Addis Ababa University

School of Graduate Studies

Faculty of Technology

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EFFECT OF LOW TEMPERATURE PRESERVATION ON THE PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITIEIS OF SELECTED FISH SPECIES OF LAKE ZIWAY

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Engineering

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Acronyms

AAU	Addis Ababa University
ANOVA	Analysis of Variance
APC	Aerobic Plate Count
BGLB	Brilliant Green Lactose Bile
DMA	Di-Methyl Amine
EC/E.Coli	*Escherichia Coli*
FAO	Food and Agricultural Organization
FC	Faecal Coliform
FFA	Free Fatty Acid
FPME	Fish Production and Marketing Enterprise
ISO	International Organization for Standardization
LST	Lauryl Sulphate Tryptose
MPN	Most Probable Number
ND	Not Detected
PFS	Partial Freezing Storage
SD	Standard Deviation
TC	Total Coliform
TPC	Total Plate count
TMA	Tri-Methyl Amine
TMAO	Tri-Methyl Amine Oxide
TVB-N	Total Volatile Base Nitrogen
TVC	Total Viable Count
TVN	Total Volatile Nitrogen

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Abstract

With the purpose of determining the effect of frozen storage on quality of fish fillet and evaluating the existing low temperature preservation technique in the case of Fish Production and Marketing Enterprise, samples from the same lot of commercially harvested and processed tilapia fish (*Oreochromis niloticus*) fillets were frozen at -18 ± 2^{o}c. The physicochemical and microbiological analyses were carried out at regular 15 days interval on tilapia fish fillets stored for up to 90 days. The fresh fish fillets were found to contain 18.52±0.08% protein, 0.37 ± 0.01% fat, 79.87 ± 0.01% moisture and 0.98 ± 0.01% ash contents. During the entire period of storage, the protein, moisture and ash contents were significantly ($p < 0.05$) decreased to 17.25 ± 0.088%, 78.50 ± 0.71%, and 0.88 ± 0.02% respectively. However, the fat content of the fish fillets increased significantly ($p < 0.05$) to 0.56 ± 0.01 after 90 days of frozen storage. The TVB-N and pH values were also increased significantly ($p < 0.05$) from 12.04 ± 0.48 mg N/100g and 6.43 ± 0.01 to 21.75 ± 0.35 mg N/100g and 6.61 ± 0.01 respectively. The total bacterial load in fresh fish fillets was reduced from $2.57x10^{6}$ to $8.2x10^{5}$ CFU/g after 90 days of frozen storage. The total coliforms and faecal coliforms were also decreased from 460 MPN/g and 23 MPN/g to 23 MPN/g and undetectable level, respectively. From these results it was concluded that freezing, if not properly used, has a negative effect on reduction of nutritional value of fish products. The storage of tilapia fish fillets under frozen condition showed a significantly ($p < 0.05$) higher deterioration of product quality. The nutritional quality, as estimated by the proximate composition analysis, underwent a gradual loss of nutrients until day 90, in agreement with loss of freshness of the fish fillets observed for the TVB-N. Furthermore, the results revealed that gradual biochemical changes reduce the quality of frozen fish fillets as the duration of storage increases. Under frozen condition, the overall nutritional quality of tilapia fish fillets was found to depend on duration of frozen storage.

1. Introduction

1.1 Background

Fish is one of the best protein sources available to the human body in quality and quantity. It is an important food in countries with long coastlines and extensive inland water resources. It is also a valuable source of vitamin A and B, iodine and oils containing polyunsaturated fatty acids. Food from the sea in general has given man satisfaction for both his nutritional needs and his gustatory appetites (Msangi and Griffin, 1974). There is considerable scope for increasing the production and consumption of fish in many developing countries. Global production from capture fisheries as well as aquaculture and the fish food supply is currently the highest on record and remains very significant for global food security, providing more than 15% of total animal protein supply (FAO, 2002). The total world capture production in 2004 was about 95 million tones and of this about 0.9 million tones were from inland waters (FAO, 2006). Most of the global total capture in inland waters came from the catches of Asia and Africa (About 64% and 25%, respectively). Inland fisheries are of great importance in Africa. In 2004, the total capture production in Africa was approximately 4.99 million tones of this 15,681(0.33%) tones were from Ethiopia (FAO, 2006).

Ethiopia is endowed with 7400 km^2 of lakes that harbour various fish species which are ecologically and economically important to the country (Tedla, 1973). Some of these species include Nile tilapia (*Oreochromis niloticus),* African catfish (*Clarias gariepinus),* Barb fish *(Barbus sp),* Nile perch *(Lates niloticus)* and others. The current total annual average commercial catch from some lakes in Ethiopia estimated at 13000 tones (LFDP, 1996). Rough estimates based on commercial catch and other parameters have shown that the country's inland water can support 30 to 40 thousand metric tones of fish per year (FAO, 1993). The estimates of the country's total fishery available so far are less certain because the data used for estimation were quite crude and also were based on short-lived surveys (Alem, 1993). However, it roughly shows the potential of the fishery resource, which can alleviate shortage of food (protein) in the country.

The potential yield compared with, the current fishing rate is low and accounted for only 30-40% (FAO, 1993). Considering the current growth of demand for fish products in Ethiopia, it is vital to systematically develop the fishery based on knowledge of the ecosystem to sustain the yield. The average per capita fish consumption in Ethiopia currently is about 240 g per person per year (LFDP, 1998). Since the methods used for fishing in most Ethiopian lakes are still primitive, decline in the fish catch of some commercially important fish such as tilapia, Nile perch, catfish barbus and labeo have been reported from Lakes Zeway, Awassa and Chammo (Alem, 1993). Thus, where there is an acute shortage of food (protein), there is an urgent need to use the country's fishery resource sustainably based on data obtained from scientific studies.

Fresh waters carry a surprisingly large number (40%) of fish in the river systems and lakes of the tropics (Lowe-Mc Connell, 1987). The fish community in the tropics is generally more diverse than fish in the temperate regions, particularly the tilapia (family *Cichlidae*) are the most successful fish found widely spread in the tropical and subtropical waters. They can tolerate a wide range of temperature, salinity and oxygen profiles (Balarin and Hatton, 1979). The majority of such fisheries are in Africa, but accidental and deliberate introductions of tilapia into freshwater lakes in Asia have allowed large fisheries to develop in countries with a tropical climate such as Papua New Guinea, the Philippines, and Indonesia (De Silva *et al*., 2004). These fish are economically the most important and are more accepted as food fish in Ethiopia because of their palatability and history of use from inland fisheries in the country (LFDP, 1996). In Ethiopia, tilapia *(Oreochromis niloticus)* is found in most rivers and lakes. Current estimates have shown that this species alone accounts for about 60% of the country's fishery and it is estimated at 80% of the total sales in 1996 (LFDP, 1998).

Ethiopia has a great potential for aquaculture development and the initiation of aquaculture seems to be one means of providing animal protein to the people. The current increasing market demand for fish protein in Ethiopia can be met only when the capture fishery is supplemented by aquaculture. The nutritive value of dishes prepared from fish is comparable to that of animal meat, but in some cases fish-based meals are advisable. Thus, consumers have become increasingly interested in fish as a source of dietary protein. However, of all flesh foods, fish is the most susceptible to tissue decomposition, development of rancidity, and microbial spoilage. Freshly caught fish spoil easily and need to be properly preserved. Fish begin to deteriorate as soon as they leave the water. Since fish spoil easily in a short period of time in the tropics if kept at room temperature, they will have to be preserved by freezing or by dehydration and other suitable processing techniques so that they can be transported over long distances and used over a period of time (Swaminathan, 1974).

Post harvest losses of fish reach 35% of the world's fishing catch (FAO, 1986). The Food and Agriculture Organization of the United Nations (FAO) has estimated that in some developing countries, post harvest losses of fish exceed those of any other commodity, often surpassing 50% of the landed catch (FAO, 1986). Reducing these losses could increase protein availability, improve nutritional status, and eliminate some of the need to import food. Simple preservation techniques, common in one part of the world, could be employed in others to reduce post harvest losses. Food preservation is the process of treating and handling food in such a way as to stop or greatly slowdown spoilage to prevent food borne illness while maintaining nutritional value, texture and flavor. In other words, food preservation aims at preventing the microbial spoilage of food products and the growth of food borne pathogens. Thus, the two principal goals of food preservation methods are: (i) increasing the shelf life of the food and (ii) ensuring the safety for human consumption (Andress and Harrison, 1999). The increased separation of the producer and consumer in an industrial society added another reason: food must be safe and palatable as it reaches the consumer (Fryer *et al*., 1997).

Many different techniques have been used to preserve fish quality and increase their shelf life. They are designed to inhibit or reduce the metabolic changes that lead to fish spoilage by controlling specific parameters of the fish and/or its environment. Low temperature storage is one of the different ways of preserving foods at industry level. Freezing, particularly, is one of the simplest and least time-consuming ways to preserve foods at home. The extreme cold in freezing simply retards the growth of microorganisms and slows down changes that affect quality or cause spoilage in foods. Freezing cannot improve the flavor or texture of any food, but when properly done it can preserve most of the quality of the fresh product (Andress, and Harrison, 1999). It is the best way of processing flesh foods so that they retain their fresh eating qualities (Kordylas, 1990). Proper preservation begins the moment the fish is hooked and pulled from the water. How you initially handle a fish can greatly affect its quality, taste and storage life.

1.2 Statement of the Problem

The main challenge that fish industries of the developing countries face is to comply with consumer expectations, particularly product quality. Lack of adequate infrastructure and technical expertise often attributed to quality defects. These in turn result in significant financial losses because of rejection and low prices for exports. The quality of raw material is the key factor that governs the final product quality. Therefore, the first critical control point for a fish plant's quality assurance system is the control of raw material at reception. Fishing in Ethiopia is constrained by lack of efficient infrastructure and marketing network. The infrastructure at fishing sites is underdeveloped as well as inadequate, and is devoid of transport facilities to link remote water bodies with the major consumers at distant. Harvesting and processing technologies are not in place, thus limiting the scope of marketing to the nearest local outlets where fish can be sold fresh immediately after catching. The Fisheries Resource Development Department and Fish Production and Marketing Enterprise are the government agencies vested with the responsibility of development and extension of inland fisheries processing and marketing. The FPME was established in February 1978 with the main objective of providing market security for fishermen so as to encourage the fishing market. The fish processing plant, which is located 10 Km from the center of Addis Ababa on the way to Debrezeit, is equipped with modern cold storage and has the capacity to process up to 15,000 Kg per day.

Nowadays, the company is exporting its products to Sudan and Saudi Arabia and preparations are underway to export products to the European markets. Although the fish products to be exported bear manufacturing and expiry dates, the dates are not in accordance with the Ethiopian Fish Standards set by Quality and Standard Authority of Ethiopia (QSAE). The cold storage life of white frozen fish at a storage temperature of $-20^{o}c$ is about four months (Ethiopian standard, ES 2825:2006). However, in the case of Ethiopian Fish Production and Marketing Enterprise without any scientific basis the shelf life labeled on the fish products is nine months. On the other hand, the fish products to be sold in the domestic markets have no manufacturing and expiry dates. They also lack proper labeling and packaging.

The other major and intractable problem in the field of fish technology which requires to be resolved is the variation in the stability of commercially important fish species (LFDP, 1998). The instability resulting from the denaturation and aggregation of proteins especially during frozen storage results in texture changes such as toughening of the muscle which makes the fish unpalatable. The responsibility for research in fisheries and living aquatic resources lies with the Sebeta Fish and Other Living Aquatic Resources Research Center within the Ethiopian Institute of Agricultural Research. However, the research center mainly concentrates on identification of fish species, and fish production and breeding. Some regional states have also their own agricultural research organization, but only two regions, Amhara and Oromiya, have organized fishery research center within their research organization. The Biology Departments of Addis Ababa University, Haramaya University, and Hawassa University also undertake basic research in fisheries. However, most of the researches carried out in the past have been fragmented with limited relevance for practical fisheries development and management.

No research has been attempted in the field of fish processing particularly preserving the qualities of fish except that the Sebeta Fish and Other Living Aquatic Resources Research Center is currently conducting a research on fish preservation by traditional drying methods such as salt drying, sun drying, and smoking.

On the basis of these problems, the rationale of this study is to evaluate the present low temperature preservation technology and come-up with baseline information that would help the Fish Production and Marketing Enterprise and other fish processing companies in Ethiopia in order to preserve fish products without deterioration of the quality. Additionally, the study can contribute to improve the existing preservation techniques that may help penetrate new market outlets with quality products.

