Food Microbiology

CONTENT

- Microbial Contamination of Fresh Food
- Contamination of Animal Food Products
- Microbial Contamination of Processed Foods
- Microbial Food Spoilage
- Types of Food Spoilage and Concerned Micro-Organisms
- Spoilage of Canned Food
- Food Preservation
- Low Temperature Treatment
- Chemical Treatment
- Treatment by Radiation
- Food-Borne Diseases Caused By Microorganisms
- Source
- Symptoms
- Prevention
- Fungal Food-poisoning (Mycointoxications)
- Food Infections
- Non-bacterial Food Infections
- Fermented Food Items
- Non-bacteria Fermented Food Items Bread

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Introduction

The diet of many people is supplemented with food items preserved by special methods and available in a variety of conditions and stages of preparation. Such food may be frozen, canned or dehydrated; it may be partly or completely baked or pre-cooked, ready for heating and serving. During preparation, heterotrophic microorganisms for meeting their nutritional requirement can attack such foods. The unrestricted growth and multiplication of these microorganisms in food may render it unfit for consumption and can result in spoilage or deterioration.

1.0 Microbial Contaminations Of Fresh Foods

The inner tissues of healthy plants and animals are free of microorganisms. They become contaminated when exposed to the microorganisms. The magnitude of this microorganism contamination depends upon various factors such as the microbial population of the environment from which the food was taken, the condition of the raw product, the method of handling the food and the conditions of storage.

1.1 Contamination of Plant Food Products:

1.1.1. Fruits and Vegetables:

Fruits and vegetables are generally contaminated by bacteria including species of *Bacillus*, *Enterobacter*, *Lactobacillus*, *Leuconostoc, Pseudomonas, Sarcina, Staphylococcus*, *Streptococcus* etc. Various molds and yeasts also inhabit the fruits and vegetables.

Contamination through infection:

Fruits and vegetables are normally susceptible to bacterial, fungal and viral infections. These infections invade the fruit and vegetable tissue uring various stages of their development and result in the subsequent spoilage.

Contamination through post-harvest handling:

Usually, mechanical handling of fruits and vegetables during post-harvest period produces 'breaks' in them which invite microbial invasion. Since the pH of the fruits is relatively acidic (i.e. high in sugar), they are more susceptible to fungi in contrast to vegetables, which are more susceptible to bacteria because of their pH being slightly higher (5.0 to 7.0; less in sugar).

1.1.2. Cereals:

Cereals and cereal products contain microorganisms from insects, soil and other sources. *Bacillus, Lactobacillus, Micrococcus, Pseudomonas* etc. are the bacteria, which are generally found on freshly harvested grains.

Mostly bacteria such as species of *Bacillus, Lactobacillus, Micrococcus, Sarcinq, Serratia*, *coliforms* etc

contaminate wheat flours. Molds like *Aspergiltus, Penicilltum* are also very common

Contamination of Animal Food Products:

Meats:

The interior portions of meat are usually free of microbial contaminations if healthy animal is properly slaughtered. The fresh cut meat gets immediately contaminated with microorganisms derived from globes, hands, implements used to cut the meat, hides, hairs, intestines of the animals and the air of the slaughter house. Each new surface of meat, resulting from a new cut, adds more microorganisms to the exposed tissue. The more common microorganisms occurring on fresh, meats include both bacteria and molds. Bacteria such as species of *Bacillus, Clostridium, Escherichia, Pseudomonas, Lactobacillus, Micrococcus, Streptococcus. Sarcina, Salmonella* occur most commonly. Molds that contaminate fresh meat include *Cladosporium, Geotrichum, Mucor, and Penicillium Sporotrichum* etc. Yeasts are less commonly occurring.

1.2.2. Eggs:

Clean eggs with uncracked shell normally do not contain microorganisms within. Poor sanitary and storage conditions under which it is held determine its subsequent microbial content. Bacteria and molds may enter the egg through cracks in the shell. The microbial flora recovered from the eggshells generally includes the species of bacteria *Micrococcus, Pseudomonas*, *Streptococcus, Staphylococcus, Sarcina*; and the molds.

1.2.3. Poultry:

The surface of freshly dressed eviscerated poultry has microbial flora, which is derived from the live birds or from the manipulations during killing, defeathering and evisceration. Species of *Bacillus, Enterobacter, Escherichia, Proteus, Pseudomonas, Salmonella; and Staphylococcus* constitute the major microbial flora on the skin of freshly dressed eviscerated poultry.

Prof.M.Ghosal

1.2.4.Fish:

The microbial flora of freshly caught fish usually reflects the microbial conditions of water from where they are harvested. Fish micro flora includes bacteria like *Alcaligenes, Micrococcus, Pseudomonas, Serratia, and Vibrio* etc. When the fish are cleaned and cut on shipboard under poor handling conditions, they invite more microorganisms to grow on it. These micro-organisms can be exemplified by the species of *Achromonobacter, Bacillus, Micrococcus*, and *Pseudomonas* etc.

Microbial Contaminations Of Processed Foods

The quality and quantity of microorganisms associated with processed food including baked and fermented ones depends upon the ingredients used and processing methods. Microorganisms present

Prof.M.Ghosal

Prof.M.Ghosal

in flour, sugar, fat, milk, egg, water, colors, man handling and the instruments etc. may contaminate the baked food products. Spore forming bacteria may escape destruction and become responsible for ropiness in breaked bread during baking process. The ropiness of the bread is caused by *Bacillus subtilis* or *Bacillus licheniformis*. Further, the baked products are subject to contaminate by molds such as *Mucor, Rhizopus, and Aspergillus* etc. Microorganisms through air, man and equipment contaminate the fermented foods like pickles. Most of these organisms do not multiply, as the reaction of the medium is considerably acid. Some yeasts and yeast-like forms such as Torula, Oidium etc., which are acid, tolerate establish in these foods on exposure.

3.0 Microbial Food Spoilage

Foods used by human being and animals are contaminated, as stated earlier, by heterotrophic microorganisms to meet their nutritional requirements. The undesirable alterations brought about in foods by such microorganisms are referred to as 'spoilage'.

