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Isolation and identification of Bacteria from Spoiled fishes collected from the fish vendors of Visakhapatnam harbour, North Andhra Pradesh

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Abstract
Fish are classified as any of the cold-blooded aquatic vertebrates of the super class Pisces typically Showing gills, fins and a streamline body. In addition, 'fish' also refers to the flesh of such animals used as food. This super class of vertebrates includes all the bony and cartilaginous finfish, and excludes Molluscs and Crustacea. We most often like to consume sea food, and in this sea food fishes Play an important role and are important part of the daily diet for human being. Sometimes we find spoiled Fish, the spoilage is due to different types of bacteria that grow on the slime Of fish. An attempt was made to isolate and identify different types of genera in bacteria that at spoil the fish. The most commonly found genera include pseudomonas, Photobacterium. To identify and isolate this type of Bacteria, Samples were collected from Vishakhapatnam harbour from ten different species of fish. The Isolation and identification were done with the help of streak plate method. Different types of bacteria were identified which includes staphylococcus aureus, pseudomonas, Klebsiella, proteus species. The Bacteria were isolated based on the gram staining.

Keywords: Isolation and identification**,** Bacteria, Spoiled fishes Visakhapatnam harbour, North Andhra Pradesh.

1. Introduction

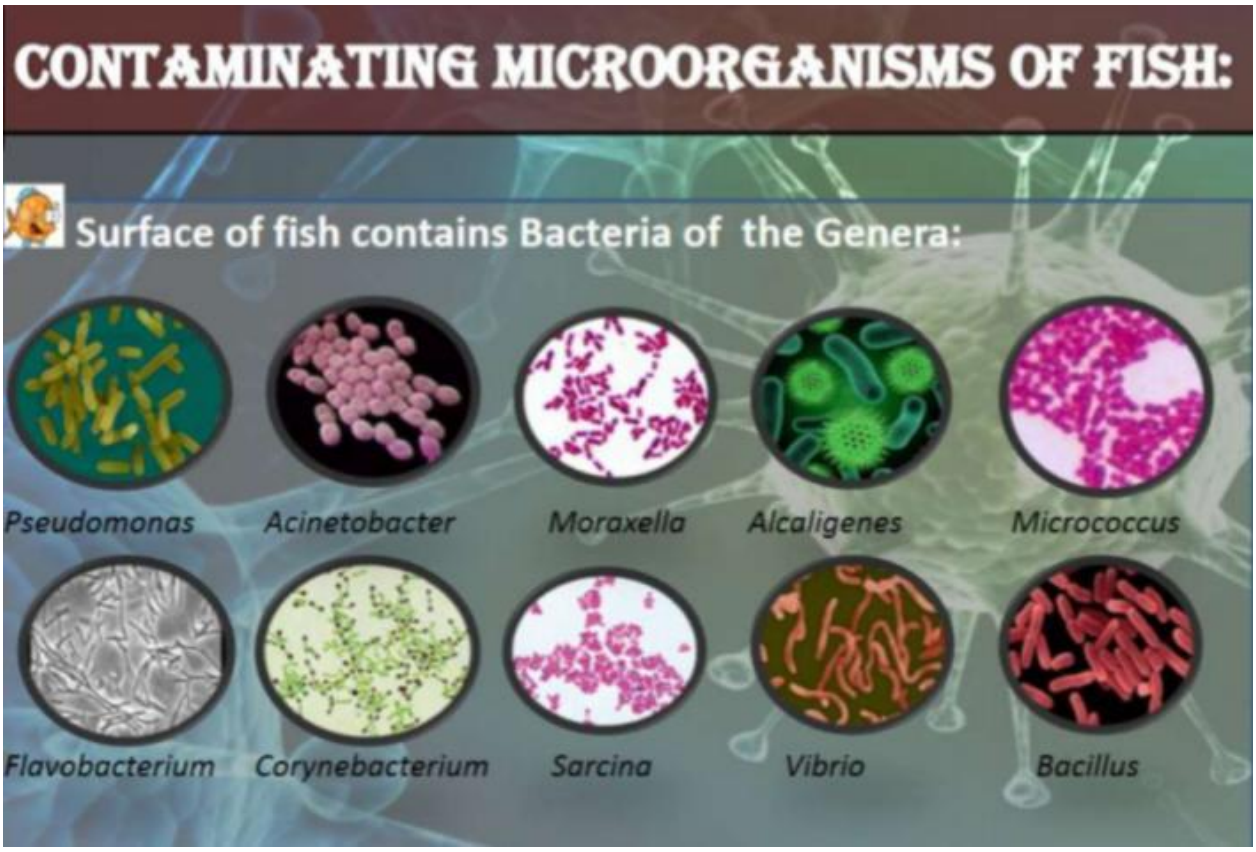
Fish are cold-blooded, gill-bearing aquatic organisms that lack limbs. Fish have great diversity, including armoured fish, jawless fish, lobe-finned fish, cartilaginous fish, and ray-finned fish. In the taxonomic hierarchy, fishes belong to the kingdom Animalia, phylum Chordata and class Pisces. Fish is one of the most consumed seafood and it is a highly perishable food product. Fish and fish products are widely consumed as it is a good nutrition source due to their high protein content, unsaturated fatty acids, especially omega-3 fatty acids.The biological and chemical nature of fish leads to its deterioration after it is caught. The spoilage process (Rigor mortis) will start within 12 h.The deterioration occurs very quickly due to the metabolic activity of microorganisms, endogenous enzymatic activity (autolysis), and the chemical oxidation of lipids.Spoilage of fish is a process of deterioration in the quality of fish, which changes its appearance, odour and taste. The breakdown of biomolecules like proteins, amino acids and

fats in the fish are the factors responsible for fish spoilage. Thus, a fish can be spoiled by either chemical or biological degradation

In chemical degradation, protein, fats, amino acids etc., are decomposed, whereas microorganisms carry out the biological degradation. Other than bacterial and chemical degradation, enzymatic and mechanical damage can also cause fish spoilage. There are certain factors like high moisture, protein and fat content, improper handling etc., that favouring the spoilage in fish. The common causes of fish spoilage are bacterial contamination and chemical oxidation (protein, fats etc.). The microorganisms involved in fish spoilage refer to the SSOs (specific spoilage organisms) that result in the formation of numerous unwanted metabolites, which adds undesirable appearance, flavour, and odour to the fish.

MICROBIAL FLORA OF FISH:-

Microorganisms are found on all the outer surfaces (skin and gills) and in the intestines of live and newly caught fish. The total number of organisms vary enormously and Liston (1980) states a normal range of 102-107 cfu (colony forming units)/cm2 on the skin surface. The gills and the intestines both contain between 103 and 109 cfu/g (Shewan, 1962). The bacterial flora on newly-caught fish depends on the environment in which it is caught rather than on the fish species (Shewan, 1977). Fish caught in very cold, clean waters carry the lower numbers whereas fish caught in warm waters have slightly higher counts. Very high numbers, i.e., 107 cfu/cm2 are found on fish from polluted warm waters. Many different bacterial species can be found on the fish surfaces. The bacteria on temperate water fish are all classified according to their growth temperature range as either psychrotrophs or psychrophiles. Psychrotrophs (cold-tolerant) are bacteria capable of growth at 0°C but with optimum around 25°C. Psychrophiles (cold-loving) are bacteria with maximum growth temperature around 20°C and optimum temperature at 15°C (Morita, 1975). In warmer waters, higher numbers of mesophiles can be isolated. The microflora on temperate water fish is dominated by psychrotrophic Gram-negative rod-shaped bacteria belonging to the genera Pseudomonas, Moraxella, Acinetobacter, Shewanella and Flavobacterium. Members of the Vibrionaceae (Vibrio and Photobacterium) and the Aeromonadaceae (Aeromonas spp) are also common aquatic bacteria and typical of the fish flora (Table 5.4). Gram-positive organisms as Bacillus, Micrococcus, Clostridium, Lactobacillus and coryneforms can also be found in varying proportions, but in general, Gram-negative bacteria dominate the microflora. Shewan (1977) concluded that Gram-positive Bacillus and Micrococcus dominate on fish from tropical waters. However, this conclusion has later been challenged by several studies which have found that the microflora on tropical fish species is very similar to the flora on temperate species (A cuff et al., 1984; Gram et al., 1990; Lima dos Santos 1978; Surendran et al., 1989). A microflora consisting of Pseudomonas, Acinetobacter, Moraxella and Vibrio has been found on newly-caught fish in several Indian studies (Surendran et al., 1989). Several authors conclude, as Liston (1980), that the microflora on tropical fish often carry a slightly higher load of Gram-positives and enteric bacteria but otherwise is similar to the flora on temperate-water fish.



Aeromonas spp. Are typical of freshwater fish, whereas a number of bacteria require sodium for growth and are thus typical of marine waters. These include Vibrio, Photobacterium and Shewanella. However, although Shewanella putrefaciens is characterized as sodium- requiring, strains of S. putrefaciens can also be isolated from freshwater environments (DiChristina and DeLong, 1993; Gram et al., 1990; Spanggaard et al, 1993). Although S. putrefaciens has been isolated from tropical freshwaters, it is not important in the spoilage of freshwater fish (Lima dos Santos, 1978; Gram, 1990).

Changes in the microflora during storage and spoilage/Specific spoilage organisms:-

Bacteria on fish caught in temperate waters will-enter the exponential growth phase almost immediately after the fish have died. This is also true when the fish are iced, probably because the microflora is already adapted to the chill temperatures. During ice storage, the bacteria will grow with a doubling time of approximately 1 day and will, after 2-3 weeks, reach numbers of 108-109 cfu/g flesh or cm^2 skin. During ambient storage, a slightly lower level of 107-108 cfu/g is reached in 24 hours. The bacteria on fish caught in tropical waters will often pass through a lag-phase of 1-2 weeks if the fish are stored in ice, where after exponential growth begins. At spoilage, the bacterial level on tropical fish is similar to the levels found on temperate fish species (Gram, 1990; Gram et al., 1990). If iced fish are stored under anaerobic conditions or if stored in CO2 containing atmosphere, the number of the normal psychrotrophic bacteria such as S. putrefaciens and Pseudomonas is often much lower, i.e., 106-107 cfu/g than on the aerobically stored fish. However, the level of bacteria of psychrophilic character such as P. phosphoreum reaches a level of 107-108 cfu/g when the fish spoil (Dalgaard et al., 1993).

