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Biochemical and Microbial changes in fish

Unit -1

Biochemical Composition of Raw fish:

The **biochemical** and isotopic **composition** of nonliving organic matter carries a detailed molecular history of the biogeochemical system that produced it, including its biological and geochemical sources, fluid residence times, and the ultimate origins of elemental building blocks.

The percentage composition of the four major constituents of fish viz. water, protein, lipid and ash (minerals) is referred to as proximate composition (it may be noted that the term does not indicate any degree of inaccuracy in the analysis). These four components account for about 96-98% of total tissue constituents in most cases. The range of values for these constituents in the edible portion of common fish species from
Indian coastal waters are Carbohydrates, vitamins, nucleotides, other non-protein nitrogenous compounds etc. are also present in small quantities. Though quantitatively minor components, these play vital roles in maintaining the system and thus are essential for growth and development of the organisms.

Protein: Proteins play three major roles in nutrition. They provide both essential and non essential aminoacids which are building blocks for protein biosynthesis. Protein biosynthesis is necessary both for growth of infants and children and also for the constant replacement and turnover of body proteins in adults. Secondly, amino acids are precursors of hormones (e.g. adrenaline, nor-adrenaline), porphyrins, many other biomolecules and secondary metabolites. Thirdly, the amino acids contribute a minor but significant fraction of the total daily energy requirement of the body via oxidation of its carbon skeletons. Thus, proteins are important for growth and development of the body, maintenance and repairing of worn out tissues and for production of enzymes and hormones required for many body processes.

Fish is an important source of quality animal proteins and it has been reported that fish protein has greater satiety effect than other sources of animal proteins like beef and chicken. In comparison to the other sources of dietary animal proteins, consumers have wide choice for fish as far as affordability is concerned as there are many varieties and species of fishes available, especially in the tropical countries (FAO 2013). Two forms of child under nutrition Kwashiorkor (chronic protein deficiency) and marasmus (chronic deficiency of calories), often occurring together are world health problems, In this, context,

fish, being one of the cheapest sources of quality animal protein, is playing a big role and can still play a bigger role in preventing protein-calorie malnutrition (PCM).

The importance of fish in providing easily digested protein of high biological value is well documented. In comparison to the other sources of dietary proteins of animal origin, such as chicken, mutton, pork, beef etc. the unit cost of production of fish is much cheaper. Fish also come in a wide range of prices making it affordable to the poor. A common man can afford to meet the family large species of fish available. Calorie half of one meet the family hood from malnutrition. Is a fairly large number of fish specda's dietary requirementy protein requirement of this explains how fish plays an import amines available.

In the past this has served as a justification for promoting fisheries and aquaculture activities in several countries. On a fresh-weight basis, fish contains a good quantity of protein, about 18-20%, and contains all the essential amino acids including the sulphur containing amino acids cysteine and methionine.

Fatty acids (Fish Oils)

There are mainly three types of fatty acids: (1) saturated fatty acids (SFAs), (2) monounsaturated fatty acids (MUFAs) and (3) polyunsaturated fatty acids (PUFAs). The first two are synthesized endogenously, but the third one cannot be synthesized by the humans from other components by any known biochemical pathways, and therefore must be obtained from the diet. Fatty acids (FAs) are highly complex bimolecular and it is important to know their nomenclature to understand them. n-3 fatty acids which are important in human nutrition are: α-linolenic acid (18:3, n-3; ALA), leicosapentaenoic acid (20:5, n-3; EPA), and docosahexaenoic acid (22:6, n-3; DHA). These three PUFAs have either 3, 5 or 6 double bonds in a carbon chain of 18, 20 or 22 carbon atoms, respectively. All double bonds are in the cis-configuration, i.e. the two hydrogen atoms are on the same side of the double bond. Most naturally-produced fatty acids (created or transformed in animalia or plant cells with an even number of carbon in chains) are in cis-configuration where they are more easily transformable. n-3 compounds are more fragile than n-6 because the last double bond is geometrically and electrically more exposed, notably in the natural is configuration.

The human body cannot synthesize n-3 fatty acids de novo, but it can form 20carbon unsaturated n-3 fatty acids (like EPA) and 22-carbon unsaturated n-3 fatty acids (like DHA) from the eighteen-carbon n-3 fatty acid α-linolenic acid. These conversions occur competitively with n-6 fatty acids, which are essential closely related chemical analogues that are derived from linoleic acid (LA). Both the n-3 α-linolenic acid and n-6 linoleic acid are essential nutrients which must be obtained from food. Synthesis of the

longer n-3 fatty acids from linolenic acid within the body is competitively slowed by the n-6 analogues. Thus accumulation of long-chain n-3 fatty acids in tissues is more effective when they are obtained directly from food or when competing amounts of n-6 analogs do not greatly exceed the amounts of n-3.

Fish and Micronutrients

Micronutrients (minerals and vitamins) are the essential dietary element that is needed in small quantities which include vitamins and minerals that the body must obtain from outside sources. Micronutrients are required in small amounts as they are either components of enzyme cofactor or act as coenzymes in many biochemical reactions and metabolic processes vital for survival, growth and reproduction. The vitamins include fat soluble vitamin A, D, E, K as well as thiamine, riboflavin and niacin (vitamin B_1, B_2 and B_3) and vitamin C and minerals like copper, iron, zinc, selenium, iodine, magnesium, cobalt, manganese and macro minerals like calcium and phosphorous. Micronutrient deficiency conditions are widespread among two billion people in developing and in developed countries. These are silent epidemics of vitamin and mineral deficiencies affecting people of all genders and ages, as well as certain risk groups. They not only cause specific diseases, but they act as exacerbating factors in infectious and chronic diseases, greatly impacting morbidity, mortality, and quality of life. The small indigenous fishes. Like Amblypharyngodon mola and Pontius spore are rich in micronutrients.

Minerals

The minerals present in fish include iron, calcium, zinc, iodine (from marine fish), phosphorus, selenium and fluorine. These minerals are highly 'bioavailable' meaning that they are easily absorbed by the body. Iron is important in the synthesis of hemoglobin in red blood cells which is important for transporting oxygen to all parts of the body. Iron deficiency is associated with anemia, impaired brain function and in infants is associated with poor learning ability and poor behavior. Due to its role in the immune system, its deficiency may also be associated with increased risk of infection. Compared to other animal sources, although fish contain less iron than the amount found in red meat, iron in white fish is well absorbed and so is a useful source of iron. Calcium is required for strong bones (formation and mineralization) and for the normal functioning of muscles and the nervous system. It is also important in the blood clotting process. The intake of calcium, phosphorus when small fish are eaten with their bones rather than significant amount of zinc. Iodine, present in seafood, is important for hormones that regulate body metabolism and in children it is required for growth and normal mental development. A deficiency of iodine may lead to goiter (enlarged thyroid gland) and mental retardation in

children (cretinism). Fish is one of the few reliable sources of iodine. The UK recommended intake of iodine for adults is 140 mcg a day and a 100g portion of some fish can provide all the Fish is a particularly good source of selenium. In UK, the recommended intake for selenium is 75 mcg a day for men and 60 mcg a day for women and a 100g portion of baked cod could provide 34 mcg of selenium, which is roughly half the daily recommended intake. Selenium is a component of some of the enzymes which protect the body against damage due to oxidation (free radical damage). It is also necessary for the use of iodine in thyroid hormone production and for immune system function. Low levels of selenium intake may be associated with the increased risk of some cancers of iodine for the day.

Vitamins

Fish is a rich source of vitamins, particularly vitamins A, D and E from fatty species, as well as thiamin, riboflavin and niacin (vitamins B1, B2, B3). Vitamin A from fish is more readily available to the body than from plant foods. Among all the fish species, fatty fish contains more vitamin A than lean species. Studies have shown that mortality is reduced for children under five with a good vitamin A status. Vitamin A is also required for normal vision and for bone growth. As sun drying destroys most of the available vitamin A better processing methods are required to preserve this vitamin. The small indigenous fish Amblypharyngodon is a very rich source of vitamin A as compared to many other species. Vitamin D present in fish liver and oils is crucial for bone growth since it is essential for the absorption and metabolism of calcium. It also plays a role in immune function and may offer protection against cancer. Oily fish is the best food source of unfortified vitamin D. Vitamin D is not found in many foods and tends to be a vitamin that many vulnerable groups go shot of, such as teenage girls and the elderly. Fish is also a good source of the B vitamins and can provide a useful contribution to the diet for this group of vitamins, as does red meat. The B group of vitamins is responsible for converting food to energy in the cells of the body and they help with the function of nerve tissue. If eaten fresh, fish also contains a little vitamin C which is important for proper healing of wounds, normal health of body tissues and aids in the absorption of iron in the human body.

Nutritional value of fish:

Fish is an important component of human diet. More than 50% of Indian population is fish eating and in some states like Assam and other North Eastern states, West Bengal, Odessa, Goa and Kerala, more than 90% of the population consume fish. Fish contains proteins and other nitrogenous compounds, lipids, minerals and vitamins and very low level of carbohydrates. Protein content of fish varies from 15 to 20% of the live body weight. Fish proteins contain the essential amino acids in the required proportion and thus, improve the overall protein quality of a mixed diet. The superior nutritional quality of

fish lipids (oils) is well known. Fish lipids differ greatly from mammalian lipids in that they include up to 40% of long-chain fatty acids (C14 - C22) that are highly unsaturated and contain 5 or 6 double bonds. Fish is a good source of vitamin B complex and the species with good amount of liver oils are good source of fat soluble vitamins A and D. Fish is particularly a good source of minerals like calcium, phosphorus, iron, copper and trace elements like selenium and zinc. Besides, saltwater fish contains high levels of iodine also. In fact, fish is a good source of all nutrients except carbohydrates and vitamin C. Some inland fish species like singhi (*Heteropnestus fossilis*), magur (*Clarias batrachus*), Murrells (*Channa sp.)*, and Koi (*Anabas testudineus*) are known to have therapeutic properties.

Nutritional value of preserved and processed fish:

The nutritive value of preserved and processed fish and fishery products is generally lower than that of raw fish. This is due to loss of some of the nutritive value during various stages and processes of fish preservation. Handling and preparation. Following are the chief changes encountered.

1. Exudation :

"Drip" occurring in unfrozen fish is increased during freezing and defrosting or thawing. The drip is a free liquid containing 4% Protein. Which is lost, Similar loses in protein also occur during precooking in canning. Water soluble vitamins and minerals are also lost in the exudates.

2. Cooking:

A Cooking at 60° C causes loss in weight due to exudation of liquid. Undergone fish is more digestible than raw or overdone and fried fish. Broiling, Boiling, baking and simmering have little effect. but frying cause heavy loss of essential amino acid. Heating destroy creatine. Steam or pressure cooking causes loss in protein and minerals. Broiling or deep fat frying renders fish less digestible due to formation of additional difficult to split linkages among amino acid and protein.

3. Canning:

Canning does not affect protein and fat as to their nutritional value, nor does it destroy iodine, heat stable vitamins and essential amino acid. O. The other hand, canning causes considerable loss in such vitamins as B1, pantothenic acid, Pteroylgtamic acid, vitaminC.

4. Salting and smoking:

Curing of fish with salting or salting combined with smoking results in substantial loss of protein about 1 to 5 % due to salting and 8 to 30% due to smoking. Salting of course

accelerate oxidative rancidity of fats which ultimately reduces the digestibility of the fat of the product

5. Freezing :

Freezing and lyophilizing do not affect the amino acids content, but encourage rancidity of fat, on one hand, and desiccation and denaturation of protein. on the other, such undesirable look as freezer burn and rusting reduce the acceptability of the product although no actual loss in nutritional value of protein occur. Among the vitamins, vitamin E is destroyed by freezing..

