

**Introduction:**

Except for a few sterile foods, all foods harbor one or more types of microorganisms. Some of them have desirable roles in food, such as in the production of naturally fermented food, whereas others cause food spoilage and foodborne diseases. To study the role of microorganisms in food and to control them when necessary, it is important to isolate them in pure culture and study their morphological, physiological, biochemical, and genetic characteristics.

The food-producing period dates from about 8,000 to 10,000 years ago and, includes the present time. It is presumed that the problems of spoilage and food poisoning were encountered early in this period. With the advent of prepared foods, the problems of disease transmission by foods and of faster spoilage caused by improper storage made their appearance. Spoilage of prepared foods apparently dates from around 6000 BC. The practice of making pottery was brought to Western Europe about 5000 BC from the Near East. The first boiler pots are thought to have originated in the Near East about 8,000 years ago. The arts of cereal cookery, brewing, and food storage were either started at about this time or stimulated by this new development.

Few advances were apparently made toward understanding the nature of food poisoning and food spoilage between the time of the birth of Christ and AD 1100.

The first person to appreciate and understand the presence and role of microorganisms in food was Pasteur. In 1837, he showed that the souring of milk was caused by microorganisms, and in about 1860 he used heat for the first time to destroy undesirable organisms in wine and beer. This process is now known as pasteurization.

## **Food Microbiology: Current Status:**

In the early 20th century, studies continued to understand the association and importance of microorganisms, especially pathogenic bacteria in food. Specific methods were developed for their isolation and identification. The importance of sanitation in the handling of food to reduce contamination by microorganisms was recognized. Specific methods were studied to prevent growth as well as to destroy the spoilage and pathogenic bacteria. There was also some interest to isolate beneficial bacteria associated with food fermentation, especially dairy fermentation, and study their characteristics. However, after the 1950s, food microbiology entered a new era. Availability of basic information on the physiological, biochemical, and biological characteristics of diverse types of food, microbial interactions in food environments and microbial physiology, biochemistry, genetics, and immunology has helped open new frontiers in food microbiology. Among these are:

### **A. Food Fermentation/Probiotics**

- Development of strains with desirable metabolic activities by genetic transfer among strains.
- Development of bacteriophage-resistant lactic acid bacteria
- Metabolic engineering of strains for overproduction of desirable metabolites
- Development of methods to use lactic acid bacteria to deliver immunity proteins
- Sequencing genomes of important lactic acid bacteria and bacteriophages for better understanding of their characteristics
- Food biopreservation with desirable bacteria and their antimicrobial metabolites

- Understanding of important characteristics of probiotic bacteria and development of desirable strains
- Effective methods to produce starter cultures for direct use in food processing

## **B. Food Spoilage**

- Identification and control of new spoilage bacteria associated with the current changes in food processing and preservation methods.
- Spoilage due to bacterial enzymes of frozen and refrigerated foods with extended shelf life.
- Development of molecular methods (nanotechnology) to identify metabolites of spoilage bacteria and predict potential shelf life of foods.
- Importance of environmental stress on the resistance of spoilage bacteria to antimicrobial preservatives.

## **C. Foodborne Diseases**

- Methods to detect emerging foodborne pathogenic bacteria from contaminated foods
- Application of molecular biology techniques (nanotechnology) for rapid detection of pathogenic bacteria in food and environment
- Effective detection and control methods of foodborne pathogenic viruses
- Transmission potentials of prion diseases from food animals to humans
- Importance of environmental stress on the detection and destruction of pathogens
- Factors associated with the increase in antibiotic-resistant pathogens in food
- Adherence of foodborne pathogens on food and equipment surfaces
- Mechanisms of pathogenicity of foodborne pathogens
- Effective methods for epidemiology study of foodborne diseases
- Control of pathogenic parasites in food.

## **Contamination of Foods:**

The internal tissues of healthy plants (fruits and vegetables) and animals (meat) are essentially sterile. Yet raw and processed (except sterile) foods contain different types of molds, yeasts, bacteria, and viruses. Microorganisms get into foods from both natural (including internal) sources and from external sources to which a food comes into contact from the time of production until the time of consumption.