The composition of the microflora also changes quite dramatically during storage. Thus, under aerobic iced storage, the flora is composed almost exclusively of Pseudomonas spp. And S. putrefaciens after 1-2 weeks. This is believed to be due to their relatively short generation time at chill temperatures (Morita, 1975; Devaraju and Setty, 1985) and is true for all studies carried out whether on tropical or temperate-water fish. At ambient temperature (25°C), the microflora at the point of spoilage is dominated by mesophilic Vibrionaceae and, particularly if the fish are caught in polluted waters, Enterobacteriaceae.

Definition of Fish Spoilage:-

It refers to the contamination of fish, resulting in an undesirable change in the colour, texture, flavour, odour, appearance, etc. Spoilage of fish is also called “Putrefaction”. Fish spoilage can occur due to enzymatic degradation, bacterial degradation, chemical decomposition and mechanical damage. We can characterize the spoiled fish by observing the colour change, fishy smell, sliminess in the skin and scales, firmness of the flesh, discolouration of the backbone etc.

BACKGROUND

SPOILAGE:- Spoilage is the degradation of food such that the food becomes unfit for human consumption. Food can be spoiled by a number of means, including physical and chemical means. However, the most prevalent cause of food spoilage is microbial growth and residence in the food, which results in numerous undesirable metabolites being produced in the food that cause unwanted flavour and odor.

The main culprits are microbial organisms known as specific spoilage organisms (SSOs). The concept of SSOs arises from the fact that not all bacteria cause food spoilage; indeed, the degree of food spoilage is not proportional to the amount of microbes present on the food. SSOs are solely responsible for spoilage of the food and the typical characteristics associated with that spoilage. They are typically present in very low numbers and comprise a low percentage of the microflora present on the food.

Identification of SSOs is done by comparisons of the physical and chemical features of the collective spoiled products with the individual products left behind by each organism in the spoilage microflora. In particular, the qualitative ability of each organism to produce off-odors (spoilage potential) and the quantitative ability of each organism to produce spoilage metabolites (spoilage activity) are examined. This simple phenotypic identification scheme, along with a 16S rDNA gene sequencing to confirm results, allows scientists to discover which organism or organisms in the spoilage microflora are directly responsible for the spoilage.

Each unique environment has its own unique SSOs, because each different environment selects for particular organisms to thrive. The spoilage domain for an SSO is identified based upon the conditions (pH, temperature, water activity, and atmosphere) under which that SSO can grow and produce the metabolites that cause spoilage.

SPOILAGE IN FISH:-

Fish spoilage manifests itself physically in numerous ways. In terms of smell, spoiled fish will generally have a fishy, sour, or ammonia-like stench. Appearance-wise, spoiled fish may appear to be dry or mushy in certain areas, and the gills may have slime. Spoiled fish will also have flesh that is soft, or does not spring back when pressed upon. Typically, spoiled fish will also have a green or yellowish

discoloration; however, this arises not from spoilage metabolites, but rather oxidation of the oxygen transporters in fish blood (myoglobin to metamyoglobin) during frozen storage or from prolonged or unnecessary exposure of the fish to air.

Compared to other foods, fish is unique as a substrate for microbial growth. This uniqueness stems from several important factors: the poikilotherm nature of fish, a high post mortem pH in the flesh (typically greater than 6.0), the presence of non-protein-nitrogen (NPN) in large quantities, and the presence of trimethylamine oxide (TMAO).

The poikilotherm nature of fish selects for bacteria that can thrive in a wide range of temperatures. For example, the microflora of temperate water fish is dominated by psychrotrophic Gram-negative, rod-shaped bacteria such as those found in the genera Pseudomonas and Moraxella, with only varying proportions of Gram-positive organisms such as Bacillus.

The high post mortem pH of fish flesh is caused by the fact that fish flesh is low in carbohydrates (less than 0.5%) in the muscle tissue and that only small amounts of lactic acid are produced after death. This allows pH sensitive organisms such as Shewanella putrefaciens to grow in seafood but not in other meats. (5)

The NPN fraction of the fish flesh consists of low-molecular-weight water-soluble nitrogen contains compounds, particularly free amino acids and nucleotides, that allow it to serve as a readily available bacterial growth substrate. Decomposition of these compounds is responsible for many of the off-odors and off-flavours typically found in spoilage. For example, the breakdown of cysteine and methionine by certain microbes, both sulfur-containing amino acids, forms hydrogen sulfides and methylmercaptane respectively which causes undesirable odors to emanate from spoiled fish.

The presence of TMAO in fish is well-established, and it is known to cause a high redox potential in the fish flesh, although the significance of this is not clear. The spoilage of fish is influenced most by the presence of TMAO in conditions where oxygen is not present. Some anaerobic bacteria are able to utilize TMAO as the terminal electron acceptor in an anaerobic respiration process with trimethylamine (TMA) as the primary product; TMA contributes to the characteristic ammonia-like and fishy off-flavours in spoiled fish.

There are three modes of fish spoilage: Oxidation, Enzymatic and Microbial spoilage.

1.Oxidative spoilage: Lipid oxidation is a major cause of deterioration and spoilage of fish that contain high oil/fat content stored fat in their flesh. Oxidation typically involves the reaction of oxygen with the double bonds of fatty acids. In fish, lipid oxidation can occur enzymatically or non-enzymatically. Lipid oxidation promotes protein denaturation, modification of the protein, electrophoretic profiles, nutritional losses, and endogenous antioxidant systems losses. Lipid hydrolysis and oxidation cause “belly burst” in fish in which the enzymes and microorganisms of the digestive tract cause massive gas development.

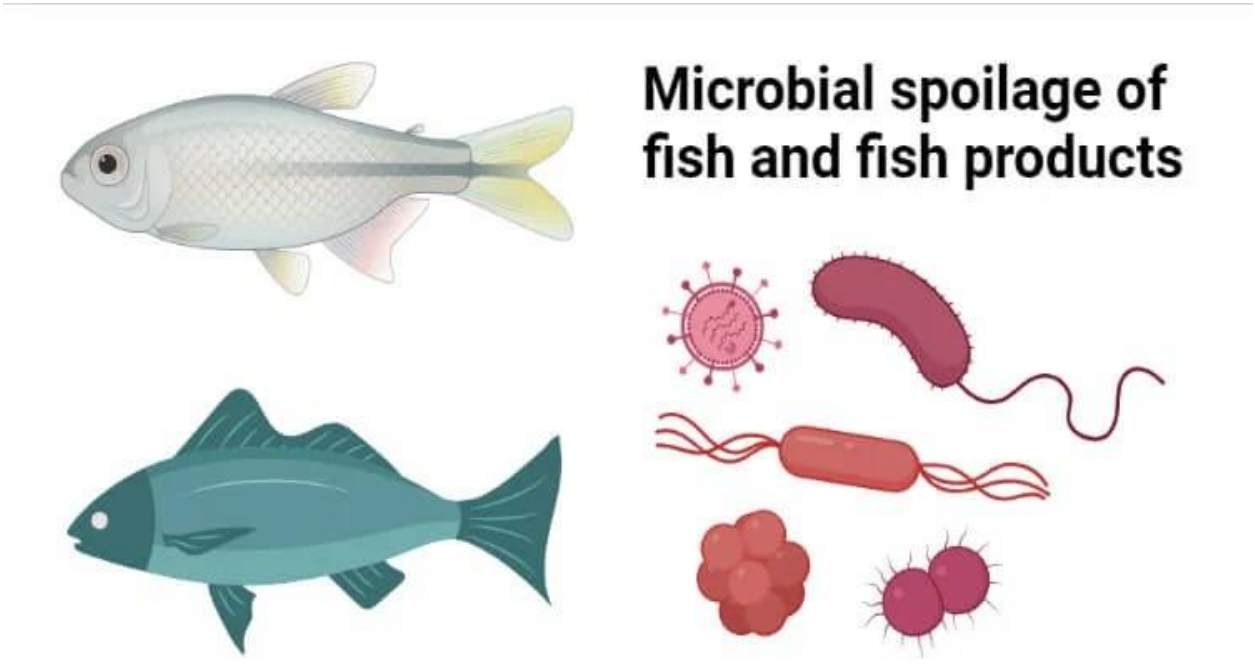
2.Enzymatic spoilage: After capture, biological and chemical changes take place in dead fish due to the action of various enzymes found in fish. The digestive enzymes cause extensive autolysis which results in fish muscle softening, rupture of the belly wall, and drain out of the blood. Several proteolytic enzymes are found in fish that contribute to degradation in fish muscle and fish products during storage and processing. Proteolysis is responsible for the degradation of proteins which leads to fish spoilage by microbial growth.

3.Microbial Spoilage: Fish flesh is composed of protein, fats, carbohydrates, water, and amino acid compounds such as trimethylamine oxide (TMAO), urea, taurine, creatine, free amino acids, and trace glucose, etc. The Internal tissue of fish is generally considered sterile. Bacteria are present on the slime layer of the skin, gill surfaces, and the intestine. The microbial growth In fish is the main cause of fish spoilage and produces amines, biogenic amines, organic acids, alcohols, aldehydes, and ketones with unpleasant and off-flavors. The high water activity, low acidity (pH > 6) of fish result in the fast growth of microorganisms that leads to undesirable changes in appearance, texture, flavor, and odor, reducing its quality. At room temperature, Bacillus, Clostridium, Escherichia, Micrococcus, Proteus, Sarcina, and Serratia may predominate. For unpreserved fish, spoilage is caused by Gram-negative, fermentative bacteria (such as Vibrionaceae), whereas psychrotolerant Gram-negative bacteria (such as Pseudomonas spp. And Shewanella spp.) tend to spoil chilled fish.

