Chapter 2

Literature Review

Fermentation is a method of preservation of food by using microorganisms that have been utilized by humans for thousands of years. The food or its products where the controlled microbial growth performs the biotransformation into new edible forms with enhanced flavors and aromas are called fermented foods (Xiang et al., 2019). The microbial population in fermented food products causes chemical and textural changes (Kavitake et al., 2018). According to Steinkraus (1993), fermented food is a fascinating relationship between man, microbes, and food. The microbial population in fermented food can cause spoilage or produce a food product acceptable to man. So, there is a very thin line between rotten and fermented food, which is demarked by taste and tradition (Steinkraus, 2004). The first one is undesirable, whereas the latter is considered a delicacy. Fermented food helped to sustain the human population in the past and provided proteins, vitamins, minerals, and calories. The co-evolution of food and human civilization is a known fact, and so is the fermentation technique adopted to preserve perishable food items (Anal et al., 2019).

2.1 History of fermented food

Alcohols were the first fermented product consumed by man, and its scanty evidence dates back at least 30,000 years ago. The first Neolithic jars consisting of a chemically fermented mixture discovered in Jiahu (Hunan Province, China) date back to 6500-5500 B.C (McGovern et al., 2004). Dominy, 2015 stated that alcoholic fermentation is a natural process where primates, birds, and insects also partake in its consumption unintentionally. He opined that human beings and the food habit consisting of fermented food co-evolved in due process of human civilization. Kindstedt (2012) in his book “Cheese and Culture: A History of Cheese and Its Place in Western Civilization” reported that fermented product known in the Neolithic age was fermented milk products like curd and cheese, and the art of cheese-making was developed as far back as 8000 years ago in the Fertile Crescent between Tigris and Euphrates Rivers in Iraq. The Egyptians developed dough fermentations used in leavened bread in 4000–3500 BCE (Prajapati & Nair, 2003). The Harappans (3200 – 1500 BC) appeared to have known the process of alcoholic fermentation and its distillation (Mahdihassan, 1979). Mahdi Hassan reported that even before the advent of Aryans, the earliest distilled liquor in India was Parisrut prepared from the flowers of Mahua (*Bassia latifolia*) which dates back to 3000 B.C. (Mahdihassan, 1981). Alcoholic beverages are still known as *Mahua* in Bihar. In the Vedic age, there is ample evidence of the use of fermentation technology in 1500 BC-600 BC as the Rigveda mentions the names of three fermented beverages *somrasa*, *sura*, and *parisrut* (Chaudhary, 2019).

2.2 Types of fermented food

There are several types of fermented food products and beverages consumed worldwide (viz: *kefir*, *sauerkrant*, *miso*, *salami*, *cheese*, *idli*, *dosa*, *tempe*, *natto*, beer wines, pickles, etc.). Tamang (2010) classified fermented foods based on the pattern of fermentation, use of micro-organisms, and substrate in fermentation. 1) Based on the pattern of fermentation, they grouped the products into solid (*e.g., Tempe),* submerged (e.g., soy sauce), and moist types. 2) Based on raw materials or substrates, there are eight major groups, viz. fermented vegetable, fermented soybean, and non-soybean legumes, fermented cereals, fermented milk, fermented fish, fermented roots, and tubers, and miscellaneous. 3) Based on the micro-organism, fermented food may be naturally fermented or by the addition of external 'starter culture'. Most fermented food products are acidic, but some are alkaline (Wang & Fung, 1996). Alkaline-fermented foods constitute a group of food products widely consumed in Southeast Asia and African countries. In alkaline fermented food products, as the name suggests, the pH tends to increase during fermentation (Nout, 2014). Most alkaline fermentations are achieved spontaneously by mixed bacterial cultures, principally dominated by *Bacillus subtilis* (Wang & Fung, 1996). Some examples of Alkaline fermented foods are *natto*, *thua-nao*, and *kinema* prepared by fermenting cooked soybeans; *dawadawa* from African locust beans; *ogiri* from melon seeds; *ugba* from African oil beans; *kawal* from fresh legale leaves; *owoh* from cotton seeds; and *pidan* from fresh poultry eggs (Sumi et al., 1987; Chukeatirote, 2015; Sarkar et al.,1994; Campbell□Platt, 1980; Odunfa,1981; Njoku & Okemadu 1989; Dirar, 1984; Wang & Fung, 1996; Ganasen & Benjakul, 2010).

