FOOD AS SUBSTRATE FOR MICROBES

1. **Introduction**
2. **Factors that affect the growth of microorganisms**
3. **Intrinsic factors that affect the growth of microorganisms**
4. **Extrinsic factors that affect the growth of microorganisms**

1. Introduction

The food for us is also a food for microorganism. Microorganisms are associated with plants and animals in nature. They play important role for survival of plants and animals. Our food mainly consists of materials from plants and animals and it is obvious that food can contain microorganisms. Some microorganisms serve us as food, some present in food are helpful and some others are harmful to our health. Microorganisms present in food use them as source of nutrients or their growth media and grow. Growth of microorganisms in food can result in improving the quality of food and in some cases can deteriorate their quality depending on the type of microorganism. Growth of harmful microorganisms in food can result in spoilage and sometimes cause several diseases on consumption of such food. Food spoilage by microorganisms is due to increase in their numbers, utilizing nutrients, causing enzymatic changes resulting in bad flavors due to breakdown of some food materials or synthesis of new compounds. Due to such microbial activities, food become unfit for human consumption. Microorganisms bring about oxidation of reduced carbon, nitrogen, sulfur compounds present in dead plants and animals in nature and become important part in recycling of these elements.

Food acts as good medium for transmission of many diseases. If the food is contaminated by pathogenic microorganisms, they can grow and increase their population and cause diseases on consumption of such food. Some time microorganisms may not grow in food but they are transported through food. Therefore, food act as good medium for spread of diseases. Several food born diseases are the result of microorganism present in food or their growth in them.

Growth of microorganisms in nature is dependent on various factors. The factors influencing the growth of microorganisms are physical, chemical and biological in nature. The important factors which contribute to the growth of microorganisms in food are temperature, pH, moisture content, redox potential, nutrient content, inhibiting substances and other microorganisms present in food.

2. Factors that affect the growth of microorganisms

Several factors related to the environment and the conditions in which food is stored influence the growth of micro-organisms in food. These factors can be divided into intrinsic and extrinsic elements. Intrinsic factors are those that are characteristic of the food itself; extrinsic factors are those that refer to the environment surrounding the food.

The parameters of plant and animal tissues that are an inherent part of the tissues are referred as *intrinsic parameter*. These parameters are as follows:

- Nutrient content

- pH
- Moisture content and water activity (a_w)
- Oxidation–reduction potential (Eh)
- Antimicrobial constituents
- Biological structures

The extrinsic parameters of foods are not substrate dependent. They are those properties of the storage environment that affect both the foods and their microorganisms. Those of greatest importance to the welfare of food borne organisms are as follows:

- Temperature
- Relative humidity
- Presence and concentration of gases
- Presence and activities of other microorganisms

3. Intrinsic factors that affect the growth of microorganisms

Nutrient content

Nutritional quality of food in terms of their chemical composition, nutritive value or nutrients, their proportion and growth promoting ability are important for growth of microorganisms. Food for energy have more carbohydrates and other carbon compounds like esters, alcohols, peptides, amino acids, organic acids and their salts. Carbohydrates may include polysaccharides like cellulose, starch and different sugars. Depending on the type of carbohydrate and other energy compounds present, there will be variation in terms of microorganisms growing in food. Most of the sugary food have growth of yeasts and bacteria and starchy food promote growth of molds which are able to hydrolyze starch by producing amylases. Cellulolytic and pectinolytic molds grow on food containing these materials. Carbon for growth usually comes from carbohydrates and other organic compounds in most of the heterotrophic microorganisms.

