### Dear viewers, Namaskar

## Welcome to the lecture series on Food technology

Today the topic Microbial spoilage of fish, meat, poultry and egg shall be discussed under the following sub headings

- 1. Introduction
- 2. Microbial spoilage of fish
- 3. Microbial spoilage of meat
- 4. Microbial spoilage of poultry
- 5. Microbial spoilage of egg

#### 1. Introduction

Foods and microorganisms have long and interesting associations which developed long before the beginning of recorded history. Foods are not only nutritious to consumers, but are also excellent source of nutrients for microbial growth. Depending upon the microorganisms present, foods may spoil or preserved by fermentation. Food spoilage means the original nutritional value, texture, flavour of the food are damaged, the food become harmful to people and unsuitable to eat.

Spoilage of food involves any change which renders food unacceptable for human consumption and may result from a variety of causes, which includes i) insect damage; ii) physical injury due to freezing, drying, burning, pressure, drying, radiation etc; iii) activity of indigenous enzymes in plant and animal tissues; iv) chemical changes not induced by microbial or naturally occurring enzymes.

A food unfit for consumption may not necessarily be spoiled and may contain high number of food poisoning causing bacteria. Microbial deterioration of food is evidenced by alteration in the appearance (color changes, pockets of gas/ swelling), texture (soft & mushy), color, odor, and flavor or slime formation.

There are three types of microorganisms that cause food spoilage -- yeasts, moulds and bacteria.

- Yeasts growth causes fermentation which is the result of yeast metabolism. There are two types of yeasts true yeast and false yeast. True yeast metabolizes sugar producing alcohol and carbon dioxide gas. This is known as fermentation. False yeast grows as a dry film on a food surface, such as on pickle brine. False yeast occurs in foods that have a high sugar or high acid environment.
- Moulds grow in filaments forming a tough mass which is visible as 'mould growth'. Moulds form spores which, when dry, float through the air to find suitable conditions where they can start the growth cycle again. Mould can cause illness, especially if the person is allergic to molds. Usually though, the main symptoms from eating mouldy food will be nausea or vomiting from the bad taste and smell of the mouldy food.

Both yeasts and moulds can thrive in high acid foods like fruit, tomatoes, jams, jellies and pickles. Both are easily destroyed by heat. Processing high acid foods at a temperature of 100°C (212°F) in a boiling water canner for the appropriate length of time destroys yeasts and moulds.

**Bacteria** are round, rod or spiral shaped microorganisms. Bacteria may grow under a wide variety of conditions. There are many types of bacteria that cause spoilage. They can be divided into: *spore-forming* and *nonspore-forming*. Bacteria generally prefer low acid foods like vegetables and meat. In order to destroy bacteria spores in a relatively short period of time, low acid foods must be processed for the appropriate length of time at 116°C (240°F) in a pressure canner.

Keeping above points into considerations, in this section we will discuss how spoilage of fish, meat poultry and egg takes place and what kind of microorganisms involved in this unfavorable process.

# 2. Microbial spoilage of fish

Fresh fish spoilage can be very rapid after it is caught. The spoilage process will start within 12 h of their catch in the high ambient temperatures of the tropics. Spoilage is the process through which fish loses its flexibility due to stiffening of fish mussels after few hour of its death. Most fish species degrade as a result of digestive enzymes and lipases, microbial spoilage from surface bacteria and oxidation. During fish spoilage, there is a breakdown of

various components and the formation of new compounds. These new compounds are responsible for the changes in odour, flavor and texture of the fish meat.

Contamination of fish from enteric bacteria of human and animal origin may also be responsible for various food spoilages. During handling and storage, quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product. The quality of fish degrades, due to a complex process in which physical, chemical and microbiological forms of deterioration are implicated.

Some reports on the storage quality of frozen/chilled fishes were still not comprehensive on spoilage mechanism and quality assessment. Degradation of lipids in fatty fish produces rancid odors. In addition, marine fish and some freshwater fish contain trimethylamine oxide that is degraded by several spoilage bacteria to trimethylamine (TMA), the compound responsible for fishy off odors. Iron is a limiting nutrient in fish, and this favors growth of bacteria such as pseudomonads that produce siderophores that bind iron.

