

Growth and survival of microorganisms in food

Expected Learning Outcome

- Factors that affect the growth of microorganisms in food.
- Categorising these factors as intrinsic and extrinsic based on whether these are internal to the food or outside the food.
- Nature of these factors and their role in inhibiting or promoting microbial activity in food.

2.1. Factors affecting the growth of microorganisms in fish

Foods meant for human consumption are rarely sterile, and contain several microorganisms. Microorganisms associated with food include natural micro-flora of raw material and organisms that are introduced during harvesting / handling, processing, storage and distribution.

The microbial load in food depends on factors such as

- Nature of the food
- Storage environment
- Properties of organisms in foods
- Effects of processing

Generally, micro-flora associated with food has no effect and food is consumed without objection and no adverse consequences. But in some instances associated microorganisms manifest their presence leading to spoilage, food borne illness or Transformation of properties of food in a beneficial way (Ex. Fermentation).

Factors affecting microbial growth in foods

Microorganisms associated with food and their growth is affected by several factors. These are broadly grouped into 2 types.

1. Intrinsic factors :Includes physicochemical properties of food such as,

- Nutrients
- pH and buffering capacity
- Redox potential
- Water activity / moisture content
- Antimicrobial constituents

2. Extrinsic factors:Include conditions of storage environment such as,

- Relative avidity
- Temperature of storage
- Gaseous atmosphere
- Presence / activities of other organisms

2.1.1. Nutrient content

Microorganisms associated with foods derive nutrients as source of energy, nitrogen, water, vitamins, minerals etc. for their growth and energy needs from foods. Nature of nutrient

availability favor growth of different microorganisms. Ability of microorganisms to utilize nutrients favors their growth on foods, while inability to utilize nutrients reduces microbial growth.

Ex. Saccharolytic microorganisms grow well on cereals using carbohydrates as carbon source.

- Proteolytic bacteria grow well on fish and meat.
- Diverse microbial group are associated with fruits containing variety of sugars (sucrose, fructose etc) favoring a variety of microorganisms and yeasts.
- Generally, foods where nutrients are easily available (fish/meat) support high microbial load and activity. Foods with low water content are protected against microbial invasion.

2.1.2 pH and buffering capacity

The acidity and alkalinity of an environment affects growth and metabolism of microorganisms as the activity and stability of macromolecules, enzymes and nutrient transport is influenced by pH. Generally, bacteria grow fast at pH 6-8. But bacteria that produce acids have optimum pH between pH 5 and 6 (Ex: Lactobacillus and Acetic acid bacteria). Yeast grows best at pH 4.5-6.0 and Fungi at 3.5 - 4.0.

In low pH foods (Ex. Fruits), spoilage is mainly by yeasts and fungi than bacteria. Fishes with pH around neutrality (6.5-7.5) favour bacterial growth and spoil rapidly than meat (pH: 5.5 - 6.5). Ability of low pH to restrict microbial growth has been employed as a method of food preservation (Ex: use of acetic and lactic acid).

Buffering capacity refers to the ability of foods to withstand pH changes. Microorganisms have ability to change pH of the surrounding environment to their optimal level by their metabolic activity. Decarboxylation of aminoacids releases amines which increases surrounding pH. Deamination of aminoacids by enzyme deaminases release organic acids causing decrease in pH. Thus, protein rich foods like fish and meat have better buffering capacity than carbohydrate rich foods.

2.1.3. Moisture content (a_w)

Moisture content of the food affects microorganisms in foods, and the microbial types present in foods depends on the amount of water available. Water requirement for microorganisms is described in terms of water activity

(a_w) in the environment and is defined as the ratio of the water vapor pressure of food substrate to the vapor pressure of pure water at same temperature.

$$a_w = P/P_o$$

P = vapour pressure of water in substrate

P_o = vapour pressure of solvent (pure water)

a_w is related to relative humidity (RH)

$$RH = 100 \times a_w$$

Water activity of solutes and requirements of certain microorganisms

- a_w of pure water: 1.0
- NaCl solution (22 %): 0.86
- Saturated NaCl solution: 0.75
- Bacteria generally require higher value of a_w than fungi
- G^{-ve} bacteria require higher a_w than G^{+ve} bacteria
- Most spoilage bacteria do not grow at a_w below 0.91

