Thai fermented fish *som-fug* (Feng et al., 2021).

There were three bacteriophage species: *Staphylococcus phagepSco-10*, *Staphylococcus virus Sextaec,* and *uncultured Caudoviralesphage* in S1. In S2, *Staphylococcus phagepSco-10, Staphylococcus phagevB_Sau_Clo6, Staphylococcus virus Sextaec, Staphylococcus virusP108, Staphylococcus phagevB_Sau_S24* were detected. Bacteriophages are the viruses that infect bacteria and are omnipresent in all ecosystems. Because of their ability to infect bacteria, phages are utilized as an important agent for combating pathogenic bacteria in clinical treatments. Phage-related research nowadays gained momentum in biotechnology, such as biosensors, therapeutic medicine, food preservation, aquaculture diseases, pollution remediation, wastewater treatment, and issues related to limitations of phage-based remedies (Mahony et al., 2020). However, Phage also causes the risk to any process requiring bacterial growth. Its presence may lead to manufacturing delays, lower quality products, and contamination (Samson & Moineau 2013). Jun et al., 2017 reported a complete genome sequence of the *Staphylococcus*

Myoviridae phage pSco-10 and infected *Staphylococcus cohnii. Staphylococcus virus Sextaec* is also a Staphylococcus infecting virus. The occurrence of Staphylococcus and other phage viruses in *napham* indicated that *Staphylococcus* and other bacterial population were controlled naturally by the phage viruses (Jung et al., 2011). The 16S metagenomic analysis of *kimchi*, traditional fermented food in Korea, shows the presence of phage viruses in its microbiome, and a correlation is drawn between the relative abundance of Staphylococcus phage virus and Staphylococcus (Jung et al., 2011).

The SEED function study was performed only on samples S1 and S2. As the *napham* fermentation progressed from the first month in S1 to the second month in S2, the metagenomic sequence read to SEED functional categories increased. In S1 the total functional sequence read was 4083, and this increased to 4530 in S2. The increase is because of the increase in microbial abundance in S2 compared to S1. Open Reading Frame in S1 was assigned as 2940704 sequences, and in S2 there were 532641 Open Reading Frame sequences. Similar result was observed in fermented food *kimchi*, a fermented food in Korea (Jung et al., 2011).

The top 5 functional classes in S1 belong to core metabolic functions that included 'Carbohydrates'(18.91%), 'Cofactors, Vitamins, Prosthetic Groups, Pigments'(15.82%), 'Amino Acids and Derivatives' (12.86%), 'Protein Metabolism'(6.245%), and 'Unclassified functions'(4.55%). sequence read. The top 5 functional classes in S2 also belong to core metabolic functions consisting of 'Carbohydrates' (19.35%), 'Cofactors, Vitamins, Prosthetic Groups, Pigments' (16.82%), 'Amino Acids and Derivatives (11.65%), 'Protein Metabolism' (6.00%), and 'Unclassified functions' (4.50%). In S1, 1333 proteins were identified, whereas in S2 1556 proteins were identified.

In *kimchi*, the carbohydrate metabolism yielded 14.49% (Jung et al., 2011). The fraction of the *napham* microbiome reads in the carbohydrate was higher than those of microbiomes of *kimchi*. The results showed that the *napham* microbiome had high metabolic versatility concerning amino acid and protein metabolism.

During this study, both strategies were used to study the *napham* metagenomes. WGS showed a better result in detecting reads up-to the species level. From the studies comparing the output between 16S metagenomic sequencing and long-read sequencing technologies like WGS it was shown that the latter produce greater taxonomic classification at the genus and species level as found in studies

(Pearman et al., 2020 and Nygaard.et al., 2020). .However, long uninterrupted sequences are required for WGS study as mentioned by Pearman et al. (2020) in their research. When such DNA is not obtained, the procedure involving the amplification of gene (16S amplification) may be useful in revealing the Metagenome.

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