LECTURE-1
Microbial spoilage of foods.

Cause of spoilage:
Spoilage may be due to one or more of the following:-
1. Growth and activity of micro organisms
2. Insects
3. Action of the enzymes of the plant or animal food.
4. Purely chemical reactions i.e., those not catalysed by enzymes of the tissues or of micro organisms
5. Physical changes such as those caused by freezing, burning, drying, pressure etc.

Classification of foods by ease of spoilage:
1. Stable or non perishable foods:
These foods which do not spoil unless handled carelessly include such products as sugar,flour and dry beans.
2. Semi perishable foods:
If these foods are properly handled and stored, they will remain unspoiled for a fairly long period.
Ex: Potatoes, apples etc.
3. Perishable foods:
It includes most important daily foods that spoil readily
Ex: Meats, fish, poultry, milk, eggs, fruits and vegetables.

Factors affecting the growth of micro organisms in food:
Associative growth:
Associations of micro organisms with each other are involved in spoilage or fermentation of most foods.
Competition between the different kinds of bacteria, yeasts and molds in a food ordinarily determines which one will outgrow the others and cause its characteristic type of spoilage.If conditions are favourable for all, bacteria usually grow faster than yeasts and yeasts faster than molds.
Micro organisms are not always **antagonistic** or antibiotic to each other, however and may sometimes be symbiotic i.e., mutually helpful.
Some times they are **synergistic** i.e., when growing together they may be able to bring about changes such as fermentations.

Ex: *Pseudomonas syncyanea* growing alone in milk produce light

brownish tinge and
streptococcus lactis no color change in milk. When two organisms grow together, a bright blue color develops resulting from PH effect on the brown pigment produced by *P. syncyanea*.

Metabiosis in which when one organism makes conditions favourable for growth of the second. Both organisms may be growing at the same time, but more commonly one succeed the other.
Ex: Raw milk at room temperature normally first supports an acid fermentation by *streptococcus lactis* and *coli form bacteria* until the bacteria are inhibited by the acid they have produced. Next the acid folerant *lactobacilli* increase the acidity further until they are stopped. Then film yeasts and molds grow over the top, finally reducing the acidity so that proteolytic *bacteria* can become active.

Effect of environmental conditions:
Environment determines which the different kinds of micro organisms in a food will
outgrow the others and causes its characteristic type of change or spoilage. Chief among these factors are the intrinsic or chemical properties and extrinsic or physical properties of the food.

Factors affecting the growth and survival of micro-organisms in foods.

Intrinsic parameters:
The parameters of plant and animal tissues that are inherent part of the tissues are referred
to as intrinsic parameter. These parameters are as follows:

1. **pH:**
PH: It is the negative logarithm of the hydrogen ion activity.

pH = Hydrogen ion activity
Every micro organism has a minimal, a maximal and an optimal pH for growth.
Bacteria grow fastest in the pH range 6.0 – 8.0, yeasts 4.5 – 6.0 and filamentous fungi 3.5 – 4.0.

Inherent acidity:
Some foods have a low pH because of inherent property of the food. Ex: Fruits &
vegetables.

Biological acidity:

Some foods develop acidity from the accumulation of acid daring fermentation. Ex: curd, sauerkraut, pickles etc.
Molds can grow over a wide range of pH vales than the yeast and bacteria. Film yeasts grow well on acid foods such as sauerkraut and pickles. Most yeasts do not grow well in alkaline substrates. Bacteria which are acid formers are favoured by moderate acidity. Active proteolytic bacteria, can grow in media with a high pH (alkaline.) Ex: Egg white. The compounds that resist changes in pH are important not only for their buffering capacity but also for their ability to be especially effective within a certain pH range.but also for their ability to be especially effective within a certain pH range.

Vegetable juices have low buffering power, permitting an appreciable decrease in pH with the production of small amount of acid by lactic acid bacteria during the early part of sauerkraut and pickle fermentations. This enables the lactics to suppress the undesirable pectin –hydrolyzing and proteolytic competing organisms. Low buffering power makes for a more rapidly appearing succession of micro-organisms during fermentation than high buffering power.
Egg white where the pH increases to around 9.2 as CO2 is lost from the egg after laying. **Fish spoil more rapidly than meat under chill conditions.** The pH of mammalian muscle, round 5.6 and it is lower than that of fish (6.2 - 6.5) and this contributes to the longer storage life of meat.
The ability of low pH to restrict microbial growth has been employed since the earliest times in the presentation of foods with acetic and lactic acids.
Fruits are acidic than vegetables pH of milk – neutral. Fruits generally undergo mold and yeast spoilage than vegetables.

2. **Redox potential (Eh): - Oxidation – reduction potential:**

Oxygen tension or partial pressure of oxygen about a food and the O R potential or
reducing and oxidising power of the food itself, influence the type of organisms which will grow and hence the changes produced in the food. The O R potential of the food is determined by:
1. Characteristic O R potential of the original food.
2. The poising capacity i.e., the resistance to change in potential of the food.

3. The oxygen tension of the atmosphere about the food.
4. The access which the atmosphere has to the food.
Head space in an “evacuated” can of food contain low oxygen tension compared to air.
Micro organisms are classified as aerobic, anaerobic, and facultative based on the Acinetobacter requirement of O2.
Molds – aerobic
Yeasts – Aerobic and facultative.
Bacteria – Aerobic, anaerobic and facultative.

High O - R potential favours aerobes and facultative organisms.
Low O-R potential favours anaerobic and facultative organisms.

However some aerobes grow at low O-R potential O-R potential of a system is usually written Eh and measured and expressed in terms of millivolts (mv).
Highly oxidised substrate would have a positive Eh and a reduced substrate have a negative Eh. Aerobic microorganisms require positive Eh. Ex: Bacillus, Micrococcus, Pseudomonas and.
Anaerobic micro organisms required negative Eh. Ex: Clostridium
Most fresh plant and animal foods have a low and well poised O – R potential in their interior because plants contain reducing substances like ascorbic acid and reducing sugars where as animal tissues contain –SH (Sulf hydryl) and other reducing groups. As long as the plant or animal cells respire and remain active, they have low level of O-R potential. Meat could support the aerobic growth of shine forming or souring bacteria at the same time that anaerobic putrefaction was proceeding in the interior.Heating and processing may alter the reducing and oxidising substances of food.
Ex: Fruit juices lost reducing substances by their removal during extraction and filtration by their removal during extraction and filtration and therefore have become more favourable for the growth of yeasts.

3. Nutrient content:
Food is required for energy and growth of micro organisms. Carbohydrates especially the sugars are commonly used as an energy source. Complex carbohydrates such as cellulose can be utilized by few organisms and starch can be hydrolysed by any a limited number of organisms. Many organisms cannot use the disaccharide lactose (Milk sugar) and therefore do not grow well in milk.

Maltose is not attacked by some yeasts. Some micro organisms hydrolyze pectin of the fruits and vegetables.
Limited number of micro organisms can obtain their energy from fats by producing lipases. Aerobic their energy from fats by producing lipases. Fats are hydrolyzed to glycerol and fatty acids. Aerobic micro organisms are more commonly involved in the decomposition of fats than are anaerobic ones and the lipolytic organisms usually are also proteolytic. Hydrolysis products of proteins, peptides and amino acids serve as an energy source for many proteolytic organisms when a better energy source is lacking. Meats are decomposed by proteolytic sps Ex: *Pseudomonas sps.* Concentration of food in solution increases the osmotic effect and amount of available moisture. Molds & yeasts can grow in the highest concentrations of sugars. Bacteria can grow best in low concentration of sugars.Micro organisms differ in their ability to use various nitrogenous compounds as a source of nitrogen for growth. Many organisms are unable to hydrolyze proteins and hence cannot get nitrogen from them. Peptides, aminoacids, urea, ammonia and other simpler nitrogenous compounds may be available to some organisms but not to others. These compounds may be used under some environmental conditions but not under other conditions.
Ex: Some lactic acid bacteria grow best with polypeptides as nitrogen foods, cannot attack casein.
Some microorganisms use fermentable carbohydrates and results in acid production which suppresses the proteolytic bacteria and hence it is called **sparing action** on the nitrogen compounds. Many kinds of molds are proteolytic but very few yeasts are actively proteolytic. Proteolytic bacteria grow best at pH values near neutrality and are inhibited by acidity. Carbon for growth may come partly from CO2 and also from organic compounds. Minerals required by microorganisms are always present in low level. Sometimes an essential mineral may be unavailable, lacking or present in insufficient amounts. Ex: Milk contains insufficient iron for pigmentation of the spores of *Penicillium roqueforti.*
Accessory food substances or vitamins needed by the organisms. Some micro organisms are unable to manufacture some vitamins.Meats are high in B vitamins and fruits are low, but fruits are high in ascorbic acid. Egg white contains biotin but also contains avidin. This avidin ties up biotin mating it unavailable to microorganisms and eliminating possible spoilage organisms. Thiamine, pantothenic acid, folic acid group and ascorbic acid are heat labile and drying causes loss in these compounds.

Storage of foods for long periods may result in decrease in level of the accessory growth factors. Each kind of bacterium has a definite range of food requirements. Some micro organisms can use other carbon compounds such as organic acids and their salts, alcohols and esters.
Pseudomonas spp may be satisfied by simple compounds such as ammonia or nitrates or more complex compounds such as amino acids, peptides or proteins.

LECTURE-2

4-Inhibitory substances and biological structure:

Inhibitory substances: These originally present in the food or added purposely to prevent growth of micro organisms. The stability of some foods against attack by microorganisms is due to the presence of certain naturally occurring substances that possess and express antimicrobial activity. Some plant species are known to contain **essential oils** that possess antimicrobial activity. Among these are eugenol in cloves,

allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allyl isothiocyanate in mustard, eugenol and thymol in sage, and carvacrol (isothymol) and thymol in oregano.

Cow's milk contains several antimicrobial substances, including lactoferrin, conglutinin, and the lactoperoxidase system (see below). Milk casein as well as some free fatty acids have been shown to be antimicrobial under certain conditions.

Lysozyme present in **milk and egg** ,it is most active against gram positive bacteria.

Egg is contains Ovotransferrin, avidin and ovolflaroprotein, Ovoflavo protein and avidin in egg white which sequester biotin and riboflavin restricting the growth of those bacteria.

Propionic acid produced by the propionibacteria in a swiss cheese is inhibitory to molds. Nisin produced by certain strains of *Streptococcus lactis* may be useful in inhibiting lactate fermenting, gas forming clostridia in curing cheese. Heating foods may result in the formation of inhibitory substances. Ex: Heating lipids may hasten auto oxidation and make them inhibitory.

Browning concentrated sugar syrups may result in the production of furfural and hydroxyl methyl furfural which are inhibitory to fermenting organisms.

Lactoperoxidase System

This is an inhibitory system that occurs naturally in bovine milk, and it consists of three components: lactoperoxidase, thiocyanate, and H_2O_2.

The milk enzyme lactoperoxidase will catalyse the oxidation of thiocyanate by H2O2 to produce Hypo-thiocyanate. This can kill gram negative bacteria and inhibit gram positives.

Biological structures of food on the protection of foods against spoilage has been observed.

Ex:

1) Inner parts of healthy tissues of living plants and animals are sterile or low in microbial

content.

2) Protective covering on the food like shell on egg, skin on poultry, shell on nuts, rind or skin on

fruits and vegetables, artificial coating like plastic or wax.

3) Layers of fat over meat may protect the part of the flesh or scales may protect the outer part of

the fish.

5-Water activity:

Micro organisms have an absolute demand for water. Without water, no growth can occur. The exact amount of water needed for growth of micro organisms varies. This water requirement is best expressed in terms of available water or water activity (a_w).

a_w for pure water is 1.00

The water activity (a_w) of most fresh foods is above 0.99.

Low aw – decrease in the rate of growth of organisms.