- Spoilage molds grow at a_w of 0.80
- Halophilic bacteria grow at a_w of 0.75
- Xerophilic and osmophilic yeasts grow at of 0.61

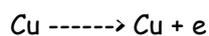
Microorganisms like halophiles, osmophiles and xerophiles grow better at reduced a_w . Microorganisms can not grow below a_w 0.60, and in such situations spoilage of food is not microbiological but due to chemical reactions (Ex: oxidation).

Relationship between a_w , temperature and nutrition

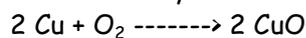
- Growth of microorganisms decreases with lowering of a_w
- The range of a_w at which the growth is greatest occurs at optimum temperature for growth
- The presence of nutrients increases the range of a_w over which the organisms can survive

2.1.4. Redox potential (Oxidation - Reduction potential / O-R potential/ Eh)

Microorganisms show varying degree of sensitivity to O-R potential (Eh) of growth medium. Redox reaction occurs as a result of transfer of electrons between atoms or molecules. O-R potential of a substrate is defined as the ease with which the substrate loses or gains electrons. Substrate is oxidized when it loses electrons and is called as good reducing agent. Substrates that gain electrons become reduced and are a good oxidizing agents.



Oxidation may also achieved by addition of oxygen.



Transfer of electro from one compound to another creates a potential difference between two compounds and is measured by an instrument and expressed as millivolts (mv). Highly oxidized substances have more electric potential (positive potential) and reduced substances negative electrical potential. Zero electric potential when oxidation and reduction are equal. O/R potential of a system is expressed by Eh. Aerobic microorganisms require positive Eh for growth and anaerobes, negative Eh. Reducing conditions in food is maintained by -SH groups in meat and ascorbic acid and reducing sugars in fruits and vegetables.

Factors influencing O/R potential of a food

- The characteristic of O/R potential of the original food.
- Poising capacity - (Resistance to change in potential of food)
- Oxygen tension of the storage atmosphere of food
- Access that the atmosphere has to the food
- Microbial activity

Eh requirement of microorganisms

- Aerobic microorganisms require oxidized condition (+ Eh) for growth. Ex. Bacillus sp
- Anaerobes require reduced condition (-Eh). Ex. Clostridium sp
- Microaerophils are aerobes growing at slightly reduced condition. Ex. Lactobacillus, Campylobacter.
- Facultative anaerobes have capacity to grow both under reduced and oxidized condition. Eg. Yeasts.

- Plant foods have positive Eh (fruits, vegetables) and spoilage is mainly caused by aerobes (bacteria and molds).
- Solid meat and fish have negative Eh (-200 mv), and minced meat positive Eh (+200 mv).

Microorganisms and Eh of food

- Microorganisms affect Eh of food during growth. Aerobes reduce the Eh of environment due to oxygen utilization. Growth medium becomes poorer in oxidizing and richer in reducing substances.
- Microorganisms reduce Eh by releasing metabolites. Hydrogen sulphide released by anaerobic microorganisms reacts with oxygen and creates reduced condition.
- Presence or absence of appropriate quantity of oxidizing/ reducing agents in the medium influences growth and activity of all microorganisms.

2.1.5. Antimicrobial constituents and barriers

All foods have one or the other mechanism to prevent or limit potentially damaging effects by microorganisms through protective physical barriers to infection (Ex. skin, shell, and husk) and antimicrobial components. Natural covering of some foods provide excellent protection against entry and subsequent damage by spoilage microorganisms. These include outer covering of fruits, outer shell of egg, skin covering of fish and meats.

The outer covering is usually composed of macromolecules and these are resistant to degradation and create inhospitable environment for microorganisms due to low a_w and shortage of readily available nutrients. The antimicrobial substances such as short chain fatty acids in animal skin and essential oils in plant surfaces help to prevent entry of microorganisms.