3.1. Factors (conditions) That Invite Food-spoilage:

As stated earlier, the variety of variety of microorganisms contaminates natural food substances. The type of food substances and the methods by which they are processed and preserved favours the contamination. Most of the microorganisms prove to be potential contaminants and most of the foods serve as good media for microbial growth. These microorganisms, when given a chance to grow, bring changes in natural properties such as appearance, flavour, odour etc. of the contaminated food thus causing spoilage. Two major factors that invite spoilage of foods are the storage conditions and the chemical properties of the food.

3.1.1. Spoilage Due to Storage Conditions:

Temperature and oxygen are considered two most important factors that invite microbial contamination resulting in' spoilage of foods in storage conditions.

a. Role of temperature:

Foods stored at below - 17°C remain free from microbial growth and a slew decrease in their population may even take place. Above this temperature the presence and multiplication of microorganisms in food remain in existence. This is the reason why refrigerated foods are subject to spoilage by microorganisms. Foods and food-items stored at room temperature or in warm conditions remain open for spoilage by rnesophilic and thermophilic microorganisms.

b. Role of oxygen:

Aerobic and anaerobic condition plays an important role in determining the kinds of microorganisms, which can multiply and spoil various food and food-items in storage conditions. If oxygen is available, various aerobic bacteria and molds cause spoilage chiefly surface spoilage whereas if the conditions are anaerobic anaerobic bacteria like *Clostridium spp*. Etc.

Prof.M.Ghosal

Prof.M.Ghosal

3.1.2. Spoilage due to Food's Own Chemical Properties:

The chemical conditions of foods influence the type of microorganisms which can grow over it and. hence determine the nature of changes that would be brought by the spoilage action of contaminating microorganisms. Four major chemical conditions of food, e.g., composition, acidity, moisture and osmotic concentration are of the major importance in this type of spoilage.

a. Chemical composition:

(i) Foods rich in proteins are degraded by Proteolytic microorganisms. Proteins are degraded into its various components due to the action of especially gram-negative, spore forming bacteria, e.g., *Proteus, Pseudomonas*, some *cocci* etc.

Protein foods + Proteolytic Amino + Amines + Ammonia

microbes acids

(NH3) + Hydrogen sulfide (H2S).

(ii) Foods rich in carbohydrates are degraded by carbohydrate fermenting

microorganisms, particularly yeasts and molds. Bacteria like *Micrococcus, Leuconostoc* and *Streptococcus* can also degraded carbohydrates.

Carbohydrate + Carbohydrateà Acids + Alcohols +Gases

Foods Farmenting microorganisms

(iii) Foods rich in fats are attacked by relatively few microorganisms such as mold and some gram-negative bacteria. These microorganisms are therefore, lipolytic in nature.

Fatty foods + Lipolytic microorganisms à Fatty acids+ Glycerol

b. Acidity:

Generally the fruits are acid foods (pH below 4.5) while nearly all vegetables, fish, meats and milk-products are non-acid (pH above 4.5). Since the pH of the acid foods (fruits) is sufficiently low, they do not allow bacterial growth and subsequent spoilage. Mainly yeasts and molds spoil them. Contrary to this, non-acid foods have sufficiently high pH, they are spoiled mainly by bacteria.

c. Moisture and Osmotic concentration:

Average 13% free water is required in food for usual microbial growth. This is the reason why the foods

Prof.M.Ghosal

of high sugar and salt concentration do not allow most of the microorganisms to grow. But, specific microbial growths cannot be over-ruled. 65-70% sugar concentration is required to prevent mold-growth and 50% to prevent bacterial and yeast growth.

Types of Food-spoilage and Concerned Microorganisms:

Spoilage of Noncanned Foods

1. Fresh Fruits and vegetables **Soft rot** ***Rhizopus nigricans, Erwinia carocovera,***

***pseudomonus spp*.**

Black mold rot ***Aspergillus niger*,**

Gray mold rot ***Botrytis cinerea***

Blue mold lot ***Penicillium italjcum***

2. Pickles **Pink yeasts** ***Rhodotorula***

Black pickles ***Bacillus nigrieans***

Suit pickles ***Bacillus spp*.**

3. sugar products, Honey, Syrop **Ropy** ***Enteiobactet aeroqans***

Yeasty

a.Torula

b.Saccharomyces

c.Zygosaccharomyces

Moldy ***Asperqillus, Penicillium***

Pink ***Micrococcus roseus***

Prof.M.Ghosal

		Green	*Pseudomonas fluorescens*
4. Concentrated, Orange Juice	"Off" flavour		*Acetobacter, Lactobacters, Leuconostoc*
5. Bread		ropy	*Bacillus subtilis*
6. Bloody			*Serretis morcescens*
7. Moldy			*Aspergillus niger, Penicillium*
8. Fresh Meat	Putrefaction		*Aicaiiqenes, Clostridiurn , Chromobacterium*, *Proteus vulgaris, Pseudomonas fluorescens*
		Souring	*Chromobacterium*, *Chromobacterium*, *Pseudomonas*
9. Cured Meat	Moldy		*Asoerqillus, Penicillium, Rhizopus*
		Greening	*Lactobacillus, Pediococcus, Streptococcus*
		Souring	*Micrococcus, Pseudomonas*
		Slimy	*Leuconostoc*
10. Poultry	Slimy		*Alcaligenes, Xanthomonas*
		Odour	*Pseudomonas*

Prof.M.Ghosal

11. **Fish Putrefaction Alcaligenes** ***Bacillus,Micrococcus*****,**

Chromobacterium,Flavobacterium

Discolouration

Pseudomonas

12. **Eggs Green rot** ***Pseudomonas,fluorescens***

Colourlessrot

Pseudomonas,Alcaligenes**,**

Chromobacterium,Coliform bacteria

Fungal rote

Cladosporium,Mucor, Penicillium**,**

Sporotrichum

Black rot ***Proteus***

Spoilage of Canned Foods:

Canned foods may spoil either, due to biological or chemical reasons. We would discuss only the biological spoilage as it is the point at issue.