## **Sources of contamination:**

### **A. Plants (Fruits and Vegetables)**

The inside tissue of foods from plant sources are essentially sterile, except for a few porous vegetables (e.g., radishes and onions) and leafy vegetables (e.g., cabbage and Brussels sprouts). Some plants produce natural antimicrobial metabolites that can limit the presence of microorganisms. Fruits and vegetables harbor microorganisms on the surface; their type and level vary with soil condition, type of fertilizers and water used, and air quality. Molds, yeasts, lactic acid bacteria, and bacteria from genera *Pseudomonas*, *Alcaligenes*, *Micrococcus*, *Erwinia*, *Bacillus*, *Clostridium*, and *Enterobacter* can be expected from this source. Pathogens, especially of enteric types, can be present if the soil is contaminated with untreated sewage. Diseases of the plants, damage of the surface (before, during, and after harvest), long delay between harvesting and washing, and unfavorable storage and transport conditions after harvesting and before processing can greatly increase microbial numbers as well as predominant types. Improper storage conditions following processing can also increase their numbers.

### **B. Animals, Birds, Fish, and Shellfish**

Food animals and birds normally carry many types of indigenous microorganisms in the digestive, respiratory, and urinogenital tracts, the teat canal in the udder, as well as in the skin, hooves, hair, and feathers.

Many, as carriers, can harbor pathogens such as *Salmonella* serovars, pathogenic *Escherichia coli*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Listeria monocytogenes* without showing symptoms. Laying birds have been carrying *Salmonella* Enteritidis in the ovaries and contaminating the yolk during ovulation.

Fish and shellfish also carry normal microflora in the scales, skin, and digestive tracts. Water quality, feeding habits, and diseases can change the normal microbial types and level. Pathogens such as *Vibrio parahaemolyticus*, and *V.cholerae* are of major concern from these sources.

In addition to enteric pathogens from fecal materials, meat from food animals and birds can be contaminated with several spoilage and pathogenic microorganisms from skin, hair, and feathers, namely *Staphylococcus aureus*, *Micrococcus* spp., *Propionibacterium* spp., *Corynebacterium* spp., and molds and yeasts.

### **C. Air**

Microorganisms are present in dust and moisture droplets in the air. They do not grow in dust, but are transient and variable, depending on the environment. Their level is controlled by the degree of humidity, size and level of dust particles, temperature and air velocity, and resistance of microorganisms to drying. Generally, dry air with low dust content and higher temperature has a low microbial level. Spores of *Bacillus* spp., *Clostridium* spp., and molds, and cells of some Gram positive bacteria (e.g., *Micrococcus* spp. and *Sarcina* spp.), as well as yeasts, can be predominantly present in air. If the surroundings contain a source of pathogens(e.g., animal and poultry farms or a sewage-treatment plant), different types of bacteria, including pathogens and viruses (including bacteriophages), can be transmitted via the air. Microbial contamination of food from the air can be reduced by removing

the potential sources, controlling dust particles in the air (using filtered air), using positive air pressure, reducing humidity level, and installing UV light.

#### **D. Soil**

Soil, especially the type used to grow agricultural produce and raise animals and birds, contains several varieties of microorganisms. Because microorganisms can multiply in soil, their numbers can be very high (billions/g). Many types of molds, yeasts, and bacterial genera (e.g., *Enterobacter*, *Pseudomonas*, *Proteus*, *Micrococcus*, *Enterococcus*, *Bacillus*, and *Clostridium*) can enter foods from the soil. Soil contaminated with fecal materials can be the source of enteric pathogenic bacteria and viruses in food. Sediments where fish and marine foods are harvested can also be a source of microorganisms, including pathogens, in those foods. Different types of parasites can also get in food from soil. Removal of soil (and sediments) by washing and avoiding soil contamination can reduce microorganisms in foods from this source.

#### **E. Sewage**

Sewage, especially when used as fertilizer in crops, can contaminate food with microorganisms, the most significant of which are different enteropathogenic bacteria and viruses. This can be a major concern with organically grown food and many imported fruits and vegetables, in which untreated sewage and manure might be used as fertilizer. Pathogenic parasites can also get in food from sewage.