2.3 Health benefits of fermented food

Steinkraus (2004) enumerated the several benefits of fermented food, which included enrichment of diet through the development of various flavors, aromas, and texture; preservation of food for longer shelf life, enrichment of food biologically with essential nutrients; detoxification of undesirable substances in food; reduction of cooking time and thereby saving the fuel. Fermented foods have been a source of supplements of essential amino acids, especially in developing countries. It can supply beneficial nutrients to undernourished obsessed people of developed and wealthy nations. In the past, the health benefits of fermented foods were not well studied, so people primarily used fermentation to preserve foods, enhance shelf life, and improve flavour. Antimicrobial metabolites such as bacteriocin are produced in fermented food by the beneficial bacterial population that inhibits foodborne pathogens (Kormin, 2001). Many organoleptic properties, vitamins, and minerals are present in fermented food products (Hwang et al., 2017). The probiotics in fermented food battle cardiovascular diseases, and gastrointestinal tract disorders, enhance the bioavailability of nutrients, improve the immune system, and fight cancer (Ishimwe et al., 2015). Some of the health benefits of fermented food reckoned by Farhad et al. (2010) are reduction of blood cholesterol, increase in immunity, fight against pathogens, fighting carcinogens, osteoporosis, diabetes, obesity, allergies, and atherosclerosis, and alleviating the symptoms of lactose intolerance. The health benefit of fermented is due to the bioactive peptides synthesized by micro-organisms during biotransformation in fermented foods (Bhat, 2015). Bioactive peptides are released during proteolysis or fermentation and play a significant role in human health by affecting the digestive, endocrine, cardiovascular, immune, and nervous systems (Bhat, 2015). Studies in the field of bioactive peptides have shown that dietary proteins could be a natural source of alpha-glucosidase and DPP-IV inhibitory peptides (Patil et al., 2015). In type 2 diabetes (T2D), Alpha-glucosidase and dipeptidyl peptidase IV (DPP-IV) enzymes play a significant role in its development. Hence, reduction or inhibition of their activity can be one of the crucial strategies in T2D management. Lule et al. (2015) reported that soy□derived bioactive peptide, lunasin, possesses antioxidative, anti□inflammatory, and anticancerous properties that could play a vital role in regulating cholesterol biosynthesis in the body. Due to the known antihypertensive effects of angiotensin-1-converting enzyme (ACE) inhibitor peptides, especially valyl-polyproline (VPP) and isoleucyl-prolyl-proline (IPP), fermented dairy items are the non-pharmacological system for the administration of hypertension (Beltran-Barrientos et al., 2016; Nejati et al., 2013; Usinger et al. 2012). Exopolysaccharides produced by certain strains of lactic acid bacteria are known for their anti-oxidant, anti-diabetic, anti-carcinogenesis, cholesterol-lowering, and immunomodulatory properties. (Pan & Mei, 2010; Wang et al., 2010; Zhang et al., 2013). Lactic acid bacteria (LAB) gained the status of GRAS (generally recognized as safe) because of the production of EPS, which has immense commercial importance due to the beneficial physicochemical properties they exhibit (Surayot et al., 2014). The bacterial exopolysaccharides can reduce total serum cholesterol by binding cholesterol, reducing cholesterol absorption, and inducing the release of bile acids similarly as executed by the dietary fiber (Mumford et al., 2010; Nampoothiri et al., 2017; Tok and Aslim 2010; Şanlier, 2019). In addition to reducing cholesterol, exopolysaccharides play a crucial role in host-microbial interactions. They are involved in microbial colonization, attachment, and immunomodulation, and they protect the bacterial wall against extreme conditions such as temperature fluctuations, osmotic stress, pH changes, or light intensity which helps bacteria to survive in extreme conditions (Caggianiello et al., 2016; Fanning et al., 2012; Şanlier, 2019).

2.4 Fermented Fish

Fish is an excellent source of low-fat proteins, omega-3 fatty acids, vitamin D, Riboflavin, essential amino acids, and minerals (Mendivil, 2020). However, the high moisture content and richness in nutrients increase the perishability of fish, and processing and preservation are mandatory for fish soon after their harvesting. Various methods like salting, drying, pickling, smoking, freezing, chemical treatment, fermentation, and sometimes a combination of all are used for the preservation of fish (Kolawole et al., 2010). In ancient times drying, salting and fermentation were the traditional methods for preserving fish (Skåra et al., 2015). These traditional methods of fish processing, collectively known as curing, were the best and cheapest method of fish preservation in the absence of modern techniques in the past, which gradually became part of the regular diet in many countries due to their characteristic texture and flavor (Kumar & Nayak, 2015). Across many countries and traditions, people developed a means to preserve food resources like fish whenever there was a seasonal scarcity, preceded by an abundant supply of fish (Katz, 2012). The earliest fermented fish sauce recorded was *garum* from the ancient Roman era (Curtis, 1991). In ancient Jordan, *Haimation* was a highly prized fish sauce. It was previously documented only in the ancient text, but the tracing of fish remains was reported in 2008 in an early Roman ceramic jar from Aila Aqaba, Jordan (Van Neer, 2008). In East and Southeast Asian countries like Korea, Japan, China, Thailand, Kampuchea, Malaysia, the Philippines, and Indonesia, the use of fermented fish has been of great cultural value from the distant past (Prajapati & Nair, 2003). In Northern Europe, and Africa fermented fish products are used mainly as condiments. In India, it is an integral part of food culture in the Northeast and Southern regions (Singh, 2014; Beddows, 1998).

