

## Freeze drying of fish, Thawing of fish, Quality changes during frozen storage, Microbial growth at low temperature

### 3.6.1. Introduction

Heat is used to dry foods or concentrates liquids by boiling to remove water and therefore preserves the food by reduction in water activity. However, heat causes a loss of sensory and nutritional qualities. In freeze drying a similar preservative effect is achieved by reduction in water activity without heating the food and sensory and nutritional qualities are consequently better retained. However both, operations (freezing & drying) are slower than conventional dehydration. Freeze drying has become an important commercial operation to dry expensive foods which have delicate aroma or texture (for example instant coffee, mushrooms, fruit juices and sea foods).

### 3.6.2. Theory of freeze drying

The boiling point of water depends on the overhead atmospheric pressure. At normal atmospheric pressure of 1 bar (i.e., 760 mm of Hg) it boils at 100°C. However, if it is held in a sealed container and overhead atmospheric pressure is lowered than one by using vacuum pumps, then it boils at less than 100°C. It is observed that the lower the pressure (or the better the vacuum) the lower is the boiling point of water but the energy needed for evaporation of the same quantity of water is more. The pressure, the corresponding boiling point of water and latent heat of evaporation are presented in the following table.

<i>Pressure (bar)</i>	<i>Boiling Point of Water °C</i>	<i>Latent heat of vaporization (kj/kg) of water</i>
1	100	2257
0.5	81	2305
0.2	60	2358
0.1	46	2392
0.01	7	2485

At 0°C water exists as ice (solid phase) and if the overhead pressure is sufficiently low, solid ice will directly sublime as water vapour without going into liquid phase. To understand the phenomenon more clearly it is essential to refer to the phase diagram of water.

#### Pressure units conversion table :

<b>Torr</b>	<b>mm Hg</b>	<b>Pascal</b>
1	1	133.3
10-1	0.1	13.33
10-2	0.01	1.333

From the phase diagram it is clear that if the temperature of water and pressure is below the triple point it exists as ice and it will directly pass into vapour phase.

If the overhead pressure of a food is held below 4.58 Torr (610.5 Pa) and the food is frozen, when the frozen food is heated solid ice sublimates directly to vapour phase without melting. Water vapour is continuously removed from the food by keeping the pressure in the freeze drier cabinet below the vapour pressure at the surface of ice by removing the water vapour with a vacuum pump and condensing it on refrigeration coils or plate or drum. As the drying proceeds, the sublimation process continues as long as latent heat of sublimation is provided/supplied. The latent heat of sublimation is either conducted through the food to the sublimation front or produced in the food by microwaves. The pressure, sublimation temperature in the frozen state and corresponding latent heat of sublimation are presented below.

In freeze drying, foods are dried in two stages, first by sublimation to approximately 15% moisture content (on wet weight basis) and then by evaporative drying of unfrozen water to 2% of moisture content (W.W.B). Desorption is achieved by raising the temperature in the drier to near ambient temperature while retaining the

low pressure.

The rate of drying depends mostly on the resistance of the food to heat transfer and on the resistance to vapour flow.

### 3.6.3. Principle of the equipment

Effectiveness of a freeze drier depends on rate of heat transfer to the sublimation front and rate of mass transfer. There are three methods of heat transfer.

Heat transfer through the frozen layer. The rate of heat transfer depends on the thickness and thermal conductivity of frozen layer. As the drying proceeds, the thickness of ice is reduced and the rate of heat transfer increases.

Heat transfer through dried layer. The rate of heat transfer to sublimation front depends on the thickness of the dried layer and the surface area of food, thermal conductivity of dry layer and the temperature difference between the surface of the food and ice front. The dried layer has very low thermal conductivity and therefore offers high resistance to heat flow. As drying proceeds, this layer becomes thicker and the resistance increases.