The fish spoilage Is also caused by psychrotrophic, aerobic, or facultative anaerobic Gram-negative bacteria such as Pseudomonas, Moraxella, Acinetobacter, Shewanella putrifaciens, Vibrio, Flavobacterium, Photobacterium, and Aeromonas.

Gram-positive bacteria such as Staphylococcus spp., Micrococcus, Bacillus, Clostridium, Corynebacterium, Brochothric thermosphacta, and Streptococcus are found in fish.

Lactic acid bacteria (LAB) can predominate in fish storage under vacuum or CO2 storage.



Defects observed on fish by microbial spoilage:-

Bacterial growth causes slime layer, discoloration of gills and eyes (in whole fish), and loss of muscle texture (softened due to proteolysis). The volatile compounds from the putrefaction of proteins will result in the formation of different types of off-odor such as fishy (due to trimethylamine) and putrid

odor. Bacteria such as Shewanella putrifaciens, Aeromonas spp., psychrotolerant Enterobacteriaceae, P.phosphoreum, and Vibrio spp. Creates ammonia-like off-flavors and fishy off-flavor. Pseudomonas putrifaciens, fluorescent pseudomonads, and other spoilage bacteria increase rapidly and produce many proteolytic and hydrolytic enzymes. The greenish-yellow color on fish is caused by Pseudomonas fluorescens; yellow color by Micrococcus; red color by Bacillus, and Sarcina. The chocolate-brown color is caused by yeasts and molds; and a musty odor by Streptomyces.

Evidence of fish spoilage: 1. Colour of fish become fade ,dirty and yellow or brown. 2. The slime of the skin increases , especially flaps and gills.3. Eyes sink and shrink.4.Pupil became cloudy.5.Cornea became opaque.6.The softening of flesh.7.Anus wet, swollen and red.8.Mean while sequence of odours is evolved.

Contamination source of fish: 1. Environmental factors. 2.Equipment used such as catch boxes, bins, holds, dressing surfaces, decks, and cutlery handles.3. Water used for washing fish and cleaning the equipment.4.Method of harvesting, season handling, and processing.

CAUSATIVE FACTORS OF SPOILAGE: Spoilage is caused by the action of enzymes, bacteria and chemicals present in the fish. In Addition, the following factors contribute to spoilage of fish. High moisture content; High fat content; High protein content; Weak muscle tissue; Ambient temperature & Unhygienic handling Action of bacteria. Generally, the freshly caught fish will be free from microbes except the surface areas. Once The fish is dead the bacteria start attacking the flesh of the fish which leads to spoilage. The action of microbes, enzymatic activity and oxidation of nutritive elements present in the fish are the common causes of fish spoilage. In addition to these, some other factors are also responsible like: 1. High moisture content. 2. High fat content.3.High protein content. 4. Weak muscle tissue. 5. Ambient temperature. 6. Unhygienic handling.

Spoilage of fish products:

Moulds and fungus: Fungus usually grows well on unsalted and salted dried fish, which has high moisture, content. Moulds usually grow at relative humidity above 75 %. The optimum temperature for growth is 30 -35 o C. In salted fish, brownish black or yellow brown spots are seen on the fleshy parts. This is mainly caused by growth of halophilic mould called Sporendonema epizoum. This gives the fish a very bad appearance. During the initial stages of appearance of moulds on the fish, it is possible to remove them manually. In advanced stages when it has penetrated the flesh nothing can be done. To avoid the mould growth it is necessary that the fish be dried properly to pack the fish in required type of packaging material and keep it in a cool and dry place from moisture. Chemical method of prevention includes dipping the fish in a 5% solution of Calcium propionate in saturated brine for 3-5 minutes depending upon the size of the fish.

Rancidity: This is caused by the oxidation of fat, which is present in the fishes. Rancidity is more pronounced in oil rich fishes like mackerel, sardine etc. The unsaturated fat in the fish reacts with the oxygen in the atmosphere forming peroxides, which are further broken down into simple and odoriferous compounds like aldehydes, ketones and hydroxy acids, which impart the characteristic

odors. At this stage the colour of the fish changes from yellowish to brown this is known as rust. This change results in an unpleasant flavour and odour to the product, thus leading to consumer rejection.

Pink /Red Halophiles: This type of spoilage is mainly due to the presence of halophilic bacteria. The source of such bacteria the salt. It is commonly found in tropical countries like India. Spoilage appears on the surface as slimy pink patches. These bacteria are not harmful by nature. They are aerobic and proteolytic in nature, grows best at 36ºC by decomposing protein and giving out an ammoniacal odour. Usage of good quality salt will avoid this. This spoilage is mostly found in heavily salted fish and absent in unsalted fish

Insect Infestation: 1. During initial drying stages 2. During storage of the dried samples.

The flies, which attack the fish during the initial drying stage are mainly blowflies belonging to the family Calliphoridae and Sarcophagidae. These flies are attracted by the smell of decaying matter and odours emitted from the deteriorating fishes. During the glut season when the fish is in plenty and some are left to rot, these flies come and lay their eggs. These eggs develop into maggots, which bury within the gill region and sand for protection from extreme heat. They develop mainly when conditions are favourable with adequate moisture and intermittent rain. This results in both economic and nutritive loss to the fish processor.

Infestation can be reduced by:

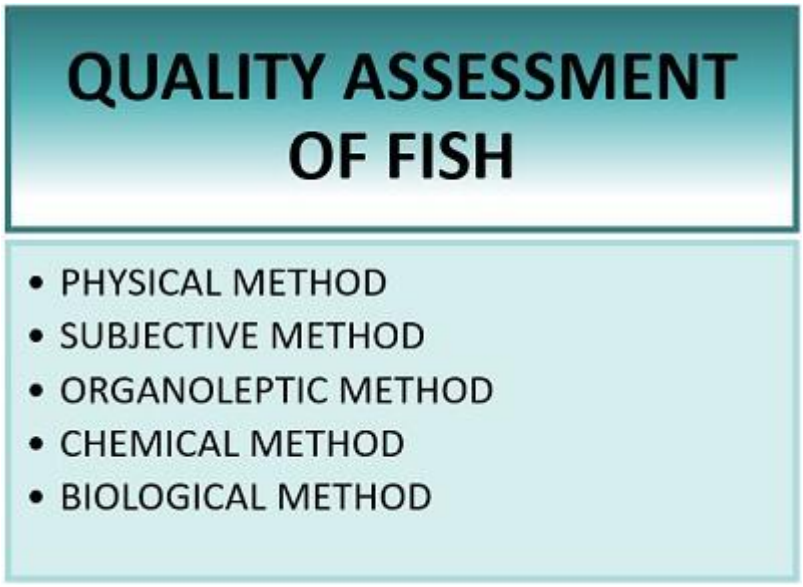
1. Proper hygiene and sanitation
2. Disposal of wastes and decaying matter
3. Use of physical barriers like screens, covers for curing tanks etc.
4. Use of heat to physically drive away the insects and kill them at 45ºC

The most commonly found pests during storage are beetles belonging to the family Dermestidae. Beetles attack when the moisture content is low and especially when the storage is for a long time. The commonly found beetles are Dermestes after, D frischii, D maculates, D carnivorous and Necrobia rufipes. The larva does most of the damage by consuming dried flesh until the bones only remain. Mites are also an important pest, which are found infesting dried and smoked products. They are very minute and bring about powdering of the product thereby giving it a white appearance. Lardoglyphus konoi is the commonly found mite in fish products.

Fragmentation: Denaturation and excess drying of fish results in breaking down of the fish during handling. Fish can become brittle and liable to physical damage when handled roughly. It is necessary that fresh fish be used as raw material to ensure a good finished product.

Assessment of fish spoilage: Quality assessment is a part of quality assurance that focuses on assessment of fulfilling Quality requirements. The quality of fish and fishery products has become a major concern in Fish industry all over the world.

We can access the quality of fish by the three consecutive methods:



Physical method: Torrymeter is a device placed vertically, and it provides a digital reading of the fish quality, whether it is aged or fresh. From the digital readings or values of the torrymeter, we can estimate the fish freshness. A low value Indicates the presence of more bacterial mass. 10 is the highest value for the freshly caught fish, and below 3 is the value of spoiled fish. The value of 6 on the torrymeter is acceptable by the consumer.

Subjective Method: It is a sensory method assessed by the sensory organs, which represent the customer view.

Organoleptic Test: It includes the quality assessment of fish by the sense of sight, smell, touch etc. We can check the quality of fish by using our sense of sight to examine the fish's eyes, gills, and skin surface. The eyes should be clear and vibrant. Any discolouration around the eyes and cloudiness in the eyes indicates that the fish is not fresh. The gills should be red-pink in colour. The skin should be shiny, not slimy.The skin or the surface of fish should be clear, and there must not be any discolouration.We can also check the quality of the fish by using our sense of touch to examine the flesh and scales of the fish:The flesh should be tight, elastic, but not slimy. The scales should be intact with the skin. We can also check the quality of the fish by using our sense of smell:

Biochemical Method: It includes the following methods:

1. Proximate testing: It is a prevalent method in which the fish components like moisture, protein, lipid etc., are regularly checked from the time of fish harvesting. This method does not give a satisfactory assessment and thereby not accepted widely.

2. Hypoxanthine value: After the death of fish, ATP (Adenosine triphosphate) splits into ADP, AMP, IMP and finally into hypoxanthine. The value of hypoxanthine increases during the storage of fish. Hypoxanthine value gives an estimate for the freshness of fish. A fish is considered to be spoiled if a hypoxanthine value reaches 7-8 micromoles/g.

3. Trimethylamine (TMA) value: Fish contains a considerable amount of trimethylamine oxide (TMAO), but on fish spoilage, TMAO reduces into TMA. The value of TMA with a level of 1.5 mg / 100 g indicates that the fish is moderately spoilt.
4. Ammonia production: The production of ammonia indicates the extent of spoilage.
5. Peroxidase value: It helps in the measurement of oxygen rancidity. Peroxidase value less than 10 (indicates the good quality of fish) and a value more than 20 (indicates rancidity).
6. Thiobarbituric acid value (TBA): It also helps us to determine the oxygen rancidity. TBA value less than 2, is accepted by the consumer.

