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Review of Literature

3.1 Introduction

Foods are the basic survival needs for human being. Since ancient times, various methods have been used to process and preserve foods. Fermentation technology has been used to develop different food products since times immemorial. The fermentation processes leading to the production of wine, beer, bread, yoghurt, cheese and pickles are probably the first biotechnologies devised by man to improve the quality and storage life of some food materials since antiquity (Battcock and Ali Azam, 1998). Holzapfel (2002) described fermented foods as palatable and wholesome foods prepared from raw or heated raw materials. Several classifications had been used to categorize the wide spectrum of fermented foods including the diversification of microorganisms, different food groups and types of fermentation involved (Yokotsuka, 1982; Campbell-Platt, 1987; Dirar, 1993; Steinkraus, 1996). In
addition to being made more shelf stable, all fermented foods have characteristics aroma and flavour, that result directly or indirectly from fermenting organisms. According to Nout and Mortarjemi (1997), fermented foods are typically unique and vary according to regions due to the variation in climate, social patterns, consumption practices and most importantly the availability of raw materials. The availability of raw materials brings about their conversion to different forms of fermented food products in order to increase the food variety as well as to maintain food security.

For many socio-economic and technical reasons, fermentation is one of most important fish preservation methods used in many parts of the world and mainly the Southeast Asian countries, including the north eastern part of India. Fermented fish processing is an artisanal activity and the processes differ from one country to another (FAO, 1981a; Anihouvi et al., 2012). Each fermented product is associated with unique group of micro flora which increases the level of protein, vitamins, essential amino acid and fatty acids (Jeyaram et al., 2009), along with an increased digestibility of the raw materials (FAO, 1971). Fermented fishery products contribute to protein intake of the people especially those in the rural hinterland, where fresh fish is not readily available. The sanitary conditions of fermented fish production were generally found to be poor and processing methods were not standardized (Suchitra and Sarojnalini, 2012).

3.2 Fermented fish products

Traditionally, the term “fermented fish” covers both enzyme hydrolyzed and microbial fermented products (Mackie et al., 1971). However, as suggested by Ozen and Mendoza (1985), only those products involving fermentative growth of microorganisms should be described as fermented. Of course, many products will involve both microbial fermentation and enzymatic activity in particular in high salt range of fermented products. Fermented fish is, therefore, any fishery product which has undergone degradative changes through enzymatic or microbiological activity either in the presence or absence of salt. According to Beddows (1985) salted, fermented and sun dried
fish is generally known as “fermented fish” since the processing methods usually involved salting, fermentation and drying.

Processing of fermented fish and fishery products is almost exclusively confined to the Southeast Asian region (Adam et al., 1985; Cooke et al., 1993). Fish fermentation in the Southeast Asian sub-region normally lasts for several months (three to nine months) and the fish flesh may liquefy or turn into a paste (Huss and Valdimarson, 1990). Some of these products include Nuoc-mam of Vietnam and Cambodia, Nam-pla of Thailand, Sushi of Japan and Patis of the Philippines. Among the many other fermented fish, fish paste and the fish sauces products like Mam-tom of China, Mam-ruoc of Comodia, Bladchan of Indonesia, Shiokara of Philippines and Pla-mam of Thailand of Southeast Asia have been reviewed and discussed (Van Veen, 1965; Crisan and Sands, 1975). In Africa, salting and drying of fish for preservation is accompanied by fermentation, but the period is short (a few days). It was also observed in the study that, unlike in Southeast Asian countries, fermented fishery products in Africa are usually whole or in cut pieces, and are not a paste or sauce. Fermented fish is used in Africa, both as a condiment and as food fish (FAO, 1992; Anihouvi et al., 2012). The products are all characterized by a strong odour (Watts, 1965). Different fermented fish products of Africa were reported, such as Lanhouin in Benin and in Togo (Anihouvi et al., 2005; Dossou-Yovo et al., 2011), Momone, Koobi, Kako and Ewule in Ghana (Essuman et al., 1992; Nerquaye-Tetteh et al., 1978; Abbey et al., 1994; Yankah, 1988), Guedj in Gambia, Tambadiang, Yet and Guedj in Senegal, Djege and Jalan in Mali, Fessiekh, Kejeick, Terkeen and Mindeshi in Sudan, Dagaa in Uganda, Gyagawere, Adjonfa and Adjuevan in Ivory Coast, and Salanga in Chad (Dirar, 1993; Koffi-Nevry et al., 2011). Watanabe (1982) described the fermented fishery products of Senegal as highly salted and semi-dried fishery products with an obnoxious odour and a cheesy but rich fishy flavour reminiscent of Kusaya from Japan. Fermented fishery products in Africa may either be soft with a high moisture content, semi-dry or very dry. Some products are also heavily salted and dried whilst others are dried without any salting. Some types of fermented products have a rancid taste (FAO, 1981a).
Indigenous fermented foods contribute a large portion of daily food intake in the north eastern states of India. Fermentation is an obsolete and outmoded conventional means of preservation of fish in the north eastern region of India and small fishes are mostly subjected to fermentation (Barua and Goswami, 2005). In Bangladesh, the use of freshwater minor carp like *puthi* (*Puntius* spp.) was reported to be used in the production of semi fermented product locally called *Chepa Shutki* (Mansur *et al*., 2000). Many indigenous traditional fermented fish products of northeast India like *Ngari* and *Hentak* of Manipur, *Tungtap* of Meghalaya, *Numsing/Hukoti* of Assam and *Lona ilish* have been reviewed (Sarojnalini and Vishwanath, 1988; Muzaddadi *et al*., 2003; Thapa *et al*., 2004; Majumdar and Basu, 2010; Tamang *et al*., 2012; Kakati and Goswami, 2012).

### 3.3 Microbial diversity of fermented fish products

Fish in its natural environment has its own micro-flora in the slime on its body, gut and in its gills. These micro-organisms as well as the enzymes in the tissues of the fish, bring about putrefactive changes in fish when it dies. Microorganisms play an important role in the later stage of fermentation, and the protein degradation by these organisms lead to production of volatile compounds from amino acids and small peptides (Lopetcharat and Park, 2002). Each fermented product is associated with a unique group of microflora which increases the level of protein, vitamins, essential amino acids and fatty acids (Jeyaram *et al*., 2009).

Furthermore, the micro-organisms generally present in the salt used for salting also contribute to the degradative changes in the fish. Solar salt, which is the most widely used in fish curing, has been found to contain the largest amount of micro-organisms. The general bacterial flora of solar salt mostly comprises *Bacillus* types (75%) with the remainder being *Micrococcus* and *Sarcina* types. The most important spoilage organisms always present in solar salt are the red halophilic bacteria.

Processing caused considerable changes in the composition of the microflora in counts as well as in species found (Pivovarou *et al*., 1988). Suchitra and Sarojnalini (2012) reported that gram positive rod, *Bacillus* and
gram negative cocci, \textit{Micrococci} species predominates the bacterial flora of the fermented fish product \textit{Ngari}. The bacterial flora comprises strain of \textit{Bacillus cereus}, \textit{B. coagulans}, \textit{B. pumilis}, \textit{B. subtilis}, \textit{B. Panthothenticus}, \textit{Staphylococcus} and \textit{Micrococcus} species. The occurrence of this spore-forming \textit{Bacillus} spp. in the completely fermented product may reflect the resistant nature of these microorganisms (Crisan and Sand, 1975). The presence of large percentage of \textit{Bacillus} species during initial and end of fermentation process of \textit{Ngari} suggested that spore-forming \textit{bacilli} might play an active role during fermentation. The presence of 3 species of \textit{Micrococcus} throughout the fermentation also indicates the possible involvement of nonsporing microorganisms in every stage of fermentation (Rose, 1982).

\textit{Aryanta et al.}, (1991) studied the occurrence and growth of microorganisms during the fermentation of fish sausage. The microbial fermentation of fish has precedents in the production of various fish sauces and paste (Beddows, 1985). But, the role of fermentating microorganisms in the fermented fish products of Southeast Asia appears to be minimal.

