Unit -2

Food Microbiology

- **<u>Food as a substrate for microorganisms</u>: -**

The interactions between microorganisms, plants and animals are natural and constant. Since the human food supply consists basically of plants and animals or products derived from them, it is understandable that our food supply can contain microorganisms in interaction with the food.

In most cases microorganisms use our food supply as a source of nutrients for their own growth. This can result in deterioration of the food. Microorganisms can 'spoil' food by increasing their numbers, utilizing nutrients, producing enzymatic changes and contributing off-flavours. Microorganisms breakdown food products or synthesize new compounds during spoilage of food. This is one of their functions in nature to convert reduced forms of carbon, nitrogen, and sulphur in dead plants and animals to the oxidized forms.

To prevent spoilage of food we minimize the contact between microorganisms and our foods (prevent contamination) and also eliminate microorganisms from our foods, or at least adjust conditions of storage to prevent their growth (preservation).

When the microorganisms involved are pathogenic, food infection and food poisoning may result.

Interactions between microorganisms and our foods are sometimes beneficial. We use them for the production of fermented food products like curd, idli, dosa, sauerkraut, pickles etc.

Then questions arise—

What are the governing factors in these interactions?

Why is this interaction beneficial at some times and not at others?

Why do some foods support the growth of microorganisms more readily than others?

Why are some foods very stable in regard to microbial deterioration?

Food is the substrate and so the characteristics of the food are an important consideration. The type of microorganisms present and the environmental conditions also are important. However, the food or substrate dictates what can or cannot grow. By understanding the characteristics of the food or substrate one can make predictions about the microbial flora that may develop.

- **<u>Major groups of bacteria, fungi, yeasts important in food microbiology</u>.**

Food microbiologists must know about the microorganisms, which are important in foods; at least to the extent that will enable them to identify the main types.

Generally—

1) Bacteria

2) Fungi (Molds)

3) Yeasts

are important in food microbiology.

1) Bacteria

Morphological characteristics: - These are prokaryotic, single celled, oval, rod, comma, or spiral shaped. Some have capsule, flagella, or endospores.

Encapsulation: - The presence of capsules or slime may account for sliminess or ropiness of a food. In addition, capsules serve to increase the resistance of bacteria to adverse conditions, such as heat or chemicals.

Endospores: - Bacteria of the genera *Bacillus, Clostridium, Desulfatomaculum, Sporolactobacillus* (rods), *and sporosarcina* (cocci) have the ability to form endospores. These are very refractile and resistant to heat, ultraviolet light and desiccation.

Formation of cell aggregates: - It is a characteristic of some bacteria to form long chains and of others to clump under certain conditions. It is more difficult to kill all bacteria in intertwined chains or sizable clumps than to destroy separate cells.

Cultural characteristics important in food bacteriology: -

Bacterial growth in and on foods often is extensive enough to make the food unattractive in appearance or otherwise objectionable. Pigmented bacteria cause discoloration on the surface of foods; films may cover the surfaces of the liquids, growth may make surface slimy; or growth throughout the liquids may result in undesirable cloudiness or sediment.

Physiological characteristics important in food bacteriology: -

The chemical changes occur when bacteria grow in food like hydrolysis of complex carbohydrates to simple ones; hydrolysis of proteins to polypeptides, amino acids, and ammonia or amines and hydrolysis of fats to glycerol and fatty acids. O-R reactions, which are utilized by bacteria to obtain energy from foods (carbohydrates, other carbon compounds, simple nitrogen-carbon compounds etc.) yield such products as organic acids, alcohols, aldehydes, ketones, and gases.

Genera of bacteria important in food bacteriology

Sr. No.	**Name of bacterial genus**	**Characters**	**Importance**
1	Acetobactor	Rod shaped, motile	1. Oxidize ethyl alcohol to acetic acid. 2. Found on fruits, vegetables, souring fruits, and alcoholic beverages. 3. Cause spoilages in alcoholic beverages.
2	Aeromonas	Rods, Gram negative, facultative anaerobic	1. Found in aquatic environment. 2. Pathogenic to fish, frogs.
3	Alcaligenes	Produce alkaline condition	1. *A. viscolactis* causes ropiness in milk and *A. metalcaligenes* gives a slimy growth to cheese.
4	Alteromonas	Several former species of *Pseudomonas* are now classified as	1. Found in marine environment and are important in sea foods.

		Alteromonas	
5	Arthrobacter	Soil organisms, psycrophilic	Enter into food through soil and spoil food
6	Bacillus	Form endospores, aerobic and facultative, mesophilic or thermophilic, may or may not be proteolytic, gas forming or not, lipolytic or not	1. *B. cereus* is proteolytic and cause spoilage in proteinaceous food. 2. *B. polymyxa*, *B. macerans* produce acid and gas in food. 3. *B. coagulans* produce lactic acid from sugar and sour the food and it is used in production of lactic acid. 4. *B. pumilus* (ATCC 27142) is used for testing of sterilization by gamma radiation. 5. *B. stearotherophilus* (ATCC 7953) and *B. subtilis* (ATCC 6633) are used for testing of sterilization by steam. 6. *B. subtilis* var. *niger* (ATCC 9372) is used for testing of sterilization by ethylene oxide.
7	Brevibacteri-um		*B. linens* is important in surface smear of certain cheeses, e.g., Brick or Limburger, where they produce an orange-red pigmentation and help ripening.
8	Brochotrix	Gram-positive rods, temp. range 0 to 45 ^{0} C, pH range 5 to 9, grow in 6 to 10% NaCl.	They can spoil a wide variety of meats and meat products.
9	Campyloba-cter	Were originally classified in the genus *Vibrio*, Gram negtive curved rods	*C. fetus* subspecies *jejuni* associated with gastroenteritis
10	Clostridium	Anaerobic to microaerophilic, produce, endospores, many species are proteolytic, all are catalase negative	1. Cause gaseous spoilage of canned vegetables. 2. *C. putrefaciens & C. lentoputrescens* cause putrefaction in protein food. 3. *C. perfringens* causes

			violent disruption or stormy fermentation of the curd. 4. *C. butyricum* cause late gas in cured cheese. 5. *C. botulinum* causes botulism (food poisoning)
11	Corynebacte-rium	Slender, barred or clubbed rods	1. *C. diptheriae* cause diptheria 2. *C. bovis* cause bovine mastitis
12	Desulfatoma-culum	Gram negative rods, endospore forming, sulfur compounds serve as terminal electron acceptor in respiration and reduced to hydrogen sulfide	*D. nigrificans* responsible for sulfide stinker spoilage in canned foods.
13	Enterobacter	Were formerly classified as *Aerobacter*	Widely distributed in nature and member of coliform group and contaminate food.
14	Erwinia	Plant pathogens	1. Cause necrosis, galls, wilts, or soft rots in plants and damage vegetables and fruits. 2. *E. carotovora* causes soft rot and rotting of plants. 3. *E. carotovora* subspecies *atroseptica* produces black rots in potatoes. 4. Subspecies *betavasculorum* causes soft rot in sugar beets
15	Escherichia	Found in feces, Gram negative rods, present in intestinal tract, one of the coliform group.	1. Normal flora of intestine but many serotypes can be pathogenic. 2. Associated with water hence contaminate food.
16	Flavobacterium	Produce yellow to orange pigment	1. Cause discolouration on the surface of meats. 2. Involved in spoilage of shellfish, poultry, eggs, butter and milk. 3. Psycrophilic species grow on thawing vegetables
17	Gluconobacter	Oxydize ethanol to acetic acid	*G. oxydans* causes ropiness in beer following viscous growth in beer or wort.
18	Halobacterium	Halophiles, chromogenic	Grow and cause discolourations on foods high

			in salt, such as salted fish.
19	Klebsiella	Capsulated	*K. pneumoniae* causes pneumonia
20	Lactobacillus	Long slender Rods, microaerophilic, catalase negative, Gram positive, Homofermentative ferment sugars to lactic acids, acetic acid, carbon dioxide, Heterofermentative produce lactic acid, alcohol and volatile products.	1. Homofermentatives which grow at 37 ^{0}C and above are *L. bulgaris, L. helveticus, L. lactis, L. acidophilus, L. thermophilus.* Homofermentative with lower optimal temp. are *L. casei, L. plantarum, L. leichmannii.* 2. Heterofermentative with higher optimal temp. are *L. delbrueckii and L. fermentum.* Heterofermentative with lower optimal temp. are *L. brevis, L. buchneri, L. pastorianus, L. hilgardi and L. trichodes* 3. These are used to produce curd and fermented milk products, butter, cheese, sauerkrauts and ghee. 4. Used for production of lactic acid. 4. Some spoil foods, beers, wines etc. 5. Grow in refrigerated meats, cause greening of sausages.
21	Leuconostoc	Also called *Betacoccus,* Heterofermentative lactic streptococci, produce lactic acid, acetic acid, ethanol and carbon dioxide, produce flavouring agent diacetyl, acetoin and 2, 3 butanediol.	1. Produce flavouring agent in curd and fermented milk products from citric acid in milk like diacetyl, acetoin and 2,3 butanediol. 2. Used as starter cultures in production of buttermilk, butter and cheese. 3. Used in sauerkrauts, vegetable fermentation products. 4. Tolerate about 60% sugar concentrations hence grow in syrups, cakes, and ice creams. 5. Produce carbon dioxide in cheeses, spoil foods high in sugars.
22	Microbacterium	Resist adverse conditions, produce vitamins, nonmotile,	1. Survive pasteurization of milk hence give high counts in these milks 2. Optimum

		Gram positive, rods, asporogenous, aerobic, homofermentative, lactic acid producers	temperature 30 ^{0}C.
23	Micrococcus	Spherical, Gram positive, aerobic, catalase positive, optimum temperature 25 to 30 ^{0} C	1. Tolerate high concentration of salts. 2. Acid-proteolytic. 3. Produce pigment and discolour the surface of foods e.g. *M. luteus* produce yellow and *M. roseus* pink pigments.
24	Mycobacterium	Tubercle bacilli	Causes tuberculosis, present in raw milk of infected animals
25	Pediococcus	Cocci, Gram positive, catalase negative, microaerophilic	1. Salt tolerant, acid producers, wide range of temperature. 2. Spoil alcoholic beverages. *p. damnosus* spoil beer. 3. *P. cerevisiae* used as starter culture in sausages
26	Photobacterium	Coccobacilli, luminescent	Cause phosphorescence of meats and fish.
27	Propionibacterium	Rods or coccoid, small, nonmotile, Gram positive, asporogenus, catalase positive, anaerobic to aerotolerant	1. *Propionibacterium freudenreichii* ferment lactates to produce the gas that helps to form holes, or eyes, and also contribute to flavour in Swiss cheese. 2. Pigmented propionobacteria can cause colour defects in cheese.
28	Proteus		1. Involved in spoilage of meat, seafood, and eggs. 2. Cause food poisoning in nonrefrigerated foods.
29	Pseudomonas	Gram negative, motile, asporogenus rods	1. Utilize large variety of noncarbohydrate carbon compounds, unable to use most carbohydrates. 2. Produce variety of products that affect flavour of food. 3. Use simple nitrogenous products and produce own growth factors and vitamins. 4. Proteolytic and lipolytic. 5. Produce oxidised products and slime at the surface of foods. 6. Grow

			in refrigerated foods. 7. Produce white, creamy, reddish; brown pigment *P. fluorescens* produces green (pyoverdin), and *P. nigrificans* produces black fluorescence.
30	Salmonella		Enteric pathogens, grow in foods, cause food infections and typhoid
31	Serratia		Produce pink or magenta pigment to cause red discolouration on the surface of foods.
32	Shigella		Cause bacillary dysenteries
33	Sporolactobacil lus		Form endospores and resemble lactobacillus
34	Staphylococcus	Gram positive, cluster forming cocci, beta haemolytic, coagulase positive	*S. aureus* gives yellow to orange colour and produce enterotoxin which causes food poisoning
35	Streptococcus	Cocci, form chains, Gram positive	1. Pyogenic (pus producing) group *S. agalactiae* causes mastitis in cow. *S. pyogenes* causes human septic sore throat and scarlet fever. 2. Viridans group includes *S. thermophilus* important in cheeses, yoghurt, in pasteurized milk. 3. Lactic group *S. lactis, L. cremoris* are used as starter cultures for cheese, buttermilk, butter, curd etc. 4. Enterococcus group *S. faecalis* found in raw foods, originate in intestinal tracts of human and animals and used as indicator organisms as faecal pollution.
36	Vibrio		Widely distributed in fresh and salt water, soil, and alimentary canal of humans and animals, cause cholera.