Biological Method: It includes the total plate count method (TPC). The biological method involves a quality assessment of fish by the bacterial cell count. First, you need to grind the fish and then dilute the sample by following serial dilution. Prepare media for the growth of microorganisms present in the fish, where we can use both ordinary and selective media.
We can use agar media to enumerate the microbial mass in marine fish and tryptone glucose beef extract agar media to calculate the cell count in processed fish. Other than this, selective media like SS-agar can be used for the detection of coliform bacteria (E.coli, Shigella sp, etc.) in the fish. After media preparation, perform the pouring method and incubate the plates for 24 hours at 35-37 degrees Celsius.
Count the number of bacteria per plate by multiplying with the dilution factor. Thus, the total plate count method gives a count for the bacterial (pathogenic and non-pathogenic) population present in the fish. Hence, the total plate count method does not determine the edibility of the fish.

MICROORGANISMS RESPONSIBLE FOR FISH SPOILAGE:

Staphylococcus aureus: Staph. Aureus is a Gram-positive coccus about 1 m in the diameter. The cocci are usually Arranged in grape-like clusters. The organisms are non-sporing, non-motile, and usually Non- capsulate. When grown on many types of agar for 24 hr at 37 C, individual colonies Are circular,2-3 mm in diameter, with a smooth, shiny surface; colonies appear opaque and are often pigmented(golden- yellow).

Klebsiella: Klebsiella contains a group of three species of bacteria, including K **Pneumoniae**. They are a relatively common isolate in gram-negative endophthalmitis26 and are characteristically Resistant to multiple antibiotics. Enterobacter organisms are opportunistic pathogens that Rarely produce human disease. When they function as opportunistic pathogens, however, They may be resistant to first-generation cephalosporins.
Serratia spp. Are opportunistic Pathogens that have only been recognized as capable of producing human disease since the 1960s. They are more likely to colonize the respiratory and urinary tracts of hospitalized Patients than other Enterobacteriaceae. Most hospital infections are caused by Catheterization and instrumentation of the urinary and respiratory. Klebsiella is a non-spore-Forming, non-motile, facultative anaerobic Gram-negative straight rod, 0.3–1.0 μm in Diameter and 0.6–6.0 μm in length.
The rods are arranged singly, in pairs or in short chains. The cells are capsulated.
The optimal temperature for growth is 37°C.Klebsiella sp. Are Chemoorganotrophic, having both a respiratory and a fermentative type of metabolism. Klebsiella species can contaminate various foods and contribute to disease and spoilage. The origin of the contamination is not always clear, since Klebsiella

species are widely Distributed in nature and in the gastrointestinal tracts of a wide range of animals.Klebsiella Pneumoniae, K. oxytoca, K. variicola, K. terrigena, and K. planticola are commonly found in carbohydrate-rich wastewater, surface water, cooling water,soil, plant products, fresh vegetables, sugar cane, frozen orange juice concentrate, and grains. Wood pulps, sawdustand waters receiving industrial effluents from pulp, papermills,and textile Finland textile finishing plants may release 104–106 of Klebsiella per milliliter of effluents. Klebsiella species cause spoilage of animal-derived foods (meat, fish, milk) by secreting lipases and proteases that cause the formation of sulfides and trimethylamine (off-odors) and by forming biofilm or slime on surfaces. Some strains are adapted for growth at cold temperatures and spoil these foods in the refrigerators. It has been demonstrated that an important source of Klebsiella strains causing infections may be the patient's own bowel. In addition to being a potential source of autoinfection, the acquisition of a strain in the bowel during hospitalization provides a possible source for transmission of the organism.Person-to-person spread is the most common mode of transmission of Klebsiella species in hospital infections, and hands are the main vehicles for transmission. Klebsiella species are isolated from the hospital kitchens that prepared ice creams, nasogastric feeds, cold meat, and salads.

Proteus: Proteus is a genus of Gram-negative bacteria. Proteus bacilli are widely distributed in nature As saprophytes, being found in decomposing animal matter,sewage, manure soil, the mammalian intestine, and human and animal feces. They are opportunistic pathogens, commonly responsible for urinary and septic infections, often nosocomial.

Scientific Classification

Domain: Bacteria

Phylum:Pseudomonadota

Class: Gammaproteobacteria

Order:Enterobacterales

Family: Enterobacteriaceae

Genus: Proteus

The different types of proteus species include

P. hauseri

P. mirabilis

P. myxofaciens

P. penneri

P. vulgaris

Proteus species do not usually ferment lactose, but have shown to be capable glucose fermenters depending on the species in a triple sugar iron (TSI) test.

Causative organisms for fish spoilage:

There are some important genera which infects the fish, this includes species of Pseudomonas, Photobacterium etc.

Pseudomonas.	-Frag
	-putida
	Fluoroscent
Photobacterium.	-phosphorium
	-Illipiscarium
	-kishitanis

Pseudomonas: Pseudomonas is a genus of Gram-negative, Gammaproteobacteria, belonging to the family Pseudomonadaceae and containing 191 described species. The members of the genus Demonstrate a great deal of consequently are able to colonize a wide Range of niches. in vitro and availability of an increasing number of Pseudomonas strain genome sequences has made the genus an excellent focus for scientific Research; the best studied species include P. aeruginosa in its role as an opportunistic human Pathogen, the plant pathogen P. syringae, the soil bacterium P. putida, and the plant growth-Promoting P. fluorescens, P. lini, P. migulae, and P. graminis.

Photobacterium: Photobacterium is a genus of gram-negative, oxidase positive and catalase positive bacteria In the family Vibrionaceae. Members of the genus are bioluminescent, that is they have the Ability to emit light.

Psychrobacter: Psychrobacter is a genus of Gram-negative, osmotolerant, oxidase-positive, psychrophilic Or psychrotolerant, aerobic bacteria which belong to the family Moraxellaceae and the class Gammaproteobacteria. The shape is typically cocci or coccobacilli. Some of those bacteria Were isolated from humans and can cause humans infections such as endocarditis and Peritonitis.
This genus of bacteria is able to grow at temperatures between −10 and 42°C.

Rudi Rossau found through DNA-rRNA hybridization analysis that Psychrobacter Belongs to the Moraxellaceae.

The first species (Psychrobacter immobilis) was described By Juni and Heym.

Psychrobacter occur in wide range of moist, cold saline habitats, but They also occur in warm and slightly saline habitats.

MATERIALS AND METHODS:

Culture Media:-

A special medium that is used in microbiological laboratories to identify and detect different types of microorganisms by culturing or growing. Usually, a culture medium is Composed of different nutrients

to enhance the microbial growth. Traditionally, cultural techniques have been the tests of choice for both ready-to-eat Foods and fresh produce. However, today immunoassay and PCR methods are more accepted than cultural methods, because recent developments of newer testing methods and validation Studies have demonstrated that cultural methods aren't suitable for all food groups.

Important factors: Different methods are involved in culturing techniques. For identification and detection of microorganisms in cultures, both liquid and solid Culture media are employed. Microscopes are usually used to detect microbes in cultures, and biochemical and Serological techniques are used to differentiate various organisms. Both qualitative and quantitative results of microorganisms can be obtained using Cultural methods. This means a culture media technique not only detects the presence Or absence of an organism but also provides information about the number of Organisms present in the medium. However, quantitative analysis is only possible using Solid culture media, because the individually developing colonies of organisms can be Counted only on the surface. Time to attain results can range from twelve hours to more than a week.

Sample collection:-

Samples collected from fishing harbour Visakhapatnam, from fish vendors.

In a fish harbour collect 9 different types of fish species, were collected as F1,F2,F3,F4,F5,F6,F7,F8,F9.

Then collect swab sample from the fish body surface, with the help of specimen collection tube and labell them. And store the samples with 2-3ml of peptone water,then kept for incubation for about 24hrs. After 24 hrs of incubation changes can occurs in peptone water i.e., sample become turbid due to microbial activity.

Common name	Scientific name
F1. Vanjaram (seerfish)	Scomberomorus guttatus
F2 .Kanaganta.	Rastrelliger kanagurta
F3.Saimaaringedseal	Pusa hispida saimensis
F4.Bonthu fish.	Mullet
F5.Chanduva.	Pampusargenteus
F6.Tuna fish	Thunnus
F7.Milkshark	Rhizoprionodonacutus
F8.Ribbon fish	Trichiuruslepturus
F9.Jella	Arius jella

Media preparation: Media used for further studies is solid media they are Blood agar, MacConkey's a Chocolate agar, Solid media are used to grow microorganisms in their full physical form, pare bacterial pure cultures, or isolate bacteria to study colony characteristics. The Bacterial growth on solid media varies in appearance as mucoid, round, smooth, rough, Filamentous, irregular, and punctiform.