Spoilage bacteria differ somewhat for freshwater and marine fish and for temperate and tropical water fish. Storage and processing conditions also affect microbial growth. *Pseudomonas* and *Shewanella* are the predominant species on chilled fresh fish under aerobic conditions. Packing under carbon dioxide and addition of low concentrations of sodium chloride favor growth of lactic acid bacteria and *Photobacterium phosphoreum*. Heavily wet-salted fish support growth of yeasts while dried and salted fish are spoiled by molds. Addition of organic acids selects for lactic acid bacteria and yeasts. Pasteurization kills vegetative bacteria but spores of *Clostridium* and *Bacillus* survive and may grow, particularly in unsalted fish. Spore-forming bacteria are usually associated with spoilage of heat-treated foods because their spores can survive high processing temperatures. These Gram-positive bacteria may be strict anaerobes or facultative, which commonly associated with fish spoilage.

## 3. Microbial spoilage of meat

A number of animal species are used for red meat production, including cattle, swine, sheep, goat, deer, buffalo, camel, and horse. Meats are subject

to spoilage by a wide range of microorganisms, including Gram-positive and Gram-negative bacteria, yeasts, and molds.

There are a number of factors that influence the composition of the meat spoilage microflora:

- Pre-slaughter husbandry practices (e.g., free range vs. intensive rearing)
- Age of the animal at the time of slaughter
- Sanitary handling during slaughter, and processing
- Temperature controls during slaughter, processing, and distribution
- Preservation controls
- Type of packaging
- Consumer handling and storage

The most frequent bacteria to occur on fresh meat are bacteria of the genera *Acinetobacter, Pseudomonas, Brochothrix, Flavobacterium, Psychrobacter, Moraxella, Staphylococcus, Micrococcus,* lactic acid bacteria and various genera of the *Enterobacteriaceae* family. The survival and growth of these microbes is influenced, to a great extent, by the composition of the atmosphere surrounding the meat. Among these most frequently caused by the following groups of bacteria:

- 1) Pseudomonas spp.
- 2) Enterobacteriaceae
- 3) Brochothrix thermosphacta
- 4) Lactic acid bacteria

It is common knowledge that meat can be spoiled quickly under aerobic conditions (in the presence of an atmosphere comprised of air). This is caused by the rapid growth of pseudomonades. Psychrotrophic species such as *Pseudomonas fragi*, *P. lundensis*, *P. putida* and *P. fluorescens* can be isolated from unpacked meat showing signs of spoilage. *P. fluorescens* occurs more frequently on fresh meat, though during longer periods of storage *P. fragi* becomes dominant. Higher concentrations of CO<sub>2</sub> (10%) inhibit the growth of both *P. fluorescens* and *P. fragi* on red meat. *P. fragi* plays a significant role in meat spoilage; meat is even considered the ecological niche for this species.

*P. fragi* represents the dominant species among the pseudomonades regardless of the packaging of the meat. All other species occur primarily on unpacked meat, i.e. under aerobic conditions.

A population of pseudomonades of  $10^7 - 10^8$  CFU/g causes slime to form on meat and a bad smell to appear. Both these deviations appear, however, when pseudomonades exhaust the glucose and lactic acid in the meat and begin to metabolise nitrogenous compounds, particularly amino acids. When the diffusion gradient of glucose from lower layers of the meat towards the surface no longer serves to cover the needs of a large number of bacteria, the degradation of amino acids and proteins begins, accompanied by the release of ammonia, amines and sulphides. The characteristic aroma of spoiling meat appears.

The proteolytic activity of pseudomonades assists their penetration into the meat. In such case, the capabilities of proteolytic bacteria enable them to gain a competitive advantage over other bacterial groups or species, as they are able to gain access to new sources of nutrients that are not available in this way to microbes with weaker or no proteolytic properties.

The genus *Shewanella*, which is also frequently present on meat, is a genus of microbes related to *Pseudomonas* spp. The species *Shewanella putrefaciens* releases substances characteristic of meat spoilage and also produces hydrogen sulphide (sulphane). This compound, in combination with muscle pigment, causes greening of meat. *Shewanella putrefaciens* is considered the primary cause of spoilage in vacuum-packed meat stored at refrigerated temperatures, particularly at higher pH values.

