FOOD AND INDUSTRIAL MICROBIOLOGY

FACTORS AFFECTING GROWTH AND SURVIVAL OF MICROORGANISMS IN FOODS

Introduction

Food spoilage means the original nutritional value, texture, colour, flavour, taste etc., of the food are changed / damaged, the food become harmful to people and unsuitable to consume. Food can deteriorate as a result of two main factors:

1) Growth of microorganisms usually from surface contamination especially important in processed food

2) Action of enzymes from within cells part of normal life processes. It is important to note that many plants fresh vegetables and fruit are still alive when bought and even when eaten raw and meat from animals undergoes gradual chemical changes after slaughter. The various sources through which microorganisms gain entry into the foods are shown in Table- 1.

Table -1 Primary sources of microorganisms found in foods

Microflora present in soil and water
Microflora present in air
Microflora present on plant and plant products
Microflora present on food utensils and equipments
Microflora present in animal feeds
Microflora present on animal hides
Microflora present in intestinal tracts of humans and animals
Food handlers

Microorganisms involved in food spoilage (other than Canned Foods) with some examples of causative organisms are enlisted in Table – 2. A variety of intrinsic and extrinsic factors determine whether microbial growth will preserve or spoil foods, as shown in Table - 3.

Intrinsic or food related parameters are those parameters of plants and animal tissues which are inherent part of the tissue. e.g., pH, water activity (aw), oxidation reduction potential (Eh), nutrient content, antimicrobial constituents and biological structures. Extrinsic or environmental parameters are properties of storage environments which affect both foods as well as microorganisms and include temperature of storage, relative humidity of storage environment, and concentration of gases in environment.

Table - 2 Microorganisms involved in food spoilage (other than canned foods) with some examples of causative organisms

Food	Type of Spoilage	Microorganisms involved
Bread	Mouldy	*Rhizopus nigricans* *Penicillium* spp. *Aspergillus niger*
	Ropy	*Bacillus subtilis*
Maple sap and syrup	Ropy	*Enterobacter aerogenes*
	Yeasty	*Saccharomyces* *Zygosaccharomyces*
	Pink	*Micrococcus roseus*
	Mouldy	*Aspergillus* *Penicillium*
Fresh fruits and vegetables	Soft rot	*Rhizopus* *Erwinia*
	Gray mold rot	*Botrytis*
	Black mold rot	*A. niger*
Fresh meat	Putrefaction	*Alcaligenes* *Clostridium* *Proteus vulgaris* *Pseudomonas fluorescens*
Cured meat	Mouldy	*Aspergillus* *Rhizopus* *Penicillium*
	Greening, slime	*Lactobacillus* *Leuconostoc*
Fish	Discoloration	*Pseudomonas*
	Putrefaction	*Alcaligenes*
Eggs	Green rot	*P. fluorescens*
	Colorless rots	*Pseudomonas* *Alcaligenes*
	Black rots	*Proteus*
Concentrated orange juice	"Off" flavor	*Lactobacillus* *Leuconostoc* *Acetobacter*
Poultry	Slime, odor	*Pseudomonas* *Alcaligenes*

Table -3 Factors affecting the development of microorganisms in foods

Intrinsic Factors	Extrinsic factors	Implicit factors	Processing factors
Nutrient content pH Redox potential Water activity Antimicrobial constituents & barriers	Temperature Relative humidity Gaseous atmosphere Growth rate	Synergism Antagonism Commensalism	Irradiation Washing Slicing Pasteurization Packaging

Intrinsic Parameters

Nutrient content

Like all other living beings, microorganisms need water, a source of carbon, an energy source, a source of nitrogen, minerals, vitamins and growth factors in order to grow and function normally. Since foods are rich source of these compounds, they can be used by microorganisms also. It is because of these reasons that various food products like malt extracts, peptone, tryptone, tomato juice, sugar and starch are incorporated in microbial media. The inability to utilize a major component of the food material will limit its growth and put it at a competitive disadvantage compared to those that can. In general, molds have the lowest requirement, followed by yeasts, Gram negative bacteria, and Gram-positive bacteria. Many food microorganisms have the ability to utilize sugars, alcohols, and amino acids as sources of energy. Few others are able to utilize complex carbohydrates such as starches and cellulose as sources of energy. Some microorganisms can also use fats as the source of energy, but their number is quite less. The primary nitrogen sources utilized by heterotrophic microorganisms are amino acids. Also, other nitrogenous compounds which can serve this function are proteins, peptides and nucleotides. In general, simple compounds such as amino acids are utilized first by a majority of microorganisms.

