Chapter 1
Microbes of Food and Factors Affecting Microbial Growth in Food

A food microflora mainly depends on microbial type, characteristics of a food type, contamination, processing and storage conditions. The microbial groups associated with foods are bacteria, fungi, protozoa, algae and viruses. Most foods contain sufficient nutrients to support microbial growth. Several factors encourage, prevent, or limit the growth of microorganisms in foods, the most important are aw, pH and temperature. aw: (Water Activity or Water Availability).

Types of Microorganism Associated with Food

Sources of Microorganisms in foods

The primary sources of microorganisms in the food are

I. Soil microbial flora

The soil contains the greatest variety of microorganisms of any source of contamination. The fertile soil contains large number of microorganisms which are contaminating the surfaces of plants growing on it and animals roaming over the land. Soil dust whipped by air current and soil particles are carried by running water to get into or on to foods.

The soil is important source of heat resistant spore forming bacteria. The most important types of organisms contaminating through soil are *Bacillus*, *Clostridium*, *E. coli*, *Enterobacter*, *Flavobacterium*, *Pseudomonas*, *Proteus*, *Leuconostoc*, *Acetobacter*, *Chromobacterium* etc.

II. Waterborne microorganisms

Water is an important source of microorganisms especially Coliforms which are indicator organisms for fecal contamination. Natural water contains the natural flora as well as the microorganisms from soil, animals and sewage. *Proteus*, *Micrococcus*, *Streptococcus*, *Pseudomonas*, *Bacillus, E. coli*, *Enterobacter* and *Chromobacterium* are the important kinds

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of bacteria found in natural water. Coliforms are called the common waterborne bacteria. These are gram-negative, non-sporulating rods indicate water contamination. Hence they are called indicator organisms.

III. Microorganisms in Air

Air is another source of contamination in food. Because it contributes dust, droplets, droplet nuclei, aerosols and suspended particle. Disease organisms especially those causing respiratory infections may spread by air. The microorganisms in the air will not grow because of the lack of nutrients but they will suspend in air for very long time. Fungal spores and bacterial spores are predominant in air, mold spores are more resistant to dry and persisting for very long time. Among bacteria cocci are predominant than rods. Yeasts are also present in air. The number of microorganisms in the air may be depending on so many factors such as sunshine, location and the amount of suspended dust or spray. Dry air contents more organisms than moist air. Rain or snow removes the organisms from the air.

IV. Microorganisms in vegetation

The natural surface flora of plants varies with the plant but generally include the species of *Bacillus*, *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Coliforms*, *Micrococcus* and Lactic acid bacteria. The number of bacteria will depends on the plant and its environment and may range from a few hundred or thousand per square centimetre of surface to million. Exposed surface of plants become contaminated from soil, water, sewage, air and animal so that microorganisms from these sources are added to the natural flora. Plants are associated with the bacterial plant pathogens such as *Corynebacterium*, *Curtobacterium, Pectobacterium, Pseudomonas* and *Xanthomonas* and fungal pathogens among several genera of molds.

V. Microorganisms from Animal

The source of microorganisms from animals includes the surface flora, the flora of respiratory tract and the flora of the gastrointestinal tract. Hides, hooves and hair of animal contain large number of microorganisms from soil, manure feed and water. Similarly feathers and feet of poultry carry heavy contamination from soil and other sources. The skin of many meat animals contain *Micrococcus*, *Staphylococcus* and β- hemolytic *Streptococcus*. The feces and fecal contaminated products of animals can contain many enteric pathogens. Animal contributes their wastes and finally their bodies to the soil and water which contain large group of microbial flora. So many pathogenic organisms are present in animal and animal products including *Brucella* sps, *Mycobacterium tuberculosis, Coxiella burnetii, Salmonella typhi* and *paratyphi*, *Listeria monocytogens*, *Campylobacter jejuni*, enteropathogenic *E. coli, Clostridium perfringens* and *Clostridium botulinum*.