To reduce incidence of microbial contamination of foods from sewage, it is better not to use sewage as fertilizer. If used, it should be efficiently treated to kill the pathogens. Also, effective washing of foods following harvesting is important.

## **F. Water**

Water is used to produce, process, and, under certain conditions, store foods. It is used for irrigation of crops, drinking by food animals and birds, raising fishery and marine products, washing foods, processing (pasteurization, canning, and cooling of heated foods) and storage of foods (e.g., fish on ice), washing and sanitation of equipment, and processing and transportation facilities. Water is also used as an ingredient in many processed foods. Thus, water quality can greatly influence microbial quality of foods. Contamination of foods with pathogenic bacteria, viruses, and parasites from water has been recorded.

Potable water does not contain coliforms and pathogens (mainly enteric types), it can contain other bacteria capable of causing food spoilage, such as *Pseudomonas*, *Alcaligenes*, and *Flavobacterium*. Improperly treated water can contain pathogenic and spoilage microorganisms. To overcome the problems, many food processors use water, especially as an ingredient, that has a higher microbial quality than that of potable water.

## **G. Humans**

Between production and consumption, foods come in contact with different people handling the foods. They include not only people working in farms and food processing plants, but also those handling foods at restaurants, catering services, retail stores, and at home. Human carriers have been the source of pathogenic microorganisms in foods that later caused foodborne diseases, especially with ready to eat foods. Improperly cleaned hands, lack of aesthetic sense and personal hygiene, and dirty clothes and hair can be major sources of microbial contamination in foods.

The presence of minor cuts and infection in hands and face and mild generalized diseases (e.g., flu, strep throat, or hepatitis A in an early stage) can amplify the situation. In addition to spoilage bacteria, pathogens such as

*Staphylococcus. aureus*, *Salmonella* serovars, *Shigella* spp., pathogenic *E. coli*, and hepatitis A can be introduced into foods from human sources.

Proper training of personnel in personal hygiene, regular checking of health, and maintaining efficient sanitary and aesthetic standards are necessary to reduce contamination from this source.

## **H. Food Ingredients**

In prepared foods, many ingredients or additives are included in different quantities. Many of these ingredients can be the source of both spoilage and pathogenic microorganisms. Various spices generally have very high populations of mold and bacterial spores. Starch, sugar, and flour might have spores of thermophilic bacteria. Pathogens have been isolated from dried coconut, egg, and chocolate. The ingredients should be produced under sanitary conditions and given antimicrobial treatments. In addition, setting up acceptable microbial specifications for the ingredients will be important in reducing microorganisms in food from this source.

## **I. Equipment**

A wide variety of equipment is used in harvesting, slaughtering, transporting, processing, and storing foods. Many types of microorganisms from air, raw foods, water, and personnel can get into the equipment and contaminate foods. Depending on the environment (moisture, nutrients, and temperature) and time, microorganisms can multiply and, even from a low initial population, reach a high level and contaminate large volumes of foods. Also, when processing equipment is used continuously for a long period of time, microorganisms initially present can multiply and act as a continuous source of contamination in the product produced subsequently.

Small equipment, such as cutting boards, knives, spoons, and similar articles, because of improper cleaning, can be sources of cross contamination.



*Salmonella, Listeria, Escherichia, Enterococcus, Micrococcus, Pseudomonas, Lactobacillus, Leuconostoc, Clostridium, Bacillus* spp., and yeasts and molds can get in food from equipment . Proper cleaning and sanitation of equipment are important to reduce microbial levels in food.

## **J. Miscellaneous**

Foods might be contaminated with microorganisms from several other sources, namely packaging and wrapping materials, containers, flies, vermin, birds, house pets, and rodents. Many types of packaging materials are used in food.

Flies, vermin, birds, and rodents in food processing and food preparation and storage facilities should be viewed with concern as they can carry pathogenic microorganisms. House pets can also harbor pathogens.