2.5 Origin and cultural perspective of fermented fish

The origin of fermented fish took place independently in different locations of the world (Ishige, 1993). (i) The center of salt production; (ii) ecological distribution of irrigated rice cultivation, and; (iii) seasonal behavior of fish stocks in the locality were the three criteria that led to the origin of the fermented fish product (Ishige, 1993). Ruddle and Ishige (2010) tried to trace the likely origin and courses of distribution, together with the development of fermented fish culture in South East Asia. From the extensive field survey of the various fermented fishery products in countries of Southeast Asian countries, they concluded that the widest varieties of fermented fish products were found in Southeast Asia (Rudle & Ishige, 2005 & 2010). The fermented fish formed the principal dietary role in their food habit and hence, they regarded Southeast Asia as the region of one center of origin of fermented fish products. Freshwater fish species naturally occurring in local hydrological systems would have been fermented for preservation (Molinos et al., 2016). In many such regions with a seasonal flood during monsoon and parchedness during the rest of the season, the populace exploited the seasonal availability of fish to be preserved for the lean period through the technique of fermentation (Ruddle &Ishige, 2010)).

2.6 Manufacturing process and diversity of fermented fish

Fermented fish products like fish sauce and fish pastes are regarded as the mother of all condiments. Fish sauce is produced due to changes in physical and chemical characteristics by microbial activity in high salt concentrations and low oxygen levels (Lopetcharat et al., 2001). Traditional and non-traditional fish products are classified, in different ways, according to the method of processing, substrates used, salt concentration, mechanism of fermented products, and forms of final products.

Traditional and non-traditional fermented foods may be classified into three categories (Amano, 1962). In the first category, fish fermentation is a natural and traditional process and the main task is performed by the enzymes present in the fish flesh and entrails. A little amount of salt is added in this process. In the second category, 'starter cultures' are used on the flesh of fish and entrails. The ‘starter culture’ is made of selective microbial enzymes and salt. The third category consists of non-traditional products manufactured by artificially induced accelerated fermentation. Saisithi (1987) suggested the classification of traditional fermented fish products into three groups:

1) first, the carbohydrate source is not used as an additive and the fermented product is obtained by natural fermentation by the activity of the fish enzyme, bacterial enzyme already present in fish and salt mixture; 2) Second, the carbohydrate is used as an additive, and the fish and carbohydrates are fermented by the activity of bacterial enzymes present in fish and carbohydrate mixture; 3) Third, 'starter culture' is used that bring about fermentation. Ruddle & Ishige (2005) gave a precise generic classification and method of preparations of fermented fish in East Asia and classified the fermented fish into two groups. One consists of fermented fish products from freshwater fish and the other from marine finfish, shellfish, Crustaceae, etc. Ruddle & Ishige (2005) classified fermented fish into a high salted product (with 20% salt), low salt product (with 6-8% salt), and no salt product. Sometimes fermented fishery products are also categorized according to the form, structure, and composition of the final product (SubbaRao, 1967; Beddows, 1998). Accordingly, fermented fish are of three main types:

a. The fermented fish where the original form is retained. Eg: *Buro* (Phillipines), *makassar* (Indonesia), *pedah-kemburg* (Thailand), *makassar* (Indonesia), *ngari* (India), Colombo cured mackerel (India). Salt is added to the fish and such type of fermented fish product is also called the salted fish (Beddows, 1998). As the original form of this type of product is retained, the process of fermentation may or may not take place. In *pedah-siam* or *pedah-kemburg*, whole fish (*Rastrelliger* sp., *Scomber neglectus* and *Scomber kangurtal*) which have 15-16% fat with the entrails removed is salted in ratio 3:1(3 fish: 1salt) and stored for 24 hours (Beddows, 1998). The salt desiccates the water and fish is packed carefully in crates in which the ripening takes place in anaerobic conditions. ‘Colombo cure’ method is a prevalent practice in

Southern India and Sri-Lanka. In this method usually mackerel fish and salt in the ratio of 3: 1 are mixed in a concrete tank and dried tamarind fruit is added. Improvements in the quality are obtained if 5% acetic acid is added in place of tamarind (Balachandran &Vijayan, 1975; Narzary et al., 2021).

b. Fish sauce: *Budu* (Malaysia), *patis* (Philippines), *nuoc-mam* (Vietnam), *nampla* (Thailand) are some of the examples of fish sauce. It is the most well know product amongst all the fermented fish products in Southeast Asian, Europe and North America (Narzary et al., 2021). Fish sauce provides a good amount of daily nitrogen intake, vitamins like vitamin B12 and minerals (Saisithi, 1994). The fish tissue during fermentation process in fish sauce is subjected to extensive liquefaction and the resultant fluid part is collected as the sauce (Kumar & Nayak, 2015; Narzary et al., 2021).
c. Fish paste: *Ngapi* (Burma), *bagoong* (Philippines), *prahoc* (Kampuchea), *trassi* (Indonesia) and *belachan* (Malaysia) are some example of fish paste. The duration of fermentation in fish paste is generally shorter and the final product is in paste form (Narzary et al., 2021).