VI. Microorganisms from food handlers and food equipment

The microbial flora on the hands and outer garments of handlers generally reflect the environment and habits of individuals and the organisms may be those from soil, water, dust and other environmental sources, additional important sources are those that are common in nasal cavities, the mouth and on the skin and those from the gastrointestinal tract that may enter food through poor personal hygiene practices. When vegetables are harvested in containers and utensils, it is expected to find some of the surface organisms on the products to contaminate contact surfaces.

Common bacteria present in food

The bacteria which are important in food biotechnology are

- *Acetobacter*

  They are gram-negative bacteria oxidize ethanol to acetic acid. They are rod shaped motile bacteria found on fruits and vegetables.

- *Aeromonas*

  They are gram-negative, facultative anaerobic, psychrophilic rods and which are commonly found in fish, frog and other mammals.

- *Alcaligenes*

  They are gram-negative rod shaped bacteria present in feeds, soil, water and dust. The important species are *Alcaligenes viscolactis*, which produces ropiness in milk and *Alcaligenes metalcaligenes* gives a slimy growth on cottage cheese.

- *Alteromonas*

  These organisms are gram-negative aerobic rods. The important species is *Alteromonas putrefaciens*.

- *Arthrobacter*

  It is a gram-positive bacterium predominant in soil.

- *Bacillus*

  It is a gram-positive spore forming aerobic or facultative anaerobic organisms. The important species are *Bacillus subtilis* and *Bacillus stearotheromphilus* (these are hyper thermophilic and spore producing bacteria).

- *Brevibacterium*

  It is also a gram-positive bacteria produces orange red pigment and helps ripening.

- *Brothrothrix*

  These are gram-positive non spore forming bacteria found in many food items.

- *Campylobacter*

  It is gram-negative, spiral shaped rods. The species *Campylobacter jejuni* is associated with gastroenteritis in human.

- *Clostridium*

  There are gram-positive spore forming rods. There are obligate (strict) anaerobes. Many species are capable of fermenting carbohydrate and produce acids and gases. *Clostridium botulinum* (causes botulism) and *Clostridium perfringens* (causes gas of gangrene and food poisoning) are the most important species in food biotechnology.

- *Corynebacterium*

  There are gram-positive, rod shaped bacteria that are sometimes involved in the spoilage of vegetables and meat products. The important species, *Corynebacterium diphtheriae* causes diphtheria in humans.

- *Citrobacter*

  These enteric bacteria are slow lactose-fermenting, gram-negative rods that typically produce yellow colonies.

- *Desulfotomaculum*

  It is a gram-negative rod and inhabitants of the soil, fresh water and the rumen. It is a sulphur oxidizing bacteria.

- *Escherichia*

  It is a gram-negative, non-sporulating, motile and facultative anaerobic bacteria commonly referred as coliform and which are indicator organisms.

- *Enterobacter*

  These are enteric gram-negative coliform bacteria like *E. coli*.

- *Erwinia*

  These are gram-negative enteric rods especially associated with plants. *Erwinia carotovora* is the most important organism responsible for spoilage of food.

- *Flavobacterium*

  These are gram-negative rods characterized by their production of yellow to red pigments on agar and by their association with plants. Some are mesotrophs and others are psychrotrophs, where they participate in the spoilage of refrigerated meats and vegetables.

- *Gluconobacter*

  These are gram-negative rod shaped bacterium which can oxidize ethanol to acetic acid and can cause ropiness in beer.

- *Hafnia*

  These are gram-negative enteric rods important in the spoilage of refrigerated meat and vegetable products.

- *Halobacterium*

  These are obligate halophiles and causes discoloration on the foods high in salt such as salted fish.

- *Klebsiella*

  These are gram-negative non-sporulating, non-motile and facultative anaerobic bacteria commonly referred as coilforms and which are indicator organisms. *Klebsiella pneumoniae* is the causative organism for bacterial pneumonia in human.

- *Lactobacillus*

  These are gram-positive, rod shaped, microaerophilic bacteria typically occur on most vegetables, along with some of the other lactic acid bacteria. Their occurrence in dairy products is common. It can ferment the carbohydrate lactose and can produce acids and gas. The important species are *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii* and *Lactobacillus pentosus*.