Biological spoilage of canned foods:

Biological spoilage of canned foods occurs due to the action of various microorganisms. Spore forming bacteria, e.g., *Clostridium, Bacillus* represent the most important group of canned food spoiling microorganisms because of their heat resistant nature (thermophilic nature). In addition, there are other microorganisms, which are not heat resistant (mesophilic) but enter through the leakage of the container during cooling and spoil the food. In this way we can divide biological spoilage of canned foods, into two following categories.

a. Biological spoilage by thermophilic bacteria:

Under processing of canned foods result in spoilage by thermophilic bacteria, the

bacteria that grow best at temperature of 50 degree C or higher. Five types of this spoilage can be recognized.

(i) Flat sour spoilage:

In this, type the spoilage can, not be detected unless the can is opened, the spoilage is caused by *Bacillus spp*, such as *B.coagulans* and *S.stearothermophilus* resulting in sour, abnormal odour, some times cloudy liquor in the food content of the can.

(ii) Thermophilic anaerobic (TA) spoilage:

Clostridium thermosaccharolyticum, an obligate thermophile, causes spoilage. The can swells and may burst due to production of CO2 and H2. The food becomes fermented, sour, cheesy and develops butyric odour.

(iii) Sulfide spoilage:

Clostridium nigricans is involved in this spoilage. It produces H2S gas which is absorbed by the food product which is usually blackened and gives “rotten egg” odour

(iv) Putrefactive anaerobic spoilage:

Clostridium sporogenes causes spoilage through putrefaction. The can swells and may burst. Putrefaction may result from partial digestion of the food. The latter develops typical "putrid" odour.

(v) Aerobic sporeformers’ spoilage:

Bacillus spp., the aerobic bacteria, causes spoilage. If the canned food is cured meat, swelling of the can is observed.

b. Biological spoilage by mesophilic microorganisms:

Bacillus spp., Clostridium spp., yeasts and other fungi, which are mesophilic (an organism growing best at moderate temperature range ± 25 to 40oC) are mainly responsible for this type of canned food spoilage. As stated earlier, these organisms enter through the leakage of the container during cooling.

Clostridium butyricum and *C. pasteurianum* result in butyric acid type of fermentation in acidic (tomato juice, fruits, fruit juices etc.) or medium acidic (corn, peas, spinach etc.) foods with swelling of the container due to the production of CO2 and H2.

Bacillus subtilis and *B.mesentroides* have been reported spoiling canned sea foods, meats etc. Other mesophilic bacteria, which have been reported in cans, are *Bacillus polymixa*, *B.macerans, Streptococcus sp., Pseudomonas, Proteus* etc. Yeasts and molds have also been found present in canned foods. Yeasts result in CO2 production and swelling of the cans.

4.0 Food Preservation

Foods are readily decomposed unless special methods are used for their preservation. All methods of food preservation are based upon one or more of the following principles -----

(i) Prevention or removal of contamination.

(ii) Inhibition of microbial growth and their metabolism.

(iii) Killing of microorganisms.

Various practices are used for food preservation now the days. These practices can be summarized as under:

- Heat treatment
- Low temperature treatment
- Dehydration
- Osmotic pressure treatment
- Chemical treatment
- Treatment by radiation

4.1. Heat Treatment:

High temperature is one of the most reliable and safest methods of food preservation. This treatment can be summarized under following three heads:

a. Pasteurization:

Pasteurization is used specially when the aim is to kill pathogenic organisms and where the spoilage organisms are not very heat resistant and the product cannot stand high temperatures. Since pasteurization does not kill all the microorganisms, it is necessary to store these products at low temperatures. Two methods of pasteurization are used: **high temperature short time (HTST) method and low temperature long time (LTLT) method**. The minimal heat treatment for market milk in 62.8°C for 30 minutes in the holding method and 71.7°C for about 15 seconds in the HTST method. Grape wines may be pasteurized for one minute at 81 to 85°C and grape juice at 76.7°C for 30 minutes.

b. Steam under pressure (Canning):

Steam under pressure, such as in pressure cooker, is the most effective method, since it kills all vegetative cells and spores. Such heatings to high temperatures by steam injection is followed by rapid cooling. The whole process of canning includes, cleaning, blanching, filling of cans or jars, sealing, autoclaving and cooling.

(i) Cleaning

Fresh vegetables, fruits or meat of high quality are selected and freed of all dirt and undesirable parts in order to reduce bacterial load and to preserve the best portion of the product.

(ii) Blanching

The cleaned food is subjected to hot water or steam treatment in order to affect blanching or scalding. This treatment kills part of microorganisms, fixes the natural colour of the product, and removes air within the product by expansion, which might otherwise help in decomposition. It does not allow effective autoclaving, and stops the activity of autolytic enzymes.

(iii) Filling of cans or jars

The hot food is then filled into cans or jars which are also kept hot in order to expand the food and remove air or gas trapped within. In fact steam occupies all the space that is not occupied by food. The containers are soon sealed.

(iv) Autoclaving

The cans so filled and sealed are subjected to, heat treatment in, the form of steam under pressure. Acid foods may require only immersion of containers in boiling water. Non-acid foods, however, need to be subjected to 15 lb pressure (116°C - 121°C) for one to two, hours. Meats may take longer time. The amount of time will also depend upon the load of the autoclave. Heavily loaded autoclaves will take more time.

(v) Cooling

As soon-as autoclaving is done, if is better to cool the container immediately. By doing so overcooking an undesirable changes in texture and flavour of the product may be checked. In addition thermophilic bacteria surviving heat treatment will not be allowed to germinate. The only dangerous spore forming bacterium, which survives the treatment is *Clostridium botulinum*

4.2 Low Temperature Treatment:

Temperature approaching 0° C and lower retard the growth and metabolic activities of microorganisms. Modern refrigeration and freezing equipment has made it possible to transport and store perishable foods for longer periods of time. Refrigerated trucks and railway cars, ship's storage vaults, and the home refrigerator and freezer have improved the quality of the human diet had increased the variety of foods available.