\textit{Fujii} (1994) reported that \textit{Staphylococcus} and \textit{Micrococcus} were known to prevail during \textit{Shiokara} fermentation and \textit{Bacillus} spp., halophilic cocci, etc. were detected in fish sauce. Other microbial roles were evident in the case of \textit{Kusaya} (special salt-dried fish), \textit{Funazushi} (fermented crucian carp with rice) and \textit{Nukazuke} (fermented sardine with rice-bran) were some of the fermented fish products of Japan. Bacteria tentatively classified as "Corynebacterium" produce antibiotics in \textit{Kusaya} gravy which is successively used for \textit{Kusaya} production and contribute to the good preservability of the product.

Some lactic acid bacteria became dominant during the fermentation of \textit{Funazushi} and \textit{Nukazuke}, contributing to the special taste and flavor, and inhibit the growth of spoilage/pathogenic bacteria by lowering the pH (Fujii, 1994). \textit{Pediococcus penosaceus} and \textit{Lactobacillus plantarum} (indigenous lactic acid bacteria) dominated the indigenous fermentation, achieving populations of \(10^7 - 10^8\) cfu/g by 48 hours and decreasing the pH of the product to 4.5 - 4.7 (Tamang, 1998). Lactic Acid Bacteria (LAB) was reported
to be important in food flavour development. The presence of LAB in the sample was also expected to contribute significantly to the flavour of fish sauce (Gibbs, 1987). Thapa et al., (2004) reported the counts of lactic acid bacteria, endospore-forming rods, yeasts and aerobic mesophilic counts ranged from 4.0-7.2, 3.3-4.6, <1–3.5 and 4.3–7.3 log cfu/g in Ngari, Hentak and Tungtap - traditional fermented fish products of northeast India, respectively.

Many authors have reported a large range of microorganisms involved in fish fermentation in African regions. The microbial population of Lanhouin - a fermented fish product of Benin, consisted of a variety of gram-positive and gram-negative bacteria. The gram-positive were largely halophilic types: *Bacillus* spp., *Staphylococcus* spp., *Micrococcus* spp., *Streptococcus* spp. and *Corynebacterium* spp. However, *Bacillus* spp. and *Staphylococcus* spp. were the predominant genera identified (Anihouvi et al., 2006; Anihouvi et al., 2007). These organisms could also have come from the salt used to treat the fish. *Streptococcus* and *Corynebacterium* species were present in very few numbers and this could be due to the high salt concentration. The high salt concentration leaves only salt tolerant microorganisms to survive; salt concentration up to 7% results in the inhibition of lactic acid bacteria (Horner, 1997). Most *Bacillus* isolates from Lanhouin were identified as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus mycoides* and *Bacillus cereus*; *Staphylococcus* species consisted mainly of *Staphylococcus lentus* and *Staphylococcus xylosus*. Similarly to Lanhouin, various species of microorganisms including *Bacillus*, *Lactobacillus*, *Pseudomonas*, *Pediococcus*, *Staphylococcus*, *Klebsiella*, *Debaryomyces*, *Hansenula* and *Aspergillus* involve in the fermentation of Momone (Nerquaye-Tetteh et al., 1978; Yankah, 1988; Oronsaye, 1991; Essuman, 1992; Sanni et al., 2002). But as for Lanhouin, the predominant ones were *Bacillus* ssp. and *Staphylococcus* species. Among the *Bacillus* species, *B. subtilis*, *B. licheniformis*, *B. megaterium*, *B. cereus* and *B. mycoides* have also been reported for Momone (Sanni et al., 2002). However in contrast to Momone, species such as *Klebsiella*, *Debaryomyces*, *Hansenula* or moulds such as *Aspergillus* were not detected in Lanhouin. The predominant microbial populations associated with
Guedj fermentation were *Proteus* spp., *Shewanella putrefaciens*, and *Bacillus* spp. (Diop, 2008).

Since the solid substrate fermentation of fish is usually of an alkaline type, microorganisms such as *Bacillus* spp., *Staphylococcus* spp., *Micrococcus* spp., which constituted the predominant genera involved in Lanhouin, Momone and Guedj are expected (Oronsaye, 1991). The presence of similar genera of microorganisms had been reported for various other fermented products obtained by alkaline fermentation.

In contrast, the recent work carried out by Koffi-Nevry *et al.*, (2011) on Adjuevan a fermented fish from Côte d'Ivoire showed that the fermentation was dominated by lactic acid bacteria, and the genera and species isolated and identified were *Leuconostoc lactis*, *Lactobacillus fermentum*, *Pediococcus* spp. and *Streptococcus* species. These results agreed with the findings of various authors for fermented fish products obtained with a mixture of fish and carbohydrate source such as rice (Adams, 1987), but not for fish fermented without source of carbohydrate. Indeed, Asian fermented fish products were usually prepared by mixing fresh fish, salt and rice. For instance, *Pla-ra* and *Pla-chom* two fermented fish products from Thailand are prepared by mixing freshwater fish, salt and roaster rice and this mixture is allowed to ferment at room temperature for 6-12 months (Phithakpol, 1995; Yachai, 2008). According to Tanasupawat *et al.*, (1998) the microorganisms responsible for the fermentation of *Pla-ra* and *Pla-chom* were *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Enterococcus* and *Staphylococcus*. Similarly, Yachai *et al.*, (2008) isolated 11 strains of homofermentative, rod shaped lactic acid bacteria and five strains of heterofermentative, sphere-shaped lactic acid bacteria from *Pla-ra* and *Pla-chom*. They were identified as new species and named *Latobacillus acidipiscis* spp. nov. and *Weissella thailandensis* spp. nov., respectively. Such lactic acid bacteria are expected in both *Pla-ra* and *Pla-chom*, since these fermented fish products were prepared from a mixture of fresh fish and roaster rice.

The roaster rice added to fish meat was considered to be a good source of carbohydrate, and consequently enhanced the development of lactic acid
bacteria and the production of organic acids, mainly lactic acid which limited the survival of non-acid tolerant bacteria. So, with the exception of *Streptococcus* spp. reported by Anihouvi *et al.*, (2007) in *Lanhouin*, the results of Koffi-Nevry *et al.*, (2011) disagreed with the findings of various authors who reported that the microbial populations of various African fermented fish products are mainly *Bacillus* spp. and Micrococcaceae, since the type of fermentation was an alkaline one. Zakhia and Cuq (1991) reported that the organic acids produced during the fermentation of fish in Mali were mainly acetic acids, whereas it would appear that in Asia mainly lactic acid is produced.

The total population of microbes increased during the early stage of fermentation and then decreased. Normally food that is produced, ripened or fermented by the actions of bacteria will yield high total counts and a known flora of non pathogenic organisms should be present (Hall *et al.*, 1967). Nagao (1951) observed an increase in the bacterial load from $10^4$ to $10^7$ g$^{-1}$ in *Shiokara* during 41 days of fermentation and indicate the possibility of bacterial role in the ripening of the product which finally resulted in the production of flavours.

According to Lonsane *et al.*, (1985), a temperature of $25^\circ$C - $35^\circ$C is generally employed in solid substrate fermentation. Fluctuation in the temperature prevents proper growth of fermenting bacteria. *Ngari*, a fermented fish product of Manipur, subjected to $20^\circ$C required longer period of time than the normal period (Suchitra and Sarojnalini, 2012).

**3.4 Physico-chemical aspects of fermented fish products**

The chemical compounds which are produced in fish muscle by autolytic enzymes, putrefactive microorganisms or by chemical reactions like lipid oxidation, gradually accumulate in the fish muscle during spoilage and their determination provide a measure of the progress of spoilage (Lakshmanan, 2000). In physico-chemical assessment of quality, the various products of spoilage in fish muscle are quantitatively determined and correlated with sensory characteristics.
Many authors have studied the biochemical pattern occurring during the spontaneous fermentation process of fish. In this respect, changes in moisture, protein, fat, free fatty acids, total volatile nitrogen, and histamine were observed during the fermentation period. Several studies have been carried out to study the biochemical pathways followed during the degradation process of fish fermentation.