- *Leuconostoc*

  They are gram-positive bacterium. The most important species is *Leuconostoc mesenteroides*.

- *Listeria*

  They are gram-positive and non-sporing rods. The most important organism is *Listeria monocytogens* which causes the spoilage of fish and fish products.

- *Micrococcus*

  These are gram-positive cocci and are inhabitants of mammalian skin and can grow in the presence of high level of salt.

- *Moraxella*

  These are short gram-negative rods they do not form acid from glucose. The most important species is *Moraxella bovis*.

- *Mycobacterium*

  These are gram-positive rods. The most important species are *Mycobacterium tuberculosis* (causative agent of tuberculosis), *Mycobacterium leprae* (causative agent of leprosy) and *Mycobacterium bovis*.

- *Pediococcus*

  These are gram-positive homofermentative cocci. These are lactose fermenting bacteria.

- *Proteus*

  This enteric gram-negative rods are aerobes that often display pleomorphism. These organisms are motile and typically produce swarming growth on the surface of moist ager plates. They are typical of enteric bacteria. One important species is *Proteus vulgaris*.

- *Pseudomonas*

  These are gram-negative rod shaped motile bacteria. They are typical soil and water bacteria and they are widely distributed among fresh food especially vegetables, meats, poultry and sea food products. These are one of the largest foodborne bacteria.

- *Photobacterium*

  These are Coccobacilli and can cause phosphorescence of meat and fish.

- *Propionibacterium*

  These are gram-positive, small, non motile rod shaped bacteria can ferment carbohydrates. These bacteria are commonly present is cheese and cheese related products.

- *Salmonella*

  These are gram-negative enteric bacteria. They are considered to be human pathogens. The most important pathogen is *Salmonella typhi* and *Salmonella paratyphi*. These organism cause enteric fever.

- *Serratia*

  These are gram-negative rods that belong to the family Enterobacteriaceae are aerobic, proteolytic and produce red pigments on culture media and in certain food. *Serratia liquefaciens* and *Serratia marcescens* are the most prevalent of the foodborne species. It causes spoilage of refrigerated vegetables and meat products.

- *Shigella*

  These are gram-negative, non-sporulating motive and facultative anaerobic bacteria and can cause bacillary dysentery in humans. *Shigella dysenteriae* is the causative organism other *Shigella* species are *Shigella flexneri*, *Shigella sonnei* and *Shigella boydii*.

- *Staphylococcus*

  These are gram-positive cocci occur in the form of grape like clusters and which include *Staphylococcus aureus*, causes several diseases in human including foodborne gastroenteritis.

- *Streptococcus*

  These are gram-positive cocci which occur in the form of long or short chains. It will cause pyogenic infection in human (*Streptococcus pyogens*).

- *Vibrio*

  These are gram-negative straight or curved rods. The most important organism is *Vibrio cholerae* which causes cholera disease.

- *Yesinia*

  These are gram-negative rods and include the causative agent of human plague, *Yersinia pestis*. Some species are causing foodborne gastroenteritis.

Molds and Yeast in Food

Fungi are eukaryotic spore producing organisms capable of reproducing both sexual and asexual manner. Fungi are commonly classified into two: unicellular fungi-yeast and multicellular fungi-mold. Fungi belong to plant Kingdom–Myceteae. These are multicellular, nonphotosynthetic organism with chemoheterotrophic mode of nutrition. Fungi lacks roots, stem or and devoid of chlorophyll.

Molds regarded as eumycetes or true fungi. Molds are filamentous fungi that grow in the form of a tangled mass that spreads rapidly and may cover several inches of area in 2-3 days. The total of the mass or any large portion of it is referred to as mycelium. Mycelium is composed of branches or filaments referred to as hyphae. Groups of hyphae are referred to as mycelium. Fungi are greatest importance in food and it multiply by ascospores, zygospores and conidiospores.

Major Molds Present in Food

Rhizopus, *Mucor*, *Aspergillus*, *Penicillium*, *Neurospora*, *Eupenicillium*, *Eurotium*, *Fusarium*, *Alternaria*, *Geortrichum*, *Helminthosporium*, *Trichothecium*, *Cephalosporium*, *Aureobasidium* (*Pullularia*) and *Botrytis* etc.