**Important Microorganisms in food****A. Important Bacterial Groups in Foods**

Among the microorganisms found in foods, bacteria constitute major important groups. This is not only because many different species can be present in foods but also because of their rapid growth rate, ability to utilize food nutrients, and ability to grow under a wide range of temperatures, aerobiosis, pH, and water activity, as well as to better survive adverse situations, such as survival of spores at high temperature. For convenience, bacteria important in foods have been divided into several groups on the basis of similarities in certain characteristics. Some of these groups and their importance in foods are listed here.

**1. Lactic Acid Bacteria**

They are bacteria that produce relatively large quantities of lactic acid from carbohydrates. Species mainly from genera *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Lactobacillus*, and *Streptococcus thermophilus* are included in this group.

**2. Acetic Acid Bacteria**

Most of the acetic acid belong to two genera *Acetobacter* and *Gluconobacter*. Both oxidize ethyl alcohol to acetic acid, but *Acetobacter* is capable of oxidizing acetic acid further to CO<sub>2</sub>.

**3. Propionic Acid Bacteria**

They are bacteria that produce propionic acid and are used in dairy fermentation. Species such as *Propionibacterium shermanii* are included in this group.

#### **4. Butyric Acid Bacteria**

They are bacteria that produce butyric acid in relatively large amounts. Some *Clostridium* spp. such as *Clostridium butyricum* are included in this group.

#### **5. Proteolytic Bacteria**

They are bacteria that can hydrolyze proteins because they produce extracellular proteinases. Species in genera *Micrococcus*, *Staphylococcus*, *Bacillus*, *Clostridium*, *Pseudomonas*, *Alteromonas*, *Flavobacterium*, *Alcaligenes*, some in *Enterobacteriaceae*, and *Brevibacterium* are included in this group. Some bacteria are putrefactive, they decompose proteins anaerobically to produce foul smelling compounds such as H<sub>2</sub>S, mercaptans, amines, indole. Ex: *Clostridium*, *Proteus*, *Pseudomonas*.

#### **6. Lipolytic Bacteria**

They are bacteria that are able to produce lipases which catalyze the hydrolysis of fats to fatty acids and glycerol. Many of the aerobic, actively proteolytic bacteria also are lipolytic.

Species in genera *Micrococcus*, *Staphylococcus*, *Pseudomonas*, *Alcaligenes*, *Serratia*, *Alteromonas*, and *Flavobacterium* are included in this group. *Pseudomonas fluorescens* – Strongly lipolytic

#### **7. Saccharolytic Bacteria**

They are bacteria that are able to hydrolyze complex carbohydrates (disaccharides or polysaccharides) to simpler sugars. Species in genera *Bacillus*, *Clostridium*, *Aeromonas*, *Pseudomonas*, and *Enterobacter* are included in this group. Amylolytic bacteria possess amylase to bring about the hydrolysis of starch outside the cell. Amylolytic bacteria are *Bacillus subtilis* and *Clostridium butyricum*.

#### **8. Pectinolytic Bacteria:**

Pectins are complex carbohydrates that are responsible for cell wall rigidity in vegetables and fruits. Species in genera *Erwinia*, *Bacillus*, *Clostridium*, *Achromobacter*, *Aeromonas*, *Arthrobacter*, *Flavobacterium* are included in this group.

## **9. Thermophilic Bacteria**

Optimum temperature required for these bacteria 45°C - 55°C .Species from genera *Bacillus*, *Clostridium*, *Pediococcus*, *Streptococcus*, and *Lactobacillus* are included in this group.. *Bacillus stearothermophilus* – thermophilic flat sour spoilage of low acid canned foods.

## **10. Thermoduric Bacteria**

They are bacteria that are able to survive pasteurization temperature treatment. Some species from *Micrococcus*, *Enterococcus*, *Lactobacillus*, *Pediococcus*, *Bacillus* (spores), and *Clostridium* (spores) are included in this group.

Some thermoduric bacteria like *Bacillus* and enterococci can also be psychrotrophic.

## **11. Psychrotrophic Bacteria**

They are bacteria that are able to grow at refrigeration temperature. Unlike psychrophiles, psychrotrophs do not have their optimal temperature for growth at refrigeration temperature and their optimum between 25°C and 30°C.

