

**Sub Name: FOOD MICROWBIOLOGY**

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## **SPOILAGE OF FISH AND SHELLFISH**

### **INTRODUCTION**

Owing to their high nutritive value the spoilage of fish and fishery products proceeds at a faster rate. Spoilage of fish and shellfish is the result activities of autolytic enzymes, oxidation and associated microorganisms.

- ✓ **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.
- ✓ **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.

### **FACTORS AFFECTING SPOILAGE**

The kind and rate of spoilage of fish is affected by several factors.

#### **1. Kind of fish**

Fishes differ considerably in perishability. Some flat fishes spoil more readily than round fish because they pass through rigor mortis more rapidly. Certain fatty fishes (oil sardine) deteriorate rapidly because of oxidation of unsaturated fat/oil. Fishes high in trimethyl amine oxide (TMAO) spoil quickly and produce stale fishy smell by producing TMA.

#### **2. Conditions of the fish when caught**

Fishes that are exhausted due to struggle while capture (Ex: gill netting, long lining), lack of oxygen and excessive handling spoil rapidly. This is because of exhaustion of glycogen during struggling and causing smaller drop in pH. Feedy fish (fishes with full of food in stomach) are more easily perishable than those with empty intestine.

#### **3. Kind and extent of contamination of fish**

Contamination of fish with bacteria from various sources (mud, water, handlers, contact surfaces, slime etc.) increase bacterial load. Bacteria from slime, gill and intestine invade the flesh and cause spoilage. In general, greater the load of bacteria of fish the more rapid the spoilage. In ungutted fish (whole fish) decay of food in the gut may release odorous substances enabling diffusion of decomposition products into the flesh. Gutting the fish on boat spreads intestinal and surface slime bacteria to flesh. But, thorough cleaning will remove most bacteria, and adequate chilling will inhibit bacterial growth. Any damage to fish skin or mucous membrane will reduce the keeping quality of the product.

#### 4. Temperature

Warmer the temperature faster will be the bacterial growth and quicker will be the spoilage. Reducing the temperature of fish by chilling will delay bacterial growth, hence, spoilage slows. Cooling temperature around zero degree Celsius (0°C), helps to delay spoilage.

#### 5. Use of preservatives

Use of preservatives including antibiotics will prevent bacterial build up thus extend shelf life of fish.

### **CHARACTERS OF SPOILING FISH**

- Change in external characteristics as the fish spoilage progresses can be used to indicate spoilage. The sequences of changes taking place as the spoilage proceeds are,
- Bright characteristic colour of fish fades, and fish becomes discoloured (appear dirty yellow or brown).
- Increase in slime on skin especially on gills.
- Eyes gradually sink and shrink, pupil becomes cloudy and cornea turns opaque.
- Gills turn light pink and finally to pale yellow colour.
- Softening of the flesh and exude juice when squeezed and easily indented by pressing with fingers.
- Flesh can be easily stripped from along the back bone / vertebral column.
- Release of odorous substances- the normal, fresh, seaweedy odour will change to sickly sweet, stale fishy odour due to TMA and other malodorous substances. Fatty fishes also show rancid odour.

### **SPOILAGE OF CRUSTACEANS (SHRIMPS, CRABS AND LOBSTERS)**

Spoilage of crustaceans is essentially same as fishes. Spoilage differs depending on the handling and chemical composition. Crustaceans differ from fish in having carbohydrate (about 0.5%), higher content of free aminoacids than fish, and enzymes that rapidly break down proteins. Bacterial flora of crustaceans includes bacteria from the water from which harvested and also contaminants acquired during fishing, handling, transportation, processing etc).

Spoilage generally begins at the body surface. Presence of higher concentration of free aminoacids and nitrogenous extractives make them susceptible to rapid attack by spoilage bacteria. Initial spoilage is by production of large amounts of volatile base nitrogen (VBN). Some amount of VBN is also produced from the reduction of TMA. Subsequent spoilage results in production of off-odour substances, making it unfit for consumption.