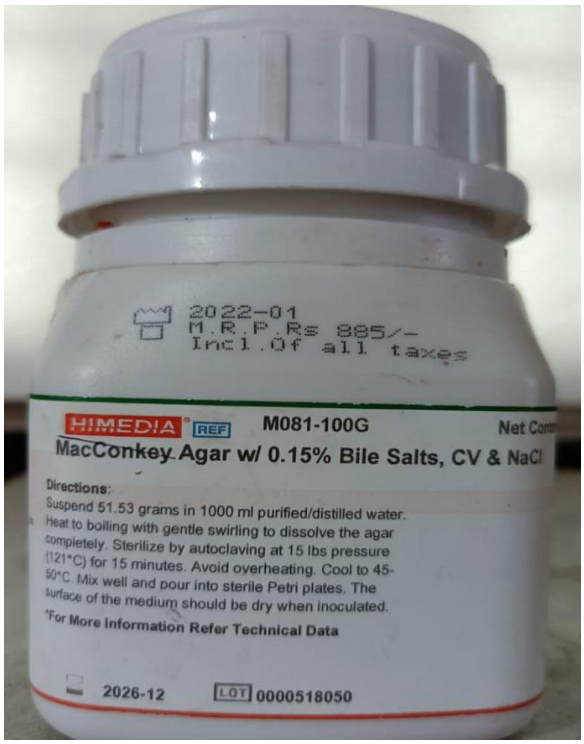
Types of media used:-

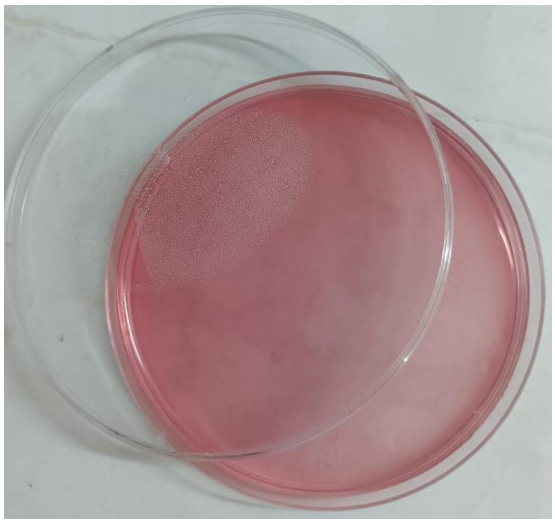
- Mecconkyagar
- Blood agar

Mecconkyagar: MacConkey agar (MAC) was the first solid differential media to be formulated which Was developed at 20thCentury by Alfred Theodore MacConkey. MacConkey agar is a Selective and differential media used for the isolation and differentiation of non-fastidious Gram negative rods, particularly members of the family Enterobacteriaceae and the genus Pseudomonas. MacConkey agar is used for the isolation of gram-negative enteric bacteria And the differentiation of lactose fermenting from lactose non-fermenting gram-negative Bacteria. Pancreatic digest of gelatin and peptones (meat and casein) provide the essential Nutrients, vitamins and nitrogenous factors required for growth of microorganisms. Lactose Monohydrate is the fermentable source of carbohydrate. The selective action of this Medium is attributed to crystal violet and bile salts, which are inhibitory to most species Of gram positive bacteria. Sodium chloride maintains the osmotic balance in the medium. Neutral red is a pH

indicator that turns red at a pH below 6.8 and is colourless at anypH greater than 6.8. Agar is the solidifying agent.

MacConkey agar (MAC) is a bacterial culture medium named after bacteriologist Alfred T. MacConkey (1861-1931). MacConkey agar is a selective and differentiating agar that only grows gram-negative bacterial species; it can further differentiate the gram-negative organisms based on their lactose metabolism.





Contents:- It contains bile salts (to inhibit most Gram-positive bacteria), crystal violet dye (which also inhibits certain Gram-positive bacteria), and neutral red dye (which turns pink if the microbes are fermenting lactose).

Composition of MacConkey Agar

Ingredients	Amount
Peptone (pancreatic digest of gelatin)	17gm
Proteosepeptone **(**meatandcasein**)**	3 gm
Lactose monohydrate	10gm
Bile salts	1.5 gms

Sodium chloride	5gm
Neutral red	0.03gm
Crystal Violet	0.01gm
Agar	13.5gm

Distilled Water Add to make 1 Liter.

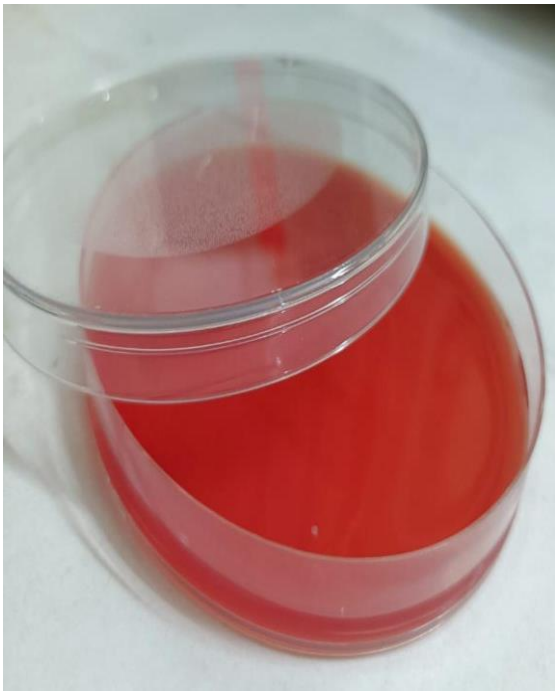
PH -7.1

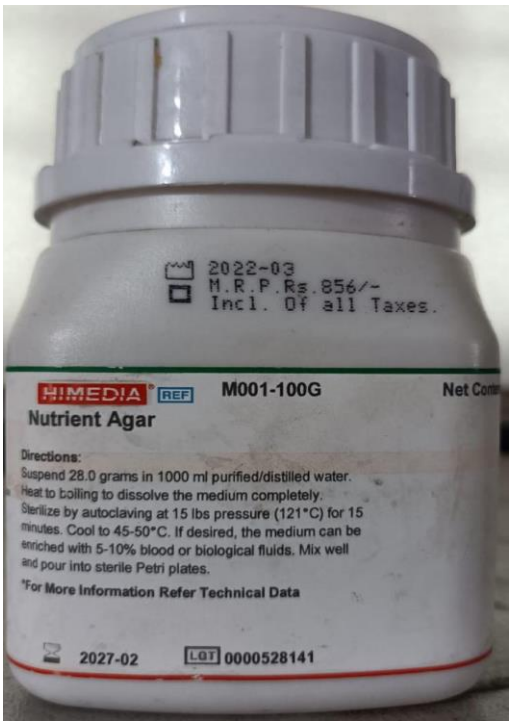
Mecconkyagar media preparation:- Suspend 49.53 grams of dehydrated medium in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well before pouring into sterile Petri plates. And then store at cool temprature

Uses:- MacConkey agar is used for the isolation of gram-negative enteric bacteria. It is used in the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria. It is used for the isolation of coliforms and intestinal pathogens in water, dairy products and biological specimens.

Blood agar: composition, preparation and uses: In general purpose, enriched and differential soild medium, which supports the growth of Most ordinary bacteria .It is also useful to detect and differentiate haemolytic bacteria, Especially streptococcus species. Blood supplies a number of required number of Substances for growth of fastidious organisms.

Blood agar is used to grow a wide range of pathogenic organisms particularly those which do not grow in ordinary culture media. Microorganisms such as Haemophilus influenza, Streptococcus pneumonia and Neissaria species require Blood agar to grow. It is also used to detect haemolytic bacteria especially Streptococcus species.





Composition of blood agar:

Nutrient agar ……. 500 ml

Sterile defibrinated blood ……. 25 ml

Choice of blood: For most pathogens, haemolysis free defibrinated horse, sheep, goat or rabbit blood can be used. Sheep blood cannot be used for Haemophilus influenzae because it contains inhibitors for H. influenza. Oxalated horse blood is used in blood agar for testing Satellitium. Human blood (expired citrated blood from blood bank) should not be used because it contains inhibitors for pathogenic organisms. Also human blood inhibits Streptococcus and interfere with haemolysis pattern.

Composition

Composition per litre :

0.5% peptone

0.3% beef extract / yeast extract

1.5% agar

0.5% NaCl

Distilled water

(since blood agar is made from Nutrient agar, above is the composition of nutrient agar)

5% sheep blood

pH should be from 7.2 to 7.6 .

Preparation of blood agar: Suspend 28g of nutrient agar powder in 1litre of distilled water. Heat this mixture while stirring to fully dissolve all components. Autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes. Once the nutrient agar has been autoclaved, allow it to cool but not solidify. When the agar has cooled to 45-50 degrees C, add 5% (vol/vol) sterile defibrinated Blood that been warmed to room temperature and mix gently but well. Avoid air bubbles. Dispense ascetically in 15 ml amount in sterile plates while liquid. Allow to solidified at room temperature. Label the petri dishes with date and batch numbers. Store the petri dishes at 4 °C in sealed plastic bag to prevent loss of moisture. It can be used up to 4 weeks.

Uses: Used to culture Streptococcus pyogens and Streptococcus pneumonia. Used for Satellitium test of Hemophiuls infuenzae. Inoculate the Blood agar with infulenzae and then streak Staphylococcus aureus on the plate. Incubate the plate in CO2 incubator at 35-37 C for 18-24 hours Observe characteristic satellitium of influenza. Used to culture Neisseria species

Blood agar can be made selective medium for certain pathogens by adding antibiotics, dyes, chemicals or by heating Crystal violet blood agar is selective for Streptococcus pyogens Kanamycin or neomycin blood agar is selectives for anaerobes such as Neisseria species Chocolate agar (heated blood agar) is selective for Haemophilus influenzae.

Peptone water: Peptone Water is formulated as per Shread, Donovan, and Lee. It is a broth medium used for the growth of the organism and a base for determining carbohydrate fermentation patterns of non-fastidious organisms. In addition, it is also used for the detection of indole production by the organism.

Composition of Peptone Water

Ingredients	Gms/L
Peptone	10.0
Sodium chloride	5.0
PH	7.2

Preparation: Suspend 2grams in 1000 ml distilled water. Mix thoroughly and distribute into the final containers. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. And then store at cool temprature for further use.

Result on Peptone Water

A positive result (inoculated medium): Growth, turbidity seen

A negative result (uninoculated medium): No growth

Inoculation:-

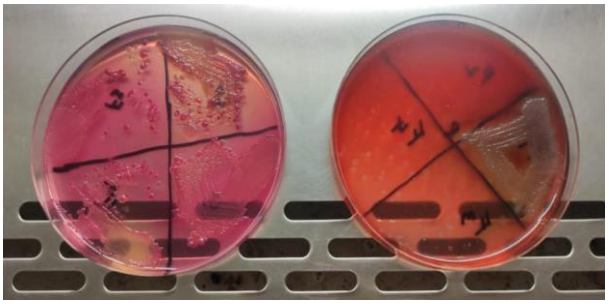
Streak plate method: Streaking is a technique used in microbiology for the isolation of single colonies of microorganisms, either from a mixed species or from the same species. This technique is mostly applicable to bacteria but is also used for some yeasts. It is an old technique that has been in use since the time of Robert Koch. It was first demonstrated by Loeffler and Gaffky in Koch's laboratory.