Physical damage to outer barrier allows microbial invasion and cause spoilage. Some foods are resistant to attack by microorganisms and remain stable due to the presence of naturally occurring substances which have antimicrobial property. Many plant species possess essential oils which are antimicrobial.

2.2 Extrinsic parameters

Extrinsic parameters refer to those properties of the environment to which the food is exposed. This includes the characteristics of the storage environment of the food which have profound effect on the food as well as the microorganisms associated with the food. The intrinsic factors of the food are influenced by the conditions of storage environment, and there by affect the quality of food. The environmental factors which have influence on the food associated microorganisms are;

- Storage temperature
- Relative humidity of storage environment
- Presence or absence of gases
- Presence and activities of other microorganisms.

2.2.1. Temperature of storage

Storage temperature refers to temperature at which the food is handled and stored.

Microorganisms growing over a wide range of temperature have been reported from food. The minimum temperature supporting the growth of microorganism was found to be well below the freezing temperature of water, and the highest temperature close to boiling temperature of water. However, no single microorganism is capable of growing over this wide temperature range. Thus, selecting a proper temperature for the storage of different food types helps in maintaining

quality. Each microorganism exhibits minimum, optimum and maximum temperature at which growth occurs, called cardinal temperature. This temperature is characteristic for an organism and is influenced by factors such as nutrient availability, pH of growth medium, water activity etc.

Grouping of microorganisms based on temperature requirement for growth

Microorganisms are grouped in to 4 broad types based on cardinal temperature.

Groups	Minimum temperature for growth (°C)	Optimum temperature for growth (°C)	Maximum temperature for growth (°C)
Psychrotrophs	-5 to +5	25-30	30-35
Psychrophiles	-5 to +5	12-15	15-20
Mesophiles	5-15	30-40	40-47
Themophiles	40-45	55-75	60-90

Psychrophiles

Microorganisms capable of growing at low temperature are called psychrophiles. These are further divided in to 2 types based on their optimum temperature for growth.

Obligate psychrophiles (cold loving) - These have temperature optima of 12-15°C, but unable to grow above 20°C. These are confined to polar regions and deep marine environment.

Psychrotrophs (facultative psychrophiles)

These have same minimum temperature for growth as psychrophiles but have higher optimum and maximum growth temperature. Thus are found in most diverse habitats, grow well in refrigerated temperature and cause spoilage of chilled food.

Ex: *Alcaligenes*, *Cornybacterium*, *Flavobacterium*, *lactobacillus*, *Pseudomonas*, *Enterococcus* etc. These bacteria grow well at 5-7°C (refrigerator temperature) and cause spoilage of meat, fish, poultry, eggs etc.

Mesophiles

These grow well under normal temperature conditions of 30-40°C. As a rule mesophiles grow quickly at their optimum temperature than psychrotrophs. Thus the spoilage of food at mesophilic temperature range is rapid than at chill temperature. Mesophiles are found in most genera of bacteria.

Thermophiles

These are high temperature loving microorganisms with the optimum temperature in the range of 55- 75°C. Thermophiles are generally less important in food microbiology. But thermophilic spore formers of the genus *Bacillus* and *Clostridium* cause spoilage of canned foods.

Molds and yeasts

Like bacteria, many molds and yeasts are associated with food. Many spoilage molds are able to grow over a range of temperature. Some molds grow at refrigerated temperature (*Aspergillus*,

Cladosporium, Thamnidium Sp).

Yeasts also grow and involve in spoilage of food under appropriate conditions. These grow over psychrophilic and mesophilic temperature range.

Storage temperature and spoilage

- Temperature of storage is most important parameter affecting the spoilage of highly perishable foods (Ex. fish).
- Nature of food need to be considered while selecting storage temperature. Maintaining all foods at refrigerated temperature is not advisable as it affects the quality of food.

Ideal temperature for storage of most vegetables is 10°C , banana: 13-17°C and refrigerator temperature for most foods.