In general, bacteria require higher values of *a*w for growth than fungi, with Gram-negative bacteria having higher requirements than Gram positives. Most spoilage bacteria do not grow below *a*w = 0.91, whereas spoilage molds can grow as low as 0.80.

Effects of Low a_w

The general effect of lowering a_w below optimum is to increase the length of the lag phase of growth and to decrease the growth rate and size of final population. This effect may be expected to result from adverse influences of lowered water on all metabolic activities because all chemical reactions of cells require an aqueous environment.

Factors that may affect water activity (aw). include the following.

1. The kind of solute employed to reduce aw. Potassium chloride usually less toxic than NaCl.

And less inhibitory than sodium sulphate.

2. The nutritive valve of the culture medium. The better the medium for growth, the lower the

limiting aw.

3. Temperature: Most organisms have the greatest tolerance to low aw at about optimal tsemperatures.

4. Oxygen supply: Growth of aerobes takes place at lower aw in the presence of air than in its absence.

5. pH Most organisms are more tolerant of low aw at PH valves near neutrality than in acid or alkaline media.

6. Inhibitors: The presence of inhibitors narrows the range of aw for growth of micro organisms.

Some general conclusions related to water requirement of micro

organisms are

1. Each organism has its own characteristic optimal aw.

2. Bacteria require more moisture than yeasts and yeasts more than molds.

3. Micro organisms that can grow in high concentrations of solutes e.g. sugar and salt have low water activity (aw). Osmophilic yeasts grow best in high concentrations of sugar.

LECTURE-3

Extrinsic factors

The extrinsic parameters of foods are not substrate dependent. They are those properties of the storage environment that affect both the foods and their microorganisms. Those of greatest importance to the welfare of food borne organisms are as follows:
1. temperature of storage
2. relative humidity of environment
3. presence and concentration of gases
4. Changes caused by microorganisms.

Temperature of Storage
Microbial growth can occur over a temperature range from about -8°C up to 100°C. at atmospheric pressure.Micro organisms can be classified into several physiological groups based on their cardinal temperatures
Thermophiles have optimum 55-75°C
Mesophile have optimum 30 -40°C
Psychrophiles (Obligate psychrophiles) 12 - 15
Psychotroph (facultative) 25-30
Low temperature affects the uptake and supply of nutrients to enzyme systems within the cell. Many micrograngisms responds to growth at lower temperature by increasing the amount of unsaturated fatty acids in their membrane lipids and that psychrotrophs generally have higher level of unsaturation in a fatty acid decreases its melting point so that membranes containing higher levels of unsaturated fatly acid will remain fluid and hence functional at lower temperatures. As the temperature increases above the optimum, the growth rate declines as a result of denaturation of proteins.

Relative humidity: (RH)
Relative humidity and water activity are interrelated. When foods with low *a*w values are placed in environments of high RH, the foods pick up moisture from air until equilibrium has been established. Likewise, foods with a high *a*w lose moisture when placed in
an environment of low RH.
There is a relationship between RH and temperature that should be borne
in mind in selecting proper storage environments for foods. In

general, the higher the temperature, the lower the RH, and vice versa.

Gaseous atmosphere:
Oxygen comprises 21% of the earth's atmosphere and is the most important gas in contact with food under normal circumstances. The inhibitory effect of CO_2 on microbial growth isapplied in modified atmosphere packing of food and is an advantage in carbonated mineral
waters and soft drinks. Growth inhibition is usually greater under aerobic conditions than anaerobic and the inhibitory effect increases with decrease of temperature, presumably due to the increased solubility of CO_2 at lower temperatures. CO_2 dissolves in water to produce carbonic acid which decreases PH and partially dissociates into bicarbonate anions and protons. CO_2 also affects solute transport, inhibition of key enzymes involving carboxylation, decarboxylation reactions in which CO_2 is a reactant and reaction with protein amino groups causing change in their properties and activity.

Changes caused by microorganisms
Different chemical changes can occur because great variety of organic compounds are present in foods and numerous kinds of micro organisms that can decompose them may grow in the food.
Following changes are observed in foods.
Changes in Nitrogenous organic compounds:
Most of the nitrogen in foods is in the form of proteins. Proteins are hydrolysed to polypeptides, simpler peptides or amino acids before they can serve as nitrogenous food for most organisms.
Proteinases catalyze the hydrolysis of proteins to peptides gives bitter taste to foods, **Peptidases** catalyze the hydrolysis of peptides to simpler peptides and finally to amino acids.

$$\text{Proteins} \xrightarrow{\text{Proteinases}} \text{Peptides} \xrightarrow{\text{Peptidases}} \text{Polypeptide} \xrightarrow{\text{Peptidases}} \text{amino acids}$$

Anaerobic decomposition of proteins, peptides or aminoacids result in the production of obnoxious odors called putrefaction

Changes in Non nitrogenous organic compounds:
Main non nitrogenous foods for micro organisms, mostly used to

obtain energy but possibly serving as source of carbon, include carbohydrates, organic acids, aldehydes and ketones, alcohols, glycosides, cyclic compounds and lipids.

Carbohydrates:
Carbohydrates act as energy source by micro organisms. Complex, di, tri or npolysaccharides usually are hydrolyzed to simple sugars before utilization. A monosaccharide (glucose) aerobically would be oxidised to carbon-dioxide and water. Glucose anaerobically decompose to

a) An alcoholic fermentation by yeasts with ethanol and CO2 as the principal products.
b) A simple lactic fermentation as by homo-fermentative lactic acid bacteria.
c) A mixed lactic fermentation by hetero-fermentative lactic acid bacteria with lactic and acetic acids, ethanol, glycerol and CO2 as the chief products.
d) The coli type of fermentation as by *coliform bacteria* with lactic, acetic formic acids, ethanol, CO2, H2 etc.
e) The propionic acid fermentation by propionic bacterium
f) Butyric – butyl – isopropyl fermentations yields butyric and acetic acids, CO2 & H2.

Lipids:
Fats are hydrolysed to glycerol and fatty acids by lipase. Phospholipids may be degraded to their constituent phosphate, glycerol, fatty acids and nitrogenous base.

Other compounds:
Alcohols usually oxidised to the corresponding organic acids.Ethanol to acetic acid; Acetaldehyde to acetic acid.

LECTURE-4

Contamination of Foods

Microorganisms from various natural sources act as **source of contamination**.

1-From green plants and fruits

Natural surface flora of plants varies with the plant but usually includes species of *Pseudomonas, Alcaligenes, Flavobacterium, Micrococcus,* coliforms and lactic acid bacteria.
The no. of bacteria will depend **on the plant** and **its environment** and may range from a few hundred or thousand per square centimeter of surface to millions.
Ex: Surface of well washed tomato contains 400-700 micro organisms per square centimeter.

2. From animals

Sources of micro organisms from animals include the surface flora, the flora of the respiratory tract, and the flora of the gastro intestinal tract. Hides, hooves, and hair contain microorganisms from soil, manure, feed and water but contain spoilage organisms.Feathers, feet of poultry carry heavy contamination of micro organisms.
Skin of many meat animals may contain micrococci, Staphylococci and beta haemolytic streptococci. Pig or beef carcasses may be contaminated with salmonellae.
Insects and birds cause mechanical damage to fruits and vegetables, introduce microorganisms and open the way for microbial spoilage.

3. From sewage:

When untreated domestic sewage is used to fertilize plant crops, there is a chance that raw plant foods will be contaminated with human pathogens especially those causing gastrointestinal diseases. Natural water contaminated with sewage contributes their micro organisms to shell fish, fish, and other seafood.

4-From soil:

Soil contains greatest variety of micro organisms. They are ready to contaminate the surfaces of plants growing on or in them and the surfaces of animals roaming over the land. Soil dust is whipped up by air currents and soil particles are carried by running water to get into or onto foods. Soil is an important source of heat resistant spore forming bacteria.

5-**From water:**

Natural water contain not only their natural flora but also microorganisms from soil and possibly from animals or sewage. Kinds of bacteria in natural waters are chiefly of in *Pseudomonas*, *Chromobacterium*,
Proteus, Micrococcus, Bacillus, Streptococcus, Enterobacter and *Escherichia coli*. The water commonly is chlorinated but there have been presence of chlorine resistant flora. Efficient filtration greatly reduces the microbial content.

6-From Air:

Air does not contain a natural flora of microorganisms, but accidentally they are present on suspended solid material or in moisture droplets. Micro organisms get into air on dust, dry soil, spray from stream, lakes or oceans, droplets of moisture from coughing, etc.

Mold spores because of their small size, resistance to drying and large numbers of per mold plant are usually present in air.

Cocci are more numerous than rod shaped bacteria. Yeasts especially asporogenous chromogenic ones are found in most samples of air.

Number of microorganisms in air at any given time depend on factors like **amount of movement, sunshine, humidity, location and the amount of suspended dust or spray**.

7-During handling and processing:

Additional contamination may come from equipment coming in contact with foods, from packaging materials and from personnel.

LECTURE-5

Food Preservation

Principles of Food Preservation:
In accomplishing the preservation of foods by the various methods, the following principles are
involved:
1. Prevention or delay of microbial decomposition
a. By keeping out microorganisms (asepsis)
b. By removal of microorganisms, e.g., by filtration
c. By hindering the growth and activity of microorganisms, e.g., by low temperature, drying, anaerobic conditions, or chemicals.
d. By killing the microorganisms, e.g., by heat or radiation
2. Prevention or delay of self-decomposition of the food.
a. By destruction or inactivation of food enzymes, e.g., by blanching
b. By prevention or delay of purely chemical reactions, e.g., prevention of oxidation by means of an antioxidant
3. Prevention of damage because of insects, animals, mechanical caused, etc.,

Asepsis:
It is the keeping out micro organisms as a preservative factor.
-The inner tissues of healthy plants and animals usually are free from micro organisms. If there is a protective covering about the food, microbial decomposition is delayed or prevented.
Ex: Shells of nuts, skins of fruits & vegetables, shells of egg. Fat on meat or fish.
-In food industries an increasing amount of attention is being given to the prevention of the contamination of foods, from the raw material to the finished product.
*Packaging of foods is a widely used application of asepsis.
*In dairy industry, contamination with micro organisms is avoided from the production to handling of milk in the market.

*In the meat – packing industry sanitary methods of slaughter, handling and processing reduce the load and thus improve the keeping quality of the meat or meat products.
*In industries involving controlled food fermentation e.g., in cheese making, the fewer the competing organisms in the fermenting material, the more likely the success of the fermentation.

Removal of Micro organisms:
Removal of micro organisms is not very effective in food preservation but under special conditions it may be helpful. Removal may be accomplished by means of filtration, centrifugation, washing, trimming.

a) Filtration:
It is the only successful method for the complete removal of organisms and its use is limited to clear liquids. The liquid is filtered through a previously sterilized "bacterial proof" filtered made of sintered glass, diatomaceous earth, material and the liquid is forced through by positive or negative pressure. This method has been used successfully with fruit juices, beer, soft drinks, wine and water.

b) Centrifugation or Sedimentation:
It is not very effective. All micro organisms are not removed.
Ex: 1) Used in the treatment of drinking water but it is insufficient to remove micro organisms
2) In the milk, main purpose of centrifugation is not to remove bacteria but to take out other suspended materials.

c) Washing:
Washing raw foods remove spoilage micro organisms.
Ex: Cabbage heads or cucumbers before their fermentation into sauerkraut and pickles, removes most of the soil micro organisms on the surface.Washing fresh fruits and vegetables removes soil organisms that may be resistant to the beat process during canning.
Washing foods is dangerous if water adds spoilage micro organisms and increases the moisture so that growth of spoilage organisms is encouraged.

d) Trimming:
Spoiled portions of a food removed by trimming. Large no. of spoilage organisms are removed by this way.
Ex: Trimming the outer leaves of cabbage heads is recommended for the manufacture of sauerkraut.

