Chapter 1

Introduction

Fermentation is the process by which microorganisms can convert a wide range of compounds into desirable products. It is the pathway for breaking down complex compounds with the help of enzymes present in certain microorganisms (Schwan, 2004). Fermentation pathways are named after the acid or alcohol produced during the process, viz., Lactic acid fermentation, alcoholic fermentation, and mixed acid fermentation. Microbes have a unique transformative ability that has been utilized by humans in the production of fermented beverages & foods, preserving food, making the food more digestible, and reducing the toxicity of food substances (Katz, 2012). Lactic acid fermentation of vegetables, milk, pickles, cereal, legumes, meat, and fish preserve the food substance for a prolonged period and produce a broad range of fermented foods like *kimchi, yogurt, cheese, bread, idli, dosa, tempe, miso*, etc. (National Council Research,1992). Some fermented food may be alkaline, where the protein is degraded to amino acids and peptides. During this process of alkaline fermentation, ammonia releases, pH increases, and a pungent ammoniacal smell are produced (Parkouda et al., 2009). Many fermented food products of legumes, pulses, animal proteins, fish proteins, and seafood are considered alkaline fermented foods for their unique sensory characteristics (Ugwuanyi et al., 2019). In alcoholic fermentation, the substrate is sugar, and byproducts are ethanol and CO_2 (Toivola, 1984). Alcoholic fermentation is carried out by some fungi and bacteria. Fermentation is a natural technique that can help make food progressively edible, nutritious, and delightful. The fermented food contains benefits to health that stretch beyond food preservation. The bacteria, known as probiotics or good bacteria live in our body and confer benefits to several health issues related to the digestive system. Presently the emphasis is on using more fermented food in our eating regimens to help, by and large, wellbeing and sustenance of health. However, many of the traditional fermented foods have become less popular. Fish is an excellent source of nutrition with beneficial nutrients like easily digestible proteins, omega-3 fatty acids, essential amino acids, and vitamins but at the same time, it is one of the highly perishable food items. Traditionally the fishes have been preserved through salting, drying, pickling, smoking, marinating, and fermentation which are collectively known as fish curing.

Before the advent of modern technology like canning, and refrigeration, fermentation was the chief method of preserving fish. Fermented fish has been consumed since ancient times and is a significant source of protein for the world population (Prajapati & Nair, 2003). It is a part of the staple diet in Southeast Asian countries such as Cambodia, Indonesia, Thailand, Malaysia, and the Philippines (Beddows, 1998). Besides Southeast Asian countries, fermented fish products are familiar in East Asia, Central Asia, South Asia, Africa, and European countries (Rattagool, 1985). It has a unique fragrance, flavor, and texture, liked by people of various communities all over the world. The fermentation of fish depends on the naturally found enzymes (in the muscle of the intestinal tract) and microorganisms present in the raw fish (Giyatmi & Irianto, 2017). Endogenous enzymes like trypsin, chymotrypsin, elastase, and aminopeptidase are the key ingredients in fish fermentation. Muscle tissue enzymes like cathepsins, peptidases, transaminases, amidases, amino acid decarboxylases, glutamic dehydrogenases, and related enzymes may also play a vital role in the fermentation of fish (Giyatmi & Irianto, 2017).

Natural microbes are present in the body parts like slime, guts, and gills of fish. These micro-organisms, and the enzymes present in the tissues of the fish, bring about putrefactive changes when the fish dies. In fermented fish, salt-tolerant microbes play a significant role in the degradative changes of fish (Mackie, 1971). It is found that most fermented fish are made from fatty fish because fish oils are highly unsaturated and prone to oxidation. Certain pro-oxidants, such as haem, in the proteins, catalyze the oxidation reaction (Mackie, 1971; Giyatami & Iranto, 2017).

The fermentation of fish produces desirable organoleptic properties and increases the bioavailability of minerals (Şanlier et al., 2019). Dincer et al. (2010), reported the presence of essential amino acids and a high ratio of docosahexaenoic acid (DHA) & eicosapentaenoic acid (EPA) in the fermented fish sauce prepared from sardines. In fermented fish, the enzymatic hydrolysis causes large bio-molecules to break down into small bioactive peptides, and these peptides were reported to have an anti-oxidant property, antiproliferative effect against human lymphoma cells (Lee et al., 2004), ACE inhibitor property (Kleekayai et al., 2015), and anti-hypertensive property (Ryan et al., 2011). Natural antioxidants were reported from fermented fish *ngari* and *hentak* (Majumdar et al., 2015).

1.1 Fermented fish products

Some ethnic fermented fish products found in different countries of world are *jeotgal* (Guan et al., 2011; Jung et al., 2013), *plaa-som* (Saithong et al., 2010), *shiokara* (Fujii et al., 1999), *patis* (Steinkraus, 1993), *pedah* (Indriati et al., 2006), *budu*, *nam-pla* (Wongkhalaung, 2004 & Saisithi, 1987), *surströmming* (Kobayashi et al., 2000), *ndagala*, *salanga*, *guedj, tanbadiangyeet, mamone, kako, kobiewurefua*, *djegue, djadan*, *fessiekh, terkeen, mideshi, kejeick* (Essuman, 1992).