Some species from *Pseudomonas*, *Alteromonas*, *Alcaligenes*, *Flavobacterium*, *Serratia*, *Bacillus*, *Clostridium*, *Lactobacillus*, *Leuconostoc*, *Carnobacterium*, *Brochothrix*, *Listeria*, *Yersinia*, and *Aeromonas* are included in this group.

## **12. Halophilic Bacteria**

Halophilic Bacteria require certain minimal concentrations of dissolved sodium chloride for growth. Ex: *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Flavobacterium*, *Vibrio* spp. which grow best in media with 0.5-3.0 % salt. These are **slightly halophilic**. These bacteria are isolated from fish, shell fish.

**Moderate halophiles** are grown in the media containing 3.0 – 15% salt, such as salted fish, brined fish, brined meats and some salted vegetables. **Extreme halophiles** grow in the heavily brined foods 15 – 30% salt. EX: *Halobacterium*, *Halococcus*.

### **13. Halotolerant Bacteria**

Halotolerant bacteria can grow with or without salt. Usually they are capable of growing in foods containing (0.5%) salt or more. Ex: *Bacillus*, *Micrococcus*, *Corynebacterium*, *pediococcus*.

### **14. Aciduric Bacteria**

Acidoduric bacteria are able to survive at low pH (<4.0). Some species from *Lactobacillus*, *Pediococcus*, *Lactococcus*, *Enterococcus*, and *Streptococcus* are included in this group.

### **15. Osmophilic or Saccharophilic Bacteria:**

Osmophilic bacteria are those which grow in high concentrations of sugar. Ex: *Leuconostoc*.

### **16. Gas-Producing Bacteria**

They are bacteria that produce gas (CO<sub>2</sub>, H<sub>2</sub>, H<sub>2</sub>S) during metabolism of nutrients.

Species from genera *Leuconostoc*, *Lactobacillus* (heterofermentative), *Propionibacterium*, *Escherichia*, *Enterobacter*, *Clostridium*, *Bacillus* and *Desulfotomaculum* are included in this group.

*Leuconostoc*, *Lactobacillus*, *Propionibacterium*, produces only CO<sub>2</sub>. *Desulfotomaculum* produce H<sub>2</sub>S. Other genera produce both CO<sub>2</sub> and H<sub>2</sub>.

### **17. Slime forming bacteria:**

They are bacteria that produce slime because they synthesize polysaccharides. Some species or strains from *Xanthomonas*, *Leuconostoc*, *Alcaligenes*, *Enterobacter*, *Lactococcus*, and *Lactobacillus* are included in this group.

*Alcaligenes viscolactis*, *Enterobacter aerogenes* producing slime in milk and *Leuconostoc* spp. producing slime in sucrose solutions . *Micrococcus* makes curing solutions for meats ropy. *Lactobacillus plantarum* and Lactobacilli may produce slime in various fruit, vegetable and grain products e.g. in cider, sauerkraut and beer.

### **18. Pigmented Bacteria:**

Colors produced by pigmented bacteria growing on or in foods.

*Flavobacterium*-Yellow to orange; *Serratia*-Red; *Halobacterium*-Pink

### **19. Coliform and Fecal coliform group:**

Coliforms are short rods that are defined as aerobic and facultative anaerobic, gram negative, non-spore forming bacteria. Ex: *Escherichia coli*, *Enterobacter aerogenes*.

Fecal coliform group includes coliforms capable of growing at 44 - 45°C. They are used as an index of sanitation.

Some of the characteristics that make the coliform bacteria important in food spoilage are:

1. Their ability to grow well in a variety of substrates and synthesize most of the necessary vitamins.
2. Their ability of the group to grow well over a fairly wide range of temperatures from below 10°C to about 46°C.
3. Their ability to produce considerable amounts of acid and gas from sugars.
4. Their ability to cause off – flavours often described as unclean or barny.

5. Their ability of *E. aerogenes* to cause sliminess or ropiness of foods.

## 20. Enteric Pathogenic bacteria

Pathogenic *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Escherichia*, *Vibrio*, *Listeria* and others that can cause gastrointestinal infection are included in this group.