UNIT-2

Fish decomposition:

A study of the decomposition of the food fishes presents an interesting field for both the chemist and the bacteriologist. While the chemistry of fish decomposition has been investigated to some extent, comparatively little has been reported regarding the bacteriology of the problem. Browne (1917) reported the results obtained from his work on the decomposition of various fish during storage in ice stating that autolysis, rather than bacterial action, seems to play the most important part in the initial stages of decomposition. The part played by bacteria in the decomposition of sardines has been studied and reported by Obst (1919). The bacteriology of canned or preserved fish has received the attention of several workers. The question of whether or not the Bacterium coli is an inhabitant of the intestines of fish has been investigated by Browne, (1917), Eyre (1904), Houston (1903-04), Amyot (1901), and others but the papers of Browne and of Obst already mentioned seem to be the only studies made of the part played by bacteria in the actual decomposition of the flesh of fish before preserving.

Postmodern changes and Rigor mortis:

Fish once it is caught and removed from water cannot survive. It soon dies of suffocation, unable to breathe. A common symptom of such death in blood congestion on gills and gill covers. Blood capillaries often burst and cause the appearance of blood stains. In a freshly dead fish, muscles continue to show irritability for some time when stim Rigorulated by electric current, although blood circulation ceases. This actively of muscles is also soon lost, though gradually. Now the body of fish enters a state called rigor mortis.

Rigor mortis:

Rigor mortis is due to a biochemical change in the muscles that occurs several hours after death, though the time of its onset after death depends on the ambient temperature. Without ATP, myosin molecules adhere to Actins filaments and the muscles become rigid.

Rigor mortis literally, the stiffness of death. The rigidity of a body after death. Rigor mortis is a good example of a Latin term (one in this case that was coined in the 19th century) remaining intact in contemporary medical usage (and crime writing).

The most dramatic change is onset of rigor mortis. Immediately after death the muscle is totally relaxed and the limp elastic texture usually persists for some hours, where after the muscle will contract. When it becomes hard and stiff the whole body becomes inflexible and the fish is in rigor mortis. This condition usually lasts for a day or more and then rigor resolves. The resolution of rigor mortis makes the muscle relax again and it

becomes limp, but no longer as elastic as before rigor. The rate in onset and resolution of rigor varies from species to species and is affected by temperature, handling, size and physical condition of the fish.

The effect of temperature on rigor is not uniform. In the case of cod, high temperatures give a fast onset and a very strong rigor mortis. This should be avoided as strong rigor tensions may cause gaping, i.e., weakening of the connective tissue and rupture of the fillet.

Rigor mortis starts immediately or shortly after death if the fish is starved and the glycogen reserves are depleted, or if the fish is stressed. The method used for stunning and killing the fish also influences the onset of rigor. Stunning and killing by hypothermia (the fish is killed in iced water) give the fastest onset of rigor, while a blow on the head gives a delay of up to 18 hours (Azam *et al.*, 1990; Proctor *et al.*, 1992).

The technological significance of rigor mortis is of major importance when the fish is filleted before or in rigor. In rigor the fish body will be completely stiff; the filleting yield will be very poor, and rough handling can cause gaping. If the fillets are removed from the bone pre-rigor the muscle can contract freely and the fillets will shorten following the onset of rigor. Dark muscle may shrink up to 52 % and white muscle up to 15 % of the original length (Buttkus, 1963). If the fish is cooked pre-rigor the texture will be very soft and pasty. In contrast, the texture is tough but not dry when the fish is cooked in rigor. Post-rigor the flesh will become firm, succulent and elastic.

Causes of Rigor mortis:

The hardening of the muscles and stiffening of the body that begins 3 to 4 hours after death. It occurs partly because the deteriorating SR releases calcium into the cytosol, and the deteriorating sarcolemma admits more calcium from the extracellular fluid.

In rigor mortis, the body becomes stiff and completely unpliable, as all the muscles tense due to changes that occur in them at a cellular level. Rigor mortis settles in at 2–6 hours after death and can last for 24–84 hours. After this, the muscles become limp and pliable once more.

Fully developed rigor mortis is an easily identifiable and reliable indicator that death has occurred. The time of onset is variable but it is usually considered to appear between 1 and 6 hours (average 2–4 hours) after death. Depending on the circumstances, rigor mortis may last for a few hours.

The stiffening of the muscles in an animal shortly after it has been slaughtered too soon after slaughtering and prior to complete Rigor Mortis, the meat will become tougher than normal. If the carcass is frozen prior to complete Rigor Mortis, the meat will be extremely tough several days.

Post –rigor decay and spoilage of fish:

The initial steps in post mortem decomposition of fish upto rigor mortis are thus certainly not undesirable. On the contrary some breakdown products contribute to improvement in taste and quality. It is a common knowledge that fish preserved prior to rigor mortis is inferior in taste and quality to fish preserved after the settings in of rigor mortis.

However, immediately following passage of rigor mortis. The fish undergoes intense and rapid determination and becomes inedible, Alkaline foul smelling and limp. The causes of this spoilage are many important among them include enzymatic, microbial and chemical factors.

Enzymatic spoilage:

Enzymatic spoilage is caused by the autolytic fish enzymes. Fishes are highly perishable than meat because of more rapid autolysis by fish enzymes, and favorable conditions for microbial growth due to less acid reactions. The autolytic spoilage can be prevented by reducing the activity of enzymes by lowering the temperature.

Oxidation or non-enzymatic spoilage

Oxidation or non-enzymatic spoilage is caused by the oxidation of fish fat. The oxidative deterioration of many unsaturated fish oils leads to spoilage of fish. Thus, the fatty fishes spoil much faster than lean fishes.

Microbial spoilage:

Fish products with high salt contents may spoil due to growth of halophilic bacteria (salted fish) or growth of anaerobic bacteria and yeasts (barrel salted fish). It is concluded that the spoilage is probably caused by lactic acid bacteria, certain *psychotrophic Enterobacteriaceae and/or Photobacterium phosphoreum. The predominant bacteria associated with spoilage are Brochothrix thermosphacta, Carnobacterium spp., Lactobacillus spp., Lactococcus spp., Leuconostoc spp., Pediococcus spp., Stretococcus spp., Kurthia zopfii, and Weisella spp.*

Composition of the microflora on newly caught fish depends on the microbial contents of the water in which the fish live. Fish microflora includes bacterial species such as

Pseudomonas, Alcaligenes, Vibrio, Serratia and Micrococcus (Gram and Huss, 2000) Microbial growth and metabolism is a major cause of fish spoilage which produce amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketenes' with unpleasant and unacceptable off-flavors (Dalgaard et al., 2006; Emborg et al., 2005; Gram and Dalgaard, 2002). For unpreserved fish, spoilage is a result of Gram-negative, fermentative bacteria (such as Vibrionaceae), whereas psychrotolerant Gram-negative bacteria (such as Pseudomonas spp. and Shewanella spp.) tend to spoil chilled fish (Gram and Huss, 2000). It is, therefore, important to distinguish non spoilage microflora from spoilage bacteria as many of the bacteria present do not actually contribute to spoilage (Huss, 1995).

Bacterial flora of fish:

In microbiology, collective bacteria and other microorganisms in a host are known as flora. Although microflora is commonly used, the term microbiota is becoming more common as microflora is a misnomer. Flora pertains to the Kingdom Plantae.

Bacterial floras isolated from eggs, skin, gills, and intestines have been described for a limited number of fish species. Generally, the range of bacterial genera isolated is related to the aquatic habitat of the fish and varies with factors such as the salinity of the habitat and thebacterial load in the water.

The bacteria most frequently described as fish pathogens are Aeromonas, Edwardsiella, Pseudomonas, Shewanella, Mycobacterium, Streptococcus, and lavobacterium, of which some are common in Polish waters. Bacterial flora plays an important role in host health in a variety of tissues and organ systems such as the skin, gastrointestinal tract, and urogenital system, as well as the respiratory system.

Bacterial spoilage:

Bacterial spoilage is caused by the activities of microorganism associated with the fish. Bacterial spoilage of fish begins only after the completion of rigor mortis, which results in the release of products of protein denaturation due to decrease in pH, which is utilizable by bacteria. Thus, prolonging rigor mortis helps to delay spoilage and thereby keeps fish fresh.

Rigor mortis is hastened by struggling of the fish, lack of oxygen and warm temperature. However, rigor mortis can be delayed by reducing enzyme activities by lowering pH and adequate cooling of fish. The pH of the fish has important influence on perishability because of its influence on growth of bacteria. Lower the pH of fish, slower will be bacterial

decomposition of fish. Lowering of pH occurs during rigor mortis when muscle glycogen is converted to lactic acid.

Spoilage of both marine and fresh water fish occurs in the same manner. Fish contain high levels of protein and non- protein nitrogenous constituents (16~20 %), lack carbohydrate, and have varying amounts of fat depending on the species of fish. The non-protein nitrogenous compounds in fish include free aminoacids, volatile nitrogen bases-ammonia and trimethyl amine (TMA), creatine, taurine, betaines, uric acid, anserine, carnosine and histamine. Spoilage of fish begins from the surface, gill and intestine because of high bacterial load. From gills, intestine and surface microorganisms ¬gradually migrate to adjacent tissue and cause spoilage. Spoilage organism first utilizes simpler compounds and later fish protein releasing various off-odour compounds converted to lactic acid.

Chemical spoilage:

The most common chemical action which causes spoilage is the oxidative rancidity in fatty fishes. The levels of peroxide value and free fatty acid content both a measure of oxidative rancidity are considered an index of quality of fat fishes. The spoilage in fish is accompanied by the change in physical characteristic. This includes rancidity and autolysis.

Rancidity:

Spoilage is caused by reactions in the fat of fish giving rise to unpleasant odors and flavors. This spoilage is generally called rancidity; fish oil is more liable to spoilage than other oils due to the greater number of unsaturated fatty acids as shown by the lower saponification number and higher iodine value. The greater the degree of unsaturation the greater the tendency for rancidity, the biochemical change to the production of rancid flavor and odor can best be understood by examining the mechanism of the reactions that give rise to chemical rancidity (Eyo, 2001).

Rancidification is the process of complete or incomplete oxidation or hydrolysis of fats and oils when exposed to air, light, or moisture or by bacterial action, resulting in unpleasant taste and odor. When fats and oils are left open, they get oxidized in the presence of air, their smell and taste change. This means the oxidation is the main cause for rancidity in fats and oil. The substances which prevent oxidation of the food items are called antioxidants.

There are two basic types or causes of rancidity that cause and/or contribute to the degradation of stored edible oils: oxidative and hydrolytic. Oxidative rancidity, known as autoxidation, occurs when oxygen is absorbed from the environment.

Rancidity is a condition in which the substance with oil and fats get oxidized when they are exposed to air. A substance is said to be rancid when there is a change in smell, taste, and colour. An example of rancidity is when a chips pack is exposed to atmospheric air which results in a change in taste and odor.

Rancidity can be prevented using the following methods: Adding antioxidants (substances which prevent oxidation) to food. Storing food in airtight containers to slow the process of rancidification. Refrigerating food also helps to slow down rancidification.

This process eventually results in rancidity and creates a bad smell, changes in color, and the negative change called oxidation. Eating rancid fat may not make you sick over the short term, but consuming rancid fat over time can negatively affect your health.

Rancid foods have the same look and texture as when they were purchased, but their smell and taste have changed. The odour is akin to wet cardboard, oil paint, wood varnish or play dough. Some people are offended by it and know to throw the product out, while others think that's just how the product normally smells.

Autolysis:

Autolysis means "self-digestion". It has been known for many years that there are at least two types of fish spoilage: bacterial and enzymatic. In others,autolysis contributes to varying degrees to the overall quality loss in addition to microbially- mediated processes.

The autolytic enzymes activity and the enzyme activity due to bacterial multiplication are prime factors in fish spoilage. The former often makes fish susceptible to bacterial attack. Bacterial activity hastens decomposition. The proteins are broken down by steps into amino acid and other nitrogenous end products such as ureides and Xanthine bases. Further decomposition takes place and final products are produced as ammonia and carbon dioxide, on other hand and volatile basic compounds and fowl smelling products such as indole,skatole andhydrogen sulphide, on the other hand. Ammonia and carbon dioxide render the fish alkaline (pH rises to 7.6). Foul smelling compounds are responsible for the disagreeable odourof rotten fish. Histamine is another product which imparts a bitter or pungent taste with advanced spoilage. The above enzymatic decomposition brings about great structural changes leading to softening of fish flesh. This is the basis of touch test in the organoleptic criteria for estimation of freshness of fish. Certain end products like indole and tyrosine or pH measurements also similarly form basis for estimation of extent of fish spoilage.