Common Foodborne Yeasts

Yeasts are unicellular fungus. It can be differentiated from bacteria by their larger size, oval, elongated-spherical cell shape. Yeasts are classified into two:

1. True yeast - Ascomycotina
2. False Yeast - Deuteromycetes or fungi imperfecti

Major Foodborne Yeasts

True Yeast

This type of yeast produces ascospores and arthrospores. Vegetative reproduction of such types of yeast is taking place by fission and budding.

eg:- *Pichia*, *Saccharomyces – S. cerevisiae*, *Debaryomyces*, *Torulospora – T. delbrueckii* and *Zygosaccharomyces*

False Yeast

Reproduction of false yeast is by budding. These are fungi imperfecti because their sexual stages of cell division are not yet identified.

eg:- *Candida – C. albicans*, *C. lipolytica* and *C. guilliermondii*

Rhodotorula (*R. mucilaginosa* - pigment producing yeast) and *R. glutinis* and *Trichospora*

Microbes of curd

Curd has lactic acid bacteria or *Lactobacillus*. This bacteria multiplies itself in the ambient temperature of 30-40^{0}C and in few hours ferments the milk to form curd. Curd is a rich source of calcium and protein and is suitable for lactose intolerant people. Curd is a probiotic, it makes the gut healthy. Curd contains a number of bacteria like *Lactococcus lactis*, *Lactococcus lactis cremoris*, *L. acidophilus* etc. Therefore, the exact knowledge about the different strains that exist in curd is lacking. A report was published in the European Journal of Clinical Nutrition that suggests that the type of needful bacteria present in curd vary considerably between places. It was also demonstrated that the Indians curd contains nearly 250 different strains of *Lactobacillus*.

Microbes of wheat

Wheat is naturally exposed to microbial organisms as it grows in the field and as it is stored and transported. The microbial content of wheat flour is lower than that of wheat itself as microorganisms reside on the outer bran layers of the wheat grain which are removed during the milling process.

Bacteria, fungi and actinomycetes were found in most wheat samples. Organisms common to both wheat and flour includes Psychrotrophic bacteria, fecal *Streptococci*, catalase negative bacteria, aerobic thermophilic spore-forming bacteria, Flat sour bacteria, *Aureobasidium pullulans* and *Streptomyces albus* survived the milling process.

Microbiology of Cereals and Cereal products

Cereals and cereal products are significant and important human food resources and livestock feeds worldwide. The main cereal grains used for foods include corn (maize), wheat, barley, rice, oats, rye, millet and sorghum. Because of their extensive use as human foods and livestock feeds, the microbiology and safety of cereal grains and cereal products is a very important area. The source of microbial contamination of cereals are many, but all traceable to the environment in which grains are grown, handled and processed. Microorganisms that contaminate cereal grains may come from air, dust, soil, water, insects, rodents, birds, animals, humans and processing equipment. Many factors that are a part of the environment influence microbial contamination of cereals including rainfall, drought, humidity, temperature, sunlight, frost, soil conditions, wind, insect, bird and rodent activity, harvesting equipment, use of chemicals in production versus organic production, storage and handling and moisture control.

The microflora of cereals and cereal products are varied and includes molds, yeasts, bacteria (Psychrotrophic, mesophilic, thermophilic, thermoduric), lactic acid bacteria, rope-forming bacteria (*Bacillus* spp.), bacterial pathogens, coliforms and *Enterococci*. Bacterial pathogens that contaminate cereal grains and cereal products and cause problems include *Bacillus cereus*, *Clostridium botulinum*, *C. perfringens*, *E. coli*, *Salmonella* and *Staphylococcus aureus*. Coliforms and *enterococci* also occur as indicators of unsanitary handling and processing conditions and possible fecal contamination.

Bacteria are frequent surface contaminants of cereal grains. For the bacteria to grow in cereal grains, they require high moisture or water activity (a_w) in equilibrium, with high relative humidity. Generally, bacteria are not significantly involved in the spoilage of dry grain and become a spoilage factor only after extensive deterioration of the grain has occurred and high moisture conditions exist. Lactic bacteria may also be present in the raw grain and carry over into flour and cornmeal and spoil dough prepared with them. Yeasts present on cereal grains may also carry through into processed products. The main spoilage organisms in cereal grains, however are molds.

There are more than 150 species of filamentous fungi and yeasts on cereal grain. The filamentous fungi that occur on cereal grains are divided into two groups, depending on when they predominate in grain in relation to available moisture in the grain. These groups have been referred to as field fungi and storage fungi. Field fungi invade grain in the field when the grain is high in moisture (18 to 30% i.e, at high a_w)

and at high relative humidities (90 to 100%). Field fungi include species of *Alternaria*, *Cladosporium*, *Fusarium* and *Helminthosporium*. Storage fungi invade grain in storage at lower moisture contents (14 to 16%), lower a_w and lower relative humidities (65 to 90%). These main storage fungi are species of *Eurotium*, *Aspergillus* and *Penicillium.*

Microbes of Meat

The growth of microbes in meat is governed by a number of intrinsic and extrinsic factors. Intrinsic properties of meat such as pH and moisture can promote microbial growth, whereas temperature is an extrinsic factor. Fresh meat has a high water content that is favorable for the growth of microorganisms. It also generally contains bacteria, including those that can cause diseases. The animals naturally carry bacterial species like *Salmonella* and *E. coli* in their intestines and raw meat can become contaminated during the slaughter process. Equipments and tools used in the processing of meat can also become contaminated with microbes and spread those to the raw meat. Raw meat should be cooked thoroughly before consumption.

Beef

The most common pathogenic bacteria found in beef is *E. coli*. The *E. coli* strain 0157:47 is a rare, dangerous bacterium that can cause severe damage to the intestinal lining. *Salmonella*, *Staphylococcus aureus* and *Listeria monocytogenes* are also common contaminants in beef. All these organisms can be destroyed by cooking.

Pork

In Pork, *E. coli*, *Salmonella*, *S. aureus* and *Yersinia enterocolitica* are the most common bacterial contaminants. Chitterlings (intestine of pig) can be contaminated with *Y. enterocolitica* leading to a diarrhoeal illness known as Yersiniosis. Microbial contaminants in pork can be destroyed by cooking to an internal temperature of 145^0F.

Chicken

Chicken is often contaminated with *Salmonella enteritidis*, *S. aureus*, *Campylobacter jejuni*, *L. monocytogenes* and *E. coli* can also be found in chicken. Chicken should be cooked to an internal temperature of 165^0F to kill the microbes.

Bacteria in cooked meat

Thorough cooking can generally destroy most bacteria on raw meat, including pathogenic ones. Nevertheless, if there are subsequent lapses in food safety practices, food poisoning may still occur. Raw meat may be contaminated with spores of certain pathogenic bacteria (eg:- *Clostridium perfringens*) and spores are not readily destroyed by normal cooking temperature. Heat of cooking can rather activate the spores to germinate and develop into vegetative cells which can multiply rapidly in foods that are placed at ambient temperature for a long period. Consuming foods that contain high levels of *Clostridium perfringens* vegetative cells may lead to foodborne illness.

In addition, pathogenic bacteria may be introduced into the ready-to eat cooked meat through cross-contamination and multiply to larger amount as a result of time and temperature abuse of the food, causing foodborne illness in consumers.

Microbes of fish

The population of microorganisms associated with living fish reflects the microflora of the environment at the time of capture of harvest, but is modified by the ability of different microorganisms (mainly bacteria) to multiply in the sub-environments provided by the skin/shell surfaces, gill areas and the alimentary canal. Shell fish taken from water near human habitations will tend to have higher bacterial loads and a more diverse microflora compared with those taken from isolated areas. The muscle tissue and internal organs of freshly caught, healthy finish and molluscan shellfish are normally sterile, but bacteria may be found on the skin, chitinous shell, gills of fish, as well as in their intestinal tract. The circulatory system of some crustaceans is not "closed" and the hemolymph of crabs can harbor substantial levels of bacteria, particularly members of the genus *Vibrio*. Microbial levels vary depending on water conditions and temperature.

Typically, bacteria from skin and gills are predominantly aerobic although facultative bacteria, particularly *Vibrio* spp., may occur in high numbers on pelagic fish. Obligately anaerobic bacteria are uncommon on the surface of fish but can occur in significant numbers in the intestine. Lactic acid bacteria in particular *Carnobacteria* are also commonly isolated from fish gut.

The bacteria on finfish and shellfish are predominantly gram-negative for fish from temperate waters. A higher proportion of gram-positive cocci and bacillus spp. can be found on some fish from warm, tropical water and some studies report as much as 50-60% of the microflora being of these types. However, the microflora of fish from warm, tropical water may also be dominated by gram-negative bacteria. The microflora of living fish from temperature water is remarkably consistent and commonly includes members of the genera *Psychrobacter*, *Moraxella*, *Pseudomonas*, *Acinetobacter*, *Shewanella* (previously *Alteromonas*), *Flavobacterium*, *Cytophaga*, *Vibrio*, *Aeromonas*, *Corynebacterium* and *Micrococcus*.

In addition to bacteria, yeast such as *Rhodotorula*, *Torulopsis*, *Candida* spp. and occasionally fungi are reported from finish and shellfish.

Microbes of Egg

Freshly laid eggs are generally sterile particularly the inner contents. However the shells get contaminated from the environmental sources such as fecal matter of the bird, beddings, by the handlers and wash water and also the packaging materials in which the eggs are packed. There are several extrinsic and intrinsic mechanisms through which the egg protects itself from the microbial invasion. Waxy shell membrane retards the entry of microorganisms. Further, the shell also prevents the entry of microorganisms. The membranes inside the shell behave as mechanical barriers to the entry of microorganisms. Further lysozymes present in the egg white is effective against gram-positive bacteria and avidin in the egg white forms a complex with biotin, thus making it unavailable for the microorganisms. Also high pH (pH 9-10) of albumin inhibits the microbial growth. Binding of riboflavin by the apo protein and chelation of iron by conalbumin further helps in hindering the growth of microorganisms that might have gained entry inside the egg.

Microflora in Rice Soils

Flooding of rice soils provides a favorable environment for anaerobic microbes and the biochemical changes are varied and numerous. However, a thin surface layer of lowland soil generally remains oxidized and sustains aerobic microbes. The main biochemical processes in flooded soil, however, can be regarded as a series of successive oxidation-reduction reactions mediated by different types of bacteria. Three major types of microbes are present in lowland rice soils in variable proportion.

- Obligate aerobes that grow only in the presence of molecular oxygen
- Obligate anaerobes that grow only in the absence of oxygen
- Facultative anaerobes that can grow either with molecular oxygen or an aerobically when supplied with a suitable electron acceptor other than molecular oxygen

Results of studies in Japan, India, Egypt and the Philippines suggest that bacteria predominate in flooded soils, whereas fungi and actinomycetes are more abundant in upland soils. Bacteria such as *Mycobacteria*, *Bacillus*, *Pseudomonas* and other biologically active bacteria are present in greater numbers in the rhizosphere than in the soil farther away. Some aerobic microbes including fungi, nematodes, yeast and protozoa have occasionally been found inside the root tissue of the rice plant. Flooding causes changes in the character of the microbial flora in soils.

There are many kinds of nitrogen fixing microbes in lowland rice soils. A study in Thailand identified *Azotobacter* (0-10^4/g soil), *Beijerinckia* (0-10/g soil), *Clostridia* (10^4 – 10^6/g soil), non sulphur purple bacteria (10^2-10^6/g soil) and blue green algae (10^2-10^3/g soil). The population density of these nitrogen fixers depends primarily on specific soil properties such as pH, organic matter content and available phosphorus.