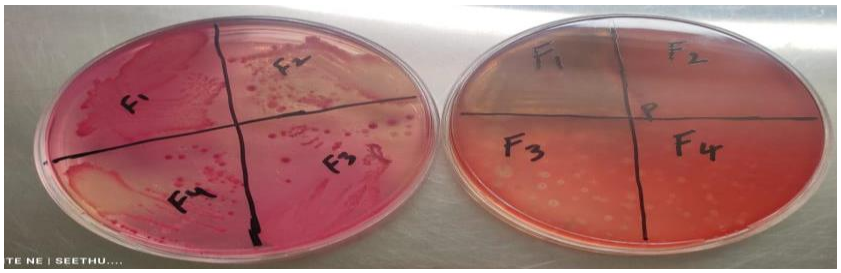
Streak plate method principle: The streak plate method is based on the principle of dilution. It can be described as a rapid qualitative isolation technique. The main criterion of isolation is to obtain a reduced number of colonies. In this technique, a loopful of culture is spread on an agar plate to get individual cells far apart enough from each other. The streaking method gradually dilutes the inoculum such that the bacterial cells can be counted as colony forming units (CFUs).

Process of streaking:-

Step by step procedure of streak plate method:

1. Take prepared 2ml peptone water into 4 tubes.
2. Take the samples of four different species of fish.
3. Collect the small amount of mucous sample from 4 different species of fish with the Sterile swab sticks.
4. Mix the small amount of mucous sample into 2ml of peptone water.
5. Mix the tubes continuously until the sample is completely dissolved in the peptone Water and incubate for 24hr.
6. Take MacConkey agar, blood agar, sterile plates.
7. Mark the plates as sample F1, F2, F3, F4, F5, F6, F7, F8, F9.
8. Red heat the nichrome loops and after cooling it, transfers aseptically a loopfull of Culture on the MacConkey, blood agar.
9. Sterilize the loop again on the gas flame to cool it , test it by touching the agar.
10. Now pick the inoculums from the peptone water and subculture on macconkey,Chocolate agar plates by steak it perpendicular to turning the plate half way around the Streak.
11. Make a single streak cross the Centre. Then, spread the inoculums evenly distributed In a cross-zigzag arrangement to the primary streak.
12. Sterilize the loop in between the streaks holding the inoculating media up so the edge of the loop touches the agar.
13. Repeat the streaking in MacConkey, blood agar plates.
14. Place the plate at 37 c in an incubator observe the results after 24hrs.
15. Take prepared 2ml peptone water into tube.





Liquid broth culture:- Broth cultures are liquid cultures used to grow bacteria in laboratories. To create a broth Culture, a sterile liquid growth medium is required. The medium is inoculated with bacteria And placed in an incubator at the appropriate temperature.

Preparation of liquid broth culture : Add 13g of nutrient broth powder (CM0001B) in 1L of distilled water. Mix and dissolve them completely. Pour them into the final containers. Sterilizelaving at 121℃ for 15 minutes.

Identification of microbes: After incubation period, remove the cultured petri plates from incubator and observe the Plates.Numerous numbers of colonies are formed on media plates. Each colony Represents a population of cells that are genetically identical. From the collected nine Samples, microbial growth is observed on all the nine samples on both the MacConkey Agar and blood agar.

Sample	MacConkey Agar	Blood Agar
(F1)Scomberomorus guttatus	Non lactose fermenting	White pin point colonies
(F2)Rastrelliger kanagurta	Non lactose fermenting	White pin point colonies
(F3)Pusa hispida saimensis	Non lactose fermenting and Lactose fermenting	Hemolytic
(F4)Mullet	Non lactose fermenting and lactose fermenting	Hemolytic
(F5)Pampus argenteus	Non lactose fermenting and lactose fermenting	Hemolytic
(F6)Thunnus	Non lactose fermenting and lactose fermenting	Hemolytic
(F7)Rhizoprionodon acutus	Non lactose fermenting and lactose fermenting	Hemolytic
(F8)Ribbon fish	Non lactose fermenting and	Hemolytic

	lactose fermenting	
(F9)Arius jella	Non lactose fermenting and lactose fermenting	Hemolytic and white pinpointed colonies

Microscopic examination of samples:-This staining technique helps to determine

1) Gross morphology of the bacteria and
2) Differentiation of bacteria into groups determining the subsequent biochemical tests.

A) Gram positive and
B) Gram negative

This differentiation is helpful in determining the subsequent biochemical tests and media for their culture the laboratory.

Procedure: Take a clean, and grease free side. Prepare the smear of suspension on the clean slide with a loopful of sample. Air dry and heat fix. Crystal violet was poured and kept for about 30 seconds to 1 minute and rinse With water. Flood the grams iodine for 1 minute and wash with water. Then, wash with 95% alcohol or acetone for about 10 – 20 seconds and rinse with Water. Add safranin for about 1 minute and wash with water. Air dry, blot dry and observe under microscope.at both 400x and 1,000x oil immersion.
The staining results of Gram stain are as follows

Gram positive: Blue purple colour

Gram negative: Pink colour
Occasionally Gram-positive organisms lose their ability to retain crystal violet and stain Gram negatively. The reasons may be as follows.

1) Over decolonization of the Smear
2) Use of iodine solution which is too old (yellow instead of brown)
3) Preparation of smear from old culture.
4) Cell wall damage due to excessive heat fixation of the smear or due to antibiotic therapy.

Gram Positive Bacteria: Actinomyces, Bacillus, Clostridium, Corynebacterium, Enterococcus, Gardnerella, Lactobacillus, Listeria, Mycoplasma, Nocardia, Staphylococcus, Streptococcus, Streptomyces.
Gram Negative Bacteria: Escherichia coli (E. coli), Salmonella, Shigella, and other Enterobacteriaceae, Pseudomonas, Moratic acid bacteria, Legionella etc.

Results :-

Biochemical tests: Biochemical tests are the tests that are performed on different bacteria for their identification on the basis of their biochemical activities towards different biochemical compounds.

Biochemical tests are one of the traditional methods for the identification of microorganisms, usually performed with phenotypic identification.For many years these methods were employed extensively, and they continue to be used nowadays, especially in some laboratory routines where a particular type of microorganism has to be identified rapidly. One of the traditional methods commonly used is a simple visual detection of the growth of the organism in the presence of essential nutrients by increased turbidity in the liquid medium. Microorganisms can be classified into different groups on the basis of their reaction to such tests. Some tests even allow the distinction of microorganisms to the species level. Microorganisms undergo the metabolism process to maintain nutritional and functional cellular activities either catabolically or anabolically. Both processes occur simultaneously with the biochemical reaction which is mediated by various types of enzymes. The enzymes produced by an organism helps in the identification of bacteria by observing their enzymatic activities using a specific media with the inoculation of a pure culture of the organism where under suitable incubation period releases their respective enzymes. The enzyme produced reacts with the biochemical compounds present in the media and exhibits specific color change which is the major key for the identification of bacterial species.

*After 24 hours of incubation for methyl red and Voges Proskauer we have to add Indole into methyl red and Voges Proskauer reagent into Voges Proskauer media.

*For oxidase test by using oxidase discs spread the inoculums on oxidase disc observe Results after 5-10 hours.

If it is blue colour, it is positive. If there is no blue purple Color, it is negative.

Basic biochemical tests with principle, procedure, and examples that can be analyzed:-

OXIDASE TEST: This test is used to help in the identification of the organisms which produce the Enzyme oxidase . Examples: Pseudomonas, Neisseria, Vibrio and Pasteurella species.

Procedure: Place a piece of filter paper in a clean petri dish. Add 2 to 3 drops of freshly prepared oxidase reagent.Smear a colony of the organisms on the filter paper by using a glass rod. Observe the reaction.

Observation: 1).Blue purple colour positive test. 2).No blue purple colour negative test.

UREASE TEST: Proteus strains are strong urease producers. Salmonellae and Shigellae do not produce Urease.This test helps in differentiating enterobacteria.

Procedure: Inoculate MIU medium with a colony of the test organism. Incubate at 35°C overnight. Examine the medium by looking for a red-pink color.

Observations: 1). Red-pink medium : Positive test. 2).No-red -pink color : Negative test

BIOCHEMICAL REACTIONS ON TRIPLE SUGAR IRON AGAR (TSI) SLANTS:

TSI slants are useful in the identification of Entero-bacteria by their specific reactions on the slants.

Procedure: Streak the TSI slant with a loop and stab with a straight needle. Incubate at 37°C for 18-24 hours. The various reactions observed on the slants are as follows

CITRATE TEST: This test is performed in the identification of enterobacteria

Procedure: Inoculate 3-4ml of sterile Simmon’s citrate medium with a broth culture of test Organisms. Incubate at 35°C up to 4 days.

Results: 1). Turbidity and blue color : Positive test.

2). No growth (non-turbidity and persistence of original color) : Negative test.

COAGULASE TEST : This test is used to differentiate Staphylococcus aureus from S. epidermidis and S, and S. Saprophyticus.

Procedure: Place a drop of physiological saline on each end of a slide. Make thick suspensions of the organism in each drop. Add a drop of plasma to one of the suspensions. Mix gently and look for Clumping of the organisms within 10 seconds.

Results:

1) Clumping within 10 seconds: Staphylococcus aureus.
2) No clumping within 10 seconds : No production of coagulase.

INDOLE TEST: This test is important in the identification of enterobacteria such as E. coli, P. vulgaris,P. rettgeri, etc.

Procedure: Inoculate MIU medium with test organism colonies. Incubate at 37℃ by placing Kovac's reagent strip in the neck of the MIU tube and Stopper the tube (or add Kovac's reagent about 0.5ml). Look for reddening of the lower part of the test strip (or formation of the red Color of the reaction mixture).