**Heating by microwaves :** Heat is generated at the ice front and the rate of heat transfer is not influenced by thermal conductivity of ice or dry matter or the thickness of dry layer. However it is difficult to control microwave heating.

**Rate of mass transfer:** It depends on the water vapour pressure gradient inside the drying chamber which is controlled by

1. The pressure in drying chamber
2. Temperature of the vapour condenser – both should be as low as possible.
3. Temperature of ice at the sublimation front and it should be as high as possible (at 0°C) without melting.

**Equipment :** freeze drier consists of a vacuum chamber containing trays to hold food during drying, source of heat to supply latent heat of sublimation, refrigeration coils to condense vapour with automatic devices to keep maximum area of the coil free of ice for vapour condensations and vacuum pump to remove non expensible vapour.

**Types of equipments :**

1. Accelerated freeze driers : Food is held between expanded metal mesh for heat transfer.
2. Radiation freeze driers : In paired radiations from radiant are used. Heating is more uniform. Close contact between food and the heat is not necessary; float trays can be used and cheaper. Easier to clean.
3. Microwave and dielectric freeze driers

### 3.6.4. Freeze drying process

Freeze drying cycle consists of 3 stages.

1. Freezing.
2. Primary drying to remove water ice.
3. Secondary drying to remove bound water molecules from the primary dried materials.

1) **Freezing :** Freezing the product temperature is reduced to -50°C at one atmospheric pressure to ensure that it is below the eutectic point.

2) **Primary drying :** This is a dynamic process involving transits of ice phase to vapour phase directly. This leaves the solid matrix formed in the freezing phase but without the ice crystals. During drying, an interface is set up which comes from the exposed surface to vacuum system to the surface where heat source is in contact or moves deeper in radiant heat. At this interface, actual drying takes place. There are number of factors which will indicate the completions of primary drying process.

1) **Pressure stability :** During drying, pressure changes slowly until it stabilizes at an ultimate low level. The process is considered as complete where the constant pressure is maintained for 1 to 2 hours.

2) **Product temperature rise.** When heat energy is not required for sublimation, the overall product temperature will rise until equal or close to the chamber temperature. This can be detected with a temperature probe and

displayed.

3) Pressure rise test. If an isolation valve is available, it will be possible to isolate the drying chamber. If the material is not dry, then the pressure will rise quickly (due to vapour pressure of the product).

**Secondary Drying:** This is part of the process where remaining small proportion of “locked in” water is removed ( Usually 2.5% by volume). This is not always considered necessary and depends on the product. The locked in or bound water is removed from the products by supplying additional heat energy to the system. This is achieved either by increasing the temperature or by improving the thermal contact between the product and the existing of heat source.

**Effect of freeze drying on food :** Freeze dried foods have high retention of sensory and nutritional qualities and shelf life of more than 12 months when correctly packed. Aroma retention is 80-100%. Shrinkage is very little and texture is well maintained. Allows rapid and full rehydration but the product is fragile and require protection from mechanical pressure. However, the open porous structure of the food may allow oxygen to enter and cause oxidative deterioration of lipids and subsequent browning. Changes in thiamine and ascorbic acid content during freeze drying are moderate and there are negligible losses of other vitamins. Therefore vacuum or inert gas packaging is required.

## Thawing of fish

### Chapter 1: Methods of fish thawing

#### 4.1.1 Introduction

Thawing is a process whereby heat is introduced into the frozen product. In order to raise its temperature above freezing point, the rate of heat input required to thaw 1 ton of fish per hour is approximately 85 KW. Thawers or methods of thawing can be divided into two broad classes.

1) Those which depend only on the conduction of heat through the thawing material.

E.g. Thawing in air, thawing in water and thawing between heated plates.

2) Those which do not depend on the conduction of heat through the thawing material.

E.g. Dielectric thawing and electric resistance thawing.