2) Fungi (Molds)

Mold growth on foods, with its fuzzy or cottony appearance, sometimes coloured, is familiar to everyone. Usually such food is considered unfit to eat. Some molds are involved in the spoilage of many kinds of foods, while special molds are used in the manufacture of certain foods.

Some kinds of cheese are mold ripened like Blue, Roquefort, Camembert, Gammelost etc., some are used in making oriental foods, e.g. soy sauce, miso, sonti etc. Molds have been grown as food or feed and are used to produce products used in foods, such as **amylase** for bread making or **citric acid** used in soft drinks.

General Characters of molds: -

The term **'mold'** is a common one applied to certain multicellular, filamentous fungi whose growth on foods usually is readily recognized by its fuzzy or cottony appearance. The main part of the growth commonly appears white but may be coloured or dark or smoky. Coloured spores are typical of mature mold of some kinds and give colour to part or all of the growth. The thallus or vegetative body is characteristic of thallophytes, which lack true roots, stems, and leaves.

Morphological Characters of molds: -

The morphology, i.e. the form and structure of molds is used in their identification and classification.

Hyphae and Mycelium: -

The mold thallus consists of a mass of branching, intertwined filaments called **hyphae** (singular **hypha**), and whole masses of these hyphae are known as the **mycelium.** The haphae may be **submerged,** or growing within the food, or **aerial,** or growing into the air above the food. Hyphae may be classified as **vegetative**, **or growing,** and hence involved chiefly in the nutrition of the mold, or **fertile,** involved in the production of reproductive parts.

In most molds the fertile hyphae are aerial, but in some molds they may be submerged. The hyphae of some molds are full and smooth, but in others are thin and ragged. A few kinds of molds produce **sclerota** (singular **sclerotum**), which are tightly packed masses of modified hyphae, often thick walled, within the mycelium.

Molds are divided into 2 groups—

Septate: - with cross walls dividing the hyphae into cells and nucleus in each cell
Nonseptate: - without cross walls and nuclei scattered throughout their length.

Some hyphae are clear, but some are dark or smoky or coloured. Special mycelial structures help in the identification of molds e.g. **the rhizoids** of *Rhizopus and Absidia*, **the foot cell** in *Aspergillus,* and **the dichotomous, or Y-shaped** branching in *Geotrichum.*

Reproductive parts in molds: -

Molds can grow from a transplanted piece of mycelium. Reproduction of molds is chiefly by means of asexual spores. Some molds also form sexual spores. Such molds are termed **'perfect'** and are classified as either ***Oomycetes or Zygomycetes*** if nonseptate, or ***Ascomycetes or Basidiomycetes*** if septate. The asexual spores producing septate molds are termed as **'imperfect'** and are classified as ***Fungi imperfecti.***

I) Asexual spores: -

These are produced in large numbers and are small, light, and resistant to drying. They spread readily through air and start new mold thallus where conditions are favourable. There are 4 types of asexual spores—

1) **Conidia (**singular conidium)
2) **Arthrospores or oidia (**singular oidium)
3) **Sporangiospores**
4) **Chlamydospores**

II) Sexual spores: -

There are 4 types of sexual spores—

1. Oospores produced by ***Oomycetes***
2. Zygospores produced by ***Zygomycetes***
3. Ascospores produced by ***Ascomyctes***
4. Basidiospores produced by ***Basidiomycetes***

 # Important molds in food and industries

Sr. No.	**Name of mold**	**Importance**
1	Mucor	1. Involved in spoilage of foods. 2. *M. rouxii* used for the saccharification of starch 3. Ripening of cheeses (Gammelost) 4. Making Oriental foods
2	Rhizopus	*R. stolonifer* a bread mold involved in spoilage of bread, berries, fruits, vegetables, etc.
3	Aspergillus	1. *A. repens* spoil many foods and foods with high sugar and salt concentrations. 2. *A. niger* used for production of citric and gluconic acids. 3. *A. flavus & A. oryzae* are used in production of oriental foods and enzymes.
4	Penicillium	1. *P. expansum* blue-green mold causes soft rots of fruits 2. *P. digitatum* yellowish-green mold and *P. italicum* blue mold causes soft rot of citrus fruits 3. *P. camemberti* gray mold useful in ripening of Camembert cheese 4. *P. roqueforti* bluish-green mold used in ripening of blue cheeses e.g. Roquefort. 5. Cause trouble in canned acid foods.
5	Trichothecium	*T. roseum* a pink mold grows on wood, paper, fruits such as apples and peaches, and vegetables such as cucumbers.
6	Geotrichum	*G. candidum* produce white, yellow, orange or red growth in dairy products
7	Neurospora (Monilia)	1. *N. sitophila* called 'red bread mold' because its pink, loose textured growth often occurs on bread. 2. It also grows on sugarcane bagasse and on various foods.
8	Sporotrichum	*S. carnis* found growing on chilled meats causing white spots.
9	Botrytis	*B. cinerea* causes disease of grapes.

10	Trichoderma	*T. viride* forms bright green colour on foods.
11	Cladosporium	Dark mold causes 'black spots' on may foods.
12	Helminthosporium	Most species are plant pathogens and spoil many vegetables.
13	Alternaria	1. *A. citri* causes rotting of citrus fruits 2. Other species dirty grey-green growth on foods.
14	Fusarium	Spoil many kinds of foods.
15	Monascus	Reddish-purple in colour found on dairy products.
16	Sclerotinia	Cause rots of vegetables and fruits.

3) Yeasts

Yeasts are fungi which are generally not filamentous but unicellular and ovoid or spheroid and reproduce by budding or fission.

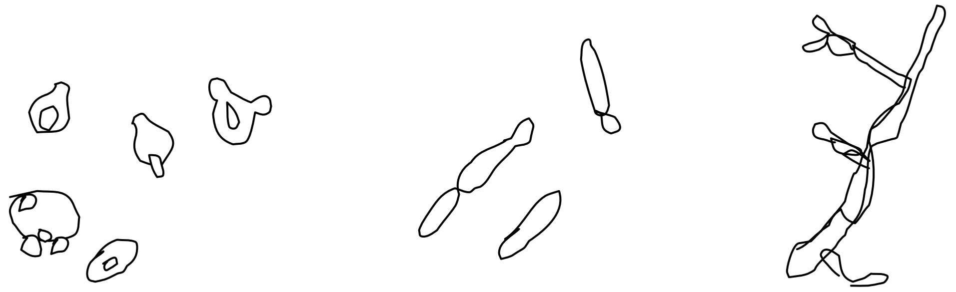
Yeasts may be useful or harmful in foods. Yeasts fermentations are involved in the manufacture of foods such as bread, beer, wines, vinegar, enzymes, single cell protein food and surface ripened cheese.

Yeasts are undesirable when they cause spoilage of sauerkraut, fruit juices, syrups, molasses, honey, jellies, meats, wine, beer, and other foods.

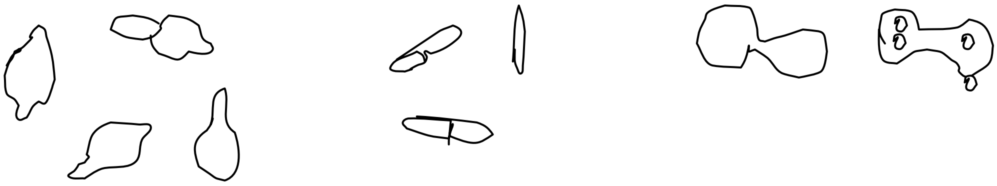
General characters of yeasts

Morphological characters

The yeasts may be spherical to ovoid, lemon shaped, pear shaped, cylindrical, triangular, or even elongated into a false or true mycelium. They also differ in size. Visible parts of the structure are the cell wall, cytoplasm, water vacuoles, fat globules, and granules.



a) b) c)



d) e) f)

a) *Saccharomyces cerevisiae*, with budding cells and one ascus with 4 ascus

b) Candida yeast, with elongated cells

c) Candida yeast, showing pseudomycelium

d) Lemon shaped and flask shaped yeast

e) Schizosaccharomyces, multiplying by fission

f) Zygosaccharomyces, showing conjugation with ascus and 4 ascospores

Reproduction of yeasts

Asexual reproduction: -

Budding: - Most yeasts reproduce asexually by multilateral or polar budding, a process in which some of the protoplasm bulges out the cell wall; the bulge grows in size and finally walls off as a new yeast cell. In some yeasts, the bud appears to grow from a tube like projection from the mother cell. Replicated nuclear material is divided between the mother and daughter cells.

Fission: - A few species of yeasts reproduce by fission and some reproduce by a combination of fission and budding.

Sexual reproduction: -

Ascospores: - Sexual reproduction of **'true' yeasts (*Ascomycotina)*** results in the production of ascospores, the yeast cell serving as the ascus. The formation of ascospores follows conjugation of two cells in most species of true yeasts, but some may produce ascospores without conjugation, followed by conjugation of ascospores or small daughter cells. The usual number of spores per ascus and the appearance of the ascospores are characteristics of the kind of yeast. The ascospores may differ in colour, in smoothness or roughness of their walls, and in their shape (round, oval, reniform, bean or sickle shaped, saturn or hat shaped, hemispherical, angular, fusiform, or needle shaped).

'False' yeasts, which reproduce no ascospores or other sexual spores, belong to the ***Fungi imperfecti.*** Cells of some yeasts become **chlamydospores** by formation of a thick wall about the cell, for example, ***Candida, Rhodotorula, and Cryptococcus.***

 # Important yeasts in food and industries

Sr. No.	**Name of mold**	**Importance**
A	**True yeasts**	
1	Saccharomyces	1. *S. cerevisiae* used in leavening of bread, in production of alcohol, glycerol, invertase. 2. *S. cerevisiae* var. *ellipsoideus* is a high alcohol yielding variety used to produce industrial alcohol, wine, and distilled liquors. 3. *S. uvarum*, bottom yeast is used in making beer. 4. *S. fragilis* and *S. lactis*, are important in milk products.
2	Schizosaccharomyces	Are found in fruits, molasses, soil, honey etc.
3	Zygosaccharomyces	1. Involved in spoilage of honey, syrups, and molasses 2. Some are used in fermentation of wines and soy sauce.
4	Pichia	*P. membranaefaciens* grows as a pellicle on beers or wines.
5	Hanensula	Grows as a pellicle on beers or wines.
6	Debaryomyces	Form pellicles on meat brines, grows on cheese and sausage.
7	Hanseniaspora	Grow in fruit juices.
B	**False yeasts (Fungi imperfecti)**	
1	Torulopsis	Spoil milk and milk products, concentrated fruit juices, and acid foods.
2.	Candida.	1. Many form films and can spoil foods high in acid and salt. 2. *C. utilis* is grown for food and

		feed. 3. *C. krusei* has been grown with dairy starter cultures to maintain the activity and increase the longevity of the lactic acid bacteria. 4. *C. lipolytica* can spoil butter.
3	Brettanomyces	1. *B.* bruxellansis and *B. lambicus* produce acid and used in late fermentation of Belgian lambic beer and English beers, French wines.
4	Trichosporon	Grow best at low temperatures and are found in breweries and on chilled beef
5	Rhodotorula	The red, pink, or yellow yeasts may cause discolouration on foods e.g. coloured spots on meats or pink areas in sauerkraut.

==

- **<u>Sources of contamination of food</u>**

Foods may be contaminated by microorganisms at any time during harvest, storage, processing, distribution, handling, or preparation. The primary sources of microbial contamination are soil, air, animal feed, animal hides and intestines, plant surfaces, sewage, and food processing machinery or utensils.

1. **Air and dust**
2. **Soil, water and plants**
3. **Gastrointestinal tract**
4. **Animals**
5. **Animal feeds**
6. **Food handlers**
7. **Food utensils**
8. **Cross-contamination**
9. **Unsafe temperature**
10. **Poor personal hygiene**
11. **Pests**

1. Air and dust

Microorganisms are found everywhere in our environment. Many types can be found in air and dust, and can contaminate food at any time during food preparation or when food is left uncovered. Imagine a kitchen where food is prepared and stored in rural communities, and think how easily microorganisms in the air and dust could contaminate the food.

2. Soil, water and plants

Many microorganisms present in soil and water may contaminate foods. Microorganisms also grow on plants and can contaminate food if care is not taken to remove them by washing or inactivate them by cooking. Soil is a particularly rich source of *Clostridium* bacteria. Water may be contaminated by faeces. Plants may also be contaminated by faeces if untreated sewage has been used as a fertilizer.

3. Gastrointestinal tract

The intestines of all humans and animals are full of microorganisms, some of which are beneficial but others are pathogenic. Bacterial pathogens such as *Salmonella, Campylobacter* and *Escherichia coli* (strain O157:H7) are common examples. Contamination of foods by faecal material is the major cause of food poisoning events. This includes indirect contamination, for example from people's hands if they prepare food without washing their hands after visiting the latrine/toilet.