Maintenance of Anaerobic conditions:
Scaled, packaged foods may have the anaerobic conditions in the container.
A complete fill, evacuation of the unfilled space (the head space in a can) or replacement of the air by CO_2 or by an inert gas such as nitrogen will bring about anaerobic conditions.
Spores of aerobic bacteria may resistant to heat and survive in canned foods but unable to grow in the absence of O_2.

LECTURE-6

Food Preservation by use of high temperature.

Killing of micro organisms by heat is supposed to be caused by the denaturation of the proteins and by the inactivation of enzymes required for metabolis.

Factors affecting heat resistance (Thermal death time):

Cells and spores of microorganisms differ widely in their
Certain factors are known to resistance to high temperatures.
affect the heat resistance of cells or spores. Chief known factors are as follows.

1. The temperature – time relationship: The time for killing cells or spores under a given set of conditions decreases as the temperature is increased.

2. Initial concentration of spores (or cells): The more spores or cells present, the greater the heat treatment necessary to kill all of them.

3. Previous history of the vegetative cells or spores: The conditions under which the cells have been grown and spores have been produced and their treatment thereafter will influence their resistance to heat.

a) Culture medium
b) Temperature of incubation
c) Phases of growth or age
d) Dessication

4. Composition of the substrate in which cells or spores are heated.

a) Moisture: Moist heat is a much more effective killing agent than dry heat. Dry materials require more heat for sterilization than moist ones.

b) Hydrogen – ion concentration (pH) : Cells or spores are most heat resistant in a substrate that is at or near neutrality.

Acid food – PH < 4.5, Fruit & Certain vegetable products
Low acid foods – PH > 4.5 , Meat, seafood, milk and most of the common vegetables.

C) Other constituents of the substrate: Sugar and salt.

Heat resistance of bacteria and bacterial spores:

Cocci usually are more resistant than rods although there are many exceptions. The higher the **optimal and maximal temperatures** for growth, the greater the resistance to heat is likely to be. The bacteria that **clump** considerably or form **capsules** are more difficult to kill than those which do not form capsules. The cells high in **lipid** content are harder to kill than other cells.

Heat resistance of Enzymes:
Although most food and microbial enzymes are destroyed at 79.4°C, some may withstand higher temperatures, especially if high temperature short time heating is employed. Thermal processes designed to inactivate micro organisms will also inactivate enzymes of concern.
Some hydrolases (proteinases and lipases) will retain a substantial level of activity after an ultra high temperature process. The residual activity of these enzymes may spoil the processed product during long term storage.
Detection of the bovine phosphatase enzyme in processed milk usually indicates that the milk was not properly pasteurized.
Heat penetration:
The rate of penetration of heat into food must be known in order to calculate the thermal process necessary for its preservation. Since every part of the food in a can or other container must receive an adequate heat treatment to prevent spoilage.
Heat penetration from an external source to the center of the can may take place by **conduction**, where heat passes from molecule to molecule, by **convection** where heat is transferred by movement of liquids or gases or some times by **combination of conduction and convection** .conduction is slow in foods and rapid in metals. When both conduction and convection are involved in the heating of foods, they may function simultaneously or successively. When solid particles of food are suspended in a liquid, the particles heat by conduction and the liquid heats by convection. Some foods change in consistency during heating, and a broken heating curve results.
Ex: Sugar syrups, Brine packed whole grain corn, certain thick soups and tomato juices.

Factors that determine the time required to bring the center of the container of food up to the sterilizing temperature are as follows:

1. The material of which the container is made. Glass has a slower rate of heat penetration than a metal can.

2. The size and shape of the container. The larger a can is, the longer it will take to reach a given temperature at the center because the distance to the center of the larger can is greater and it has less surface per volume or weight. Hence larger cans are heated longer proportionally but not to as high a temperature at the center.

3. Initial temperature of the food.

The temperature of the food in a can when it goes into the retort (steam sterilizer) makes no difference in time required for the center of the can to reach the retort temperature. A high initial temperature is important in processing canned foods that heat slowly,such as cream style corn and meat.

4. Retort temperature:

Food cans placed in steam sterilizer of different temperatures reach the respective temperatures at the same time.

5. Consistency of can contents and size and shape of pieces:

All of these are important in their effect on heat penetration. Pieces that retain their identity i.e., do not cook apart. Ex: Peas, plums, beets, Asparagus whole grain corn. If the pieces are large, heating is delayed, because the heat must penetrate to the center of the pieces before the liquor can reach the retort temperature. Large beets or large stalks of Asparagus heat more slowly than small ones.

6. Rotation and agitation:

Rotation or agitation of the container of food during heat processing will hasten heat penetration if the food is fluid, but it may also cause physical changes in foods. Rotation is used successfully with canned evaporated milk, and shaking is used in foods in the form of pastes or purees.

TDP (Thermal Death Point):

Temperature required to kill known no. of micro organisms at a given time. Here temperature is unknown.

TDT (Thermal Death Time):

Time required to kill known no. of micro organisms at a given temperature.

DRT (Decimal Reduction Time):

Time required to reduce the microbial population at specified temperature. It is ndesignated as “D”

Heat treatments employed in processing foods:

The various degrees of heating used on foods might be classified as

1. Pasteurization
2. Heating at about 100°C
3. Heating above 100°C

1. Pasteurization:

Pasteurization is a heat treatment that kills part but not all of the microorganisms present in foods and the temperature applied is below 100°C. Heating may be by steam, hot water, dry heat or electric currents. Products are cooled promptly after the heat treatment.

Pasteurization is used

1. When more rigorous heat treatments might harm the quality of the product.
2. To kill pathogens in milk.
3. When the main spoilage organisms are not very heat resistant (Ex Yeasts in fruit juices).
4. When competiting microorganisms are to be killed, allowing a desired fermentation

Preservative methods used to supplement pasteurization include
Refrigeration Ex: Milk
Keeping out micro organisms Ex: Packaging
Maintenance of anaerobic conditions Ex: Sealed containers
Addition of high concentrations of sugar. Ex: Sweetened condensed milk
Presence or addition of chemical preservatives. Ex: Organic acids on pickle

Methods of pasteurization:

HTST Method: (High temperature short time)

In this method, high temperature is employed for a short time. Temperature is 71.7°C and time is 15 sec.

LTLT Method: (Low temperature long time)

In this method, low temperature is employed for a longer time. Temperature is 62.8°C and time is 30 minutes time. Ex: Milk

In this method, the temperature applied is **.Ultra pasteurization**
137.8°C for at least 2 seconds. Earlier pasteurization temperature was set based on pathogenic organism present in milk
Mycobacterium tuberculosis. This bacterium killed at 61.7°C, but
burnetti, a rickettsia causing Q fever is other organism C*oxiella*
,survived pasteurization temperature. Hence to kill *Coxiella burnetti*

Pasteurization temperature was raised to 62.8°C. *Coxiella burnetti* is transmitted by milk

2-Heating at about 100°C:
This treatment was sufficient to kill everything. Many acid foods can be processed successfully at 100°C or less. Ex: sauerkraut & highly acid fruits. 100°C temperature is obtained by boiling a liquid food or by immersion of the container of food in boiling water or by exposure to following steam.

3-Heating above 100°C:
Temperatures above 100°C usually are obtained by means of steam under pressure in steam pressure sterilizers or retorts. The temperature in the retorts increases with rising steam pressures. Ex: Milk can be heated to temperatures up to 150°C by use of steam injection or steam infusion followed by flash evaporation of the condensed steam and rapid cooling. Processes such as this for milk have been referred to as **ultrahigh temperature or UHT processes**.

Canning:
Canning is defined as the preservation of foods in sealed containers and usually implies heat treatment to prevent spoilage. Canning is done in “tin cans” made of tin coated steel or in glass containers, aluminum, plastics as pouches or solid containers, or of a composite of materials.

Appertization:
Preservation of foods by canning with the application of heat treatments in cork stoppered, wide mouthed glass bottles.

Pressurized Packaged Foods:
Pressurized packaging liquids or pastes called aerosols, are packed under pressure of a propellant gas, usually CO_2, N_2 or nitrous oxide, so as to dispense the food as a foam, spray or liquid. Many foods are now being so packaged, e.g., whipped cream

LECTURE-7

Preservation by use of low temperatures.

Common or cellar storage:
The temperature in common or cellar storage usually lower than 15°C. Root crops, potatoes, cabbage, celery, apples stored for limited periods. The deterioration of such fruits and vegetables by their own enzymes and by micro organisms is not prevented but is slower than at atmospheric temperatures.

Chilling or cold storage:
Chilling storage is at temperatures not far above freezing and usually involves cooling by ice or mechanical refrigeration. Eggs, dairy products, meats, seafood, vegetables and fruits may be held in chilling storage for a limited time. Enzymatic and microbial changes in the foods are not prevented but are slowed considerably. Chilling temperature is selected on the basis **of the kind of food** and **the time** and **conditions of storage.**
Changes in humidity as well as in temperature during storage may cause “sweating” or precipitation of moisture on the food. A moist surface favours microbial spoilage.
In “Gas storage” of foods, where the composition of the atmosphere has been controlled by the introduction of CO2, Ozone or other gas or the removal of CO2.
Gas storage ordinarily is combined with chilling storage. In the presence of optimal concentrations of carbon dioxide or ozone the following **advantages** are present .
-A food will remain unspoiled for a longer period.
-Higher relative humidity can be maintained without harm to the keeping quality of certain foods.
-Higher storage temperature can be used without shortening the keeping time of the food.
Combination of U.V. irradiation with chilling storage helps preserve some foods **and may permit the use of a higher humidity or storage temperature than with chilling along.** U.V. lamps have been installed in rooms for the storage of **meat and cheese.**

Freezing or frozen storage:
The storage of foods in the frozen condition has been an important preservative method for centuries? **Under frozen storage, microbial growth is prevented entirely and the action of food**

enzymes is greatly retarded. The lower the storage temperature, the slower will be any chemical or enzymatic reactions.

Fruits and vegetables are selected on the basis of their suitability for freezing and their maturity and are washed, trimmed, cut or otherwise pretreated as desired.

Vegetables are scalded or blanched.

Fruits are packed in syrup.

Most foods are packaged before freezing but strawberries are frozen before packaging.

Scalding or blanching of vegetables is done with hot water or steam and has the following advantages

1-Inactivation of most of the plant enzymes which cause toughness, change in color, mustiness, loss in flavor, softening and loss in nutritive valve.

2. Reduction in the no. of micro organisms on the food.
3. Enhancement of the green color of vegetables such as spinach.
4. Displacement of air entrapped in the tissues.

Types of freezing:

1. Sharp freezing or slow freezing; 2. Quick freezing; 3. Dehydro freezing

Sharp freezing	**Quick freezing**
1. It refers to freezing in air with only natural air circulation or with electric fans. 2. Temperature usually -23.3°C or lower but may vary from -15 to -29°C. 3. Time required to achieve sharp freezing is 3-72 hrs. 4. Large ice crystals are formed. More mechanical damage of food is observed. 5. Longer period of solidification.	1. It refers to freezing foods in relatively short time (30 min). 2. Temperature usually between -17.8 to 45.6°C if it is indirect contact with refrigerant. (or) -17.8°C to -34.4°C if it is done with air blast freezing. 3. Time required to achieve above temperatures is 30 min or lower. 4. Small ice crystals are formed hence there is less mechanical damage of food. 5. Shorter period of

	solidification and therefore less time for diffusion of soluble materials and separation of ice.

Advantages of Quick Freezing over slow freezing:
1. Smaller ice crystals are formed; hence there is less mechanical destruction of infact cells of the food.
2. There is a shorter period of solidification of ice
3. There is more prompt prevention of microbial growth
4. There is more rapid slowing of enzyme action.
5. Quick frozen foods are supposed to bring to the room temperature before cooking or consumption. This process is called thawing. Ex: Vegetables, meat.