2.7 Fermented fish of Northern Europe

Fermented fish products in Nordic countries like Iceland, Norway, and Sweden are significant part of their traditional cuisine. *Hákarl*, *rakfisk*, *surströmming*, and Barrel-salted ripened herring and sprats are the common fermented fish product of Nordic European countries (Skåra et al., 2015). *Hákarl* is a delicacy of Iceland prepared by fermenting Greenland Shark. The meat of Greenland Shark is cut into small pieces and fermented underground in pits near the sea then hung to dry for several months. This method of curing reduces the toxicity in Greenland Shark which is otherwise poisonous when consumed fresh (https://travelfoodatlas.com/hakarl-iceland-smelly-fermented-shark-delicacy/ accesse on 5/3/2020). *Rakfisk* is produced from arctic charr and lake trout. It is a Norwegian delicacy and is prepared by salting and fermenting fish in tight lid containers at low temperature for 3-12 months (Skåra et al., 2015). *Surströmming* is a part of Swedish traditional dish. For its production Baltic herring (*Clupea harengus* var. *membras*) found in North Sea of Atlantic ocean is caught during May to July just before the spawning. The caught are then dipped in saturated salt solution for 4 hours. After that the fish are beheaded, gutted and put in barrels in weak salt solution of 17 % (Alm, 2012). For Barrel-salted ripened herring and sprats, Fjord herring, Baltic herring, sprats (*Clupea sprattus*), anchovies (*Engraulis* sp.), sardines (*Sardina pilchardus*) and different stocks of herring are used. In traditional method, herrings are beheaded and put in barrels with salt, sugar and some spices (Timberg et al., 2014; Skåra, 2015).

2.8 Fermented fish products of Africa

Drying, salting and fish fermentation are three artisanal methods used for fish preservation in African countries. The fermentation process employed in fermented fish products of Africa is of short duration where the final product retains its original form and is not transformed into fish paste or sauce (Essuman, 1992). In Ghana fermented fish is called '*mamone*' which means stinking fish in Akan language of Ghana (Nerquaye-Tettch et al., 1978). *Guedj, koobi, ewule* are the fishery products that are hard dried or semi-dried and used as fish food that are found in many parts of Africa. Soft texture fishery products are found in Côte d'Ivoire, Gambia, Ghana and Sudan which are used as condiment. *Feseekh* of Sudan is used both as condiment and fish food (El Sheikha et al., 2014). Some of the popular African fermented fishery products are *ndagala* (Barundi), *salanga* (Chad), *guedj,* (Gambia, Senegal), *djegue* (Mali), *fessiekh, terkeen, mideshi, kejeick* (Sudan) (Essuman, 1992).

2.9 Fermented fish products of Asia

Because of its unique savor and taste, the popularity of fermented fish in Asian countries is far reaching (Farnworth, 2008). All three types of fermented fishery products are found in Asian countries. The fermented fish products found in Thailand are *budu, kapi* and *pra-la* (Phithakpol, 1987). *Budu* is a popular fermented fish product with colloidal consistency. Sometimes carbohydrate source is also added in fermented fish product and *Pra-la* is such fermented fish paste where rice bran is added (Rattagool, 1985). The technology used for preparation may be similar, but the additives are different in different products. The additives rice and fruits are used as raw materials in these fermented fish products. This allows the growth of fermentative Lactic Acid Bacteria thereby reducing its pH. Sometimes the similar product may have divergent names in different localities of Thailand (Narzary et al., 2021). In Malaysia fermented fish like *pakesan* is well-liked which is prepared from fresh water fish mixed with tamarind and salt. *Belacan* (shrimp paste), *cinkaluk, budu* and *kikapikan* are the diversity of fermented fish products found in Malayasia. In Phillipines fermented fish products fermented by LAB are consumed (Rattagool, 1985). *Bagoong* (fish paste), *balao-balao* (fermented shrimps), *burongishida* (fermented rice and fish), *bagoong alamang* (shrimp paste), and *paite* (fish sauce) are some of the regular fermented fish product of the Philippine (National Research Council, 1992*).* Olympia (1992) reported that many fishes including herring, anchovie, shrimp etc. and shell fishes that are used for the preparation of *bagoong. Bagoong* has a characteristic salty taste, with a faint cheesy odor, and prepared from fish or shrimp. The fish sauce in Phillipines is called *paite* and obtained from thoroughly fermented *bagoong.* In Indonesia, the popular fermented fish products are *trasi* (shrimp paste), *pedah* (fatty, partly dried, salty fish), *jambal roti* (fermented dry salted marine fish), *bekasang* and *bekasam* (fermented freshwater fish) *budu*, *cincaluk*, *naniura*, *petis*, *picungan*, *pudu*, *rusip*, and *tukai* (Putro, 1993; Huda, 2012). Fermented fish products *terasi, peda,* and *kecapikan,* contribute to less than 2% of the total processed fermented fish products (Huda, 2012). In many fermented fish products of Southeast Asia, koji (*Aspergillus* sp.) is added to speeden the fermentation (Steinkraus, 1993). Burma has traditional fermented fish *ngapi* made from salted fish or shrimp and is considered the national food of Burma (Steinkraus, 2004). *Nuoc-mam* is a fish sauce consumed by a large group of people in Vietnam. It is prepared from small fishes and shrimps. The fishes are layered with salt and kept for fermentation. After the aging process, the supernatant liquor decanted off to give first-quality *nuoc-mam* (Nghia, 2017). The fish tissue during the fermentation process in fish sauce is subjected to extensive liquefaction, and the resultant fluid part is collected as the sauce (Kumar & Nayak, 2015). *Yu-lu* is a Chinese traditional fermented fish sauce widely consumed as a condiment for cooking. Anchovies (or the other small marine fish) are allowed to ferment by adding salt at the level of 30–40%, salt/fish (*w/w*) in this product. The period of fermentation is 12–18 months (Jiang et al., 2007).