## B. Important Mold Genera

Molds are important in foods because they can grow even in conditions in which many bacteria cannot grow, such as low pH, low water activity (*aw*), and high osmotic pressure. Many types of molds are found in foods. They are important spoilage microorganisms. Many strains also produce mycotoxins and have been implicated in foodborne intoxication. Many are used in food bioprocessing. Finally, many are used to produce food additives and enzymes. Some of the most common genera of molds found in food are listed here.

- 1- *Aspergillus*. It is widely distributed and contains many species important in food. Many are xerophilic (able to grow in low *aw*) and can grow in grains, causing spoilage. They are also involved in spoilage of foods such as jams, nuts, and fruits and vegetables (rot). Some species or strains produce mycotoxins (e.g., *Aspergillus flavus* produces aflatoxin). Many species or strains are also used in food and food additive processing. *A. niger* is used to process citric acid from sucrose and to produce enzymes such as B-galactosidase.
- 2- *Alternaria*. Members are septate and form dark-colored spores on conidia. They cause rot in tomatoes and rancid flavor in dairy products. Some species or strains produce mycotoxins.
- 3- *Fusarium*. Many types are associated with rot in citrus fruits, potatoes, and grains. They form cottony growth.

- 4- *Geotrichum*. They grow, forming a yeast like cottony, creamy colony. They establish easily in equipment and often grow on dairy products (dairy mold). Species: *Geotrichum candidum*.
- 5- *Mucor*. They produce cottony colonies. Some species are used in food fermentation and as a source of enzymes. They cause spoilage of vegetables. Species: *Mucor rouxii*.
- 6- *Penicillium*. It is widely distributed and contains many species. Some species are used in food production, such as *Penicillium roquefortii* and *Pen. camembertii* in cheese. Many species cause fungal rot in fruits and vegetables. They also cause spoilage of grains, breads, and meat. Some strains produce mycotoxins
- 7- *Rhizopus*. They cause spoilage of many fruits and vegetables. *Rhizopus* spp. is the common black bread mold.

### **C. Important Yeast Genera**

Yeasts are important in food because of their ability to cause spoilage. Many are also used in food bioprocessing. Some are used to produce food additives.

- 1- *Saccharomyces*. Cells are round, oval, or elongated. It is the most important genus. *Saccharomyces cerevisiae* are used in baking for leavening bread and in alcoholic fermentation. They also cause spoilage of food, producing alcohol and CO<sub>2</sub>.
- 2- *Pichia*. They form pellicles in beer, wine, and brine to cause spoilage. Some are also used in food fermentation.
- 3- *Rhodotorula*. They are pigment-forming yeasts and can cause discoloration of foods such as meat, fish, and sauerkraut.
- 4- *Torulopsis*. They cause spoilage of milk because they can ferment lactose. They also spoil fruit juice concentrates and acid foods.



- 5- *Candida*. Many species spoil foods with high acid, salt, and sugar and form pellicles on the surface of liquids. Some can cause rancidity in butter and dairy products (e.g., *Candida lipolyticum*).
- 6- *Zygosaccharomyces*. Cause spoilage of high-acid foods, such as sauces, ketchups, pickles, mustards, mayonnaise.

#### **D. Important Viruses**

Viruses are important in foods, some are able to cause enteric disease, and thus, if present in a food, can cause foodborne diseases. Hepatitis A have been implicated in foodborne outbreaks. Several other enteric viruses, such as poliovirus, echo virus, and Coxsackie virus, can cause foodborne diseases. In some countries where the level of sanitation is not very high, they can contaminate foods and cause disease.

Some bacterial viruses (bacteriophages) are used to identify some pathogens (*Salmonella* spp., *Staphylococcus aureus* strains) on the basis of the sensitivity of the cells to a series of bacteriophages at appropriate dilutions. Bacteriophages are used to transfer genetic traits in some bacterial species or strains by a process called transduction (e.g., in *Escherichia coli* or *Lactococcus lactis*).

Finally, some bacteriophages can be very important because they can cause fermentation failure. Many lactic acid bacteria, used as starter cultures in food fermentation, are sensitive to different bacteriophages. They can infect and destroy starter-culture bacteria, causing product failure.