### 3.4.1 pH

The pH of the fish muscle is considered as an index of its freshness. The increase in pH may be attributed to the production of volatile base compounds by bacterial activity (Cann et al., 1983). Higher pH values i.e. 7, were reported in case of some fermented fish products of Africa such as momone, lanhouin (Yankah, 1988; Abbey et al., 1994; Nerquaye-Tetteh et al., 1978). Similar fermented fish known as Pedah siam was processed in Thailand. The standard pH requirement for Pedah siam is 6.0-6.4 with a pH of 6.5 or higher considered as indicative of poor quality (FAO, 1971). In contrast for Adjuevan, pH values ranging between 5.2 and 6.1 were reported by Koffi-Nevry et al., (2011). No literature on the recommended pH range of African fermented fish products is available (Anihouvi et al., 2012). But, since in the processing of African fermented fish products, a seemingly deteriorated fish must be used, the high pH values around 7 or above 7 obtained is expected and may be considered as the usual pH value for these fermented fish products. Such pH fit well with the strong but not repugnant smell which characterize the majority of African fermented fish products (Essuman, 1992; Sanni et al., 2002; Anihouvi et al., 2009).

The pH of fresh hilsa fish (Tenualosa ilisha) decreased significantly ($P<0.05$) from 6.24 to 5.88 after dry salting and thereafter slowly declined to 5.17 after 120 days and then slightly increased and reached to 5.28 at the end of 150 days of fermentation (Majumdar and Basu, 2010). Increase in pH during the later part of fermentation has been attributed to the formation of volatile basic compounds (Yatsunami and Takenaka, 1996). Lactobacilli are the major producers of lactic acid, responsible for the decrease in pH and the increase in acidity during the fermentation (Valyasevi et al., 2001). The pH,
moisture and salt contents of the finished product of Hatahata-zushi, a fermented fish product of sandfish (*Arctoscopus japonicus*) and boiled rice were 4-1, 552 g kg\(^{-1}\) and 41-0 g kg\(^{-1}\), respectively on a salt-free dry weight basis (Chang *et al.*, 1994).

Suchitra and Sarojnalini (2012) reported that the pH values were increased during natural fermentation of Ngari - a fermented fish product of Manipur under different temperature, due to non-involvement of lactic acid bacteria. It might also be related to the decrease in the population of fungi, since they prefer to grow satisfactorily in slightly acidic pH. The increased in pH allows bacteria to become dominant mainly the *Bacillus* spp. in Ngari.

### 3.4.2 Moisture

It was observed that during fermentation, the moisture content of fermenting fish varied considerably according to the type of fish (lean or fatty fish), the duration of the fermentation, the quality of salt used and the amount of salt as well. Anihouvi *et al.*, (2009) observed that the moisture content in cassava fish decreased from 73% to 46.9% after 8 days of fermentation for *Lanhouin*, while a decrease range from 78% to 57.6% was noted for *Momone*. The decrease in moisture content is emphasized during the sun drying which was the final step of the processing except for certain types of wet fermented fish products. Sun-drying step was not always sufficient to stabilize the fermented fish products. This step which was normally combined with salting (by addition of NaCl) had dual effects such as the lowering of the water activity (aw) level and a specific inhibitory effect on the growth of some species of microorganisms through the Na\(^+\) ion. So, the two steps (salting and drying) were interrelated to reduce the moisture sufficiently. The decrease in moisture was due to osmotic migration of salt into and water out of the fish (Horner, 1997; Itou and Akahane, 2000). Decrease in moisture led to increase in salt content and consequently extend shelf life of the products (Horner, 1997; Lopez, 1987; Kingley-Ekow, 1999). Majumdar and Basu (2010) reported that the moisture content decreased significantly (*P*<0.05) from 55% of fresh fish to 46% during fermentation hilsa steak after dry salting and further decreased to 42% after first 15 days in saturated brine. However, after
15 days of fermentation, the moisture increased slightly and reached to about 50%. Suchitra and Sarojnalini (2012) also reported the effect of temperature and time of fermentation on the moisture content during the fermentation of Ngari a fermented fish product of Manipur. The moisture content was reported in Chepa Shutki, a semi fermented fishery product prepared from Puntius spp. collected from the markets of Bangladesh in ranges from 39.62% to 46.89% (Nayeem et al., 2010).

Products with high moisture content (above 35%) were susceptible to attack by blowflies especially if the salt level in the product is low. This results in the development of maggots in some fermented fishery products during storage. At low moisture content (below 15%) the product was brittle and prone to fragmentation and attack by insects such as dermestes (FAO, 1992).

3.4.3 Protein

Variation in protein content was observed and depends on enzymatic and microbial activities during the fermentation. Decrease in protein content has been reported by various authors during the spontaneous fermentation of various fish products including Lanhouin, Momone, Guedj and Adjuevan and other fermented fish products (Yankah, 1988; Essuman, 1992; Abbey et al., 1994; Anihouvi et al., 2009; Koffi-Nevry et al., 2011).

In this regard, Anihouvi et al., (2009) reported that the protein content in cassava fish decreased from 75.6% to 54.8% dried weight basis after 8 days of fermentation during Lanhouin processing. This revealed a loss of 27.5% of the initial protein content of fresh fish. Similar decrease had also been reported by Abbey et al., (1994) for Momone. Regarding Adjuevan, protein content of the fermenting fish decreasing from 53.93% to 25.66% was observed (Koffi-Nevry et al., 2011). The decrease in protein content of the fermenting fish samples was explained by proteolysis effect during which proteins are broken down into peptides and amino acids which could be lost in the exudates (extracted water) from the fish. In this respect, Abbey et al., (1994) had reported a protein content of 12% in the exudates collected during the fermentation of Momone. Majumdar et al., (2005) reported the mean value for
moisture, ash, protein and lipid content of *Lona ilish* a salt fermented fish product were 54.35%, 16.73%, 17.56% and 9.41%, respectively. Analysis showed that *Tungtap* - a traditional fermented fish product of Meghalaya was a good source of protein (40.6g/100g) (Agrahar-Murugkar and Subbulakshmi, 2006).

The protein content was reported in *Chepa Shutki*, a semi fermented fishery product prepared from *Puntius* spp. collected from the markets of Bangladesh in ranges from 58.26% to 65.75% (Nayeem *et al.*, 2010). Amano (1962) observed a loss of 30% nitrogen in the fermenting fish products of fermented fish, *Shiokario*. The fraction of the decomposed proteins remains in the fish muscles as free amino acids content increased steadily indicating that some polypeptide was being formed in addition to amino acids. This degradative process however brings out certain characteristics flavour that is essential for the quality of the final product (Amano, 1962; Ito and Sato, 1963).

The changes in nitrogenous compounds of the fish during fermentation are associated with denaturation of proteins, increase of free amino acids and other forms of non-protein nitrogen in the muscle tissue of the fish (Majumdar and Basu, 2010). Increase of protein nitrogen (PN) after dry salting may be due to the fact that the loss of water has been more than the loss of PN during this time.

3.4.3 (i) Total volatile base nitrogen (TVB-N)

Chemical compounds such as total volatile nitrogen (TVN) and biogenic amines (e.g. histamine) which normally did not exist in fish muscle are mostly formed in the fermented fish products as the result of autolysis and microbial spoilage of fish. In this respect, total volatile nitrogen (TVN) contents varying between 294.5 - 374.5 mg N/100g and 295.4 - 394.8 mg N/100g were recorded in *Lanhouin* and *Momone*, respectively (Anihouvi *et al.*, 2006). High level in TVN resulted from the formation of nitrogenous basic compounds, such as ammonia, due to the protein degradation through microbial and enzymatic activities.
Spoilage of fish is accompanied by the release of several volatile compounds like dimethylamine, trimethylamine, ammonia, trimethylamineoxide etc. which are produced by both bacterial and endogenous enzymes (Lannelongue, 1980). The concentration of these compounds in the tissue may indicate the degree of spoilage, particularly in the later stage of spoilage. TVB-N content was in the range of 15 to 18 mg% in fresh fish, molluscs and crustaceans and it is one of the most common indices of quality used universally for deciding the state of freshness of seafood.