There are also other methods such as infra red and microwave heating, but they are not of interest for thawing on a large scale

#### 4.1.1.1 Thawing in air

##### a) *Natural convection:*

It is sometime convenient to thaw at room condition, if conditions are suitable. Temperatures above 18°C should not be used. Lot of space and labour is required for thawing on a large scale by this method and the introduction of humidification to prevent slight drying of the fish is difficult.

##### b) *Air blast:*

In this method, a current of air is directed on the frozen block and the increased air velocity reduces thawing time. The rate of thawing is mainly dependent on the resistances of the fish itself to the flow of heat. At the air velocity of 6m/sec, there is very little resistance, which is optimum. In modern air-blast thawers with the recirculation of humid air at 20°C (the maximum, temperature that should be employed), the 10cm block of frozen fish requires about 5 hours for thawing before they can be filleted. The fish can be separated easily after about 3 hours, which reduces the thawing time to about 4 hours and gives more even thawing. Complete humidification of the air is necessary in order to prevent drying of the fish and to achieve ideal condition for heat transfer. Weight losses in the fish normally do not exceed 1%. The problem of cleaning is greater with the air blast thawers when compared with most other types. Two types of air blast thawers, i) continuous ii) batch.

##### i) *Continuous:*

Continuous air-blast thawers have been built in two designs. Cross flow and parallel flow. In the cross flow design, the direction of air flow is across the conveyor at right angle. Typically there are five conveyors, one above the other. The fish travels horizontally along the top conveyor until when it reaches the end, it falls to the conveyor below which is traveling in the opposite direction and so on. In order to achieve high velocity, capacity of the fan should be high with this arrangement and use of fan power is more than 30 KW for a unit fish. Thus a large portion of the heat for thawing is obtained from the fan.

In the parallel flow thawers, the air travels in the same direction as the conveyor. With humid air, most of the heat transfer to the fish takes place by condensation of water vapour and an air temperature drop at 30°C can be tolerated. The fan capacity is relatively low and therefore, the fan power is about 7.5 KW for the 1 ton/hour unit and the face area of the heater and humidifier in conveyor is, in a single line occupying a greater

floor area, than a cross flow thawers. In the other, the conveyor is divided into three sections, one above the other.

**ii) Batch:**

The largest model of batch air-blast thawers has five compartments each holding 1 ton of 10cm blocks of whole fish on two trolleys with suitably- spaced shelves, giving an output at about 1 ton/hour. Air flow is in series through the five compartments in a line. Periodic reversal at air flow can be employed to even out differences between compartments. The thawers can also be used on a semi continuous basis by inserting and withdrawing trolleys at intervals of 1 hours. More labour for loading and unloading is required than for continuous thawers, but the batch thawer is simple and compact. The 20 KW fan heaters and humidifier are located above the fish compartments. Batch thawers with small fans giving moderate velocities, in which blocks of fish are placed on racks or shelves, have been used for thawing frozen fish for over periods of up to 10 hours.

#### 4.1.1.2 Water thawing

**a) Immersion:**

The thawing of blocks of whole in water has been shown to give good results, but has been used only to a limited extent for thawing on a large scale. The thawed product can be as high in overall quality and suitable for processing. Pump capacities for water thawers need not be great, because the water velocity required for good heat transfer is 0.5 cm/sec, giving thawing rates equal to those for air-blast. It is probably necessary, to use somewhat higher velocities, perhaps 2cm/sec or more, in order to give a good degree of mixing of the water, preventing stratification and to provide reasonably quick response to the heater, particularly for warm up with initially cold water. Too high velocities induce turbulence, causing foaming at the surface of the water and possibly lead to water logging. The temperature of the water should conserve heat, it is better to change the water once or twice in 24 hour so as to limit the degree of contamination of the water.