Escherichia coli (abbreviated to *E.coli*) exists in many harmless varieties or 'strains', but some strains are pathogenic. The strain called *E.coli* O157:H7 causes a potentially fatal foodborne disease in humans.

4. Animals

Many food borne microorganisms are present in healthy animals raised for food, usually in their intestines, hides, feathers, etc. Meat and poultry carcasses can be contaminated during slaughter by contact with small amounts of intestinal contents. For example, in animals slaughtered in rural communities without any safety measures, microorganisms present in the animals' intestines can easily contaminate the meat.

Animal hides are an important source of contamination of the general environment, the hands of meat workers, and skinned meat carcasses.

5. Animal feeds

Animal feeds are a source of microorganisms, especially *Salmonella*, which can contaminate poultry and other farm animals. The organisms in dry animal feed spread throughout the local environment and may get on to animal hides, hair and feathers, as well as on people who handle the feeds.

6. Food handlers

The term **food handler** can be applied to anyone who touches or handles food, and this includes people who process, transport, prepare, cook and serve food. The presence of microorganisms on the hands and outer garments of food handlers reflects the standard of hygiene in the environment and the individuals' personal hygiene. The microorganisms transmitted to foods by food handlers may come from the hides of animals, soil, water, dust, gastrointestinal tracts and other environmental sources. In food preparation at home, food borne microorganisms can be introduced from the unwashed hands of people who are infected by bacteria and viruses, and who cook and serve the food to family members.

7. Food utensils

Food utensils are cutting boards, knives, spoons, bowls and other equipment used in food preparation, which may become contaminated during food processing and preparation. For example, in families where there is no access to running water, the food utensils may not be properly cleaned, stored and handled, and may become a major route of food contamination.

8. Cross-contamination

Cross-contamination of food is the transfer of harmful microorganisms between food items and food contact surfaces. Prepared food, utensils and surfaces may become contaminated by raw food products and microorganisms. These can be transferred from one food to another by using the same knife, cutting board or other utensil without washing it between uses. A food that is fully cooked can become re-contaminated if it touches raw foods or contaminated surfaces or utensils that contain pathogens.

9. Unsafe temperature

An unsafe temperature for food storage is a major factor in food contamination. Many microorganisms need to multiply to a very large number before enough are present in food to cause disease in someone who eats it. However, if bacteria can have warm, moist conditions and an ample supply of nutrients, one bacterium can reproduce by dividing (on average) every half an hour and can produce 17 million bacteria in 12 hours! So, if you leave lightly contaminated food out overnight, it will be highly contaminated and infectious by the next day.

10.Poor personal hygiene

Poor personal hygiene of food handlers is another major factor in food contamination. The most important contaminants of food are the microorganisms excreted with faeces from the intestinal tract of humans. These pathogens are transferred to the food from faecal matter present on the hands.

Hands should be washed before starting work on preparing food, and after touching any food, surface or equipment that may be contaminated. Bad personal habits like scratching your hair and nose with your fingers also contribute to food contamination. Sneezing and coughing spreads contaminants and microorganisms through the air and onto uncovered food, and onto surfaces and hands that can transfer the infectious agents into food.

11.Pests

Foods can be damaged and also contaminated by pests. Many stored grains are lost through the damage done by pests, including termites (*mist*) (Walavi), beetles (shenkida), locusts (Naktoda), cockroaches (Zural), flies (Masha) and rodents such as rats and mice. Pests can damage and contaminate foods in various ways, such as boring into and feeding on the insides of grains, or tunneling into stems and roots of food plants.

Pests also damage the protective skin of foods allowing microorganisms to get inside the food and causing it to rot more quickly. Pests can pollute food with their excreta and with bodies and body fragments when they die. They also transfer microorganisms on to food while walking on it. Flies and cockroaches readily move between wastes and foods, transporting microorganisms with them as they go.

==

- **<u>Factors affecting kind and number of microorganisms in food.</u>**

Knowledge of the factors that favour or inhibit the growth of microorganisms is essential to an understanding of the principles of food spoilage and preservation. The main factors of a food that influence microbial activity are as follows—

1. **Hydrogen ion concentration (pH)**
2. **Moisture (Water activity)**
3. **Oxidation – reduction potential (O-R)**
4. **Nutrients**
5. **Presence of inhibitory substances in food**

1. Hydrogen ion concentration (pH): -

Every microorganism has a minimal, a maximal, and an optimal pH for growth. Microbial cells are significantly affected by the pH of food because they apparently have no mechanism for adjusting their internal pH.

In general, yeasts and molds are more acid-tolerant than bacteria. Foods with low pH values (below 4.5) usually are not readily spoiled by bacteria and are more susceptible to spoilage by yeasts and molds. A food with low pH would therefore tend to be more stable microbiologically than a neutral food. Hence foods of low pH such as fruits, soft drinks, fermented milks, sauerkraut and pickles can be kept for long time.

Some foods have a low pH of inherent acidity e.g. the fermented products have a low pH because of developed acidity from the accumulation of lactic acid during fermentation.

Molds can grow over a wide range of pH values than most yeasts and bacteria. Most yeast are favoured by a pH about 4 to 4.5, on the other hand most yeasts do not grow well in alkaline substrates. Most bacteria are favoured by a pH near neutrality (ph 7), although some bacteria, such as the acid formers are favoured by moderate acidity. The proteolytic bacteria can grow in alkaline pH, as found in the white of a stored egg.

The buffers in a food i.e. 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. Vegetable juices have low buffering power, permitting an appreciable decrease in pH. Low buffering power makes for a more rapidly appearing succession of microorganisms during fermentation than does high buffering power. Milk, on the other hand, is fairly high in protein (a good buffer) and therefore permits considerable growth and acid production by lactic acid bacteria in the manufacture of fermented milks before growth of the starter culture is finally suppressed.

Particularly organic acids are more inhibitory than others. E.g. acetic, benzoic, citric, lactic, propionic and sorbic acids, hence they are widely used as acidulants or preservatives in foods.

Not only are the rates of growth of microorganisms affected by pH, so are the rates of survival during storage, heating, drying, and other forms of processing. Also, the initial pH may be suitable, but because of competitive flora or growth of the organism itself, the pH may become unfavourable. Conversely, the initial pH may be unfavourable, but the growth of microorganisms may alter the PH to a range that is more favourable for the growth of many other microorganisms.

2. Moisture (The concept of Water activity a_w): -

Microorganisms have an absolute demand for water, without water no growth can occur. Exact amount of water needed for growth of microorganisms varies. This water requirement is best expressed in terms of available water or water activity $\mathbf{a_w}$ –

$$\textbf{Water activity } a_w = \frac{\textbf{Vapour pressure of the solution}}{\textbf{Vapour pressure of the solute}}$$

$$\textbf{Equilibrium Relative Humidity (ERH)} = a_w \times 100$$

$$a_w = \frac{ERH}{100}$$

The a_w for pure water would be 1.00.

The a_w for 1.0 m solution of the ideal solute would be 0.9823.

A relative humidity about a food corresponding to an a_w lower than that of the food would tend to dry the surface of the food. The a_w for many groups of foods is summarized as follows—

	a_w values	**Food**
1.	0.98 and above	Fresh meat and fish, Fresh fruits and vegetables, Milk and most beverages, Canned fruits in light syrup
2.	0.93 – 0.98	Evaporated milk, Tomato paste, Processed cheese, Bread, Canned cured meats, canned fruits in syrups
3.	0.85 – 0.93	Dry or fermented sausage, Dried beef, Sweetened Condensed milk
4.	0.60 – 0.85	Dried fruits, flour, cereals, Jams and Jellies, Nuts
5.	Below 0.60	Chocolate, Confectionary, Honey, Biscuits, Potato Chips. Milk powder

Water is made unavailable in various ways—

1. Solutes and ions tie up water in solution. Therefore, an increase in the concentration of dissolved substances such as sugars and salts is in effect a drying of the material. Not only is water tied up by solutes, but also water tends to leave the microbial cells by osmosis if there is a higher concentration of solute outside the cells than inside.

2. Hydrophilic colloids (gels) make water unavailable. As little as 3 to 4 % agar in the medium may prevent bacterial growth by leaving too little available moisture.

3. Water of crystallization or hydration is usually unavailable to microorganisms. Water it, when crystallized as ice, no longer can be used by microbial cells. The a_w of water ice mixtures (vapour pressure of ice divided by vapour pressure of water) decreases with a decrease in temperature below 0 ^{0} C.

The a_w values	at temperature
1.00	0 C
0.953	- 5 C
0.907	- 10 C
0.846	- 15 C
0.823	- 20 C

In a food, as more ice is formed, the concentration of solutes in the unfrozen water is increased, lowering its a_w. The reduction of a a_w by a solute depends primarily on the total concentration of dissolved molecules and ions, each of which is surrounded by water molecules held more or less firmly.

Each microorganism has a maximal, optimal and minimal a_w for growth. As the a_w is reduced below the optimal level, there is a lengthening of the lag phase of growth, a decrease in the rate of growth and a decrease in the amount of cell substance synthesized.

Factors that may affect a_w requirements of microorganisms include the following—

1. The kind of solute employed to reduce a_w. For molds the lowest a_w for growth is particularly independent of the kind of solute used. E.g. KCl, NaCl, Na_2SO_4.

2. *The nutritive value of the culture medium:* - In general, the better the medium for growth, the lower the limiting a_w.
3. *Temperature:* - Most organisms have the greatest tolerance to low a_w at about optimal temperatures.
4. *Oxygen supply:* - Growth of aerobes takes place at a lower a_w in the presence of air than in its absence, and the reverse is true of anaerobes.
5. *PH:* - Most organisms are more tolerant of low a_w at pH values near neutrality than in acid or alkaline media.
6. *Inhibitors:* - The presence of inhibitors narrows the range of a_w for growth of microorganisms.

Most bacteria grow well in a medium with a water activity a_w approaching 1.00 (at 0.995 to 0.998) i.e. they grow best in low concentrations of sugar or salts. Culture media for most bacteria contain not more than 1 % sugar and 0.85 % NaCl.

In general, bacteria require more moisture than yeasts and yeasts require more moisture than molds. The minimal a_w for spore germination is low as 0.62 for some molds and as high as 0.93 for others.

Group of microorganism	**Minimal a_w value**
Many bacteria	0.91
Many yeasts	0.88
Many molds	0.80
Halophilic bacteria	0.75
Xerophilic fungi	0.65
Osmophilic yeasts	0.60

3. Oxidation – reduction potential (O-R): -

The oxygen tension or partial pressure of oxygen about a food and the O-R potential, or reducing and oxidizing 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. The 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 and
4. The access, which the atmosphere has to the food.

Air has a high oxygen tension. From the standpoint of ability to use free oxygen, microorganisms have been classified as—

Aerobic – When they require free oxygen

Anaerobic – When they grow best in the absence of free oxygen
Facultative – When they grow well either aerobically or anaerobically

Molds are aerobic, most yeasts grow best aerobically, and bacteria of different kinds may be aerobic, anaerobic, or facultative. From the standpoint of O-R potential, a high (oxidizing) potential favours aerobes but will permit the growth of facultative organisms, and a low (reducing) potential favours anaerobic or facultative organisms. Growth of an organism may alter the O-R potential of a food enough to restrain other organisms. Anaerobes, for example, may lower the O-R potential to a level inhibitory to aerobes.

The O-R potential of a system is usually written ***Eh*** and measured and expressed in terms of **millivolts (mV)**. A highly oxidised substrate would have a positive Eh, and a reduced substrate a negative Eh. Aerobic microorganisms require positive ***Eh*** values or positive mV O-R potentials, e.g. Bacilli, Micrococci, Pseudomonads, and Acenetobacters etc. Conversely, anaerobes require negative ***Eh*** values or negative mV O-R potentials, e.g. Bacteriodes and Clostridia etc.

Most fresh plants and animal foods have a low and well-poised O-R potential in their interior. In plants it is because of reducing substances such as ascorbic acid and reducing sugars and in animal tissues it is because of -SH (sulfhydryl) and other reducing groups. As long as the plant or animal cells respire and remain active, they tend to poise the O-R system at a low level, resisting the effect of oxygen diffusing from the outside. Therefore, a piece of fresh meat or a fresh whole fruit would have aerobic conditions only at and near the surface. The meat could support aerobic growth of slime-forming or souring bacteria at the surface at the same time that anaerobic putrefaction proceeds in the interior. Heating may reduce the poising power of the food by means of destruction or alteration of reducing and oxidizing substances and also allow more rapid diffusion of oxygen inward.