### **SPOILAGE OF MOLLUSCS**

Spoilage of molluscs is different from fish/shrimp because of difference in chemical composition. These have high carbohydrate content and low total nitrogen when compared to fish/shrimps. Carbohydrate content, mostly in the form of glycogen, is noticed in clams (3.40%) and oysters (5.60%). Fermentative type of microbial spoilage is noticed because of presence of glycogen. These also contain high levels of nitrogen bases (free arginine, aspartic acid, glutamic acid) than fish. Higher carbohydrate content is responsible for different spoilage pattern of molluscs over other seafood. The filter feeding molluscs have high bacterial load and are involved in spoilage.

The fermentative type of spoilage causes reduction in pH as spoilage progresses. This decrease in pH is used as indicator of extent of spoilage. Thus, pH is used as best objective criteria for examining microbial quality of oysters. Besides pH, organoleptic quality and microbial load are also desired as microbial quality indices. Using pH scale as microbial quality indicator, oysters can be grouped as good (pH 6.5 – 5.9), off (pH 5.8), musty (pH 5.7 – 5.5), and sour/putrid (pH 5.2 or below).

### **SPOILAGE OF SEMI-PROCESSED AND PROCESSED FISHERY PRODUCTS**

The native microbial floras as well as the microorganisms entering in to the food through various stages of handling and processing play an important role in spoilage of fresh as well as processed fish and fishery products. Though the post- harvest handling and treatment of fish is expected to reduce microbial proliferation, some of the process-tolerant and surviving microorganisms grow and cause spoilage of the product.

### **MICROBIOLOGY AND SPOILAGE OF FRESH FISH**

The microbial load and types of microorganisms associated with the freshly harvested fish directly reflects on the microflora of the immediate environment from which fish is harvested. Further, several microorganisms are added to the fish from harvesting gear and net, from the boat deck, fish contact surface, fish holds and fishermen. Once the fish dies, microorganisms present on the body surface, gills and the intestine start multiplying and increase in numbers causing deteriorative changes in fish. As the time lapses the spoilage proceeds faster making the fish unfit for human consumption. The spoiling fish is generally characterized by the loss of bright body coloration, fading of gill colour, sunken eyes and development of off odour metabolites of spoilage bacteria. The spoilage flora of fresh fish is generally dominated by the Gram negative bacteria.

The effective way to prevent or delay spoilage of fresh fish is by reducing the activity of spoilage microorganisms which can be achieved by lowering the temperature of holding the harvested fish.

### **MICROBIOLOGY AND SPOILAGE OF CHILLED FISH**

Holding the fish in low temperature close to freezing point of water affects the microflora of fish. The low temperature reduces microbial and enzymatic activity resulting in extension of shelf life of fish. Factors such as quality of fish, method and duration of chilling and efficiency of storage method influence the quality of chilled fish. Many microorganisms associated with fish survive low temperature and spoilage is mainly caused by psychrotrophs. Among the bacterial flora dominance of Gram negatives is observed over Gram positive bacteria. Chill storage brings about changes in composition of microflora, and mesophiles are gradually dominated by psychrophiles. The low temperature, however, helps to maintain fish in good condition only for a short time. The prolonged storage under chill condition leads to growth of psychrophilic microorganisms resulting in spoilage of fish.

### **MICROBIOLOGY AND SPOILAGE OF FROZEN FISH**

The bacteriological quality of frozen products depends on the bacterial load of the raw material, contamination during handling and processing and extent of removal of these contaminants during processing. The freezing and storage under frozen condition has detrimental effect on surviving microorganisms and reduction in count is highly variable.

### **GROWTH AND SURVIVAL OF MICROORGANISMS ASSOCIATED WITH FROZEN FOODS**

The psychrotrophic bacteria in fish are sensitive to freezing stress, and the sensitivity is strain dependent. Spoilage microorganisms generally grow and cause spoilage when the raw fish product is held long time before freezing, frozen at a very slow rate (slow freezing conditions), thawed too slowly or held under thawed condition for a long time. However, the ultimate activity of microorganisms depends on the time duration and temperature of holding the product. As most microorganisms are unable to grow below -10 °C or -12 °C, increase in temperature above this limit results in dramatic increase in growth rate. The changes in microflora and biochemical alterations observed in frozen fish products are similar to that of raw chilled fish.