Water activity (aw)

Water is often the major constituent in foods. Even relatively ‘dry’ foods like bread and cheese usually contain more than 35% water. The state of water in a food can be most usefully described in terms of water activity. Water activity of a food is the ratio between the vapour pressure of the food, when in a completely undisturbed balance with the surrounding air, and the vapour pressure of pure water under identical conditions. Water activity, in practice, is measured as Equilibrium Relative Humidity (ERH) and is given by the formula:

Water Activity (aW) = ERH / 100

Water activity scale extends from 0 (bone dry) to 1.00 (pure water). But most foods have a water activity in the range of 0.2 for very dry foods to 0.99 for moist fresh foods. Based on regulations, if a food has a water activity value of 0.85 or below, it is generally considered as non-hazardous. This is because below a water activity of 0.91, most bacteria including the pathogens such as *Clostridium botulinum* cannot grow. But an exception is *Staphylococcus aureus* which can be inhibited by water activity value of 0.91 under anaerobic conditions but under aerobic conditions, it requires a minimum water activity value of 0.86. Most molds and yeasts can grow at a minimum water activity value of 0.80. Thus a dry food like bread is generally spoiled by molds and not bacteria. In general, the water activity requirement of microorganisms decreases in the following order: Bacteria > Yeast > Mold. Below 0.60, no microbiological growth is possible. Thus, the dried foods like milk powder, cookies, biscuits etc are more shelf stable and safe as compared to moist or semi moist foods.

Factors that affect water activity requirements of microorganisms include the following kind of solute added, nutritive value of culture medium, temperature, oxygen supply, pH, inhibitors, etc. Each microorganism has a minimal water activity for growth as shown in Table - 4.

Table - 4 Minimum water activity values of spoilage microorganisms

Microbial group	Minimum a_w	Examples
Most bacteria	0.91	*Salmonella* spp. *Clostridium botulinum*
Most yeasts	0.88	*Torulopsis* spp.
Most molds	0.80	*Aspergillus flavus*
Halophilic bacteria	0.75	*Wallemia sebi*
Xerophilic molds	0.65	*Aspergillus echinulats*
Osmophilic yeasts	0.60	*Saccharomyces bisporus*

Water acts as an essential solvent that is needed for most biochemical reactions by the microorganisms. Water activity of the foods can be reduced by several methods: by the addition of solutes or hydrophilic colloids, cooking, drying and dehydration: (e.g. egg powder, pasta), or by concentration (e.g. condensed milk) which restrict microbial growth so as to make the food microbiologically stable and safe.

A wide variety of foods are preserved by restricting their water activity. These include:

Dried or low moisture foods

These contain less than 25% moisture and have a final water activity between 0.0 and 0.60. e.g., Dried egg powder, milk powder, crackers, and cereals. These products are stored at room temperature without any secondary method of preservation. These are shelf stable and do not spoil as long as moisture content is kept low.

Intermediate moisture foods

These foods contain between 15% and 50% moisture content and have a water activity between 0.60 and 0.85. These foods normally require added protection by secondary methods such as pasteurization, pH control, refrigeration, preservatives, but they can also be stored at room temperature. These include dried fruits, cakes, pastries, fruit cake, jams, syrups and some fermented sausages. These products are usually spoiled by surface mold growth.

pH and buffering capacity

The pH, or hydrogen ion concentration, [H+], of natural environments varies from about 0.5 in the most acidic soils to about 10.5 in the most alkaline lakes. Since the pH is measured on a logarithmic scale, the [H+] of natural environments varies over a billion-fold and some microorganisms are living at the extremes, as well as every point between the extremes. The range of pH over which an organism grows is defined by three cardinal points: the minimum pH, below which the organism cannot grow, the maximum pH, above which the organism cannot grow, and the optimum pH, at which the organism grows the best. Microorganisms which grow at an optimum pH well below neutrality (7.0) are called acidophiles. Those which grow best at neutral pH are called neutrophiles and those that grow best under alkaline conditions are called alkalophiles. In general, bacteria grow faster in the pH range of 6.0-8.0, yeasts 4.5-6.5 and filamentous fungi 3.5-6.8, with the exception of lactobacilli and acetic acid bacteria with optima between pH 5.0 and 6.0 (Table - 5). The approximate pH ranges of some common food commodities are shown in Table -5.