Dehydrofreezing:
Fruits and vegetables have about half their moisture removed before freezing. Certain foods like fruits, vegetables, fish, shrimp and mushrooms now are being frozen by means of liquid nitrogen.

Changes during Freezing:
Quick freezing process rapidly slows chemical and enzymatic reactions in the foods and stops microbial growth. A similar effect is produced by sharp or slow freezing but with less rapidity. Physical effects like expansion in volume of the frozen food and ice crystals form and grow in size. Ice crystals form and grow in size.
Ice crystals are usually larger with slow freezing and more ice accumulates between tissues cells than with quick freezing and may crush cells.
Water is drawn from the cells to form such ice and results in increase in the concentration of solutes in the unfrozen liquor and leads to salting out dehydration, denaturation of proteins and causes irreversible changes in colloidal systems such as the syneresis of hydrophilic colloids.

Effect of freezing on microorganisms
Bacteria differ in their capacity to survive during freezing, with cocci being generally more resistant than Gram-negative rods. Of the food-poisoning bacteria, salmonellae are less resistant than *Staphylococcus*
aureus or vegetative cells of clostridia, whereas endospores and

food-poisoning toxins are apparently unaffected by low temperatures. From the strict standpoint of food preservation, freezing should not be regarded as a means of destroying foodborne microorganisms. The type of organisms that lose their viability in this state differ from strain to strain and depend on the type of freezing employed, the nature and composition of the food in question, the length of time of freezer storage, and other factors, such as temperature of freezing. Low freezing temperatures of about -20° C are less harmful to microorganisms than the median range of temperatures, such as -10° C. For example, more microorganisms are destroyed at -4° C than at -15° C or below. Consider some of the events that are known to occur when cells freeze:

1. Freezing results in an increase in the viscosity of cellular matter, a direct consequence of water being concentrated in the form of ice crystals.
2. Freezing results in a loss of cytoplasmic gases such as O_2 and CO_2. A loss of O_2 to aerobic cells suppresses respiratory reactions. Also, the more diffuse state of O_2 may make for greater oxidative activities within the cell.
3. Freezing causes changes in pH of cellular matter. Various investigators have reported changes ranging from 0.3 to 2.0 pH units.
4. Freezing effects concentration of cellular electrolytes. This effect is also a consequence of the concentration of water in the form of ice crystals.
5. Freezing causes a general alteration of the colloidal state of cellular protoplasm. Many of the constituents of cellular protoplasm such as proteins exist in a dynamic colloidal state in living cells. A proper amount of water is necessary to the well-being of this state.
6. Freezing causes some denaturation of cellular proteins. Precisely how this effect is achieved is not clear, but it is known that upon freezing, some –SH groups disappear and such groups as lipoproteins break apart from others. The lowered water content, along with the concentration of electrolytes, no doubt affects this change in state of cellular proteins.
7. Freezing induces temperature shock in some microorganisms. This is true more for thermophiles and mesophiles than for psychrophiles. More cells die when the temperature decline above freezing is sudden than when it is slow.
8. Freezing causes metabolic injury to some microbial cells such

as certain *Pseudomonas* spp. Some bacteria have increased nutritional requirements upon thawing from the frozen state and as much as 40% of a culture may be affected in this way.

Preservation by Drying

Drying usually is accomplished by the removal of water, but any method that reduces the amount of available moisture i.e., lowers the $\mathbf{a_w}$ in a food is a form of drying. Ex: 1. Dried fish heavily salted to remove moisture.

Sun dried food:
Moisture removed by exposure to the sun's rays without any artificially produced heat and without controlled temperatures.

Dehydrated or dessicated food:
Dehydrated or desiccated food has been dried by artificially produced heat under controlled conditions of temperature.

Methods of Drying:
-Solar Drying: Solar drying is limited to climates with a hot sun and a dry atmosphere certain fruits such as raisins, prunes, figs, apricots, nectarines, pears and peaches are dried by solar. Fish, rice and other grains are also dried.
-Drying by mechanical dryers: Artificial drying involves the passage of heated air with controlled relative humidity over the food to be dried. The simplest dryer is the evaporator, sometimes used in the farm home.
Forced – draft drying systems employ currents of heated air that move across the food usually in tunnels. Alternative method is moving the food on conveyor belt or on trays in carts through the

heated air. Liquid foods like milk, juices and soups may be evaporated by the use of low temperature and vacuum.
Drum drying is the passage of food over a heated drum with or without vacuum. Spray drying is spraying the liquid into a current of dry, heated air.
Smoking :
Certain foods are dried by smoking. Smoking of foods usually has two purposes .Adding desired flavours and has preservation effect. Ex: Meat.

-Other methods:
Electronic heating – Removal of still more moisture from food already fairly well dried.
Foam mat drying – liquid food is whipped to foam dried with warm air and crushed to a powder.
Pressure gun puffing – Partially dried foods to give a porous structure that facilitates further drying.
Tower drying – Drying by dehumified air at 30°C or lower used for tomato concentrate, milk and potatoes.
Freeze Drying- Freeze-drying, also known as lyophilisation, or Cryodesiccation, is a dehydration process typically used to preserve a perishable material or make the material more convenient for transport
Freeze-drying works by freezing the material and then reducing the surrounding pressure to allow the frozen water in the material to sublimate directly from the solid phase to the gas phase.

Sun drying

Solar dryer

Freeze dryer

Microbiology of specific Dried foods:

Dried fruits: The numbers of micro organisms on most fresh fruits range from comparatively few to many depending on pretreatments. Most dried fruits may; vary from a few hundred per gram of fruit to thousands. They are mostly on the outer surfaces. Spores of bacteria and molds are likely to be the most numerous.

Dried vegetables:
Range from small numbers to millions per gram. If drying trays are improperly loaded, souring of such vegetables as onions and potatoes by lactic acid bacteria with a marked increases in numbers of bacteria may take place during the drying process. Risk is greater with onions because they are not blanched.
Ex: Chiefly bacteria are *Escherichia, Enterobacter, Bacillus, Clostridium, Micrococcus, Pseudomonas, Streptococcus*.

Dried Eggs:
Powdered eggs are fully dehydrated eggs.they are made using spray drying in the same way that powdered milk is made.Dried eggs may contain from a few hundred micro organisms, mostly bacteria per gram up to over 100 million, depending on the eggs broken and the methods employed. A variety of kinds of organisms have been found in dried eggs includes Microcci, Streptococci, Coliforms, Spore formers and molds.

Dried Milk:
No. of micro organisms in dry milk may vary from a few hundred per gram to millions. Predominant kinds of organisms in dry milk are thermoduric Streptococci, Micrococci and spore formers.

Intermediate moisture Foods:
Numerous commercially prepared foods which contain 20 – 40% moisture and have non refrigerated shelf stability have been refined to as intermediate moisture products.
Ex: jams, jellies, honey, many dried fruits, , meat products like pepperoni, country ham, and some dried fish.
These products are also referred as “reduced – water activity products”.

LECTURE-8

Preservation by food additives

A food additive is a substance or mixture of :**Food Additives**
substances, other than the basic food stuff, which is present in food as a result of a production, processing, storage or packaging. Definition given The term does not include chance contamination. by W.H.O

Chemical preservatives: The food additives which are specifically added to prevent the decomposition of a food have been referred to as chemical preservatives.

The properties of Antimicrobial preservative:

1. It should have wide range of antimicrobial activity.
2. It should be non toxic to human or animals.
3. It should be economical.
4. It should not have an effect on the flavor, taste of the original food.
5. It should not be inactivated by the food.
6. It should not encourage the development of resistant strains.

Preservatives may be bacteriocidal and kill the target organisms or they may be bacteriostatic in which case they simply prevent them growing. This is very often a dose-dependent feature; higher levels of
an antimicrobial proving lethal while the lower concentrations that are
generally permitted in foods tend to be bacteriostatic. For this reason
chemical preservatives are useful only in controlling low levels of contamination and are not a substitute for good hygiene practices

Lactic, acetic, propionic and citric :**Organic acids and their salts**
acids or their salts may be added to foods **Citric acid** is used in

syrups, drinks, jams and jellies as a substitute for fruit flavors and for preservation.

Lactic and acetic acids are added to brines of various kinds, green olives etc. **Propianates – (sodium or calcium propionate)** are used most extensively in the prevention of mold growth and rope development in backed goods and for mold inhibition in many cheese foods. Propionic acid is found naturally in Swiss cheese up to 1%.

Benzoates:

Sodium salt of benzoic acid has been used extensively as an antimicrobial agent in foods. It is incorporated into jams, jellies, carbonated beverages, pickles etc.

Acetates:

Sodium diacetate has been used in cheese spreads and malt syrups and as treatment for wrappers used on butter.

Nitrites and Nitrates:

Nitrites can react with secondary and tertiary amines to form nitrosamines, which are known to be carcinogenic. Nitrites are currently added in the form of sodium nitrite potassium nitrite, sodium nitrate and potassium nitrate. Inhibits *C. botulinum* in meat products.

Sulfur dioxide and sulfites:

Egyptians and Romans burned sulphur to form sulphur dioxide a means of sanitizing wine making equipment and storage vessels.

Sulphur dioxide and sulphites are used in the wine industry to sanitize equipment and to reduce the normal flora of the grape They form sulphurous acid and effectiveness is enhanced at must. -low PH .The effect of sulfurous acid against microbial cells by:**1 reduction of disulfide** linkages**,2- formation of carbonyl compounds,3- reaction with ketone groups and 4- inhibition of respiratory mechanisms**. In addition to antimicrobial action of sulfites, they are also used to prevent enzymatic and non enzymatic changes or discoloration in some foods. Fumes of burning sulphur are used to treat most light colored dehydrated fruits. SO2 also used in syrups, fruit juices and wine making.

Sugar and salt:

These compounds lower the water activity (aw) and have an adverse effect on microorganisms .Sodium chloride is used in brines and curing solutions or applied directly to the food. Salt has been reported to have the following effects

1. It causes high osmotic pressure and hence plasmolysis of cells.

2. It dehydrates foods by drying out and tying up moisture as it dehydrates microbial cells.

3. It ionizes to yield the chlorine ion.

4. It reduces the solubility of oxygen in the moisture

5. It senitizes the cell against CO2.

6. It interferes with the action of proteolytic enzymes.

Sugar such as glucose or sucrose has ability to make water unavailable to organisms by osmotic effect.

Alcohol:

Ethanol, a coagulant and denaturized of cell proteins. Flavoring extracts e.g., vanilla and lemon extracts are preserved by their content of alcohol.

Formaldehyde:

Addition of formaldehyde to foods is not permitted, except as a minor constituent of wood smoke. This compound is effective against molds, bacteria, and viruses.

Antioxidants

They are prevent rancidity in fats and food containing fats. fats exposed to light ,moisture ,heat or heavy metal ions become activated and oxidize to peroxides .the most used antioxidants are butylated hydroxyl anisole(BHA), butylated hydroxyl toluence(BHT), ascorbic acid and lecithin. The BHA and BHT are most frequency used in variety of products because they are relatively stable to heat .

Wood smoke:

Smoking of foods usually has two main purposes:

Adding desired flavours and Preservation effect .Smoking process helps preservation by combined action of the heat and chemical preservatives from the smoke and by the drying effects, especially Smoking temperatures for meat vary from 43 - 71°C at the surface. and the smoking period lasts from a few hours to several days. Wood smoke contains a large number of volatile compounds that may have bacteriostatic and bactericidal effect. Formaldehyde is considered the most effective with phenols and cresols.

Spices and other condiments:

Spices and other condiments do not have any marked bacteriostatic effect in the concentrations used, but help other against in preventing the growth of organisms in food. Mustard flour and volatile oil of mustard are very effective against *Saccharomyces cerevisiae*. Cinnamon and cloves containing cinnamic aldehyde & eugenol respectively usually are more bacteriostatic than are other spices. Extracts of onion ,garlic and horseradish as well as of cabbage and turnip have been shown to be inhibitory to *Bacillus subtilis* and *Escherichia coli*. Acrolein is the active material in onions and garlic and butyl thiocyanate in horse radish.