The total nitrogen content and different nitrogenous nutrients in food may vary. Microorganisms differ in their ability to use various nitrogenous compounds as source of nitrogen for growth. Many organisms are unable to hydrolyze proteins due to lack of proteolytic enzymes. Proteolytic organisms produce proteases and hydrolyze proteins to peptides and amino acids which are used as source of nitrogen for growth by them and others. Proteolytic property is different among microorganisms depending on the type of protease enzyme produced. Bacteria are more proteolytic than fungi in general. Protein rich food promote more growth of bacteria than molds and yeasts. Some of the lactic acid bacteria grow best with polypeptides as nitrogen source, cannot attack casein, and do not grow well with only a limited number and kinds of amino acids present. Presence of fermentable carbohydrates in a food results in an acid fermentation and suppression of proteolytic bacteria preventing production of obnoxious nitrogenous products. Many proteolytic bacteria grow best at pH values near neutrality and are inhibited by acidity.

Some microorganisms are not able to produce vitamins and other growth factors. They need to be provided in growth medium. Food contain different vitamins, minerals and other growth factors and their composition and content may vary. Fresh plant and animal food contain B

complex group of vitamins but stale food lack some of these vitamins. Meats are high in B vitamins and fruits are low, but fruits are high in ascorbic acid. Processing of food often reduces the vitamin content. Thiamine, pantothenic acid, folic acid and ascorbic acid are heat-labile and drying causes loss in vitamins such as thiamine and ascorbic acid. Storage of food for long may also result in decrease in vitamins and other growth factors. Some microorganisms produce vitamins and other growth factors which support growth of others organisms present in food. Each kind of microorganisms has a range of food requirements. Some have wide range and growth takes place in variety of substrates, such as coliform bacteria. Some pathogenic bacteria have very narrow range of substrates and require presence of specific kinds of substrates for growth. Generally molds can utilize many kinds of food ranging from simple to complex substrates by producing variety of hydrolytic enzymes. Depending on the composition, complexity and nutritive value, different food support growth of different microorganisms which are responsible for food contamination and spoilage.

pH

Biocatylatic activities are influenced by pH. Growth of microorganisms is affected by the pH of growth environments in food (ie. growth medium) which is the result of large number of enzymes responsible for metabolism and growth. Every organism or enzyme has a minimum, optimum and maximum pH requirements for their survival, growth or catalytic activity. Influence of pH of food not only has effect on growth of microorganisms but also on processing conditions. Food having acidic contents promote growth of acid loving microorganisms such as yeasts, molds and some bacteria. Most of the spoilage bacteria grow in food at neutral pH. Food are preserved with the addition of preservatives such as citric acid, acetic acid etc. which create acidic environment and protect food to some extent from the growth of bacteria and other microorganisms requiring neutral or alkaline environment. However there may be chances of contamination and growth of acidophilic molds or yeasts. Therefore, growth of molds is frequently observed in fruits, fruit juices and other processed acidic food. Food acidification by fermentation in home food preparations is the oldest practice man has been doing. It is due to production of organic acids in food by growth and fermentation of microorganisms such as lactic and acetic acid bacteria. Lactic acid and acetic acid are considered as antibiotic in earlier scientific observations.

Moisture Content and Water Activity (a_w)

Life exists only with water. Every living organism has the demand for water, without which no growth can occur. Similarly, microorganisms require water for their growth. Water is an excellent solvent for all life processes in every living organism for biocatalytic activity. The amount of water required varies for different organisms. Water requirement of microorganisms is expressed as available water or water activity. Water activity is the vapor pressure of the solution (of solutes in water in most food) divided by the vapor pressure of the solvent (usually water). Available water for microorganisms varies depending on microorganism and the type of food. In general, bacteria require more water activity than molds and yeasts. Most bacteria grow well in a medium with a water activity approaching 1.0. They grow well in low concentrations of sugar or

salt, although there are exceptions. Gram negative bacteria have higher water requirements than gram positive bacteria. Most of the food spoilage bacteria do not grow below a_w 0.91, while spoilage molds can grow even at a_w 0.80. The aerobic food poisoning bacterium, *Staphylococcus aureus* is found to grow at a_w as low as 0.86 while anaerobic *Clostridium botulinum* does not grow below a_w 0.94. Different molds differ considerably in optimal a_w for vegetative growth and spore germination. Each mold also has an optimal a_w and range of a_w for growth.

The effect of lowering a_w below optimum is to increase the length of the lag phase of growth and to decrease the growth rate and size of final population of microorganisms. This is due to adverse influences of lowered water on all metabolic activities in microorganisms since all chemical reactions in cells require an aqueous environment. The a_w is influenced by other environmental parameters such as pH, Eh (redox potential) and growth temperature required for microorganisms. The strategy employed by microorganisms as protection against osmotic stress is the intracellular accumulation of compatible solutes. Generally, the effect of lowered a_w on the nutrition of microorganisms is of a general nature where cell requirements that must be mediated through an aqueous environment are progressively shut off. In addition to the effect on nutrients, lowered a_w undoubtedly has adverse effects on the functioning the cell membrane, which must be kept in a fluid state.

Redox potential (Eh)

Prevailing oxygen concentration of food in their environment, chemical composition and type of microorganisms associated, contribute to the oxidation-reduction (O-R) potential of food and affect growth of microorganisms in them. The O-R potential of food is determined by characters such as

- oxygen tension of atmosphere above the food,
- access of atmosphere to the food,
- resistance of food to the changes occurring and
- O-R state of materials present in food.

Oxygen content in head space of the food contributes to the growth of microorganisms as microorganisms are aerobic, micro-aerophilic, facultative and anaerobic for oxygen requirement to grow. Aerobic and micro-aerophilic organisms require free oxygen in air but in different concentrations, facultatives grow in presence or absence of oxygen and anaerobes grow only in the absence of oxygen. Most of the fungi, bacteria and yeasts grow in food under aerobic conditions and anaerobic bacteria such as *Clostridium botulinum* require anaerobic conditions. Oxygen concentration of head space on food depends on its access to air. Food stored under evacuation or canned promote growth of anaerobic bacteria.

Redox potential of a system is expressed as *Eh* and expressed in millivolts (mV). Highly oxidized substrates have positive *Eh* and reduced substrates have negative *Eh*. Therefore aerobic organisms require positive *Eh* and anaerobic ones require negative *Eh* values for their growth. Most of the fresh plant and animal food have low redox potential because of reducing substances present in them. Fresh vegetables and fruits contain reducing substances such as ascorbic acid, reducing sugars and animal tissues have sulfhydryl (-SH) and other reducing group compounds

considered as anti-oxidants. Fresh vegetables, fruits and meat support growth of aerobic microorganisms at their surfaces because of positive redox potential. However, the anaerobic microorganisms grow in inner parts of vegetables, fruits and meat because of negative redox potential. Most of processed plant and animal food gain positive redox potential therefore promote growth of aerobic organisms. Food subjected to heat and/or packed under negative pressure promote growth of anaerobic organisms. Microorganisms growing in food may create different redox potential states depending on their oxygen requirement and redox changes they bring about in food contents. Quality of processed food in terms of redox status depends on the changes brought about by processing methods, type of microorganisms and chemical changes.

Antimicrobial Constituents

The stability of some foods against attack by microorganisms is due to the presence of certain naturally occurring substances that possess and express antimicrobial activity. Some plant species are known to contain essential oils that possess antimicrobial activity. Among these are eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allyl isothiocyanate in mustard, eugenol and thymol in sage, and carvacrol (isothymol) and thymol in oregano. Cow's milk contains several antimicrobial substances, including lactoferrin, conglutinin, and the lactoperoxidase system. Raw milk has been reported to contain a rotavirus inhibitor that can inhibit up to 10^6 pfu (plaqueforming units)/ml. It is destroyed by pasteurization. Milk casein as well as some free fatty acids have been shown to be antimicrobial under certain conditions. Eggs contain lysozyme, as does milk, and this enzyme, along with conalbumin, provides fresh eggs with a fairly efficient antimicrobial system. The hydroxycinnamic acid derivatives (*p*-coumaric, ferulic, caffeic, and chlorogenic acids) found in fruits, vegetables, tea, molasses, and other plant sources all show antibacterial and some antifungal activity.

4. Extrinsic factors that affect the growth of microorganisms

Temperature

Microorganisms, individually and as a group, grow over a very wide range of temperatures. Therefore, it is well to consider at this point the temperature growth ranges for organisms of importance in foods as an aid in selecting the proper temperature for the storage of different types of foods. The lowest temperature at which a microorganism has been reported to grow is −34°C; the highest is somewhere in excess of 100°C. It is customary to place microorganisms into three groups based on their temperature requirements for growth. Those organisms that grow well at or below 7◦C and have their optimum between 20°C and 30°C are referred to as *psychrotrophs*. Those that grow well between 20°C and 45°C with optima between 30°C and 40°C are referred to as *mesophiles*, whereas those that grow well at and above 45°C with optima between 55°C and 65°C are referred to as *thermophiles*.

With regard to bacteria *Alcaligenes, Shewanella, Brochothrix, Corynebacterium, Flavobacterium, Lactobacillus, Micrococcus, Pectobacterium, Pseudomonas, Psychrobacter, Enterococcus*, and others. The psychrotrophs found most commonly on foods are those that belong to the genera *Pseudomonas* and *Enterococcus*. These organisms grow well at refrigerator

temperatures and cause spoilage at 5–7°C of meats, fish, poultry, eggs, and other foods normally held at this temperature. Standard plate counts of viable organisms on such foods are generally higher when the plates are incubated at about 7°C for at least 7 days than when incubated at 30°C and above. They apparently do not grow at this temperature but do grow at temperatures within the mesophilic range if other conditions are suitable. It should be pointed out that some organisms can grow over a range from 0◦C to 40◦C. One such organism is *Enterococcus faecalis*.

Most thermophilic bacteria of importance in foods belong to the genera *Bacillus*, *Clostridium, Geobacillus,* and *Thermoanaerobacter*. Although not all species of these genera are thermophilic, they are of great interest to the food microbiologist and food technologist in the canning industry. Just as molds are able to grow over wider ranges of pH, osmotic pressure, and nutrient content, they are also able to grow over wide ranges of temperature as do bacteria. Many molds are able to grow at refrigerator temperatures, notably some strains of *Aspergillus, Cladosporium*, and *Thamnidium,* which may be found growing on eggs, beef, and fruits. Yeasts grow over the psychrotrophic and mesophilic temperature ranges but generally not within the thermophilic range.

Relative humidity (RH)

The RH of the storage environment is important both from the standpoint of water activity within foods and the growth of microorganisms at the surfaces. When the a_w of a food is set at 0.60, it is important that this food be stored under conditions of RH that do not allow the food to pick up moisture from the air and thereby increase its own surface and subsurface a_w to a point where microbial growth can occur. When foods with low a_w values are placed in environments of high RH, the foods pick up moisture until equilibrium has been established. Likewise, foods with a high a_w lose moisture when placed in an environment of low RH. There is a relationship between RH and temperature that should be borne in mind in selecting proper storage environments for foods. In general, the higher the temperature, the lower the RH, and vice versa.

Presence and Concentration of Gases

Carbon dioxide (CO_2) is the single most important atmospheric gas that is used to control microorganisms in foods. Ozone (O_3) is the other atmospheric gas that has antimicrobial properties, and it has been tried over a number of decades as an agent to extend the shelf life of certain foods. It has been shown to be effective against a variety of microorganisms, but because it is a strong oxidizing agent, it should not be used on high-lipid-content foods since it would cause an increase in rancidity.

Presence and Activities of Other Microorganisms

Some foodborne organisms produce substances that are either inhibitory or lethal to others; these include antibiotics, bacteriocins, hydrogen peroxide, and organic acids.

Conclusion: The foods that support the growth of all human beings also support the growth of microorganisms. Taken together, all the intrinsic and extrinsic parameters represent nature's way of preserving/supporting plant and animal based foods from microorganisms. By determining the

extent to which each exists in a given food, one can predict the general types of microorganisms that are likely to grow.