In Japan, the fermented fish is called *shikora* (preserves the whole or partial of the fish shape), and *shikora* paste (yielded by pounding or grating). When the additives like rice, vegetables or salt is used along with fish, then it is known as *narezushi. Shikora* and *shikora* pastes are mostly used as condiments and side dishes (Ishige, 1993). Korea also possesses a rich dietary tradition of fermented food and fish. The fermented fish products of Korea are *jeotkal, eo-ganjang,* and *sikhae* (Lee, 1993).

2.10 Fermented fish products of India

In the coastal part of India, methods like drying, salting, pit curing and 'colombo curing' are used. Colombo curing is similar to salting and pickling practiced in Sri Lanka. The practice of pit-curing is common in certain parts of Tamil Nadu and the neighboring region of Travancore. In this curing method the salted fishes are buried in pits lined with mat for a period ranging from a few days to a fortnight or more. The final product is partially dry and possesses a distinct flavour and taste much liked in the eastern parts of Tamil Nadu (Narzary et al., 2021; Rao et al., 1958). In some process of curing, Malabar tamarind (*Garginia cambogea*) is put inside the gut of gutted and cleaned fish and stacked in barrels containing in brine solution. The fish curing industry is much neglected in India (Pillai, 1956). However, fermented fish products are quite popular in North East India and West Bengal. It is interesting to note that many products do not use salt (Majumdar et al., 2016). In place of salt, sometimes local alkali called *kharwi*/*khar* is used. The type of fermented food products found in this region is unique and one of a kind (Narzary et al., 2021). The fermented fish pastes prepared in this region are pounded with various herbaceous plants like taro and allowed to age inside bamboo stems. Two types of fermented fish products are reported in North East India: one that retains solid forms after fermentation and the other in a paste form. They are used as condiments or with other vegetables for their texture and flavor. The local people associate these products with some health benefits. Some of the fermented fish products of North East India found are: a) *Shidal* is a popular fermented fish product found in different states of North East India. It is prepared from *Puntius* sp. (generally *Puntius sophore*) and *Stipinna phasa* and is known by different names across the five states of North East India (Majumdar et al., 2015) of North East India. b) *Ngari* is a traditional dry non-salted fermented fish product of Manipur produced exclusively from sun dried *Puntius sophore* Ham. (Jeyaram et al., 2009). *Tungtap* is traditional fermented fish in Meghalaya which are prepared from *Puntius* sp. and *Danio* sp. (Rapsang and Joshi, 2012). *Hentak, numsing, sukakomacha, sukati, napham, nichow, nakham*are some other fermented fish prepared by different ethnic communities found in North East India (Thapa et al., 2006; Narzary et al., 2016). *Lona ilish*is salted and prepared exclusively from Indian shad (*Tenualosa ilisha*) popular in both Bangladesh and parts of India bordering Bangladesh (Majumdar, 2016).