Natural' Food Preservatives

The uncertainty voiced by consumer organisations and pressure groups over the use of food additives including preservatives has already been referred to. The use of natural food components possessing antimicrobial activity such as essential oils and the lactoperoxidase system in milk have attracted some attention in this respect. Attention has also been paid to the bacteriocins produced by food-grade micro-organisms such as the lactic acid bacteria. Nisin (see Section 9.4.1) is an already well established example and its use can be extended by expedients such as inclusion of whey fermented by a nisin-producing strain of Lactococcus lactis as an ingredient in formulated products like prepared sauces. Food fermentations may serve either or both of two purpose.

1. To produce new and desired flavours;
2. 2. To help preserve the food.
   Ex: Fermented milks, sauerkraut.

LECTURE -9

Food Preservation by Radiation

Electromagnetic (e.m.) radiation is a way in which energy can be propagated through space. It is characterized in terms of its wavelength l, or its frequency n,
The range of frequencies that e.m. radiation can have is known as the electromagnetic spectrum and is grouped into a number of regions, visible light being only one small region .
The energy carried by e.m. radiation is transmitted in discrete packets or quanta. As far as food microbiology is concerned, only three areas of the e.m. spectrum concern us; microwaves, the UV region and gamma rays.

Ultraviolet Irradiation

UV radiation has wavelengths below 450nm.The quanta contain energy sufficient to excite electrons in molecules from their ground state into higher energy orbitals making the molecules more reactive. Chemical reactions thus induced in micro-organisms can cause the failure of critical metabolic processes leading to injury or death.
Only quanta providing energy sufficient to induce these photochemical reactions will inhibit micro-organisms, so those wavelengths that are most effective give us an indication of the sensitive chemical targets within the cell. The greatest lethality is shown by wavelengths around 260nm which correspond to a strong absorption by nucleic acid bases. The pyrimidine bases appear particularly sensitive, and UV light at this wavelength will, among other things, induce the formation of covalently linked dimers between adjacent thymine bases in DNA. If left intact these will prevent transcription and DNA replication in affected cells. The resistance of micro-organisms to UV is largely determined by their ability to repair such damage, although some organisms such as micrococci also synthesize protective

pigments. Generally, the resistance to UV irradiation follows the pattern:

Gram-negatives<Gram-positives =yeasts<bacterial spores <mould spores<viruses

Germicidal Lamps

Low-pressure mercury lamps are used: 80% of their
UV emission is at a wavelength of 254nm which has 85% of the biological activity of 260 nm. Wavelengths around 200nm are absorbed by oxygen in the air producing ozone which is harmful. Food containers are sometimes treated in use UV and some meat chill store rooms have UV lamps to retard surface growth. UV can however induce spoilage of products containing unsaturated fatty acids where it accelerates the development of rancidity. Process workers must also be
protected from UV since the wavelengths used can cause burning of
the skin and eye disorders.

Factors Influencing Effectiveness

factors that influence the effectiveness of ultraviolet rays are as follows:

1. Time. The longer the time of exposure, the more effective the treatment.
2. Intensity. The intensity of the rays reaching an object will depend on the power of the lamp, the intensity will increase with the power of the lamp.
3. Penetration. The nature of the object or material being irradiated has an important influence on the effectiveness of the process. Penetration is reduced even by clear water, which also exerts a protective effect on microorganisms. Dissolved mineral salts, especially of iron, and cloudiness greatly reduce the effectiveness of the rays. Even a thin layer of fatty or greasy material cuts off the rays. Therefore, the rays affect only the outer surface of most irradiated foods directly exposed to the lamp and do not penetrate

to microorganisms inside the food. The lamps reduce the number of viable organisms in the air surrounding the food.

Ionizing Radiations

Radiation classified as ionizing includes x-rays or gamma rays, cathode or beta rays, protons, neutrons, and alpha particles. In practice three different types are used.
1) High-energy electrons. in the form of b particles produced by)

radioactive decay or machine generated electrons. Strictly speaking they are particles rather than electromagnetic radiation, although in some of their behaviour they do exhibit the properties of waves. Because of their mass and charge, electrons tend to be less penetrating than ionizing e.m. radiation.
(2) X-rays generated by impinging high energy electrons on a suitable target. They are not currently considered economical for use in the food industry.
(3) Gamma g rays produced by the decay of radioactive isotopes. The most commonly used isotope cobalt 60, It emits high-energy g-rays which can penetrate food up to a depth of 20 cm .

Effects on Microorganisms

Ionizing radiation can affect micro-organisms directly by interacting with key molecules within the microbial cell, or indirectly through the inhibitory effects of free radicals produced by the radiolysis of water. These indirect effects play the more important role since in the absence of water, doses 2–3 times higher are required to obtain the same lethality. Removal of oxygen also increases microbial resistance 2–4 fold and it is thought that this may be due to the ability of oxygen to participate in free radical reactions and prevent the repair of radiation induced lesions. As with UV irradiation, the main site of damage in cells is the chromosome. Hydroxyl radicals cause single- and double-strand breaks in the DNA molecule as a result of hydrogen abstraction from deoxyribose followed by b-elimination of phosphate which cleaves the molecule. They can also hydroxylate purine and pyrimidine bases.

Resistance to ionizing radiation depends on the ability of the organism to repair the damage caused. Resistance generally follows the sequence:

Gram-negativeo <Gram-positive = moulds<spores = yeasts<viruses

Food-associated organisms do not generally display exceptional resistance, although spores of some strains of *Clostridium botulinum* type A have the most radiation resistant spores. Since studies on food irradiation started, a number of bacteria which are highly resistant to radiation have been isolated and described. Although one of these, *Deinococcus radiodurans*, was first isolated from meat, their role in foods is not significant in the normal course of events.

The bactericidal efficacy of a given dose of irradiation depends on the following:

1 The kind and species of organism:
2 The numbers of organisms (or spores) originally present: The more organisms there are, the less effective a given dose will be.
3 The composition of the food: Some constituents, e.g., proteins, catalase, and reducing substances (nitrites, sulphites, and sulfhydryl compounds), may be protective. Compounds that combine with the SH groups would be sensitizing. Products of ionization may be harmful to the organisms.
4 The presence or absence of oxygen. The effect of free oxygen varies with the organism,ranging from no effect to sensitization of the organism. Undesirable "side reactions," are likely are intensified in the presence of oxygen and to be less frequent in a vacuum or an atmosphere of nitrogen.

5 The physical state of the food during irradiation. Both moisture content and temperature affect different organisms in different ways.
6 The condition of the organisms. Age, temperature of growth and sporulation, and state vegetative or spore-may affect the sensitivity of the organisms.

Microwave Processing

Microwave heating and processing of foods is becoming increasingly popular, particularly at the consumer level. Microwaves are electromagnetic waves between infrared and radio waves. Unlike the other forms of radiation we will discuss, microwaves act
indirectly on micro-organisms through the generation of heat. The energy or heat produced by microwaves as they pass through a food is a result of the extremely rapid oscillation of the food molecules to align themselves with the electromagnetic field being produced. This rapid oscillation, or intermolecule friction, generates heat. The preservative effect of microwaves or the bactericidal effect produced is really a function of the heat that is generated.

High Hydrostatic Pressure (HHP). (Pascalization)

Also called as pascalization. This technique is applied to foods, which can be liquid or solid, packaged or unpackaged, to high pressure (which varies depending upon application) usually for 5 minutes or less. HHP can be used on many foods such as raw and cooked meats, fish and shellfish, fruit and vegetable products, cheeses, salads, dips, grains and grain products, and liquids including juices, sauces, and soups. The high pressure does not destroy the food. Microorganisms living on the surface and in the interior of the food are inactivated. Inactivation is accomplished by affecting the molecular structure of chemical compounds necessary for metabolic metabolism in the microorganisms. HHP is equally effective on molds, bacteria, viruses, and parasites.

Lecture 10

Microbiology of milk

Milk is a good growth medium for many microorganisms
near-neutral pH, and **available** ,because of its **high water content**
nutrients. Milk, however, is not an ideal growth medium for some
microorganisms due to **insufficient amounts of amino acids,** for
Milk does example, lysine, arginine, isoleucine, and glutamic acid.
possess a number of antimicrobial features mentioned in previous
lecture , present either to protect the udder from infection or to
protect the newborn calf. Stimulation of lactoperoxidase activity
through the addition of exogenous hydrogen peroxide has been
investigated as a means of preserving raw milk in developing
countries where ambient temperatures are high and refrigeration is
not often available .

Three sources contribute to the micro-organisms found in milk: **the**
the teat exterior and its immediate surroundings, and ,**udder interior**
the milking and milk-handling equipment.
Aseptically taken milk from a healthy cow normally contains low
numbers of organisms, typically fewer than 102–103 cfu ml_1, and
milk drawn from some quarters may be sterile. The organisms most
commonly isolated are micrococci, streptococci and the diptheroid
Corynebacterium bovis. Counts are frequently higher though due to
mastitis.

Mastitis:- an inflammatory disease of the mammary tissue, which is
a major cause of economic loss in the dairy industry. Mastitis is
also diagnosed by the presence of high numbers of
polymorphonuclear leukocytes which can rise to levels of 107 ml_1
in infected milk. Many organisms can cause mastitis, the most
important being Staphylococcus aureus, Escherichia coli,
Streptococcus agalactiae, Strep. dysgalactiae, Strep. uberis,
Pseudomonas aeruginosa and Corynebacterium
pyogenes.

In most developed countries milk is chilled almost immediately after
it
issues from the cow and is held at a low temperature thereafter. It
is
stored in refrigerated holding tanks before being transported by a
refrigerated or insulated lorry to the dairy where it is kept in chill

storage
tanks until use. Throughout this time, its temperature remains below 7 1C and the only organisms capable of growing will be psychrotrophs.
There are many psychrotrophic species, but those most commonly found
in raw milk include Gram-negative rods of the genera Pseudomonas, Acinetobacter, Alcaligenes, Flavobacterium, psychrotrophic coliforms,
predominantly Aerobacter spp., and Gram-positive Bacillus spp.

Preservation:

-Asepsis:
The prevention of the contamination of milk is important in its preservation. Cleaning of dairy utensils, cow, milk-contact surfaces like pipelines, tanks, pumps, valves, separators, stirrers, and fillers is maintained to reduce the microbial contamination. Packaging serves to keep microorganisms from bottled milk, fermented milks, and other diary product.
-Removal of microorganisms:
After microorganisms have entered milk, it is difficult to remove them effectively. The process of centrifugation, will remove some microorganisms from milk. **Bactofugation:** The centrifugal procedure used for removing bacteria from milk, known as bactofugation. It is not used extensively on a commercial basis.
-**Use of heat**
The four types of heat treatment applied to milk are described in the following table:-
Heat treatment of milk

Low Temperature Holding (LTH)	62.8 1C for 30 min
High Temperature Short Time (HTST)	71.7 1C for 15 s
Ultra High Temperature	135 1C for 1 s
‘Sterilized’	4100 1C typically 20–40 min

The most popular of the UHT systems Ultrahigh temperature (UHT): are the direct-heating methods like:

1. Steam-injection technique: It includes a steam is injected into milk process.
2. Steam infusion technique: It includes milk is injected into the steam process.
The combination of this type of heat treatment with aseptic packaging results in a category of products usually referred to as "sterilized milk" or "sterilized cream." This designation does not imply absolute sterility but a sterile product in the commercial sense. These products have an extremely long shelf life around 6 weeks.

-Use of low temperatures:
Newly pasteurized milk is to be cooled to 7 C **Refrigerated Storage:** or less and maintained there.
Freezing
Ice cream and other frozen dairy desserts are frozen as part of the manufacturing process and are stored at low temperatures in the frozen state, where microbial multiplication is impossible.
Butter in storage is held at -17 to -180C or lower, where no microbial growth can take place. Frozen cream is stored in considerable amounts at a similar temperature.