1.3 General Objective

The general objective of the thesis work is to study the effect of low temperature preservation on the physicochemical and microbiological qualities of tilapia fish fillet of Lake Ziway

1.4 Specific Objectives

1. To assess the proximate composition and microbiological qualities of fresh and preserved fish.
2. To determine total volatile base nitrogen, which is an indicator of quality loss in fresh and preserved fish.
3. To evaluate post-harvest changes occurring in microbial load and physicochemical quality of fish products during low temperature storage.
4. To evaluate the existing low temperature preservation technique used by Fish Production and Marketing Enterprise.

2. Literature Review

2.1 Fish Production and Marketing in Ethiopia

Aquaculture is a food production technology whereby fish or other aquatic organisms are grown in managed systems that produce greater harvest than would naturally occur. Aquaculture is still virtually non-existent in Ethiopia, despite favorable physical conditions. Over 20,000 fish species are known globally. In Ethiopia, over 200 species of fish are known to occur in lakes, rivers and reservoirs (FAO, 1993). The potential yield estimates from the major lakes in Ethiopia, based on the survey of FAO is summarized in table 2.1.

Table 2.1 Empirical estimates of fish yield of major Ethiopian lakes based on surface area, mean depth (MD) and morpho edaphic index (MEI)

Lake	Potential annual yield (tons) based on:		
	Area	MD	MEI
Chamo	5500	3500	Not available
Abaya	10100	9800	18200
Awassa	1200	600	1200
Shala	4300	1100	12200
Langano	2700	1300	3400
Abijata	2500	1700	2650
Ziway	4500	5900	8600
Koka	2400	1000	1500
Tana	24900	28000	22400
Fincha	1700	Not available	Not available
Total	59800	_	_

Source: FAO (1993)

A conservative estimated potential yield between 30,000 and 40,000 tones per year can be given for the main water bodies of Ethiopia. Furthermore, the rivers fishery potential yield is roughly estimated at about 5,000 tones per year (FAO, 1993). Estimates of the potential yield have been calculated for individual lakes based on surface area, mean depth and conductivity (Table 2.1). Due to inadequate data collection system, these estimates should be regarded as conservative.

Development and management of aquatic resources in Ethiopia is largely at the infant stage. Commercial fishing is new practice in the Ethiopian lakes and it was started in the 1950s.The production in most Ethiopian water bodies is far below the estimated potential yield (30 to 40% on average). The total estimated landing from the major lakes, for example, in 1996/97 was about 8855 tons (FAO, 1993). The bulk of the production is made of Nile tilapia, Nile perch, Barb, Catfish, African catfish, and Labeo species. The most important species is tilapia which accounts for nearly 60% of the landing. In Tana, Chamo and Abaya, Nile perch is also caught in significant quantities. Ethiopia has less fish production as compared with the 70 million people.

Most of the landing is consumed within Ethiopia. Only very little amount of the landing is exported to middle-east markets. Since 1990, the FPME has tried to develop fresh water fish export to middle-east markets, particularly to Saudi Arabia and Sudan. The maximum amount so far exported was about 245 tones valued US$698,012 (FPME's unpublished data, 2007).

2.2 Fish Handling and Processing

In modern fish processing plants, especially the small-scale ones, flake ice generators dominate as flake ice ensures major contact surface with fish. Products are delivered direct to shops where they should be placed in cold stores and if necessary ice should be added. Good trade practice indicates that retailers should only keep a one-day stock of cooled fish or fish products such as fillets, deheaded and gutted fish (FAO, 1994).

The traditional methods of fish handling, processing and marketing in Ethiopia continue to flourish. Those groups of traders from Addis Ababa, who hold licenses from the Fisheries Resource Development Department of the Ministry of Agriculture to sell fish in their retail shops, carry whole fresh fish without ice by rented open truck from Lakes Ziway and Koka to major towns such as Addis Ababa, Debrezeit and Nazret (Adama), especially during fasting periods. Smaller traders regularly use horses or donkeys to reach less accessible destinations.

Informal fishermen on Lake Chamo have to walk daily 10 Km to Arba Minch, carrying fish in sacks on their heads, in order to bring their catch to the traditional markets, as there is no road access to the lake shore. In the case of Lake Tana, women gut and skin the fish, remove the gills and tie about 2 Kg in a bundle by threading a reed through the eyes of the fish. Then, they sell these products either to hotel owners or direct to the consumers in the street (FAO, 1993).

Traditional processing of fish includes only sun drying, while smoking has been tried to meet the market demand of the expatriate community in Addis Ababa. Dried fish, known locally as ‘’kuanta’’, is prepared by fishermen. Fish is filleted, cut into large strips and hung up to dry on strings for two to three days. The dried fish is packed in to sacks for storage on the ground for several weeks if necessary. Although fresh fish is more advantageous for producers, drying has become a method frequently used to preserve excess catches. The dried fish is however prepared in poor hygienic standards, mostly with insufficient time of drying and stored directly on the ground. This leads to a significant deterioration of the dried product and some times to physical losses (FAO, 1986).

Regarding fresh fish handling and distribution in Ethiopia, most of private traders do not use even basic cold chains because of lack of ice as well as inappropriate amount of ice for the given catch (FAO, 1993). Ethiopian consumers have preference in general for whole fresh fish. The FPME uses modern preservation method, particularly freezing.

2.3 Quality Deterioration of Fish and Preservation Techniques

Animal food products deteriorate rapidly at ambient temperatures, and aquatic foods are among the most highly valued and most perishable of all food products due to the effect of a wide variety of biochemical and microbial degradation mechanisms (Smith & Hui, 2004) and food quality depends upon a number of factors including storage time and storage temperature (Fatima *et al*., 1988). According to the standard ISO 8402 (1994), Quality is "the totality of features and characteristics of a product or service that bear on its ability to satisfy stated or implied needs". This working definition applies to all goods and services. In the case of fish products, it entails aspects related to gastronomic delights, purity, nutrition, safety, consistency, and product excellence.

The principal components of the fish muscle - water, fat and protein - must be preserved with little or no changes. The protein content is usually in the range of 15-20 percent, whereas the fat content varies widely from species to species and from season to season; it can be as low as 0.5 percent in lean and starved fatty fish, and can reach over 20 percent in some species (FAO, 1994).

Although fish is a good source of protein, fat and minerals, the quality of fish is best before frozen storage and that quality is better achieved in the first ten days of storage (Srinivasan *et al*., 1997). They showed that deterioration increases as the duration of storage increases. Good quality proteins are not universally available in sufficient quantities in the form of agricultural products. In view of this, it is not surprising that, in their quest for food scientists and industrialists all over the world have again focused their attention upon the seas as a most potential source of nutrients. The most important function of dietary protein is to supply amino acids either directly or indirectly. These amino acids are then used by an organism to synthesise its own proteins. The well-known fact that proteins differ in their nutritive values, is due to the variability of amino acid composition. The quality of fish protein is very high due to the essential amino acid composition.

Off-odours and off-flavours, slime formation, gas production, discoloration and changes in texture are obvious signs of spoilage (Huss, 1994). The development of these spoilage conditions in fish and fishery products is due to a combination of chemical, autolytic and microbiological changes, but the spoilage rate can be reduced by taking preventive measures like icing or keeping at low temperature during storage. Swaminathan (1974) also discussed that spoilage of fish occurs due to the action of bacteria, moulds and also enzymes present in the fish. Due to the action of endogenous enzymes present in the fish, ammonia and trimethylamine (TMA) are produced; as a result the fish become soft and spoilt. The rate of alteration has been shown to depend on factors such as the nature of the fish species, size, lipid content, feeding state at the moment of capture, importance and nature of microbial load and storage temperature.

Dehydration of frozen seafood products is a common cause of quality loss, but it is much more easily prevented than protein denaturation and lipid oxidation. Moisture loss results in undesirable changes in texture and flavor due to loss of volatile components. Lipid oxidation is a major cause of deterioration for many foods containing fats and oils. The large amount of polyunsaturated fatty acid moieties (Smith and Hui, 2004) found in fish lipids makes them highly susceptible to oxidation by an autocatalytic mechanism. Lipid deterioration limits shelf life of fish (Aubourg and Medina, 1999). Lipid oxidation causes loss of flavor as well as nutrition and creates toughening and texture problems (Aubourg and Medina, 1999). The result is unpleasant odor and flavor called rancidity.

Various methods of reducing spoilage in fresh fish have been investigated including early evisceration (Townley &Lanier, 1981) and the application of chemical dips. After death, fish pass several stages: rigor mortis, dissolution of rigor mortis, autolysis and bacterial spoilage. Autolytic changes occur as a result of enzymatic changes within the muscle. Sigholt *et al*. (1997) described that handling and processing of fish during rigor can result in loss of quality and lower fillet yield. Low temperature preservation is one of the best methods to preserve the fresh quality of the fish products. Fish should be chilled quickly to the temperature of melting ice soon after capture and maintained at that temperature. Studies have clearly demonstrated that as the storage temperature of fishery products is lowered, the storage life is increased (Fatima *et al*., 1988).

Considerable interest has been shown in holding fishery products at -1 to -3^{o}C to permit a lengthening of the keeping time without the cost of processing and freezing at sea. Storage of fishery products at these temperatures is known as super chilling or partial freezing. FDA's (FDA, 2001: cited by Smith & Huil, 2004) report also showed that reducing temperature slows the growth of pathogenic and spoilage microorganisms and reduces the rate of deteriorative, and biochemical and chemical reactions in the muscle and other edible tissues. Thus, one can reduce deterioration and decomposition by lowering the product temperature ensuring that any heat generated by the product is promptly removed.

Freezing is an efficient preservation process because of the transformation of liquid water into ice which reduces greatly microbial and enzymatic activities. But ice crystals formed during freezing have a great influence on the quality of foodstuffs after thawing causing drip losses, textural and colour modifications (Fellows, 2000). Freezing is a common practice in the meat, fish and other animal protein based industries, because it preserves the quality for an extended time and offers several advantages such as insignificant alterations in the product dimensions, and deterioration in the products color, flavor and texture (Obuz and Dikeman, 2003). However, there are some disadvantages associated with frozen storage (Kropf & Browers, 1992) including freezer burn, product dehydration, rancidity, drip loss and product bleaching which can have an overall effect on the quality of the frozen foods, and some researchers also reviewed that even though frozen storage is an important preservation method for sea food, quality deterioration in muscle tissues occur during frozen storage (Connel, 1964; Matsumoto, 1979). Although freezing changes a food product less than other preservation methods, there may be some dehydration or water loss leading to quality losses and therefore economic losses. This water transfer should be minimised in order to maintain the quality of the product.

Giddings & Hill (1978) reported that the extent of quality loss is dependent upon many factors including storage temperature, rate of freezing and thawing, temperature fluctuations, and freeze-thaw abuse during storage. A recent study showed that storage time and temperature are the major factors affecting the rate of loss of quality and shelf life of fish (Srinivasan *et al*., 1997). As reported by FAO/WHO (1977) and FAO (1982) storage temperature is the main factor affecting freshness and quality of fish. According to De Koning and Mol (1991), controlling the temperature of fish is perhaps the most important element in the preservation of fresh fish. The proper cooling of fish has a number of advantages. Firstly, bacterial activity depends very much on temperature; the lower the temperature, the slower the rate of bacterial spoilage; likewise, enzyme activity also decreases as temperature falls, so the rate of autolytic spoilage is significantly slowed (De Koning and Mol, 1991).

2.4 Quality Changes during Frozen Storage

Many researchers (Disney, 1976; Shewan, 1977) showed that much variation exists in the pattern of spoilage of fish species during frozen storage. According to these studies, tropical fish apparently have much longer shelf lives in ice than those from temperate or cold water. This is because the microorganisms found in fish from cold water can easily adapt the low temperature of ice. Most researches on the spoilage of fish in ice have been carried out on marine species. Less is published on spoilage characteristics of fresh water fish. However, these studies do indicate that, although spoilage patterns of freshwater and marine fish are similar (Shewan, 1977), the storage life of freshwater fish tends to be longer.

Fish decomposition is mainly due to bacterial growth resulting in the production of various volatile substances. Some of theses substances are not normally found in live muscle tissue, while others, which are already present in the muscle, increase logarithmically in parallel with microbial growth (Malle and Poumeyrol, 1989). The total number of microorgansims, named total viable counts (TVC) or aerobic plate counts (APC) have been used in mandatory seafood standards in many developed countries. The assay of some of these substances usually provides useful data for the evaluation of fish freshness or microbial quality.