Seafood held at elevated frozen storage temperatures (-10 °C to -5 °C) are likely to support mold growth though at very slow rate. Some molds and yeasts can grow in that range. Though bacteria do not grow in frozen foods, they are able to survive freezing and frozen storage to a certain extent, so that thawed fish spoils about as fast as fish that has never been frozen. Gram negative bacteria are more readily killed by the freezing conditions than Gram positive bacteria while, spores are least sensitive and thus show better survival.

Microbial analysis of frozen product gives some information about the quality of the fish before it was frozen. However, all strains of microorganisms are to some extent killed by freezing and frozen storage. Hence, the bacterial load in frozen fish is always lower than that observed before the product was frozen. For frozen foods use of *E. coli* as indicator of sanitary quality during processing is not suitable, and instead fecal streptococci are preferred owing to their greater ability to survive freezing.

## **PATHOGENIC MICROORGANISMS AND FROZEN FOODS**

Among human pathogenic microorganisms, *Vibrio parahaemolyticus* is encountered in small numbers in frozen foods since it is quite sensitive to conditions of freezing and thawing. However, these potentially dangerous bacteria get destroyed on cooking, but pose threat only under the conditions of recontamination of the cooked food and abuse of holding time and temperature.

The potentially toxigenic *Clostridium botulinum* is not affected by freezing conditions and presents no hazard unless conditions for its outgrowth and toxin production are provided. However, toxins that might be present in the raw product or produced as a result of bacterial growth in seafood prior to freezing would not be inactivated by the freezing process. Generally, conditions permitting the development of large populations of spoilage bacteria also favour toxigenesis of *C. botulinum*. The poor temperature control which is commonly encountered during the distribution of frozen seafood favours bacterial growth leading to spoilage. Staphylococci are reasonably resistant to the effects of freezing hence their load in frozen food gives an indication on the extent of handling/human contact that the food had received prior to freezing.

## **MICROBIOLOGY AND SPOILAGE OF CANNED FISHERY PRODUCTS**

Canned seafoods are expected to be commercially sterile and free from spoilage and potentially pathogenic microorganisms. The bacteriological hazards of canned food mainly results from improper or inadequate processing or leakage of cans. The inadequate heat processing causes survival and growth of heat resistant clostridial spores responsible for botulism. The mesophilic spoilage organisms entering the cans through leakage caused due to improper seaming of cans grow during storage eventually resulting in spoilage of canned product.

The semi-processed canned seafood products are often subjected to bacteriological problems. The stability and safety of these products depend on the factors such as combination of preservatives used and pasteurization process applied. In most pickled products that depend on salt (eg. anchovies) or a low pH (eg. mussel) for stability, the heat process given destroys both hazardous and spoilage microorganisms. Yeast like *Pichia fermentans* often cause illness after growing in semi-processed canned seafood.

In some smoked products which are canned using minimal heat treatment, the storage stability is attributed to presence of salt, smoke constituents and a low water activity. Thus, production of safe final product can be achieved only by having good control of microbiology and spoilage of cured fishery products

Salting and drying are the most common methods used for curing of fish. The preservative effect of salted and or dried fishery products is due to lowering of water activity. Bacterial counts of fully dried seafood are generally low, unless there has been extensive surface contamination. Spoilage of such products is mainly caused by halophilic bacteria which can persist on the surfaces of contaminated cured seafood. However, the microbiological hazards due to pathogenic microorganisms are negligible in cured or salted seafood products.

Smoked seafood products vary widely in microbial stability depending on the nature and degree of severity of processing. Heavily salted, hot smoked products are microbiologically similar to the fully dried products since their water content is too low to support bacterial growth and hence pose little or no hazard. Lightly smoked products that are brined only enough to improve a flavour carry a mixed microbial population and are only slightly more stable than unprocessed fish. Generally, Gram positive bacteria dominate the microflora of such products soon after preparation but Gram negative bacteria gradually become more numerous during refrigerated storage and are ultimately responsible for their spoilage. The hot smoked fishery products that have not been adequately dried are of high risk due to selective outgrowth of *C. botulinum* resulting from favourable Eh and elimination of competing bacteria. er processing parameters.