## **Intrinsic and Extrinsic Parameters of Foods That Affect Microbial Growth**

The ability of microorganisms to grow or multiply in a food is determined by the food environment (intrinsic environment of food) as well as the environment in which the food is stored (extrinsic environment).

### **INTRINSIC PARAMETERS**

- **pH**

Most microorganisms grow best at pH values around 7.0 (6.6-7.5). In general, molds and yeasts are able to grow at lower pH than bacteria, and Gram-negative bacteria are more sensitive to low pH than are Gram-positive bacteria and pathogenic bacteria being the most fastidious.

On the basis of pH, foods can be grouped as high-acid foods (pH below 4.6) and low-acid foods (pH 4.6 and above). Fruits, soft drinks and vinegar all fall below the point at which bacteria normally grow. Fruits generally undergo mold and yeast spoilage, and this is due to the capacity of these organisms to grow at pH values <3.5, which is below the minima for most food spoilage and all food poisoning bacteria, most of the meats and seafoods have a pH of about 5.6 and above. This makes these products susceptible to bacteria as well as to mold and yeast spoilage. Most vegetables have higher pH values than fruits and vegetables should be subject more to bacterial than fungal spoilage.

- **Water activity**

Water activity ( $a_w$ ) is a measure of the availability of water for biological functions and relates to water present in a food in free form. The free water in a food is necessary for microbial growth. It is necessary to transport nutrients and remove waste materials, carry out enzymatic reactions, synthesize cellular materials, and take part in other biochemical reactions, such as hydrolysis of proteins to amino acids. The water requirements of

microorganisms should be described in terms of the water activity ( $a_w$ ) in the environment. This parameter is defined by the ratio of the water vapor pressure of food substrate ( $P$ , which is  $<1$ ) to the vapor pressure of pure water ( $P_o$ , which is 1), at the same temperature.

$a_w = p/p_o$ , where  $p$  is the vapor pressure of the solution and  $p_o$  is the vapor pressure of the solvent (usually water). This concept is related to relative humidity (RH) in the following way:

$$\text{RH} = 100 \times a_w.$$

In general, bacteria require higher values of  $a_w$  for growth than fungi, with gram-negative bacteria having higher requirements than gram positives. Each microbial species (or group) has an optimum, maximum, and minimum  $a_w$  level for growth

In general, the minimum  $a_w$  values for growth of microbial groups are as follows: Most Gram-positive bacteria, 0.90; and Gram-negative bacteria, 0.93. , whereas most molds, 0.80 and most yeasts ,0.88.

### • **Nutrient Content**

The microorganisms of importance in foods require :

- water
- source of energy
- source of nitrogen
- vitamins and related growth factors
- minerals

The importance of water to the growth of microorganisms was presented above. To the other four groups of substances, molds have the lowest requirement, followed by yeasts, gram-negative bacteria, and gram positive bacteria. As sources of energy, foodborne microorganisms may utilize sugars, alcohols, and amino acids. Some few microorganisms are able to utilize complex carbohydrates such as starches and cellulose as sources of

energy by first degrading these compounds to simple sugars. Fats are used also by microorganisms as sources of energy, but these compounds are attacked by small number of microbes in foods.

Microorganisms may require B vitamins in low quantities, and almost all natural foods have a large quantity for those organisms that are unable to synthesize their essential requirements. In general, gram-positive bacteria are the least synthetic and must therefore be supplied with one or more of these compounds before they will grow. The gram-negative bacteria and molds are able to synthesize most or all of their requirements. Consequently, these two groups of organisms may be found growing on foods low in B vitamins.

- **Oxidation-Reduction Potential**

The O/R potential of a substrate may be defined generally as the ease with which the substrate loses or gains electrons. When an element or compound loses electrons, the substrate is said to be oxidized, whereas a substrate that gains electrons becomes reduced. Therefore, a substance that readily gives up electrons is a good reducing agent, and one that readily takes up electrons is a good oxidizing agent. The O/R potential of a system is expressed by the symbol Eh.

Aerobic microorganisms require positive Eh values (oxidized) for growth such as some members of the genus *Bacillus*., whereas anaerobes require negative Eh values (reduced) such as the genus *Clostridium*.