Spoilage due to other factors:

Mention may be made here of heavy losses from spoilage, mackerel in America from a copepod planter (Clamus app), which forms their food. These copepods called" red pepper", and present in the stomach and cause spoilage within 24 hours even of iced fish.

Spoilage in marine fish:

Marine fish contain in their flesh a compound called trimethylamine oxide. It is a nitrogenous compound which does not occur in fresh water fish. Infect the ammonia which must be removed from body is converted in marine fish into trimethylamine oxide and stored in flesh, because in this form the toxic effect of ammonia is neutralized. However, during enzymatic decomposition particularly due to bacteria, this compound is broken down into its amine – the trimethylamine.

This is a basic compound and is responsible for the sharp characteristics foul odour of putrefyingmarine fish. The estimation of formation of trimethylamine is used as a measure of fish spoilage in case of marine fish. The test is popularly known as TMA test. The method of TMA test involves calorimetric or chromatographic bprocedures. It is one of the most effective criteria for determining the extent of bacterial decomposition. It has proved very useful for many fishes such as cod, haddock and flounder. Also the TMA test holds good particularly in case of estimating spoilage in chilled or iced fish. It must be noted however, that the TMA test may not apply equally well to other species of fish or to fish preserved by other means, because the TMA formation from trimethylamine oxide (TMAO) may be affected by a number of other factors besides bacterial deterioration. Such factors, include different chemical composition of fish: some enzymes may reduce TMAO into TMA : preservatives or antioxidants used in fish preservation may also reduce TMAO into TMA.

Spoilage of freshwater fish:

The spoilage of freshwater fish responsible that ofarine fish. The spoilage is mainly due to combined effect of autolytic and bacterial decomposition. Break down of certain aminoacids produce substance which cause bad taste and disagreeable off odour in spoiling freshwater fish. The presence of the volatile nitrogen containing breakdown products in consumption. The enzymes of fish under normal conditions can single day. The skin of fish decomposes earlier than the flesh. It is the guanine of fish which undergoes swift enzymatic decomposition resulting in deamination to ammonia. Gill spoilage an organoleptic symptoms of state fish is due to the enzyme catalase from erythrocytes in blood. ATP ase is responsible for the disappearance of ATP, within a short period from the muscles of carp. The rate of glycolysis is however slower in freshwater in freshwater fish that in marine fish. The bacterial spoilage is common in freshwater fish, and the bacteria involved do not belong to the

freshwater but appear by contamination from exposure of fish to soil, air, plant or the food of fish or from handling and processing.

Unit – 3

Fish preservation:

Fish preservation is the method of increasing the shelf life of fish and other products by applying the principles of different branches of science in order to keep the fish, after it has landed, in a condition wholesome and fit for human consumption. Ancient methods of preserving fish included drying, salting, pickling and smoking. All of these techniques are still used today but the more modern techniques of freezing and canning have taken on a large importance.

Fish is one of the protein foods that needs careful handling (Eyo, 2004). This is because fish spoils easily after capture due to the high tropical temperature which accelerates the activities of bacteria, enzymes and chemical oxidation of fat in the fish. Due to poor handling, about 30 – 50% of fish harvested are wasted in Nigeria. These losses could be minimized by the application of proper handling, processing and preservation techniques (Bate and Bendall, 2010).The purpose of processing and preserving fish is to get fish to an ultimate consumer in good, usable condition. The steps necessary to accomplish this begin before the fishing expedition starts, and do not end until the fish in eaten or processed into oil, meal, or a feed (Karube et al., 2001). Fish begins to spoil as soon as it is caught, perhaps even before it is taken out of the water. Therefore, the key to delivering a high quality product is close attention to small details throughout the entire process of preparation, catching, landing, handling, storage, and transport.

Fish that becomes spoiled or putrid is obviously unusable (Gopakumar, 2000). Fish that is poorly cared for may not be so obviously bad, but it loses value because of off-flavors, mushy texture, or bad color that discourage (Burt, 2003), a potential purchaser from buying. If customers have bought one bad fish, they probably won't buy another. On the other hand, if you consistently deliver good quality at a fair price, people will become loyal customers. Spoilage proceeds as a series of complex enzymatic bacterial and chemical changes that begin when the fish is netted or hooked (Burt, 2003). This process begins as soon as the fish dies. The rate of spoilage is accelerated in warm climates. The fish's gut is a rich source of enzymes that allow the living fish to digest its food (Lima Dos Santos et al., 2011). Once the fish is dead, these enzymes begin digesting the stomach itself. Eventually the enzymes migrate into the fish flesh and digest it too. This is why the fish becomes soft and the smell of the fish becomes more noticeable.

Principles of preservation:

Most commonly used fish preservation methods are; chilling, freezing, curing (drying, salting and smoking), canning, marinating, boiling and fermentation. The other methods such as preservation by irradiation, freeze-drying, modified atmospheric packaging, retort pouch packaging are also used for preserving fish.

At present different methods are used to preserve the fish and fishery products based on the desirable end product properties. Most commonly used fish preservation methods are; chilling, freezing, curing (drying, salting and smoking), canning, marinating, boiling and fermentation. The other methods such as preservation by irradiation, freeze-drying, modified atmospheric packaging, retort pouch packaging are also used for preserving fish.

Top quality fresh fish are essential for fish preservation. Of all flesh foods, fish is the most susceptible to tissue decomposition, development of rancidity and microbial spoilage. Safe handling of fish is important to reduce your risk of food borne illness and to produce a quality meal.

Following are the principles involved in fish preservation.

1. Cleaning :

The objective is to prevent spoilage of fish from contamination and unhygienic conditions. Landed fish Carries a number of microbes, particularly bacteria, both on surface of body as well as in the intestine and body cavity. There are chances of contamination with dirt, dirty water or dirty hands during handling. Poor sanitary conditions and careless handling of fish, particularly in tropical regions, greatly increase speed of spoilage. Bleeding of fish due to injury during capture or when fish is gutted, leave blood stains and smears leading to unhygienic conditions. Therefore cleaning of fish greatly inhibits spoilage activity, so also keeps hygienic conditions at all levels of handling of fish.

2. Lowering the temperature:

This is very effective in delaying the onset and prolongation of the duration of rigor mortis and in slowing down. At normal room temperature prevailing in tropics, autolysis is fairly rapid. It is well known that adenosinopolyphosphates are active causing rapid breakdown of ATP and ADP into adenine and adenosine. But at 6° C. Already their activity is slowed down and only AMP is the breakdown product. At -8°C ie in frozen state there is a further curb and only IMP is the end product. At the temperature of liquid air there is no activity of the adenosinopolyphosphates and consequently no breakdown of ATP and ADP. Further at room temperature the lag phase of few bacteria lasts only a few hours. Lag phase is the period

during which bacterial multiplication is very slow, and it is followed by very rapid multiplication of bacteria.

3. Raising the temperature :

Heat decomposes autolytic enzymes and thereby brings their activity to a stop. Further, moist heat under pressure or a temperature in the range of 110°C – 121°C under a steam pressure of 7 kg.per 6.45 square cm kills all such bacteria which are more resistant than the bacteria themselves are also destroyed. Thus even the application of this principle is very effective in stopping all chances of spoilage and in ensuring a very long term preservation. However, some thermophilic strains of bacteria survive even under such conditions and have been the cause of botulism type of food poisoning.

4. Dehydration :

Removal of water content enhances greatly the keeping quality and period of fish. Presence of moisture is very favorable for bacterial growth and multiplication. Loss of moisture therefore curbs bacterial activity and even kills them. The length of keeping period of fish depend upon the degree of dehydration achieved.

5. Use of salt :

The effectiveness is for two reasons. First salting brings about removal of moisture from fish by osmosis, secondly salt enters tissue and increase salt concentration to saturation point. The presence of high salt concentration destroys autolytic enzymes and halts bacterial activity. The bacteria slow down their multiplication and are forced to spore formation.

6. Use of fish preservation :

A number of drugs and chemicals are effective in preventing spoilage of fish. Some popular for industrial uses are mentioned below :

a. Ascorbic acid prevent rancidity in fatty fish

b. Chemical ice containing small concentration of sodium nitrate, may be enhance keeping period of fish at this temperature by a week over the period achieved with ordinary ice.

c. Auremycin is an antibiotic obtained from the fungus S.aureofascines.It is very effective in preventing fish spoilage. Even at room temperature, a fish dipped in 50ppmStrength solutions of this antibiotic will keep fresh for a few hours longer than untreated one. When it is used in small concentration in ice it enhances the keeping period by a week than period achieved with ordinary ice.

d. Phenolic components of smoke viz. guaiacol,cresol,pyrogallol,catechol from certain woods have antiseptic properties and are thus helpful in preventing fish spoilage. These are particularly bactericidak, resulting in heavy kill off in the bacterial load of fish.

e. Vinegar is a powerful preservatives.
f. Detergent like sodium ricinoleate as thrive in high salt concentration.
7. Exposure to low radiation of gamma rays :

This helps in destroying completely both bacteria as well as the Pseudomo as group of microbes,there by preserving the fish wholesome.

8. Electrocuting by “ion wind” :

It is a recent technique developed in Britain. Electrically charged air particles, blowing as “ ion wind” kill the microbes. Air ions are produced by a high voltage generator and discharged from a conductor maintained at higher electrical potential than earth. Negative ions formed are repelled at the negative potential, giving rise to the “ion wind”which is then attracted towards the object maintained at relatively positive potential.

Methods of preservation:

Firstly, we already know that, fish is highly perishable commodity, which spoiled very rapidly due to the infection of the various types of micro-organism.

So, for this, we have to manage strong and peculiar method fish preservation, so that its frequency of spoiling can be greatly decreases. For the prevention of fish spoilage, there are many types of process in which some are very popular methods of preservation like, drying, canning, icing, etc. All this process are categorized under physical and chemical method of fish preservation and they are as following,

Type of preservation:-

1) Physical method
2) Chemical method

Chemical methods of preservation is nothing but the preservation of fish like commodity by the application of different types of chemical substance, which we are using from earlier day of preservation like, benzoic acid and sodium benzoate etc. Similarly physical method of preservation is also very common and some of them are very much popular to fisherman and their crew member.

Physical methods of preservation:-

1) Drying and dehydration
2) Canning
3) Freezing / icing
4) Salt curing

5) Smoking / smoke curing
6) Marinades

These are some of the method of physically preservation of fish.

1) Drying and dehydration:-

It is the method of fish preservation in which the moisture content of fish is removed by drying and then process called dehydration. As we know that, for any microbial reaction their must a wet / moist medium to flourish them. So they cannot perform these types of activity after dehydration process. By this action of dehydration almost 90-95% of moisture is removed from the fish. Then they are packed in the specific packaging material.

2) Canning:-

It is very popular type of physical preservation in which selected food material are prepared for the consumer table, packed in tin or glass container capable of being sealed air tight, heated sufficiently to destroy the spoilage organism within the container and cooled rapidly. It is similar to the drying, but in it food is heated in a sealed and air tight metal chamber to destroy the micro-organism and rapidly cool down.

Unlike other method of preservation canning alter the nature of material significantly forming almost new products because of various treatment, the raw material are subjected to and various additives used in processing. Containers for canned foods are normally made of tinplate, but aluminum and other modification are now a day's popular.

3) Icing / Freezing:-

It is a method of food preservation employing low temperature, icing and chilling can maintain fish fresh only for a very limited period. Quality deterioration takes place in chilled fish. Freezing is a method low temperature preservation of fish that can ensure very long shelf life and can also provide a processed product very much similar to fresh fish. Most methods of long term preservation bring about major changes in the physical, chemical, textural and organoleptic characteristic of fish.