Results: 1). Reddening of strip: Positive test. 2). No red color : Negative test

CATALASE TEST: This test is used to differentiate catalase producing bacteria such, as Staphylococci from Non-catalase producing bacteria such as Streptococci.

Procedure : Pour 2 to 3 ml of hydrogen peroxide solution in a test tube. Immerse growth of organisms in the test-tube solution by using sterile wooden Stick (or a sterile glass rod). Look for immediate bubbling.

Observations: Appearance of bubbles: Presence of catalase producing organisms. No formation of bubbles: Presence of non-catalase producing organisms. The culture should not be more than 24 hours. This test should be performed from a blood free culture medium (nutrient agar) Since catalase is also present in red blood cells.

Catalase producing organisms are: Staphylococcus aureus, Escherichia coli, Mycobacterium tuberculosis, Legionella pneumophila.

Identification of sample 1

Test	Mecconkyagar	Blood agar
INDOLE	Negative	Negative
TSIA	Acid/alkaline slant	Acid/alkaline slant
UREASE	Positive	Positive
CITRATE	Positive	Positive
OXIDASE	Positive	Positive
B.ESCULIN	Positive	Positive
CATALASE	Positive	Positive
COAGULASE	Negative	Negative
Organism confirmed as	Pseudomonasaeuroginosa	Pseudomonas aeuroginosa

Identification of sample 2

Test	Mecconkyagar	Blood agar
INDOLE	Negative	Negative
TSIA	Acid /alkaline slant	Acid/alkaline slant
UREASE	Positive	Positive
`CITRATE	Positive	Positive
OXIDASE	Positive	Positive
B.ESCULIN	Positive	Positive
COAGULASE	Negative	Negative
CATALASE	Positive	Positive
Organism confirmed as	Pseudomonas aeuroginosa	Pseudomonas aeuroginosa

Identification of sample 3:-

Test	Mecconkyagar		Blood agar	
	Lactose fermenting mucoid	Non lactose fermenting	White mucoid	Swarming growth
INDOLE	Negative	Positive	Negative	Positive
TSIA	Alkaline slant	Acid alkaline slant with H2S production	Alkaline slant	Acid alkaline slant with H2S production
UREASE	Positive	Positive	Positive	Positive
CITRATE	Positive	Positive	Positive	Positive
OXIDASE	Negative	Negative	Negative	Negative

Test	Mecconkyagar		Blood agar	
B.ESCULIN	Negative	Negative	Negative	Negative
CATALASE	Positive	Positive	Positive	Positive
COAGULASE	Negative	Negative	Negative	Negative
Organism confirmed as	Klebsiella	Proteus	Klebsiella	Proteus

Identification of sample 4:-

Test	Mecconkyagar	Blood agar	
	Lactose fermenting mucoid	White rough colonies	Small hemolytic pinpointed colonies
INDOLE	Positive	Positive	Negative
TSIA	Acid/acid	Acid/acid	Acid/acid
UREASE	Positive	Positive	Negative
CITRATE	Positive	Positive	Negative
OXIDASE	Negative	Negative	Negative
B.ESCULIN	Negative	Negative	Negative
COAGULASE	Negative	Negative	Positive
CATALASE	Positive	Positive	Positive
Organism confirmed as	E.coli	E.coli	Staphylococcus aureus

Identification of sample 5:-

	Mecconkyagar		Blood agar	
Test				
	Lactose fermenting	Non lactose fermenting	White rough colonies	White transparent colonies
INDOLE	Positive	Positive	Positive	Positive
TSIA	Acid/alkaline slant with H2S production	Acid/acid	Acid alkaline slant with H2S production	Acid/acid
UREASE	Positive	Positive	Positive	Positive
CITRATE	Positive	Positive	Positive	Positive
OXIDASE	Negative	Negative	Negative	Negative
B.ESCULIN	Negative	Negative	Negative	Negative
COAGULASE	Negative	Negative	Negative	Negative
CATALASE	Positive	Positive	Positive	Positive
Organism	Proteus	E.coli	Proteus	E.coli

confirmed as				

Identification of sample 6:-

Test	**Mecconkyagar**		**Blood agar**	
	Lactose fermenting	Non lactose fermenting	White transparent colonies	White transparent colonies
INDOLE	Positive	Negative	Positive	Negative
TSIA	Acid/acid	Acid alkaline slant	Acid/acid	Acid alkaline slant
UREASE	Positive	Positive	Positive	Positive
CITRATE	Positive	Positive	Positive	Positive
OXIDASE	Negative	Negative	Negative	Negative
B.ESCULIN	Negative	Positive	Negative	Positive
COAGULASE	Negative	Negative	Negative	Negative
CATALASE	Positive	Positive	Positive	Positive
Organism confirmed as	E.coli	Pseudomonas	E.coli	Pseudomonas

Identification of sample 7:-

Test	**Mecconkyagar**	**Blood agar**
INDOLE	Positive	Positive
TSIA	Acid/acid	Acid/acid
UREASE	Positive	Positive
CITRATE	Positive	Positive
OXIDASE	Negative	Negative
B.ESCULIN	Negative	Negative
COAGULASE	Negative	Negative
CATALASE	Positive	Positive
Organism confirmed as	E.coli	E.coli

Identification of sample 8:-

Test	**Mecconkyagar**		**Blood agar**	
	Lactose fermenting	Non lactose fermenting	White transparent colonies	White transparent colonies
INDOLE	Positive	Negative	Positive	Negative
TSIA	Acid/acid	Acid alkaline slant	Acid/acid	Acid alkaline slant

UREASE	Positive	Positive	Positive	Positive
CITRATE	Positive	Positive	Positive	Positive
OXIDASE	Negative	Positive	Negative	Positive
B.ESCULIN	Negative	Positive	Negative	Positive
COAGULASE	Negative	Negative	Negative	Negative
CATALASE	Positive	Positive	Positive	Positive
Organism confirmed as	E.coli	Pseudomonas	E.coli	Pseudomonas

Identification of sample 9:-

Test	**Mecconkyagar**	**Blood agar**
INDOLE	Negative	Negative
TSIA	Alkaline slant	Alkaline slant
UREASE	Positive	Positive
CITRATE	Positive	Positive
OXIDASE	Negative	Negative
B.ESCULIN	Negative	Negative
COAGULASE	Negative	Negative
CATALASE	Positive	Positive
Organism confirmed as	Klebsiella	Klebsiella

SAMPLE	MACCONKEYAGAR	BLOODAGAR	MICROSCOPICOBSERVATION	INDOLE	TSI	UREASE	CITRATE	OXIDASE	B.ESCULIN	CATALASE	CAGULASE	ORGANISMSIDENTIFIED
F1	NLF	Whitetransparentcolonies	GNB	_	Acid/alkalineslant	+	+	+	+	+	_	Pseudomonas aeuroginase
F2	NLF	Whitetransparent colonies	Gnb	_	Acid/alkalineslant	+	+	+	+	+	_	Pseudomonas aeuroginase
F3	LF Mucoid	Whitemucoid	Gnb	_	Alkalineslant	+	+	_	_	+	_	klebsiella
	NLF	Swarminggrowth	Gnb	+	AcidalkalineslatwithH2Sproduction	+	+	_	_	+	_	Proteus
F4	LF Mucoid	Whiteroughcolonies	Gnb	+	Acid/acid	+	+	_	_	+	_	E.coli
	-	Smallhemol	GPCinclusters	_	Acid/acid	_	_	_	_	+	+	Stapylococcus
		yticpinpoint colonies										Aureus
F5	LF	Whiterough colonies	Gnb	+	AcidalkalineslantwithH2S production	+	+	_	_	+	_	Proteus
	LF	Whitetransparent colonies	Gnb	+	Acid/acid	+	+	_	_	+	_	E.coli
F6	LF	Whitetransparentcolonies	Gnb	+	Acid/acid	+	+	_	_	+	_	E.coli
	NLF	Whitetransparent colonies	Gnb	_	Acid/alkaline slant	+	+	+	+	+	_	pseudomonas
F7	LF	Whitetransparentcolonies	Gnb	+	Acid/acid	+	+	_	_	+	_	E.coli
F8	LF	White	Gnb	+	Acid	+	+	_	_	+	_	E.coli
		transparentcolonies			/acid							
	NLF	Whitetransparent colonies	Gnb	_	Acid/alkaline slant	+	+	+	+	+	_	pseudomnas
F9	LF mucoid	Whitemucoi d d	Gnb	_	Alkalineslant	+	+	_	_	+	_	Klebsiella

RESULT:-From the nine collected samples fishes the microbial growth is observed from nine Samples. E.Coli, Staphylococcus aureus , Proteus, Klebesilla , Pesudomonas aeuroginosa, are Different species of bacteria that were isolated and identified from the collected sample Fishes . The growth is observed on two different agar plates. On agar plates the colony Morphology identified is that pinpoint colonies, non-lactose fermenting colonies, Fermenting colonies, swarming growth for proteus species on blood agar, mucoid Colonies. For the isolated bacteria biochemical test were performed, the biochemical test For bacteria gave some positive result and some negative result, alkaline slant.