4. Nutrients: -

The kinds and proportions of nutrients in the food are all important in determining what organism is most likely to grow. Consideration must be given to—

A) Foods for energy
B) Foods for growth
C) Accessory food substances, or vitamins

A) Foods for energy: -

The carbohydrates, especially the sugars, are most commonly used as an energy source. Other compounds e.g. alcohols; amino acids, esters, peptides and organic acids etc may serve as an energy source. Few organisms can utilize complex carbohydrates such as starch, cellulose. Many organisms cannot use the disaccharide lactose (milk sugar) and therefore do not grow well in milk. Yeasts do not attack maltose. Bacteria often are identified and classified on the basis of their ability or inability to utilize various sugars and alcohols. Most organisms utilize sugar glucose for energy.

The ability of few microorganisms to hydrolyse pectin is important in the softening fruits and vegetables. The limited number of microorganisms obtains energy from fats. Hydrolysis products of proteins, peptides and amino acids serve as energy source for many proteolytic organisms.

All these complex substances are used as energy source when there is a limited supply of simple carbohydrates. Not only is the kind of energy food important but also its concentration in solution and hence it's osmotic effect and the amount of available moisture. For a given percentage of sugar in solution, the osmotic pressure will vary with the weight of the sugar molecule. Therefore, a 10% solution of glucose has about twice the osmotic pressure of a 10% solution of sucrose or maltose; i.e. it ties up twice as much as moisture.

In general, molds can grow in the highest concentration of sugars and yeasts in fairly high concentrations, but most bacteria grow best in fairly low concentrations. There are notable exceptions: Osmophilic yeasts grow in as high concentrations of sugar as molds, and some bacteria can grow in fairly high concentrations of sugars.

Of course, an adequate supply of foods for growth will favour utilization of the foods for energy. More carbohydrate will be used if a good nitrogen food is present in sufficient quantity.

B) Foods for growth: -

Microorganisms differ in their ability to use various nitrogenous compounds as a source of nitrogen for growth. Many microorganisms are unable to hydrolyse proteins and hence cannot get nitrogen from them without kelp from a proteolytic organism. One protein may be a better source of nitrogenous food than another because of different products formed during hydrolysis, especially peptides and amino acids.

Peptides, amino acids, urea, ammonia, and other simpler nitrogenous compounds may be available to some organisms but not to others or may be usable under some environmental conditions but not under others. The presence of fermentable carbohydrate in a substrate usually results in an acid fermentation and suppression of proteolytic bacteria and hence in what is called a **'sparing'** action on the nitrogen compounds. Also, the production of obnoxious nitrogenous products is prevented or inhibited.

Many kinds of molds are proteolytic, but comparatively few genera and species of bacteria and very few types of yeast are actively proteolytic. Carbon for growth may come partly from carbon dioxide, but more often it comes from organic compounds.

The minerals required by microorganisms are nearly always present at the low levels required, but occasionally present in insufficient amounts. An example is milk, which contains insufficient iron for pigmentation of the spores of *Penicillium roqueforti.*

C) Accessory food substances or Vitamins: -

Some microorganisms are unable to manufacture some or all of the vitamins needed and must have them furnished. Most natural plant and animal foodstuffs contain an array of these vitamins, but some may be low in amount or lacking. Thus meats are high in **B** vitamins and fruits are low, but fruits are high in **C** vitamin (Ascorbic acid). Egg white contains **biotin.**

The processing of foods often reduces the vitamin content. Thiamine, pantothenic acid, folic acid, ascorbic acid are heat labile, and drying causes a loss in vitamins. Even storage of foods for long periods may result in a decrease in the level of some of the accessory growth factors.

Each kind of microorganism has a definite range of food requirements. For some species range is wide, and growth takes place in a variety of substrates. For some species range is narrow and they can grow in only a limited number of kinds of substrates. Some can use a variety of carbohydrates e.g. the *coliform bacteria and Clostridium spp,* and others only one or two, while some can use other carbon compounds such as organic acids and their salts, alcohols and esters (*Pseudomonas spp).* Some can hydrolyse complex carbohydrates, although others cannot. Likewise, the nitrogen requirement of microorganisms is different. Some require simple compounds such as ammonia or nitrates and some require complex compounds such as amino acids, peptides, or proteins.

Microorganisms also vary in their need for vitamins or accessory growth factors. Some synthesize part (*Staph. aureus)* and others synthesize all factors (*Pseudomonas, E. coli)*. Some do not synthesize all growth factors; they have to furnish all (*Lactic acid formers and pathogens).*

It should be emphasized that in general, the better the medium for an organism, the wider the ranges of temperature, pH, and a_w over which growth can takes place.

5. Presence of inhibitory substances in food: -

Inhibitory substances—

- Originally present in the food
- Added purposely or accidentally
- Developed there by growth of microorganisms
- Developed by processing methods

may prevent growth of microorganisms.

Examples of inhibitors naturally present are the *lactenins and anticoliform factor* in freshly drawn milk, *lysozyme* in egg white and *benzoic acid* in cranberries.

A microorganism growing in a food may produce one or more substances inhibitory to other microorganism e.g. *acids, alcohols, peroxides and antibiotics.*

e.g. *Propionic acid* produced by the propionibacteria in Swiss cheese is inhibitory to molds, *Alcohol* formed by yeasts in wine inhibits competitors and *nisin* produced by *Streptococcus lactis* inhibit lactate-fermenting, gas forming clostridia in curing cheese.

There also is possibility of the destruction of inhibitory compounds in foods by microorganisms. Certain molds and bacteria destroy *phenol compounds, benzoic acid, sulphur dioxide* that are added in meat, fish or other foods.

Heating of foods may result in the formation of inhibitory substances-

e.g. heating of lipids results autooxidation, browning of sugar syrups may result in the production of furfural and hydroxymethyl furfural, which are inhibitory to organisms.

The effect of the *biological structure* of food on the protection of foods against spoilage has been noted. The inner parts of whole, healthy tissues of living plants and animals are either sterile or low in microbial content. Often there is a protective covering about the food e.g. the shell on eggs, the skin on poultry, the shell on nuts, skin on fruits & vegetables. Also we surround the food with an artificial coating e.g. plastic or wax. This physical protection is of the food not only helps its preservation but also of determine the kind, rate, and course of spoilage. On the other hand, an increase in exposed surface brought about by peeling, skinning, chopping, or comminution may serve not only to distribute spoilage organisms but also to release juices containing food materials for the microorganisms. The same results are obtained when freezing of meat disintegrates tissues.

In meat the growth of spoilage bacteria takes place mostly in the fluid between the small meat fibres, and it is only after rigor mortis that much of this food material is released from the fibres to become available to spoilage organisms.

 # Combined effects of factors affecting growth: -

Each of the compositional factors of foods—$a_{w,}$ pH, O-R potential and nutrient content—can significantly affect the resulting microbial flora. Many of these factors interact. E.g. a microorganism growing near its optimal pH will be more tolerant to changes in a_w than will one growing close to its minimal or maximal pH. Therefore, a combined inhibitory effect of an unfavourable pH and a_w can be noted. To prevent or retard growth, several of these factors can be manipulated rather than adjusting one to an inhibitory level.

- ## <u>Principles food preservation</u>

In this section we outline the microbiological principles involved in the various methods of food preservation. As a result of improved methods of preservation and transportation, our diet has become more varied and better balanced. Perishable foods have been made available year-round instead of only seasonally. The preparation of meals has been made easier, and foods in general are being produced in a cleaner and more sanitary manner than before.

Principles of food preservation: -

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 antioxidants.

3. Prevention of damage because of insects, animals, mechanical causes, etc.

==

- **<u>Microbiostatic and microbicidal methods of food preservation: -</u>**

The chief methods of food preservation are as follows—

1. **Asepsis, or keeping out microorganisms**
2. **Removal of microorganisms**
3. **Maintenance of anaerobic conditions e.g. in a sealed, evacuated container**
4. **Use of high temperature**
5. **Use of low temperature**
6. **Drying (This includes the tying up of water by solutes, hydrophilic colloids)**
7. **Use of chemical preservatives (Food additives), either developed by microorganisms or added**
8. **Irradiation**
9. **Mechanical destruction of microorganisms e.g. by grinding, high pressures**
10. **Combination of two or more of the above methods**

1. Asepsis (Keeping out microorganisms): -

In nature, there are numerous examples of asepsis or keeping out microorganisms as a preservative factor. The inner tissues of healthy plants and animals usually are free from microorganisms. If there is a protective covering about the food, microbial decomposition is delayed or prevented.

e.g. Shells of nuts, skins of fruits and vegetables, husks of corns, shells of eggs, skin on meat or fish.

When outer covering gets damaged, microorganisms contaminate inner part and they spoil the inner part of food. Packaging of the foods is a widely used application of asepsis. The coverings may range from a loose carton or wrapping, which prevents primarily contamination during handling. The sealed container of canned foods protects the food from contamination by microorganisms.

In the dairy industry, contamination with microorganisms is avoided as much as possible during handling and packaging of it.

In the canning industry the bio burden, or load of microorganisms determines the heat process necessary for the preservation of food.

In the meat packing industry, sanitary methods of slaughter, handling and processing reduce the load of microorganisms.

2. Removal of microorganisms: -

For the most part the removal of microorganisms is not very effective in food preservation, but under special conditions it may be helpful. Removal may be accomplished by means of filtration, centrifugation (sedimentation or clarification), washing, or trimming.

i) Filtration: - 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 "bacteria proof" filter made of sintered glass, diatomaceous earth, unglazed porcelain, membrane pads, or similar 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.

ii) Centrifugation or sedimentation: - It is not very effective generally. In that some but not all of the microorganisms are removed. It is used in the treatment of drinking water but is insufficient by itself. When centrifugation (clarification) is applied to milk, the main purpose is not to remove bacteria but to take out other suspended materials.

iii) Washing: - It can be helpful in their preservation but may be harmful under some conditions. Washing cabbage heads or cucumbers before their fermentation into sauerkraut and pickles, respectively, removes most of the soil microorganisms on the surface. Washing fresh fruits and vegetables removes soil microorganisms that may be resistant to the heat process during the canning of these foods. Washing and germicidal treatment of the equipment used for the storage of food is effective procedure during the handling of all kinds of foods. Washing foods may be dangerous if the water adds spoilage organisms or increases the moisture so that growth of spoilage organisms is encouraged.

iv) Trimming: - Trimming away spoiled portions of a food or discarding spoiled samples is important from the standpoint of food laws and may be helpful in food preservation. Although large numbers of spoilage organisms are removed in this way, heavy contamination of the remaining food may take place. Trimming the outer leaves of cabbage heads is recommended for the manufacture of sauerkraut.

3. Maintenance of anaerobic conditions: -

A preservative factor in sealed, packaged foods may be 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 carbon dioxide or by inert gas such as nitrogen will bring about anaerobic conditions. Spores of some of the aerobic spore formers are especially resistant to heat and may survive in canned food but be unable to germinate or grow in the absence of oxygen.

4. Use of high temperature: -

The killing of microorganisms by heat is supposed to be caused by the denaturation of the proteins and especially by the inactivation of enzymes required for metabolism. The heat treatment necessary to kill organisms or their spores varies with the kind of organism, its state, and the environment during heating. Depending on the heat treatment employed, only some of the vegetative cells, most or all of the cells, part of the bacterial spores, or all of them may be killed. The heat treatment selected will depend upon the kinds of organisms to be killed, other preservative methods to be employed, and the effect of heat on the food.

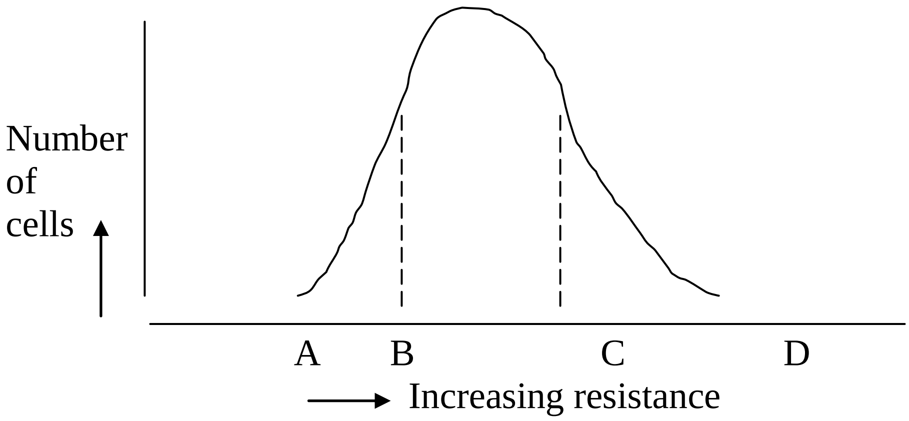
Heat resistance (Thermal Death Time, TDT): -

The heat resistance of microorganisms is usually expressed in terms of their **Thermal Death Time (TDT).**

Definition of TDT: - It is defined as the time it takes at a certain temperature to kill a stated number of organisms (or spores) under specified conditions.