Numerous species of the *Enterobacteriaceae* family have been found on beef, lamb, pork and chicken meat and offals. The genera *Serratia, Enterobacter, Pantoea, Klebsiella, Proteus* and *Hafnia* all contribute to spoilage. In view of its potential to spoil meat, the most important in this regard are *Serratia liquefaciens, Hafnia alvei* and *Enterobacter agglomerans*. The genus *Serratia* is the most frequent member of the *Enterobacteriaceae* family to be found on meat. *Serratia liquefaciens*, in particular, is isolated from meat stored in various atmospheres. *Serratia grimesii* and *Serratia proteamaculans* are other such species.

The species *Citrobacter freundii* and *Proteus vulgaris* have been detected in minced beef stored in an aerobic or modified atmosphere. *Hafnia alvei* is often found in minced meat in a modified atmosphere or in a vacuum. Representatives of the genus *Rahnella*, which also plays a role in the spoilage process, have also been isolated from vacuum packed beef and beef stored in modified atmosphere in the later stages of storage.

Vacuum packaging and modified atmosphere favour facultative anaerobic bacteria, including lactic acid bacteria and *Brochothrix thermosphacta*. Lactic acid bacteria, in particular, are highly competitive in a modified atmosphere. The genera *Lactobacillus, Carnobacterium* and *Leuconostoc* may be associated with the spoilage of chilled meat.

The psychrotrophic lactic acid bacteria occurring most frequently on meat include *Lactobacillus sakei*, which is considered an agent of spoilage of vacuum-packed meat and meat packed in modified atmosphere. It is clear from the expert literature that only psychrotrophic lactic acid bacteria, such as the species *Lactobacillus species*, *Carnobacterium divergens*, *C. maltaromaticum* and *Leuconostoc* spp., can attain large numbers of cells in meat in modified atmosphere and vacuum-packed meat stored at refrigeration temperatures. Species such as *Lactobacillus sakei* and *L. algidus* have been isolated from vacuum packed meat at 4 °C. If the temperature falls to 1 °C, *Lactobacillus* spp., *Weissella* spp. and *Leuconostoc mesenteroides* become the dominant species. The effect of temperature on the composition of the microflora under conditions that are otherwise identical is evident in this change.

On meat in storage, the genus *Carnobacterium* is represented by the species *Carnobacterium divergens* and *Carnobacterium maltaromaticum*. *Carnobacterium divergens* has been isolated from samples of unpacked meat and vacuum-packed meat. Psychrotrophic *Brochothrix thermosphacta* is an important agent of meat spoilage. This species grows under aerobic conditions and on vacuum-packed meat. While this microbe has been isolated from samples of beef throughout the storage period, it has been isolated from vacuum-packed pork only up to the middle of the period of its shelf life.

Lactic acid bacteria then became dominant, which points to their better competitive capabilities.

Spoilage of vacuum-packed beef may also be caused by psychrotrophic clostridia. This type of spoilage is known as "blown pack spoilage" (BPS), which emphasises the characteristic feature accompanying this kind of spoilage – a strongly inflated package Cases of spoilage of this kind have been registered in the USA, Canada, Brazil, New Zealand, South Africa, the United Kingdom and Ireland. The species *Clostridium* is considered the most frequent agent of this type of spoilage.

## 4. Microbial spoilage of poultry

The consumption of poultry and poultry products has increased markedly during the past decade.

Poultry is the second most widely eaten type of meat globally and, along with eggs, provides nutritionally beneficial food containing high-quality protein accompanied by a low proportion of fat. All poultry meat should be properly handled and sufficiently cooked in order to reduce the risk of food poisoning. Growing demand in poultry consumption has led to the evolution of intensive broiler flock production and mechanized slaughter practices, which influence the microbiology of the raw products.

Each step in the processing of raw poultry influences the level of spoilage microflora on the product. In general, whole poultry carcasses have lower microbial populations than cut-up poultry. Poultry carcasses receive several washing steps during the slaughter process.

More than 50 strains of spoilage bacteria representing 25 genera have been reported as part of the initial microflora in raw chicken. At refrigerated temperatures, pseudomonads represent the largest genus represented. Cell numbers of nonpigmented pseudomonads increase substantially during spoilage, and storage conditions greatly influence the cell numbers of pigmented pseudomonads. Modified atmospheric storage dramatically changes the predominant spoilage microflora compared to aerobic storage, as it prevents the growth of obligately aerobic microorganisms. Psychrotrophic spoilage microorganisms isolated from chicken carcasses are Pseudomonads,

Acinetobacter spp., Flavobacterium spp., Corynebacterium, Yeasts, enterics, and others.