**b) Spray:**

In this, larger thawers are used, in which heated water is sprayed directly on the fish. The blocks of fish travel on a conveyor, which is made up of a series of open mesh, wire baskets in a housing ventilated by a small fan. The water is recirculated through a special filter and cleaner section. The thawing time is claimed to be at least as short as in air, blast thawing. Hygienic condition is maintained in the thawers because of the working action of the sprays of clean water. It takes about 5 hours for the 10cm block of whole fish to be thawed completely, in a thawer with are output of 1ton/hour.

#### 4.1.1.3 Thawing between heated plates

This type of thawer is similar to a plate freezer except that warm fluid, normally at 20°C is circulated throughout the (metal) plate. The product is held in contact with the plates throughout the thawing period which is about 8 hours for complete thawing of the 10cm block of whole fish. Thawing is more even than on air blast thawing, with the result that the fish can be filleted sooner, typically 7 hours after an initial period at 4 hours in the thawer as opposed to 12 hours in air-blast thawing. Little power is required excluding the heat for thawing, the plant is compact and robust

**a) Dielectric thawing:**

In this method, the frozen material is placed in a high frequency electrical field between two flat metal plates. An alternating voltage at 5,000 V and 40MHz is applied to the plates and hence heat is produced in the electrically charged particles in the food. The plates are fed from a generator and suitably shielded to confirm the field. Unless the food is homogenous and regular in shape, there is possibility for uneven heating so that the parts of the material are cooked while other parts remain frozen. This can be minimized in block at fish continuously conveying them through the field and restricting the power density according to the degree of thawing. Thus, a typical industrial thawer for blocks at fish includes a conveyor belt and six generators each with a rating of 20KW giving a nominal output of 1 ton /hr with 10 cm blocks of whole fish. Thawing is very rapid so that the total length of conveyor has been much shorter than that for continuous air-blast thawers. The thawing time for blocks of large white fish is about 80 min, and for blocks of small fatty fish is about 20min.

**b) Dielectric and water:**

The dielectric thawer has a thermal efficiency of about 70%. Cooling water for the generators absorbs the waste

heat. It has been suggested that heated water be used for the thawing either before or after the dielectric thawer, a hybrid system, in order to increase the output and thermal efficiency and reduce the risk of overheating. Since the heat conduction is rapid during the early stages, the 10 cm block of whole fish can be 30% thawed in less than 30min in water at 15°C but the complete thawing takes up to 80 min at the temperature at 15°C.

#### **c) Electric resistance thawing:**

In this method, fish can be heated by placing it between two electrodes used applying a low voltage so that a current flows through. Like dielectric heating, it is difficult to achieve even heating unless the item is homogenous and regular in shape. It is convenient to partially heat the fish by a heat conduction method such as immersion in circulating tap water and thawing by heat conduction is rapid during the early stage. Usually the time taken for water immersion and electric heating should be roughly equal. The total thawing time is between 20 and 60 min depending on the type at blocks, at least twice as fast as thawing in air or water. A complete batch thawer of this type has plates and blocks of fish arranged in layers one above the other in the same way as a horizontal plate freezer. The plates are heated electrically or by passing worm fluid through them during the early stage at thawing when heat conduction is employed and act as electrodes during the stage of electric resistance thawing.

#### **d) Vacuum thawing**

Vacuum thawers consist of airtight chambers in to which the fish is loaded using trolleys. The temperature of the water vapour is typically between 18°C and 20°C and the vapour condenses on the cold surface of the fish where the latent heat of vaporization is absorbed by the fish meat transfer to the product is much more rapid than the previous method of thawing. Equivalent thawing fishes may be reduced from 20hr in air to less than 1 hr in a vacuum thawing unit and the water usage at such units is low. However, care must be taken that gases released from the fish as thawing proceed do not cause rupturing at flesh.

The advantages of this method of defrosting are that with the exception at a vacuum pump there are no moving parts, hence the possibility of mechanical breakdown is reduced compared to the previous methods. Also the vapour is distilled from water in the tank it condenses on to the fish in a pure state, so there is no risk of contamination from bacteria which may accumulate within the system.