-Drying:
Condensed Products:
Evaporated milk is made by removing about 60 percent of the water from whole milk, so that about 11.5 percent lactose would be in solution, plus twice the amount of soluble inorganic salts in whole milk. This high concentration of sugar is inhibitory to the growth of some bacteria
and prevents the growth of some bacteria. Bulk condensed milk is more condensed than evaporated milk and is a still poorer culture medium for organisms not tolerant of high sugar concentrations. Condensed whey, called **whey semisolids**, is another concentrated dairy product, as is condensed buttermilk, called **Semisolid buttermilk**, which has its concentration of acid and other solutes increased by the condensation process. High concentrations of sugar and the increased percentage of soluble inorganic salts tie up the moisture, making it unavailable to any microorganism except osmophilic microorganisms. Therefore, drying, both by removal of

water and by tying it up, is a main
preservative factor.

Dry Products

Among the dairy products prepared in the dry form are milk, skim milk, cream, whey, buttermilk, ice cream mix, and malted milk. Usually the milk is preheated before drying (to 65 to 85 C for the roller process and to 68.8 to 93.3 C for the spray process). This preheating process pasteurizes the milk and kills the less heat-resistant microorganisms. Some of the drying processes involve an instantization step, in which the dry powder is wetted and then redried. In this process the product is actually exposed to contact with the air at nine different stages. A large salmonellosis outbreak involving powdered milk was shown to have resulted from air contamination during this instantizing process. The microbial content of the heat-dried dairy product depends on the content of the liquid product to be dried, the temperature and time of preheating, the evaporation process, contamination and growth in storage tanks and pipes.The method of drying, and contamination from air sources. Preheating kills organisms as pasteurization would and hence destroys all but the thermodurics. Evaporation, especially a continuous process, may result in increases in thermodurics and bacteria that are thermophilic. The high temperature of the roller process without vacuum destroys almost all organisms except bacterial spores.

-Added Preservatives:

The addition of preservatives to dairy products is permitted only to a limited extent. The use of sorbic or propionic acid or one of their salts is permitted in cottage cheese. Added sugar acts as a preservative of sweetened condensed milk; Sodium chloride or common salt is added in the manufacture of various kinds of cheese but usually it is more for flavor or for controlling the growth of microorganisms.

Lecture 11

Spoilage of milk

-Gas Production
Gas production by bacteria usually is accompanied by acid formation and it is undesirable in milk and milk products.
In raw milk the coliform bacteria are most apt to be the main gas formers.
Heterofermentative lactics also may produce gas, but usually not enough to be evident in the milk. Yeasts (lactose-fermenting) usually are absent or in low numbers in milk and do not compete well with the bacteria.
Gas-forming *Clostridium* and *Bacillus* do not compete well with acid formers at higher temperatures but may function if the acid formers are absent or comparatively inactive. Milk heated at pasteurizing temperatures or above, the chief acid formers will be killed, the spores of Clostridium and *Bacillus* species will survive, and gas formation by the spore formers
may take place.

Proteolysis:
The hydrolysis of milk proteins by microorganisms usually is accompanied by the production of a bitter flavor caused by some of the peptides released. Proteolysis is favored by :
1. Storage at a low temperature.
2. by the destruction of lactic and other acid formers by heat.
3. By the destruction of formed acid in the milk by molds and film yeasts.
4. The neutralization of acids by products of other organisms.

The types of change produced by proteolytic microorganisms include
(1) Acid proteolysis, in which acid production and proteolysis occur together. Acid proteolysis may be caused by several species of *Micrococcus*, some of which grow in the udder of the cow and cause acid proteolysis of aseptically drawn milk.

(2) Proteolysis with little acidity or even with alkalinity.
(3) Sweet curdling, caused by rennin like enzymes of the bacteria at an early stage of proteolysis.
(4) Slow proteolysis by intracellular enzymes of bacteria after their autolysis. Slow proteolysis by intracellular enzymes of bacteria after their autolysis is of no significance in milk under ordinary circumstances but is significant when a long time is allowed for their action
(5) Residual proteolytic activity of heat-stable proteinase. ex., *Pseudomonas fluorescens* produces a proteinase that will survive pasteurization even though the bacterium does not.

Ropiness:
Ropiness and sliminess can occur in milk, cream, or whey but are important mostly in market milk and cream.
Nonbacterial ropiness or sliminess may be due to:
(1) Stringiness caused by mastitis and in particular by fibrin and leukocytes from the cow's blood
(2) Sliminess resulting from the thickness of cream, e.g., at the top of a bottle.
(3) Stringiness due to thin mms of casein or lactalbumin during cooling, as sometimes is observed on surface coolers. This effect is only temporary.
Bacterial ropiness is caused by slimy capsular material from the cells, usually gums or mucins, and develops best at low storage temperatures. The ropiness decreases as the acidity of the milk or cream increases.

There are two main types of bacterial ropiness, one in which the milk is most ropy at the top and the other in which the milk becomes ropy throughout.
Surface ropiness is caused most often by *Alcaligenes viscolactis*, an organism chiefly from water or soil that can grow fairly well in the vicinity of 100C. Some of the thermoduric microococci, e.g., *Micrococcus freudenreichii*, can cause surface ropiness.
Ropiness throughout the milk may be caused by any of a number of kinds of bacteria
1- *Enterobacter aerogenes*, *E. cloacae*, *Klebsiella oxytoca*, and rarely *Escherichia coli*. Ropiness caused by *Enterobacter* usually is worse near the top of the milk.
2- Certain strains of some of the common species of lactic acid bacteria. *Streptococcus lactis var. hollandicus* causes ropiness in milk, and is used in making Scandinavian fermented milk.

Lactobacillus casei, L. bulgaricus , and *L. plantarum* occasionally produce ropiness, as do strains of *Streptococcus cremoris*. Most of these lactic bacteria can grow in long chains, a characteristic that supposedly contributes to the stringy condition of the milk.

3. Miscellaneous other bacteria among the alkali formers, micrococci, streptococci, and bacilli. Ordinarily these bacteria would be suppressed by the acid formers.

Since the sources of the bacteria causing ropiness are water, manure, utensils, and feed, the reduction or elimination of contamination from these sources helps prevent ropiness. Adequate pasteurization of milk readily destroys most of these kinds of bacteria.

The following changes in the milk fat take place:

1. Oxidation of the unsaturated fatty acids, which, coupled with other decomposition, yields aldehydes, acids, and ketones and results in tallowy odors and tastes. The reaction is favored by metals, sunlight, and oxidizing microorganisms.
2. Hydrolysis of the butterfat to fatty acids and glycerol by the enzyme lipase. The lipase may have been in the original milk or may be microbial.
3. Combined oxidation and hydrolysis to produce rancidity.

Species of lipase-forming bacteria are found in many of the bacterial genera, e.g., *Pseudomonas, Proteus, Alcaligenes, Bacillus, Micrococcus, Clostridium*, and others. Many of the molds and some species of yeasts are lipolytic. ***Pseudomonas fragi*** **and *Staphylococcus aureus* produce fairly heat-resistant lipases which may survive pasteurization if present in the raw milk.**

Alkali Production:

The group of alkali formers includes bacteria which cause an alkaline reaction in milk without any evidence of proteolysis. The alkaline reaction may result from the formation of ammonia, as from urea, or of carbonates. Most of these bacteria grow from moderate to low temperatures, and many can survive pasteurization. Ex. of alkali formers are *Pseudomonas fluorescens* and *A. viscolactis*.

Flavor Changes:

As drawn from the cow, milk may be abnormal in flavor because of the individual cow, mastitis, the stage of lactation of the cow, or feed.

Some of the off-flavors caused by microorganisms are described

as follows:
1-**Sour or Acid Flavor:** The acidity may be described as
"clean," as produced by *Streptococcus lactis* and other lactic.
"aromatic;" when lactic streptococci and aroma-forming *Leuconostoc* species are growing together.
"sharp," when appreciable amounts of volatile fatty acids (formic, acetic, or butyric) are produced by coliform bacteria, *Clostridium* spp., and other organisms.

Clean and aromatic flavors are desired in fermented milk products, but sharp flavors are undesirable.

2-Bitter Flavors:

Bitterness usually results from proteolysis but may follow lipolysis or even fermentation of lactose. Milk from cows late in their lactation period sometimes is slightly bitter. some organisms can cause bitterness such a certain strains of coliform bacteria and of asporogenous yeasts.

3-Caramel Flavor:

Certain strains of *Streptococcus lactis var. maltigenes* produce this flavor, which resembles the cooked flavor of overheated milk.

Color Changes

Color changes caused by microorganisms may occur along with other changes. The color may be due to the surface growth of pigmented bacteria or molds in the form of a scum or ring or may be present throughout the milk.

1. Blue Milk: *Pseudomonas syncyanea* produces a bluish-gray to brownish color in milk in pure culture but when growing with an acid former like *Streptococcus lactis* causes a deep-blue color. This defect and the blue colour produced by actinomycetes or species of the mold *Geotrichum* are rare.

2. Yellow Milk: *Pseudomonas synxantha* may cause a yellow color in the cream layer of milk, coincident to lipolysis and proteolysis. Species of *Flavobacterium* can also give yellowness.

3. Red Milk: Red milk usually is caused by species of *Serratia*, e.g., *S. marcescens*, but is rare because other bacteria ordinarily outgrow the red-pigmented species. *Brevibacterium erythrogenes* produces a red layer at the top of the milk, followed by proteolysis. *Micrococcus roseus* may grow and produce red sediment, and yeast may produce pink or red colonies on the surface of sour milk or cream. Blood in milk will give it a red color. The red blood cells settle out or can be centrifuged out.

4. Brown Milk: A brown color may result from *Pseudomonas putrefaciens* or by the enzymic oxidation of tyrosine by *P.*

fluorescens.

Sources of contamination :
1)On the Farm
• Milk contains relatively few bacteria when it leaves the udder of the healthy cow and generally these bacteria do not grow in milk.
• During milking, the contamination of milk is by the exterior of the udder and adjacent areas. Bacteria found in the manure, soil and water may enter from this source.
• Two most significant sources of contamination are dairy utensils and milk-contact surface, including the milk pail or milking machines. • Undesirable bacteria from these sources include lactic streptococci, coliform bacteria, psychrotrophic gram-negative rods, and thermodurics, those which survive pasteurization, e.g., micrococci, enterococci, bacilli, and brevibacteria.
• Other possible sources of contamination are the hands and arms of the milker or dairy workers.
The number of bacteria per milliliter of milk added from various sources depends on the care taken to avoid contamination.

2)In Transit and at Manufacturing Level
• After milk is left in the farm, the possible contamination include the tanker truck, transfer pipes, sampling utensils, and the equipment at the market-milk plant or other processing plant.The amount or level of contamination from each of these sources depends on cleaning and sanitizing methods.
• Hands and arms of the employees are a possible source of contamination and pathogens.

Lecture 12

Microbiology of fruits and vegetables

Fruits

Despite the high water activity of most fruits, the low pH leads to their spoilage being dominated by fungi, both yeasts and moulds but especially the latter. The degree of specificity shown by many species of moulds, active in the spoilage of harvested fruits in the market place or the domestic fruit bowl, reflects their possible role as pathogens or endophytes of the plant before harvest. Thus *Penicillium italicum* and *P. digitatum* show considerable specificity for **citrus fruits**, being the blue mould and green mould respectively of oranges, lemons and other citrus fruits. ***Penicillium expansum* causes a soft rot of apples** and, although the rot itself is typically soft and pale brown, the emergence of a ring of tightly packed conidiophores bearing enormous numbers of blue conidiospores, has led to this species being referred to as **the blue mould of apples**.
An especially widespread mould on both fruits and vegetables is the
grey mould ***Botrytis cinerea*,** Infection of grapes on the vine by this same mould can lead to drying out of the grape and an increase in sugar concentration and wines made from such contaminated fruit are considered to be very special.
To avoid excessive mould spoilage of harvested fruit during storage and transport it is necessary to harvest at the right stage of maturity and avoid damage and bruising. Mouldy fruit should be removed and destroyed and good hygiene of containers and packaging equipment is essential to prevent a build-up of mould growth.

