Factors influencing microbial growth in food

By- Dr Ekta Khare

Department of Microbiology,

Chhatrapati Shahu Ji Maharaj University, Kanpur

Factors influencing microbial growth in food

- The most important factors that influence microbial growth in foods can be summarized in the following categories:
- **Intrinsic factors** - factors related to the food itself
- **Extrinsic factors** - factors related to the environment in which the food is stored
- **Implicit factors** - factors related to the microorganisms themselves,
- **Processing factors**,
- Interactions between factors, as mentioned above, can also affect the growth of microbes in foods in a complicated way; the combined effects of these interactions may be additive or synergistic.

Table 1 Factors influencing the development of microbes in food

Intrinsic factors	Extrinsic factors	Implicit factors	Process factors
Nutrient content	Relative humidity	Metabiotic	Physical treatments
pH	Storage temperature	Symbiotic	Use of chemicals
Oxidation–reduction potential	Gaseous atmosphere	Commensalism	Contamination
Water activity	Presence of other microorganisms	Antagonism	
Biological structure		Predation	
Antimicrobial content		Competition	

Intrinsic Factor: pH

- In general, molds and yeasts can grow at lower pH compared to bacteria and Gram-negative bacteria are more sensitive to low pH than Gram-positive bacteria.
    - The pH range of growth for molds is 1.5 to 9.0;
    - for yeasts 2.0 to 8.5;
    - for Gram-positive bacteria 4.0 to 8.5; and
    - for Gram-negative bacteria 4.5 to 9.0.
- According to the pH values of the foods, they are generally divided into four groups:
- **Group 1: low-acid foods** with a pH of more than 5.3 include corn, meat, fish, and milk. All types of microorganisms can grow on them, especially bacteria.
- **Group 2: medium-acid foods** with a pH range of 5.3–4.5 include bananas, yogurt, and pumpkin. All types of microorganisms can grow on them.
- **Group 3: acid foods with a pH range** of 4.5–3.7 include tomatoes, orange juice, sugar beet, and grapes; they are susceptible to the growth of yeasts and molds, and a few types of bacteria. Most pathogenic bacteria do not grow on these foods.
- **Group 4: high-acid foods** with a pH below 3.7 include apples, grapefruit juice, and limes; they are susceptible to the growth of yeasts and molds. Most bacteria, particularly the pathogenic ones, do not grow on such foods.

Table 3 Approximate pH values for the Growth of Selected Microbes in Food

Products	pH
Vegetables	
Eggplant	4.5
Potatoes (tubers and sweet)	5.3 to 5.6
Tomatoes (whole)	4.2 to 4.3
Cabbage	5.2-6.3
Spinach	5.1-6.S
Brussels sprouts	6.3-6.6
Beets	4.9-5.S
Asparagus	5.0-6.1
Carrots	4.9 to 6.3
Fruits	
Watermelons	5.2 to 5.6
Bananas	4.5 to 4.7
Limes	1.8 to 2.0
Grapes	3.3-4.5
Cherries	3.2-4.7
Pineapple	3.2-4.1
Strawberries	3.0-4.2
Raspberries	2.9-3.7
Apples	2.9-3.5
Oranges	2.8-4.0
Dairy products	
Milk	6.3 to 6.5
Butter	6.1 to 6.4
Camembert cheese	6.1-7.0
Cottage	4.1-5.4
Gouda	4.7
Meat	
Chicken	6.2 to 6.4
Beef	5.1 to 6.2
Turkey	5.6 - 6.0
Pork	5.3 - 6.4
Dry sausages	4.4 - 5.6
Sea food	
Shrimp	6.8 - 8.2
Crab	6.8 - 8.0
Catfish	6.6 - 7.0