Main principle behind this method is cool temperature do not favors the all activities of microorganism and also its enzymatic activity becomes inactivates, due to low temperature. Icing is done in 1:1 ratio, means one layer ice and one layer fish and so on. So that it does not make contact in other fish and also the weight should not be increased so much to lower side of fish. It is done in a plastic caret of about 40 Kg of its capacity.

4) Salt curing:-

It is traditional method of preservation of fish plasticized as such or in combination with drying or smoking. Salt curing is an important method of preservation. Main concept regarding this techniques, is when the some amount of salt solution is entered into the fish flesh, it can delay the activity of microorganism or even inactivate them by reducing the water activity by process called “exosmosis” this forms the basis of the salting preservation. Moisture is very necessary to flourish the bacteria at any place.

Concentration of salt at 4-10% level in fish flesh is known to prevent the action of most spoilage bacteria as well as autolytic decomposition. When the concentration of salt is 20% or more in flesh, the decomposition process in the fish proceeds only very slowly.

5) Smoking / smoke curing:-

Smoking or smoke curing, like drying and salt curing is ancient methods of preservation of fish. In early method of smoking, heavily salted fish used to smoked for long duration and even few weeks and the resultant products were called “hard cures”. This product has long shelf life at ambient temperature. Owing to the high salt concentration and long smoking and drying period, this lowers the water activity considerably.

Smoking is also employed as an intermediate step in processing canned smoked fish. Lightly smoked fish is considered as an alternate to fresh fish having slightly pleasant smoke flavor. In course of time the “hard cures” gave a way to milder production with less salt and lower duration of smoking. Such products however, have short shelf life.

6) Marinades:-

It is nothing but the preservation of the fish or fish protein processed by treatment with edible acid (acetic acid) and salt and put up in brine, sauce or oils. Fish marinades are characterized by the typical marinades odour and flavor. Treatment with acetic acid and salt brings about a short refinement in fish simultaneously developing typical marinated odors and flavor. Further improvement brought by addition of various spices and covering liquids. Generally pelagic fatty fishes such as sardine, herring etc. used for this preservation method.

Chemical methods of preservation:-

Chemical methods of preservation is mainly based on the various type of chemical substance either it is organic acid or oxide of metal or non-metal etc. this chemical substance generally acts on the internal physiology of the micro-organism and alter them greatly.

These chemical substances are as follows,

1) Organic acid and esters

2) Nitrite
3) Sulphur dioxide

1) Organic acid and esters:-

The most important organic and esters that are used as food preservatives are organic acid such acetic and lactic acid are used for retarding the growth and antimicrobial effect of spoilage organism.

Organic acid and esters are as follows,

1) Ascorbic acid
2) Benzoic acid
3) Acetic acid
4) Lactic acid
5) Propionic acid

2) Nitrite:-

The antibacterial action of nitrite was first described in the 1920. Nitrite is inhibitory to a range of bacteria. Early works showed that a level of 200 mg/ Kg at pH 6.0 is sufficient to stain of *E. coli, Micrococcus, pseudomonas* and nitrite have ability to inhibit spore forming bacteria such as *C. botolinum* bacteria inhibition by nitrite increases with decreasing pH. Nitrite also contributes to the typical cured meat flavor.

3) **Sulphur dioxide:-**

Sulphur dioxide has a reputation for its disinfecting properties and its earliest use in the food industry was when sulphur candle were burn to disinfectant the vessel use to produce and store wine. It is also used as an antioxidant and inhibits enzymic and nonenzymic browning reaction in food product.

SO_2 is a reactive molecule can disrupt microbial metabolism in number of ways. As a reducing agent, it can break disulphide linkage in protein and interferes with redox process

Special problems in fish preservation:

1. Denaturation due to freezing of fish :

It is common knowledge that a frozen fish is less valued than a fresh fish. This is so because a frozen fish loses much of the flavour band taste. The flesh becoming dry, tough and dehydrated. In short frozen fish shows “denaturation” of the flesh. Technically, denaturation of flesh is described as a changed condition of flesh protein in which the protein cannot be extracted any longer by the known methods of solubility in various salt solutions as

applied to normal fish muscle for protein extraction. The deterioration in quality of fish flesh is directly associated with degree of insolubility produced during freezing.

The rate and extent of protein denaturation in frozen fish muscle juice have been determined. The extent of denaturation is greater at −2 °C., approximately 25 per cent, of the total protein being precipitated in 84 days. At lower temperatures denaturation diminishes at −20° C., only 3 per cent, protein being precipitated after 84 days. Hydrogen ion concentrations on the acid side of pH 5.8 (20°) lead to a marked increase and on the alkaline side to a marked decrease in denaturation. The amount of water appearing as ice when the juice is in equilibrium at various temperatures down to −9 °C. has been determined.

2. Problems arising out of industrial processes in fish preservation industries :

Certain process, if carried out carefully in unhygienic conditions or without adequate precautions, do more harm than good in fish preservation. Washing is good in reducing bacterial flora of the fish surface considerably but if care is not exercised, and washing is followed by treatment with contaminated crushed ice it becomes not only useless but may even add to the bacterial load. Like wise evisceration,aimed at removing organs with high metabolic rate and hence more readily autolyzed after death,if not exercised with care may help in contamination of flesh with bacteria rather than in its avoidance. Gutting of fish is aimed at preventing autolysis of flesh by the enzymes of the gut. However, gutting often helps in concentration of flesh with bacteria if the intestinal fluid. Handling of fish at different stages such as on board, on shore and in the market has the risk of helping the bacterial spoilage by contamination with unhygienic surfaces. Filleting of flesh, particularly in fat fishes, may only increase chances of rancidity.

Fish autolyzates are deliberately obtained from controlled spoilage of fish under experimental conditions. Fish autolyzates are useful in studying the growth of spoilage causing bacteria as these serve as excellent substrate. The aminoacid constituents of fish autolyzates are severaltimes more abundant than in Normal fish flesh. To obtain fish autodigested by the addition of desired bacterial strains or particular enzymes, at 52°C for several hours under controlled pH. Fish silage is a particular controlled spoilage preparation obtained from the activity of the bacteria Lactobacillus plantarum which causes formation of large quantities of lactic acid.

Unit – 4

Food poisoning

Food poisoning occurs when you swallow food or water that contains bacteria, parasites, viruses, or toxins made by these germs. Most cases of food poisoning are from common bacteria such as Staphylococcus or E. coli. Food poisoning, also known as acute gastroenteritis, is an acute inflammation of the lining of the stomach and small bowel. Food poisoning is a common, usually mild, but sometimes deadly illness that occur suddenly (within 48 hours) after consuming a contaminated food or drink. Most of the common contaminants cause nausea, vomiting, diarrhea, and abdominal cramping. Depending on the contaminant, fever and chills, bloody stools, dehydration, and nervous system damage may follow. Food poisoning comes from eating foods that contain germs like bad bacteria or toxins, which are poisonous substances. Bacteria are all around us, so mild cases of food poisoning are common.

Histamine poisoning from badly preserved fish:

Histamine fish poisoning results from the consumption of inadequately preserved and improperly refrigerated fish. It resembles an allergic reaction but is actually caused by bacterially-generated toxins in the fish's tissues. Previous terms for histamine fish poisoning were scombroid fish poisoning, pseudoallergic fish poisoning, histamine overdose, or mahi-mahi flush. The term scombroid was used because the first fish species implicated in this poisoning were from the suborder Scombridae, which includes mackerel, tuna, marlin, swordfish, albacore, bonito, skipjack, and almost 100 other species. The term histamine fish poisoning is now considered more appropriate because many cases are from nonscombroid fish. Examples include mahi-mahi (dolphin fish), amberjack, herring, sardine, anchovy, and bluefish.

Histamine is a biogenic amine that naturally occurs in the human body and has an important physiological role as neurotransmitter messenger. There are two origins of histamine: the first is endogenous and the second exogenous by ingestion of certain foods such as fish and fermented products. In healthy people, amine oxidase rapidly detoxify histamine, but consumers can develop severe symptoms of histamine intoxication if high amounts are ingested.

The incubation time for histamine intoxication is short, often ranges from 2 min to 2 h, and people usually develop symptoms while they are still eating. The main symptoms of histamine intoxication are cutaneous (rash, urticaria, oedema, and localized inflammation), gastrointestinal (nausea, vomiting and diarrhoea), haemodynamic (hypotension) and

neurological (headache, tingling, oral burning sensation, flushing and itching). Symptoms are often mild, rarely severe and frequently disappear after 24 h.

Histamine is formed in fish and fishery products due to certain bacteria capable of producing an enzyme, histidine decarboxylase. This enzyme will convert free histidine to histamine. Temperature abuse during post-harvest chilling, storage and/or processing is one of the main factors influencing the production of histamine.

The main bacteria producing histamine are members of the family Enterobacteriaceae. However, Histamine is also produced, but to a lesser extent, by bacteria that can grow at refrigeration temperatures. The implementation of hygienic measures and maintaining a cold chain at each step of the production and distribution are essential to avoid or limit the production of histamine.

European Regulation (EC) No. 2073/2005 of 15 November 2005 specifies fish families associated with high levels of histidine. These are Scombridae, Clupeidae, Engraulidae, Coryphaenidae, Pomatomidae and Scombrocidae. A Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Meeting on the Public Health Risks of Histamine and other Biogenic Amines from Fish and Fishery Products presented a global list of fish species associated with scombroid poisoning.

Little scientific data concerning histamine intoxications are available in Morocco. During 2016, the official authority in Morocco recorded 2723 cases of food poisoning, of which 53.5% are collective cases. These intoxications represents 17.2% of all intoxication in Morocco, occupying third position. Following the consumption of fish, five histamine-poisoning outbreaks were recorded in 2016 and 2 in 2017.

A survey conducted in Morocco on fish consumption and frequency of fish poisoning showed that 68.5% of respondents had never encountered a poisoning problem and 28.2% had rarely. Risk analysis is a process that has three components: risk assessment, risk management, and risk communication. Risk assessment is the scientific evaluation of known or potential adverse health effects resulting from human exposure to food borne hazards. The Codex Alimentarius risk assessment incorporates four steps which are hazard identification, hazard characterization, exposure assessment and risk characterization. The Codex states that risk assessment should be based on the most relevant national scientific data and should use the available quantitative and qualitative data.

Several tools and methods can be used to perform a risk assessment. The Risk Ranger is a risk calculation tool developed by the Food Safety Authority of Australia. It helps to determine the relative risks of various products/contaminants/treatments. It takes the form of a Microsoft Excel spreadsheet and incorporates principles of health risk assessment. It

combines exposure probability to a food-borne hazard, magnitude of the hazard in a food if present, and severity consequences that may result from the exposure level and frequency.

Disease severity is affected by the intrinsic characteristics of the pathogen/toxin and a consumer's susceptibility. Risk Ranger incorporates all factors that affect risk from a hazard in a particular product including:

- Severity of the hazard and susceptibility of the population of interest;
- The probability of a disease-causing dose of the hazard being present in a meal;
- The number of meals consumed by a population of interest in a given period.

The user is required to answer 11 questions related to hazard severity, the probability that a pathogenic dose of the hazard is present in a meal, and the probability of exposure to the hazard.

Risk Ranger uses risk assessment principles, as it incorporates the probability of exposure to a food risk, the prevalence of hazards in a food product where they exist, and the probability and the severity of the consequences of a level of contamination exposure.

The objective of this study is to assess the risk of histamine in the various categories of fresh or processed fishery products (canned fish, semi-preserved fish, and frozen fish) and to evaluate this risk following the consumption of various fish species known to contain high levels of histidine such as sardine, anchovy, mackerel and horse mackerel.

Food poisoning from eating a poisonous fish species:

CIGUATERA FISH POISONING

Ciguatera fish poisoning occurs after eating reef fish contaminated with toxins such as ciguatoxin or maitotoxin. These potent toxins originate from Gambierdiscus toxics, a small marine organism (dinoflagellate) that grows on and around coral reefs. Dinoflagellates are ingested by herbivorous fish. The toxins produced by G.toxicus are then modified and concentrated as they pass up the marine food chain to carnivorous fish and finally to humans. Ciguatoxins are concentrated in fish liver, intestines, roe, and heads.