Definition of Thermal Death Point (TDP): - It is the temperature necessary to kill all the organisms in 10 minutes.

Cells and spores of microorganisms differ widely in their resistance to high temperatures. There are differences in heat resistance within a population of cells or spores, as illustrated by the frequency distribution curve in the following graph.



Frequency distribution curve showing heat resistance in a culture

Points A to B: - Small numbers of cells have low resistance
Points B to C: - Most of the cells have a medium resistance
Points C to D: - Small numbers of cells have high resistance

*** Factors affecting heat resistance of the cells: -**

1. The temperature time relationship
2. Initial concentration of cells or spores
3. Previous history of the vegetative cells or spores
    a) Culture medium
    b) Temperature of incubation
    c) Phase of growth or age
    d) Desiccation

4. Composition of the substrate in which cells or spores are heated
    a) Moisture
    b) pH (Hydrogen ion concentration)
    c) Other constituents of the substrates

1. The temperature time relationship: -

The time for killing cells or spores under a given set of conditions decreases as the temperature is increased. This is illustrated in the following table by the results of 115,000 spores of bacteria / ml in corn juice at pH 6.1

Temperature Degree Celsius	Thermal Death Time, or time to destroy all spores in minutes
100	1200
105	600
110	190
115	70
120	19
125	7
130	3
135	1

2. Initial concentration of cells or spores: -

The more cells or spores present, the greater the heat treatment necessary to kill all of them. Following is the table showing TDT of spores in corn juice at pH 6.0 at 120 ^{0} C.

Initial concentration of spores. Number / ml	Thermal Death Time or time required to kill all spores, minutes at 120 ^{0}C
50,000	14
5,000	10
500	9
50	8

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.

Following are the conditions affecting the resistance in they are grown

a) Culture medium
b) Temperature of incubation
c) Phase of growth or age
d) Desiccation

a) Culture medium: -

The medium in which growth takes place is especially important. In general the better the medium for growth, the more resistant the cells or spores. The presence of an adequate supply of accessory growth factors usually favours the production of heat resistant cells or spores.

b) Temperature of incubation: -

In general, resistance increases as the incubation temperature is raised towards the optimum for the organism and for many organisms increases further as the temperature approaches the maximum for growth. E.g. *Escherichia coli* is considerably more heat resistant when grown at 38.5 ^{0} C, which is near its optimal temperature, than at 28 ^{0} C.

c) Phase of growth or age: -

The heat resistance of vegetative cells varies with the stage of growth and spores with their age. Bacterial cells show their greatest resistance during the late lag phase but almost as great resistance during their maximum stationary phase, followed by decline in resistance. The cells are least resistant during their phase of logarithmic growth. Very young (immature) spores are less resistant than are mature ones.

d) Desiccation: -

Dried spores of some bacteria are harder to kill by heat than are those kept moist, but this apparently does not hold for all bacterial spores.

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

The material in which the spores or cells are heated is so important that it must be stated if a thermal death time is to have meaning. It is dependent on following factors.

a) Moisture
b) pH (Hydrogen ion concentration
c) Other constituents of the substrates

a) Moisture: -

Moist heat is much more effective killing agent than dry heat. For sterilization of liquid material 121 ^{0}C for 15 minutes are required and for solid material 160 to 180 ^{0}C for 3 to 4 hours are required.

b) Hydrogen ion concentration (pH): -

In general, cells or spores are most heat resistant in a substrate that is at or near neutrality. An increase in acidity or alkalinity hastens killing by heat.

c) Other constituents of the substrate: -

Sodium chloride present in food has a protective effect on some spores.

Sugars seem to protect some organisms or spores but not others. The optimal concentration for protection varies with the organism. It is high for some osmophilic organisms and low for others, high for spores and low for non-osmophilic cells. The protective effect of sugar may be related to a resulting decrease in a_w. A reduced a_w does result in an increase in observed heat resistance.

Solutes differ in their effect on bacteria. Glucose e.g. protects *E. coli* and *Pseudomonas fluorescens* against heat better than sodium chloride. On the other hand, glucose affords no protection or is even harmful to *staphylococcus aureus*, where as sodium chloride is very protective. Concentration of solutes may affect the heat process necessary for sterilization.

Colloidal materials, especially proteins and fats are protective against heat. This is well illustrated in the following table by the data of Brown and Peiser (1916) who used *Thermal death points.*

Sr.No.	Substance	*S. lactis* ^0C	*E. coli* ^0C	*L.bulgaricus* ^0C
1	Cream	69-71	73	95
2	Whole milk	63-65	69	91
3	Skim milk	59-63	65	89
4	Whey	57-61	63	83
5	Broth	55-57	61	81

*** Effect of protective substances on heat resistance of bacteria**

Heat resistance (TDT) of yeasts and yeast spores: -

In general the ascospores of yeasts need only 5 to 10^0 C more heat for their destruction than the vegetative cells from which they are formed. Most ascospores are killed by 60^0 C for 10 to 15 min; a few are more resistant, but none can survive even a brief heating at 100^0 C. Vegetative yeasts usually are killed by 50 to 58^0 C for 10 to 15 minutes. Both yeasts and their spores are killed pasteurization treatments given milk (62.8^0 C for 30 min or 71.7^0 C for 15 sec), and yeasts are readily killed in the baking of bread, where the temperature of the interior reaches about 97^0 C.

Heat resistance (TDT) of molds and mold spores: -

Most molds and their spores are killed by moist heat at 60 in 5 to 10 min, but some species are considerably more heat-resistant. The asexual spores are more resistant than mycelium. Many species of *Aspergillus, Penicillium and Mucor* are more resistant to heat than are other molds. Sclerotia are especially difficult to kill by heat. Some can survive a heat treatment of 90 to 100^0 C for a brief period and have been known to cause spoilage in canned fruits. It was found that 1,000 min at 82.2^0 C or 300 min at 85^0 C was necessary to destroy Sclerotia from a species of *Penicillium.*

Molds spores are fairly resistant to dry heat. Dry heat at 120 ^{0} C for 30 min will not kill some of the more resistant spores.

Heat resistance (TDT) of Bacteria and Bacterial spores: -

The heat resistance of vegetative cells of bacteria varies widely with the species. Cocci are usually more resistant than rods. The higher the optimal and maximal temperatures for growth, the greater the resistance to heat. Bacteria that clump or form capsules are more difficult to kill than those which do not. Bacteria high in lipid content are harder to kill than are other cells.

A few examples of TDT of bacterial cells are shown in following table: -

Sr. No.	Bacterium	Time in Min	Temperature C
1	*Neisseria gonorrhoeae*	2-3	50
2	*Salmonella typhi*	4.3	60
3	*Staphylococcus aureus*	18.8	60
4	*Escherichia coli*	20-30	57.3
5	*Streptococcus thermophilus*	15	70-75
6	*Lactobacillus bulgaricus*	30	71

The heat resistance of bacterial spores varies greatly with the species of bacterium and conditions during sporulation. Resistance at 100^0 C may vary from less than 1 min to over 20 hours.

A few examples of TDT of bacterial spores are shown in following table: -

Sr. No.	Spores of Bacterium	Time to kill at 100^0 C in Min
1	*Bacillus anthracis*	1.7
2	*Bacillus subtilis*	15-20
3	*Clostridium botulinum*	100-330
4	*Clostridium calidotolerans*	520
5	*Flat sour bacteria*	Over 1030

Heat treatments employed in processing foods: -

The temperature and time used in heat processing a food will depend on what effect heat has on the food and what other preservative methods are to be employed. Some foods, such as milk can be heated to only a limited extent without undesirable changes in appearance or loss in palatability (tastyness), where others like corn or pumpkin can undergo a more rigorous heat treatment without marked changes. The greater the heat treatment, the more organisms will be killed. The heating must destroy all potential spoilage organisms. In canning, an attempt is made to kill organisms that could spoil the food during later handling. In pasteurization, most of the spoilage organisms are killed but others survive and must be inhibited by low temperatures or some other preservative methods. Following are some methods in which various degrees of heating is used on foods—

1) Pasteurization
2) Heating at about 100^0 C
3) Heating above 100^0 C

1)Pasteurization: -

It is a heat treatment that kills part but not all of the microorganisms present and usually involves the application of temperatures below 100 C.

The heating may be by means of steam, hot water, dry heat, or electric currents, and products are cooled promptly after the heat treatment.

Pasteurization is used when—
--> More rigorous heat treatments might harm the quality of the product, as with market milk.
--> Aim is to kill pathogens, as with market milk
--> The main spoilage organisms are not very heat resistant, such as the yeasts in fruit juices.
--> Any surviving spoilage organisms will be taken care of by additional preservative methods, as in the chilling of market milk.

Preservative methods used to supplement pasteurization include --> Refrigeration e.g. milk
--> Keeping out microorganisms, usually by packaging the product in a sealed container
--> Maintenance of anaerobic conditions, as in sealed containers
--> Addition of high concentrations of sugar, as in sweetened condensed milk
--> Presence or addition of chemical preservatives e.g. the organic acids in pickles.

Time and temperatures used in the pasteurization process depend on the method employed and the product treated. There are 2 methods commonly used in pasteurization—

I) High Temperature Short Time (HTST) method: -

For milk 71.7 ^{0} C for 15 seconds, for Ice-cream 82.2^0 C for 20 seconds, for beer 80^0 C for 20 seconds, for wines 82 to 85^0 C for 1 minute, for fruit juices 85 to 87^0 C for 30 to 60 seconds heat treatments are used.

II) Low Temperature Holding (LTH) method: -

For milk 62.8^0 C for 30 minutes, for Ice-cream 71.1^0 C for 30 min, for beer 60^0 C for 30 minutes, for wines 65 to 70^0 C for 20 minutes, for fruit juices 65 to 75^0 C for 30 minutes heat treatments are used.

2) Heating at about 100^0 C: -

Formerly, home canners processed all foods for varying lengths of time at 100^0 C or less. But nowadays they use pressure cookers. During **baking** the internal temperature of bread, cake etc approaches but never reaches 100^0 C due to which bacterial spores that survive the baking of bread may cause ropiness. **Simmering** is gentle boiling with temperature about 100^0 C. In **roasting** meat the internal temperature reaches about 80^0 C. **Frying** gets the outside of the food very hot, but the centre does not reach 100^0 C. **Cooking** is a indefinite term.

3) Heating above 100^0 C: -

Temperatures above 100^0 C usually are obtained by means of steam under pressure in steam-pressure sterilizers or retorts. The temperature in the retorts increases with rising stem pressures.

Pressure lb	**Temperature ^{0} C**
0	100
5	109
10	115.5
15	121.5

When liquid foods are to be sterilized before their introduction into sterile cans, high stem pressures are used to apply a high temperature for a few seconds.

Ultrapasteurization or Ultra High Temperature: -

Heating of milk at 137.8^0 C for 2 seconds by use of steam injection or steam infusion followed by **“flash evaporation’** of the condensed steam and rapid cooling is called as ultrapasteurization.

Canning: -

Definition: -It is defined as the preservation of foods in sealed containers and usually implies heat treatment as the principal factor in the preservation of spoilage.

Most canning is in **‘tin cans’,** which are made of tin-coated steel, but glass containers, aluminium, or composite material may be used.

The canning procedure: -

Raw food for canning should be freshly harvested, properly prepared, inspected, graded if desired, and thoroughly washed before introduction into the can.

Many vegetable foods are **blanched** or **scaled** briefly by hot water or steam before packaging. The blanching washes the food further, sets the colour, softens the tissues to aid packing, helps to form vacuum, and kills some microorganisms.

A brine, consisting of salt solution or salt plus sugar, is added to some canned vegetables; and sugar syrups may be added to fruits.

The container is evacuated before sealing, usually by heating the headspace, or unfilled part of the container, but often by mechanical means.

The heat process in canning: -

The canner aims for complete sterilization of most foods but does not always attain it. The canner may kill all that could spoil the food under normal conditions of storage and may leave some that are unable to grow.

The heat process necessary for the canning depends on the factors that influence the heat resistance of the most resistant spoilage organisms and those which affect heat penetration.

The heating is ordinarily done in retorts, with or without steam pressure as food demands.