Among post-harvest changes, degradation of fish muscle caused by endogenous proteases is a primary cause of quality loss during cold storage or handling (Haard *et al*., 1994). The freezing process induces muscle tissue changes by the formation and accretion of iced crystals, dehydration and increases in solute (Shenouda, 1980). Freezing and frozen storage of particular species of fish result in eventual detrimental changes in texture and functional properties which determine the end of their practical storage life. Such changes include decrease in solubility and extractability of the myofibrillar proteins. Factors which may contribute to these changes include partial dehydration due to freezing of water and associated concentration of solutes in the tissue. Research findings showed that frozen fish which has undergone deterioration during storage appear to accumulate high molecular weight protein aggregates, stabilized by hydrophobic interactions as well as by disulfide bonds and other covalent cross links (Haard *et al*., 1994). According to Benjakul *et al*. (1997), the degradation of muscle structure results from many proteases. The effects of individual proteases on degradation are difficult to estimate during iced storage. Factors that cause denaturation or degradation of the protein can affect ATPase activities. Such activities have been widely used to monitor postmortem changes during iced or frozen storage (Mac Donald & Lanier, 1994).

In order to assess the trend and degree of freeze-induced denaturation of any fish or seafood material, there are numerous well-known methods, such as determination of basic volatile nitrogen, K-value, and ATPase assay. Although there are reports on the amino acid composition of fish (Iwasaki and Harada, 1985), little information is available on changes of amino acid content of fish fillets stored as frozen. In a previous study (Careche and Li-chan, 1997), it was reported that frozen storage at -18^{o}C has a significant effect on changes in protein solubility of cod myosin. They showed that the soluble protein concentration decreased by about 20% and 90% after 2 and 7 days frozen storage, respectively. Smith and Hui (2004) described that fatty fish have relatively high concentration of polyunsaturated fatty acids compared with terrestrial animals, and these fatty acids are very much susceptible to oxidation. Oxidation of these fatty acids can be initiated prior to freezing by endogenous or microbial enzymes. They also discussed that freezing does not stop lipid oxidation, and aquatic food products can become highly oxidized during frozen storage, with the development of rancid, fishy flavors and discoloration.

Fish tissues have lipases and phospholipases that remain active at frozen storage temperature yielding free fatty acids (FFA) during enzymatic hydrolysis. The FFA formation itself does not lead to nutritional losses. However, it has been proved that (Shenouda, 1980; Rehbein, 1988) accumulation of FFA in frozen fish is related to some extent to lack of acceptability of frozen fish, because FFAs are known to cause texture deterioration by interacting with proteins and have been shown to be strongly interrelated with lipid oxidation (Han & Liston, 1988). Lipid hydrolysis and oxidation have been shown to occur during lean fish frozen storage and become an important factor of fish acceptance, influencing protein denaturation, texture changes, loss of functionality and fluorescence development (Aubourg and Medina, 1999).

Certain fish have novel ways of cycling nitrogen that can lead to quality problems during frozen storage. Total Volatile Bases Nitrogen (TVB-N) is one of the most widely used methods today to estimate the degree of decomposition of fish. It includes the measurement of trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile nitrogenous compounds associated with seafood spoilage (Malle and Poumeyrol, 1989). Chemical tests usually measure the amounts of breakdown product derived from enzymatic, bacterial or oxidative activity and have trimethylamine, histamine, ammonia and several other breakdown products which can be chemically recorded to evaluate the frozen fish quality. The level of TVB-N for white fish is generally considered to be fresh if the TVB-N is less than 20 mg N/100 g sample. If the TVB-N reaches 30 mg N/100 g most regulatory authorities consider the fish to be stale, whilst at level of 35 mg N/100 g the fish is regarded as unfit for consumption (Egan *et al.*, 1981).

The pattern of increase in TMA, a component of TVB-N found in small quantities in fresh fish flesh, and TVB-N during ice storage and PFS is consistent with the pattern of changes in bacterial counts (Fatima *et al*., 1988). The levels of these compounds are primarily responsible for the fishy odors which increase as spoilage proceeds. The degree of spoilage in fish, as characterized by the production of TMA or other volatile bases, is different for each species and related to the type of processing and duration of storage. This is so because the composition of fish muscle varies from species to species, which is evidently reflected in differences in the course of decomposition (Sadok *et al*., 1996).

3. Materials and Methods

3.1 Description of Study Area

Lake Ziway is the most northerly rift valley lakes of Ethiopia. The lake contains five main Islands (Debretsion, Debresina, Gelila, Tsedecha, and Funduro) of volcanic origin (Schroder, 1984). It lies between East Shoa and Arsi Administrative zones of Oromia Region about 145 Km due south of Addis Ababa, on the left side of Addis-Awassa highway. The lake is located 7^o 52' to 8^o 8' N latitude and 38^o 40' to 38^o56' E longitude (Makin *et al*., 1975). The altitude of Lake Ziway is 1636 m above sea level. It has an area of 440 Km^2, and a medium depth of 2.5 m (Tenalem, 1998). The lake is fed by the two major rivers: Meki and Katar, which drain from the Northwestern and Southeastern plateaus respectively. Lake Ziway is being used for irrigation purposes and much of the land around it is under continuous cultivation, which may cause contamination of fish. The fish community of the lake is composed of both native and introduced species. The native species comprise *O.niloticus* and some *Barbus* species whereas the introduced ones are *Tilapia zilli*, *Clarias gariepinus* and *Carassius auratus*. The potential yield of the lake fishery is estimated in the range of 1000 to 6000 tones per year (LFDP, 1996).

The water chemistry of the lake is very similar to other lakes in the Ethiopian rift valley where Na^+ and $HCO_3^- + CO_3^{2-}$ are the dominant ions (Table 3.1).

Table 3.1 Physical and chemical characteristics of Lake Ziway

Characteristics	**Quantity**
Conductivity (K= 25μ. $S.Cm^{-1}$)	410
pH	8.50
Na^+(mg/l)	2.87
K^+(mg/l)	0.31
Ca^{2+}(mg/l)	0.56
Mg^{2+}(mg/l)	0.64
$HCO_3^- + CO_3^{2-}$ (mg/l)	4.0
Cl^- (mg/l)	0.32
SO_4^{2-} (mg/l)	0.32
Total Phosphorus (mg/l)	219
SiO_2 (mg/l)	37.0

Source: Kebede *et al*., 1994.

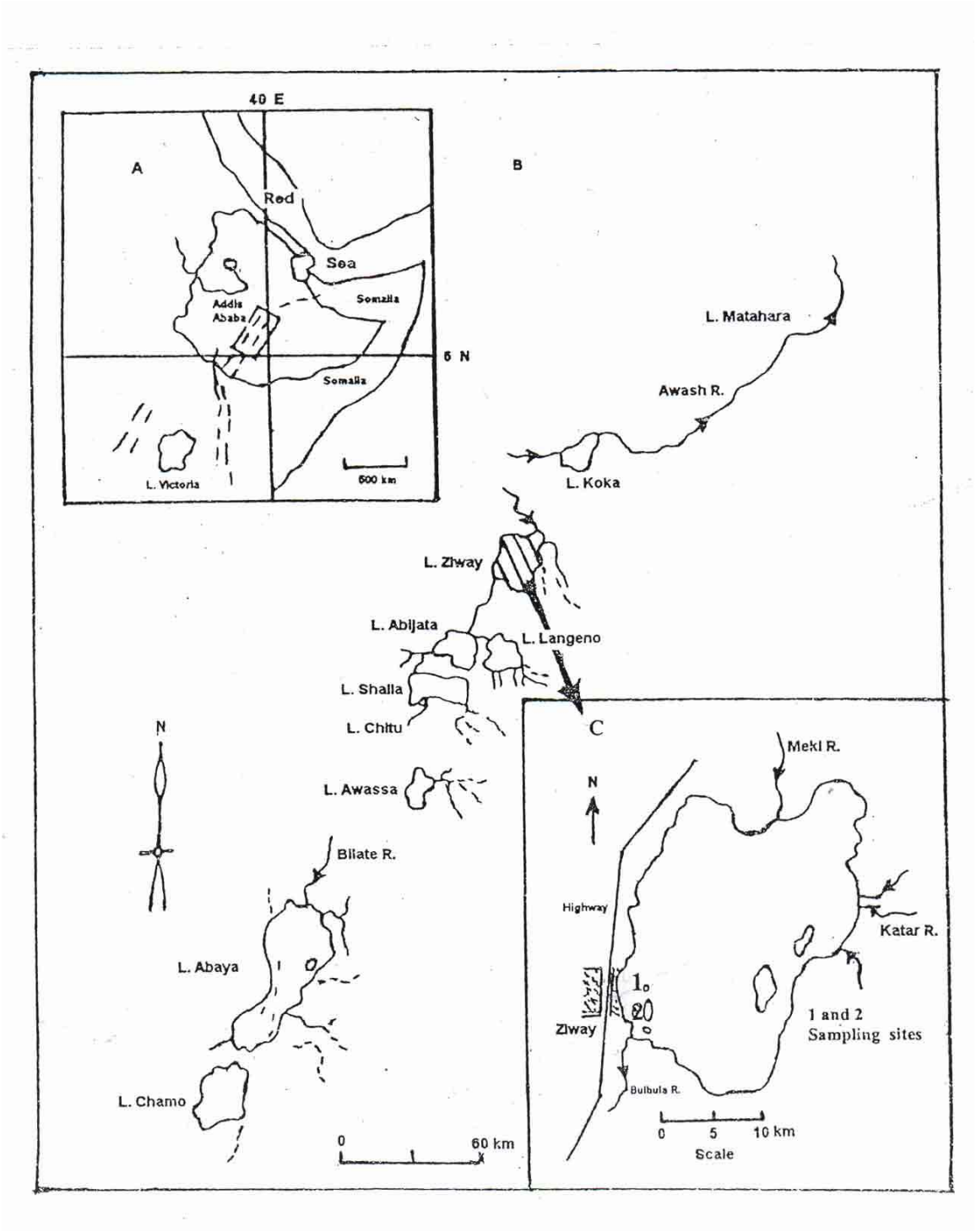

Figure 3.1 Map of A) Ethiopia, B) Rift Valley Lakes of Ethiopia, and C) Lake Ziway with sampling sites.

3.2. Study Design

A causal study has been conducted to determine the effect of low temperature preservation on quality of commercially important fish species. The study attempted to determine the effect of cold storage on fish product quality and evaluate the existing low temperature preservation technique used by the Ethiopian Fish Production and Marketing Enterprise. The study has been conducted between March and June 2007.

3.3. Collection and Preparation of Samples

For this study, Nile Tilapia (*Oreochromis niloticus*) known locally as 'Koroso', which has been caught by seine net and gill net from Lake Ziway, was purchased in March from Fish Production and Marketing Enterprise at the source, Lake Ziway, 12 hours after catching. The fish samples were transported in ice (Ethiopian standard, ES 2823, 2006) to processing unit where they were immediately washed, filleted, and packed in polyethylene bag (25 cm x 25 cm). A portion of the sample was transported in ice to laboratory, Addis Ababa, for analysis and the remaining portions were stored in cold storage (-18 ± 2^{o}C) at Ziway center for three days. After three days, the samples were transported to the main store, Addis Ababa, and stored in the Fish Production and Marketing Enterprise cold storage for three months at a temperature used by the company (-18±2^{o}C) to preserve the fish products. Storing the fish samples under this condition was aimed at evaluating the existing low temperature preservation technique of FPME. At regular interval of 15 days, frozen fish samples were drawn from the cold store and they were defrosted by exposure to a temperature of 2-5 ^{o}C for about 12 hours for analysis. All processes starting from fish catching to withdrawal of the fish sample from cold store for analysis have been performed according to the methods used by the Ethiopian FPME. The reason for this choice of method was to evaluate the fish processing technique used by the Ethiopian FPME. The flow diagram for fish processing used by the FPME is depicted as follows.