Kimura and Kiamakura (1934) suggested that volatile base nitrogen level of 10 mg% or less for fresh fish, 20 to 30 mg% at the beginning of spoilage and over 30 mg% for spoil fish. The recommended range for cured fishery products is 200 mg%. The wide variation in the levels of TVB-N are due to differences in the composition, bacterial flora, handling methods etc. (Balachandran, 2001). The gradual increase of TVB-N at the later stage of maturation is probably due to the enzymatic and bacterial action of the fish (Connell, 1995; Oetterer et al., 2003). The high value of NPN and TVB-N might be attributed to the subsequent microbiological and biochemical changes in the fish muscle during the drying and fermentation process. The value of NPN (540 mg%), AAN (163 mg%) and TVB-N (48 mg%) of *Lona ilish* indicate degradation of tissue protein that may possibly be responsible for the generation of typical flavor and aroma of the final product (Majumdar et al., 2005).

The similar high value of total volatile nitrogen (TVN) contents were recorded by 9 other workers (Nerquaye-Tetteh et al., 1978; Abbey et al., 1994). Level of total volatile nitrogen (TVN) in fish was commonly used as a spoilage indicator (Silva et al., 1998; Pearson, 1976; Kerr et al., 2002). TVN measurements indicate the extent of the breakdown of proteins due to bacterial and enzymatic action, leading to amines production and thus a low nutritional value of the product (Kerr et al., 2002; FAO, 1971; Pearson, 1976). Pearson (1976) suggests that for white–fleshed fish, TVN levels below 200 mg N/kg indicate that the fish is fresh, whereas the fish would be rejected for human
consumption when the TVN level exceeds approximately 500 mg N/kg (Silva et al., 1998).

Anihouvi et al., (2006) reported the moisture, protein, salt, total volatile nitrogen and histamine contents ranged between 50.1- 56.6%, 24.6 - 26.5%, 5.2 - 7.3%, 294.5 - 374.5 mg N/100g and 21.4 -33.1 mg /100g, respectively in case of a fermented fish product lanhouin processed from cassava croaker/cassava fish (Speudotolithus spp.) or Spanish mackerel/king fish (Scomberomorus tritor), widely used as condiment in Benin, Togo, and Ghana. Gradual increases in total volatile nitrogen and thiobarbituric number contents and pH values were recorded during the processing of momone, a lanhouin - like fermented fish product (Yankah, 1988; Abbey et al., 1994).

1.4. 3 (ii) Amino acid composition

Amino acid composition of a fish product contributes significantly to its taste and also decides the quality of the protein. Glycine, alanine, serine and threonine taste sweet, while arginine, leucine, valine, phenylalanine, histidine and isoleucine give a bitter taste (Sikorski and Kolakowska, 1990).

Dincer et al., (2010) mentioned that fish sauce was produced by incubating mixtures of sardine (Sardina pilchardus) at 6 different concentrations of sodium chloride and glucose at 37°C for 57 days. High amounts of glutamic acid, alanine, lysine, leucine and aspartic acid was recorded in case of all groups of sauces. The comparison of amino acid composition of fish sauce in different groups showed that lysine was the most abundant essential amino acid in the study. When the contents of non-essential amino acids in the raw material were compared with sauces, the content of aspartic acid was found to be higher in each group. After fermentation, the contents of aspartic acid, glutamic acid, histidine and hidoksil-L-proline were increased, whereas, the content of others were significantly decreased in fish sauce group in a comparison with raw sardine.

In the degradative changes occurring during fermentation, no significant changes were observed in the amino-acids particularly the essential ones. The degradation process, however, brings out certain characteristic
flavours which are essential for the quality of the final product (Amano, 1962; Ito and Sato, 1963).

Ijong and Ohta (1995) stated that the traditional product fermented under variable temperature has lower total amino acid content than the laboratory products. Also, glutamic acid, lysine and isoleucine are found to be the predominant amino acids in Bakasang produced with 100g/kg salt. They also noticed a lower liberation of amino acids under variable temperature than when fermented at constant temperature. Majumdar et al., (2005) reported the significance decrease in the proportions of amino acids in salt fermented fish product Lona ilish compared to raw hilsa fish. Lysine has been reduced to a greater extent in the Lona ilish as compared to the raw hilsa fish. Meister (1965) reported disappearance of cysteine and found taurine in the fish sauce. Majumdar et al., (2005) mentioned that the loss of amino acid content during fermentation might be probably due to the formation of derivatives of amino acids such as amines and gluconeogenic substances.

Rabei et al., (2009) observed that the total concentration of free amino acids increased in Egyptian salted-fermented fish (Feseekh) during ripening (20 days) and storage (40–60 days) from 8 to 72 g/kg (dry weight) after 60 days of storage. The predominant free amino acids were leucine, glutamic acid, lysine, alanine, valine, aspartic acid, isoleucine and citrulline.

Fermented blue mussel sauces (FBMSs) (Mytilus edulis) were prepared with 25% NaCl (w/w) at 20°C for different fermentation periods and it was observed that content of protein increased and carbohydrate decreased throughout the fermentation. In addition, the levels of amino acids such as glycine, alanine, proline, aspartic acid and glutamic acid were higher in case of, which may be important for the taste of fish and shellfish sauce (Park et al., 2005).

Amino acids analysis showed that the glutamic acid was the highest among all other amino acids in both fresh and salted-fermented fish. Whereas the levels of glycine, alanine, isoleucine, phenylalanine, lysine and proline were significantly increased in salted-fermented fish, those of tyrosine, histidine and arginine were reduced in salted compared to fresh fish.
Glutamic acid contents was also found to be quite high in *Mola* (*Amblypharyngodon mola*), *Chela* (*Chela phulo*), *Chapila* (*Gudusia chapra*) and *Punti* (*Puntius stigma*) ranging from 5.76% to 6.96% with the highest in *chela* and the lowest in *punti*. On the other hand, cysteine content was quite low ranging from 0.20 to 0.31% with the highest in *chela* and the lowest in *mola* (Nurullah *et al.*, 2003).

The World Health Organisation recommended leucine and isoleucine requirements for adults of 14 and 19 mg amino acids/kg body weight per day (FAO, 1986).

### 3.4.4 Lipids

Fish with high levels of lipids are prone to oxidation and become rancid as microbial spoilage occurs (Jay, 1992). Because of the unsaturated nature, fish body oils are susceptible to oxidation and also easily develop rancid and unacceptable odors and flavors during storage (Waterman, 1976). Once fatty compounds are oxidized, the breakdown products of lipid oxidation potentially can react with proteins and vitamins, leading to a loss of nutritional value and quality of the fish and fish products.

#### 3.4.4 (i) Peroxide value (PV)

Oxidative rancidity is one of the most important factors that determine the acceptability of the fish during processing and storage. Balachandran (2001) stated that the peroxide value is a measure of the first stage of oxidative rancidity. Lakshmanan (2000) mentioned that if the PV is above 10 to 20 miliequivalent O$_2$/kg of fat, then the fish will smell and taste rancid in all probabilities. Certain pro-oxidants, such as haem, in the proteins catalyze the oxidation reaction. Similarly, iron impurities in the crude solar salt used for curing also accelerate auto-oxidation (Saisithi *et al.*, 2006). Oxidized fish oils have a characteristic taste and paint-like smell, but the acceptability of products having the typical taste and flavour of oxidized fats depends very much on local preferences. The products of fat oxidation take part in further reactions especially with amines and with other decomposition products of proteins to produce coloured compounds as well as substances with odour (Saisithi *et al.*, 2006). Lipases present in the fish flesh also hydrolyze the lipids.
(Lovern, 1962), but the extent is dependent on the level of salting and fermentation (Amano, 1962).