Any food that needs to be preserved must, be freed as best as possible from microorganisms before subjecting it to this method as low temperatures do not kill them but only inhibit their activity. Low temperature preservation includes two different methods viz, chilling and freezing.

a. Chilling:

This involves preservation of foods like meat, eggs, fish, vegetables etc. only for few days at a.

temperature between 4°C to 7°C. If foods are kept for longer periods undesirable changes due to active enzymes arid psychrophilic organism such as Pseudomonas fluorescence and some Micrococcus spp. take place-causing spoilage.

b. Freezing:

This process is used for preserving perishable plant and animal, products for long periods, from weeks to months, Before freezing, the foods are stored, trimmed washed and, blanched. Blanching consists, of immersing the food in boiling water or exposing it to live steam for minutes. Blanching destroys most of the microorganisms and inactivates enzymes that would alter the product even at low temperatures. The food is then immediately packaged and frozen. Quick, freezing which is preferred to slow freezing implies a freezing time of 30 minutes or less and the temperature between -180C to -34°C. Quick freezing produces smaller ice crystals and less damage, to the food tissues. Slow freezing, produce large crystals of ice, which rupture cell structures and causes extensive drip, or loss of fluid upon thawing.

Frozen fruits may be stored between -1°C and -18°C with little further change. It should be emphasized that freezing cannot be relied upon to kill all microorganisms no matter what the temperature. The number and types of viable and non-viable microorganisms present in frozen foods reflect the degree of contamination of the raw product, the sanitation in the processing plants, and the speed and care with which the product was processed. The microbial count of most frozen, foods decreases during storage; but many organisms, including pathogens, e g., and species of Salmonella, a survive for long periods of time at –9 and -17°C. However frozen food should be immediately used after thawing because the surviving microorganisms begin to multiply as soon as they are warmed. Frozen foods are not expected to lose their nutritional value but the flavour and aroma of fresh food is lost with the length .of storage period.

4.3. Dehydration:

Dried foods have been used for centuries, and they are more common through out the world than frozen foods. The removal of water by drying in the sun and air or with applied heat causes dehydration. Food products containing 10% or less of free moisture are not subjected to spoilage by microorganisms as their activity is suspended for want of moisture. The preservative effect of dehydration, is due mainly to microbistasis. The microorganisms are necessarily killed; their growth can be prevented, by reducing the moisture content of their environment below a critical level. The critical level is determined by the characteristics of the particular organism and the capacity of the food item to bind water so that it is not available as free moisture. Once dehydrated, the food should be kept in air-tight containers so that it is not exposed to fluctuations in humidity content of the atmosphere. Slight increase in moisture contents will permit growth of varies microorganisms such as molds and yeasts first and bacteria later, for dry foods are not free from contamination by microorganisms.

4.4. Osmotic Pressure Treatment:

When high osmotic pressures are built in a food by increasing its solute concentration, microorganisms having osmotic pressure relatively lower, are plasmolysed and eventually die.

Salt is widely used to preserve certain foods. The slating and bringing of fish, corning of beef, and bringing of green olives are examples of the use of high salt concentration. With the exception of halophiles, practically no multiplication of organisms occurs in salt concentration of 25% preservation of jellies, jams, maple syrup and honey is because of high sugar content. It is not uncommon to find mold growth on the surface of jelly which has been exposed to air. This may also be due to the condensation of evaporated water on the surface of the jelly to produce a layer of less concentrated sugar solution. Osmophilic yeasts occasionally grow in honey and produce sufficient carbon dioxide to burst the jar.

The food preserved by addition of salts or sugars should be kept in air-tight containers and stored in cool locations to avoid contamination by high osmotic pressure tolerating microorganisms such as yeasts and molds.

4.5 Chemical Treatment :

Chemical preservatives are added to kill or inhibit microorganisms in food. The may be incorporated into the foods or only their surface or the wrappers used for them may be treated, or they may be used as gas or vapours around the food. Some chemicals may be effective on selected group of microorganisms while others on a wide variety of them. Chemical preservatives may be harmless if they are added during the storage period and are removed before the food is consumed. But if they are consumed as such, they may be poisonous to man or animal, as well as to microorganisms.

4.5.1. Organic acids and their salts:

I. Several organic acids and their salts are common preservatives as they have marked microbiostatic and microbicidal action.

II. Benzoic acid and benzoate are used for the preservation of vegetables. Sodium benzoate is used in the preservation of jellies, jams, fruit juice and other acid for example Salicylic acid and salicylates are used as preservatives of fruits and vegetables in place of benzoate. However, it is considered to be deleterious to health of consumer.

III. Sorbic acid is recommended for foods susceptible to spoilage fungi, e.g., it inhibits mold growth in bread. Wrapping material for cheese may be treated with it. It is also used in sweet pickles and for control of lactic fermentations of olives and cucumbers.

IV. Foods prepared by fermentation processes, e.g. milk products etc. are preserved mainly by lactic, acetic and propionic acids.

V. Flavouring extracts of vanilla, lemons are preserved in 50 - 70% alcohol as it coagulates cell proteins.

4.5.2. Inorganic acids and their salts:

Most common among the inorganic acids and their salts are, sodium chloride, hypochlorites, sulphurous acids and sulphites, sulphor dioxide, sodium nitrate and sodium nitrite.

a. Sodium chloride

Sodium chloride produces high osmotic pressure and therefore causes destruction of many microorganisms by plasmolysis. It causes dehydration of food as well as microorganisms, releases disinfecting chlorine ion by ionization, reduces solubility of oxygen in the moisture, sensitizes microbial cells against carbon dioxide and interferes with the action of proteolitic enzymes. These are the reasons why this common salt is used widely for preservation either directly or in brine or curing solutions.

b. Hypochlorites

The hypochlorous acid liberated by these salts is an effective germicide provided the organic matter content of the medium is not high. It is oxidative in its action. The commonly used forms are sodium and calcium hypochlorites. Drinking water or water used for washing foods or icing them may be dissolved with hypochlorites.

c. Sulphurous acids and Sulphites

Sulphurous acids and sulphites are added to wines as preservatives. Sulphurous acid is used especially in the preservation of dry fruits. It helps in retention of original colour of the preserve and inhibition of molds more than either yeasts or bacteria. Potassium metabisulphite is used in canning.

d. Sulphur dioxide

Sulphur dioxide has a bleaching effect desired in some fruits, and also suppresses the growth of yeast and molds. It is used as a gas to treat drying fruits and is also used in molasses.

e. Nitrates and Nitrites

Nitrates and nitrites produce an inhibitory effect on bacterial growth and are used usually together in meat and fish preservation and for retention of red-colour of the meat. Nitrate is changed to nitrous acid which reacts with myoglobin to give nitric oxide myoglobin. It is the latter which gives a bright red colour to the meat making it more attractive in appearance. However, both nitrite and nitrate are poisonous, if present in potable water or food products in more than minimal amounts. It is why the generous use of these chemicals as preservative in meat and fish products has been questioned.