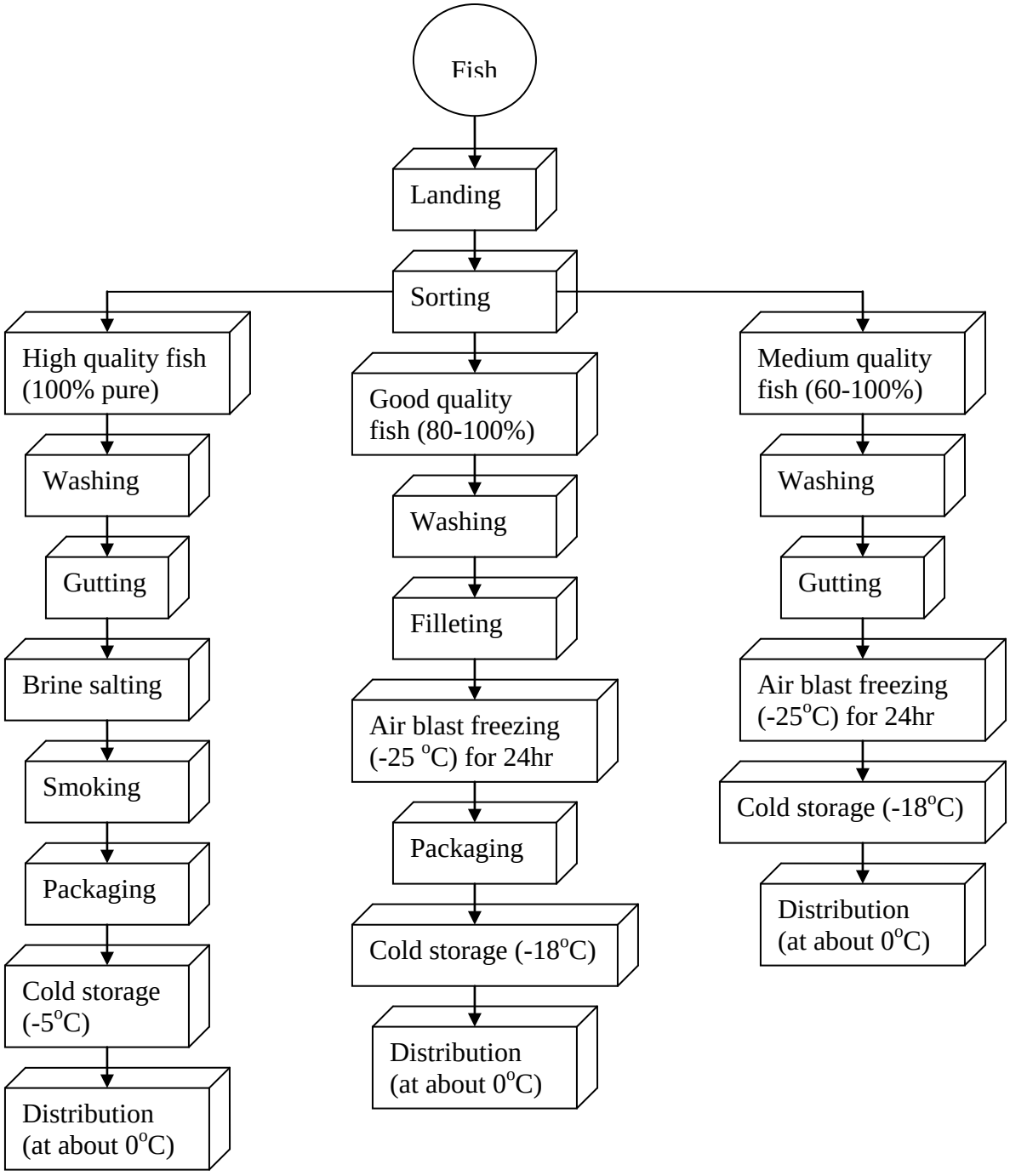

Figure 3.2 Process flow diagram for fish processing used by FPME

The FPME does not have any scientific basis for grading the catch. It is performed by simple observation. For this study, the fish considered as good quality (80 – 100% pure) was used as fillet. This is because fillets are the most commercialized forms of the fish products in the domestic and export markets.

3.4. Sample Analyses

All fish product samples (fresh and preserved) were analyzed for total plate count, total coliforms, faecal coliforms, pH, moisture content, ash content, fat content, protein content, and total volatile bases nitrogen (TVB-N) in the Ethiopian Health and Nutrition Research Institute (Food Chemistry and Food Microbiology Laboratories) and at Addis Ababa University, Faculty of Technology (Chemical Engineering Laboratory).

Distilled water and analytical grade reagents were used through out the experiments. Each time either duplicate (for protein content, fat content, TVB-N, total plate count, total coliform and faecal coliform) or triplicate (for pH, moisture content, and ash content) representative and homogeneous samples were taken from both the fresh and preserved fish products at regular interval of 15 days for analysis during the period of 90 days.

3.5. Analytical Procedures

Measurement of Moisture Content

A clean dried and covered, flat aluminium dish containing about 6 g sample was dried to a constant weight at 103 ± 2^{o}C in a drying oven for 6 hrs and it was cooled in a desiccator (silica gel as a desiccant) and weighed (AOAC, 2000). Then the moisture content was estimated by the formula:

$$\text{Moisture content (\%)} = \frac{\text{Weight of original sample} - \text{Weight of dry sample}}{\text{Weight of original sample}} \times 100 \quad (1)$$

Measurement of Ash content

A dry, tarred porcelain dish containing 2 g wet sample was placed in an open oven in order to carbonize the sample. The carbonized sample was placed in a muffle furnace set at 550^{o}C for 1 hr and by allowing to cool in a desiccator and weighing by an analytical balance it, the ash content was determined (AOAC, 2000) using a formula :

$$\% \text{ Ash} = \frac{W_2 - W_1}{W_3} \times 100\% \quad (2)$$

Where: W_1 = mass of empty porcelain dish

W_2 = mass of dish with ash

W_3 = mass of original product sample

Measurement of Protein Content

A digestion flask containing about 0.5 g dried sample, to which some amount of acid mixture (concentrated sulphuric acid and orthophosphuric acid) and catalyst mixture (K_2SO_4 and Selenium) were added. The mixture was exposed to about 370^oC in order to allow digestion. Then it was distilled with Kjeldahl apparatus (Kjeltech system, Hoganas, Sweden) by adding 40% NaOH and the distillate was collected in saturated boric acid solution containing a mixture of bromocresol green and methyl red as an indicator. Finally, the distillate was titrated with 1.02 N sulphuric acid to a reddish color (Egan *et.al*., 1981). The protein content was estimated using the formula:

$$\text{Nitrogen Content (\%)} = \frac{\text{mL of acid added} \times \text{Normality of acid} \times 100 \times 0.014}{\text{Weight of wet product sample (g)}} \quad (3)$$

$$\text{Therefore, Protein content (\%)} = \text{\% Nitrogen content} \times 6.25 \quad (4)$$

Measurement of Fat Content

A clean and dried thimble containing about 5 g of dried sample which was covered with fat free cotton at the bottom and top was placed in the extraction chamber. Then extraction has been performed for about 6 hours by soxhlet apparatus using AOAC (2000) method. Finally, the fat content was estimated by using the formula:

$$\text{Weight of fat } (W_f) = W_a - W_b \quad (5)$$

Where: W_a = Weight of extraction flask after extraction

W_b = Weight of extraction flask before extraction

$$\text{Fat g/100g fresh sample} = \frac{W_f \times (100 - \text{Moisture \%})}{W_D} \quad (6)$$

Where: W_D = Dried sample obtained after determination of moisture.

Measurement of pH

The sample was homogenized in distilled water in the ratio 1:10 (w/v) and the pH was measured using a digital pH meter by introducing the electrodes, in which the glass indicator electrode and the Ag/AgCl reference electrode are joined in one shaft, in to the prepared homogeneous sample extract while stirring with magnetic stirrer (ISO, 1999).

Determination of TVB-N

About 300 mL of 0.6M perchloric acid was added to about 100 g of fish sample. After homogenization by using food sample homogenizer (Waring commercial, New Hartford, USA), the mixture was filtered using Whatman filter paper No.42. Fifty mL of the extract was transferred to distillation flask and 10 mL of 20% NaOH was added to it. Then, it was distilled until about 100 mL of the distillate has been collected in to 25 mL of 4% boric acid with methyl red indicator. Finally, the distillate was titrated with 1.02 N H_2SO_4 (Malle and Tao, 1987). The amount of TVB-N was calculated by using the equation:

$$\text{TVB in mg-N/100g} = \frac{T \times 14 \times V_1}{V} \qquad (7)$$

Where: T = Milliliters of 1N standard acid.

V_1= the water content in the fish sample + mL of Perchloric acid solution.

V = volume of aliquot

Total Plate Count

About 5 mL of the mixture prepared from about 20 g fish sample and 180 mL sterile saline solution (0.85% NaCl) was transferred to a test tube labeled as 10^{-1}. About 0.5 mL of the diluted solution from the test tube labeled as 10^{-1} was transferred to a test tube containing 4.5 mL sterile diluent and labeled as 10^{-2}. Again about 0.5 mL diluted solution from the test tube labeled as 10^{-2} was transferred to a test tube containing 4.5 mL sterile diluent and labeled as 10^{-3} and a ten-fold serial dilution was repeated till the last tube labeled as 10^{-7} (Dilution 10^{7}) (Gunasekaran, 1995).

One mL of the diluted sample was then transferred to each nutrient agar plate labeled as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} from the corresponding test tubes. The plates were rotated by hand at least 5 times in the clockwise direction and 5 times in anticlockwise direction. Finally several times crosswise rotation was made for equal distribution of the media. After spreading the samples on the agar plates using L-rod, the plates were inverted and placed in an incubator at 37^{o}C for 48 hours (Gunasekaran, 1995). The plates containing countable number of colonies (between 30-300 per plate) were selected and the number of colonies was calculated using the formula:

For tube 1 (10^{-1})

No. of CFU/ml = No. of colonies x 10^{1} x 1

For tube 2 (10^{-2})

No. of CFU/ml = No. of colonies x 10^{2} x 1 (8)

For tube 3 (10^{-3})

No. of CFU/ml = No. of colonies x 10^{3} x 1

Where: 10^{1}, 10^{2}, and 10^{3} are dilution factors for the corresponding tubes.

: 1 indicates that the sample volume was 1 mL. Similarly, number of cells for higher dilutions can be calculated using corresponding formulas.

Total coliforms and Faecal coliforms by the most probable number (MPN) method

Three tubes of Lauryl Sulphate Tryptose (LST) broth were used for each dilution (1:10, 1:10^{2}, 1:10^{3}, 1:10^{4}, 1:10^{5} and 1:10^{6}) and the tubes were incubated at 35^{o}C for 48 ± 2 hr for gas formation. After primary incubation one loopful of positive tubes (gas formation tubes) were transferred to Brilliant Green Lactose Bile (BGLB) media for total coliforms (incubated at 35^{o}C for 48 hr) and *E. coli* broth for faecal coliforms (incubated at 44^{o}C for 24 hr) according to AOAC (2000) method.

4. Results and Discussion

Fish and shellfish are highly perishable foods and susceptible to faster postmortem deterioration (Smith & Hui, 2004). Deterioration in quality of seafoods is attributed to the highly sensitive proteins and fats present in aquatic organisms (Haard *et al*., 1994). The major deteriorative processes that affect the texture, color and flavor of seafood are microbial spoilage, autolysis (Lund *et al.*, 2000), polymerization, deamination, decarboxylation and biochemical reactions (Haard *et al*., 1994). However, the loss of quality depends directly on the nature of fish species and on the handling and storage conditions. The rates at which microbial and autolytic spoilage occurs vary according to the species of fish, area of catch, method of catch and, above all, processing and storage temperature (Shewan, 1977). Once the fish are caught, on-board storage conditions exert a strong effect on the quality of manufactured fish products and, accordingly, on their commercial value.

Freezing is an efficient preservation process because of the transformation of liquid water into ice which reduces greatly microbial and enzymatic activities due to reduction in water activity. The ice crystals formed during freezing have a great influence on quality deterioration of foodstuffs. This results in drip losses, textural and colour modifications (Fellows, 2000). The freezing process (pre-freezing treatments, freezing, frozen storage, and thawing), if properly conducted, is generally regarded as the best method of long term food preservation when judged on the basis of retention of sensory attributes and nutrients (Karmas and Harris, 1988). The rate of food freezing changes with the freezing conditions and the food product properties. Changes in product properties during freezing are due to the continuous reduction of the temperature and therefore the continuous changing of the unfrozen water in to ice that increase solute and reduce water activity. The lower the water activity the longer the keeping quality of the food stuffs.