The PV had been reported as 41.3 meq O$_2$/kg fat in salted anchovy after 9 weeks of fermentation (Hernandaz-Herrero et al., 1999). The PV for Lona ilish, a traditional salt fermented fish product of northeast India was also recorded as 40.0 meq O$_2$/kg (Majumdar and Basu, 2010).

3.4.4 (ii) Free fatty acid (FFA)

The free fatty acid content is the most popular measure of lipolysis in fish. It correlates closely with time and temperature of storage and depends on fish species (Sikorski et al., 1987). Free fatty acids value is a measure of the extent of oxidative deterioration in oily fish, but it can fall further at latter stages of fish spoilage (FAO/SIFAR, 2001). A high level of FFA is characteristic of product that have undergone both microbial and biochemical spoilage (Pearson, 1976; Huss, 1988; Horner, 1997; Tungkawachara et al., 2003).

FFA contents ranging from 11 to 14% oleic acid, and 27.2 to 36.6 % oleic acid were recorded on market samples of Lanhouin obtained from cassava fish (Pseudotolithus spp.) and king fish (Scomberomorus tritor), respectively. Similar increase in FFA contents during the fermentation of Momone and the ripening of salted Anchovies has also been reported by Abbey et al., (1994) and Hernández-Herrero et al., (1999). The increase in FFA showed that salt did not inhibit lipases responsible for the liberation of free fatty acids. Such liberation has been indicated by Roldan et al., (1985) and Perez-Villareal and Pozo (1992). This may indicate that greater proportions of unsaturated fatty acids were liberated and were subjected to oxidative splitting at the double bonds. The resulting substances, mostly ketones and aldehydes, appear to be largely responsible for flavor, odour and taste of the fermented fish products. Most fat acidity begins to be noticeable to the palate when the free fatty acid values calculated as oleic acid is about 0.5 – 1.5% (Pearson, 1976). The FFA value had been reported as 31.84% oleic acid in salted anchovy after 9 weeks of fermentation (Hernandez-Herrero et al.,
1999) and as 18.22 % oleic acid in case of Lona ilish, a traditional salt fermented fish product of northeast India (Majumdar and Basu, 2010).

3.4.4. (iii) Fatty acids composition

The lipid in fish muscle can influence product quality through interaction with other components. The degradative changes like lipolysis and auto-oxidative deterioration of unsaturated fatty acids, resulting in product deterioration and undesirable aroma and flavours (Ackman and Takeuchi, 1986). Lipolysis constitutes the prior step to free fatty acid autooxidation. Following the release of fatty acids, secondary reactions of fatty acids result in the development of numerous oxidation products, such as aldehydes, ketones and alcohols that are responsible for the flavor characteristic of meat products (Berger et al., 1990; Bolzoni et al., 1996; Flores et al., 1997; Garcia et al., 1991; Lopez et al., 1992). Lipolysis and oxidation have been widely studied in dry sausage (Dainty and Blom, 1995) and dry-cured ham (Buscailhon et al., 1994; Moltilva et al., 1994; Toldra et al., 1997). Changes in the fatty acid composition in intramuscular fat during processing have been reported for French (Buscailhon et al., 1994), Serrano (Flores et al., 1997; Moltilva et al., 1994) and Iberian dry cured hams (Cava et al., 1997; Ordonez et al., 1996).

Recently, the lipids in fish muscle have received much interest as a source of EPA and DHA fatty acids in human diets. Lipid and fatty acids compositions of many marine fish and shell fish as well as the effect of different diets on lipid compositions of these marine species have been investigated (Ackman and Takeuchi, 1986; Viswanathan and Gopakumar, 1984; Halver, 1980). Suzuki et al., (1986), Viola et al., (1988) and Bieniarz et al., (2000) have investigated some of the factors causing changes in the composition of fatty acids in various fish species. Data on fatty acid composition aid food scientists and nutritionists in dietary formulation, processing and product development (Jadranka et al., 2003).

Detailed information about lipid components and their fatty acids constituents is needed to understand how to diminish oxidative or hydrolytic factors which affect quality of fish (Ugoala et al., 2008). Also, fatty acids composition is the surest method of determining the selectivity of a
hydrogenation reaction since fatty acids profile will aid in determining oils suitable for the production of solid fats for industrial uses (Buckley et al., 1989).

Fish lipids are valuable products, which have well documented health benefits (Calder, 2003; Vanschoonbeek et al., 2003). Marine fish oil preparations contain considerable amounts of unsaturated fatty acids of >20 carbon atoms like eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6); while, most of the freshwater fish lipids contain fatty acids of <20 carbon atoms (Bligh et al., 1988). EPA and DHA contents of total fatty acids vary from 5 to 20% in most marine fish and from 3 to 5% in shellfish (Amit et al., 2011) which gives marine fish an edge over freshwater fishes.

Visessanguan et al., (2006) reported that, as the fermentation proceeded, increase of fatty acid contents, in both total and non-polar lipid fractions, was observed, with a corresponding decrease in the quantity of fatty acids of the polar lipid fraction. Even though the effect on the composition of fatty acids was almost negligible. An increased amount of fatty acid in non-polar lipid fractions may contribute to the greater free fatty acid content and might partly come from the hydrolysis of phospholipids. Besides the lipolytic activity of both muscle and microbial lipases, the curing process generally resulted in an increase in saturated fatty acids (SFA), such as myristic, palmitic, and stearic acids, and decreases in monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), e.g. oleic and linoleic acids (Martíń et al., 1999).

Dincer et al., (2010) reported that the major fatty acids were observed to be palmitic acid (16:0), oleic acid (18:1n-9), Eicosapentaenoic acid (EPA, 20:5n-3) and Docosahexaenoic Acid (DHA, 22:6n-3) in fish sauce produced by incubating mixtures of sardine (Sardina pilchardus) at 6 different concentrations of sodium chloride and glucose at 37°C for 57 days. Montano et al., (2001) also reported the presence of a good amount of docosahexanoic acid in traditional shrimp paste condiment of Philippines prepared from small shrimp Acates spp. These varieties as well as the quantity and quality of fatty
acids noticed might be due to differences in sub-species, diet, spawning cycle, season and environment.

3.5 Microbiological quality of fermented fish products

Over the years, a number of spoilage and pathogenic microorganisms, including lactic acid bacteria, *Pseudomonas* spp., *Staphylococcus* spp., *Salmonella* spp., *Clostridium perfringens*, *Clostridium botulinum*, *Escherichia coli* O157:H7 and *Listeria monocytogenes*, have been associated with fish and fish products. *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, *Clostridium botulinum* type A and B are the pathogenic bacteria of particular concern in fermented fish products. The presence of *Staphylococcus aureus*, *Streptococcus* spp. and *Escherichia coli* in fermented fish products might be attributed to poor handling practices and faecal contamination during processing and storage (Hazan, 1988; ICMSF, 1996). *Staphylococcus aureus* was regarded as a poor competitor and its growth in fermented food is generally associated with a failure of the normal micro flora (Nychas and Arkoudelos, 1990).

Pathogenic contaminants were detected in traditional fermented fish products of northeast India. It was reported that the count of *Bacillus cereus* was $<10^2$ cfu/g, whereas the number of enterobacteriaecae and *Staphylococcus aureus* were $< 10^3$ cfu/g in traditional fermented fish products *Hentak, Ngari* and *Tungtap* (Thapa et al., 2004). Approximately, 70% of the bacterial isolates from a nine-month-old Thai fish sauce were halophiles of *Bacillus* types (Saisithi et al., 2006).