4.5.3. Antibiotics:

Aureomycin (chlorotetracycline) is the most commonly used antibiotic for the preservation of animal products under chilling conditions. It is extensively used for the preservation of poultry, meat and fish. The antibiotic is applied to the surface of the fresh meat by dipping it in a solution of the antibiotic or it may be fed to the animal, by mixing it with feed or water, for one to several days before slaughter. Fish are treated by adding the antibiotic in the ice or water in which they are to be transported.

The indiscriminate use of antibiotics as preservatives, however, should be prevented or the antibiotics used should be such that it is demobilised on cooking so that the internal flora of man using such food is not constantly exposed to the effect of the antibiotic. It is important for otherwise the use would lead to the development of the antibiotic resistant strains of microorganisms in the body. Aside from this, some individuals sensitive to antibiotics become exposed constantly to allergy.

4.6 Treatment By Radiation :

4.6.1 Gamma rays and high-energy electron beams:

Gamma rays and high-energy electron beams have been used for the preservation of fresh perishable canned and packaged foods. They have good penetration and are effective to a depth of about 15 cm in most foods. Food preservation by such radiation dosage is called ''cold-sterilization" as it produces only a few degrees rise in temperature of the product.

4.6.2.Ultraviolet ray:

Ultraviolet rays are short Waves and are used to sterilize the surface of foods. These rays have been successfully used for the treatment of water for beverages, aging meat's packaging pf sliced bacon, treatment, of knives for slicing bread, for sterilizing utensils, for prevention of spoilage by organisms on the surface of preserved pickles, cheese and prevention of air contamination. Gold-storage rooms of meat-processing plants are sometimes equipped with germicidal lamps which reduce the surface contamination and permit longer periods of spoilage-free storage.

Radiation pasteurization or sterilization represents a term which describes the killing of over 98% but not 100% of the microorganisms by intermediate dosage of radiation. This method increases the storage life of some meats, sea-foods, certain fruits and vegetables when stored at low temperature? Radiation pasteurization provides the possibility of an entirely new approach to food preservation and could bring about a radical change in industrial methods of food processing.

However, the effect of radiation on colour, flavour nutritional quality of food, odour, texture needs to be more carefully understood. Similarly, chemical changes in food products brought about by radiations may cause bad effects on animal and human subjects and need to be more adequately investigated.

5.0 Food-Borne Diseases Caused By Microorganisms :

"Food poisoning" is a term customarily applied to represent the illness caused both by the ingestion of toxins produced by the organisms in the food as well as resulting from the infection of the host by the organisms carried in by the food. But, more correctly, all food-borne diseases can be classified into two categories: 'food-poisoning' or 'food-intoxications' and 'food-infections'. Food-poisoning or food-intoxication diseases are those which are caused by the consumption of toxins produced by organisms in the food whereas food-infection diseases are those trial are caused by the organisms which enter into the body through ingestion of contaminated food.

5.1.Microbial 'Food-poisoning' or 'Food-intoxications':

5.1.1. Bacterial "Food-Poisoning" (Bacterial food-intoxications) :

There are two major food-poisonings or food-intoxications caused by bacteria. These are: **Bolutism and Staphylococoal-poisoning.**

a. Bolutism :

Bolutism is caused by the ingestion of food containing the neurotoxin (toxin that affects the nervous system) produced by Clostridium bolutinum, an anaerobic spore forming bacterium. Sixty to seventy percent-cases of bolutism die. There are 7 types (type A,B,C, D,E,F,G) of these neurotoxins recognized on the basis of serological specificity. The neurotoxin of C. bolutinum is a protein. It has been purified and crystallized and is so powerful that only a does as low as 0.01 mg is said to be fatal to human being. The toxin is absorbed mostly in the small intestine and paralyzes the involuntary muscles of the body.

Serological Types of Bolutism Neutotoxins :

1. Type A : It commonly causes human bolutism in the western part of the United States and is more toxic than type B.
2. Type B : This type occurs more often then type A in most soils of the world end is less toxic to human being.
3. Type C : So far as is known, this type causes bolutism of cattle, fowls, and other animals but not of human beings.
4. Typed D : This type has been reported causing forage poisoning of cattle in South African Countries.
5. Type E : This type ties been obtained chiefly from fish and fish products and is toxic for human being.
6. Type F : This type has been isolated in Denmark end causes human bolutism.
7. Type G : It has recently been isolated from the soil in Argentina. It does not concern with human bolutism.

Source :

The main sources of bolutism are canned meat, fish, string beans, sweet corn, beets and other low medium acid foods. The foods implicated are generally those of a type that have undergone some treatment intended for the preservation of the product such as canning, pickling or smoking, but one which failed to destroy the spores of this bacterium. When the intended preservative treatment is inadequate and is followed by storage conditions which permit the germination and growth of the microorganisms, one of the most lethal toxins known to humanity is produced, The toxin has been known to persist in foods for long periods, especially when storage has been at low temperatures. It is unstable at pH value above 6.8.

Temperature is considered to be the most important factor in determining whether toxin production will take place and what the rate of production will be. Various strains of *C.bolutinum* types A and B vary in their temperature requirements; a few strains grow at 10 to 11 C.However, the lowest temperature for germination of spores of most of the strains is 15°C and maximum of 48°C.

Symptoms :

Symptoms generally occur within 12 to 36 hours after consumption of the spoiled food. Early symptoms are digestive disturbances followed by nausea, vomiting, diarrhoea together with dizziness and headache. Double vision may occur early and there may be difficulty in speaking. Mouth may become dry, throat constricted, tounge may get swollen, and coated. Involuntary Muscles become paralysed and paralysis spreads to the respiratory system and to the heart. Death normally results from respiratory failure

Prevention :

- Canned food should be properly processed by using approved heat processes.
- Avoiding food that has been cooked but not well heated.
- Raw foods, frozen foods thawed and held at room temperature should be avoided.
- Gassy and spoiled canned foods should be rejected.
- Boiling of suspected food for at least 15 minutes.