Factors affecting microbial growth in food

Intrinsic factors

These are inherent in the food. They include pH, moisture content and nutrient content of the food, antimicrobial substances and biological structures.

pH (Hydrogen ion concentration)

The most of the bacteria grow best at neutral or weakly alkaline pH usually between 6.8 & 7.5. Some bacteria can grow within a narrow pH range of 4.5 and 9.0 eg: *Salmonella*. Other microorganisms especially yeast and molds and some bacteria grow within a wide pH range, eg: molds grow between 1.5 to 11.0, while yeasts grow between 1.5 and 8.5.

The microorganisms that are able to grow in acid environment are called acidophilic microorganisms. These microorganisms are able to grow at pH of around 2.0. Yeasts and molds grow under acid conditions. Other microorganisms such as *Vibrio cholerae* are sensitive to acids and prefer alkaline conditions. Most bacteria are killed in strong acid or strong alkaline environment except *Mycobacteria*.

The minimum and maximum pH for growth of some specific microorganisms are given below

Microorganisms	Minimum	Maximum
E. coli	4.4	9.0
Salmonella enterica serovar Typhi	4.5	8.8
All bacteria	4.0	9.0
Molds	1.5	11.0
Yeast	1.5	8.5

The pH values of some food products are given below

Food type	**Range of pH values**
Beef	5.1-6.2
Chicken	6.2-6.4
Milk	6.3-6.8
Cheese	4.9-5.9
Fish	6.6-6.8

Fruits	<4.5 (most <3.5)
Vegetables	3.0-6.1

Moisture Content

Water activity (a_w) is a term describing the availability of water to microorganisms. It is only roughly related to percent moisture. Pure water has an a_w of 1.00.

The water activity of some food products are given below

Food product	**a_w**
Raw meat and milk	0.99-1.0
Luncheon meat	0.95
Boiled ham, sliced bacon	0.90
Dried grains	0.80

The water activity limits for growth of principal food borne disease organisms are given below

Microorganisms	**Minimum a_w for growth**	**Reference**
Salmonella	0.945	Christian and Scott, 1953
C. botulinum	0.95	Scott, 1957
C. perfringens	0.93	Kang *et al.*,1969
Vibrio parahaemolyticus	0.94	Benchat, 1974

Certain molds and bacteria can grow on fish immersed in saturated salt solution where the a_w is about 0.75. Some molds can grow in foods with a_w 0.62 -0.65. At these lower limits, growths are very slow. The a_w of fully dried foods, such as crackers or sugar, is about 0.10 and such products are microbiologically stable because of this factor alone. The stability of intermediate moisture foods (a_w 0.75-0.90), such as dried fruits, jams and soft moist pet foods depends on combinations of factors, such as low a_w, low pH, pasteurization, chemical additives and impervious packaging.

Oxygen

The oxygen is essential for growth of some microorganism, these are called aerobes. Others can't grow in its presence and are called anaerobes. Still others can grow either with or without oxygen and are called microaerophilic. Strict aerobes grow only on food surfaces and can't grow in foods stored in cans or in other evacuated, hermetically sealed containers. Anaerobes grow only beneath the surface of foods or inside containers. Aerobic growth is faster than anaerobic. Therefore, in products where both conditions exist, such as in fresh meat, the surface growth is promptly evident, whereas sub surface growth is not.

Lethal effects of Temperature

Heat is the most practical and effective means to destroy microorganisms. Microbial cell reduction occurs slowly just above maximal growth temperatures. However, the rate of death increases markedly as the temperature is raised. Pasteurization, the destruction of vegetative cells of disease producing microorganisms, consists of a temperature of 140^0F for 30 minutes, or about 161^0F for 16 seconds. Yeasts, molds and the vegetative cells of spoilage bacteria also die at pasteurization temperatures. To render long-acid foods commercially sterile requires a retort capable of operating at temperatures above 212^0F. The rate of thermal destruction is greater in foods with high a_w than in those with low a_w. Microbial contaminants in dry foods, such as chocolate or dried bone meal are hard to destroy with heat.