In India, various traditional methods of fish curing are used for the preservation of fish. In the Southern Coastal States, 'colombo curing' and 'pit curing' methods are used for fermenting fish products (Parvathy, 2018). Northeast India is also a culturally diverse region inhabited by more than 200 tribes with unique and distinct cultural practices. Northeast India consists of an immense range of indigenous fermented fish products with distinctive taste, texture, and organoleptic characters. Some of the well-known fermented fish products of North East India are *ngari* and *hentak* from Manipur, *sheedal* in Tripura and Assam, *ngawum* in Mizoram, and *tungtap* in Meghalaya (Thapa et al., 2004; Jeyaram et al., 2009; Rapsang & Joshi, 2012). *Namsing*, *sukakomacha*, *bordia*, *lashim*, *sukati*, *karati*, *shidal*, *lonailish*, *napham*, *nichow* and *nakham*, (Thapa et al., 2006; Majumdar & Basu, 2010; Majumdar et al. 2016; Narzary et al., 2016) *sukka ko macha*, *sidra*, *gnuchi* and *sukuti* (Thapa et al., 2016;) are some other fermented fish products found in North East India.

1.2 Microorganisms isolated and studied in fermented fish

The microorganisms during the fermentation break down and utilize various constituents of food like carbohydrates, fats and proteins. Louis Pasteur established a link between fermentation and microorganisms when he discovered the activity of microbes in alcoholic fermentation (Manchester, 2007). Since then, microbes present in various fermented food were evaluated and the scientific, as well as industrial interests in food microbiology, grew manifold. Microorganisms in ethnic fermented fish of the world mainly consisted of *Bacillus* spp., *Staphylococcus* spp., *Micrococcus* spp., and *Pediococcus* spp. *Enterococcus faecalis*, *Lactobacillus plantarum*, *Lactobacillus reuteri* and *Streptococcus salivarius*. The yeasts including species of *Candida* and *Saccharomyces* were reported from fermented fish products in Thailand

(Saithong et al., 2010; Hwanhlem et al., 2011). *Micrococcus* and *Staphylococcus* were dominant bacterial genera during the ripening of *shiokara* (Wu et al., 2000). *Haloanaerobium praevalens* was reported from *surströmming*, the fermented herrings of Sweden (Kobayashi et al., 2000). *Haloanaerobium fermentans*, *Tetragenococcus muriaticus* and *Tetragenococcus halophilus* from the Japanese puffer-fish ovaries were reported by Kobayashi et al., 2000. *Bacillus subtilis*, *Bacillus pumilus*, *Eenterococcus faecalis*, *Enterococcus faecium*, *Lactococcus lactis*, *Lactococcus plantarum, Lactobacillus amylophilus, Lactobacillus fructosus, Lactobacillus confusus, Lactobacillus corynifomis*, *Lactobacillus plantarum, Leuconostoc mesenteroides, Pediococcus pentosaceus, Micrococcus*; yeasts—*Candida bombicola*, *Candida chiropterorum*, and *Saccharomycopsis* sp. were reported from Indian fermented and sun-dried fish products (Thapa et al., 2004, 2006, 2007).

1.3 Traditional fermented fish *napham*

Napham is a traditional fermented fish product of the Bodo tribe of Assam (Narzary et al., 2016). *Napham* is a lesser-known fermented fish product of Assam and prepared in rural areas inhabited by Bodo ethnic groups. This fermented fish product is popular due to its characteristic flavor. It is an economical way of fish preservation and processing known to the villagers. Generally, it is prepared at home and not on a commercial scale. Traditional foods are co-related to their environment. The ethnic food habits of indigenous inhabitants of Assam also have a unique relationship with its ecosystem. Assam is known for its wetlands, freshwater lakes (locally called beel), oxbow lakes, and meanders, marshy and swampy areas. These water reservoirs provide a suitable ecosystem and are breeding grounds for many fishes. The populace exploits the seasonal availability of fish and preserves it for a lean period through techniques of drying or fermentation. Kokrajhar district comprises some 280 recorded wetlands that have a large number of ichthyofaunal diversity. The indigenous Bodo tribe of the Kokrajhar area uses the fish from these wetlands, small bogs, streams, or other water sources and prepares this culturally significant fermented fish product. The raw material used in the preparation of *napham* consists of small fishes, tender shoots of Arum (*Colocasia esculanta*), a hollow cylinder of the matured bamboo stem, kharwi (local alkali), and banana leaf/lemon leaf. The fish are gutted, cleaned, and then dried under the sun. The semi-dried fishes are further smoked under low flame till they are dried. The dried fishes are pounded with the help of mortar and

pestle, known as *uwal* and *gaihen,* in the local *Bodo* dialect. The stems of arum are added at the time of pounding, and a paste is prepared. The paste is inserted into the container made of a hollow bamboo stem called *hasung* open from one side and closed from the other side by an inter-node. The raw material is covered with a layer of ash of *kharwi* (alkali) and then with banana leaf (*Musa*). The opening of the bamboo container is sealed with ash and clay paste. It is sealed tightly to ensure the anaerobic condition. The whole preparation is left to age for a minimum of three months for fermentation.

Preservation is a part of Bodo culture, and the production of *napham* shows the skill of food preservation by the Bodo tribe. Fermented fish has a huge market all over the world and *napham* also has a potential for creating good market and a livelihood generation for the economically weaker sections of indigenous Bodo community. There is a pertinent research gap in the indepth functional investigation of *napham*. The literatures which are available are only on the making process of *napham* and this is the first study on diverse aspects of *napham.*

This research work is a preliminary study of biochemical, nutritional and microbial characteristics of *napham* and will help in preservation of age-old traditional knowledge of the Bodo tribe. The market products do not ensure the hygiene level, and the proper study can reveal the harmful and beneficial microbial load present in *napham*. Up to now, there is no standardized production technology to produce quality *napham* using starter culture technology. The present study may help to formulate and standardize the quality of *napham* in future endeavors. With these research gaps, the present study aims to do an investigation of the biochemical and microbial composition of *napham* with following aim and objectives.

1.4 Aim and objectives

- Documentation of the method of preparation of *napham.*
- Biochemical analysis of *napham.*
- Microbial analysis of *napham* by culture-dependent and culture-independent methods (metagenomics).