### **4.1.1.4 Choice of method**

Many factors should be considered when choosing between the various methods. The choice depends largely on the type of product and the form in which it was frozen. In some uses, it is difficult to provide sufficient shielding in the dielectric thawer. The air-blast thawer might be at a disadvantage in the amount of cleaning required to keep bacterial counts within the statutory limits. All the main methods described above can produce satisfactory results with blocks of whole fish. Both heat conduction and electric methods fail with large fish. The electric methods give more uniform thawing with compact block at small fish. 'Continuous thawers' would appear to have advantage over batch thawers for blocks at whole fish. For blocks of fillets, water thawing is unsuitable and block thickness probably should not exceed 5 cm for thawing in humid air blast and between heated plates. The electric methods give good results with blocks of fillets. Block upto 10cm thick have been thawed successfully dielectric thawer using only three generators. Fillets, unprotected by the skin of the fish, are more susceptible than whole fish to deterioration on thawing, particularly shrinkage, discolouration and dehydration. Pronounced thaw-rigor accompanied by shrinkage and loss of fluid sometimes can be produced when thawing is rapid.

## **Quality changes during frozen storage**

### **Chapter 1: Quality changes in fish during freezing & frozen storage-physical changes**

#### **5.1.1 Quality changes in fish during freezing**

The physical changes which occur during freezing & storage of frozen products comprise crystallization of ice with expansion of the volume, and desiccation starting from the surface of the frozen fish.

##### **5.1.1.1 Ice formation**

The crystallization of a ice is initiated when the temperature of the fish is lowered about  $-1^{\circ}\text{C}$ . At the

same time, concentration of various inorganic salts and organic components present in the fluid of the fish occurs and consequently the freezing point falls. The temperature of the fish must be lowered further before additional water freezes. There is also an increase in the volume of the fish when the water is converted to ice. At  $-3^{\circ}\text{C}$  about 70% of the water is frozen. At  $-5^{\circ}\text{C}$ , about 85% is frozen; at  $-25^{\circ}\text{C}$  about 95% and at  $-50^{\circ}\text{C}$  to  $-60^{\circ}\text{C}$ , almost all the water in the fish is frozen. The larger part of the water consequently freezes between  $-1^{\circ}\text{C}$  and  $-5^{\circ}\text{C}$  and it is the rate of cooling this temperature interval determines the size of the ice crystals. The preserving effect of freezing however is due to the extent of reduction in the rate of chemical and biological processes, when the temperature of the fish is lowered. The crystallization or freezing of the water has certain unfavorable effects. Rapid freezing, results in small ice crystals, and the quality of quick frozen fish may be practically equivalent to that of fresh unfrozen fish.

Microscopic examination of frozen fish reveals that the changes occurring in freezing and thawing are caused rather by irreversible changes in the colloidal state of the proteins induced by the increased concentration of dehydrates in the unfrozen part of the fluid in the fish tissue. Small ice crystals are certainly a feature of quick freezing whereas large ice crystals can be the result not only of slow freezing but also of considerable post mortem changes in the tissue prior to freezing. Histological investigations on frozen fish revealed that when freezing fish with considerable post mortem changes, the ice crystals destroy the sarcolemma more easily and grow large sizes causing more histological changes to the tissue. The rate of freezing therefore has been considered of vital importance for obtaining a high quality product. The DNA content in the drip also varied with rate of freezing. With more rapid freezing, there is a drop in DNA content in the expressible fluid. In ultra rapid freezing, however, there is a rise in expressible DNA, but probably due to different kind of cell damage.