2.2.2 Relative humidity (RH)

Relative humidity of the food storage environment refers to percentage of moisture present in the atmosphere. RH and water activity are closely related and RH is measure of a_w of the gas phase of atmosphere.

$$RH = 100 \times a_w$$

Effect of RH on food being stored

RH of storage environment influences a_w of foods and growth of microorganisms on food surface. Foods of low a_w stored in high RH environment leads to transfer of water from environment to the food, increasing a_w of food until equilibrium is reached. Condensation of moisture on food surface results in localized regions of high a_w on surface and subsurface. This favors growth of microorganisms which were viable but unable to grow due to low a_w , increases a_w of immediate environment due to metabolic activity of microorganisms which produce water as end product of respiration. Thus microorganisms grow and causes spoilage of food which was considered safe microbiologically due to the growth of microorganisms requiring high a_w .

Foods of high a_w when stored in low RH environment lose moisture, become flaccid and unfit for consumption due to loss of quality.

RH and spoilage of food

- RH and temperature of environment are related, and as the temperature increases RH decreases.
- Foods that are susceptible for spoilage by yeasts, molds and certain bacteria should be stored under low RH conditions.
- Improperly wrapped animal meat kept in refrigerator (high RH) undergone surface spoilage.
- Selection of suitable RH condition for storage is necessary to avoid surface microbial growth and maintain desirable qualities of food as food may lose/take up moisture under improper RH condition and lose its quality.

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2.2.3. Presence and concentration of gases in the environment

Exposure of foods to gases in the storage environment (gaseous environment) affects growth and survival of microorganisms in foods. Since exposure of food to oxygen favors growth of aerobic microorganisms, gaseous environment need to be modified to ensure

reduced microbial activity and resultant spoilage. This approach is commonly employed in the preservation of fruits and vegetables.

Gases used to control microorganisms in foods

Carbon dioxide, ozone and nitrogen are most important gases used to control microorganisms in food. Several ready to eat foods are packed in the presence of these gases to reduce microbial activity and extend shelf life of packed foods. Such foods are referred to as Modified Atmosphere Package (MAP) foods.

Carbon dioxide is single most important atmospheric gas used to control microorganisms in foods and is used in varying concentration depending on the type of food. Carbon dioxide in elevated pressure is also used in carbonated water and soft drinks. Molds and Gram negative microorganisms are more sensitive to CO_2 than Gram positive bacteria. *Lactobacilli* are resistant to CO_2 . Yeasts show considerable resistance and tolerate high CO_2 level but can cause spoilage of carbonated beverage (Ex. *Brettanomyces sp.*)

Mechanism of inhibition

Carbon dioxide mainly acts as bacteriostatic agent. But some microorganisms are killed by prolonged exposure. Mechanism of inhibition of CO_2 is due to the formation of carbonic acid which lowers pH. Lowered pH affects physical properties of plasma membrane of microorganisms and affects solute transport, inhibits key enzymes, and reacts with amino group of proteins causing changes in their property and activity.

Ozone (O_3) is also has antimicrobial properties and extends shelf life of certain fruits and vegetables foods. O_3 concentration of 0.15-5 ppm is known to double the shelf life by inhibiting spoilage bacteria and yeast.

2.2.4. Presence and activities of other microorganisms

The nature of microorganisms (microbial associations) encountered in foods can affect microflora of food which are called implicit factors. These include

- Properties of organisms present in food
- Response of these organisms to their environment
- Interaction with other organisms in food

Effect of activities of microorganisms on food micro-flora

- Among several organisms present in food, microorganisms which find condition suitable (in food) for growth dominate over other organisms. Ex. Molds can grow on dry fish, but slowly, than bacteria. In fresh fish bacteria overgrow molds as conditions are most favourable. Faster growing bacterial growth is inhibited by low a_w or low pH where moulds grow and cause spoilage.
- Some food borne microorganisms produce metabolite /substances such as antibiotics, bacteriocins, hydrogen peroxide, organic acids etc which are inhibitory /lethal to other microorganisms.
- Spoilage microorganisms can interact wherein growth of one favours the growth of others. Ex: In low a_w food (grain) growth of few molds increases a_w leading to growth of other xerophilic molds.

- One organism may increase nutrient availability to others by degrading complex food substrates to simple forms.
- Some microorganisms may remove inhibitory substances thereby permit the growth of others.