Generally, fish has been widely accepted as a good source of protein and other elements necessary for the maintenance of healthy body (Arannilewa *et al*, 2005). Inadequate storage techniques would imply a substantial shortfall in fish availability thereby affecting the animal protein intake of the people in the tropics whose protein intake from fish ranges between 17.5-50% (Arannilewa *et al*, 2005). Many factors such as type of species, size of fish, temperature of the storage, physical condition, and methods of catching and handling can affect the shelf life of fish during storage (Huss, 1988). Several methods are available for assessing fish and fish product quality and deterioration. However, there is much interspecies variation with respect to chemical, bacteriological and sensory quality changes, depending on storage temperature, whether the product is fresh or processed, and the method of processing. Therefore, acceptable limits for each quality criterion may vary greatly between different species (Huss, 1988). The quality of fish in general decreases after death due to chemical reactions (changes in protein and lipid fractions, the formation of biogenic amines and hypoxanthine) and microbiological spoilage.

4.1 Proximate composition

The results of proximate composition of tilapia fish fillets that was stored for up to three months at -18 ± 2^{o}c are presented in Table 4.1. The values are either means of triplicate (for moisture and ash contents) or duplicate (for protein and fat) determination ± SD. The sample for analysis was taken on fresh weight basis (g/100g fresh weight). In cases where dry sample was taken, it was converted to fresh weight basis for calculation purpose. All data were subjected to analysis of variance (one-way ANOVA) at 95% confidence interval using the software SPSS 13.0 for windows.

Table 4.1 The proximate composition of Nile Tilapia (*Oreochromis niloticus*) fish fillets stored at -18 ± 2^{o}C for three months

Storage time (Days)	**Moisture Content (%)**	**Ash Content (%)**	**Protein Content (%)**	**Fat Content (%)**
0	79.87 ± 0.01	0.98 ± .01	18.52 ± 0.08	0.37 ± 0.01
15	80.37 ± 0.23	0.95 ± 0.01*	18.05 ± 0.01*	0.57 ± 0.03*
30	80.01 ± 0.25	0.94 ± 0.02*	17.63 ± 0.09*	0.37 ± 0.01
45	78.83 ± 0.01*	0.94 ± 0.01*	17.64 ± 0.08*	0.63 ± 0.01*
60	78.97 ± 0.19*	0.91 ± 0.03*	17.65 ± 0.13*	0.47 ± 0.01*
75	79.06 ± 0.02	0.89 ± 0.01*	17.28 ± 0.044*	0.51 ± 0.014*
90	78.50 ± 0.71*	0.88 ± 0.02*	17.25 ± 0.088*	0.56 ± 0.01*

* Means significantly different from that of the fresh sample at 0.05 level.

In the present study, the trend in changes of moisture, protein and fat contents of tilapia fish fillets stored under frozen condition was found to be similar to the findings of Arannilewa *et al*. (2005). Slight differences in the values of these parameters may be due to the differences in the catching season, geographical location, fish size, fish age (or maturity) and water composition. One-way ANOVA showed that the proximate composition (protein, fat, ash, and moisture contents) of the fish fillet was significantly affected by the frozen condition.

4.1.1 Moisture and Ash contents

Statistically significant differences ($p < 0.05$) were observed from catching of the fish to 90 days of storage, for the moisture and ash contents of tilapia fish fillets stored at -18 ± 2^{o}c (Table 4.1). The initial moisture and ash contents decreased significantly ($p < 0.05$) from 79.87% and 0.98% to 78.50% and 0.88% respectively at the end of the storage period (Table 4.1).

A decrease in ash content was also reported by Beklevik *et al*. (2005) for sea bass filets during frozen storage. Drip loss during the thawing process might be one of the reasons for the decrease in the whole moisture and ash contents. The loss of moisture content of fish might have been also due to desiccation as well as temperature fluctuation during frozen storage. It has been clearly established that fluctuating cold store temperature (temperature abuse) is a major cause of dehydration (FAO, 1994). Fluctuating temperature in the freezer can cause the migration of water vapor from the product to the surface of the container. This defect is sometimes found in commercially frozen foods, which have been improperly handled.

Sublimation of ice can also lead to the loss of moisture. Some transfer of moisture from the product is unavoidable during freezing and frozen storage, which leads to dehydration of the fish. Good operating conditions are essential in order to keep dehydration to a minimum. Such losses could lead to freezer burn, textural changes and overall quality deterioration (Srinivasan *et al*., 1997). Frozen fish that have suffered severe drying in cold store have a dry, wrinkled, toughened and pale appearance on the surface that is characteristic of the condition known as freezer burn. The development of toughness and dryness, which is related to protein denaturation, is one of the major losses of quality in fish muscle during frozen storage. The cause of toughness is protein denaturation and aggregation accompanied by loss of water-holding capacity. The sum up of these changes on the fish muscle results in high thaw drip loss. Although glazing can prevent loss of moisture and lipid oxidation, sublimation of ice can lead to the loss of moisture.

Similar results were reported by Pawar and Magar (1969) for pomphrets, mackerel and sardines stored under frozen condition. Sawant and Magar (1961) also reported a decrease in moisture content of fish during frozen storage. Dehydration of frozen seafood products is a common cause of quality loss, but it is much more easily prevented than protein denaturation and lipid oxidation. Moisture loss results in undesirable changes in texture and flavor defect due to loss of volatile components. Weight loss is also an economic loss. Even though processed and packed in good operating conditions, frozen fish may dry slowly in cold store. This is undesirable as most obvious reason that the product will lose weight and it also negatively alters its texture and appearance. Most importantly, drying speed up denaturation of the protein and oxidation of the fat which affect shelf life of frozen fish. Dehydration is of particular interest because it is less obvious, harder to quantify and often has a large economic impact. It is the result of the inevitable loss of water vapor that occurs when a product is exposed to air or another gaseous medium when the product is not tightly packed. Although freezing changes a food product less than other preservation methods, there may be some dehydration or water loss leading to quality defects and therefore economic losses. This water transfer should be minimised in order to maintain the quality of the product.

Generally speaking, water loss by evaporation is greater during the pre-cooling (or pre-freezing) period, since the mass transfer rate is reduced when the product freezes. In general, high initial temperatures cause high dehydration losses. As dehydration is related to heat transfer efficiency, the surface/weight ratio plays an important role.

4.1.2 Protein content

The highest protein content (18.52 ± 0.08) was recorded for fresh fish sample and the least (17.25 ± 0.088) was recorded for frozen fish sample stored for 90 days (Table 4.1). The decrease in protein content may be attributed to denaturation of fish protein, i.e., due to the changes in the proportion of chemical composition and protein breakdown. As a result there may be leaching of nitrogen during thawing which in turn causes a reduction in the protein content. It is not desirable to have low protein content owing to long-term frozen storage, causing a decrease in the nutritional value in frozen fish product.

Denaturation of protein involves the destruction of its secondary, tertiary and quaternary structure, reducing the protein to a simple polypeptide chain (Careche and Li-Chan, 1997). A number of factors, including slow freezing and variability of storage conditions, cause this denaturation. Rate at which denaturation occurs depend largely on the rate of temperature reduction at which it was frozen. In the present study, the protein content of the fish sample was changed significantly ($p < 0.05$) from 18.52 ± 0.08 to 17.25 ± 0.088 (Table 4.1). Following death, fish undergo rapid protein degradation as a result of endogenous and bacterial enzymes.

Destabilizing forces on proteins increase with changes such as temperature abuse and eventually result in protein unfolding (FAO, 1994). A denatured protein has not only lost its ability to function as an enzyme, but also its "water-holding" ability. This results in denatured fish flesh dripping excessively when thawed (a situation known as "drip-thaw"), and appearing white, dull and spongy, and upon chewing becoming fibrous and tasteless. The rate at which protein denaturation takes place in frozen fish depends largely on the freezing temperature and will slow down as the temperature is reduced (FAO, 1994). The other possible reason for the decrease in protein content might be the loss of NH_3, volatile amines, and conversion nitrogen to other non-protein nitrogen molecules.

Fish proteins can also be injured by rancidity. During the early stages of autoxidation, free radicals and relatively stable hydroperoxides are formed; these subsequently react with proteins causing polymerization of proteins and catabolic (or enzymatic) destruction of amino acids (Danopoulos and Ninni, 1972). Deterioration of fish quality due to protein denaturation can therefore be checked by using as low temperature as possible, preferably -18°C.

Formaldehyde and dimethylamine (DMA) are produced by enzymatic (tri-methyl amine oxidase activity) degradation of trimethylamine oxide (TMAO), a natural constituent in the muscle of a large number of marine fish and shellfish (Rehbein, 1988). During freezing of the products, the formaldehyde produced is a highly reactive molecule, leading to inter- and intramolecular linkages between protein chains (Aubourg, 1998). As a result, protein denaturation and the loss of quality of the frozen fish may also be associated with the formation of formaldehyde.

In addition, FFA and their oxidation products would have an effect on muscle texture and functionality since they interact with myofibrillar proteins and promote protein aggregation. Myofibrillar proteins constitute approximately 66-77 per cent of the total proteins in fresh fish (Connel, 1964). As a result of proteolysis and improper storage of whole fish, degradation of these proteins occurs, resulting in solubilization, and subsequent loss of gases, reducing the protein content and furthermore causing undesirable changes in the texture and taste of many fishes. Previous reports by other authors have shown that the stabilisation of myofibrilar proteins is directly related to better fish quality (Careche and Li-Chan, 1997).

4.1.3 Fat content

Lipid oxidation is a major cause of deterioration for many foods containing fats and oils. The large amount of polyunsaturated fatty acid moieties (Smith and Hui, 2004) found in fish lipids makes them highly susceptible to oxidation by an autocatalytic mechanism. Lipid deterioration limits shelf life of fish (Aubourg and Medina, 1999). Lipid oxidation causes loss of flavor and nutrition and creates toughening and texture problems (Aubourg and Medina, 1999). The result is unpleasant odor and flavor called rancidity. The oxidation of lipids plays a very important role in the spoilage of both lean and fatty fishes. In the present study the fat content of tilapia fish fillets stored under frozen condition was significantly ($p < 0.05$) increased from 0.37% for fresh fish sample to 0.56% for the frozen fish sample stored for 90 days (Table 4.1). It is due to the fact that there is an inverse relationship between the moisture and lipid contents of fish flesh. According to the results reported by Beklevik *et al*. (2005), the lipid ratio of sea bass fillets was 1.22% at the beginning of the storage and it was reported as 2.28%, 2.86%, and 3.58% in the 3rd, 6th, and 9th months of storage, respectively. Tokur (2000) also stated that an increase in lipid content occurred during frozen (-18 ^{o}C) storage of rainbow trout.

The fat content of tilapia fish fillets stored under frozen condition showed an increasing trend during the storage period. Except for the slight differences which may be due to the differences in type of fish species, fish maturity, lipid content, feeding state at the moment of capture, the catching season, and geographical location, the observed changes in fat content occurring during tilapia fish fillets stored under frozen condition were consistent with descriptions presented by other authors (Beklevik *et al*., 2005).

Rancidity in fats caused by lipid oxidation may occur by several mechanisms. A major pathway follows the free radical mechanism which includes three stages: initiation, propagation, and termination. During initiation, catalysts such as heat, metal ions, and irradiation cause lipid molecules to form lipid free radicals (R*). These free radicals react with oxygen to form peroxyl radicals (ROO*), which then may react with other lipid molecules to form hydroperoxides (ROOH) and new free radicals (R*), resulting in self propagating chain reactions. When free radicals increase in a system, they interact to form non-radical end products thus terminating the chain reaction. Peroxides are not stable compounds and they break down to aldehydes, ketones and alcohols which are the volatile products causing off-flavour in products. These compounds are responsible for off-flavour and off-odour of fish (Huss, 1994)

Seafood products are very susceptible to rancidity caused by oxidation of fish lipids which contain highly unsaturated fatty acids that are easily attacked by oxygen free radicals. Oxidation of fish lipids not only produces off flavor but also reduces their nutritional value. This problem is important not only for fatty fish species but also for lean fish species. In some cases lipid oxidation may be more severe in lean fish. This is because the fatty acids of phospholipids, main lipids in lean fish, are normally more susceptible to oxidation. The internal portion of an intact animal muscle is normally anaerobic. The oxygen required to promote lipid oxidation in foods diffuses from food surfaces into the interior thus, lipid oxidation starts from the surface of the food (Smith & Hui, 2004).