#### 5.1.1.2 Desiccation

Desiccation from the surface of the frozen fish takes place during freezing and storage because there is always transport of water vapour from the fish to the evaporator or cooling coils. The greater the temperature difference between fish and evaporator, and greater the velocity of the circulating air, the more moisture will be transported and the more frost will be formed resulting in the decreased efficacy of the evaporator. The loss of water from the product and the formation of snow on the evaporator coils are greater at high storage temperature, because the warmer air will take up more moisture. Change in fluctuation in storage temperature influences the desiccation, loss of weight and quality of the fish contributes to a poor appearance or results in "freezer burn". The critical limit of dehydration or rate of weight loss of frozen fish in cold storage is  $50\text{g/m}^2/24\text{h}$ .

Medium sized white fish stored in a rather dry room lost 7, 3.5 & 1.5% in weight per month at  $-9$ ,  $-21$  &  $-29^{\circ}\text{C}$ , respectively. The air surrounding the frozen product is usually at a lower moisture vapour pressure than the product and therefore acts as a sponge in removing moisture from the product. The loss of moisture results in the dehydration of the flesh to a point where chalky and fibrous texture develops, discolouration takes place and off flavours develops. The phenomenon is also known as "Freeze burn". When frozen fish is in contact with air, oxidation of fat or oil in the fish takes place and these results in "rusting" or discolouration of the flesh and development of rancid odours and flavours.

If the desiccation is pronounced, the fish surface may become dry and fibrous. In some cases the skin may change the colour, several other factors influence the loss are weight, the kind of wrapping, its sealing and moisture transmission characteristics. The rate of moisture loss or weight loss or water vapour transmission rate (WVTR) from fish in cold storage is expressed in  $\text{g/m}^2/\text{day}$ . The denaturation of the protein or reduced protein extractability is also due to the electrostatic interaction between protein and fatty acid. Freezing increases toughness of fish muscle, which proceeds progressively during subsequent storage. Toughness can not be measured in terms of percentage of soluble protein in the cold stored fish. The proportion of fish muscle protein soluble in 5% NaCl solution was not correlated with the development of toughness during cold storage at or below  $-20^{\circ}\text{C}$ . Assessment by a taste panel is time consuming and inaccurate. Since fish muscle fibres are extremely short and interspaces. Instruments such as penetrometers have limited usefulness. But by using a new method of treating the fish muscle with 1% formaldehyde, it was found that the unfrozen fish muscle fibres were broken up & there by reducing the light transmission. Frozen fish showed a higher proportion of intact fibres, letting through more light.

#### **Toughness:**

Toughness or changes in texture can be due to denaturation of proteins. The denaturation may be due to the concentration of inorganic salts which results in damage to the flesh proteins or due to lipid hydrolysis and myoglobin in the cells, are the main colouring pigments in fish flesh. The dark parts of the muscles contain much more pigment than the light or white muscle.

### 5.1.1.3 Discolouration

In freshly caught fish the blood has a brick red color of oxy hemoglobin. This color gradually changes to the red brown or brown color of met hemoglobin. Myoglobin suffers the same kind of changes. Frozen tuna and sword fish may exhibit green and brown discolouration on looking. Uncooked fish meat contains three derivatives of myoglobin. The pigment responsible for the pink colour in normal cooked meat of tuna is hemochrome, derived from the reaction of myoglobin with non-heme constituents. Greening is due to pigments resulting from the oxidation of hemochrome that occurs when the meat is unduly exposed to oxidative condition during and after cooking. Greening of frozen sword fish may be related to take H<sub>2</sub>S produced by putrefactive bacteria. Proper evisceration and removal of blood immediately after the catch reduce the risk of discolouration. The undesirable discolouration in yellow fin tuna meat can be averted, if the fish is frozen at full rigor, stored at a temperature of -23<sup>0</sup>C to -27<sup>0</sup>C, and defrosted by still air at 10<sup>0</sup>C.

#### **a. Carotenoid pigments:**

Some fishes like salmon, and some crustaceans, contain carotenoid pigments in the flesh. The red color astaxanthin tends to fade away during frozen storage. In crustacean, like lobster, pigment is limited to the surface of the meat, the changes during frozen storage lead to yellow discolorations.