I) HTST heat process: - It is now used for some liquid foods’ requiring special equipment for sterilizing the food in bulk, sterilizing the containers and lids, and filling and sealing the sterile containers under aseptic conditions. Examples of HTST process are as follows—

 # The Dole process: - In which Heat-Cool-Fill (HCF) system is used.
 # Martin HTST System: - Mixed liquid and solid pieces are heated directly by contact with high temperature steam before aseptic canning.
 # Sterilizing and Closing (SC) method: - sterilization of the food is accomplished before the can is sealed.
 # Pressure Filler Cooker method: - The food is sterilized by high-pressure steam and filled into the can' then the can is sealed and the heat processing is continued as long as necessary before cooling.
 # Dehydrocanning: - The food e.g. the apple slices are dried to about half its original weight before canning.

II) By direct gas flame

III) By steam injection

IV) By heating in a fluidised bed of granular solids

V) Hydrostatic sterilizers: - It consists of a vertical tank with conveyors that carry cans down through a water leg up into live steam, and then up and out through a second water leg.

VI) Flash 18 method: - Canning is done in a high-pressure (18-psi) chamber. The product is given an HTST treatment to bring it to processing temperature, and the cans are filled, closed and partially cooled in the chamber.

VII) Heat plus other combination methods: - Heat plus antibiotics, irradiation or chemicals are used.

Pressurized packaging of foods in canning: -

Pressurized packaged liquids or pastes, called aerosols, are packed under pressure of a propellant gas, usually carbon dioxide, nitrogen, or nitrous oxide, so as to dispense the food as a foam, spray, or liquid. E.g. whipped cream and other toppings, beverage concentrates, salad dressings, condiments, oils, jellies, and flavouring substances.

The pressurized foods are subject to microbial spoilage unless adequate preservatives methods are employed. Acids foods may be heated, canned, and then gassed.

The gas used as a propellant may have an influence on the kinds of organisms likely to grow. E.g. Nitrogen would not inhibit aerobes if a little oxygen was present, but carbon dioxide would be inhibitory under the same condition. Carbon dioxide inhibits many microorganisms, but does not inhibit lactic acid bacteria, *Bacillus coagulans*, *Streptococcus faecalis or yeasts.*

The cooling process in canning: -

Following the application of heat, the containers of food are cooled as rapidly as is practicable. The cans may be cooled by immersion in cold water or by a spray of water.

5. Preservation of food by use of low temperature

Low temperatures are used to retard chemical reactions and the action of food enzyme and to slow down or stop the growth and activity of Microorganisms in food. The lower the temperature, the slower will be chemical reactions, enzyme reaction, and microbial growth.

Each microorganism has an optimal or best temperature for growth and a minimal temperature below, which it cannot multiply. Cooler temperatures will prevent growth, but slow metabolic activity may continue. Low temperature storage can therefore act as a significant environmental factor influencing the type of spoilage flora to predominate as illustrated in the following table.

Sr. No.	Bacterium	Spoilage flora at each temperature %		
		1 C	10 C	15 C
1	*Pseudomonas*	90	37	15
2	*Acinetobacter*	7	26	34
3	*Enterobacteriacae*	3	15	27
4	*Streptococcus*		6	8
5	*Aeromonas*		4	6
6	*Others*		12	10

The growth and metabolic reactions of microorganisms depend on enzymes, and the rate of enzyme reactions is directly affected by temperature. When the temperature is lowered, the rate of growth of a microorganism decreases.

Sr. No.	Temperature C	Average exponential generation time, in minutes
1	0	667
2	2.5	462
3	5.0	300
4	7.5	207
5	10.0	158
6	20.0	65

Growth rate of *Pseudomonas fragi* at various temperatures

Temperatures employed in low temperature storage

1) Common or Cellar storage
2) Chilling or Cold storage
3) Freezing or Frozen storage

1) Common or Cellar Storage: -

The temperature in common or cellar storage usually is not much below than that of the room temperature. It is near 15^0 C. Root crops, potatoes, cabbage, apples etc can be stored for limited periods. The deterioration of such fruits and vegetables by their own enzymes and by microorganisms is not prevented but is slower than at atmospheric temperatures.

Too low a humidity in the storage cellar results in losses of moisture from the stored food, and too high a humidity favours spoilage by microorganisms. In locations where no refrigeration is available common storage of all foods is used.

2) Chilling or Cold storage: -

Chilling storage is at temperatures not far above freezing and usually involves cooling by ice or by mechanical refrigeration. Most perishable foods, including 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.

Factors to be considered in chilling storage

a) Temperature
b) Relative humidity
c) Ventilation
d) Composition of storage atmosphere
e) Irradiation

a) Temperature: -

Most foods will keep best at a temperature just above their freezing point. The chilling temperature is selected on the basis of the kind of food and the time and conditions of storage. Certain foods have an optimal storage temperature well above the freezing point and may be damaged by lower temperatures. A well-known example is the banana, which should not be kept in the refrigerator; it keeps best at about 13 to 16.7^0 C. Some varieties of apples undergo ‘low temperature breakdown’ at temperatures near freezing, and sweet potatoes keep best at 10 to 12.8^0 C.

The temperature of a refrigerator is mechanically controlled but varies in different parts, usually between 0 and 10^0 C.

b) Relative humidity: -

The optimal relative humidity of the atmosphere in chilling storage varies with the food stored and with environmental factors such as temperature, composition of the atmosphere, and ray treatments. Too low a relative humidity results in loss of moisture and hence of weight, the wilting and softening of vegetables, and the shrinkage of fruits. Too high a relative humidity favours the growth of spoilage microorganisms.

The highest humidity is required for most bacterial growth, less moisture is needed by yeasts (90 to 92 %), and still less by molds (85 to 90 %). A moist surface favours microbial spoilage e.g. slime on the moist surface of sausage.

c) Ventilation: -

Ventilation or control of air velocities of the storage room is important in maintaining a uniform relative humidity throughout the room, removing odours, and preventing the development of stale odours and flavours. The rate of air circulation affects the rate of drying of foods. If adequate ventilation is not provided, food in local areas of high humidity may undergo microbial decomposition.

d) Composition of storage atmosphere: -

The amount and proportions of gases in the storage atmosphere influence preservation by chilling. It has been found that in the presence of optimal concentrations of CO_2 or O_3 **---**

--> a food will remain unspoiled for a longer period
--> a higher relative humidity can be maintained
--> a higher storage temperature can be used

d) Irradiation: -

The combination of ultraviolet irradiation with chilling storage helps to preserve some foods and may permit the use of a higher humidity or storage temperature than is practicable with chilling alone. Ultraviolet lamps have been installed in rooms for the storage of meat and cheese.

3) Freezing or Frozen storage

The storage of foods in the frozen condition has been an important preservative method for centuries where outdoor freezing temperatures were available. With the development of mechanical refrigeration and the quick freezing process, the frozen food industry has expanded rapidly. Even in the home, the freezing of the foods has become extensive. In this method, microbial growth is prevented entirely and the action of food enzymes is greatly retarded.

Freezing of foods: -

The rate of freezing of foods depends on number of factors, such as the method employed, the temperature, circulation of air or refrigerant, size and shape of package, and kind of food.

Sharp freezing: - Freezing in air with only natural air circulation at temperature – 23.3^0 C or lower and freezing may take from 3 to 72 hours.

Quick-freezing: - Food is frozen in short time at temperatures – 17 to -45^0 C within 30 minutes.

Nitrogen freezing: - For the overseas shipment of frozen, packaged foods involves nitrogen freezing of the cartooned foods in a special aluminium case. Certain fruits and vegetables, fish, and mushrooms now are being frozen by means of liquid nitrogen.

Dehydrofreezing: - Fruits and vegetables have about their moisture removed before freezing.

Advantages of quick freezing over slow freezing: -

i) Smaller ice crystals are formed; hence there is less mechanical destruction of intact cells of food.
ii) There is shorter period of solidification and therefore less time for diffusion of soluble materials and separation of ice.
iii) There is more prompt prevention of microbial growth
iv) There is more rapid slowing of enzyme action

 # Response of microorganisms for freezing (Factors affecting)

i) The kind of microorganism and its state
ii) The freezing rate
iii) The freezing temperature
iv) The time of frozen storage
v) The kind of food
vi) Influence of defrosting
vii) Alternate freezing and thawing
viii) Possible events during freezing of the cell

6) Preservation of food by drying

Preservation of foods by drying has been practiced for centuries. Some foods e.g. grains, are sufficiently dry as harvested or with a little drying remain unspoiled for long periods under proper storage conditions. Most foods, however, contain enough moisture to permit action by their own enzymes and by microorganisms, so that to preserve them by drying, the removal or binding of moisture is necessary.

Following table shows comparison of foods before and after drying: -

Sr. No.	Food	Moisture before drying %	Moisture after drying %
1	Milk	87	5
2	Egg	74	3
3	Beef (roasted)	60	1.5
4	Chicken (broiled)	61	1.6
5	Beans	92	12
6	Corn	76	3
7	Potatoes	80	4
8	Apple juice	86	6
9	Figs	78	4

Drying usually is accomplished by the removal of water, but any method that reduces the amount of available moisture i.e. lowers the a_w in a food is a form of drying. Thus, for example, dried fish may be heavily salted so that moisture is drawn from the flesh and bound by the solute and hence is unavailable to microorganisms. Sugar may be added, as in sweetened condensed milk, to reduce the amount of available moisture.

 # Methods of drying: -

1. **Solar or sun drying**
2. **Drying by mechanical dryers**
3. **Freeze drying**
4. **Drying during smoking**
5. **Other methods**

1. Solar or sun drying: -

Solar drying is limited to climates with a hot sun and a dry atmosphere and to certain fruits, such as raisins, figs, apricots, pears, and peaches. The fruits are spread out on trays and may be turned during drying. Fish, rice, and other grains may also be sun-dried.

2. Drying by mechanical dryers: -

Most methods of artificial drying involve the passage of heated air with controlled relative humidity over the food to be dried or the passage of the food through such air. A number of devices are used for controlled air circulation and for the reuse of the air in some processes.

Evaporator or Kiln: - It is used in the farm home, where the natural draft from the rising of heated air brings about the drying of the food. Forced-draft drying systems employ currents of heated air that move across the food, usually in tunnels. An alternative method is to move the food on conveyor belts or on trays in carts through the heated air.

Liquid foods, such as milk, juices, and soups, may be evaporated by the use of comparatively low temperatures and a vacuum in vacuum pan or similar device.

Drum drying: - Food is passed over a heated drum, with or without vacuum.

Spray drying: - Liquid food is sprayed into a current of dry, heated air.

3. Freeze drying: -

Freeze-drying or the sublimation of water from a frozen food by means of a vacuum plus heat applied at the drying shelf is being used for a number of foods, including meats, poultry, seafood, fruits and vegetables.

4. Drying during smoking: -

Foods are dried by application of smoke.

5. Other methods: -

Electronic heating has been suggested for the removal of still more moisture from a food already fairly well dried.

Foam-mat drying, in which liquid food is whipped to foam, dried with warm air, and crushed to a powder.

Tower drying in dehumidified air at 30^0 C or lower has been successful with tomato concentrate, milk, and potatoes.

 # Factors in the control of drying: -

i) The temperature employed
ii) The relative humidity of the air
iii) The velocity of the air
iv) The time of drying

7. Use of Food additives & chemical preservatives: -

Food additive: - According to **WHO**, A food additive is a substance or mixture of substances, other than the basic food stuff, which is present in the food as a result of any aspect of production, processing, storage or packaging.

Chemical preservatives: - According to **The Federal Food, Drug, and Cosmetic Act,** as amended by the Food Additives Amendment of 1958, Chemical preservative is defined as any chemical which, when added to food, tends to prevent or retard deterioration thereof; but does not include common salt, sugars, vinegars, spices, or oils extracted from spices, or substances added by wood smoke.

Most of the more common antimicrobial additives used in foods are as follows— (ON-SESA- FWSA)

i) Organic acids and their salts
ii) Nitrites and nitrates
iii) Sulfur dioxide and Sulphites
iv) Ethylene and Propylene oxide
v) Sugar and Salt
vi) Alcohol
vii) Formaldehyde
viii) Wood smoke
ix) Spices and other condiments
x) Antibiotics

i) Organic acids and their salts: -

Citric, lactic, propionic, benzoic, sorbic, acetic acids or their salts may be added to or developed in foods.

*** Citric acid: -** It is used in syrups, drinks, jams and jellies as a substitute for fruit flavours and for preservation.

*** Lactic acid: -** It is developed in curd, whey, cheese, butter or added in brines, green olives etc.

*** Propionates: -** Sodium or Calcium propionate is used most extensively in the prevention of mold growth and rope development in baked food products, cheese. In limited scale, it is used in butter, jams, jellies, figs, apple slices, and malt extract.

Mechanism of action: - It affects cell-membrane permeability. Its action on molds is not known.