Vegetables

The higher pH values of the tissues of many vegetables makes them more
susceptible to bacterial invasion than fruits although there are also a
number of important spoilage fungi of stored vegetables. The bacteria
involved are usually **pectinolytic species** of the Gram-negative genera

Pectobacterium, Pseudomonas and Xanthomonas, although pectinolytic
strains of Clostridium can also be important in the spoilage of potatoes under some circumstances, and the non-sporing Gram-positive organism
Corynebacterium sepedonicum causes a ring rot of potatoes.

Deterioration of raw vegetables and fruits due to
1. Physical factors
2. Action of their own enzymes
3. Microbial action
4. Freezing
5. Desiccation
6. Other mishandling

Some microorganisms involved in the spoilage of fresh vegetables

Microorganism	**Vegetable**	**Symptom**
Corynebacterium sepedonicum	Potato	ring rot of tubers
Ralstonia solanacearum	Potato	soft rot
Pectobacterium carotovorum var. atrosepticum	Potato	soft rot
Streptomyces scabies	Potato	scab
Xanthomonas campestris	Brassicas	black rot
Aspergillus alliaceus	Garlic,Onion	black rot
Trichothecium roseum	Tomato	pink rot
Fusarium coeruleum	Potato	dry rot
Mycocentrospora acerina	Carrot	liquorice rot

Spoilage of fruit and vegetable juices

Molds can grow on the surface of acidic fruit juices if juices are exposed to air. High moisture content favors the faster growing of yeasts. The removal of solids from the juices by extraction and sieving raises the oxidation – reduction potential and favors the growth of yeasts.
Most fruit juices are acid enough and have sufficient sugar to favor the growth of yeasts within the range of temperature that favours them from 15.6 to 35°C. Concentrates of fruit and vegetable juices contain high sugar content. It favours the growth of yeasts like acid and sugar tolerant like *Leuconostoc and Lactobacillus* species.

In addition to the usual alcoholic fermentation, fruit juices may undergo other changes caused by microorganisms:

1. **The lactic acid fermentation of sugars,** mostly by heterofermentative lactic acid bacteria such as *Lactobacillus brevis* and *Leuconostoc mesenteroides* in apple or pear juice attack by homofermentative lactic acid bacteria such as *Lactobacillus arabinosus*,

2. **The fermentation of organic acids** of the juice by lactic acid bacteria. E.x. *Lactobacillus psatorianus*, malic acid to lactic acid and succinic acid, and citric acid to lactic and acetic acids.

3. **Slime production** by *Leuconostoc mesenteroides*, Lactobacillus brevis, and *Lactobacillus plantarum* in apple juice and by *L. plantarum* and Streptocooci in grape juice.

Lecture 13

Defense Reactions

Fruits and vegetables offer a range of barriers to infection by postharvest spoilage microorganisms. Some are preformed or constitutive in the plant organ.

The cuticle barrier is the most external barrier to penetration. Removal of waxes from the cuticle has been shown to increase the vulnerability of pepper fruits and cabbage to infection by certain fungi.

Preformed antimicrobial compounds, designated "phytoanticipins," may also be involved in plant resistance, although demonstration of their actual effects on resistance is relatively hard to achieve because of difficulties in assessing inhibitory activity and in correlating changes in concentrations with decay development.

A wide range of defensive barriers may also be formed in reaction to an infection and constitute a plant immune system. **Pathogens produce pathogen-associated molecular pattern elicitors, which elicit plant defense reactions.** Plant tissues may produce small molecules of varied nature, known as **phytoalexins** .

Bramley's Seedling apples produce benzoic acid in response to the some infection , and the resistance of carrot to *B. cinerea* has been attributed to a **coumarin**.

Structural changes such as accumulation of lignin or suberin or development of callus have also been observed as defensive reactions, particularly in carrot, potato tuber, and pear .

Polygalacturonase-inhibiting proteins produced by plants and acting against endopolygalacturonases of plant-pathogenic molds that cause wall degradation and tissue maceration have been detected in a range of fruits and vegetables, including apple, pear, grape, raspberry, onion, and pepper

Controlling Spoilage

Postharvest control of **temperature, relative humidity, and**

composition of the gaseous atmosphere can reduce the physiological activity of fruits and vegetables, thereby delaying ripening and senescence and consequently prolonging shelf life. These environmental factors may act by giving less opportunity for the pathogen to develop by retaining the integrity of the plant organ and by directly inhibiting microbial growth.

-**A decrease in temperature** by 10°C reduces the respiratory activity 2- to 4-fold , and temperature close to 0°C is recommended for most commodities, with the exception of those of tropical origin and a few temperate produce items that suffer from physiological disorders (chilling
injuries) when stored at refrigeration temperatures. For these products, the optimal storage temperature is close to 10°C.

-Modified atmospheres, used in combination to **chill storage,** also reduce the physiological activity of fresh produce. Recommended CO_2 concentrations for produce storage rarely exceed 10%, and 1 to 5% O_2 is tolerated. Exposure to higher CO_2 (lower O_2) levels may result in physiological disorders, leading to loss in quality. Modified atmospheres reduce microbial spoilage of fruits and vegetables in many instances, although some spoilage microorganisms are not directly inhibited. For example, controlled atmosphere storage
of apples at 1°C in an atmosphere containing 5% CO_2 and 3% O_2 prevents the development of lesions, while in vitro this gaseous atmosphere has no effect. Generally, The lower susceptibility to postharvest pathogens of fruits and vegetables stored under controlled atmospheres is mainly due to delayed senescence.

-**Water loss** is a consequence of respiratory activity and is highly detrimental to quality. A 10% weight loss (much less for leafy vegetables) makes produce unacceptable to consumers .Storage at relative humidity higher than 90% is recommended for most commodities, with a few exceptions, e.g., garlic and onion.
High humidity also increases the availability of nutrients to spoilage microorganisms, favoring their survival and their germination.

-The possibility of using ionizing radiation to extend the shelf life of fruits and vegetables has been studied since the 1950s . Postharvest spoilage bacteria and fungi are sensitive to ionizing radiation. Doses lower than 4 kGy reduce 1,000-fold the germination of major postharvest pathogens such as *Penicillium* spp..Decimal reduction doses for *Pseudomonas* and other gram-negative bacteria are approximately 0.2 kGy. However, it appears that some fruits and vegetables are adversely affected (by tissue softening, for instance) by doses necessary to inactivate some postharvest pathogens .Treatment with ionizing radiation is consequently useful to lower initial contamination or to inhibit the growth of postharvest pathogens and to delay the development of disease, but not for complete elimination of microbial contaminants.

-**Prestorage heat treatment** has been used with some success to reduce postharvest spoilage of a wide range of fresh produce, including green pepper, apple, and citrus. Two main types of applications can be distinguished:
1- short-term exposure to heat, from a few seconds at 60 to 62°C to 60 to 120 min at about 45°C.
2- by immersion in or rinsing and spraying with hot water; or long-term exposure, also known as “curing” (for a few hours to several days), mainly in hot air.
Two kinds of effects can be observed, either **direct effects** on microbial contaminants or **indirect effects** on the treated fruit or vegetable.

Lecture 14

Microbiology of Meat, Poultry, and Seafood

Types and Origins of Initial Microfloras

It is generally accepted that bacteria are absent, undetectable, or present in very low numbers within muscle tissues of healthy live food animals (i.e., meat, poultry, and seafood). The processes of animal slaughtering and carcass dressing, or catching of seafood, allow microbial contaminants to be deposited on the exposed cut surfaces of muscle and adipose tissue. Contamination originates from the external animal surface, including the gastrointestinal tract, as well as from the environment, including air, soil, water, equipment surfaces, and humans. The exposed muscle tissues may become contaminated with a vast array of gram negative and gram-positive bacteria and fungi .

The initial prevalence and extent of microbial contamination on muscle foods may vary depending on the origin of the animal and the sanitation procedures and
hygienic practices employed during production, transport, handling, and processing of the product. Specific parameters that affect the composition and numbers
of the microflora include environmental conditions, such as wet or muddy hides, which may contain higher populations of bacteria indigenous to soil, whereas
microorganisms of fecal origin are generally more common when the hide is soiled with fecal material.

Microbial Cell Attachment and Biofilms

The first event in muscle food contamination is **the attachment of microbial cells to a surface**, which is followed by **colonization**. The composition of the initial microbiota on meat surfaces may be **affected** by differences in the rate of attachment of bacterial strains. ***Pseudomonas* is considered to attach more readily onto muscle tissue surfaces than other spoilage bacteria** . Preevisceration spray-washing or decontamination of carcasses is applied immediately after hide removal in the United States with the goal of removing contamination before strong attachment of cells occurs on the carcass surface .

In general, **bacterial cell attachment** on biotic (e.g., muscle tissue) or abiotic (e.g., equipment) surfaces may be influenced by **factors** such as **surface characteristics, properties of the substrate, and physiological stage, surface characteristics, and motility of the cells .**

Biofilms consist of bacterial cells encapsulated in an exopolysaccharide matrix that allows them to adhere to surfaces and each other and protects them from adverse conditions . Cells form microcolonies or clusters enclosed within the hydrated matrix, and pores or channels throughout the structure allow transport of oxygen, nutrients, and waste. Cell matrices form a network that facilitates formation and maintenance of the biofilm structure and increases cell resistance to sanitizers.
biofilms may form in all areas of food processing environments, including floors, walls, pipes, and drains etc. Hard-to-clean and -sanitize crevices in conveyor belts, pasteurizers, gaskets, and dead spaces become sites of biofilm establishment .Pathogens such as *Listeria monocytogenes* may persist in food plants for months and up to several years .

Mono- or multispecies biofilms are formed by spoilage as well as pathogenic bacteria, including *Pseudomonas*, *Listeria* , *Salmonella*, *Campylobacter*, *Escherichia coli*, and lactic acid bacteria (LAB).

Microbial Spoilage Of Muscle Foods

Development of the Spoilage Association

Muscle food spoilage, being the result of chemical, enzymatic, and microbial activities. As the inherent protective barriers (i.e., skin, hide, and shells) are destroyed at slaughter, or during catching of fish and shellfish, the resulting tissues become exposed to environmental microbial contamination. In addition
to reduced contamination levels (discussed in the following paragraphs), decontamination of meat with acid solutions may lead to changes in the dominating microbes associated with the tissue and, if combined with. long-term storage, may allow development of yeasts, whereas hot water or steam decontamination may select for other types of spoilage microorganisms .The levels and types of initial microbiotas also vary due to the level of hygiene, temperature, air flow, gaseous atmosphere, and other influences encountered during product handling, processing, and storage, as they select which of the initial or new contaminants will prevail and form the dominant microbiota as the product ages.

In general, relatively few microbial types dominate through selection during storage based mostly on storage temperature and the gaseous composition of the surroundings. Aerobic or facultative anaerobic gram-negative bacteria such as **Pseudomonads** dominate under cold aerobic conditions, whereas the dominant microflora consists largely of LAB in **vacuum-packaged products** . LAB, although detected in the aerobic spoilage flora of chilled meat, are considered important only in the aerobic spoilage of lamb. Muscle souring is caused by growth of LAB and *B. thermosphacta*. **LAB** are mostly responsible for spoilage of meat in restricted oxygen environments. Species of ***Leuconostoc* and *L. sakei*** have been associated with spoilage of cold stored vacuum or under modified atmosphere packaging (MAP) smeat consisting of 40% CO_2–30% O_2–30% N_2. Hence, different microbial species dominate in different environments and contribute to muscle food spoilage as they release different volatile compounds.