#### **b. Fish oils:**

The oils & fats naturally occurring in fish vary with species from colourless, as in herring through yellow to red, as in salmon. Fat herring, with a layer of oil just under the skin, may develop a yellow color during poor storage condition & which can be seen through the skin. Such fish is said to have “rusted”.

#### **c. Black spots:**

Shrimp, lobster etc, develop so called black spot when stored fresh. Bruises and rough handling increase the occurrence of this discolouration, which is caused by an enzyme, tyrosinase acting on polyphenolic compounds, present in free amino acids like tryptophan, tyrosine and form black 'melanin' pigments.

#### **Flavor changes:**

Systematic studies of flavor changes in frozen fish are lacking. Freshly processed fish, frozen and cold stored, is generally considered first to lost it's characteristic fresh fish flavour, becoming bland & insipid; there after it develops flavors variably described as “cold stored” “Salt fishy’ etc.

#### **Break down of connective tissue:**

During some occasion, fish fillets are produced in which the muscle flakes will separate when the fish is filleted. This defect, often called “gaping” appears to result from a breakdown in the connective tissue and associated components which hold the muscle cells together. Although the main myofibrillar proteins, actin and myosin are believed to remain largely intact, many of the cytoskeletal protein like connectin, nebulin and desmin are believed to be degraded. The problem is, by and large, connected with freezing at sea and fillet production from frozen whole fish.

### 5.1.1.4 Fat oxidation during freezing

The changes in lipids are directly and indirectly responsible for the quality deterioration in frozen seafoods. They involve lipolysis, lipid oxidation, and interactions of the products of these processes with non-lipid components. The endogenous fish lipases are relatively resistant to low temperatures and retain much of their activity in the frozen tissues. Some of them may even be activated in the freezing process eg. by release from lysosomes. Fish lipids undergo two main types of changes during storage of fish viz, hydrolytic changes and oxidative changes. These changes result in rancidity.

Lipid hydrolysis

Lipases bring about hydrolysis of lipids producing free fatty acids and resulting in hydrolytic rancidity. Free fatty acids trigger protein insolubilization and texture degradation in frozen stored fish.

### **Lipid oxidation**

It is a very serious problem in fish, in view of the highly unsaturated nature of fish lipids. The double bonds of unsaturated fatty acids are highly susceptible to oxidation and this leads to the production of various carbonyls and other secondary oxidation products, which impart the characteristic rancid off flavour to the product. These products, besides producing off flavour reduce the shelf life and nutritional value of the product also. Some of them are toxic in nature. These reactions are initiated by free radicals generated from unsaturated bonds, which start chain reactions resulting in the production of various undesirable compounds like peroxides, hydroperoxides, aldehydes, ketones etc. Finally, the free radicals form non-radical polymers, which terminate the chain reaction.

Oxidised lipids interact with proteins reducing the nutritive value of the proteins considerably. Malonaldehyde is one of the major oxidation products and estimation of this compound by forming the thiobarbituric acid (TBA) complex is the accepted method for monitoring the extent of lipid oxidation. In lipid oxidation, the first step leads to formation of hydroperoxides, which are tasteless but can cause brown and yellow discoloration of the fish tissue. The degradation of hydroperoxides gives rise to formation of aldehydes and ketones. These compounds have a strong rancid flavour. Lipid oxidation primarily non enzymatic in nature, recently the involvement of microsomal enzymes and lipoxygenase has been reported. This lipid oxidation takes place in fishes having more than 2% of the lipids eg. fatty fishes.

### **Factors affecting the oxidation**

1. Content and composition of unsaturated fatty acid
2. Oxygen availability
3. Light radiation
4. pH
5. Temperature
6. Moisture content
7. Content of pro and anti oxidant.