Treatment :

Successful treatment is by the administration of polyvalent antitoxin in the early stages of infection. Once the symptoms appear the fails to prove useful.

Staphylococcal-poisoning :

This is the most common type of food-poisoning caused due to the food contaminated with a potent toxin namely, enterotoxin. This toxin is produced by certain strains of *Staphylococcus aureus.* A sudden onset of illness starts usually within 3 to 6 hours after ingestion of the contaminated food.

Source :

These bacteria are commonly present on the skin, nose and other parts of human body. People who handle foods carelessly usually transfer them to the food. Foods most commonly contaminated involve those which are eaten cold, e.g., cold meat, poultry, salads, bakery products etc.

Symptoms :

As said earlier, the disease starts within 3 to 6 hours after ingestion of the contaminated food and is manifested by nausea, vomiting, abdominal pain and diarrhoea within 24 to 48 hours. If the case becomes severe, dehydration and collapse may follow. However, in usual conditions death is rare.

Control :

The disease can be controlled by preventing the entry of the bacteria to food. It is important that all susceptible foods are kept under refrigeration to restrict the growth of the bacteria; and also by the destruction of the bacteria.

Fungal Food-poisoning (Mycointoxications) :

Mycotoxins are chemical substances produced by a variety of fungi, e.g., *Aspergilli*, *Penicelli, Rhizopus, Fusarium spp.* and mushrooms. The illness that results from the ingestion of foods containing fungal toxins is called mycotoxicosis. Mycologists have come to discover a number of mycotoxins which have proved extremely harmful, sometimes lethal, to animals, and human beings. Important ones are as follows:-------

a. Aflatoxins :

"Aflatoxins are one of the most potent mycotoxins produced by Aspergiius flavus and related strains. It has been found that about 60% strains of A.flavus of produce this toxin. The discovery of aflatoxins is comparatively of recent origin. In1960, about l00,000 Turkey poults died in England within few months. It was found that the peanut meal fed to them was heavily contaminated with A.flavus. The chemical substance isolated from such peanut meal was found toxic and was named 'aflatoxin'. However, some other fungi, e.g., *Aspergillus niger, A.Oryzae, A.ochraceus, Penicilliurn citrinum* etc. have also been reported to produce aflatoxins. So the name aflatoxin is now generally used for a number of related toxins.

Aflatoxins occupy the most important position among mycotoxins because of their potent carcinogenic nature and high frequency of occurrence in nature. More specifically, aflatoxin B1 is one of the most potent aflatoxins. They are responsible for liver cancer in laboratory animals and even human beings.

b. Amatoxins and Phallotoxins :

These two mycotoxins are considered to be produced by the mushroom Amanita phalloides, the so called 'death Cup'. This mushroom is deadly poisonous and almost about 90 to 95% deaths of mushroom-eaters in Europe have been due to eating of this fungus. These two mycotoxins are chemically cyclopeptides. According to Lincoff and Mitchel (1977) the most potent amatoxins are **a-amanitin and ß-amanitin** while the phalloidin is the most potent phallotoxin. However, studies reveal the fact that these are the amatoxins which are strongly poisonous comparatively, and are responsible for producing hypoglycemia, liver distrophy and kidney-failure, leading to the death of the victim.

c. Coprine :

This mycotoxin is thought to be present in an edible mushroom namely *Coprinus atramentanius.* This chemical becomes toxic and results in gastrointestinal upsets and other physical discomforts when the mushroom eating is accompanied with alcohol.

d. Gyromitrin :

Gyromitrin, the chemical monomethylhydrazine is deadly poisonous mycotoxin reported to be present in the fruiting bodies (basidiomata) of saddle fungi (*Helvella spp*.) and false morels (*Gyromitra spp*.). This toxin is water soluble. It is thought that if the fruiting bodies be parboiled two or three times and the liquid discarded, the mushrooms become safe to eat.

e. Ochratoxins :

Ochratoxin was first isolated from the culture filtrates of *Aspergillus ochraceus* and is now produced by a number of *Aspergillus* and *Penicillum spp*., with *Penicillium viridicatum* being the dominating producer. These mycotoxins represent a group of closely related derivatives of isocoumarin linked to **L-ß-phenylalanine, an amino acid**, and are reported mainly in temperate area of North America and Europe. Ochratoxins occur mainly in grains but have also been reported in coffee, beans and peanuts and are toxic to ducklings, chicks and rats.

f. Trichothecenes :

Trichothecenes are produced by the species of *Fusarium, Cephalosporium, Myrothecium*, *Trichoderma* and *Stachybotrys*. Out of 30 known trichothecenes, T-2 toxin, nivalenol and deoxynivalenol are of common occurrence, and cause a hyperestrogenic syndrome, hemorrhage and ometimes abortion in swine.

g. Zearalenone :

This mycotoxin is produced by a number of Fusarium spp., e.g., *F.graminearum* and *F.moniliforme*. It occurs predominantly in maize and is similar to trichothecenes in its effects.

h. Algal Food-poisoning (Phycointoxications) :

Fish or Shellfish, e.g., Calms, Scallps, Mussels etc. ingest algae such as *Gymnodynlum, Gonyaulax* and others. These algae are toxic and result in fish poisoning in humans. Shellfish harvested on large scales are routinely assayed to check whether these toxic alga are present in them or not

5.2 Food Infections :

5.2.1. Bacterial Food Infections:

a. Salmonellosis:

This disease is caused through the ingestion of *Salmonella* bacteria present in food. A large number of species and serotypes are involved. An inoculum of about 600,500 cells is required to become

established and cause illness in the host. These bacteria are gram-negative, non-spore forming rods and motile by means of peritrichous flagella. Various species of *Salmonella* get ingested with improperly cooked eggs, puddings and meat that have been contaminated by the carriers. The carriers may be cats, dogs, chickens and others.

The disease appears through gastrointestinal infections as a result of the growth of the bacteria in the intestine. Typical symptoms of salmonellosis are nausea, vomiting, abdominal pain and diarrhoea. Generally the symptoms persist for 2 to 4 days. The incubation period ranges between 4 to 36 hours.