Table - 5 Approximate pH ranges of different microbial groups

Microbe	Minimum	Optimum	Maximum
Most Bacteria	4.5	6.5 – 7.5	9.0
Yeasts	1.5 – 3.5	4.0 – 6.5	4.0 – 6.5
Molds	1.5 – 3.5	4.5 – 6.8	8.0 – 11.0

Table -6 Approximate pH ranges of some common food commodities

Product	pH
Citrus fruits	2.0-5.0
Soft drinks	2.5-4.0
Apples	2.9-3.3
Bananas	4.5-4.7
Beer	3.5-4.5
Meat	5.6-6.2
Vegetables	4.0-6.5
Fish (most spp)	6.6-6.8
Milk	6.5-6.8
Wheat flour	6.2-6.8
Egg white	8.5-9.5
Fermented shark	10.5-11.5

Redox potential (Eh)

Microorganisms display varying degrees of similarity to Oxidation Reduction potential of their growth medium. The O/R potential is the measure of tendency of a revisable system to give or receive electrons. When an element or compound loses electrons, it is said to be oxidized, while a substrate that gains electrons becomes reduced. Thus, a substance that readily gives up electrons is a good reducing agent, while one that readily gains electrons is a good oxidizing agent. When electrons are transferred from one compound to another, a potential difference is created between the two compounds and is expressed in as milk volts (mV). If a substance is more highly oxidized, the more positive will be its electrical potential and vice versa. The O/ R potential of a system is expressed as Eh. Aerobic microorganisms require positive Eh values for growth while anaerobic microorganisms require negative Eh values (reduced). The redox potential we measure in a food is the result of several factors: redox couples present, ratio of oxidant to reductant, pH, poising capacity, availability of oxygen and microbial activity. With the exception of oxygen, most of the couples present in foods, e.g, glutathione, cysteine, ascorbic acid and reducing sugars tend to establish reducing conditions. pH of the food has a bearing on the redox potential and for every unit decrease in the pH the Eh increases by 58 mv. As redox conditions change there will be some resistance to change in food’s Eh and is known as poising and is similar to buffering of the medium. Poising is maximum when the two redox couples are present in equal amounts. Oxygen is the most powerful of redox couple present in food system and if the food is stored in the presence of air, high positive potential will result. Thus, increasing the exposure to oxygen in air by mincing, cutting, chopping, grinding of food will increase the Eh. Finally, microbial growth in the food reduces the Eh due to oxygen depletion.

The decrease in Eh due to microbial activity forms the basis of some tests used frequently in raw milk such as platform MBRT test.

Antimicrobial constituents

Chemical compounds having pharmacological and biological activity and produced by living organisms are called natural products. Living organisms produce primary and secondary metabolites. Primary metabolites are the products that have essential function in the organism, while secondary metabolites could simply be waste products or could have some important function in their producers. Secondary metabolites can be used as drugs against diseases such as cancer, inflammation (swelling), and so on and also have antimicrobial activity. Secondary metabolites possessing antimicrobial activity are called the natural antimicrobials and could be extracted from different sources like plants (fruits, vegetables, seeds, herb, and spices), animals (eggs, milk, and tissues), and microorganisms (fungi and bacteria). With special reference to plants, secondary metabolites are found to be healthy ingredients that work as antimicrobials or disease-controlling agents. Owing to the potential of antimicrobials against pathogenic and spoilage microorganisms, these secondary metabolites gain much importance for the application in food products. They contain the properties of antimicrobials and antioxidants at the same time and so are considered as a better option for food preservation as compared to synthetic preservatives.

Some foods can resist the attack by microorganisms due to the presence of certain naturally occurring substances which possess antimicrobial activity such as essential oils in spices (eugenol in cloves and cinnamon, allicin in garlic, cinnamic aldehyde in cinnamon, thymol in sage); lactaferrin, lactoperoxidase and lysozyme in milk; and ova transferrin, avidin, lysozyme and Ovo flavoprotein in hen’s egg albumin. Similarly, casein as well as free fatty acids found in milk also exhibit antimicrobial activity. Also, natural covering of foods like shell of eggs and nuts, outer covering of fruits and testa of seeds, hide of animals provide protection against entry and subsequent spoilage by microorganisms.

Biological Structures

The natural covering of some foods provides excellent protection against the entry and subsequent damage by spoilage organisms. 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. In the case of nuts such as pecans and walnuts, the shell or covering is sufficient to prevent the entry of all organisms. Once cracked, of course, nutmeats are subject to spoilage by molds. The outer shell and membranes of eggs, if intact, prevent the entry of nearly all microorganisms when stored under the proper conditions of humidity and temperature. Fruits and vegetables with damaged covering undergo spoilage much faster than those not damaged. The skin covering offish and meats such as beef and pork prevents the contamination and spoilage of these foods, partly because it tends to dry out faster than freshly cut surfaces.

Taken together, these six intrinsic parameters represent nature's way of preserving plant and animal tissues 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 and, consequently, the overall stability of this particular food. Their determination may also aid one in determining age and possibly the handling history of a given food.

Extrinsic Parameters

Temperature of storage

On the basis of temperature preferences microorganisms are categorized as psychrophiles, psychrotrophs, mesophiles and thermophiles. For example, organisms with an optimum temperature near 37°C are called mesophiles. Organisms with an optimum temperature between about 45°C and 70°C are thermophiles e.g, Bacillus, Clostridium etc. Some archaebacteria with an optimum temperature of 80°C or higher and a maximum temperature as high as 115°C, are now referred to as extreme thermophiles or hyperthermophiles. The cold loving organisms are psychrophiles defined by their ability to grow at 0°C. A variant of a psychrophile (which usually has an optimum temperature above 10°C) is a psychrotroph, which grows at below 7°C but displays an optimum temperature in the mesophile range, nearer room temperature. Psychrotrophs are the problematic group of microorganisms during food storage in refrigerators since they continue to grow in the refrigerated environment where they spoil the food. Of course, they grow slower at 2°C than at 25°C. In food microbiology mesophilic and psychrotrophic organisms are of greatest importance.

Microorganisms are responsible not only for spoilage of food but many of them are harmful to human health, causing food poisoning, if infected food is consumed. By understanding the dynamics of microbial growth with varying temperature and its interaction with other extrinsic factors it is possible to adjust the storage temperature, so as to enhance the shelf life of fresh or processed food. Microorganisms have been found growing in virtually all temperatures. A particular microorganism will exhibit a range of temperature over which it can grow.

Relative humidity of the storage environment

Relative humidity of air is defined as the ratio of the vapor pressure of air to its saturation vapor pressure. The equilibrium relative humidity (ERH) of a food product is defined as relative humidity of the air surrounding the food that is in equilibrium with its environment. When the equilibrium is obtained, the ERH (in percent) is equal to the water activity multiplied by 100, i.e. ERH (%) = $a_w \times 100$. When a food is exposed to a constant humidity, the product will gain or lose moisture until the ERH is reached. The moisture migration significantly affects the physical and chemical properties of the food.

Relative humidity and water activity are interrelated. When foods with low aw are stored in environment of high humidity, water will transfer from the gas phase to the food and thus increasing aw of the food leading to spoilage by the viable flora. There is a relationship between temperature and humidity which should be kept in mind. In general, the higher the temperature, lower is the relative humidity and vice versa. Foods that undergo surface spoilage from molds, yeasts, and some bacteria should be stored in conditions of low relative humidity to increase their shelf life. This can also be done by proper wrapping of the food material also. However, variations in storage temperature should be minimal to avoid surface condensation in packed foods.

Gaseous atmosphere

Modified Atmosphere Packaging (MAP) is a long established and continuously increasing technique for extending the shelf-life of fresh food products. MAP requires specialized machinery to flush out air from the packaging and replace it with a different gas or gas mixture. The MAP packaging aims to provide longer shelf-life, maintain sensory attributes like colour or appearance and achieve the food safety of the product. The normal composition of air is 21% oxygen, 78% nitrogen and less than 0.1% carbon dioxide. Modification of the atmosphere within the package by reducing the oxygen content while increasing the levels of carbon dioxide and/or nitrogen has been shown to significantly extend the shelf-life of perishable foods at chill temperatures.

Oxygen is one of the most important gases which come in contact with food influence the redox potential and finally the microbial growth. The inhibitory effect of CO_2 on the growth of microorganisms is applied in modified atmosphere packaging of foods. The storage of foods in atmosphere containing 10% of CO_2 is referred to as “Controlled Atmosphere”. This type of treatment is applied more commonly in case of fruits such as apples and pears. With regards to the effect of CO_2 on microorganisms, molds and Gram negative bacteria are the most sensitive, while the Gram positive bacteria, particularly the lactobacilli tend to be more resistant. Some yeasts such as *Bretanomyces* spp. also show considerable tolerance of high CO_2 levels and dominates the spoilage microflora of carbonated beverages. Some microorganisms are killed by prolonged exposure to CO_2 but usually its effect is bacteriostatic. Also, the presence of CO_2 tends to decrease the pH of foods and thereby inhibiting the microorganisms present in it by adversely affecting the solute transport, inhibition of key enzymes involved in carboxylation/ decarboxylation reactions. Nitrogen (N_2) is also an inert gas that is used to expel air especially Oxygen out of the packaging. It is also used as a filling gas that equalizes the effect of CO_2 absorption by the perishable food. High O_2 atmospheres were found to be particularly effective in inhibiting enzymatic browning of the tested vegetables. Also, the microbial quality was better as a reduction in yeast growth was observed. High Oxygen MAP was found to inhibit the growth of several generic groups of bacteria, yeasts and moulds and also a range of food pathogenic organisms.