#### **5.1.1.5 Factors limiting storage life**

**Protein changes:** Fish proteins undergo permanent change during freezing and cold storage and the speed at which this denaturation occurs depends upon temperature. At temperatures not very far below freezing point, -2°C for example, serious changes occur rapidly; even at -10°C, the changes are so rapid that an initially good quality product can be spoilt within a few weeks. The rate of deterioration due to protein denaturation, can be reduced by storage at as low a temperature as possible.

#### **5.1.1.6 Freeze Denaturation-I**

Unfolding of molecules due to secondary reaction between the reactive groups of different proteins and other components of fish muscle, leading to cross linking and formation of aggregates

- The proteins lose part of their solubility and reduced enzyme activity
- As a result of these changes significant deterioration of the functional properties of the fish meat may occur
- It is manifested by a decrease in water retention, gel forming ability and lipid emulsifying capacity worsening of texture and the increased dryness of fish meat
- Fish that are stored for longer period becomes tough, chewy, rubber and fibrous
- Causative factors comprise the denaturing are

1. Catalytic effect of ice and inorganic salts

2. The binding of fatty acid

3. Lipid oxidation products

- Cross linking induced by formaldehyde generated in the muscle of some fish (Gadoid) results in the formation of new covalent bonds in denatured products
- Ice crystals disturb the hydrophobic interaction in protein. Increase in concentration of salt in unfrozen food and water decrease the hydration of protein as the concentrated inorganic ions compete for the water molecules
- They participate in freeze denaturation of protein by catalyzing the hydrolysis and auto oxidation of lipids
- Free fatty acid decrease the solubility of myofibrillar protein and contribute to the formation of aggregates by binding to proteins due to hydrophobic interaction
- Oxidised fatty acid and secondary products of autoxidation especially aldehydes are much more damaging to protein solubility as they are able to form covalent bonds with reactive protein groups.

TMAO  $\rightarrow$  TMA + formaldehyde (HCHO)

So aldehydes is very reactive compound bind to some groups in protein side chain and form intra and intermolecular methylene bridges. By removing water soluble substrates and enzyme system responsible for the degradation of TMAO the undesirable protein changes can effectively be reduced.

#### 5.1.1.7 Freeze Denaturation-II

##### **Fat changes:**

Fatty fish may become unpleasantly altered during cold storage but they can be protected to some extent either by glazing or by packaging in plastic bags sealed under vacuum. These oxidation changes take place more rapidly at higher temperatures and storage at a low temperature is an effective means of slowing the rate of spoilage by this method.

##### **Color changes:**

The quality of fish is often judged by appearance, and color changes which are not otherwise significant can result in fish being downgraded. The changes in the fish flesh which bring about these color changes are also retarded at lower temperatures.

##### **Dehydration changes:**

Dehydration of the product is probably the major concern of the cold store operation and the rate of drying can be linked with a number of factors in cold store design and operation.

When fish get badly dehydrated in cold storage, the surface becomes dry, opaque and spongy. As time progresses, these conditions penetrate deeper into the fish until it becomes a fibrous, very light material. Visible effects of severe dehydration on the surface of the fish are known by the term "freezer burn". This is an unfortunate choice of term since the effect is unlikely to result from freezing in a properly designed freezer, and appears only after periods of storage in a cold store.

Frozen fish may dry slowly in cold storage even under good operating conditions. This is undesirable for reasons other than the obvious one that the product will lose weight. Drying also accelerates denaturation of the protein and oxidation of the fat in the fish. Even totally impervious wrappers used to protect the product do not give full protection if the cold store operating conditions are favorable for desiccation within the pack. In-pack desiccation prevails when there is some free space within the wrapper and temperature of the store fluctuates.

Weight loss during freezing will depend on: Type of freezer, freezing time, Type of product, Air velocity and

Freezer operating conditions.

Cold Store Weight Loss Depends on Temperature, Temperature fluctuation, Humidity, Heat transfer, Air flow over the product, Radiation effects of lighting, The product, Shape and size of the product and Type of wrapper.