4.2 pH and TVB-N Values

The pH values and TVB-N contents of tilapia fish fillet that was stored for up to three months at -18 ± 2^{o}C are presented in Table 4.2 The values are means ± SD of triplicate (for pH values) and duplicate (for TVB-N contents) analysis.

Table 4.2 The changes of pH values and TVB-N contents of Nile Tilapia (*Oreochromis niloticus*) fish fillets stored at -18 ± 2^{o}C for three months

Storage time (Days)	**pH value**	**TVB-N (mgN/100g)**
0	6.43 ± 0.01	12.04 ± 0.48
15	6.45 ± 0.01*	13.95 ± 0.00*
30	6.49 ± 0.01*	14.58 ± 0.60*
45	6.51 ± 0.01*	17.66 ± 0.76*
60	6.54 ± 0.01*	16.61 ± 0.67*
75	6.58 ± 0.02*	18.66 ± 0.11*
90	6.61 ± 0.01*	21.75 ± 0.35*

* Means significantly different from that of the fresh sample at 0.05 level.

4.2.1 pH value

The pH value of the fish sample immediately after being caught was reported to be about 6.43±0.01 (Table 4.2). The pH value increased significantly ($p < 0.05$) as the duration of storage increased. The highest pH value (6.61 ± 0.01) was obtained for fish fillets preserved for 90 days while the least value (6.43 ± 0.01) was found for fresh fish sample. Lower pH value is used as indicator of higher stress at or before the time of slaughtering of many animals (Sigholt *et. al.*, 1997). This is caused by the depletion of energy reserves during death struggle, mainly glycogen, with the production of lactate.

In the current study, the low initial pH values may indicate that fish were subjected to stress before slaughtering. This results in lack of carbohydrate for production of lactic acid that can lower the pH after death. The high pH in turn accelerates spoilage (deterioration) of fish products. The increase in pH value during frozen storage may be associated with the increase in volatile basic components. Van Den Berg (1964) reported that enzymatic activity could also contribute to pH change in frozen fish. Since enzymatic activities are not eliminated by freezing, enzymatic changes which affect the buffering capacity of the system may affect pH noticeably. pH may also be affected by solute concentration. As the temperature falls, individual solutes reach saturation point and crystallize out. An increase in solute concentration during freezing causes change in the pH (Fellows, 2000). These all factors may contribute to the change in pH value. Similar results were reported by Arannilewa *et al.* (2005) who studied effect of frozen period on the chemical, microbiological and sensory quality of frozen tilapia fish (*Sarotherodum galiaenus*). Slight differences may be due to the differences in the catching season, geographical location, fish size and water composition.

The fish products are acceptable up to a pH of 6.8 but are considered to be spoiled above a pH of 7.0 (Huss, 1988). According to these criteria, tilapia fish samples stored at -18 ± 2^{o}C were of acceptable quality during the 90 days of storage. The results of TVB-N analysis also support these results (Table 4.2).

4.2.2 Total Volatile Base Nitrogen

Even if fish are frozen at the recommended temperature of -18^{o}C (FAO, 1994) immediately after being filleted, this does not promise a longer shelf life. This is due to the chemical reactions such as hydrolysis, polymerization, deamination, decarboxylation and autolytic deterioration taking place in fish proteins and fat during frozen storage of fish, leading to irreversible changes in flavor, odor and texture (Thailambal, 2007). TVBN is a measure of the total amount of a variety of nitrogen-containing substances which are produced during storage. Level of total volatile nitrogen (TVN) in fish is commonly used as a spoilage indicator (Egan *et.al*., 1981).

In most kinds of fish species, TMA constitutes most of the total volatile bases, until spoilage (Martinsdottir and Magnusson, 2001). However, in the spoiled fish where the TMAO supplies are depleted and TMA has reached its maximum level, TVB-N levels still rise due to formation of NH_3 and other volatile amines. The 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 (Egan *et.al*., 1981).

The level of TVB-N in freshly caught fish is generally between 5 and 20 mg N/100 g muscle (Egan *et.al*., 1981). In the current study, the concentration of TVB-N increased during cold storage from 12.04 ± 0.48 to 21.75 ± 0.35 mgN/100g (Table 4.2). The TVB-N content increased progressively in the fish fillets during storage in the cold store but not attained the unacceptable limit (35 mg N/100g) for human consumption (Egan *et. al*., 1981) on the 90^{th} days of frozen storage. In the present study it was found that the tilapia fish fillet samples were acceptable for human consumption after 90 days of frozen storage. As compared with the initial value, a significant ($p < 0.05$) increase in TVB-N values was observed in this parameter on day 15, followed by an increasing trend up to the end of the storage period (Table 4.2). As for many fish species, the formation of TVB-N for tilapia fish fillets in the present study increased significantly ($p < 0.05$) with the time of storage up to day 90. The values obtained for TVN are comparable with other published data on different fish species, although no specific data for Nile tilapia exists.

Similar results were reported by Xue *et al.*, (2000) for yellowtail during refrigerated storage and by Joseph and Adnes (2004) for croaker in ice and ambient temperature storage. A similar increase in TVN has been also reported in frozen storage of cuttlefish by Thailambal (2007). Slight differences may be due to the differences in the types of species, catching season and region, age and sex of the fish (Sadok *et al*., 1996). However, the TVB-N values of tilapia fish fillets stored for 90 days under frozen condition remained at 21.75 ± 0.35 mg N/100g which is below the upper limit of acceptability.

The increase in concentration of TVB-N may be attributed to the production of DMA, TMA, ammonia and other basic nitrogenous compounds resulted from the decomposition of TMAO by endogenous enzymes present in the fish species (Egan *et al*., 1981). Thus, the increased activity of proteolytic enzymes might have contributed to the increase in TVB-N content in frozen stored tilapia fish fillets. This may also contribute for the reduction of protein content due to loss of nitrogen in the frozen stored tilapia fish fillet (Table 4.1). Oxygen or potential oxidants, such as oxidised lipids, have been reported to be inhibitors of tri-methyl oxidase (Careche, and Li-Chan, (1997).

4.3 Microbiological quality

Seafood safety is strictly dependent on the hygienic quality of the aquatic environment as well as on the different phases of the seafood production chain, from fishing to the treatments on-board and after landing (Huss,1988). Fish products, in fact, are particularly susceptible to contamination, especially those from freshwater environments characterized by slow water exchange and high anthropic contamination. In general, fish harvested from the open ocean will be relatively free of human pathogens (with the exception of certain parasites) while those from near-shore saltwater, fresh water and aquaculture sources are at greater risk for contamination (ICMSF, 1998). Sources of the microorganisms include general pollution resulting from human, animal, industries and agriculture wastes.

For the determination of microbial quality of tilapia fish before and after frozen storage, total plate count (TPC), total coliforms and feacal coliforms were analyzed. The changes in microbial flora of tilapia fish fillets during frozen storage are presented in table 4.3.

Table 4.3 Changes in microbial load of Nile Tilapia (*Oreochromis niloticus*) fish fillets during frozen storage for 90 days.

Storage time (Days)	**Total Plate Count (CFU/g)**	**Total Coliforms (MPN/g)**	**Faecal Coliforms (MPN/g)**
0	2.57×10^6	460	23
15	2.44×10^5	93	23
30	2.42×10^4	23	ND
45	4.55×10^4	9	ND
60	2.20×10^4	4	ND
75	6.00×10^4	9	ND
90	8.20×10^5	23	ND

Statistical analysis confirmed the inhibitory effect ($p < 0.05$) of frozen condition on the growth of microorganisms in tilapia fish fillets. It is evident that the microbial load decreases (Table 4.3) after freezing which is in agreement with the findings of Alam *et al.* (2004). The initial total bacterial load was found to be 2.57×10^6 CFU/g, while total coliforms and faecal coliforms were 460 MPN/g and 23 MPN/g respectively (Table 4.3).

The high microbial load in fresh fish before freezing may be accounted for the overall environmental contamination and lack of hygienic handling or poor sanitary conditions prevailing at the shore, fishing equipments and landing center. The reduction in total coliforms and feacal coliforms of tilapia fish stored at -18 ± 2^{o}C are shown in Table 4.3. The total coliform counts decreased from 460MPN/g in the fish sample up to the 75th days of frozen. The trend of increase on 90th day may be due to technical error or temperature abuse during thawing. The faecal coliform counts particularly decreased to zero on the 30th days of storage and did not reappear (Table 4.3).

On the 60th days of storage, the total bacterial load decreased by about two logs (from about 10^6 to 10^4 CFU/g) in tilapia fish fillet, which may be due to the cold shock during frozen storage under freezing condition. Similar results were reported by Thailambal (2007) for cuttlefish and crab. Since TVB-N results mainly from the reduction of TMAO by bacterial and /or their enzymatic activity (Malle and Poumeyrol, 1989), the dramatic increase in TVB-N may indicate the spoilage of fish by bacterial action.

In the current study, tilapia fish as a raw product showed higher bacterial load, which could be related to water flora poor handling practices at the time of harvest and transportation to the processing plant. The International Commission on Microbiological Specifications for Food (ICMSF) (1998) recommends that the flesh TPC should not exceed 10^6 CFU/g wet weight. This recommendation was not met by the results of this study, i.e., the total plate count for unpreserved fish sample was slightly higher (2.57 x 10^6 CFU/g) than the recommended standard. Microbial activity is responsible for spoilage of most fresh fish products. The shelf life of fish products, therefore, is markedly extended when products are stored at low temperature.

Autolysis of fish muscle proteins result in the formation of peptides and free amino acids, all of which act as suitable nutrients for microbial growth and production of biogenic amines which are known to affect the safety and taste of the fish meat. Faulty rearing, contamination of the water, harvesting, and processing practices can result in cross-contamination of fish with foodborne pathogenic bacteria (ICMSF, 1998). Minimizing the microbial load on seafood products begins prior to the harvest of the product. Good Manufacturing Practices, including holding, transporting, and processing at appropriate low temperatures, are also requisite.

The natural habitat for *E. coli* is the intestinal tract of human and vertebrate animals. In temperate waters this organism is absent from fish and crustaceans at the time of capture (except in grossly polluted waters). Moreover, fish and shellfish should always be held at temperatures below those which support growth of pathogens. This organism is therefore particularly useful as indicator (ICMSF, 1998) of fecal contamination (small numbers) or mishandling such as temperature abuse in product handling (large numbers). Contamination of food with *E. coli* implies a risk that one or more of enteric pathogens may have gained access to the food. However, failure to detect *E. coli* does not assure the absence of enteric pathogens (ICMSF, 1998). The FC and TC in the fish fillets were present in the acceptable range for fish and fish products as suggested by ICMSF (1998).

5. Strength and Limitation of the Study

This study being the first in its kind, it came up with strong findings in relation to fish processing effect on quality of tilapia fish fillets and supply of high quality fish fillets. Therefore, it may be used as scientific baseline information for subsequent studies and for different organizations engaged in fresh water fish processing and related programs in the country. The present results may add knowledge in controlling proximate composition, TVBN, pH and microbiological load so as to determine the frozen storage life of fish fillets and fish product quality. The research findings can also be used to conduct similar study in different lakes of Ethiopia which have potential on fish harvesting and processing practices.

As being causal in its design, this study shares the drawbacks of similar causal studies. In causal studies, it may be difficult to control other external factors, in this case other than temperature, which may also affect the given condition (or process). The information on the physicochemical and microbiological qualities of the fish and fish products may not exactly reflect the actual situation. Limited number of instruments were available for many kinds of analyses in the laboratories. Thus, few experiments were not performed exactly on the specified dates. This may be the reason for unexpected increase in microbial count and ash content.

6. Suggested Techniques for Improvement of the Existing Fresh Water Fish Processing Practices

There are considerable problems in maintaining fish quality during the period of post-catch handling and distribution. Proper handling of fish between capture processing and delivery to the consumer is a crucial element in assuring quality of the final fish product. Standards of sanitation, hygienic operation, method of handling and the time and temperature of holding fish are all significant factors to assure quality. Much emphasis has been placed on hygienic operation handling of the fish from the moment of catching in order to ensure good microbiological quality and long storage life of the product.

Fishing Equipments

Fishermen still mostly use one-man rafts made from papyrus and often reinforced with a very light wooden keel, whose carrying capacity is extremely limited as well as not suitable to use ice in order to keep the freshness of the fish immediately after catching. It can be suggested that the existing fishing equipments should be replaced by a new punt design (3.5 m x 1.55 m x 0.4 m) tested by LFDP (1996).