DISCUSSION

The isolated organisms from different fish species are klebsiella , staphylococcus , Pseudomonas , E.coli and proteus species . These are the most common and main bacteria Responsible for fish spoilage . The result showed that the sample F1 Scomberomorus guttatus, is Having both on MacConkey agar and blood agar, on MacConkey agar non-lactose Fermenting colonies were formed, on blood agar white transparent colonies were formed . Sample F2 Rastrelliger kanagurta is having growth on both MacConkey agar and Blood agar, on MacConkey agar non-lactose fermenting colonies were formed, on blood Agar white transparent colonies were formed. Sample F3 Pusa hispida saimensis is having growth on Both MacConkey agar and blood agar , on MacConkey agar lactose fermenting mucoid Colonies and non-lactose fermenting colonies were formed , on blood agar white mucoid Colonies and swarming growth were formed . Sample F4 Mullet is having growth on Both MacConkey agar and blood agar , on MacConkey agar lactose fermenting mucoid Colonies were formed , on blood agar white rough colonies and small hemolytic pin point Colonies were formed. Sample F5 Pampus argenteus is having growth on both MacConkey Agar and blood agar, on MacConkey agar both lactose fermenting and non-lactose Fermenting colonies were formed, on blood agar white rough colonies and white Transparent colonies were formed. Sample F6 Thunnus is having growth on both MacConkey agar and blood agar, on MacConkey agar both lactose fermenting and nonLactose fermenting colonies, on blood agar white transparent colonies were formed. Sample F7 Rhizoprionodon acutus is having growth on both MacConkey agar and blood agar, on MacConkey agar lactose fermenting colonies were formed, on blood agar white transparent Colonies were formed. Sample F8 Ribbon fish is having growth on both MacConkey agar And blood agar , on MacConkey agar both lactose fermenting and non-lactose fermenting Colonies were formed, on blood agar white transparent colonies were formed. Sample F9 Arius jella is having growth on both MacConkey agar and blood agar, on MacConkey agar Lactose fermenting mucoid colonies were formed, on blood agar white mucoid colonies were formed.

Conclusion

The present study shows that E.coli, Staphylococcus, Pseudomonas, Klebsiella and Proteus species were identified from Scomberomorus guttatus,Rastrelliger kanagurta, Pusa hispida saimensis, Mullet fish, Pampus argenteus, Thunnus, Rhizoprionodon acutus fish, Ribbon fish,Arius jella respectively. The fish is highly perishable and prone to vast variations in quality due to differences in species ‘Environmental habitats and feeding habitats, they can also function as carriers for several Microbial health hazards. Therefore maintenance of quality is most important in storage , Preservation, and transportation. Pseudomonas species was isolated from most of the collected Fishes from fish vendors. So the present

conclusion of study of bacterial species suggests that There is an urgent need to improve the quality control system in the fish market.

References

1. Alboofetileh, M., Rezaei, M., Hosseini, H. et al. Efficacy of activated alginate-based nanocomposite films to control Listeria monocytogenes and spoilage flora in rainbow trout slice. J Food Sci Technol 53, 521–530 (2016). https://doi.org/10.1007/s13197-015-2015-9
2. Alfaro, B., Nuin, M., Pin, C., Le Marc, Y. (2008). Application of "Fish shelf-life prediction (FSLP)" software for monitoring seafood quality in the cold chain. In: Cold Chain Management –Proceeding of 3rd International Workshop, J. Kreyenschmidt, ed., Institute of Animal Sciences, Bonn, pp. 90-94.
3. Baranyi, J., Tamplin, M. (2004). ComBase: A common database on microbial responses to Food environments. J. Food Prot. 67 (9), 1967-1971.
4. Dalgaard, P. (2006) Microbiology of marine muscle foods. In: Handbook of Food Science, Technology and Engineering. Vol. I. Hui, Y. H. (ed.) Taylor & Francis CRC Press, USA.Chapter 53, 53-1 – 53-20.
5. Dalgaard, P. (2009). Modelling of microbial growth. Bulletin of the International Dairy Federation, 433 45-57.
6. Dalgaard, P. Mejlholm, O., Huss, H.H. (1996) Conductance method for quantitative Determination of Photobacterium phosphoreum in fish products. J. Appl. Bact. 81, 57-64.
7. Dalgaard, P., Huss, H.H. (1997) Mathematical modelling used for evaluation and prediction Of microbial fish spoilage. In: Seafood Safety, Processing and Biotechnology. Eds. Shahidi, F.,Jones, Y and Kitts, D.D. Technomic Publishing Co. Inc., Lancaster. 73-89
8. Ziemke, F., Höfle, M., Lalucat, J., Rosselló-Mora, R. (1998). Reclassification of Shewanella Putrefaciens Owen's genomic group II as Shewanella baltica sp. Nov. Int. J. Syst. Bacteriol. 48,179-186.
9. Bate Smite, E.C and Bendall, J.R. 1956. Changes in fish muscle after death. British Medical Bulletin 12, pp.2305.
10. Gopakumar,K.2000. Enzymes and Enzyme products as Quality Indices. Seafood Enzymes, Pp 337-363. Harrd N.F and Simpspn, B.K., (Eds). Marcel Dekker, Inc.New York, Basel, U.S.A.
11. Bate Smite, E.C and Bendall, J.R. 1956. Changes in fish muscle after death. British Medical Bulletin 12, pp.2305.
12. Burt, J.R.1976. Hypoxanthine a biochemical index of fish quality. Process Biochemistry. 11(10).
13. Austin, B. (2006). The bacterial microflora of fish, revised. TheScientificWorldJournal, Vol. 6, pp. 931–945. https://doi.org/10.1100/tsw.2006.181
14. Contamination, M. (2016). Spoilage of Fish and Other Seafoods. Food Microbiology: Principles into Practice, 301–306. https://doi.org/10.1002/9781119237860.ch18
15. Doyle, M. P. (2009). Food Microbiology and Food Safety Series Editor. Retrieved from http://www.springer.com/series/7131
16. William C. Frazier; (1995) Food Microbiology, Fourth Edition.pdf.
17. Ghaly, A. E., Dave, D., Budge, S., & Brooks, M. S. (2010). Fish spoilage mechanisms and Preservation techniques: Review. American Journal of Applied Sciences, Vol. 7, pp. 846–864. https://doi.org/10.3844/ajassp.2010.859.877

18. Ikape, S. I. (2018). Octa Journal of Biosciences Fish Spoilage in the Tropiics : A Review Fish Spoilage In The Tropics : A Review. Octa Journal of Biosciences, 5(2), 34–37. Retrieved from www.sciencebeingjournal.com
19. Mahmud, A., Abraha, B., Samuel, M., Abraham, W., & Mahmud, E. (2018). Fish Preservation: a multi-dimensional approach. MOJ Food Processing & Technology, 6(3). https://doi.org/10.15406/mojfpt.2018.06.00180
20. Masniyom, P. (2011). Deterioration and shelf-life extension of fish and fishery products by Modified atmosphere packaging. In Songklanakarin J. Sci. Technol (Vol. 33). Retrieved from http://www.sjst.psu.ac.th
21. Mei, J., Ma, X., & Xie, J. (2019). Review on natural preservatives for extending fish shelf Life. Foods, Vol. 8, p. 490. https://doi.org/10.3390/foods8100490
22. Tavares, J., Martins, A., Fidalgo, L. G., Lima, V., Amaral, R. A., Pinto, C. A., … Saraiva, J. A. (2021). Fresh fish degradation and advances in preservation using physical emerginTechnologiesFoods., Vol. 10
23. https://doi.org/10.3390/foods10040780 60
24. Tayel, A. A., Bahnasy, A. G., Mazrou, K. E., Alasmari, A., El Rabey, H. A., Elboghashy, S. A., & Diab, A. M. (2021). Biopreservation and Quality Enhancement of Fish Surimi Using Colorant Plant Extracts. Journal of Food Quality, 2021. https://doi.org/10.1155/2021/6624565
25. Zhuang, S., Hong, H., Zhang, L., & Luo, Y. (2021). Spoilage-related microbiota in fish and Crustaceans during storage: Research progress and future trends. Comprehensive Reviews in Food Science and Food Safety, Vol. 20, pp. 252–288. https://doi.org/10.1111/1541-4337.12659.
26. Ryser, Elliot T., and Elmer H. Marth, eds. Listeria, Listeriosis, and Food Safety. 3RD ed. New York: Taylor & Francis Group, 2007. 636-40. Roger. UCSD, La Jolla. Keyword: Listeria.
27. Schmidt, Ronald H., and Gary E. Rodrick. Food Safety Handbood. John Wiley & Sons, 2003. 137-50. Roger. Knovel. UCSD, La Jolla. Keyword: Food and Saftey Handbook.
28. Novak, John S. Microbial Safety of Minimally Processed Foods. New York: C R C P LLC, 2002. Ch.3. Roger. UCSD, La Jolla. Keyword: Listeria monocytogenes.
29. [6] Van Wagner, L.R. 1989. FDA takes action to combat seafood contamination. Food Process. 50: 8–12.
30. Abbas, K.A., A.M. Saleh, A., Mohamed and O. Lasekan, 2009. The relationship between Water activity and fish spoilage during cold storage: A review. J. Food, Agric. Environ., 7: 86-90.
31. Adebowale, B.A., L.N. Dongo, C.O. Jayeola and S.B. Orisajo, 2008. Comparative quality Assessment of fish (Clarias gariepnius) smoked with cocoa pod husk and three other different Smoking materials. J. Food Technol., 6: 5-8. DOI: 10.3923/jftech.2008.5.8
32. Akinola, O.A., A.A. Akinyemi and B.O. Bolaji, 2006. Evaluation of traditional and solar Drying systems towards enhancing fish storage and preservation in Nigeria Abeokuta local Government as a case study. J. Fisheries Int., 1: 44-49. DOI: 10.3923/jfish.2006.44.4
33. AMEC., 2003. Management of wastes from Atlantic seafood processing operations. AMEC Earth and Environmental Limited, Dartmouth, Nova Scotia, anada. http://aczisc.dal.ca/nparpt.pdf
34. Engvang, K. and H.H. Nielsen, 2001. Proteolysis in fresh and cold-smoked salmon during Cold storage: Effects of storage time and smoking process. J. Food Biochem., 25: 379-395. 10.1111/j.1745- 4514.2001.tb00747.x

35. Erkan, N. and O. Ozden, 2006. Gutted and un-gutted sea bass (Dicentrarchus labrax) stored In ice: Influence on fish quality and shelf-life. Int. J. Food Properties, 9: 331-345. DOI: 10.1080/10942910600596373
36. Jones, N.R., 1963. Chemical change in fish muscle during storage. Proceed. Nutr. Soc., 22: 172-176. DOI: 10.1079/PNS19630036 Kader, A.A., 2005. Increasing food availability by Reducing postharvest losses of fresh produce. Acta Horticulturae, 3: 2169-2176.