G.toxicus may proliferate on dead coral reefs more effectively than other dinoflagellates. The risk of ciguatera poisoning is likely to increase as coral reefs deteriorate because of climate change, ocean acidification, offshore construction, and nutrient runoff.

Risk for Travelers

Up to 50,000 cases of ciguatera poisoning get reported annually worldwide. Because the disease is underrecognized and underreported, this is likely a significant underestimate. The incidence in travelers to highly endemic areas has been estimated as high as 3 per 100. Ciguatera is widespread in tropical and subtropical waters, usually between the latitudes of 35°N and 35°S; it is particularly common in the Pacific and Indian Oceans and the Caribbean Sea. The incidence and geographic distribution of ciguatera poisoning are increasing. Newly recognized areas of risk include the Canary Islands, the eastern Mediterranean, and the western Gulf of Mexico. Medical practitioners must be aware that cases of ciguatera fish poisoning acquired by travelers in endemic areas may present in nonendemic (temperate) areas. In addition, cases of ciguatera fish poisoning are seen with increasing frequency in nonendemic areas as a result of the increasing global trade in seafood products.

Fish that are most likely to cause ciguatera poisoning are large carnivorous reef fish, such as barracuda, grouper, moray eel, amberjack, sea bass, or sturgeon. Omnivorous and herbivorous fish such as parrot fish, surgeonfish, and red snapper can also be a risk.

Clinical Presentation

Ciguatera poisoning may cause gastrointestinal, cardiovascular, neurologic, and neuropsychiatric illness. The first symptoms usually develop within 3–6 hours after eating contaminated fish but may be delayed for up to 30 hours. Adverse health effects referable to the above-named organ systems include:

- Diarrhea, nausea, vomiting, and abdominal pain
- Bradycardia, heart block, hypotension
- Paresthesias, weakness, pain in the teeth or a sensation that the teeth are loose, burning or metallic taste in the mouth, generalized itching, sweating, and blurred vision. Cold allodynia (abnormal sensation when touching cold water or objects) has been reported as characteristic, but there can be acute sensitivity to both hot and cold. Neurologic symptoms usually last a few days to several weeks but may persist for months or even years.
- Fatigue, general malaise, insomnia

The overall death rate from ciguatera poisoning is <0.1% but varies according to the toxin dose and availability of medical care to deal with complications. The diagnosis of ciguatera poisoning is based on the characteristic signs and symptoms and a history of eating fish species known to carry ciguatera toxin. Fish testing can be done by the US Food and Drug

Administration (FDA) in their laboratory at Dauphin Island. There is no readily available test for ciguatera toxins in human clinical specimens.

Prevention

Travelers can take the following precautions to prevent ciguatera fish poisoning:

- ✓ Avoid or limit consumption of reef fish.
- ✓ Never eat high-risk fish such as barracuda or moray eel.
- ✓ Avoid eating the parts of the fish that concentrate ciguatera toxin: liver, intestines, roe, and head.

Ciguatera toxins do not affect the texture, taste, or smell of fish, nor are they destroyed by gastric acid, cooking, smoking, freezing, canning, salting, or pickling.

Treatment

There is no specific antidote for ciguatoxin or maitotoxin poisonings. Symptomatic treatment may include gabapentin or pregabalin (neuropathic symptoms), amitriptyline (chronic paresthesias, depression, and pruritus), fluoxetine (chronic fatigue), and nifedipine or acetaminophen (headaches). Intravenous mannitol has been reported in uncontrolled studies to reduce the severity and duration of neurologic symptoms, particularly if given within 48 hours of the appearance of symptoms. It should only be given to hemodynamically stable, well-hydrated patients.

After recovering from ciguatera poisoning, patients may want to avoid consuming fish, nuts, alcohol, or caffeine for at least 6 months, as they may cause a relapse in symptoms.

SCOMBROID

Scombroid occurs worldwide in both temperate and tropical waters. One of the most common fish poisonings, it occurs after eating improperly refrigerated or preserved fish containing high levels of histamine and often resembles a moderate to severe allergic reaction.

Fish typically associated with scombroid have naturally high levels of histidine in the flesh and include tuna, mackerel, mahi (dolphin fish), sardine, anchovy, herring, bluefish, amberjack, and marlin. Histidine is converted to histamine by bacterial overgrowth in fish improperly stored after capture. Histamine and other scombrotoxins are resistant to cooking, smoking, canning, or freezing.

Clinical Presentation

Scombroid poisoning resembles an acute allergic reaction, usually appearing 10–60 minutes after eating contaminated fish. Symptoms include flushing of the face and upper

body (resembling sunburn), severe headache, palpitations, itching, blurred vision, abdominal cramps, and diarrhea. Untreated, symptoms usually resolve within 12 hours but may last up to 48 hours. Rarely, there may be respiratory compromise, malignant arrhythmias, and hypotension requiring hospitalization. There are no long-term sequelae. Diagnosis is usually clinical. Clustering of cases helps exclude the possibility of true fish allergy.

Prevention

Fish contaminated with histamine may have a peppery, sharp, salty, taste or "bubbly" feel but will usually look, smell, and taste normal. The key to prevention is to make sure that the fish is properly iced or refrigerated at temperatures <38°F (<3.3°C) or immediately frozen after being caught. Cooking, smoking, canning, or freezing will not destroy histamine in contaminated fish.

Treatment

Scombroid poisoning usually responds well to antihistamines (H1-receptor blockers, although H2-receptor blockers may also provide some benefit).

SHELLFISH POISONING

Several forms of poisoning may occur after ingesting toxin-containing shellfish, including filter-feeding bivalve mollusks (mussels, oysters, clams, scallops, and cockles), gastropod mollusks (abalone, whelks, and moon snails), or crustaceans (Dungeness crab, shrimp, and lobster). Toxins originate in small marine organisms (dinoflagellates or diatoms) that are ingested and are concentrated by shellfish.

Risk for Travelers

Contaminated (toxic) shellfish may be found in temperate and tropical waters, typically during or after phytoplankton blooms, also called harmful algal blooms (HABs). One example of a HAB is the Florida red tide caused by Karenia brevis.

Clinical Presentation

Poisoning results in gastrointestinal and neurologic illness of varying severity. Symptoms typically appear 30–60 minutes after ingesting toxic shellfish but can be delayed for several hours. Diagnosis is usually one of exclusion and typically is made clinically in patients who have recently eaten shellfish.

PARALYTIC SHELLFISH POISONING

Paralytic shellfish poisoning (PSP) is the most common and most severe form of shellfish poisoning. PSP is caused by eating shellfish contaminated with saxitoxins. These potent neurotoxins are produced by various dinoflagellates. A wide range of shellfish may cause PSP, but most cases occur after eating mussels or clams.

PSP occurs worldwide but is most common in temperate waters, especially off the Pacific and Atlantic Coasts of North America, including Alaska. The Philippines, China, Chile, Scotland, Ireland, New Zealand, and Australia have all reported cases.

Symptoms usually appear 30–60 minutes after eating toxic shellfish and include numbness and tingling of the face, lips, tongue, arms, and legs. There may be headache, nausea, vomiting, and diarrhea. Severe cases are associated with ingestion of large doses of toxin and clinical features such as ataxia, dysphagia, mental status changes, flaccid paralysis, and respiratory failure. The case-fatality ratio is dependent on the availability of modern medical care, including mechanical ventilation. The death rate may be particularly high in children.

NEUROTOXIC SHELLFISH POISONING

Neurotoxic shellfish poisoning (NSP) is caused by eating shellfish contaminated with brevetoxins produced by the dinoflagellate K. brevis. Predominately an illness of the Western Hemisphere (southeastern coast of the United States, the Gulf of Mexico, and the Caribbean), there are also reports of the disease from New Zealand.

NSP usually presents as a gastroenteritis accompanied by neurologic symptoms resembling mild ciguatera or paralytic shellfish poisoning, 30 minutes to 3 hours after a shellfish meal. A syndrome known as aerosolized red tide respiratory irritation (ARTRI) occurs when aerosolized brevetoxins are inhaled in sea spray. This has been reported in association with a red tide (K. brevis HAB) in Florida. It can induce bronchoconstriction and may cause acute, temporary respiratory discomfort in healthy people. People with asthma may experience more severe and prolonged respiratory effects.

Food poisoning of bacterial origin:

Food borne illness is an ever-present threat that can be prevented with proper care and handling of food products. It is estimated that between 24 and 81 million cases of food borne diarrhea disease occur each year in the United States, costing between $5 billion and $17 billion in medical care and lost productivity.

Chemicals, heavy metals, parasites, fungi, viruses and bacteria can cause food borne illness. Bacteria related food poisoning is the most common, but fewer than 20 of the many thousands of different bacteria actually are the culprits. More than 90 percent of the cases of food poisoning each year are caused by *Staphylococcus aureus, Salmonella, Clostridium perfringens, Campylobacter, Listeria monocytogenes*, *Vibrio parahaemolyticus, Bacillus cereus,* and Entero-pathogenic *Escherichia coli*. These bacteria are commonly found on many raw foods. Normally a large number of food-poisoning bacteria must be present to

cause illness. Therefore, illness can be prevented by (1) controlling the initial number of bacteria present, (2) preventing the small number from growing, (3) destroying the bacteria by proper cooking and (4) avoiding re-contamination.

Poor personal hygiene, improper cleaning of storage and preparation areas and unclean utensils cause contamination of raw and cooked foods. Mishandling of raw and cooked foods allows bacteria to grow. The temperature range in which most bacteria grow is between 40 degrees F (5 degrees C) and 140 degrees F (60 degrees C). Raw and cooked foods should not be kept in this danger zone any longer than absolutely necessary. Undercooking or improper processing of home-canned foods can cause very serious food poisoning.

Since food-poisoning bacteria are often present on many foods, knowing the characteristics of such bacteria is essential to an effective control program.

Staphylococcus aureus

Man's respiratory passages, skin and superficial wounds are common sources of *S. aureus*. When *S. aureus* is allowed to grow in foods, it can produce a toxin that causes illness. Although cooking destroys the bacteria, the toxin produced is heat stable and may not be destroyed. Staphylococcal food poisoning occurs most often in foods that require hand preparation, such as potato salad, ham salad and sandwich spreads. Sometimes these types of foods are left at room temperature for long periods of time, allowing the bacteria to grow and produce toxin. Good personal hygiene while handling foods will help keep *S. aureus* out of foods, and refrigeration of raw and cooked foods will prevent the growth of these bacteria if any are present.

Salmonella

The gastrointestinal tracts of animals and man are common sources of *Salmonella*. High protein foods such as meat, poultry, fish and eggs are most commonly associated with *Salmonella*. However, any food that becomes contaminated and is then held at improper temperatures can cause salmonellosis. *Salmonella* are destroyed at cooking temperatures above 150 degrees F. The major causes of salmonellosis are contamination of cooked foods and insufficient cooking. contamination of cooked foods occurs from contact with surfaces or utensils that were not properly washed after use with raw products. If *Salmonella* is present on raw or cooked foods, its growth can be controlled by refrigeration below 40 degrees F.

Clostridium perfringens

C. perfringens is found in soil, dust and the gastrointestinal tracts of animals and man. When food containing a large number of *C. perfringens* is consumed, the bacteria produce a toxin in the intestinal tract that causes illness. *C. perfringens* can exist as a heat-resistant spore, so it may survive cooking and grow to large numbers if the cooked food is held between 40 degrees F and 140 degrees F for an extensive time period. Meat and poultry dishes, sauces and gravies are the foods most frequently involved. Hot foods should be served immediately or held above 140 degrees F. When refrigerating large volumes of gravies, meat dishes, etc., divide them into small portions so they will cool rapidly. The food should be reheated to 165^{o} F. prior to serving.