Methods of Catching

Both kinds of fishing gears (passive and active) are used to catch the fish. Passive gears such as gill nets and long lines are common in all Ethiopian lakes. However, active gears such as beach seines, and hook and line are not common in most of the lakes. In the case of passive gears, it is only after a certain time, for instance the next morning, that they are checked and the fish caught removed. Thus, it is recommended to commonly use an active gear particularly seine net as a means of fish catching wherein the fish caught removed immediately. This may reduce unnecessary microbial and physicochemical changes occurring on fresh fish and their effect on the final fish products.

Catch Handling on Board

Different catching methods presumably involve different stress levels. Fish caught in fishing nets or with fishing hooks usually struggle for a long time to get loose. In fishing nets, the fish may also suffer from a considerable scale loss, which may result in physiological stress and increased mortality. Fish which get scraped or bruised during handling can lose their protective slime coating, thereby reducing their natural defense against invasion of pathogens and spoilage microorganisms. Loss of scales or cuts is even a more dramatic invitation to infection or direct mortality due to injury. Rough handling will result in a faster rate of spoilage. Fish landed by the artisanal fishermen are generally not iced. On arrival at landing sites, fish have been exposed to high ambient tropical temperatures which lead to rapid quality deterioration. The time lapse before icing and the exposure to ambient temperatures encourage proliferation of microorganisms, resulting in an enhanced bacterial activity and an induced enzymatic spoilage. Stowage and handling conditions before reaching the Ziway receiving station are unsatisfactory, with fish on the ground without ice awaiting collection.

Indeed it is suggested that proper handling should begin when landing the fish. Bruising caused by contact with hard surfaces (decks, gunwales, etc.) should be minimized. Exposure to the sun and summertime temperatures can cause quality problems in less than an hour. However, simply chilling fish can prevent quality deterioration and reduces health risks that can result from elevated temperatures. Thus, fish should be iced immediately after catching. The fish should be washed immediately, either by hosing down or by bucket rinses to remove slime and reduce spoilage bacteria. Fish tissue is almost sterile, but the skin surface and viscera contain many types of bacteria. The skin slime and viscera also provide food for bacterial growth. Thus, rough treatment should be avoided while cleaning the fish.

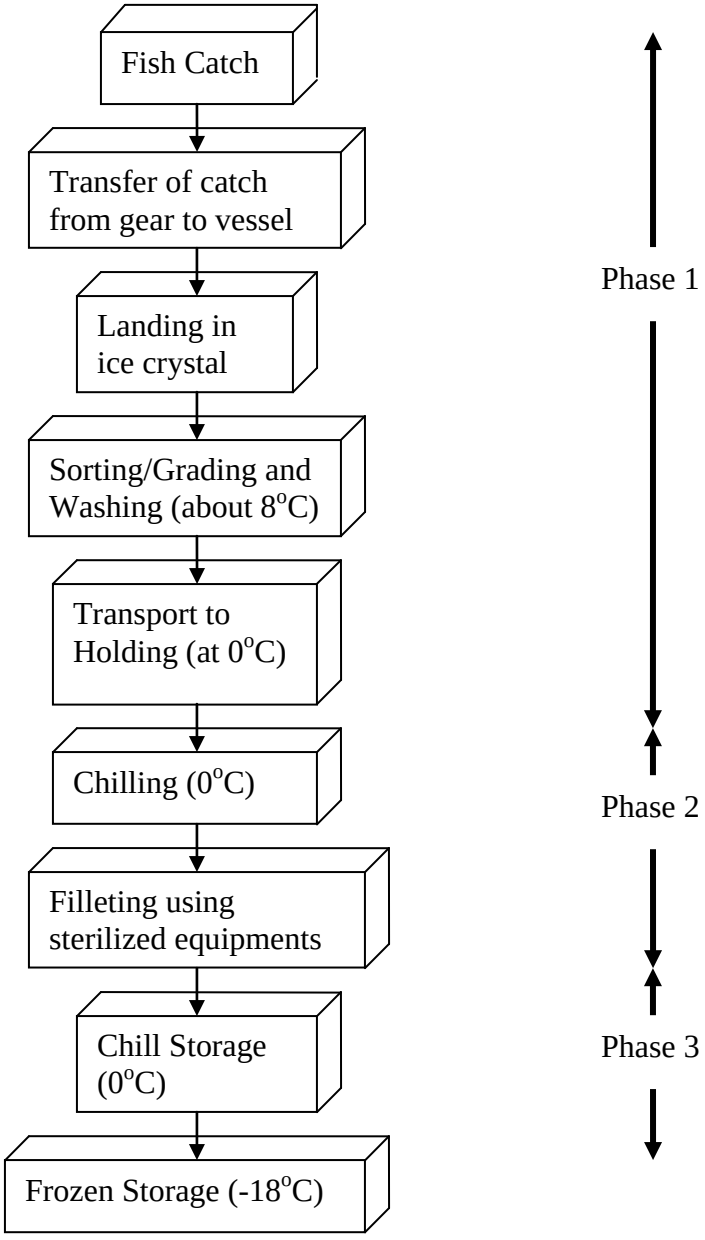

Figure 6.1 Suggested typical unit operations in fish catch, catch handling and frozen storage of tilapia fish fillet

Important aspects in the catch handling (FAO, 1994) are:

- ✓ Phase one covers the time used for the necessary handling onboard, i.e., until the fish is placed in chilling medium. It must be as short as possible. The fish temperature at time of capture is high enough to initiate fast spoilage rate.

- ✓ Phase two - the chilling process - must be arranged so that a fast chilling rate is obtained for the whole catch. Maximum chilling rate will be obtained by a homogeneous mixing of fish and ice, where the individual fish is completely surrounded by ice and the heat transfer is therefore maximum, controlled by the conduction of heat through the flesh to the surface. The appearance of fish completely surrounded by ice is often deteriorated due to discolorations and impression-marks (Thailambal, 2007). In practical life, icing is therefore often done by placing a single layer of fish on top of a layer of ice in the box even if it is bad practice from a temperature control and shelf life point of view. Cooling is primarily achieved by melt-ice dripping from the box stacked on top. This type of chilling will only function satisfactorily if fish boxes are shallow and have a perforated bottom.
- ✓ Phase three, which covers the chilled storage period, is important where an evenly distribution of temperature is maintained in the fish until transferred to the main frozen storage at $-18 \pm 2^{o}C$. This period may be extended for several days and it has to be given top priority.

Amount of Ice Used to Chill the Catch

Although ice is used to chill the catch while transporting the fish to the processing hall, the amount of ice provided by the FPME is usually not sufficient. For any given container the ice:water:fish ratio can be calculated from a knowledge of ambient temperature, water temperature, the insulative properties of the container and the period of holding. The requirement of the ice is to cool the water and fish to 0°C, to counter the heat input from aeration and to counter against the heat gain through the container. Theoretically, the amount of ice necessary to cool down fish from a temperature T_f to 0°C can easily be calculated from the following energy balance:

$$L \times M_{io} = M_f \times cp_f \times (T_f - 0) \qquad (9)$$

Where: L = latent heat of fusion of ice (80 kcal/kg)

M_{io} = mass of ice to be melted (kg)

M_f = mass of fish to be cooled (kg)

cp_f = specific heat capacity of fish (kcal/kg · °C)

From equation (9) it emerges that:

$$M_{io} = M_f \times cp_f \times T_f / L \qquad (10)$$

The specific heat capacity of lean fish is approximately 0.8 kcal/kg · °C (FAO, 1994). This means that as a first approximation:

$$M_{io} = M_f \times T_f /100 \qquad (11)$$

This is a very convenient formula, easy to remember, to quickly estimate the quantity of ice needed to cool fish to 0°C and to plan the amount of catch. The theoretical quantity necessary to cool fish to 0°C is relatively small and in practice much more ice may be used to keep chilled fish. The main reason for using more ice is because of losses caused by different factors. There are losses due to wet ice and ice spilt during fish handling, but by far the most important losses are thermal losses that may be aggravated when processing unit site is far from the source.

In principle, the energy balance between the energy taken by the melted ice to compensate heat from outside the box or container could be expressed as follows:

$$L \times (dM_i/dt) = U \times A \times (Te - T_i) \qquad (12)$$

Where: M_i = mass of ice melted to compensate for thermal losses (kg)

U = overall heat transfer coefficient (kcal/hour · m^2 · °C)

A = surface area of the container (m^2)

T_e = external temperature

T_i = ice temperature (usually taken as 0 °C)

t = time (hours)

Integrating equation (12) (assuming T_e = constant) results in:

$$\int dM_i = (U \times A \times T_e/L) \int dt$$

$$M_i = M_{io} - (U \times A \times T_e / L) \times t \qquad (13)$$

It is possible to estimate thermal losses, calculating U and measuring A. However, this type of calculation will seldom give an accurate indication of ice requirements for a number of practical factors (lack of reliable data on materials and conditions, irregularities in the construction of containers, irregular geometric shape of boxes and containers, influence of lid and drainage, radiation effect, and type of stack). However, this can be planned in advance and managed.

Sanitation of Harvesting Vessels

The traditional vessels (the papyrus reed boats), the wooden punts used by some fishermen as well as the motorized boats used by the FPME to transport the catch to the processing hall are not cleaned regularly. In some cases they clean these equipments using the water from the lake itself which may not be safe. On a harvest vessel, a good sanitation program that include regular cleaning and sanitizing of nets, equipment (harvest and processing), holds, totes, baskets, boxes, and bins must be in place. It is also critical that water, refrigeration and freezing media, such as refrigerated lake-water and brines, and ice are as free of microorganisms as possible. Thus, appropriately treated water should be used in processing, ice production, fish food contact surface cleaning and rinsing, and in other applications whereby contamination is possible. In general, good sanitation procedures should be applied throughout the harvest, transport, storage, and post-harvest handling of the fish products.

Processing

Manual deboning, as performed by the FPME, causes disruption of the tissue and exposure of the flesh to air, which accelerates several deteriorating processes, such as lipid oxidation and bacterial proliferation which in turn result in biochemical changes. It may not be possible to protect lipid oxidation caused by exposure to air during deboning. However, the microbiological contamination and spoilage can be minimized by using sterilized and/or hygienic equipments.

Rate of Freezing

Freezing in the air blast freezer takes place for a long period of time (for 24 hours). Slow freezing of the fish tissues leads to defects in sensory attributes and formation of larger ice crystals in the extra-cellular spaces. The large ice crystals, particularly needle shaped ones, mechanically damage the cellular structure and upon thawing, the food material will have a poorer texture due to loss of structural components.

The freezing time should be as short as possible. Fast cooling and freezing greatly reduce dehydration for two reasons. First, the temperature of the product is reduced quickly, which minimizes the evaporation rate. Second, fast freezing minimizes the length of product holding time that results in defects of sensory attribute. There is some quality loss during freezing, even with quick freezing. However, if the initial quality is very high, double freezing can still result in very good quality. Double freezing refers to storage and processing operation in which the product is frozen, then thawed or partly thawed (preferably microwave) to facilitate processing, then refrozen. A complete mathematical solution of freezing rate is not possible (Fellows, 2000). However, an approximate solution based on the modified Plank's equation for one-dimensional objects suggested by Pham (1986) with the modification for the effect of object shape proposed by Cleland (1991) is adequate (Rotstein *et al*., 1997).

$$t_f = \frac{1}{E} X \left[\frac{\Delta H_1}{\Delta T_1} + \frac{\Delta H_2}{\Delta T_2} \right] X \left[\frac{R}{h} + \frac{R^2}{2K_f} \right] \qquad (14)$$

Where $\Delta H_1 = \rho C_u (\theta_i - \theta_{fm})$

$\Delta H_2 = \rho L + \rho C_f (\theta_{fm} - \theta_{fin})$

$\theta_{fm} = 1.8 + 0.263\ \theta_{fin} + 0.105\ \theta_a$

$\Delta T_1 = \left(\frac{\theta_1 + \theta_{fm}}{2} \right) - \theta_a$

$\Delta T_2 = \theta_{fm} - \theta_a$

t_f = freezing time (S)

ρ = density of the product

K_f = thermal conductivity

θ_i = the temperature at which the product enters the freezer

θ_{fin} = the temperature at which the product exits the freezer

θ_a = cooling air

θ_{fm}= mean freezing temperature

L = latent heat of freezing

C_f = specific heat capacity of frozen food

C_u = specific heat capacity of unfrozen food

h = surface heat transfer coefficient

R = the shortest distance from the thermal center (slowest cooling point) of the product to the product surface

E = constant; E = 1 for infinite slab

E = 2 for cylindrical shape

E = 3 for sphere

✓ Operators should be aware of the factors which affect freezing time, which include:

- Freezer type
- Operating temperatures
- Product type
- Product temperatures
- Product thickness and
- Contact between the product and the freezing surface.