Nutrient Content

- Some basic nutrients are required by microorganisms for the growth and maintenance of metabolic functions.
- These nutrients include water, a source of energy, nitrogen, vitamins, and minerals.
- Foodborne microorganisms can procure energy from carbohydrates, alcohols, and amino acids.
- Phosphorus, iron, magnesium, sulfur, manganese, calcium, and potassium are some examples of minerals required for microbial growth.
- Many bacterial species, especially Gram-negative rods such as Pseudomonas, Acinetobacter, Moraxella, Shewanella, and Aeromonas, as well as pathogenic spore formers like Clostridium botulinum, are proteolytic and can grow well in protein-rich foods and spoil them rapidly.
- Foods rich in carbohydrates can be spoiled by carbohydrate-fermenting microorganisms, particularly by yeasts and molds.
- Bacterial species of the genera Bacillus, Clostridium, Aeromonas, Pseudomonas, Leuconostoc and Enterobacter are saccharolytic and can also attack carbohydrates.
- Gram-positive bacteria are the least synthetic and must, therefore, be supplied with one or more of B vitamins in food before they will grow.

Table 5 Groups of Microorganism based on the Way they Satisfy their Energy Requirements

Sources	Description
Carbon Sources	
Autotrophs	CO_2 sole or principal biosynthetic carbon source
Heterotrophs	Reduced, preformed, organic molecules from other organisms
Energy Sources	
Phototrophs	Use light as their energy source
Chemotrophs	Use energy from the oxidation of chemical compounds (either organic or inorganic)
Electron Sources	
Lithotrophs	Uses reduced inorganic molecules as their electron source
Organotrophs	Extract electrons from organic molecules
photoautotrophs /photolithoautotrophs	Utilize light energy and have CO2 as their carbon source

Moisture Content

- Microorganisms require water in an available form for their growth in food products.
- The water requirements of microorganisms are described in terms of the water activity (aw) of the food or environment.
- According to Chichester et al., 1963, water activity can be defined as the ratio of the water vapor pressure of the food to the vapor pressure of pure water at the same temperature.
- Mathematically, it can be written as

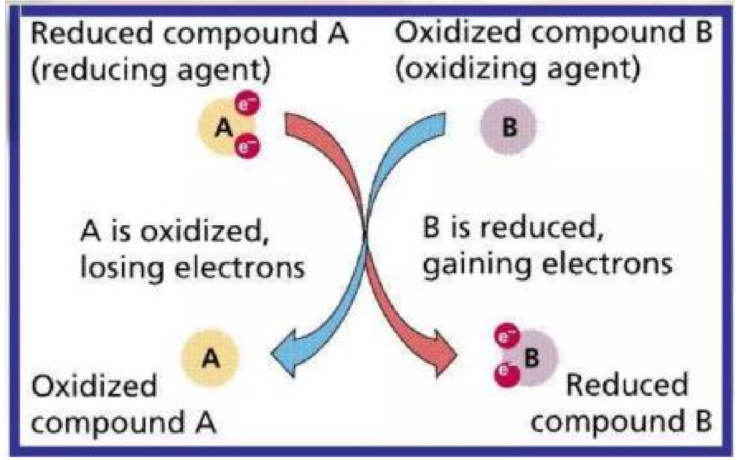
  $$aw = P/P°$$

- Where, P denotes the Vapour pressure of the food;
- P° denotes the Vapour pressure of pure water.
- The water activity of pure water is 1.00, and that of completely dehydrated food is 0.00.
- The food is classified into three categories based on it’s aw range:
  - High-moisture foods (aw above 0.85);
  - Intermediate-moisture foods, (aw 0.60–0.85);
  - Low-moisture foods (aw below 0.60).
- High-moisture foods with water activity above 0.85 are highly perishable foods as they are susceptible to the growth of spoilage and pathogenic microorganisms, especially the fast-growing bacteria.
- Microbial spoilage of intermediatemoisture foods is a relatively slow process, caused mainly by yeasts and molds. Low moisture foods are not spoiled by microorganisms unless their moisture content is raised by any means.

