

Intrinsic and Extrinsic Parameters of Foods That Affect Microbial Growth

Growth of microorganisms in food is dependent on various parameters. The factors influencing the growth of microorganisms are physical, chemical and biological in nature. The factors can be generally classified as intrinsic and extrinsic factors.

The intrinsic and extrinsic factors affecting the growth of microorganisms in food are explained below:

Intrinsic Parameters in Food:

The parameters present in substrates in which the microorganisms are growing, that are internal parts of the substrate are called as intrinsic parameters.

1. Hydrogen Ion

Concentration (pH):

All the microorganisms have a minimal, maximal and optimal pH for their growth, survival and activity of their enzymes. Growth of microorganisms is affected by the pH of growth environments in food (growth medium) resulting large number of enzymes responsible for metabolism and growth. Influence of pH of food not only has effect on growth of microorganisms but also on processing conditions. Food having acidic contents promotes growth of acid loving microorganisms such as yeasts, moulds and some acidophilic bacteria.

Mould can grow over a wider range of acidic pH than bacteria and yeast. Most of the fermentative yeasts can grow at pH of about 4.0 to 4.5, as in fruit juices and acid food such as sauerkraut and pickles. A food with an acid pH would tend to be more microbiologically stable than neutral or alkaline food. Because of this restrictive pH the food such as fruits, soft drinks, fermented milks, sauerkraut and pickles

Most of the bacteria, except acid fermenters are favored alkaline or neutral pH. Most of the bacteria preferred a pH range between 7.0-7.5 but some proteolytic bacteria can grow on food substrate with high pH. The buffer content in the food is important to maintain the stability against microbial spoilage.

Buffers permit an acid (or alkali) fermentation to go on longer with a greater yield of products and organisms. Vegetable juices have low buffering capacity permitting a decrease in pH with the production of only small amount of acid by the lactic acid bacteria during the early stage of sauerkraut and pickle fermentation. This helps to inhibit the growth of pectin hydrolyzing and proteolytic competing bacteria in 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. The inhibitory properties of

2. Water Activity or Moisture Content (a_w):

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 a_w . 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).

In other words it is defined by the ratio of the water vapor pressure of food substrate to the vapor pressure p of pure water at the same temperature – $a_w = p/p_o$, where P is the vapor pressure of the solution and P_o is the vapor pressure of the solvent (usually water). The a_w content is very well related to relative humidity (RH) in the following way: $RH = 100 \times a_w$.

Pure water has an a_w of 1.00, a 22% NaCl solution (w/v) has an a_w of 0.86, and a saturated solution of NaCl has an a_w of 0.75. The water activity (a_w) of most fresh foods is above 0.99. In general, bacteria require more water activity than moulds and yeasts. 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 moulds 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_K 0.94. Moulds differ considerably in optimal a_w for vegetative growth and spore germination.

The lowest a_w value for foodborne bacteria is 0.75 for halophiles (“salt-loving”), whereas xerophilic (“dry-loving”) moulds and osmophilic (preferring high osmotic pressures) yeasts have been reported to grow at a_w values of 0.65 and 0.61. The lowest water activity values

permitting growth of spoilage microorganisms is given in the Table 3.1.

Table 3.1: Lowest a_w values for different types of microorganisms spoiling food

Group of Microorganism	Minimal (a_w) value
Bacteria	0.91
Yeasts	0.88
Moulds	0.80
Halophilic bacteria	0.75
Xerophilic fungi	0.65
Osmophilic yeasts	0.60

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 other factors which influence the water activity are the kinds of solute employed to reduce water activity, the nutritive significance of culture medium, temperature, supply of

3. Redox Potential (Eh):

The reducing and oxidizing power of the food will influence the type of organism and chemical changes produced in the food. The concentration of oxygen in food, 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 a food may be defined as the ease with which the substrate loses or gains electrons.

The Redox potential of food is determined by characters such as:

- (a) Oxygen tension of atmosphere above the food,
- (b) Access of atmosphere to the food,
- (c) Resistance of food to the changes occurring and
- (d) O-R state of materials present in food.

On the basis of the ability of microorganism to utilize oxygen,

organisms are classified as aerobic, anaerobic and facultative anaerobes. Aerobes require free oxygen and anaerobes don't prefer oxygen as it is toxic to them, hence, it is grow in the absence of molecular oxygen. Facultative may grow both aerobic and anaerobic conditions.

Generally fungi- mould and yeasts are aerobic. But bacteria are variables of these aspects. Some are aerobic, some are anaerobics and others are facultative anaerobes. If oxidation potential is high then aerobes will grow better than anaerobes, but if conditions become more reduced then anaerobes will be the predominant organisms.

The O-R potential is written as Eh and measured and expressed as millivolts (mV). If the substrate is highly oxidized would have a positive Eh and substrate is reduced is a negative Eh. Aerobic microorganisms such as bacilli, cocci, micrococci, pseudomonas, acinetobacters require and grow at positive O-R potential and anaerobe such as Clostridia and bacteriodes require negative O-R potential 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 antioxidants.

Fresh vegetables, fruits and meat promote growth of aerobic microorganisms in the surface regions 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

4. Composition of Nutrients:

Nutrients are one of the most important compounds for the growth and functioning of microorganism. Nutritional quality of food depends on the chemical composition, nutritive value or nutrients, their proportion and growth promoting ability to the microorganisms.

The most important factors which have to be considered are the energy substances in food, nitrogen substances, growth promoting substances, accessory food substances or vitamins, minerals, and water content which all are very essential for growth or energy production of organisms.

The most energy sources of organisms are carbohydrates. Complex carbohydrates such as cellulose, hemicelluloses, starch, pectin, etc. can be utilized by various types of microorganisms. At the same time other carbon compounds such as esters, alcohols, peptides, amino acids, organic acid and their salt are also serving as energy sources for many organisms.

Bacteria are identified and classified based on their ability or inability to utilize various sugars and alcohols. Most organisms can hydrolyse complex carbohydrates and can use glucose as energy source. Some organisms have the ability to hydrolyze pectin by producing the enzyme pectinase.

Some microorganism can hydrolyze triglycerides and other types of fats by microbial lipase and produces glycerol and smaller fatty acid. In this step triglycerides are hydrolyzed in to diglycerides then monoglycerides and glycerols under alkaline condition by microbial lipase. The glycerol and fatty acids are excellent sources of carbon and energy sources of many aerobic organisms.

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Hydrolytic products of proteins and peptides serve as sources of nitrogen for many proteolytic bacteria such as *Pseudomonas* spp. The primary nitrogen sources utilized by heterotrophic microorganisms are amino acids. A large number of other nitrogenous compounds may serve this function for various types of organisms. Some microbes are able to utilize nucleotides and free amino acids, whereas others are able to utilize peptides and proteins.

In general, simple compounds such as amino acids will be utilized by almost all organisms before any attack is made on the more complex compounds such as high-molecular-weight proteins. Protein rich food promotes more growth of bacteria than moulds and yeasts.

Some microorganisms require vitamins and other growth factors for their growth and that has to be supplied with the growth medium. Such microorganisms are called fastidious organisms. