Factors Influencing Microbial Growth on Food

Unit – I

Chapter -3

Introduction

- **Microorganisms use our food supply as a source of nutrients and energy. They increase their numbers by utilizing nutrients. This can result in a deterioration of the food. They produce enzymatic changes and off-flavours in food by breaking down a nutrient or synthesizing new compounds. Thus, they "spoil" our food and make it unfit for consumption.**

Measures Taken:

- **Reduction of the contact between microorganisms and foods (Prevent contamination)**
- **Elimination of microorganisms from our foods,**
- **Adjustment of conditions of storage (preservation)**

- **The characteristics of a food as a substrate for growth of microorganisms are important.**
- **Food or substrate will determine which microorganisms can or cannot grow on it**
- **Need to understand the characteristics of the food or substrate.**
- **Knowledge of the factors that favour or inhibit the growth of microorganisms is very important. It will help us in understanding the principles of food spoilage and preservation.**

Factors Affecting Microbial Growth on Food

Microbial growth in food depends on following factors

- **Intrinsic Factors: physical and chemical properties of the food**
- **Extrinsic Factors: Storage conditions including environmental condition where the food is stored**
- **Implicit Factors: Physiological properties of microorganisms (like hydrolytic activity)**
- **Process Factors: Heating, Cutting, Preservation**

Intrinsic Factors

1. Hydrogen-Ion Concentration (pH)

- Effect on Microbial Growth
- Effect on Microbial Ecology and Food Spoilage
- Inhibition of Microbes by Weak Acids
- Buffers in Food

2. Moisture Requirement/Water Activity

- Effect on Microbial Growth and Activity
- Ways of Reducing Water Activity
- Factors Affecting Water Requirement

3. Oxidation Reduction Potential

- Redox Couples in Food
- Effect of Microbial Growth on Redox Potential of Food
- Effect on Microbial Growth and Ecology
- Poising Capacity of Food

4. Nutrient Content

- Foods for Energy
- Foods for Growth Accessory Food Substances or Vitamins

5. Biological Structure

- Antimicrobial Barriers
- Effect of Destruction of Microbial Barriers

6. Inhibitory Substances

- Biological Inhibitory Substances
- Originally Present in Food
- Inhibitory Substances Developed/ Destroyed in Food Due to the Activity of Microorganisms
- Inhibitory Substances Developed During Processing of Food

1. Hydrogen-Ion Concentration (pH)

Effect on Microbial Growth:

- Every microorganism has a minimal, a maximal, and an optimal pH for growth.
- Bacteria grow in the pH range of 6.0–8.0
- Yeasts in the pH range of 4.5-6.0
- Filamentous fungi in the pH range of 3.5-4.0.
- Molds can grow over a wider range of pH than most yeasts and bacteria
- Many molds grow at acidities too high for yeasts and bacteria
- Most yeast do not grow well in alkaline foods and thus do not have a significant role to play in the spoilage of food products with high pH
- Activities of lactobacilli and acetic acid bacteria have pH optima between 5.0 and 6.0
- The proteolytic bacteria can grow in foods with a high (alkaline) pH

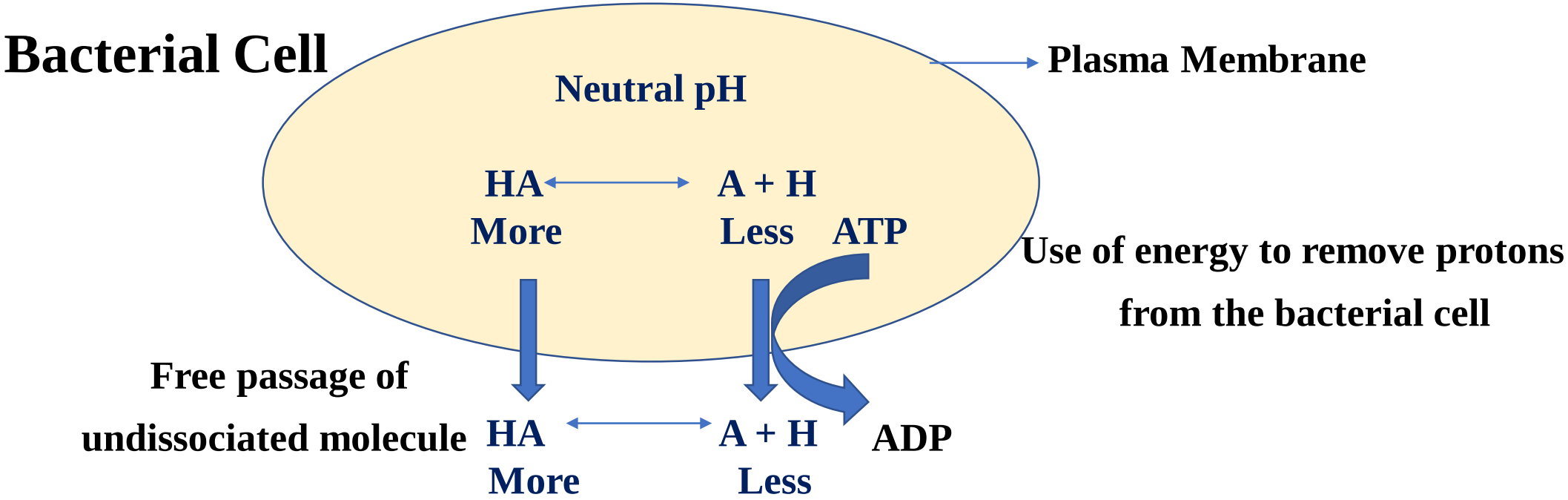
The pH ranges of some common food items		pH range of some common food microflora	
Food range	pH	Organism range	pH
• Citrus fruits	2-5	• Molds	0-11
• Soft drinks	2.5-4	• Yeasts	1.5-8.5
• Beer	3.5-4.5	• Lactic acid bacteria	3.2-10.5
• Meat	5.5-6.2	• Staphylococcus aureus	4-9.8
• Fish	6.5-7.3	• Salmonella spp.	4.1-9
• Egg white	8.6-9.6	• Escherichia coli	4.3-9
• Milk	6.5-7	• Yersinia enterocolitica	4.5-9
• Flour	6.2-7.2	• Clostridium botulinum	4.8-8.2
• Vegetables	4.8-7	• Clostridium perfringens	5.4-8.7
• Fermented shark	10-12	• Bacillus cereus	4.7-9.3

Effect on Microbial Ecology and Food Spoilage:

- The acidity of a product plays an important role in deciding the type microflora present in food and the rate and type of its spoilage
- Meats and Seafoods have a final ultimate pH of about 5.6 and above are susceptible to bacterial as well as to mold and yeast spoilage
- Most vegetables have higher pH values than fruits, and thus vegetables would be more prone to bacterial than fungal spoilage
- Meat from fatigued animals spoils faster than that from rested animals
- A well-rested meat animal, the usual 1% glycogen is converted into lactic acid, which directly causes a depression in pH values from about 7.4 to about 5.6. Most of the bacteria cannot tolerate lower pH, hence meat has a longer storage life
- Fruits generally undergo mold and yeast spoilage, due to the capacity of these organisms to grow at pH values < 3.5
- Fermented products like sauerkraut, pickles and fermented milks have a low pH and also known as biological acidity due to the accumulation of lactic acid during fermentation

Inhibition of Microbes by Weak Acids:

- The most acidic foods, contain weak organic acids causing incomplete dissociate of protons and conjugate base resulting inhibition of microbial growth
- The microbial inhibition by weak acids is directly related to the concentration of undissociated lipophilic acid molecules which can pass freely through the membrane



Inhibition of bacterial growth by weak acids

Buffers in Foods:

- Some foods are better able to resist changes in pH with their buffering capacity
- Buffers permit an acid (or alkaline) fermentation to go on longer with a greater yield of products and organisms
- Contributing to the buffering capacity of meats are their various proteins
- Vegetables are generally low in proteins and consequently lack the buffering capacity to resist changes in their pH by the growth of microorganisms
- Milk is fairly high in protein (a good buffer) and therefore permits considerable growth and acid production by lactic acid bacteria during the manufacture of fermented milks before growth is suppressed

2. Moisture Requirement/Water Activity

- This water requirement of microorganisms is best expressed in terms of available water (aw)
- The water activity depends on the number of molecules and ions present in solution

Effect on Microbial Growth and Activity:

- At a water activity of 0.6, the cytoplasm would need to contain very high concentrations of an appropriate compatible solute and the macromolecules such as DNA would no longer function properly and active growth may stop
- Bacteria require higher values of aw for growth than fungi
- Gram-negative bacteria have higher requirements than gram positives
- Yeasts and molds can grow over a wider aw range Spoilage molds can grow as low as 0.80

Minimum aw values for growth of microorganisms of importance in food

Organism	Water Activity (aw)	Organism	Water Activity (aw)
Group		Specific Organism	
Most spoilage bacteria	0.90	Pseudomonas spp.	0.97
Most spoilage yeasts	0.88	Escherichia coli	0.96
Most spoilage molds	0.80	Bacillus subtilis	0.95
Halophilic bacteria	0.75	Enterobacter aerogenes	0.945
Xerophilic molds	0.61	Clostridium botulinum	0.93
Osmophilic yeasts	0.60	Staphylococcus aureus	0.86

Ways of Reducing Water Activity:

Water is made unavailable in various ways:

1. Solutes and ions tie up water in solution- Solutes lower aw and this reduction in aw depends on the total concentration of dissolved molecules and ions

2. Hydrophilic colloids (gels) make water unavailable -

3. Water of crystallization or hydration is usually unavailable to microorganisms - In a food, as more and more ice is formed, the concentration of solutes in the unfrozen water increases, thus lowering available water and thereby it's aw is reduced.

Factors Affecting Water Requirement:

1. Kind of solute employed to reduce the aw: Potassium chloride usually is less toxic than sodium chloride, and less inhibitory than sodium sulphate
2. Nutritive value of the culture medium: The better the medium for growth, the lower the limiting aw permitting growth of microorganism
3. Temperature: Most organisms have the greatest tolerance to low aw at about optimal temperatures
4. Oxygen supply: Growth of aerobes takes place at a lower aw in the presence of air than in its absence, and the reverse is true of anaerobes
5. pH: Most organisms are more tolerant of low aw at pH values near neutrality than in acid or alkaline media
6. Inhibitors: The presence of inhibitors narrows the range of aw for growth of microorganism

3. Oxidation Reduction Potential

- The tendency of a substrate to accept or donate electrons, is termed its redox potential (Eh) or O/R potential
- When a substrate loses electrons, the substrate is oxidized (good reducing agent)while a substrate that gains electrons becomes reduced (good oxidizing agent)

Redox Couples in Food:

- Pair of oxidizing and reducing agents present in food are known as redox couples
- Foods may have a large positive Eo’ (Strong oxidizing)or a large negative Eo’ (Strong reducing) or a zero electrical potentials
- Glutathione and cysteine in meats and ascorbic acid and reducing sugars in plant products, would on their own tend to establish reducing conditions
- Oxygen, which is present in the air at a level of around 21 %, is usually has a high Eo' and is a powerful oxidizing agent
- Exclusion of air from food environment as in modified vacuum packing or canning reduce the Eh

Effect of Microbial Growth on Redox Potential of Food:

- Microbial growth in food consume oxygen and produce reducing compounds such as hydrogen
- As the oxygen content of the medium decreases, so the redox potential declines from a positive potential to a negative potential
- The decrease in Eh as a result of microbial activity is the basis of some rapid tests for determination of microbial load of food, particularly dairy products (Methylene blue reductase test)

Effect on Microbial Growth and Ecology:

- Redox potential decides the type of microorganism which can grow in that food

1. Aerobic - when they require free oxygen (Bacteria and Molds on plant juices)
2. Anaerobic - when they grow best in the absence of free oxygen (Lactic acid Bacteria)
3. Facultative - when they grow well either aerobically or anaerobically (Meat Bacteria)

- Predominance of aerobic organisms especially at food surfaces exposed to air
- At negative Eh, the anaerobic microflora requiring reduced conditions will be favoured

Poising Capacity of Food:

- Resistance to change in the redox conditions is known as poising capacity of food
- Poising is greatest when the two components of a redox couple are present in equal amounts
- Most fresh plant or animal foods have a low and well-poised O-R potential in their interior
- A piece of fresh meat or a fresh whole fruit would have aerobic conditions only at and near the surface and support aerobic growth of slime-forming or souring bacteria at the surface and anaerobic putrefaction in the interior
- Processing procedures may reduce the poising power of the food and allow more rapid diffusion of oxygen inward, due to the destruction of poising substances and changes in the physical structure of the food
- Fruit juices loose reducing substances by their removal during extraction and filtration and therefore become more favourable to the growth of yeasts than the original juice containing the pulp

4. Nutrient Content

Microorganisms use foods as a source of nutrients and energy

The food based on their nutrient composition can be classified as:

(1) foods for energy (2) foods for growth and (3)accessory food substances, or vitamins

Foods for Energy:

- **The carbohydrates, especially the sugars, are most common, other carbon compounds like esters, alcohols, peptides, amino acids, organic acids and their salts and cellulose and starch like complex carbon**
- **Bacteria differ in their ability to utilize different foods as a source of energy**
- **A limited number of microorganisms can obtain their energy from fats when sugar is absent**
- **Aerobic microorganisms are more commonly involved in the decomposition of fats than anaerobic ones, and the lipolytic organisms usually are also proteolytic**

Foods for Growth:

- Microorganisms differ in their ability to use various nitrogenous compounds as a source of nitrogen for growth
- They utilize primary nitrogen sources as amino acids and other nitrogenous compounds like nucleotides, free amino acids, peptides and proteins
- Many molds are proteolytic, but comparatively few bacteria and very few yeast are actively proteolytic

Accessory Food Substances or Vitamins:

- Some microorganisms are unable to synthesize some or all of the vitamins needed for their growth
- Most natural plant and animal foodstuffs contain an array of these vitamins
- Gram positive bacteria are the least synthetic and therefore be supplied with one or more of these compounds
- Gram negative bacteria and molds are able to synthesize most of their requirements

5. Biological Structure

The plants and animals that serve as food sources have all evolved mechanisms of defense against the invasion and proliferation of microorganisms

Antimicrobial Barriers:

- **The first barrier is the integument as a physical barrier to protect the food like the shell on eggs, the skin on poultry, the shell on nuts and the rind or skin on fruits and vegetables, or these may be surrounded by natural wax**
- **A low water activity or nutrients deficiency or antimicrobial compounds like short chain fatty acids on animal skin, essential oils on plant surfaces protect the natural food**

Effect of Destruction of Microbial Barriers:

- **Physical damage to the integument allows microbial invasion**
- **Peeling, skinning and chopping may serve not only to distribute spoilage organisms but also to release juices containing food materials for the microorganisms**

6. Inhibitory Substances

These may be originally present in the food, added purposely or accidentally, or developed there by growth of microorganisms or by processing methods

The mechanism of action for these antimicrobials may be:

(1) reaction with the cell membrane, (2) inactivation of essential enzymes, or (3) destruction or functional inactivation of genetic material

Biological Inhibitory Substances Originally Present in Food:

- The stability of some foods against attack by microorganisms is due to the presence of certain naturally occurring substances
- Antimicrobials collectively known as phytoalexins are produced by many plants in response to microbial invasion
- Some spices are known to contain essential oils that possess antimicrobial activity eugenol in cloves, allicin in garlic, cinnamic aldehyde in cinnamon, allyl isothiocyanate in mustard

Phytoalexin	Host	Pathogen
Ipomeamarone	***Ipomoea batata***	***Ceratocystis fimbriata***
Orchinol	***Orchis milliteris***	***Rhizoctonia ripens***
Pistatin	***Pisum sativum***	***Sclerotinia fructicola***
Phaseolin	***Phaseolus vulgaris***	***Sclerotinia fructicola***
Medicarpin	***Medicago sativa***	***Helminthosporium turcicum***
Rishitin	***Potato tubers***	***Phytophthora infestance***
Isocoumarin	***Daucas carota***	***Ceratocystis fimbriata***
Gossypol	***Gossypium hirsutum***	***Verticillium alboatrum***
Cicerin	***Cicer areitinum***	***Ascochyta rabiel***
Glyceolin	***Soybean, Alfalfa, Clover***	***Glomerella cingulata***
Capisidiol	***Pepper***	***Pernospora trifoliorum***
Trifolirhizin	***Trifolium pratense***	***Helminthosporium turcicum***

- **Antimicrobial substances in egg and milk**

Egg	Milk
Ovotransferrin (conalbumin)	Lactoferrin
Lysozyme	Lysozyme
Avidin	-
Ovomucoid	-
Ovoflavoprotein	-
-	Immunoglobulin
-	Lactoperoxidase

Cows' milk contains several other antimicrobial substances including conglutinin, lactenins, anticoliform factor and the lactoperoxidase system

Inhibitory Substances Developed/ Destroyed in Food Due to the Activity of Microorganisms:

- Microorganism growing in food may produce one or more substances inhibitory to other organisms such as acids, alcohols, peroxides and antibiotics
- Propionic acid produced by the propionibacteria in Swiss cheese is inhibitory to molds
- Nisin a polypeptide produced by Streptococcus lactis cause inhibition of lactate fermenting, gas-forming clostridia during curing of cheese
- Gram-negative organisms and molds are insensitive to nisin
- Certain molds and bacteria are able to destroy some of the phenolic compounds that are added to meat or fish by smoking or benzoic acid added to foods

Inhibitory Substances Developed During Processing of Food:

- Heating foods may result in the formation of inhibitory substances
- Browning of concentrated sugar syrups may result in production of furfural and hydroxymethylfurfural compounds which are inhibitory to fermenting organisms
- The milk enzyme lactoperoxidase is able to catalyse the oxidation of thiocyanate by hydrogen peroxide and produce hypothiocyanate which can kill gram-negative bacteria and inhibit gram-positives

Extrinsic Factors

These are the properties of the storage environment that affect both the foods and their microorganisms.

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

Temperature:

Psychrotrophs: Are those organisms that grow well at or below 7oC and their optimum temperature is between 20oC and 30oC. The lowest temperature at which a microorganism has been reported to grow is -34oC.; the highest somewhere in excess of 100oC.

Mesophiles: Those microorganisms that grow well between 20oC and 45oC with optima between 30oC and 40oC (E.g. Enterococcus feacalis)

Thermophiles: Those microorganisms that grow well at and above 45oC with optima between 55oC and 65oC

Relative Humidity of Environment (RH):

- When foods with low aw values are placed in environments of high R.H the foods pick up moisture until equilibrium has been established
- Likewise foods with a high aw lose moisture when subjected to high temperatures

Presence of Concentration of Gases in The Environment:

- Modified atmosphere is employed in storage of fruits
- This is the atmosphere containing increased amounts of CO2 up to about 10% applied from mechanical sources or by use of solid CO2
- Apples and pears stored under CO2 retard fungal rotting of fruits
- CO2 atmospheres extend the storage life of meat together with lower temperatures, and specific pH
- Ozone (O3) when added to food storage environment has a preservative effect on certain foods

THE HURDLE CONCEPT

- In the hurdle concept, multiple factors or techniques are employed to effect the control of microorganisms in foods, while under intrinsic and extrinsic parameters; the effect of single factors on the growth of microorganisms is presented
- The hurdle concept is applied in food preservation is also described in different ways such as; Barrier Technology, Combination Preservation or Combined Methods.
- In this technique, in order to grow the organisms must “hurdle” a series of barriers.
- A large number of factors are known that can be applied to food systems as hurdles

Some useful books

- Adams, M.R. and Moss, M.O. (1996) Food Microbiology. New Age International (P) Ltd., Publishers, New Delhi.
- McMeekin, J.N., Olley, T. Ross and Ratkowsky, D.A. (1993) ‘Predictive Microbiology: Theory and Application’, Research Studies Press Ltd., Tauton, England, 340pp.
- Stanier, R.Y., Adelberg, E.A. and Ingraham, J. (1976) The Microbial World. Prentice-Hall, Inc., Englewood Cliffs, N.J.