Packaging

Polyethylene, which does not provide a good barrier to water vapor and oxygen transmission, is used by the FPME as a packaging material. No consumer information is also labeled on the package. The packaging material must have adequate barrier properties to reduce loss of moisture due to dehydration and pick-up of taints. Chlorinated polymers such as polyvinylchloride films (like polyvinylidene chloride or saran wrap) provide very good protection against gas exchange, dehydration and oxidation attributed to their very low permeability to water vapor and oxygen. Laminated plastic aluminium foil may also be used when good vapour and moisture barriers are required, particularly with fatty fish to prevent fatty acid oxidation (Rotstein et al., 1997).

The package should give instructions on how the product should be stored and prepared, the consumer information on the ingredients and nutritional properties of the product, the 'use by' or 'best before' date, country of origin, name of the manufacturer, etc. All packages should be clearly date-stamped so that stock rotation, on the principle of first in, first out, can be strictly observed. Since migration of the plasticizers from the wrapping is a potential health hazard, the type of wrapping which can be in contact with fish should be covered by national legislation.

Heat Leakage in the Cold Room

The majority of heat leakage is through ceilings, floors, external walls and product load. The total heat load is a function of many factors viz. the temperature gradient or differential between the ambient and your set point, the quantity and thermodynamic properties of material -animate and inanimate - in the room, the air exchange rate or air cycles per unit of time, heating appliances in the room such as heat emission from electronic and electrical devices, the incandescent lamps and other illuminations, frequency at which the door and windows are open, the thermal resistance of the walls (insulation properties).

1. Heat loss through walls, ceilings, and floor.

Heat loss through walls, $Q_w = A \times \frac{K}{X} \times (T_a - T_s)$

Where A = Area

K = thermal conductivity of the insulating material

X = thickness

T_a = ambient temperature

T_s = set point -the temperature you want to achieve

$$Q_w = \frac{96.6m^2 \times 0.042\ Kcal/h.m.^oc \times (16.692-(-18))^oc}{0.1m}$$

Q_w = 1407.5238 Kcal/h

Heat loss through ceiling, $Q_c = A \times \frac{K}{X} \times (T_a - T_s)$

$$Q_c = \frac{61.562m^2 \times 0.042\ Kcal/h.m.^oc \times (16.692-(-18))^oc}{0.1m}$$

Q_c = 896.99774 Kcal/h

Heat loss through floor, $Q_f = A \times \frac{K}{X} \times (T_{sl} - T_s)$, where T_{sl} is soil temperature.

$$Q_f = \frac{61.562m^2 \times 0.042\ Kcal/h.m.^oc \times (12-(-18))^oc}{0.1}$$

Q_f = 775.6812 Kcal/h

2. Product load

$Q_p = m \times C_p \times \int dT$

$Q_p = m \times C_p \times (T_p - T_s)$, where m is mass of the product per day

C_p is specific heat capacity of the product

T_p is temperature of the product

$$Q_p = \frac{2000Kg}{24h} \times 0.8\ kcal/kg \cdot °C \times (0-(-18))^oc = \mathbf{1200\ Kcal/h}$$

3. Miscellaneous heat load

a) Lights left on during working days

$\mathbf{Q_l}$ = 6 x 60W = 360W = **309 Kcal/h**

b) Human load

Heat produced by operator at -18^{o}c is about 240W per hour.

A person spends about 24 min per day in the cold room. Thus, the heat produced by a person per day is: $Q_h = \frac{240 \times 24 \times 60}{24 \times 3600}$

Q_h = 4W

For 8 persons, $\mathbf{Q_h}$ = 8 x 4W = 32W = **27.52 Kcal/h**

c) Fan load

For 8 fans at 90W, $\mathbf{Q_{fl}}$ = 8 x 90W = 720W = **619.2 Kcal/h**

d) Defrost load

3.52 KW recovered over 4 hours.

$\mathbf{Q_d}$ = 3.52 KW/4 = 880W = **756.664 Kcal/h**

Total Heat Load, $Q_T = Q_w + Q_c + Q_f + Q_p + Q_l + Q_h + Q_{fl} + Q_d$

$\mathbf{Q_T}$ = 1407.5238 Kcal/h + 896.99774 Kcal/h + 775.6812 Kcal/h + 1200 Kcal/h + 309 Kcal/h + 27.52 Kcal/h + 619.2 Kcal/h + 756.664 Kcal/h = **5992.6 Kcal/h**

The refrigeration plant has 7.6KW, i.e, 6535Kcal/h which is suitable for the cold store. However, some precautions should be taken to protect energy loss.

The followings are suggestions on the improvement of heat leakage in the cold room:

- ✓ Use of panels with a rib profile on the external face of aluminium which is also the total external cladding - with polyurethane foam insulation and an internal face of low profile corrugated aluminium (FAO, 1994) may help to reduce heat loss through ceiling, walls and floor.
- ✓ Never stack goods directly on the floor of cold store, or close to the walls and ceilings. Leave a standard air space below and around the goods so that cold air can pass between the goods and the store structure. Pallets can be used on the floor, and vertical battens can be fixed to the walls. It is recommended that products should be at least 4 inches off the floor, 8 inches away from walls and 18 inches from roof cooling units (FAO, 1994).
- ✓ Never leave the door open for longer than necessary. In case having two doors never have more than one door open at a time as this will allow cold air go out and increase the internal temperature that accelerate enzymatic reaction and production of quality defect attributes.
- ✓ All cooling pipes in the store should be defrosted at regular interval (auto defrost freezers are preferred); efficiency of the cooler will be considerably reduced if frost is allowed to accumulate.
- ✓ Temperature of the incoming product should be as close as possible to the temperature of the cold storage room to minimize temperature fluctuation in the product. The bigger the temperature difference, the faster will water vapour leave the product to appear as frost on the cooler. Thus, freezer burn will be excessive when the temperature difference is large.
- ✓ Fluorescent lamps in the cold room and freezer should be replaced with special low temperature light sources such as high pressure sodium. This will provide: (1) better illumination levels per watt; (2) improved operational capability; (3) longer lamp life and (4) reduced lamp heat load.

Shelf Life of the Fish Products

The packages of the fish products at the FPME are not labeled with manufacturing and expiry dates. Like other foodstuffs and pre-packed foods, seafoods must bear a 'date of minimum durability' or, a 'use by date'. Clearly, realistic determination and accurate prediction of shelf life of fresh and lightly preserved seafood are important to meet consumer demands and to comply with legislative requirements. The determination of shelf life of the fish products can be achieved by estimating the product's shelf life at $0^{o}C$ and using the mathematical model suggested (Danopoulos and Ninni, 1972) for shelf life prediction of seafood stored at constant and fluctuating temperatures:

$$\text{Shelf life at T } (^{o}C) = \frac{\text{Shelf life at } 0^{o}C}{\text{Exp } [0.12 * T\ (^{o}C)]} \qquad (15)$$

The reference temperature of 0°C which is applied for the calculation of relative rate of spoilage in fresh fish may be inappropriate for lightly preserved products, and a different reference temperature (T_{ref}) can be used as shown in equation 16.

$$\text{Shelf life at T } (^{o}C) = \frac{\text{Shelf life at } T_{ref}\ (^{o}C)}{\text{Exp } [0.15 * (T - T_{ref}\ (^{o}C))]} \qquad (16)$$

7. Conclusion and Recommendations

7.1 Conclusion

The FPME is located at a distant from fish sources and the raw fish needs to be transported for a long period of time which may lead to losses. Thus, it is important to minimize loses at all levels from catch, transportation and processing to storage and distribution. The FPME needs an improved management and food preservation technologies. The increasing interest in marketing of more natural and less heavily preserved fish products is an important challenge.

The following specific conclusions are drawn from this study:

1. During frozen storage of fish fillet, significant losses in the protein, ash, and fat contents have been detected as a result of changes in chemical constituents that lead to a strong effect on the commercial value.
2. The data on proximate composition, pH value and chemical constituents revealed that the fish processing condition has significant effect on the deterioration of the physicochemical quality of the fish fillets.
3. The TVB-N content increased linearly with storage time, although it didn't reach the upper limit of 35 mgN/100g after preserving for 90 days. Thus, the products are judged fit for consumption. Based on the results, TVB-N can be used as an indicator of quality deterioration of tilapia fish fillets.
4. Bacteriological analysis indicated that freezing has significant effect on the reduction of bacterial population after 30 days preservation and then remained unchanged. A significant reduction ($p < 0.05$) in the bacterial load has been observed as a result of freezing for 30 days.

5. It was observed that the processing of fish until frozen storage has not been done under good sanitary conditions. Thus, consumption of raw fish should be avoided. This is particularly important if the fish are not properly chilled or frozen for 30 days as some bacteria may survive and cause health problems.
6. The results from this study can be used for further investigation and fish product marketing which in turn leads to boosting up fish production with good post-harvest management in the country.

7.2 Recommendations

Based on the research findings, the following recommendations are forwarded:

- Scientific knowledge is required to determine the type and level of extension packages suitable to initiate aquaculture development in Ethiopia as a strategy of food security.
- Further detailed research is required to help commercial processors to decide how long processed tilapia fish fillets can be stored under frozen condition as wholesome and safe for human consumption.
- For any long term storage of frozen fish fillet, the fish catch should at all times be kept in a cool condition before freezing. The cold store should be capable of maintaining the recommended temperature of -18°C without fluctuation which can degrade or damage the fish product and encourage dehydration.
- Selection of high quality raw fish for processing, packaging materials and use of low storage temperature with minimum fluctuation should be performed. The quality of the final fish product is highly dependent on these factors.

- In order to produce high quality fish products for the domestic and export markets, the FPME and other fish processing companies in Ethiopia are recommended to implement the suggested fish processing techniques, summarized in chapter six, properly. More detailed research should also be conducted to introduce more appropriate equipments and technologies.
- Fisheries legislation is required in Ethiopia. There are lack of legal provisions to monitor fishing and fish processing activities through fisheries regulations and lack of a clearly designated enforcement agency. The lack of fishery regulation leads to the use of inappropriate fishing materials and methods, over-fishing of the resources and inappropriate post-harvest technologies and management.

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Annex-1

Table for 3 tubes each at 0.1, 0.01, and 0.001 dilution inocula, the MPNs per gram and 95 percent confidence intervals.

Number of Positive tubes			MPN/g	Confidence limit		Number of Positive tubes			MPN/g	Confidence limit	
0.10	0.01	0.001		Lower	Higher	0.10	0.01	0.001		Lower	Higher
0	0	0	<3.0	--	9.5	2	2	0	21	4.5	42
0	0	1	3.0	0.15	9.6	2	2	1	28	8.7	94
0	1	0	3.0	0.15	11	2	2	2	35	8.7	94
0	1	1	6.1	1.2	18	2	3	0	29	8.7	94
0	2	0	6.2	1.2	18	2	3	1	36	8.7	94
0	3	0	9.4	3.6	38	3	0	0	23	4.6	94
1	0	0	3.6	0.17	18	3	0	1	38	8.7	110
1	0	1	7.2	1.3	18	3	0	2	64	17	180
1	0	2	11	3.6	38	3	1	0	43	9	180
1	1	0	7.4	1.3	20	3	1	1	75	17	200
1	1	1	11	3.6	38	3	1	2	120	37	420
1	2	0	11	3.6	42	3	1	3	160	40	420
1	2	1	15	4.5	42	3	2	0	93	18	420
1	3	0	16	4.5	42	3	2	1	150	37	420
2	0	0	9.2	1.4	38	3	2	2	210	40	430
2	0	1	14	3.6	42	3	2	3	290	90	1,000
2	0	2	20	4.5	42	3	3	0	240	42	1,000
2	1	0	15	3.7	42	3	3	1	460	90	2,000
2	1	1	20	4.5	42	3	3	2	1100	180	4,100
2	1	2	27	8.7	94	3	3	3	>1100	420	--

Declaration

I, the undersigned, declare that this is my original work, has not been presented for a degree in any University, and that all sources of materials used for the thesis have been duly acknowledged.

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Place: Addis Ababa_____

Date of submission: _____

Annex