Clostridium botulinum

Botulism accounts for fewer than one of every 400 cases of food poisoning in the U.S., but two factors make it very important. First, it has caused death in approximately 30 percent of the cases; and secondly, it occurs mostly in home-canned foods. In 1975, for example, 18 or 19 confirmed cases of botulism were caused by home-processed foods, and the other was caused by a commercial product that was mishandled in the home. *Cl. botulinum* can exist as a heat-resistant spore, and can grow and produce a neurotoxin in under processed, home-canned foods. An affected food may show signs of spoilage such as a bulging can or an off-odor. This is not true in all cases, so canned foods should not be tasted before heating. The botulinum toxin is destroyed by boiling the food for 10 minutes.

Vibrio parahaemolyticus

V. parahaemolyticus is found on sea foods, and requires the salt environment of sea water for growth. *parahaemolyticus* is very sensitive to cold and heat. Proper storage of perishable seafoods below 40 degrees F, and subsequent cooking and holding above 140 degrees F, will destroy all the *V. parahaemolyticus* on seafood. Food poisoning caused by this bacterium is a result of insufficient cooking and/or contamination of the cooked product by a raw product, followed by improper storage temperature. It is a major problem in Japan where many sea foods are consumed raw. *Vibrio vulnificus* is another member of the vibrio genus that is found in the marine environment. *V. vulnificus* is truly an emerging pathogen, but it can be controlled with proper cooking and refrigeration.

Bacillus cereus

B. cereus is found in dust, soil and spices. It can survive normal cooking as a heat-resistant spore, and then produce a large number of cells if the storage temperature is

incorrect. Starchy foods such as rice, macaroni and potato dishes are most often involved. The spores may be present on raw foods, and their ability to survive high cooking temperatures requires that cooked foods be served hot or cooled rapidly to prevent the growth of this bacteria

Listeria

Before the 1980's most problems associated with disease caused by *Listeria* were related to cattle or sheep. This changed with food related outbreaks in Nova Scotia, Massachusetts, California and Texas. As a result of its widespread distribution in the environment, its ability to survive long periods of time under adverse conditions, and its ability to grow at refrigeration temperatures, *Listeria* is now recognized as an important food-borne pathogen.

ImmunocompromisedImmunocompromised humans such as pregnant women or the elderly are highly susceptible to virulent *Listeria*.*Listeria monocytogenes* is the most consistently pathogenic species causing listeriosis. In humans, ingestion of the bacteria may be marked by a flu-like illness or symptoms may be so mild that they go unnoticed. A carrier state can develop. Death is rare in healthy adults; however, the mortality rate may approximate 30 percent in the immunocompromised, new born or very young.

As mentioned earlier *Listeria monocytogenes* is a special problem since it can survive adverse conditions. It can grow in a pH range of 5.0-9.5 in good growth medium. The organism has survived the pH 5 environment of cottage cheese and ripening cheddar. It is salt tolerant surviving concentrations as high as 30.5 percent for 100 days at 39.2 degrees F, but only 5 days if held at 98.6 degrees F.

The key point is that refrigeration temperatures don not stop growth of *Listeria*. It is capable of doubling in numbers every 1.5 days at 39.5 degrees F. Since high heat, greater than 170 degrees F, will inactivate the Listeria organisms, post-process contamination from environmental sources then becomes a critical control point for many foods. Since *Listeria* will grow slowly at refrigeration temperatures, product rotation becomes even more important.

Yersinia enterocolitica

Even though *Yersinia enterocolitica* is not a frequent cause of human infection in the U.S., it is often involved in illness with very severe symptoms. Yersiniosis, infection caused by this microorganism, occurs most commonly in the form of gastroenteritis. Children are most severely affected. Symptoms of pseudoappendicitis has resulted in many unnecessary

appendectomies. Death is rare and recovery is generally complete in 1-2 days. Arthritis has been identified as an infrequent but significant sequel of this infection.

Y. enterocolitica is commonly present in foods but with the exception of pork, most isolates do not cause disease. Like *Listeria* this organism is also one that can grow at refrigeration temperatures. It is sensitive to heat (5%) and acidity (pH 4.6), and will normally be inactivated by environmental conditions that will kill *Salmonellae*.

Campylobacter *Campylobacter jejuni*

C. jejuni was first isolated from human diarrhea stools in 1971. Since then it has continually gained recognition as a disease causing organism in humans.

C. jejuni enteritis is primarily transferred from animal origin foods to humans in developed countries. However, fecal contamination of food and water and contact with sick people or animals, predominates in developing countries.

Although milk has been most frequently identified throughout the world to be a vehicle for *Campylobacter*, one anticipates that future investigations will identify poultry and its products and meats (beef, pork, and lamb) as major reservoirs and vehicles.

C. jejuni dies off rapidly at ambient temperature and atmosphere, and grows poorly in food. The principles of animal science will play a significant role in the control of this ubiquitous organism. Hygienic slaughter and processing procedures will preclude cross-contamination while adequate cooling and aeration will cause a decrease in the microbial load. In addition, thorough cooking of meat and poultry products followed by proper storage should assist in maintaining food integrity and less contamination.

Enteropathogenic *Escherichia coli*

Enteropathoginec *E. coli* is a significant cause of diarrhea in developing countries and localities of poor sanitation. In the U.S. it has been associated with "travelers' diarrhea." However the latest outbreak in North America occurred in a nursing home in Ontario. This was a severe outbreak of *E. coli*0157:H7 associated hemorrhagic colitis.

There are at least four subgroups of enteropathogenic *E. coli*: enterotoxigenic, enterinvasive, hemorrhagic, and enteropathogenic. Each strain has different characteristics.

The major source of the bacteria in the environment is probably the feces of infected humans, but there may also be animal reservoirs. Feces and untreated water are the most likely sources for contamination of food.

Control of enteropathogenic *E.coli* and other food-borne pathogens such as *Salmonella* and *Staphylococcus aureus*can be achieved. Precautions should include adequate cooking and avoidance of recontamination of cooked meat by contaminated equipment, water or infected food handlers. Food service establishments should monitor adequacy of cooking, holding times, and temperatures as well as the personal hygiene of food handlers.

Prevention

The first step in preventing food poisoning is to assume that all foods may cause food-borne illness. Follow these steps to prevent food poisoning:

1. Wash hands, food preparation surfaces and utensils thoroughly before and after handling raw foods to prevent recontamination of cooked foods.
2. Keep refrigerated foods below 40 degrees F.
3. Serve hot foods immediately or keep them heated above 140 degrees F.
4. Divide large volumes of food into small portions for rapid cooling in the refrigerator. Hot, bulky foods in the refrigerator can raise the temperature of foods already cooled.
5. Remember the danger zone is between 40 degrees F and 140 degrees F.
6. Follow approved home-canning procedures. These can be obtained from the Extension Service or from USDA bulletins.
7. Heat canned foods thoroughly before tasting.

Infants, older persons, women who are pregnant and anyone with a compromised immune system are especially susceptible to food-borne illness. These people should never consume raw fish, raw seafood, or raw meat type products.

Pink spoilage of salted fish:

This spoilage of salted fish is common where storage is in wet stocks at 15 to 20°C. The pathogen is the halophilic bacteria often already present in the salt used for curing. Pink spoilage develops pink patches which spread over the entire fish. These patches are the growing colonies of bacteria attacking the protein of flesh and rendering it soft. In advanced stages the fleshay fall apart, develop characteristic off odours if pink which smells

offensive,sour,cheesy and Sweaty. When consumed pink causes food poisoningin mam.This spoilage may be controlled by cold storage of salted fish.

“Dun spoilage” of salted fish:

It is similar to the “pink” except that the patches or brown or black in colour and it is common in dry stacks of salt.

Unit – 5

Utilisation of fish as products and by products:

Landed fish fetches maximum price when salt in fresh condition worthy of human consumption. For several reasons including non availability of a ready Market, on nearness of market, lack of facilities for fast transport or refrigerated transport etc, an appreciable amount of fish is preserved for long term storage. This preserved fish is also meat for human consumption as food. But there are yet other utilization of fish in which processed into a number of valuable products and by products for variety of usage. Chief among these will now be dealt with, one by one.

Fish liver oil :

Fish liver oils are an important source of vitamin A and D. Two most important sources of liver oils are cod and halibut (both are cold water species). Other sources are tunas and allied species and some sharks. Although tuna's liver is small in comparison to its body weight but its oil is rich in vitamin A & D. The vitamin contents of shark liver vary greatly. Certain varieties such as tiger shark, black fin shark, hammer headed shark and saw fish are commercially important. The oil content is up to 80% of the liver. With decreasing fat content the colour of the liver changes from yellow to brown and texture from soft to firm. Presently shark liver is valued for highest unsaponifiable matter (squalene) which is present in the liver of certain deep sea sharks. Depending on the oil content and vitamin A potency, fish livers are generally classified into:

- Low oil content ---------- high vitamin A potency
- High oil content ---------- low vitamin A potency
- High oil content ---------- high vitamin A potency.

Methods of preservation of Livers for oil extraction

- By freezing : 0 – 50C for 24 h/ at -180C for several months
- By salting: 10% salt with pieces of liver
- By formalin treatment: 0.25% by weight
- By alkali treatment:

Hence steaming will not release the oil without degradation of vitamin A, protein is digested and solubilized to release the enclosed oil and vit A or a solvent is used to extract oil. Alkali such as NaOH (@1-2% by weight) or Na2CO3 is mixed with the ground liver and heated to 80-900 C by live steam with stirring. The digested liquor is centrifuged while hot to separate the oil.

Use of fish liver oil:

Nowadays liver oil is mainly used in the textile and tanning industries and in the production of cosmetics, pharmaceutical products, and lubricants.

Fish oil properties

The oils contain mainly triglycerides of fatty acids (glycerol combined with three similar or different acid molecules) with variable amounts of phospholipids, glycerol ethers and wax esters. It is characteristic of the oils that they contain a wide range of long-chain fatty acids with the number of carbon atoms ranging mainly from 14 to 22, and high degree of reactivity (unsaturation) ranging up to six double bonds per molecule. The complex nature of fish oil depends upon a number of factors. The fatty acid patterns of the oils vary widely with fish species and, to some extent, with the composition of the plankton and the time of year. These influence the properties of oils both in regard to edible as well as technical applications.
The oils contain variable, but small amounts of unsaponifiable components, such as hydrocarbons, fatty alcohols, waxes and ethers, and these also influence the properties of the oils to some extent. In order to manufacture oil of desirable properties, one should observe the following:

a. the fish should be as fresh as possible;

b. the oil should be cooled before delivery to the storage tank and should be pumped in near the bottom of the tank (not right at the bottom) and removed from the top. The sludge and water should be regularly drained from the bottom to prevent an increase in FFA during storage.

Commercial value of fish oils

The market value of fish oil depends on its chemical analysis. Normally, a basic sales value is established for oil containing a certain level of free fatty acids (2% to 3%), unsaponifiable matter (3.5%), and water and dirt (0.3%). If these levels are exceeded, the price is reduced accordingly. The price may also be reduced if the oil is dark coloured or malodorous. Standard for Fish Oils (Codex Stan 329-2017) Fish oils, fish liver oils, concentrated fish oils, and concentrated fish oils ethyl esters with comply with the following ;
Acid value $\leq$ 3 mg KOH/g

- Peroxide value $\leq$ 5 million equivalent of active oxygen/kg oil
- Anisidine value $\leq$ 20
- Total oxidation value

Fish oils with a high phospholipid concentration of 30% or more such as

krill oil shall comply with the following:

- Acid value ≤ 45 mg KOH/g
- Peroxide value ≤ 5 milli equivalent of active oxygen/kg oil
- Vitamins: Fish liver oils except of deep sea shark liver oil shall comply with following;
- Vitamin A ≥ 40 µg of retinol equivalents/ml of oil
- Vitamin D ≥ 1.0 µg/ml

The following additives may be used in Fish oil:

Additives Additive name Maximum level Antioxidant E300 Ascorbic acid, L- GMP E 304, 305 Ascorbyl esters 2500 mg/kg, as ascorbyl stearate E307a, b, c Tocopherols 6000 mg/kg, singly or in combination Emulsifier E322 Lecithin GMP E471 Mono- and di-glycerides of fatty acids GMP.