*** Benzoates: -** Sodium benzoate is used extensively as an antimicrobial in jams, jellies, margarine, carbonated beverages, fruit salads, pickles, fruit juices etc. It is effective at pH 2.5 to 4.0. It is more effective on bacteria and less effective on yeasts and molds.

Two esters of *p*-hydroxybenzoic acid, methylparaben and propylparaben are also used.

Mechanism of action: - Not known

***Sorbates: -** Calcium or Sodium or Potassium sorbate is used as a direct antimicrobial additive in foods and as a spray, dip, or coating on packaging materials. It is widely used in cheeses, baked products, beverages, syrups, fruit juices, jellies, jams, fruit cocktails, dried fruits, pickles etc.

It is more effective against yeasts and molds but is less effective against bacteria.

Mechanism of action: - Not known

*** Acetates: -** Derivatives of acetic acid e.g. monochloroacetic acid, peracetic acid, dehydroacetic acid and sodium diacetate have been recommended as preservatives but not all are approved by the FDA. Sodium diacetate is used in cheese spreads and malt syrups.

Acetic acid in the form of vinegar is used in pickles, sausages and pigs' feet. It is more effective against yeasts and bacteria than molds.

ii) Nitrites and Nitrates: -

Combinations of these various salts have been used in curing solutions and curing mixtures of meats. Nitrites decompose to nitric acid, which forms nitrosomyoglobin when it reacts with haeme pigments in meats and thereby forms a stable red colour. Nitrites can react with secondary and tertiary amines to form nitrosamines, which are known to be carcinogenic.

Nitrates only act as a reservoir for nitrites and have limited effect on microorganisms.

iii) Sulfur dioxide and Sulfites: -

These are used in the wine industry to sanitize equipment and to reduce the normal flora of the grape must. In aqueous solutions, sulfur dioxide and various sulfites, including sodium sulfite, potassium sulfite, sodium bisulfite, potassium bisulfite, sodium metabisulfite and potassium metabisulfite, form **sulfurous acid**, the active antimicrobial compound.

The fumes of burning sulfur are used to treat most light coloured dehydrated fruits, while dehydrated vegetables are exposed to a spray of neutral bisulfites and sulfites before drying. Sulfur dioxide has also been used in syrups and fruit juices and wine making.

Mechanism of action: - Many mechanisms for the action of sulfurous acid on microbial cells have been suggested, including the reduction of disulfide linkages, formation of carbonyl compounds, reaction with ketone groups, and inhibition of respiratory mechanisms.

iv) Ethylene and propylene oxide: -

These two gases are sterilants. Ethylene oxide kills all microorganisms while propylene oxide kills many microorganisms but not as effective. These are used as sterilants for packaging materials, fumigation of warehouses, and ‘cold sterilization’ of plastics, chemicals, pharmaceuticals, syringes, and hospital supplies. Also these are used in dried fruits, dried eggs, gelatin, cereals, dried yeasts and spices.

Mechanism of action: - These act as strong alkylating agents attacking labile hydrogens.

v) Sugar and salt: -

These compounds lower the a_w and thus have an adverse effect on microorganisms. NaCl is used in brines and curing solutions or is applied directly to the food. It is used in pickles.

Sugars such as glucose or sucrose make water unavailable to microorganisms. These are used in high concentrations in sweetened condensed milk, fruit syrups, jellies and candies.

Mechanism of action: - i) These cause high osmotic pressure and hence plasmolysis of cells. ii) These dehydrate foods by drawing out and tying up moisture as they dehydrate microbial cells. iii) NaCl ionizes to yield chlorine ions which are harmful to microorganisms.

vi) Alcohol: -

Ethanol is added to vanilla and lemon extracts. The alcoholic content of beer and wine is not great enough to prevent their spoilage by microorganisms. Liqueurs and distilled liquors usually contain enough alcohol to prevent spoilage by microorganisms.

Methanol is poisonous and should not be added to foods.

Glycerol is antiseptic but not important in food preservation.

Propylene glycol has been used as a mold inhibitor and as a spray to kill airborne microorganisms.

Mechanism of action: - Ethanol is a coagulant and denaturizer of cell proteins and is most germicidal in concentrations between 70 to 95 %.

vii) Formaldehyde: -

It is not added in foods. It is used in the treatment of walls, shelves, floors etc to eliminate molds and their spores. It is effective against molds, bacteria, and viruses.

Mechanism of action: - It combines with free amino groups of the proteins of cell protoplasm, injures nuclei and coagulates proteins.

viii) Wood smoke: -

The smoking of foods has two main purposes, first is to add desire flavours, colours and finishing; second is preservation.

Smoke is obtained from the burning of the wood such as hickory, apple, oak, maple, beech, birch, walnut and mahogany. Sawdust is added to the fire to give a heavy smudge. Temperature and humidity are controlled at levels favourable to the product being smoked and the duration of the smoking depends on the kind of the food. Smoking temperatures for meat vary from 43 to 71^0 C 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 the main compound of wood smoke; others are phenols, cresols, aliphatic acids from formic through caproic, primary and secondary alcohols, ketones, acetaldehyde, other aldehydes, waxes, resins, methyl & propyl isomers, catechols, pyrogallol and methyl ester.

Mechanism of action: - The smoking process helps preservation by adding the chemical preservatives developed from smoke and by drying the surface due to heat. Due to chemicals and heat coagulation of proteins of the microorganisms takes place.

ix) Spices and other condiments: -

These do not have any marked bacteriostatic effect in the concentrations customarily used. The inhibitory effect of spices differs with the kind of spice and the microorganism being tested.

- ➔ ***Mustard oil & flour (Mohari)*** are very effective against *Saccharomyces cerevisiae.*
- ➔ ***Cinnamon(Dalchni) and cloves (Lavang): -*** These contain aldehyde and eugenol respectively. These are bacteriostatic.

➔ *Ground pepper corn (Mire), mace (Jayapatri), nutmeg (Jayphal), ginger(Ale), thyme (Owa), bay leaves (Tamalpatra), marjoram, rosemary(Lal terada)* have weak inhibitory power against most organisms.

➔ *Garlic(Lasun), onion, horseradish (Mulachya shenga tikhat mul),* may be bacteriostatic. Acrolein is the active principle in onions and garlic.

X) Antibiotics: -

Most of the better-known antibiotics have been tested on raw foods like meats, fish, and poultry. **Chlortetracycline** has been found superior to other antibiotics due to its broad spectrum of activity. **Oxytetracycline** is also used. **Chloramphenicol** is also successful.

Penicillin, Streptomycin, neomycin, polymyxin, nisin, bacitracin etc are not satisfactory.

8) Preservation of food by irradiation: -

The possible utilization of radiations of various frequencies, ranging from low-frequency electrical current to high-frequency gamma rays is focused for the improved methods of food preservation.

The entire spectrum of radiation is grouped into 2 categories, one on each side of visible light.

A) **Low frequency, long-wavelength, low-quantum energy radiations: -** These range from radio waves to infrared. The effect of these radiations on microorganisms is related to their thermal agitation of the food.

B) **High-frequency, short-wavelength, high-quantum energy radiations: -** These actually excite or destroy organic compounds and microorganisms without heating the product.

 # Cold sterilization: - Microbial destruction by using radiations without the generation of high temperatures is called as **"Cold sterilization".**

 # Ionizing radiations: - Radiations of higher frequencies have high energy contents and are capable of actually breaking individual molecules into ions, hence are called as ionizing radiations.

In food industry, following radiations are used—

I) Ultra-violet radiation
II) Ionizing radiation
III) Microwave heating

I) Ultra-violet irradiation: -

Of the various electromagnetic radiations, UV irradiation has been the most widely used in the food industry. Radiation with wavelengths near 260 nm is absorbed strongly by purines and pyrimydines and is therefore the most germicidal.

*** Germicidal lamps: - The** usual source of UV radiation in the food industry is from quartz-mercury vapour lamps or low pressure mercury lamps, which emit radiation at 254 nm. The lamps are available in various sizes, shapes, and power.

*** Factors influencing effectiveness of UV treatment: -**

1) Time: - The longer the time of exposure to a given concentration, the more effective the treatment.

2) Intensity: - The intensity of the rays reaching an object will depend on the power of the lamp, the distance from the lamp to the object, and kind and amount of interfering material in the path of the rays. The intensity increases with the power of the lamp. It is measured in *microwatts per square centimetre* ($\mu W / cm^2$). A lamp is about 100 times as effective in killing microorganisms at 5 in, than at 8 ft from the irradiated object.

3) Penetration: - The nature of the object or material being irradiated has an important influence on the effectiveness of the process. Penetration is reduced by clear water, dissolved mineral salts, fatty or greasy material etc.

*** Effects on human and animals: -** Gazing at ultraviolet lamps produces irritation of the eyes within a few seconds, longer exposure of the skin results in erythema, or reddening.

*** Action on microorganisms: -** The germicidal effect of UV is determined by the intensity of the rays, time, the location of the organism and kind of the organism. Each microorganism has a characteristic resistance to UV. This can vary with the phase of growth and physiological state of the cell.

The amount of ultraviolet radiation needed to destroy several different microorganisms is summarized in the following table—

Sr.No.	Microorganism	Dose needed for 1 log cycle reduction or 1 D value (μW sec X 10^3)
1	**Gram negative bacteria**	
	Facultative anaerobes	0.8 – 6.4
	Aerobes	3.0 – 5.5
	Phototrophic	5.0 – 6.0
2	**Gram positive bacteria**	
	Bacillus	5.0 – 8.0
	Bacillus spores	8.0 – 10.0
	Micrococcus	6.0 – 20.0
	Staphylococcus	2.2 – 5.0
3	**Molds**	10.0 – 200.0
4	**Yeasts**	3.0 – 10.0

The location of the organism during the tests has a marked influence. E.G. 97 to 99 % of *Escherichia coli* in air were killed in 10 sec at 24 inch with a 15-watt lamp, but 20 sec at 11 inch was necessary for bacteria on the surface of an agar plate. Capsulation or clumping of bacteria increases their resistance. Bacterial spores usually take from 2 to 5 times as much exposure as the corresponding vegetative cells. Yeasts are 2 to 5 times and molds are 10 to 50 times as resistant as bacteria.

*** Applications in the food industry: -**

Examples of the successful use of UV rays include—

- Treatment of water used for drinking purposes and in beverages
- Aging of meats
- Treatment of knives for slicing bread
- Treatment of bread and cakes
- Packaging of the sliced bacon (meat product prepared from a pig and usually cured)
- Sanitizing of eating utensils
- Prevention of growth of slim yeast on pickles, vinegar, sauerkraut vats
- Killing of spores on sugar crystals and in syrups
- Storage and packaging of cheese
- Prevention of mold growth on walls and shelves
- Treatment of air used for or in storage and processing rooms.

II) Ionizing irradiations

* Kinds of ionizing radiations

Radiation classified as ionizing includes X-rays or Gamma rays, Cathode or Beta rays, Protons, Neutrons and Alpha particles. Neutrons result in residual radioactivity in foods, and protons and alpha particles have little penetration. Therefore these rays are not used in food preservation and will not be discussed.

X-rays: - These are penetrating electromagnetic waves which are produced by bombardment of a heavy-metal target with cathode rays within an evacuated tube. They are not currently considered economical for use in the food industry.

Gamma rays: - These are like X-rays but are emitted from by- products of atomic fission or from imitations of such by-products. Cobalt 60 and Cesium 137 have been used as source of these rays.

Beta rays: - These are streams of electrons (beta particles) emitted from radioactive material.

Electrons: - These are small, negatively charged particles of uniform mass that form part of the atom. They are deflected by magnetic and electric fields. Their penetration depends on the speed with which they hit the target. The higher the charge of the electron, the deeper its penetration.

Cathode rays: - These are streams of electrons (beta particles) from the cathode of an evacuated tube.

* Definition of terms: -

1. **roentgen (r): -** It is the quantity of gamma or x-radiation which produces one electrostatic unit of electric charge of either sign in a cubic centimetre of air under standard conditions.
2. **roentgen-equivalent-physical (rep): -** It is the quantity of ionizing energy which produces, per gram of tissue, an amount of ionisation equivalent to a roentgen. It is a measure of the absorbed energy that is effective within the food.
3. **megarep:** - It is 1 million rep. One r or 1 rep is equivalent to the absorption of 83 to 90 erg per gram of tissue.
4. **rad:** - It is the unit of radiation dose, equivalent to the absorption of 100 erg per gram of irradiated material.
5. **megarad (Mrad):** - 1 million rad.
6. **kilorad (Krad): -** 1,000 rad.
7. **electronvolt (eV): -** It is the energy gained by an electron in moving through a potential difference of 1 volt.