Pathogens rarely multiply at high salt concentrations. However, Ababouch (1988) demonstrated that *Pediococcus halophilus* was able to produce histamine during long storage at ambient temperatures of 20 - 25°C. Toxins produced by *Clostridium botulinum* in poor quality fish before salting may be stable in the salted product (Huss and Valdimarson, 1990). *Escherichia coli* and *Staphylococcus aureus* can survive for many weeks in salted fish (ICMSF, 1986). In a study conducted by Nerquaye-Tetteh et al., (1978) to isolate various micro-organisms, no *Salmonella* spp. were isolated from samples of fermented fishery products obtained from the open markets in Ghana.
Anihouvi et al., (2006) reported the total viable count of the majority (83.5%) of samples was high in case of a fermented fish product *lanhouin* processed from cassava croaker /cassava fish (*Speudotolithus* spp.) or Spanish mackerel/king fish (*Scomberomorus tritor*). Count (log cfu/g) of total aerobic mesophilic ranged from 5.4 to 6.6 and 6.2 to 7.8 for samples of cassava fish and king fish, respectively. Micrococci and bacilli ranged from 5.0 - 5.4 log cfu/g and 3.7 - 4.1 log cfu/g, respectively. Coliforms and faecal coliform counts (log cfu/g) were lower than 1 for most of the samples, however in 6% of the samples, the coliform loads (log cfu/g) ranged between 1.47 and 1.84. Mould counts (log cfu/g) were lower than 1 in the case of a fermented fish product *lanhouin* processed from cassava croaker or king fish, while *Clostridium* spp. counts varied between 1.68 and 1.80 log cfu/g. *Staphylococcus aureus* was absent in the majority (82.3%) of the samples; however *Staphylococcus* counts (log cfu/g) lower than 1 were observed in the remaining of the samples. No *Salmonella* and no yeasts were detected in all the samples. Various authors have reported similar microflora in *momone* (Yankah, 1988; Abbey et al., 1994; Nerquaye-Tetteh et al., 1978) and in other fermented fish products (Essuman, 1992). Thapa et al., (2004) reported the presence of different starins of Lactic acid bacteria, *Bacillus* spp. and *Micrococous* spp. in different fermented fish products of northeast India. Microbial deterioration was observed in *fessiekh* produced in the Sudan.

Two common defects of salted fermented fishery products called pink and dun are the result of spoilage by red halophilic bacteria and a highly osmophilic fungus respectively. The red halophilic bacteria grow in brine solutions at temperatures ranging from 15° to 55°C.

From observations of the production methods of fermented fishery products, the low level of incidence of *Clostridium botulinum* poisoning may be mainly attributed to the high level of salt usage. Since there was some proteolytic activity in the fish fermentation process, it was therefore most likely that *C. bondinum* toxins may be inactivated. There was very little information on *Salmonella* food poisoning arising from the consumption of fermented fish in Africa, despite the unhygienic fish processing practices observed in many countries.
Moulds were often associated with dried fermented fishery products. Spores of moulds which were often present in the air and soil contaminate fish during processing. Insects and mites were also known to cause mould contamination by carrying the spores on their bodies.

Yeast such as *Candida* and *Saccharomyces* were also present in *Hentak, Nagri* and *Tungtap* - a few traditional fermented fish products of northeast India (Thapa *et al.*, 2004). Similar results were also reported from *Nam-pla* and *Kapi*–Thai fermented fish products (Watanaputi *et al.*, 1983). The moulds commonly associated with dried cured fish in storage were *Aspergillus halophillus; A. restrictus; Wallemia sebi; A. glaucus group; A. candidus; A. ochraceus; A. flavus* and *Penicillum* spp. (Crisan and Sands, 1975).

### 3.6 Organoleptic quality of fermented fish products

The physical and chemical changes that occur during fermentation determine the overall sensory qualities of salted/fermented fish products (Voskresensky, 1965). These changes were induced by enzymes which break down both proteins and fats. Sensory evaluation is a tool for assessing the quality of fish and fish products, if tests are designed properly and trained personnel are selected with a meaningful statistical analysis (Kramer, 1952). Govindan (1972) reported that organoleptic evaluation is a very important method in determining the acceptability of all food products. Farber (1965) developed a numerical scoring system for the sensory assessment of freshness. In the recent years, instrumental methods have been employed to assess the freshness of fish based on physical, chemical or biological properties of fish. These are being used to assess flavour, texture and colour (Burt *et al.*, 1976; Connell *et al.*, 1976).

The complex interaction of enzymatic activity and oxidation during the fermentation, along with bacterial production of volatile fatty acids may be responsible for the characteristic flavor and aroma of fermented fish products (Beddows *et al.*, 1980). A significant role of bacteria and muscle bacterial proteases in the process of fermentation and flavor and aroma producing process was recorded (Thongthai and Siriwongpairat, 1990). The aroma in
fermented fish product has been claimed to be derived from the activity of various types of halophilic bacteria (Van Veen, 1953).

In his study, Adams (1986) concluded that micro-organisms play little or no part in aroma production. Beddows (1985) isolated halotolerant organisms, *Bacillus* spp. (cocci) and used them in pure culture but none of them produced the typical fish sauce aroma. The development of a specific aroma in fermented fish sauces and pastes may not be due to the action of micro-organisms.

Micrococci/Staphylococci and yeasts, in spite of their lower number compared to LAB, played a significant role in producing the characteristic pigmentation (Varnam and Evans, 1991) and the production of flavour compounds (Coretti, 1977). Several attempts have been made to study the changes in colour of cured products and to find suitable processing methods with the use of additives to minimize these changes (Lubis and Buckle, 1990; Dinesh *et al.*, 1994; Shetty *et al.*, 1996).

Most fermented fishery products were made from fatty fishes. Lean fishes had sometimes been noted to give a less acceptable texture and flavour. The role of fats in the fermentation process had not, however, been studied in any detail. Anihouvi *et al.*, (2009) reported that the volatile bases particularly TMA, DMA and NH are associated with changes in the organoleptic and textural quality of fish.

### 3.7 Effect of salting in fish preservation

The reduction in the physico-chemical qualities with increasing storage period could be attributed to higher activities of the spoilage agents. Salting is one of the oldest treatments in food preservation. Salting as a method of preserving fish has been used for centuries and in many places around the world such as Asia, Europe and Latin America. The simplicity of the salting process, the low cost of production and the ease with which it combines with other preservation methods, such as drying or smoking, has led to its popularity and extensive use (Berhimpon *et al.*, 1991). It decreases the water activity and consists of transporting salt into food structures and is governed by various physical and chemical factors such as diffusion, osmosis and a
series of complicated chemical and biochemical processes (Turan et al., 2007). Salt causes plasmolysis and alters protein and enzyme states in such a way that proteins become impervious to enzyme action and lose their efficacy. Salt acts as a bacteriostatic and a bacteriocidal agent when present in sufficient concentration (Beatty and Fougere, 1957; Ismail and Wootton, 1982). This property of salt has been frequently used in food processing and is the basis for the preservation of salted fish. Salting is mainly used to preserve products and also to promote important sensorial changes that make the final product appreciated by consumers (Andrés et al., 2005). Salting is usually performed by dry, brine or injection salting or a combination of these methods.

The preservative effect of salt has been recognized as being due to a decrease in water activity, less susceptibility to microbial attack and enhancement of functional properties, leading to an increase of the shelf-life time (Harris and Tall, 1994). There is usually a certain degree of fermentation involved in the salting of many fatty fishes. Sodium chloride (NaCl), also called salt, common salt, and table salt, is generally recognized as a safe (a status sometimes abbreviated by the acronym GRAS), antimicrobial and incidental food additive (Karacam et al., 2002). Salt has been used for centuries as a seasoning and flavor enhancer as well as a preservative or curing agent (Jay, 1992). Salted fish products have been shown to be safe for millennia, even in developed countries (Turan et al., 2007).

The use of salt in fish preservation is not limited to dry application. Salt is an important additive in the preparation of fermented, pickled, or processed fish or fish products. In the making of fermented fish, known concentrations of salt are added to promote degradation of proteins and retard the growth of undesirable, putrefactive microorganisms. Also, this allows desirable, NaCl-tolerant (halotolerant), fermentative species such as lactic acid bacteria to grow.