Table 7 Minimum aw Range Required for t Growth of Various Foodborne Microbes

Organism	a_w
Groups	
Most spoilage bacteria	0.90
Most spoilage yeasts	0.88
Most spoilage molds	0.80
Halophilic bacteria	0.75
Xerophillic molds	0.61
Osmophillic yeasts	0.61
Escherichia coli	0.96
Clostridium botulinum, type E	0.97
Pseudomonas spp.	0.97
Candida utilis	0.94
Rhizopus stolonifer	0.93
Enterobacter aerogenes	0.95
Enterobacter aerogenes	0.95
Staphylococcus aureus	0.86
Bacillus	0.90- 0.99
Citrobacter	0.95-0.98
C. perfringens	0.93-0.97
Lactobacillus	0.90-0.96
Leuconostoc	0.96-0.98
Aspergillus	0.68-0.88
A. niger	0.80-0.84
Mucor	0.80-0.93
Micrococcus	0.90-0.95
Fusarium	0.80-0.92
B. cereus	0.92-0.95
Salmonella	0.93-0.96

Oxidation-Reduction Potential



- The oxidation-reduction or redox potential of a substance is defined as the ratio of the total oxidizing (electron accepting) power to the total reducing (electron-donating) power of the substance.
- Aerobic organisms need food to have a positive redox potential (an oxidized state), and anaerobes need a negative potential (a reduced state) for growth.
- Each food has distinct redox potentials, and this influences the type of microbial growth typically seen in that food.
- As aerobes grow, reduce O2 in the medium, resulting in the lowering of redox potential (Eh).
- The medium becomes poorer with oxidized substrates and reaches in reduced ones.
- When the Eh of the food reaches negative values due to the growth of aerobes, then the growth of anaerobes present as contaminants in the food may start, leading to further spoilage.

Antimicrobial Constituents

- Antimicrobial substances are naturally present in certain foods that will exhibit an inhibitory action on the growth of micro-organisms.
- These could be present naturally in the food substance or formed during processing or added intentionally to the food.
- The various types of antimicrobial effects include:
  - 1. Compounds damaging structure of the cell or its function (cell wall, cytoplasmatic membrane, ribosome)
  - 2. Compounds affecting microbial enzymes (oxidative agents, chelating agents, heavy metals, antimetabolites)
  - 3. Compounds reacting with DNA (chemical mutagenes –alkylating or deaminating agents, cytostatics)
- Many organic and inorganic chemicals are added to foods as preservatives.
- Benzoic acid is an effective antimicrobial in high acid foods, fruit drinks, cider, carbonated beverages, and pickles.
- Potassium sorbate inhibits the growth of mold, yeast, and some bacteria.
- Parabens are more effective against molds and yeast than against bacteria, and more active against gram-positive than gram-negative bacteria.

Biological Structures

- Most raw foods derived from plant or animal origins normally have one or another type of natural biological structure that may hinder microorganisms from entry into the cells and tissues of the food.
- In this category are such structures as the testa of seeds, the outer covering of fruits, the shell of nuts, the hide of animals, and the shells of eggs.
- The skin does not favor microbial growth because it has low water activity, is deficient in readily available nutrients, and, often, contains antimicrobial compounds such as short-chain fatty acids in the case of animal skins or essential oils in the case of plant coatings.