2.11 Health Benefits

The advances in scientific studies revealed the significant role of micro-organisms in fermented food and fish industries, particularly of LAB, with novelties and added values. LAB isolated from 11 fermented fish samples obtained from the Northeastern (Sanriku) Satoumi region of Japan showed antioxidant activities and the isolates were identified as *Lactobacillus plantarum*, *Lactococcus lactis* and *Pediococcus pentosaceus* (Kuda et al., 2014). LAB, Bifidobacteria and many species of bacteria are conferred as probiotic. The EPS produced by probiotics enhances nutritional value, texture, shelf life of the food product and promote gut health (Korakli et al., 2002).The studies show that fermented fish also consists of several essential amino acids like arginine, isoleucine, leucine, lysine, methionine, histidine, phenylalanine, threonine, tryptophan, and valine in significant amount (Ijong & Ohta,1995; Dincer, 2010).Taurine was found in fermented sea fish of which has the ability to fight oxidative stress (Martínez-Álvarez et al., 2017). Chai et al., 2020 gave a comprehensive review on the bioactive peptides from fermented fish. Several bioactive peptides from fermented fish have been reported to have antioxidative, ACE inhibitor, antihypertensive, anti-proliferative effect against human lymphoma cell (Xiang et al., 2019). Fermentation of fish produces desirable organoleptic properties and increases the bioavailability of minerals (Şanlier et al., 2019). Jemil et al. (2016) reported 33 biopeptide compounds by fermenting Zebra blenny (*Salaria blesilisca*) muscle proteins with stater organism *Bacillus mojavensis* A21 strain. Fermented fish contained significantly less fatty acid as compared to fresh fish (Dincer et al., 2010). Another study showed that fermented fish oil contains notably high levels of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) (Han et al., 2012). Han et al. (2012) reported that fermented fish oil contains DHA (docosahexaenoic acid) which can alleviate the symptoms of atopic dermatitis. Some studies suggested that bioactive metabolites (e.g. lactoferrin and flavonoids) and polysaccharides from raw materials containing their precursors are produced in fermented fish (Ryan et al., 2011).

2.12 Health concern

One of the important processes found in fermented fish is Lipid oxidation. But sometimes because of lipid oxidation the spoilage of fermented fish also take place and compounds like hexanal, 4-hydroxy-nonanal, valeraldehyde and malondialdehyde are produced (Feng et al., 2021). These compounds were reported as carcinogenic and mutagens (Feng et al., 2021). Biogenic amines are nitrogenous compounds consisting of amine groups that are found in many fermented fish and is considered as the compounds which are of safety concern (Santos, 1996). The Biogenic amines are formed by decarboxylation of amino acids and by amination of aldehydes and ketones and can cause serious health implications when consumed in higher concentration (Stratton, 1991). BA like histamine and tyramine increases the blood pressure in sensitive people as they are vasoactive compounds. It may show the symptoms of allergy, headache, respiratory disorder, nausea and tachycardia (Durak-Dados et al., 2020). Moon et al., (2010) High concentration of histamine &tyramine was reported in 47 samples of fermented fish prepared from anchovy, squid, clam and shrimp. *Escherichia coli, Salmonella, Closteridium botulinum*, *Salmonella* sp., *Listeria* sp., and *Staphylococcus aureus* were the main food born pathogen found in fermented fish that may show various symptoms of food poisoning (Adams, 2013).

2.13 Biochemical composition of fermented fish products

Fermented fish are alkaline food products that release ammonia during the natural hydrolysis of proteins into peptides and amino acids, because of which the final pH in the product increases (Sarkar &Nout, 2014). Alkaline pH and ammonia in these products control the growth of a few dominant bacteria, which allows the anaerobic breakdown of proteins that release amine compounds (Narzary et al., 2021). The fermented fish product is well preserved and stable because of its high pH and free ammonia. The rapid growth of essential microorganisms in such food does not allow the growth of bacteria that may spoil the product (Wang & Fung, 1996; Sarkar &Nout, 2014). Most alkaline fermentations are achieved spontaneously by mixed bacteria cultures, principally dominated by *Bacillus subtilis* (Wang & Fung, 1996). The Physico-chemical characterization of different types of fermented fish depends on various factors like the nature of processing fish used, percentage of salt concentration, number of days of fermentation, microorganisms present in the product, etc. (Narzary et al., 2021).

The biochemical characteristics of *pedah*, a fermented fish of Indonesia prepared from Mackerel (*Rastrelliger brachysoma),* consist of moisture content of 46g, a protein content of 28g, fat 4g, Ca 174mg, and P316mg out of 100g sample (Irianto & Irianto, 1998).

Jeotgal, a fermented fish of Korea, is prepared from fish *Engraulis japonicas* (anchovy) or *Ammodytes* spp. It consists of moisture content 7.6 %, crude protein 7.4%, crude fat 1.1% carbohydrate 0.01 % and pH 6 %.

Budu is a fermented fish prepared from fish *Clupeoides* spp., *Sardinella* spp., and *Anchoviella commersonii.* The moisture content of *budu* is 45-60.2 %, crude protein of 12.1-3.2 %, crude fat 1.3-1.8 %, fiber 0.2%-0.4 %, the acidity of 0.4 %-0.9 % and pH 5.4-6.5 % (Saishithi et al., 1994; Phithakpol, 1993).

In *ngapi* (fermented fish of Myanmar), the moisture content found was 7.6 %, protein 41.9 %, Fat 8.4 %, NaCl 37.95 % and ash content 3.45 % (Tyn, 1993). In *ngari* (India) the raw materials are *Puntius sophore* and *Puntius ticto* and biochemical composition consisted of 36.03 % moisture content, lipid content 13.34 %, ash content 5.49, pH 6.74 %, free fatty acid 97.60 % & water soluble Nitrogen 3.62% (Sarojinalili & Suchitra, 2009).