Some bacteria actually grow better under slightly reduced conditions, and these organisms are often referred to as microaerophiles. Examples of microaerophilic bacteria are *Lactobacilli* and *Campylobacters*. Some bacteria have the capacity to grow under either aerobic or anaerobic conditions. Such types are referred to as facultative anaerobes. Most molds and yeasts encountered in and on foods are aerobic, although a few tend to be facultative anaerobes.

Plant foods, especially plant juices, tend to have positive Eh values, aerobic bacteria and molds are the common cause of spoilage of products of this type.

Solid meats have negative Eh values, an aerobic bacteria are the common cause of spoilage of products of this type.

- **Antimicrobial substances**

The stability of some foods against attack by microorganisms is due to the presence of certain naturally substances that have been shown to have antimicrobial activity. Some species are known to contain essential oils that possess antimicrobial activity. Among these are eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, cow's milk contains several antimicrobial substances, including lactoferrin, lysozyme and the lactoperoxidase system. Eggs contain lysozyme, Ovotransferrin and conalbumin, provides fresh eggs with antimicrobial system. The organic acids found in fruits, vegetables show antibacterial and some antifungal activity.

- **Biological structures**

Inner parts of healthy tissues of living plants and animals are sterile or low in microbial content. The natural covering of some foods provides excellent protection against the entry and damage by spoilage organisms. Ex: The outer covering of fruits, the shell of nuts, the hide of animals, and the shells of eggs.

### **EXTRINSIC PARAMETERS**

The extrinsic parameters of foods are those properties of the storage environment that affect both the foods and their microorganisms. Those of greatest importance to the welfare of foodborne organisms are as follows:

- **Temperature of Storage**

Microorganisms important in foods are divided into three groups on the basis of their temperature of growth : (1) thermophiles (grow at relatively

high temperature), (2) mesophiles (grow at ambient temperature), and (3) psychrophiles (grow at cold temperature). Therefore, it would be well to consider at this point the temperature growth ranges for organisms of importance in foods as an aid in selecting the suitable temperature for the storage of different types of foods. Temperature of storage is the most important parameter that affects the spoilage of highly perishable foods. Ex: The psychrotrophs found most commonly on foods are those that belong to the genera *Pseudomonas* and *Enterococcus* . These organisms grow well at refrigerator temperatures and cause spoilage of meats, fish, poultry, eggs, and other foods normally held at this temperature.

- **Relative Humidity of Environment**

The Relative Humidity (RH) of the storage environment is important both from the stand point of  $a_w$  within foods and the growth of microorganisms at the surfaces. When the  $a_w$  of a food is set at 0.60, it is important that this food be stored under conditions of RH that do not allow the food to pick up moisture from the air and increase its own surface and subsurface  $a_w$  to a point where microbial growth can occur. When foods with low  $a_w$  values are placed in environments of high RH the foods pick up moisture . Foods with a high  $a_w$  lose moisture when placed in an environment of low RH. Foods that undergo surface spoilage from molds, yeasts, and certain bacteria should be stored under conditions of low RH. Wrapped meats such as whole chickens and beef cuts tend to suffer much surface spoilage in the refrigerator before deep spoilage occurs, due to the generally high RH of the refrigerator.

- **Presence and Concentration of Gases in the Environment**

Carbon dioxide (CO<sub>2</sub>) is the single most important atmospheric gas that is used to control microorganisms . Ozone (O<sub>3</sub>) is the other atmospheric gas that has antimicrobial properties. It has been shown to be effective against

a variety of microorganisms, but because it is a strong oxidizing agent, it should not be used on high-lipid-content foods since it would cause an increase in rancidity.

- **Microbial Interference**

Microbial interference refers to the general nonspecific inhibition or destruction of one microorganism by other members of the same environment. Whereas lactic antagonism is a specific example of microbial interference. The lactic antagonism include bacteriocins, pH depression, organic acids, H<sub>2</sub>O<sub>2</sub>, diacetyl, and other products have ability to inhibit pathogens and food-spoilage organisms.

The explanations for interference are (1) competition for nutrients, (2) competition for attachment/adhesion sites, (3) unfavorable alteration of the environment, and (4) combinations of these.