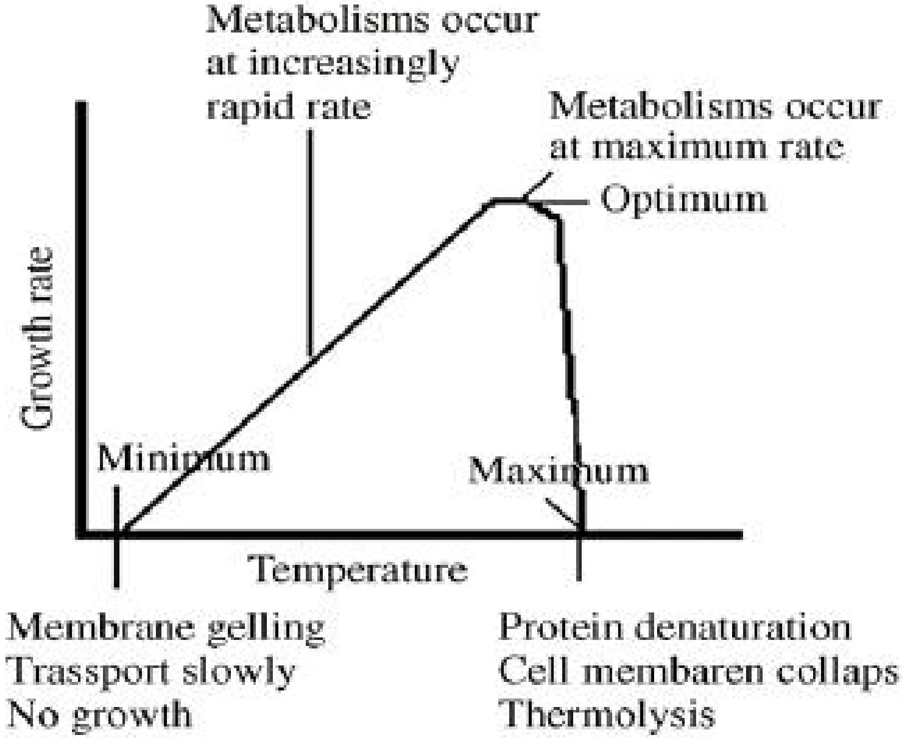
Extrinsic Factors: Storage Temperature

Table 11 Temperature Ranges for the Four Groups of Microorganism

Group	Minimum Temperature (^0C)	Optimum Temperature (^0C)	Maximum Temperature (^0C)
Thermophiles	40 to 45	55 to 75	60 to 90
Mesophiles	5 to 15	30 to 45	35 to 47
Psychrophiles	–5 to +5	12 to 15	15 to 20
Psychrotrophs	–5 to +5	25 to 30	30 to 35

- The temperature has a drastic impact on both the generation time of an organism and its lag period.
- As the temperature increases above the optimum, the growth rate declines much more sharply as a result of the irreversible denaturation of enzymes and proteins, and breakdown of the cytoplasmic membrane.
- At the temperatures above the maximum, these changes are sufficient to kill the microorganism.
- As the temperature decreases from the optimum, growth rates lows due to the slowing enzyme reactions within the cell.
- Many microorganisms grow at lower temperatures by increasing the quantity of unsaturated fatty acids in their membrane lipids, and psychrophiles usually have higher levels of unsaturated fatty acids than mesophiles.
- Membranes containing higher levels of unsaturated fatty acid will remain fluid and functional at lower temperatures.
- The expression of virulence genes in certain foodborne pathogens is regulated by growth temperature.

Figure 1 Effect of temperature on microbial growth



Effect of Concentration of Gases in the Storage Environment

- Gases inhibit microorganisms by two mechanisms.
- The first mechanism involves a direct toxic effect that can inhibit growth and proliferation.
- Carbon dioxide (CO_2), ozone (O_3), and oxygen (O_2) are gases that directly exhibit toxicity to certain microorganisms.
- This inhibitory mechanism depends upon the chemical and physical properties of the gas and its interaction with the aqueous and lipid phases of the food.
- O_3 and O_2 generate oxidizing radicals that are highly toxic to anaerobic bacteria and can have an inhibitory effect on aerobes depending on their concentration.
- Carbon dioxide is very effective against obligate aerobes and, at high levels, will deter other microorganisms.
- A second inhibitory mechanism is accomplished by modifying the gas composition, which has indirect inhibitory effects by altering the ecology of the microbial environment.
- The various technologies used to modify the gas composition in the storage environment of food are Modified Atmospheric Packaging (MAP), Controlled Atmospheric Packaging (CAP), Controlled Atmospheric Storage (CAS and vacuum packaging).