Shottsuru, a fermented fish sauce in Japan is prepared from anchovy, and opossum shrimp, wherein salt is used as raw material. The biochemical composition consisted of Nitrogen 30 to 1598mg/100ml, pH 4.54 to 5.56 %.

In *terkin* (Sudan), the moisture content consisted of 40 %, protein of 23.8 %, and pH of 6.1. The raw materials of *terkin* consisted of fish *Hydrocynus* spp., *Alestes* spp., tilapia, and Nile perch Sardines (*Sardinella* sp.) or anchovies (*Stelophorus* sp.), salt, mustard (*Brassica juncea*) and water (Abu-Hassan & Adam Sulieman, 2011; Mohammed, 2010).

Momone (Ghana) has raw materials *Barraccuda* sp., catfish, croaker, grouper, herrings, mackerel, octopus, snapper, squid, threadfin, seabream, and ribbon fish (Anihouvi, 2012), and biochemical composition of moisture content50%, protein16.8-21.9 %, free fatty acid20.8 %, pH: above 6.0 and NaCl:5.7 % (Nerquaye Tettch et al., 1978; Yankah, 1988; Sanni et al., 2002). Mizutani et al. (1991) and Saisithi (1994) reported the presence of amino acids both essential and non-essential amino acids in fermented fish products. Glutamic acid and aspartic acid were the dominant amino acid in *rusip,* a fermented fish from Indonesia. Other amino acids found in *rusip* were serine, histidine, glycine, threonine, arginine, alanine, tyrosine, methionine, and valine (Koesoemawardani, 2018). In *hatahata-zushi*, a Japanese fermented fish, the fatty acid profile consists of Eladic acid (C18:1n-9), Palmitic acid (C16:0), Decosahexanoic acid (C22:6n-3), Ecosapentanoic acid (C20:5n-3), and PalC16:1n-7 (Chang, 1994). The various literatures suggest that the production of flavor and texture of fermentation fish depends on the amino acids and fatty acids that have been formed after microbial decomposition.

2.14 Microbiota of fermented fish products

Several micro-organisms were reported from different fermented fish products. Micro-organisms are the integral part of all fermented food products and most of them are approved by FDA as GRAS (Burdock & Carabin, 2004).

In *budu,* the strains of bacteria isolated were *Lactobacillus plantarum* LP1, LP2, *Staphylococcus arlettae* SA1, *Saccharomyces cerevisiae* SC3 and *Candida glabrata* CG2 (Yuen et al., 2016), *Bacillus licheniformis*, *Tetragenococcus halophilus*, *Staphylococcus piscifermentans* (Choorit & Prasertsan, 1992; Tanasupawat & Komagata, 1995).

In *pedah*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Lactobacillus plantarum* and *Pediococcus pentosaceus* (Indriati et al., 2006) were reported.

In *nauc-man*, a fermented fish of Vietnam, *Staphylococcus* sp., *Bacillus sp.*, *Pseudomonas* sp., *Pediococcus* sp., *Lactobacillus* sp., *Streptococcus* sp, *Halobacterium cutitrubrum, Micrococcus* sp., and *Halobacterium salinarium* were isolated (Thongthai et al., 1992; Lopetcharat et al., 2001).

In *ngapi*, *Tetragenococcus halophilus*, *Staphylococcus epidermidis*, *Tetragenococcus muriaticus*, *Halanaerobium fermentans*, *Tetragenococcus muriaticus*, *Clostridium sensuaponi*, *Clostridium botulinum*, *Halanaerobium fermentans* (Kobayashi et al., 2016) were reported.

In *shidol*, *Staphylococcus aureus*, *Micrococcus* sp., *Bacillus* sp. And *Escherichia coli* (Muzzadadi & Basu, 2015) were reported.

In *mamone*, *Aspergillus, Bacillus*, *Debaryomyces*, *Hansenula, Lactobacillus*, *Pediococcus, Pseudomonas*, *Staphylococcus* and *Klebsiella*; *Bacillus* is predominant with occurrence of 37.7% (Sanni et al., 2002).