Chilling to temperature below the growth range, but above freezing, stops reproduction but kills few cells except for extremely sensitive organisms, such as vegetative cells of *C. perfringens*. Freeze kills part of a microbial population within a few hours and storage continues to be lethal at a much slower rate. The most rapid drop in aerobic plate count (total count) occurred in orange juice, which is an acid product. Bacterial spores die very slowly, if at all, during freezing and frozen storage. For example, the vegetative cells of *C. perfringens* generally all die, but the spores survive. *S. aureus* and related organisms survive well, but in most cases, there is wide variation of susceptibility among microorganisms, even among closely related species. In any case freezing is not a dependable means to destroy microorganisms since some cells of the original population almost always survive.

Some psychrotrophic microorganisms grow very slowly in foods below freezing, but usually not below 19^0F. There are a few reports of growth, usually of molds, at 14^0F, but no reliable reports of growth below that temperature. This means that the standard storage temperature for frozen foods, 0^0F does not permit microbial growth. However many microorganisms survive freezing. Most psychrotrophs have difficulty growing above 90^0F. Most foodborne disease organisms are mesophiles.

In the temperature range where both mesophilic and psychrotrophic organisms grow (about 41^0F to 90^0F), the psychrotrophs grow more rapidly causing spoilage and at the same time frequently interfering with the growth of foodborne disease organisms.

Nutrient content of the food

Microorganisms require proteins, carbohydrates, lipids, water, energy, nitrogen, sulphur, phosphorus, vitamins and minerals for growth. Various foods have specific nutrients that help in microbial growth. Foods such as milk, meat and eggs contain a number of nutrients that are required by microorganisms. These food are hence susceptible to microbial spoilage.

Antimicrobial substances

Antimicrobial substances in food inhibit microbial growth. Various foods have inherent antimicrobial substances that prevent microbial attack. Such inhibitors are like lactinin and anticoliform factors in milk and lysozyme in eggs.

Biological Structures

Some foods have biological structure that prevents microbial entry. For example, meat has fascia, skin and other membranes that prevent microbial entry. Eggs have shell and inner membranes that prevent yolk and egg white from infection.

Extrinsic factors

These are factors external to the food that affects microbial growth. They include

- Temperature of storage
- Presence and concentration of gases in the environment
- Relative humidity of food storage environment

Temperature

Temperature is the most efficient means to control microbial growth. The growth of microorganisms are affected by the environmental temperature. Various microorganisms are able to grow at certain temperature and not others. Bacteria can therefore be divided into the following groups depending upon their optimum temperature of growth.

Psychrophilic microorganism

These grow best in the temperature range of 0-15^{0}C but also down to -10^{0}C in unfrozen media. They can cause food spoilage at low temperature. Several of the microorganism found in the soil and water belong to this group. Whereas psychrotrophs thrive between 4^{0}C and 25^{0}C.

Mesophilic bacteria

These organisms grow between 25^{0}C and 40^{0}C, with an optimum growth temperature close to 37^{0}C. Some such as *Pseudomonas aeruginosa* may grow at even lower temperature between 5-43^{0}C. None of the mesophilic bacteria are able to grow below 5^{0}C or above 45^{0}C. Most pathogenic bacteria belong to this group.

Thermophilic bacteria

These grow at temperature above 45^{0}C. Often their optimum growth temperature is between 50^{0}C and 70^{0}C. Growth of some bacteria occurs at 80^{0}C. Bacteria in this group are mainly spore formers and are of importance in the food industry especially in processed foods.

Presence and Concentration of gases in the environment

This relates to the presence and concentration of gases in the food environment. Various microorganisms require for growth either high oxygen tension (aerobic), low oxygen tension (microaerobic) or absence of oxygen (anaerobic). Some microorganisms may grow either in high oxygen tension, or in the absence of oxygen (facultative anaerobes).

Foods affected by various groups

- Anaerobic or facultatively anaerobic spore formers are most likely to grow in canned foods.
- Microaerophilic bacteria are most likely to grow in vacuum packed foods since they have low oxygen tension.
- Aerobic bacteria are likely to grow on the surface of raw meat
- Aerobic molds will grow in insufficiently dried or salted products

Relative humidity

Relative humidity is the amount of moisture in the atmosphere or food environment. Foods with low water activity placed at high humidity environment take up water, increase their water activity and gets spoiled easily. For example, dry grains stored in an environment with high humidity will take up water and undergo mold spoilage.

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