- High-pressure CO_2 has a severe lethal effect on microorganisms. It has been reported that living cell numbers reduced remarkably within 24 h at CO_2 partial pressures ≥1000 kPa.
- The low solubility of oxygen in aqueous media ensures that many environments are inhabited by predominantly anaerobic organisms.
- Ozone is 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.

Relative Humidity

- The RH of the storage environment is important for the growth of microorganisms on the surface area of food.
- The relative humidity in which a food is held will influence on the water activity of that product and influence on the growth of microorganisms on the surface of a product.
- When foods with low aw values are placed in an environment of high RH, foods pickup moisture until equilibrium has been established. Likewise, food with a high aw loses moisture when placed in an environment of low RH.
- If food has low aw, it will need a storage condition of low RH to maintain that low aw at the surface of the product.
- In general, the higher the temperature, the lower the RH, and vice versa.
- At high humidity, the growth of bacteria is expected to be higher than at lower humidity.

Presence and Activities of Other Microorganisms

- Some food borne organisms generate substances that are either inhibitory or fatal to others, and these embrace antibiotics, bacteriocins, hydrogen peroxide, and organic acids.
- Antibiotics are secondary metabolites produced by microorganisms that inhibit or kill a wide spectrum of other microorganisms.
- Bacteriocins are proteinaceous or peptidic toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain.
- Phages have been shown to reduce numbers of foodborne pathogens such as *Listeria monocytogenes* on surface-ripened cheeses.
- Coliphages are very common in fresh poultry, where they reduce the numbers of viable *E. coli*.

Implicit Factors

- In **antagonism**, the growth of one organism inhibits or suppresses the growth of the second organism, and most of the associations between microorganisms are antagonistic.
- Many microorganisms generate organic acids and alcohols that are inhibitory to some of their competitors.
- A **synergistic** association exists when two or additional microorganisms grow together, producing an impact that none of the individual microbes may produce alone.
- The presence of lactic acid bacteria is required for ‘yeast cream’ effect caused by yeasts.
- This is because the coagulation of milk due to lactic acid bacteria is necessary to produce the characteristic foaming due to subsequent gas production by yeasts.

… Implicit Factors

- **Metabiosis:** Metabiotic associations are essentially "sequential synergisms," in which the growth of one microorganism produces environmental conditions favorable for the growth of a second microorganism, which in turn can create favorable conditions for a third microorganism, and so on.
- The growth of aerobic, oxidative microorganisms can remove oxygen and reduce the O/R potential of food, thereby creating anaerobic conditions that favor the growth of vastly different microbes.
- **Commensalism** is that the interaction between populations during which one gains from the interaction, and the other is unaffected.
- In the microbial world, commensalism is mostly related to nutrition that is when metabolic products of one microbial population can be used by other microbial populations with no specific gain to the first population.
- Ex. methanogens produce methane, which can be oxidized by methanotrophs, and sulfate reducers produce sulfide, which can be oxidized by a variety of sulfide-oxidizing organisms

… Implicit Factors

- A **predator** is an organism that feeds on different organisms, and plenty of varieties of protozoans are the principal predators of prokaryotes in nature.
- **Amensalism** defines a relationship in which the activity of one population is harmful to another.
- For microbes, this typically results when the products of one type of metabolism are detrimental to another.
- Examples include the production of oxygen by cyanobacteria, inhibiting anaerobic organisms
- **Competition**: Populations of microorganisms inhabiting a common environment compete for nutrients and other resources of the environment

Food Processing factors

- During the processing of foods, microorganisms can be subjected to various physical or chemical stresses.
- Such processing factors are heat, freezing, drying, osmotic effects, irradiation, and various chemicals.
- Heating helps to reduce the microbial level in food by damaging the cytoplasmic membrane, alters metabolic and enzymatic activities.
- Freezing reduces the growth of microbes by exhibiting the inhibitory effect of reduced pH and increase of Aw.
- Drying reduces microbial growth as it causes metabolic injuries that impair the proliferation of the cells.