Temperature has a dramatic affect on the development of spoilage microflora of raw poultry. The rate of microbial spoilage of broiler carcasses is twice as fast at 10°C than at 5°C, and three times more rapid at 15°C. The shelf life of cut-up poultry is 2–3 days at 10°C, 6–8 days at 4.4°C, and 15–18 days at 0°C, respectively.

The hallmarks of raw poultry spoilage are the formation of off-odors and the development of surface slime. Initially, the spoilage microflora will metabolize glucose, and then shift to substrates such as amino acids as the glucose is depleted. In particular, amino acid metabolism contributes to off-odor production. Slime formation generally follows the production of undesirable odors. During storage, small translucent drop-like colonies will appear on the tissue surface, eventually coalescing into a uniform slime layer. In general, off-odors are detected when counts reach 107–108 CFU/cm2 of tissue surface and slime formation occurs at levels more than 108 CFU/cm2.

Fungi typically play a minor role in poultry spoilage. However, when antibiotics are used to control bacterial growth, fungi can become major causes of spoilage. The principal spoilage yeasts found on raw poultry are Candida spp., Rhodotorula spp., and Torulopsis spp.

## 5. Microbial spoilage of egg

Chicken eggs are the eggs most commonly consumed by humans. Between 2000 - 2010, global egg output expanded by more than two per cent a year from 51 million tonnes to 63.8 million tonnes. This subunit will discuss the types of microbial spoilage that may occur in a variety of different egg products – shell eggs, liquid eggs, and processed eggs (cooked, frozen, dried, and baked). The microbes primarily responsible for the spoilage of shell eggs and liquid eggs are typically Gram-negative microorganisms.

A shell egg at the time of oviposition should be essentially free from microbial contamination. However, this condition is quickly changed once the exterior of the egg comes in contact with the nesting material where the egg is deposited. Dust, soil, and feces are the primary sources of contaminating microorganisms. The relatively few Gram-negative microorganisms are primarily responsible for egg spoilage. The types of spoilage or rots are sometimes characterized by the color of the spoiled eggs. For example, black rots are associated with the presence of species of Proteus and Aerobacter. Serratia species are associated with red rot and certain species of Psuedomonas with green and pink rots.

Liquid whole eggs are typically sold as blends of egg whites and yolks. The microbes responsible for the spoilage of unpasteurized liquid eggs were identified in a detailed evaluation of the spoilage microflora of unpasteurized liquid whole eggs. Gram-negative bacteria were dominant at all temperatures evaluated. Genera of the Enterobacteriaceae were predominant in the temperature range of 20–30°C, whereas the Pseudomonadaceae predominated at 5°C. Very few Gram-positive bacteria were isolated. Liquid whole egg samples were incubated at 5, 10, 20, 25, and 30°C until signs of spoilage became apparent. From these samples, the following bacteria were isolated and identified: Acinetobacter sps, Aeromonas hydrophila, Bacillus cereus, Citrobacter sps, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Serratia sps, Pseudomonas putida, Salmonella typhimurium, Streptococcus faecalis and S. lactis.

Microbial spoilage of processed eggs is not commonly a problem unless these products undergo some form of abuse. In the case of cooked or frozen eggs, this would involve a loss of temperature control. However, the spoilage of dried or baked eggs would typically involve the introduction of water into these products. In each case, the spoilage microorganisms could be either bacteria or fungi, depending upon the severity of the abuse.

Conclusion: The spoilage of food and presence of food poisoning organisms in food are very important from the point of food safety. Today the emphasis is on total quality of food which means that not only food should be nutritionally balanced but should be microbiologically safe too. Most foods serve as good growth medium for many different microorganisms. Considering the variety of foods and the methods used for processing, it is apparent that practically all kinds of microorganisms are potential contaminants. Given a chance to grow, the microorganisms will cause

changes in appearance, flavor, odour and other qualities of foods. Knowledge about the microbial biodiversity and the conditions under which bacteria are encountered as spoilage microbes is crucial for understanding spoilage mechanisms, in order to apply effective strategies for preservation.