Methods of extraction of fish liver oil from liver :

The extraction of fish oil by wet pressing is the most commonly used method for production on an industrial scale, and is basically carried out in four stages: fish cooking, pressing, decantation and centrifugation (FAO, 1986).

The livers are ground with water into a slurry, and then this is gently simmered until the oil rises to the top. The oil is skimmed off and purified. Other methods used in modern times include the Cold Flotation Process, pressure extraction, and pressure cooking.

Acipenseridae 31,600 - Comparison with enzymatic extraction, wet reduction and addition of amino compounds SFE showed the highestextraction yield (97.25 %), followed by enzymaticextraction. Less lipid oxidation was observed Hao et al.

1. Extraction of oil :

a) Method of auto fermentation :

Livers are left to undergo decomposition for several days, macerated and exposed to Sun in earthen pots. Oil released during disintegration is collected. Oil is crude and used for burning purposes or for making lights in curing yards by fishermen. The method is simple but crude.

b). Method of boiling:

Livers are chopped into pieces and boiled. Released oil is collected. Method is simple, not requiring equipments. However yield is moderate because not all oil is extracted out of liber. Generally followed small cottage scale industries is this method.

c) Method of steaming:

Minced livers are steamed under 2kg / sq- cm of pressure. Oil is collected from from top layer. The method is a quick one and is good for operation onboard of fishing vessels, where steam is readily available.

d) Method of chemical digestion :

There are three main procedures:

- Aquacide digestion :. Fresh liver is mixed with a mixture of sodium bicarbonate and paradehyde. The bill is stirred with warm water,giving an emulsion of water and released oil. As soon as the emulsion breaks, oil forms a floating layer on the top from where it is collected.
- Alkali digestion: Minced liver is mixed with caustic soda or sodium bicarbonate. It is then steamed at 82- 88°C with constant stirring. The pulp is then centrifuged to separate oil which is collected.
- Enzyme alkali digestion : In this process minced liver is brought to pH level of 1.2 to 1.5 by the addition of hydrochloric acid solution. Now commercial pepsin is mixed and the pulp digested at 43°- 49°C. Then the pH is raised by the addition of an alkali until a level of 9 is reached. Temperature is also increased to 80°C. After complete digestion the oil is run off from the top.
- Method of solvent Extraction:. This is an expensive method, but the yield is higher , however, it has not been adopted for commercial production. The method consists first dehydration of the minced liver with the application of an anhydrous salt, generally sodium sulphate. When the moisture has been removed, the pulp is mixed with the solvent is subject to distillation process and the oil is separated out. Any free fatty acids coming along during solvent extraction from f oil is removed during refinement.

e) Refinement of oil :

First, the oil collected after extraction from liver is left to stand so that water and any debris present in it settles down. Pure oil is now removed from top layer, filtered and then centrifuged. The oil thus obtained is congealing oil and has stearine present in it. To obtain non-congealing oil, it is cooled and stored at 10^0C when stearine is separated out. The oil is again filtered while still cool.

f) Standardization of vitamin A potency in the extracted oil :

Since, the liver oil is mostly used in medicine for its richness in vitamin A content. It is subject to rigorous estimation for the same. Following three methods are in vogue.

g) Biological estimation :

The standardization involving feeding with oil to experimental albino rats raised on vitamin AZ deficient food. The response in growth is matching against a similar response in a case in which such abino rat have been fed on cod liver oil with known potency of vitamin. This is how the potency or vitamin A is estimated.

h) Colorimetric estimation with tintometer :

In this process of standardization, the oil is dissolved in chloroform and then chloroformic solution of antimony trichloride is added to it. A blue colour develops. The intensity of this colour is read on tintometert and is the measure of vitamin A potency.

i) Phptoelectric spectrophotometric estimation :

The standardization uses the principles of characteristics and selective absorption of a certain zone of ultraviolet region of the spectrum. The extent of absorption is related directly to the concentration of the vitamin a . The intensity of absorption is measured with photoelectric unit and can be read on its galvanometer. For this, simply the oil is dissolved in a suitable solvent and then the solution is kept in the path of ultra violet rays.

Prototype of fish liver oil manufacturing plant :

The plant is designed on steam extraction principles. It consist of three parts ; a distingator, a steaming tank and an oil separator. The distingator is a large tank to hold large amounts of chopped liver. It is fitted with a mincing device so that finally the liver is turned into a pulp. The pulp is then pumped through a large pipe to the steaming tank. Water is added here. Steam is circulated through conducted pipes in the tank. The digested liver is then brought down to the oil separator unit where it is subject to centrifugation. The debris is processed to get a by product the fish manure or an animal feed. The yield of oil is maximum and the quality of oil is also good. The oiul may however have a fishy odour and taste. This may be deodorized during refinement. Refined oil may be blended with other vegetables oils for flavor or capsulated into concentrates of vitamin A.

Simple model of fish liver oil extractor for use in small scale cottage industry :

A simple model has been proposed by saha in 1956. It consists of an all purposes tank. It has the two sections. A lower one serving as a boiler and upper one the extracter cum separator. The bottom of the letter fits into the mouth of the former, the two sections soldered together. Two steam pipes 1.27 cm in diameter leave the boiler and entering the extractor section from above run to its bottiom, ending 3.8 cm above the bottom. There is a water inlet fitted into the boiler. One "window tube "of glass tube is fitted in each section to read the level of water in the boiler or the level of the oil in this extracter for the removal of oil. The extraction has its conical mouth closed with a removable hopper, and on outlet tube for steam and also a socket for the thermometer. The whole unit can be easily fabricated with a 24 guage G.I sheet at low cost. It is portable and handy also.

The working is simple. First boiler is filled 2/3rd with water. Now minced liver is introduced into the extractor through the hopper to fill half of it. Then water is added in a quantity ¼ of the amount of liver. Now boiler is heated over a fire. The steam is conducted into the extracter, where it heats up the liver as wells as stirs it up. Uncondensed steam leaves through the outlets pipe. Periodically a thermometer is put on this tube to record the temperature reached. Steaming is carried out for 45 minutes and then discontinued. This is enough to extract all oil and water then separate away and their levels read on the window tube of the extracter.

Fish body oil :

Fish body oil is made from tissues of fatty **fish** like sardines, sprat, salmon, and mackerel. **Fish** liver **oil** is made by pressing the cooked liver of halibut, shark or, most commonly, cod. Both types are available from high-street retailers and over the internet.

Fish body oils are usually produced during the wet reduction process. The press liquor is the oil-water emulsion containing dissolved proteins and other substances as well as particulate matter. The press liquor is passed through a series of settling tanks or a series of centrifuges. The amount of particulate matter depends on the degree of cooking, condition of the fish when processed and also the manner of pressing. Cooking is an important step because, if the cooking time and temperature is too low, the fluids (oil + water) will not be released from the protein and pressing out will be difficult. If the material is overcooked, the fish will become a soft mush and there will be an insufficient build-up of pressure in the press to expel the liquids.

Extraction by settling tank system The settling tanks are heated to assist break up of the emulsion and prevent solidification of the stearin portion of the liquids. In a series of five or more 6heated tanks the press liquor is admitted to the first at a point below the surface. Oil rises to the top and is passed to the bottom of the second tank containing water and the process is repeated in succeeding tanks. Finally oil is heated to evaporate the remaining water.

Centrifuge system of extracting fish body oil :

In this system, centrifuge is heated and water phase is spun off and almost clean oil is obtained. Further to get clean bright oil, oil is heated to about 94 degree, mixed with clean water of same temperature and passed to the polishing centrifuge. Oil produced through centrifuge system is finer, cleaner and brighter and has lower moisture content than oil from a settling tank system. Oil produced by pressing in dry reduction process is dark in colour due to contact with the metal surfaces and of poor quality.

Purification of Fish oil :

The process is also known as hardening of oil. Crude oil has number of impurities such as free fatty acids, phospholipids, diglycerides, monoglycerides, pigments, pigment decomposition products, oxidation products, sulphur compounds, proteinaceous compounds, aldehydes, ketones, pesticides residues (preferably organochlorine pesticides and polychlorinated biphenyls accumulate in fatty fish species which are generally used for fish oil production. As lipophilic compounds these molecules tend to be sequestered in lipid rich tissues and finally find place in the fish oil. Fish oil may be contaminated with toxic heavy metals (such as Cd, Hg, BP, Cu, Zn, etc. Cu and Zn are known for their distinct prooxidant effect. Spoiled raw material is responsible for the increased level of FFA, oxidative products and increased level of nitrogen and sulphur by protein degradation and increased metal contents. Oil extracted from spoiled fish is discoloured and brown and foul smelling not characteristic of fish oil. The refining is done in following five steps:

Refining :

Treatment of temperature oil with an aqueous alkaline solution which reacts with the free fatty acids to form soaps and remove any mucilages.

Bleaching

Bleaching materials commonly used are natural or activated clays and activated carbon. It removes any coloured matter, natural pigments and some of the suspended

mucilages. These adsorbent substances are also effective in the reduction of oxidation products, phosphorus, to a lesser extent sulphur compounds and heavy metals and non metals.

Hydrogenation

It is the process by which hydrogen is added directly to the unsaturated bonds in the fatty acid chain. Gaseous hydrogen, liquid oil and solid catalyst are brought into intimate contact at an elevated temperature (usually 170 – 2040 C) under a suitable pressure of hydrogen. The catalyst commonly employed is nickel, which is prepared in suspension and normally added to the oil at a level of 0.05 % to 0.1 %. When hydrogenation reach the desired point, the gas is turned off and the oil is cooled down rapidly. Lastly oil is filtered to remove catalyst. Hydrogenation process cannot be advocated where aim is to have an oil as the source of PUFAs, particularly those belonging to omega-3 family.

Further refining

It is the process of second refining of oil before deodourization, to improve 37 flavour stability

Deodourization

It is done by steam distillation under high vacuum (2 – 5 mm absolute pressure). Dry steam (free from oxygen and temperature range 170 – 2300C) is used, which is passed through the oil under vacuum for prolonged period (may be upto 5 h in a batch process). This step removes free fatty acids, decomposition products of hydroperoxides such as aldehydes and ketones, odouriferous and other volatile impurities. This step is very critical one. If time and temperature are not strictly controlled as per schedule, the most valued components of the fish oil undergo distinct deterioration. Extended exposure of α-linolenic acid at a temp of 2500 C produces geometric isomers. EPA and DHA are more susceptible to isomerisation. When retention of EPA and DHA are the concern then a temperature not exceeding 170^0 C is recommended.

Bleaching with silica gel

As an alternative to activated clay and carbon, the treatment of fish oil dissolved in hexane with silica gel gives a nearly colourless oil. It has been presumed that mono- and di-glycerides are the sources of odour and are retained in the silica gel.

Winterisation

It is a cold clearing process, additional operation for refining fish oil. When the oil is cooled sufficiently, the saturated triglycerides (have high melting points) commence to

solidify and separate out. Solid fraction is designated as ‘stearine’. Cooling must be gradual by circulating cold brine. Sudden chilling (or shock chilling) is to be avoided. The process is terminated when 38 temp of oil start rising due to release of latent heat as a result of crystallization of saturated glycerides.

Preser vation and storage of Fish oil 6 6

- Deterioration of fish oil results from the development of free fatty acids and oxidative rancidity
- Hydrolytic rancidity is caused by lipases present in the oil by¬ contaminating microorganisms
- Oxidative rancidity is caused by atmospheric oxygen and lipoxygenases present in the fish or contaminating microorganisms
- Flavour reversion also takes place due to oxidation6

6Deterioration of fish oil can be avoided by

- Controlled heating to 80 – 100 degree for 15 – 20 min⎫ By adding antioxidants⎫ By halogenation⎫ Storing under inert gas such as nitrogen⎫
- Use of Fish body oil Unlike vegetable margarines, fish margarines have an excellent¬ plastic consistency
- Fish oils can be used in manufacture of rubber, detergents,¬ lubricants, printing inks, leather and cosmetics.
- Fish oil can be used in animal feed as carriers for the oil soluble vitamin A and D

Fish meal :

Fish meal is a commercial product mostly made from fish that are not used for human consumption; fishmeal is generally used to feed farm animals in an agricultural setting. Because it is calorically dense and cheap to produce, fishmeal has played a critical role in the growth of factory farms and the number of farm animals it is possible to breed and feed.