Pickled fish are marinated in salt brine or brine containing vinegar. Curing salt (containing sodium nitrate, NO₃) can be added to the pickle to delay spoilage and control microbial activity during storage (Pederson and Meyland, 1981). A lower brine concentration is known to increase the water
holding capacity and higher yields are obtained when compared to salting with saturated brine (Slabjy et al., 1987). The yield or the weight gain of salted products depends not only on the brine concentration, but also on the brining time and temperature. The weight gain of salted herring at low brining temperature was higher than that at high brining temperature and increased weight gain seemed possible by further extension of the brining time (Birkeland et al., 2005).

In manufacturing processed fish products, adding certain amounts of NaCl assists in the extraction of salt-soluble proteins and the formation of a sticky paste of fish meat. The development of the gelled paste might be due to the formation of a protein network structure or polymerization of myosin-heavy chains (Kumazawa et al., 1995).

Salted sun-dried fish are more prone to oxidation than fish preserved by other methods because of their exposure to light and oxygen (Smith and Hole, 1991). Use of crude NaCl (which contains impurities such as chlorides, sulfates, calcium, and heavy metals) accelerates lipid oxidation during fish processing and will adversely affect the overall quality of the finished product (Yankah et al., 1996).

Ahmed et al., (2010) reported the chemical and microbiological quality changes of salted (25% of the fish weight) Kass (Hydrocynus forskalii) during storage at ambient temperature (37±1°C) and found the optimum shelf life to be three months. El Hag et al., (2012) also reported the similar result of salted (25%) Kawara (Alestes spp.) of Sudan. Majumdar et al., (2005) reported that the moisture, protein, fat, ash content and pH was 54.35%, 17.56%, 9.41%, 16.73% and 5.66, respectively in case of Lona ilish - a salt fermented fish product of northeast India. During fermentation of hilsa steak a slight increase in the moisture content was noticed during the later part of fermentation (Majumdar and Basu, 2010), which can be attributed to the absorption of water by fish tissues as they swell when immersed in salt solution during a prolonged storage (Wheaton and Lawson, 1985, Hernandaz-Herrero et al., 1999). During salting, moisture is rapidly removed from the fish and on the
other hand salt penetrates in to the flesh (Clucas, 1982). Hernandaz- Herrero et al., (2002) also observed similar trend during fermentation of salted anchovy.

The changes in nitrogenous compounds of the fish during fermentation are associated with denaturation of proteins, increase of free amino acids and other forms of non-protein nitrogen in the muscle tissue of the fish.

The salting of cod causes protein denaturation, leading to a small loss of protein during the re-hydration process, due to protein aggregation and precipitation and the short soaking time (Ito et al., 1990, Tambo et al., 1992, Thorarinsdottir et al., 2002). The salting period depends on several factors including the desired ripened characteristics in fish, the fish species, the amount of salt used and the storage temperature. Salting has no adverse effects on the value of the fish protein and bacterial growth can be significantly retarded by the presence of sufficient quantities of common salt (sodium chloride). At salt concentrations of 10% in the fish, the activity of most bacteria that cause spoilage is inhibited.

The total viable penetration of salt into the fish muscle is dependent on many factors; including the thickness of the fish, osmotic pressure, temperature, purity of the salt, freshness of the fish, and the fat content of the fish (Ingram and Kitchell, 1967). Salting of fish was usually accompanied by protein losses, as water is drawn out a meal brine is formed, some protein is dissolved into the brine as storage period continues (Clucas, 1981). Therefore, the products contain the hydrolytic product of the fish proteins as well as some unhydrolyzed substrates (Mojica et al., 2005). Generally, the quantity of protein lost depends on the exact nature and duration of the salting process and the conditions of fish when salted (Eltom, 1989). The changes in salt penetration rates closely follow changes in extractable actomyosin in muscle, indicating a dependence of the change on the degree of denaturation of fish muscle proteins. Salt causes the proteins in fish muscle to swell and the protein becomes denatured if it increases in the muscle (Hamm, 1994). Lawrie (1990) stated that crude protein decreased with storage of cured meat and this was attributed to some changes during storage that caused by maillard reaction, where in carbonyl groups of reducing sugars react with amino groups
of protein and amino acids non-enzymtically, and might also be due to an attack of myoglobin by bacteria during storage and changes in pH.

The hydrolysis of protein substances is brought about by the action of peptide hydrolases of cathepsin A, B and C types as there exists a close agreement between their optimum pH values and those in the fish muscle tissues (Shenderyuk and Bykowski, 1990). Van Claveren and Legendre (1965) reported that endo- and exo- peptidases from fish were affected differently by gradually increasing salt concentrations. They showed that when salt concentrations exceed 15%, the catheptic activity slows but continues at a reduced rate even in saturated brines. Uyenco et al., (1953) studied the influence of different salt concentrations on the hydrolysis of fish protein and pH of the fish sauce and found that the amount of amino nitrogen formed varied inversely with the salt concentrations below pH 6.

During the processing of mackerel *narezushi*, the fish body was strongly dehydrated by permeation of salt, the low pH of fish meat and pressure applied to the fish and rice mixture. In the proximate components, moisture, protein and lipid flowed out from the fish meat (Itou and Akahane, 2000).

Brine salting also had a significant effect on the microbial load of fish. This effect was also present after long time of storage for fifty six weeks in sterile closed plastic containers. At certain concentrations, salt was found to prevent growth of many microorganisms by exerting a drying effect on microbial cells and tissue, which concentrates solutes in them, creating an environment unsuitable for microbial proliferation.

Salt solubilizes the functional myofibrilar protein in meat and activates the protein to increase hydration and water-binding capacity, ultimately increasing the binding properties of protein to improve texture (Terrell, 1983; Desmond, 2006).

Improvements and advancements in technology worldwide have allowed even better use of NaCl by the food industry, such as production of processed fish products. Because salt does have its limitations and
disadvantages, its utilization conditions must be optimized to provide safe food for consumers, at the same time addressing their needs and concerns.

3.8 Irradiation preservation of fish and fishery products

The major quality problems with respect to fishery products are their contamination with microorganisms of diverse types. These organisms may be responsible for spoilage of the commodity and/or cause food borne disease to the consumers. In addition, several types of insects and parasitic worms are also encountered in fish and fish products (Devadasan, 2001).

Food irradiation is a process for the treatment of food products to improve microbial safety and to enhance their shelf life. Exposure of fishery products to ionizing radiation can effectively reduce or eliminate pathogens of public health significance, spoilage causing microorganisms, insects and parasites while maintaining wholesomeness and sensory quality of the commodity (Naik et al., 1991). This is done in a unique way, without denaturing the treated product, and without changing its palatability, as usually happens with heating (cooking, canning, frying), freezing, drying or smoking, etc. (Adam et al., 1982).

Electro-magnetic radiations, namely gamma rays and X-rays (5 MeV) having shorter wave length (<300 nm) and higher energy than the visible radiation, can cause ionization by removing electrons from the outer shell of atoms and molecules. Generally, ionizing radiation emitted by radioisotopes like cobalt-60 and caesium-137 are used for food preservation. The treatment of fish and fishery products by ionizing radiations (e.g. gamma rays from Co-60 or Cs-137) contributes potentially to the preservation of fish.

During the irradiation of food, the major effect is the splitting of water molecules to yield free radicals and Hydrogen peroxide. Free radicals are highly reactive chemical species from which the useful effects of irradiation originate. In general, the site most sensitive to irradiation is DNA and modifications to the DNA of bacteria can result in their death or inability to reproduce. Food pathogens and food spoilage organisms can be thus be destroyed by irradiation (Armstrong et al., 1994).
The safe storage life of fish can be considerably prolonged (two to three-fold at the minimum) by rather small doses (e.g. 100 - 200 krad) of ionizing radiation without any detectable change in flavour, odour, texture and appearance, i.e. the sensory quality characteristics of the fresh fish or fishery product (Devadasan, 2001). In addition, irradiation has also been advantageously combined with other usual food preservation methods, as e.g. with boiling, drying or salting, where such processed commodities have to be rendered less perishable. A further special feature of fish irradiation is that the fresh or processed product can be irradiated in the final packing because of the easy penetration of gamma rays through packaging materials.