8. **me V: -** 1 million electronvolts. It is a measure of the intensity of the irradiation.
9. **Gray (Gy): -** It is equal to 100 rads.

10. Radappertization: - It is the term used to define ‘radiation sterilization’ which would imply high dose treatments, with the resulting product being shelf-stable.

11. Radurization: - It refers to ‘radiation pasteurization’ low-dose treatments, where the intent is to extend a product’s shelf life.
12. Picowaved: - It is the term used to label foods treated with low-level ionizing radiation.

A) Use of X-rays: -

X-rays have good penetration power but the greatest drawback is the low efficiency and high cost of the production. Only 3 to 5 % of the electron energy applied is used in the production of x-rays.

B) Gamma rays and Cathode rays: -

Source: - Chief source of gamma rays are i) radioactive fission products of uranium and cobalt, ii) the cobalt circulated in nuclear reactors, and iii) other fuel elements used to operate a nuclear reactor.

Penetration: - Gamma rays have good penetration, are effective up to 20 cm in most foods. Cathode rays have poor penetration and are effective at only about 0.5 cm per meV.

Efficiency: - Because cathode rays are directional, they can be made to hit the food and therefore are used with greater efficiency than are gamma rays.

Safety: - the use of cathode rays presents fewer health problems than the use of gamma rays.

Effects on microorganisms: - The bactericidal efficacy of a given dose of irradiation depends on—

i) The kind and species of organisms.
ii) The number of organisms (or spores) originally present.
iii) The composition of the food.
iv) The presence or absence of oxygen.
v) The physical state of the food during irradiation.
vi) The condition of the organisms.

Applications: -

Currently food irradiation has been approved only in a very limited way in the United States. Low-level irradiation (1 kiloGray) can be used on fresh fruits and vegetables to kill insects and to inhibit spoilage. Dry or dehydrated vegetables (herbs and spices) can be irradiated at up to 30 kilo Gray to kill insects and bacteria.

- **Microwave processing**

Microwave heating and processing of foods is becoming increasingly popular. Microwaves are electromagnetic waves between **infrared and radio** waves. Specific frequencies are usually at either 915 megacycles or 2450 megacycles. 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 in an attempt to align them with the electromagnetic field being produced.

This rapid oscillation, or intermolecular friction generates heat. The preservative effect of microwaves or the bactericidal effect produced is really a function of the heat that is generated.

• Microbial spoilage of food

A) Classification of food by means of spoilage

On the basis of ease of spoilage, foods can be classified in 3 groups-

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, e.g. potatoes, some varieties of apples, and nutmeats.
3. **Perishable foods: -** This group includes most important daily foods that spoil readily unless special preservative methods are used. Meats, fish, poultry, most fruits and vegetables, eggs, and milk belong in this classification.

Chemical changes caused by microorganisms in food

Because of the great variety of organic compounds in foods and the numerous kinds of microorganisms that can decompose them, many different chemical changes are possible and many kinds of products can result. Following discussion is concerned only with the important types of decomposition of main constituents of foods and the chief products produced.

A) Changes in nitrogenous organic compounds
B) Changes in non-nitrogenous organic compounds
1. Carbohydrates
2. Organic acids
3. Other compounds
4. Lipids
5. Pectic substances

A) Changes in nitrogenous organic compounds

Most of the nitrogen in foods is in the form of proteins, which must be hydrolysed by enzymes of the microorganisms or of the food to polypeptides, simpler peptides, or amino acids before they can serve as nitrogenous food for most organisms. Proteinases catalyse the hydrolysis of proteins to peptides, which may give a bitter taste to foods. Peptidases catalyse the hydrolysis of polypeptides to simpler peptides and finally to amino acids. The latter give flavour of ripened cheeses. For the most part these hydrolyses do not result in particularly objectionable products.

Putrefaction: - Anaerobic decomposition of proteins, peptides, or amino acids result in the production of obnoxious odours is called putrefaction. It results in foul smelling and production of sulphur containing products such as hydrogen, methyl, and ethyl sulfides and mercaptans, plus ammonia, amines (e.g. histamine, tyramine, piperidine, putrescine, and cadaverine), indole, skatole, and fatty acids.

When microorganisms act on amino acids, they may deaminate them, decarboxylate them, or both, resulting in the following products-

Sr. No.	Chemical process	Products
1	Oxidative deamination	Keto acids + NH_3
2	Hydrolytic deamination	Hydroxy acid + NH_3
3	Reductive deamination	Saturated fatty acid + NH_3
4	Desaturation deamination	Unsaturated fatty acid + NH_3

5	Mutual O-R between pairs of amino acids	Keto acid + Fatty acid + NH_3
6	Decarboxylation	Amine + CO_2
7	Hydrolytic deamination + Decarboxylation	Primary alcohol + NH_3 + CO_2
8	Reductive deamination + Decarboxylation	Hydrocarbon + NH_3 + CO_2
9	Oxidative deamination + Decarboxylation	Fatty acid + NH_3 + CO_2

Escherichia coli produces glyoxylic acid, acetic acid, and ammonia from glycine and alanine. *Pseudomonas* produce methylamine and carbon dioxide. *Clostridia* produce acetic acid, ammonia and methane. The sulphur in sulphur bearing amino acids may be reduced to foul-smelling sulphides or mercaptans. E.g. *Desulfatomaculum nigrificans.*

Other nitrogenous compounds decomposed include—

→ Amides, Imides, and urea from which ammonia is produced

→ Guanidine and creatine, which yield urea and ammonia

→ Amines, purines, and pyrimidines, which may yield ammonia, Carbon dioxide and organic acids (mainly acetic and lactic acids)

B) Changes in non-nitrogenous organic compounds

The main non-nitrogenous foods for microorganisms serving as source of carbon and energy include carbohydrates, organic acids, aldehydes, ketones, alcohols, glycosides, cyclic compounds, and lipids.

1. Carbohydrates: -

Complex di-, tri-, or polysaccharides usually are hydrolysed to simple sugars before utilization. A monosaccharide, such as glucose, aerobically would be oxidized to carbon dioxide and water and anaerobically would undergo decomposition involving any six main types of fermentation--

i) An alcoholic fermentation, as by yeasts, with ethanol and carbon dioxide as main products

ii) A simple lactic fermentation, as by homo-fermentative lactic acid bacteria, with lactic acid as the main product
iii) A mixed lactic fermentation, as by heterofermentative lactic acid bacteria, with lactic and acetic acids, ethanol, glycerol, and carbon dioxide as the main products
iv) The coliform type of fermentation, as by coliform bacteria, with lactic, acetic, and formic acids, ethanol, carbon dioxide, hydrogen and perhaps acetoin and butanediol as likely products
v) The propionic fermentation, by propionibacteria, producing propionic, acetic, and succinic acids and carbon dioxide
vi) The butyric-butyl-isopropyl fermentations, by anaerobic bacteria, yielding butyric and acetic acids, carbon dioxide, hydrogen, and in some instances acetone, butylenes glycol, butanol, and 2-propanol.

A variety of other products are possible from sugars when different microorganisms are active, including higher fatty acids, other organic acids, aldehydes, and ketones.

2. Organic acids: -

Many of the organic acids usually occurring in foods as salts are oxidized by organisms to carbonates, causing the medium to become more alkaline. Aerobically the organic acids may be oxidized completely to carbon dioxide and water. Saturated fatty acids are degraded to acetic acid. Unsaturated or hydroxy fatty acids may be degraded partially to a saturated acid for complete beta-oxidation.

3. Other compounds: -

Alcohols usually are oxidized to the corresponding organic acid e.g. ethanol to acetic acid. Glycerol may be dissimilated to products similar to those from glucose. Glycosides after hydrolysis to release the sugar will have the sugar dissimilated characteristically. Acetaldehyde may be oxidized to acetic acid or reduced to ethanol. Cyclic compounds are not readily attacked.

4. Lipids: -

Fats are hydrolyzed by microbial lipase to glycerol and fatty acids, which are then dissimilated. Microorganisms may be involved in the oxidation of fats, but autooxidation is more common. Phospholipids may be degraded to their constituent phosphate, glycerol, fatty acids, and nitrogenous base, e.g. choline. Lipoproteins are made up of proteins, cholesterol esters, and phospholipids.

5. Pectic substances: -

Protopectin, the water insoluble parent pectic substance in plants, is converted to pectin, a water-soluble polymer of galacturonic acid, which contains methyl ester linkages. Pectinesterase causes hydrolysis of the methyl ester linkage of pectin to yield pectic acid and methanol. Polygalacturonases destroy the linkage between galacturonic acid units of pectin or pectic acid to yield smaller chains and ultimately free D-galacturonic acid, which may be degraded to simple sugars.

==

- **Types of spoilage of canned foods with organisms involved in tabular form**

Sr. No.	Type of spoilage	Microorganisms involved	Appearance of can	Contents of can
A	**Low and Medium acid foods (pH above 4.5)**			
1	Flat sour	Bacillus stearothermophilus	Possible loss of vacuum on storage	Appearance not usually altered, pH markedly lowered, Sour, abnormal odour, cloudy liquid
2	Thermophilic anaerobic (TA)	Clostridium thermosaccharolyticum	Can swells	Fermented, sour, cheesy, butyric acid odour
3	Sulfide	Desulfato-maculum nigrificans	No swelling	Usually blackened, rotten-egg odour
4	Putrefactive anaerobic (PA)	Clostridium sporogenes	Can swells	May be partially digested, pH slightly above normal, typical putrid odour

5	Aerobic endospore formers	Bacillus species	Usually no swelling, except in cured meats	Coagulated evaporated milk, sour taste
B	**High acid foods (pH below 4.5)**			
1	Flat sour	Bacillus coagulans	Can flat, change in vacuum	Slight pH change, off odour and flavour
2	Butyric anaerobic	Clostridium butyricum	Can swells	Fermented, butyric acid odour
3	Non endospore formers	Lactic acid bacteria	Can swells	Acid odour
4	Yeasts	Yeast spp.	Can swells, may burst	Fermented, yeasty odour
5	Molds	Mold spp.	Can flat	Surface growth, musty odour

Types of spoilage of non-canned foods with organisms involved in tabular form

Sr. No.	Food	Type of spoilage	Causative microorganisms
1	**Sugar products, honey, syrups**	Ropy syrup	*Aerobacter aerogenes*
		Yeasty	*Saccharomyces, Torula, Zygosaccharomyces*
		Pink syrup	*Micrococcus roseus*
		Green syrup	*Pseudomonas fluorescens*
		Mouldy	*Aspergillus, Penicillium*

2	**Bread**	Mouldy	*Rhizopus, Aspergillus*
		Ropy	*Penicillium*
		Red bread	*Bacillus spp., Serratia marcescens*
3	**Fresh fruits and vegetables**	Bacterial soft rot	*Erwinia carotovora, Pseudomonas spp.*
		Gray mold rot	*Botrytis cineria*
		Rhizopus soft rot	*Rhizopus nigricans*
		Blue mold rot	*Penicillium italicum*
		Black mold rot	*Aspergillus niger, Alternaria*
		Sliminess or souring	*Saprophytic bacteria*
4	**Pickles, sauerkraut**	Black pickle	*Bacillus nigricans*
		Soft pickles	*Bacillus spp.*
		Slimy kraut	*Lactobacillus plantarum, L. cucumeris*
		Pink kraut	*Rhodotorula*
5	**Fresh meat**	Putrefaction	*Clostridium, Pseudomonas, Proteus, Alcaligenes, Chromobacterium*
		Souring	*Chromobacterium, Lactobacillus, Pseudomonas*
6	**Cured meat**	Moldy	*Penicillium, Aspergillus, Rhizopus*
		Souring	*Pseudomonas, Micrococcus, Bacillus*
		Greening	*Lactobacillus, Streptococci, Pediococci*
		Slimy	*Leuconostoc*

7	**Fish**	Discolouration	*Pseudomonas*
		Putrefaction	*Chromobacterium, Halobacterium, Micrococcus*
8	**Poultry**	Odour, slime	*Pseudomonas, Alcaligenes, Xanthomonas*
9	**Eggs**	Green rot	*Pseudomonas fluorescens*
		Colourless rot	*Pseudomonas, Alcaligenes, Chromobacterium, Coliforms*
		Black rot	*Proteus*
		Fungal rot	*Penicillium, Mucor*
10	**Milk**	Souring	*Streptococci, Lactobacilli, Micrococci*
		Gas production	*Coliforms, Clostridia, Bacillus*
		Proteolysis	*Micrococci, Alcaligenes, pseudomonas, Proteus, Clostridia, Bacillus spp.*
		Ropiness	*Micrococci, Enterobacter, Lactobacillus, Bacillus*
		Colour & Flavour changes	*Pseudomonas, Serratia, Micrococci*