Fishmeal is made from the bones and offal left over from fish caught by commercial fisheries. The vast majority of the fish from which fishmeal is manufactured are not used for human consumption; rather, fishmeal is generally manufactured from by-catch.

Fishmeal takes the form of powder or cake. This form is obtained by drying the fish or fish trimmings, and then grinding it. If the fish used is a fatty fish it is first pressed to extract most of the fish oil.

The production and large-scale use of fishmeal are controversial. The lucrative market for fishmeal as a feed encourages corporate fisheries not to limit their yields of by-catch (from which fish meal is made), and thus leads to depletion of ecosystems, environmental damage, and the collapse of local fisheries. Its role in facilitating the breeding and over-feeding of millions of pigs and chickens on factory farms has also been criticized by animal rights and animal welfare groups. Manufacturers of fishmeal counter that fishmeal's role in the feeding and breeding of millions of farm animals leads to the production of more food and the feeding of millions of people around the world.

Others:

Fish flour:

Fish flour is a fodder of animal origin that is produced from sea-food processing waste and inedible types of sea animals. 1 ton of **fish flour** is made of 6 tons of **fish** raw material. **Fish flour** is used as food additive during feeding of domestic animals, as well as in animals breeding, birds breeding, and **fish** farming.

Fish flour is a dried powder, prepared from dressed fish and highly nutritious which contains high quality proteins with all essential amino acids and minerals. Also, it contains high amount of energy could be supplemented to the food items. In this study we have tried to establish the storage life of fish flour from Indian oil sardine by evaluating the biochemical changes in the same. This understanding will not only provide value addition but also give better economic returns to the fishing sector.

Fish silage

Fish silage as described here is defined as a liquid product made from whole fish or parts of fish that are liquefied by the action of enzymes in the fish in the presence of an added acid. The enzymes break down fish proteins into smaller soluble units, and the acid helps to speed up their activity while preventing bacterial spoilage.

Silage made from white fish offal does not contain much oil, but when it is made from fatty fish like herring it may be necessary to remove the oil at some stage.

There are other methods of making liquid fish protein, for example by adding enzymes or bacteria, but these are not described here.

How is fish silage made?

The raw material is first minced; suitably small particles can be obtained by using a hammer mill grinder fitted with a screen containing 10 mm diameter holes. Immediately

after mincing, 3·5 per cent by weight of 85 per cent formic acid is added, that is 35 kg or about 30 litres of acid to one tonne of fish. It is important to mix thoroughly so that all the fish comes into contact with acid, because pockets of untreated material will putrefy. The acidity of the mixture must be pH 4 or lower to prevent bacterial action. After the initial mixing, the silage process starts naturally, but occasional stirring helps to ensure uniformity.

The production tank can be of any size or shape provided it is acid resistant; some steel containers used for making or carrying the silage may need a polyethylene liner to prevent corrosion. Concrete tanks treated with bitumen are suitable for holding large quantities. The size and number of tanks depend on the amount and type of raw material available.

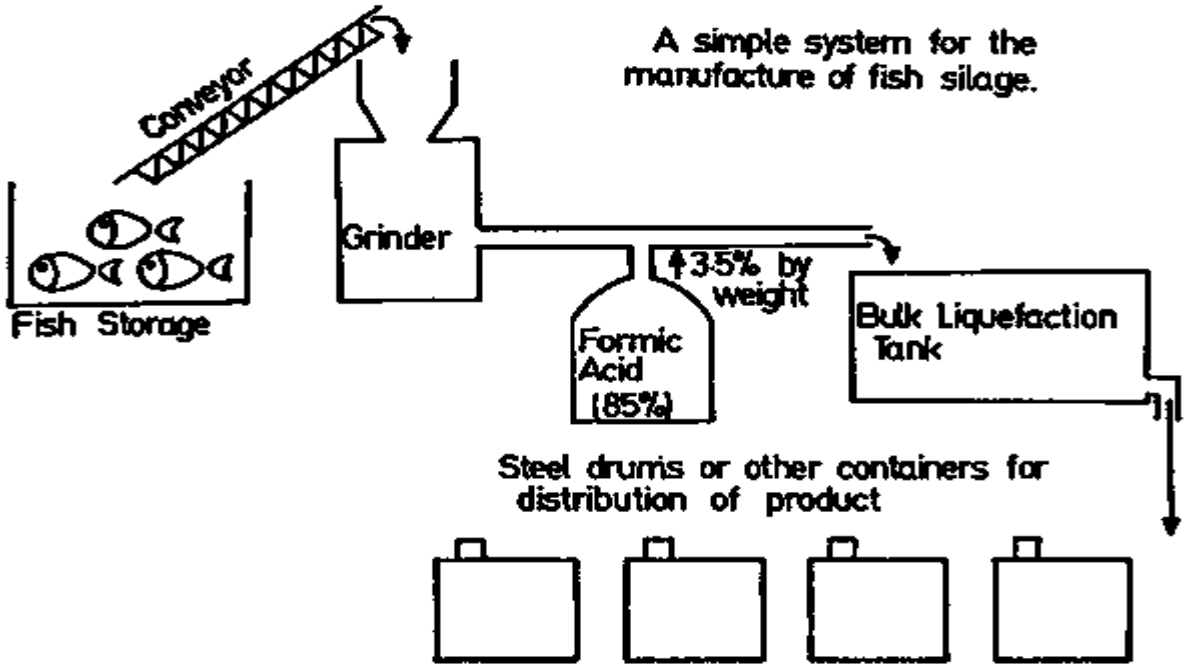
The rate of liquefaction depends on the type of raw material, its freshness, and the temperature of the process. Most species can be used, but sharks and rays are rather difficult to liquefy, and should be mixed in with other species. Fatty fish liquefy more quickly than white fish offal, and fresh fish liquefy much more quickly than stale fish. It should be possible in most installations to mince and add the acid immediately the raw material is received, thus avoiding slow liquefaction of stale fish. The warmer the mixture, the faster the process; silage made from fresh white fish offal takes about two days to liquefy at 20°C, but takes 5-10 days at 10°C, and much longer at lower temperatures. Thus in winter it would be necessary to heat the mixture initially, or to keep it in a warm area until liquid.

Minced untreated fish must be kept covered to keep out flies; once the acid has been added, flies are not attracted to the mixture.

Once the silage is prepared it can be handled like any other liquid, and transported in bulk or in containers. It can also be blended with cereals to make a semidry feed. Silage made from white fish offal should be stirred as it is removed from the production tank to obtain a uniform batch, since a bone-rich layer tends to settle at the bottom of the tank after a time. Silage made from fatty fish is more homogeneous and there is little separation even after prolonged storage, but the oil in it deteriorates very rapidly; if the oil has to be removed and used for other purposes, it can be separated by heating and centrifuging.

Fish silage can be concentrated to reduce its bulk, but more experimental work needs to be done to assess the commercial advantage of such a process.

A simple system for the manufacture of fish silage.



Fish manure

Fish manure and guano are interferior quality of fish meal products which are unfit as animal feed. Fish manure is a byproduct of curing yards., fish glue industries and oil extracting plants in which trash fish or spoilt fish have been employed. The residue after extraction of oil or poorly cured fish including sun dried fish, makes the manure. This manure is having a high content of nitrogen, phosphates and lime. The fish manure is particularly useful in raising coffee, tea, and tobacco crops.

Fish guano is the byproducts of body oil extraction plants in which oil bearing species, particularly oil sardines are used as raw material. The product being already cooked during the processing for oil extraction decomposes quickly in the soil and mixes **well.**

Fish sausage

The product is commercially manufactured in Japan, U.S.S.R .and U.S.A. These are spiced fish must preparation very much popular in Japan. Fish ham differs from fish sausages in having small pieces of solid fish meat mixed with pasted fish meat. Spices and additives are added to improve taste, flour, and keeping quality.

Fish glue

Excellent quality, in grains or flakes.

Fish glue is a traditional glue for application on organic materials such as wood.

Fish glues are by-products of desalted **fish** skins, usually cod, and have properties similar to animal skin and hide **glues**, which have largely replaced them in woodworking applications

Fish Glue is **used** in the **gluing** of wood, papers, cardboard and leather. Formulated from **fish** bones, Artisans **Fish Glue** is a natural and flexible **glue** that has a strong adhesion power. The **Fish Glue** is ideal in all creative projects of furniture and woodwork since it's reversible.

It has a very high adhesive strength, a long working time, can be reactivated with water, it is non-toxic.

Fish glue is used where strong, but also elastic bonds are required. Suited for gluing veneers, to attach different materials to wood (such as metals, metal foils, tortoiseshells), as glue for textiles and papers, glue for gilding, glue for glass and ceramic.

Properties of the fish glue:

In contrast to other animal glues, which harden on cooling, fish glue remains liquid at room temperature. Threfore, the surfaces does not have to be preheated.

Insoluble in organic solvents.

Like all other animal glues, fish glue is not waterproof. Therefore, the connections can be reverted with warm water, glue residues can easily be removed with a wet cloth. This protects valuable objects since you do not have to use tools or sanding paper to remove excess glue.

Example on how to use the fish glue:

Mix the solid fish glue with cold water (usually 100 g of fish glue and 700-1000 ml of water). Leave it to swell for 24 hours. The mixture is then heated with occasional stirring in a water bath. The water must never boil, since the glue would lose the adhesive force. The ideal temperature is 50-60 °C. After about 20-30 minutes, the fish glue has completely liquefied and is ready for use. The glue can be diluted with water to get the desired consistency.

Isinglass

Isinglass Clear, almost pure gelatin that is prepared from the air bladders of sturgeon and other sources. It is used primarily to clarify wines and beers. The name also refers to an abundant silicate material, also called muscovite, used as an insulator.

Transparent, colorless, water soluble fish glue. Isinglass was originally made from air bladders of the great Russian beluga sturgeon, *Acipenser huso*, found in the fresh waters of the Caspian and Black Seas. After restrictions were placed on Russian exports in 1939, other fish air bladders were used and isinglass became a generic term for glue derived from the swim bladder of various fish, e.g., North American isinglass is made from hake or cod. To prepare isinglass, the air bladders are removed from the fish, cleaned and air dried. The dried bladder is cut into thin translucent strips.

These strips, which are nearly 80% collagen, are dissolved in hot water then diluted and cooled into flat disks. The very strong, water soluble adhesive can be used in low concentrations. Isinglass is used as a clarifying agent in the manufacture of fish glue. It is also used as a size for handmade paper and has been used in Russia as a paint medium. Sturgeon glue is rarely available outside of Russia.

Fish leather

Fish skin is a rare type of leather. The skin surfaces are often very small. In recent years, objects made from stingray leather have grown in popularity, thanks to the glass bead-like surface structure of the skin.

Also, the proliferation of fish farms, for various species, has increased the availability of fish leather, as the skins would otherwise be wasted.

Fish leather is stronger than other leather types, if the same thicknesses are compared. This is because the fibre structure of fish skin runs crosswise, rather than parallel as in, for example, cowhide. The tensile strength of fish leather reaches up to 90 Newtons (e.g. salmon or perch).

The manufacturing principle for fish leather production was developed by the Nanai people from Eastern Siberia, who traditionally make fish leather garments. The tanning process takes about a month.

The skins of the following are suitable for making fish leather: Shark, salmon, carp, stingray, cod, sea wolf and sturgeon. Fish leather usually has a scaly structure, is thinner than calfskin and is considered to be very elastic and tear-resistant.

Fish macroni

The product is manufactured by the Mysore institute of India, The fish barbus carnaticus is minced and mixed with tapioca or sorghum flour in equal parts. The latter is partially gelatinised with hot water.

Fish biscuits

The product is manufactured in Chile and Morocco. Fish flour is blended with biscuits mixture prior to baking.