Salmonellosis can be prevented by avoiding consumption of contaminated food, by heat destruction of the bacteria, or by refrigeration to check the growth of bacteria.

b. Perfringens poisoning:

The disease caused by the strains of *Clostridium Perfringenes (=C.welchii*) is called 'perfringens poisoning' or more technically, '*Clostriclium perfringens-gastroenteritis'*. This bacterium is a gram-positive, anaerobic non-motile, spore former with an optimum growth temperature of37-43°C.

This disease has been caused by the ingestion of prepared meat, meat products and poultry. Generally, the meat that has been cooked and allowed to cool slowly before consumption allows the growth of these microorganisms. What happens is that the cooking destroys only the vegetative cells not the spores. The latter survive the heating applied during cooking and germinate into vegetative cells. It could be avoided by adequate refrigeration of the food.

Symptoms:

Symptoms appear in the form of diarrhoea, acute abdominal pain and, rarely, vomiting when the, growth of microrganisms takes place in the human intestine. Disease manifestation occurs between 8 to 22 hours after the contaminated food has been taken.

Prevention:

Prevention of the disease includes rapid cooling of cooked meats and other foods and reheating of the remaining food before further consumption.

c. *Bacillus cereus* gastroenteritis:

Bacillus cereus is a gram-positive, aerobic, rod-shaped, spore forming bacterium that causes food infections called 'gastroenteritis'. Its spores are heat resistant and remain viable even after considerable degree of cooling; germinate and produce vegetative cells. It is believed that the bacterial cells undergo lysis in the intestinal tract and release enterotoxin.

d. *Escherichia coli* gastroenteritis:

Escherichia coli bacterium is generally regarded as a part of the natural flora of the human and animal intestinal tract. In recent years, however,various serotypes of this bacterium have been thought

responsible for human and animal diarrhoeal diseases. These bacteria can be classified into two groups: one group representing enteropathogenic *E.coli* and the other representing enterotoxin producing *E.coli*.

The enteropathogenic *E.coli* are pathogenic within the intestinal tract. They have ability to penetrate epithelial cells of the intestinal mucosa, cause spithelial necrosis and ulceration resulting in the presence of red blood cells and large number of neutrophils in the stool during dysentery. This acute gastroenteritis (dysentery-like syndrom) is generally reported in the new-born and in infants upto two years of age.

The enterotoxin-producing *E.coli* fail to invade the intestinal mucosa but release an enterotoxin which causes diarrhoea-like syndrom. The latter refers to a profuse watery discharge generally from the small intestine. Since these bacteria do not penetrate and cause epithelial necrosis, red blood cells and neutrophils are not present in the diarrhoeal stool.

Foods which are highly contaminated or inadequately preserved allow the growth of such *E.coli* serotypes. The latter are heat sensitive and can be destroyed by pasteurization or by proper cooking methods.

e. Cholera:

This disease, generally called 'asiatic cholera', is caused by ***Vibrio cholerae*** and has been the cause of untold suffering and death. The symptoms include vomiting and profuse diarrhoeal (rice-water) stools which result in mineral deficiency, dehydration and increased blood acidity of the body tissues leading, finally, to the death.

Vibrio cholerae is a gram-negative, uniflagellate bacterium and is transmitted through contaminated flies, water, raw and exposed foods etc. They find their way through mouth into the intestines and produce endotoxins which disintegrate the epithelial cells of the intestines. Death rate is rather high and the course of the disease may be as short as 12 hours after the onset of the first symptoms. Individuals recovering from infection are said to be effective in controlling the disease. Cholera patients should be kept in quarantine and all materials contaminated by faeces burnt for checking infection spread.

<u>Non-bacterial Food Infections:</u>

a. Amoebiasis:

Entamoeba histolytica is the most important of various protozoa that cause intestinal diseases. These protozoa are transmitted from person to person through infected food and water, by direct contact and by flies.

Symptoms:

Disease symptoms range from abdominal discomfort with slight diarrhoea alternating with conspitation

to severe dysentery with blood and muscus in the stools. Abscesses may be developed on the lungs or lever or even in the brain.

Control:

This disease can be controlled by recognizing the chronic cases from which infective cysts of the amoeba may be transmitted and by proper sanitation and personal hygiene.

b. Viral Food Infections:

Several viruses are known to be transmitted through food and water and cause infection in humans and animals. For instance, Poliomyelitis is reported to be a food-borne viral infection carried through milk. Hepatitis virus is carried through sewage contaminated water and sea-foods causing 'viral hepatitis', a common, acute, systemic infectious disease chiefly affecting the liver that results in 'jaundice'.

6.0 Fermented Food Items :

Numerous food items are produced by fermentation processes in which one or more kinds of microbes are responsible for the characteristic flavour, texture and quality of the product. The microorganisms that produce the desirable changes may be the natural flora on the material to be fermented or may be added as 'starter' cultures.

6.1. Bacteria Fermented Food Items:

Important food items produced in whole or in part by bacterial fermentations are sauerkraut, pickles, sausage, olives etc. for humans and ensilage for animal fodder. Lactic acid bacteria are mainly responsible for the desirable type of fermentation (lactic acid fermentation) required for the production of each of these substances though other microorganisms are also involved.

a. Sauerkraut:

This food item results from a fermentation of cabbage by bacteria which are normally present on the cabbage. The cabbage is cleaned, shredded, bruised by tamping, and is packed with about 2% of salt in jars or barrels. Wooden frames are placed on the cabbage to create pressure. The combined action of salt and pressure draws from the cells of the leaves a considerable proportion of their juice. The cells of the leaf respire for a time, rapidly utilizing any oxygen present and creating anaerobic conditions. *Enterobacter* and *Erwinia sp.* initiate fermentation which is accelerated by *Leuconostoc mesenteroides* producing 0.7 to 1.0% lactic acid. *Lactobacilli* (*Lactobacillus plantarum*) then start multiplying and continue the fermentation in the final stage increasing the acidity to as much as 2.4% lactic acid. Acids, esters and diacetyl give pleasant aromas and flavours. Fermentation requires three to four weeks at 21 to 29 C. In the preparation of sauerkraut a large proportion of the antiscorbutic vitamins are preserved. Even in the early part of the eighteenth century it was urged that all ships carry a quantity of sauerkraut and that some be supplied daily to all members of the crew to prevent scurvy.

b. Dill Pickles:

Dill pickles are prepared by washing cucumbers, packing them in a cask with alternating layers of dill leaves, covering with water, and adding salt spices and sometimes sugar. The soluble sugars of the cucumber gradually diffuse out and are fermented with lactic acid bacteria. The early fermentation is initiated by *Leuconostoc mesenteroides*, *Streptococcus faecalis* and *Pedicoccus cerevisiae* and is continued in the final stage by *Lactobacillus brevis* and *L.plantarum* producing lactic acid. The latter accumulates in sufficient quantities to inhibit the growth of putrefactive bacteria. Fermentation proceeds under favourable conditions for 6 to 8 weeks finally resulting in a change in the colour, consistency and flavour of food item.

c. Sour bread:

This bread is a sour dough, from which a 'starter' is shaved to inoculate the next batch. The microorganisms appear to be *Escherichia coli* and Enterobacter species which produce a mixed lactic acid fermentation i.e., accompanying the gas there is always some lactic acid which tends to make the bread taste sour.

d. Salt-rising bread:

This type of bread is dependent upon the spontaneous fermentation of (probably) wild yeasts and common contaminating bacteria, e.g., *E.coli* and Enterobacter types. In this case salt is added to the bread, which cuts down some of the extranious contamination and one simply lets it ferment by itself.

e. Idli:

Idli is prepared from parboiled rice and decuticled black-gram dal (*Phaseolus mungo*) and represents a popular break-fast dish of South India. Soaked ingredients are thoroughly mixed, little salt is added, and then the mixture is allowed to ferment overnight for 10 to 12 hours. Predominant microorganisms that bring fermentation are *Leuconostoc mesenteroides*, *Streptococcus faecalis* and *Pedicoccus cerevisiae*. The fermented material increases in volume and gets sour. It is then cooked by baking in steam resulting in a soft, spongy product having sour flavour and good taste.

f. Green olives:

These food items are prepared by the lactic acid fermentation of olives. *Leuconostoc mesenteroides* start the early fermentation which is continued by *Lactobacilus brevis* and *L.plantarum.*

g. Sausage:

Beef and pork are fermented by lactic acid bacteria, e.g., *Pedicoccus cerevisiae* and *Microccus spp.* to prepare sausage.

h. Ensilage:

The ability of microorganisms to produce acids, particularly lactic acid, may be utilized also for the processing and preservation of feeds for animals in the preparation of ensilage (silage). Forage crops, sugarcane, sorghum, corn (maize) and potatoes are used as raw materials. Microorganisms of many

kinds, e.g., bacteria belonging to family Enterobacteriaceae, *Clostridium butyricum*, *Lactobacillus spp.* and *Streptococcus lactis* start to grow in the plant juices and ferment carbohydrates into organic acids.

Non-bacteria Fermented Food Items :

a. White or common bread:

In this bread preparation the moistened flour is mixed with yeast, *Saccharomyces cerevisiae*, and is allowed to stand for several hours in a warm place. Flour itself contains little free sugar, but there are sufficient quantities of starch splitting enzymes in it to produce some sugar during the leavening process. The sugar is rapidly fermented by the yeast with the production of alcohol and CO2, the latter causing the rising of the bread. During the baking process the alcohol is driven off.

b. Oriental Food:

In oriental countries (eastern countries of the World) large quantities of diverse food are prepared from soybean, wheat and rice through inoculation with members of mucorales, various yeasts and *Aspergillus oryzae*. Food of this type includes 'Tempeh' - a solid food prepared with soybeans processed with species of *Rhizopus*; 'Sufu'-a Chinese cheese prepared from soybeans and Actinomucor elegans and *Mucor spp*. 'Miso'(for soy-sauce) prepared from soybeans and wheat fermented with *Aspergillus oryzae* and other microorganisms.

Bread

Flours and meals for the preparation of bread are usually made from wheat or rye, occasionally from maize or barley. They are all high in starch, and the first two contain a considerable proportion of protein, commonly designated as **'gluten'**. In addition there are traces of sugar and some diastase. Flour is mixed with water to form a dough and for some types of bread a little sugar is also added.

The series of changes which occur in the flour and other constituents of the dough before baking into bread is termed 'panary fermentation'. An alcoholic fermentation by yeast is an essential step in the production of bread; this process is known as the 'leavening of bread'. A product of action of microorganisms is involved in the production of bread.

There are three basic types of rising breads –

White or common bread

In this bread preparation the moistened flour is mixed with yeast, *Saccharomyces cerevisiae*, and is allowed to stand for several hours in a warm place. Flour itself contains little free sugar, but there are sufficient quantities of starch splitting enzymes in it to produce some sugar during the leavening process. The sugar is rapidly fermented by the yeast with the production of alcohol and carbon dioxide, the latter causing the rising of the bread. During the baking process the alcohol is driven off.

Sour bread

This bread is a sour dough, from which a 'starter' is saved to inoculate the next batch. The organisms appear to be *Escherichia coli* and Enterobacter species which produce a mixed lactic acid fermentation i.e. accompanying the gas there is always some lactic acid which tends to make the bread taste sour.

Salt-rising bread

This type of bread is dependent upon the spontaneous fermentation or (probably) wild yeasts and common contaminating bacteria, *E.coli* and Enterobacter types. In this case salt is added to the bread, which cuts down some of the extraneous contamination and one simply lets it ferment by itself.

7.1. Abnormal fermentations of bread:

The most common abnormal fermentations in bread are:

Undesirable high acidity

Too high acidity may develop as the result of the growth of lactic bacteria for too long a period in the dough before baking, the bread becoming unpalatable as the result of the sourness.

Ropiness

Ropiness results from the growth, after baking, of certain highly resistant spore-producing bacteria. Several such species have been described which are mostly the varients of *Bacillus subtilis*. They are probably present in most bread, but do not develop and cause rope except when there has been too little development of acid in the leavening process and the bread has been stored at a relatively high temperature. In other words, in order to prevent rapid deterioration of the bread after baking, it is necessary that a certain amount of acid be present in the dough. This is usually formed as a result of the growth of certain lactic acid species.

Bloody bread

Some bacteria may form coloured spots or areas in the bread. *Serratia marcescens* (sometimes termed *Bacillus prodigiosus*) produces a red pigment. The red spots were interpreted before the development of modern science as spots of blood, hence the name bloody bread.