Bacillus sp., *Staphylococcus* sp., *Lactobacillus* sp., *Micrococcus* sp. plays a specific role and is present in most of the fermented fish products. In fermented fish products, factors like use of raw materials and the manufacturing process influence the type and number of micro-organisms present in them. In some cases, the addition of certain micro-organisms as 'starter culture' led to the acceleration in fermentation time, and production of better product (Sumague & Mabesa, 1994). Sumague & Mabesa, (1994) reported the addition of proteolytic enzymes of *Aspergillus oryzae* and *Aspergillus niger* hastened the production of *bagoong and* p*atis.* Yongsawatdigul et al., (2007) reported *Staphylococcus* sp. SK1-1-5 and *Virgibacillus* sp. SK 33 strains, when used as 'starter culture' increased the desired volatile compounds in the fish sauce by increased production of proteinases. Thongsanit et al., (2002) elucidated that *Tetragenococcus halophilus* improved the flavor of fish sauce. It produced volatile compounds like benzaldehyde, 2 &3- methylbutanal, and 2-methylpropanol that gave the desired flavor to the fish sauce. Fungi like *Aspergillus luchuensis* was attributed in generating flavor due to their proteolytic and lipolytic activities in low salted fermented fish in the study by Kim et al. (2016). Thus, the endogenous enzymes present in a diverse group of microbes found in fermented fish products can give desirable organoleptic properties and extended shelf life. The balanced metabolites present in the fermented fish can determine the preferable flavor and texture (Jeong et al., 2013). Furthermore, the micro-organisms also degrade the anti-nutritional and produce antimicrobial properties. Histamine is a hazardous amine reported in several fermented fishery products that has serious health implications. In a study by Kuda et al. (2012), *Tetragenococcus halophilus* isolated from *nukazu* had suppressive effect on histamine accumulated on salted fermented fish. *Weisella* strains from *plaa-som* produced antibacterial compounds and folate (Deatraksa et al., 2018).

2.15 Methodologies used for characterizing the microbiota in fermented fish

The microbial communities in fermented food consist of multi-species consortia involved in the process of biotransformation during fermentation (Babaei, 2019). Humans over the thousand years could optimize the conditions to support the growth of specific microbial communities. These microbial communities carry-out different functions that are fundamental to the texture, flavor, aroma, and safety of fermented fish (Gomes et al., 2009; Liang et al., 2016). Manipulation of different growth parameters prerequisite for the microbes, such as moisture, temperature, pH, salinity, and chemical composition of the substrate, results in a wide variety of fermented fish products (Owens et al., 1985). The analysis of microbial communities, their successional dynamics and, interactions are some of the important features to understand the process involved in fish fermentation that determines the functionality and nutritional quality of the final product (Ha et al., 2014). Broadly there are two approaches to analyze the microbial communities in fermented fish: Culture dependent method and Culture-independent method. Culture dependent method is a traditional method of microbial diversity in natural habitat that has been used by microbiologist for more than 150 years. The cultivable microbes in laboratory environment are cultured using agar or broth media of selective and enriched nature. The morphological and physiological characters of the isolated microbes are then attempted to describe and place the bacteria in taxonomic ranks (Fry, 2004). The quantification of microbial population is done by counting the serially diluted plate assay in a unit known as colony forming unit (CFU) and in chambers of optical microscopy. More recently due to advent of technological revolution sophisticated microscopy like TEM and Flow cyclometry are also used for quantification (Nguyen, 2017; Fung, 2013). The traditional phenotypic characters are often not reliable for proper phylogenetic study of isolated bacteria or fungi. Molecular techniques like sequencing of 16S rRNA and ITS inter-spacer region are nowadays used for molecular phylogenetic characterization of isolated bacteria and fungus respectively (Millar et al., 2007). The traditional culture based has limitations in providing some accuracy in total enumeration of microbes and is very resource as well as time consuming. Moreover, only the cultivable bacteria can be analyzed by culture based method which is estimated to be only 1% of the total microbial population of fermented food (Justé et al., 2008). To overcome these limitations culture-independent based molecular tools and technology is used. Metagenomics is one such approach in a genomic analysis that studies the community of micro-organism from environmental samples without the necessity of obtaining pure cultures (Ranganathan, 2019). It is the process by which metagenome is generated and involves sequencing of all DNA extracted from the sample followed by sequencing, mapping them to a reference database and annotation of genes (Lindon et al., 2018). Metagenomics has dramatically transformed the understanding of microbial diversity. Whole-genome metagenomics also promises to explore the functionality of microbial consortia in the fermentation process of food. Libraries prepared from DNA extracted from natural samples as well as food samples generate billions of nucleotide sequences. Techniques of High throughput sequencing or NGS have been used in place of traditional Sanger sequencing. Sanger sequencing with its limited throughput and high cost has limited applications in personal genome sequencing (Reuter et al., 2015). The rise of NGS technologies about a decade ago revolutionized the field of microbiology. This analyses a comprehensive description of microbial DNA content in a given sample by generating up to 10^9 sequences per reading per run (Kulski, 2016). In commercial *hongeo* product, a traditional Korean fermented fish Shotgun metagenomics approach was used to reveal the bacterial community and metabolic pathways. Four dominant bacterial genera *Psychrobacter*, *Clostrodium*, *Oblitimonas* and *Pseudomonas* were detected and alkaliphilic LAB belonging to genera *Marini lactibacillus* and *Jeotgalibaca* were also detected indicating that traditional fermented food can be good source for extracting novel bacteria (Zhao & Eun, 2020).