Spoilage differs among **cooked, cured, heat-processed, fermented, or dried products of varying water activity and pH.** Spoilage during storage of perishable, processed or cooked, uncured meats is due to surviving or **postcooking microbial contaminants**. Dominant spoilage microorganisms may include micrococci, streptococci, lactobacilli, and *B. thermosphacta* . Recontamination with nonproteolytic bacteria results in the development of sour odors, whereas recontamination with proteolytics results in putrid odors due to the breakdown of amino acids. Spoilage of canned muscle food products is usually due to spoiled raw materials, inadequate thermal processing allowing survival of heat-resistant mesophilic spore formers, slow cooling or storage at high temperatures that allow proliferation of thermophilic sporeformers, or reintroduction of microorganisms through postprocessing leakage .

Pathogens In Muscle Foods

Biological hazards associated with meat, poultry, and seafood include pathogenic bacteria, viruses, parasites, and toxigenic molds. According to the U.S. Centers for Disease Control and Prevention (CDC), pathogens (and some associated foods) responsible for most foodborne illness are *Campylobacter* (poultry), *E. coli* O157:H7 (ground beef, leafy greens, and raw milk), *L. monocytogenes* (deli meats, unpasteurized soft cheeses, and produce), *Salmonella* (eggs, poultry, meat, and produce), *Vibrio* (raw oysters), norovirus (many foods; e.g., sandwiches and salads), and *Toxoplasma* (meats). Viral pathogens are of major concern in food service, whereas bacterial pathogens such as *E. coli* O157:H7 and other Shiga toxin-producing *E. coli* (STEC), *Salmonella*, and *Campylobacter* continue to be of importance in the safety of raw meat and

poultry, as does *L. monocytogenes*
in ready-to-eat processed products .

Risks to consumers from consumption of stored muscle food products may also be due to the presence of biogenic amines such as histamine, putrescine, spermidine, and spermine. Amines found in fresh meat and fish products (primarily scombroid species such as tuna, mahimahi, and mackerel) under aerobic or vacuum/ MAP storage may lead to scombroid poisoning, a severe, and sometimes fatal, allergic reaction. Biogenic amine formation in some products has been attributed to *Enterobacteriaceae*; however, tyramine can also be formed by some strains of *Lactobacillus*. Proper sanitation and storage temperature and storage time limitation should minimize human health problems associated with biogenic amines in muscle foods.

Fermented Meats, Bacteriocins, And Probiotic Foods

Fermented foods are products of metabolic processes of various microbial types, including bacteria, yeasts, molds, or combinations thereof, that convert food commodity substrates into various types of desirable foods or ingredients such as vitamins and enzymes. There is a vast array of fermented muscle food products available throughout the world. Traditional fermented foods predate written history and were developed to upgrade plant and animal materials by producing more acceptable foods and to prevent the growth of spoilage and pathogenic microorganisms without the need for cold storage. Common fermented foods are sausages, which were invented by the Sumerians about 5,000 years ago and were popular among ancient Greeks and Romans .

Major microbial groups used in production of fermented sausages and similar products are LAB and coagulase-negative cocci. Depending on the product, groups such as yeasts and enterococci may also play a role. The fermentation process involves complex biochemical and physical reactions resulting in significant
changes in the initial product characteristics. The sensorial profile of the final product is affected by the dominating microbial strains.
In addition to their crucial role in meat fermentations, LAB are the most recognized and investigated producers of microbial antagonists, such as bacteriocins.
Bacteriocins, however, are known to exert only a transitory bactericidal effect against pathogens such as *L. monocytogenes*; often, there is regrowth of the pathogen in bacteriocin-containing foods. The regrowth may be due to factors that severely limit growth of bacteriocin producing cells

(e.g., restricted nutrient availability); decreased bacteriocin action as a result of adsorption onto
food particles, fat, and protein; the presence of curing agents; the emergence of bacteriocin-resistant cells; and or bacteriocin degradation by proteases of food and/or microbial origin. Although abundant in the environment and extensively researched in the past 30 years, the bacteriocins described above, with the exception of nisin, have not received regulatory approval in the United States and so are not commercially used in foods.

Recently, in the United States, probiotic-containing foods have gained consumer acceptance as health- promoting, functional foods. Hence, there is interest in developing fermented sausages based on probiotic microorganisms. Results of related research are still preliminary; hence, evaluations of the human health effects of fermented meats produced by probiotic bacteria are pending. There is also interest for use of LAB as probiotics in fish products.

Lecture 15

Hazardous Substances In Food

Three general classes of hazards are found in foods: (1) microbial or environmental contaminants, (2) naturally occurring toxic constituents, and (3) those resulting from intentional food additives or novel foods or ingredients. The most dangerous contaminants are those produced by infestations of bacteria or moulds in food, which can produce toxins that remain in the food even after the biological source has been destroyed.

Microbial Contamination

Pathogenic bacteria

Outbreaks of acute gastroenteritis caused by microbial pathogens are usually called food poisoning. They can be caused by foodborne intoxication (where microbes in food produce a toxin that produces the symptom) or foodborne infection (where the symptoms are caused by the activity of live bacterial cells multiplying in the gastrointestinal system).
The Table below lists the most common bacterial causes of food poisoning, in order of the rapidity of onset of symptoms. In general the intoxications have a more rapid onset.

The most important pathogens are *Clostridium botulinum*, *Staphylococcus aureus*, *Salmonella* species and *Clostridium perfringens*. The last three organisms account for about 70-80% of all reported outbreaks of foodborne illness, but there are also many others as well as some viral and protozoan agents.
The four most frequently identified factors contributing to food poisoning incidents are: improper cooling of food, lapses of 12 hours or more between preparing and eating, contamination by food handlers, and contaminated raw foods or ingredients.

Mycotoxins

Moulds, or fungi, are capable of producing a wide variety of chemicals that are biologically active. Humans have used some of these as effective antibiotics, but there are also a number of diseases resulting from accidental exposure to fungal products that contaminate food. Some examples are as follows:

Aflatoxins These are a group of highly toxic and carcinogenic compounds from the common *Aspergillus* fungus species. They are stable to heat and survive most forms of food processing. Aflatoxin contamination can occur whenever environmental conditions are suitable for mould growth, but the problem is more common in tropical and semitropical regions. Aflatoxins were first recognised in the 1960s in peanuts. On a world-wide basis, maize is the most important food contaminated with aflatoxin.

Patulin is an antibiotic that is produced by the mould *Penicillium caviforme.* It has been implicated as a possible carcinogen. Patulin in primarily associated with the apple rotting fungus and so apple juices and some baked goods with fruit can contain patulin.

Fumonisins are carcinogenic mycotoxins from the *Fusarium* fungus associated with corn. In 1990 it was reported that use of mouldy corn with high levels of fumonisins to make beer in the Transkei of South Africa was associated with a very high incidence of oesphageal cancer.

New foodborne diseases

Three of the most serious food pathogens today **(Campylobacter, Listeria and enterohemorrhagic E. coli)** were unrecognised as causes of illness 30 years ago. Some of the more important new organisms are:

Campylobacter jejuni was a well-known bacterium in veterinary medicine before it was identified as a human pathogen in 1973. It is now recognised as one of the most important causes of gastroenteritis in humans of similar importance to *Salmonella.* It is present in the flesh of cattle, sheep, pigs and poultry and can be introduced wherever raw meat is handled.
Listeria monocytogenes is a bacterium widely distributed in nature but is unusual in that it grows at refrigeration temperatures (down to 0°C). **Listeriosis** can cause abortions as well as death in the elderly and those with compromised immune systems, such as

people with AIDS. Listeria has been linked to the consumption of contaminated pâtés, milk, soft cheese and undercooked chicken, and is often found in pre-prepared chilled food.

Escherichia coli 0157:H7 is a bacterium that can damage the cells of the colon, leading to bloody diarrhoea and abdominal cramps. Raw or undercooked hamburger meat was a major vehicle of transmission in a number of well publicised outbreaks in the USA.

Salmonella typhimurium is a multi-drug resistant strain that has become a major pathogen in the UK. As well as being highly virulent it can survive at low pH and be infectious in very low numbers.

Norwalk virus is found in the faeces of humans and illness is caused by poor personal hygiene among infected food handlers. Symptoms include nausea, vomiting, diarrhoea, abdominal pain and fever. Because it is a virus, it does not reproduce in food, but remains active until the food is eaten.

'Mad cow disease' (or BSE -bovine spongiform encephalopathy) is a slowly progressive and ultimately fatal neurological disorder of adult cattle that results from infection by an unique transmission agent called a prion. Prions seem to be modified forms of normal cell surface proteins. BSE was first confirmed in Britain in 1986, but has now spread to cattle in other countries of Europe, Japan and North America. The same infective agent is also responsible for variant Creutzfeldt Jakob Disease (vCJD), a fatal disease of humans, mostly affecting young adults. By October 2009, it had killed 166 people in Britain and 44 elsewhere, with the number expected to rise because of the disease's long incubation period.

Environmental Contamination

Heavy metals and minerals

Selenium is one of the most toxic essential trace elements. The level of selenium in foods usually reflects the levels in the soil and in a few high-selenium areas, such as North Dakota and parts of China, excessive selenium intake has been associated with gastrointestinal disturbances and skin discolouration.

Mercury. Fish can contain 10-1500mg/kg of organic mercury, and even higher levels when mercury wastes are released into lake waters. Serious poisonings from mercury in fish have occurred in Japan, the most famous being that in Minamata Bay (from 1953 to

1960). Another example of widespread mercury intoxication occurred in Iraq in 1971/72 as a result of bread made from wheat treated with mercury-based pesticides. Most countries have now established maximum permitted levels on mercury in fish in the range of 0.4-1.0mg/kg.

Cadmium is a toxic element that accumulates in biological systems. Chronic exposure at excessive levels can lead to irreversible kidney failure. Plants readily take up cadmium from the soil, and there has been a slow increase in the cadmium levels in soils due to the use of phosphate fertilisers and the affect of air and water pollution.

Table. Common Bacterial Food Poisoning Organisms

Organism	**Symptoms**	**Time after food**	**Typical food sources**
Toxins			
Staphylococcus aureus	Vomiting, diarrhoea, abdominal pain	1-6 hours	Custard and cream-filled baked goods, cold meats
*Clostridium perfringen*s	Diarrhoea and severe pain, nausea	8-24 hours	Meat products incompletely cooked or reheated
Bacillus cereus	a) nausea, vomiting; b) abdominal pain, watery diarrhoea	a) 1-5 h; b) 6-16 h	Rice dishes, vegetables, sauces, puddings
Clostridium botulinum	Dry mouth,difficulty swallowing and speaking, double vision, difficulty breathing. Often fatal	2h-8 days	Home canned foods (usually meat and vegetables), and inadequately processed smoked meats
Infection			
Vibrio parahaemolyticus	Diarrhoea, abdominal cramp, nausea,	4-96 h	Fish, crustaceans

	headache, vomiting		
Salmonella spp.	Diarrhoea, fever, nausea, vomiting	8-72 h	Undercooked poultry, reheated food, cream-filled pastries
Yersinia enterocolytica	Fever, abdominal pain, diarrhoea	24-36 hours	Raw and cooked pork and beef
Escherichia coli	Fever, cramps, nausea, diarrhoea	8-44 hours	Faecal contamination of food or water
Shigella ssp.	Diarrhoea, bloody stools with mucus, fever	1-7 days	Faecal contamination of food
Campylobacter jejuni	Fever, abdominal pain, diarrhoea	1-10 days	Raw milk, poultry, eggs, meat
Listeria *monocytogenes*	Septic abortion, septicaemia, meningitis, encephalitis. Often fatal	1-7 weeks	Milk and dairy products, raw meat, poultry and eggs, vegetables and salads, seafood