A dose of 0.1 kGy can damage 2.8% of the DNA in bacterial cell, 0.14% of the enzymes and 0.005% of amino acids. Thus, irradiation can be used to improve food safety, extend shelf life and minimize losses.

The FAO/IAEA/WHO Joint Expert Committee on Food Irradiation (JECFI) has declared that the irradiation of any food up to an overall average dose of 10 KGY causes no health hazards. On the basis of JECFI’s findings the Codex Alimentarius Commission (CAC) developed general standards for irradiated foods and a recommended International Code of Practice for the operation of radiation facilities used for treatment of foods. In India, irradiation of seafoods has been approved by Government of India, as per the Gazette notification dated 2nd May, 2001.

3.8.1 Effect of irradiation on microbial quality of fish and fish products

The microbiological safety of irradiated food is fully comparable with that of foods preserved by other acceptable preservation methods. Parasites and insects can also be killed with very low doses (1 KGY) radiation without causing significant changes in the physico-chemical or sensory properties of the treated food. Mould and insects in dried fish can be destroyed at 3 to 5 KGY dose of irradiation (Devadasan, 2001).

Gram negative bacteria, including pathogens such as *Salmonella* and *Shigella* are generally more sensitive than gram positive bacteria. Vegetative cells are the most radiation sensitive whereas the bacterial spores are markedly more resistant. Low (up to 1 KGY) and medium (1-10 KGY) radiation
treatment essentially brought about suppression of spoilage-causing gram negative organisms such as *Pseudomonous, Proteus, Aeromonous* etc (Venugopal and Bongirwar, 2002). Studies on radiation survival of *E. coli, S. typhimurium, Shigella flexneri, Streptococcus faecalis* and *Staphylococcus aureus* in soft shell clam and mussel tissue have been reported. As compared to bacteria, viruses require higher radiation doses for inactivation. Irradiation does not prevent enzymatic spoilage completely. Irradiation at a dose of 2 kGy was adequate significantly eliminate different pathogens, including *Shigella* spp. and *Staphylococcus aureus* from frozen shrimp. A dose of 4 kGy has been found to be adequate enough for elimination of non spore forming pathogens in different kinds of frozen foods, including seafood.

### 3.8.2 Effect of irradiation on biochemical quality of fish and fish products

Proteins and amino acids can be affected by irradiation but not usually to the extent that they lose their nutritive value. At a dose upto 1 kGy, nutrient loss is insignificant but at doses at between 1 and 10 kGy, some losses may occur. While irradiating fatty fish, care should be taken to perform it at low temperatures and in the absence of air. Otherwise, free radicals can cause oxidative problems. Irradiation of cod and mackerel at doses up to 4.5 kGy causes no changes in the biological of value (BV) or Net Protein Utilization (NPU) of the fish which are the accepted tests for protein quality (Hafez *et al.*, 1985).

Naik *et al.*, (1991) reported that free amino acids and amino acids of proteins are sensitive to radiation. Free radicals formed by radiolysis of water, namely hydroxyl, hydrogen aqueous electron react with amino acids leading to abstraction of hydrogen and reductive deamination.

The radicals produced will react further by disproportionate. These reactions are followed by decarboxylation and deamination giving rise to ammonia and pyruvic acid, for example, in case of alanine. In the presence of oxygen, oxidative deamination replaces reductive deamination. Cystine, cysteine and methionine act as scavengers and react more readily with free radicals than the non sulphur containing aliphatic amino acids. The aromatic amino acids phenylalanine and tyrosine react readily with the transient species.
of water radiolysis, hydroxylation of the aromatic ring being the principle reaction. Phenylalanine hydroxylation to form tyrosine isomers. Hydroxylation converts these two dihydroxy phenyl alanine (DOPA) catalyzed by the phenyl oxidase. Subsequent oxidation of DOPA and polymerization can produce melanin type pigment (black spot), as observed in the case of shrimp (Javamard et al., 2006).

Oraei et al., 2011 reported the combined effect of low-dose gamma irradiation (1, 3 and 5 kGy) and frozen storage (5 months at -20°C) on chemical and sensory characteristics of rainbow trout (Oncorhynchus mykiss) fillet. The irradiation process and frozen storage time had a significant effect ($P<0.05$) on total volatile nitrogen (TVN), peroxide value (PV), thiobarbituric acid (TBA) and pH. The level of all of these factors increased with increasing frozen storage time. At the end of the fifth month of frozen storage, the lowest and the highest level of TVN, PV and TBA were corresponding to the irradiated samples at 3 and 5 kGy, respectively. In terms of the overall acceptability of their texture, odour, colour and taste, irradiated samples at 3 kGy had the best quality and remained acceptable after 5 months frozen storage. The optimum dose of gamma radiation of rainbow trout fillets according to chemical and sensory analysis was obtained at 3 kGy.

Jeevanandam et al., (2001) also reported that irradiation could significantly ($P<0.05$) extend the refrigerated shelf life of threadfin bream, salting prior to irradiation could enhance the acceptability of the irradiated fish.

The vitamins A, E, K are radiation-sensitive. Other vitamins e.g. riboflavin, niacin and Vitamin D are relatively insensitive. The changes in flavour, odour, texture and appearance of irradiated foods are small compared to those brought about by heat treatment.

Irradiation may influence the textural attributes of fish muscle. The treatment at 5 kGy enhanced the drip formation to level as high as 20% in Bombay duck, which could be reduced to 7-8% by pre-irradiation dipping in 10% solution of either sodium tri polyphosphate or sodium chloride. Treatment at a dose of 0.66 or 1.31 kGy caused a decrease in gel strength of
mince red hake (*Urophysis chuss*). The degree of textural changes in precooked lobster by irradiation at 1 kGy was comparable to that developed of storage for 3-4 months. Irradiation at 1.5 kGy did not affect the disperseability and viscosity characteristics of textural proteins of India mackerel (*Naik et al.*, 1991).

### 3.8.3 Extension of shelf life of irradiated fish and fishery products

Radiation sensitive gram negative bacteria are mostly responsible for spoilage of fishery products. Therefore, the reduction of spoilage causing microorganisms by low level of radiation leads to an extension of shelf life of fishery products. Radurization is done in the dose range of 1-3 kGy, which is sufficient reduce the initial load of spoilage causing organisms by about 1-3 log cycles (*Moini et al.*, 2009).

Research done at the Bhabha Atomic Research Centre (BARC), Mumbai, India, has shown the feasibility of radurization for a number of low and medium fatty tropical fish and shell fish. While the unirradiated controls had generally less than 10 days of acceptability at 0-2°C, the optimum dose and extended shelf life over controls at this temperature obtained for these fishery items include shrimp (dose, 1.5 kGy; 18-20 days), Bombay duck (1 kGy; 18-20 days), white pomfret (vacuum packaged to prevent oxidative rancidity) (1 kGy; 35 days), black pomfret (1 kGy; 25 days), seer steaks (1 kGy; 23 days), Indian mackerel (1.5 kGy; 25-28 days) and Indian salmon (1 kGy; 25 days). Similarly, salted and boiled chub mackerel irradiated to 2 kGy and then held at ambient temperature were reported to have a shelf life of 15 days compared to only three days for unirradiated control. Vacuum packaging was found to adversely affect the appearance of the irradiated product by drawing out muscle fluids during storage.

*Ozden and Erkan* (2010) reported 1223 studies on the wholesomeness of 278 irradiated food items including feeds and fishery products and concluded that irradiated seafoods are safe for human consumption. It is to be pointed out that the gain in microbiological or keeping quality attained by food irradiation has to be safeguarded by an effective control in the food irradiation facilities and by proper care of the product before and after processing.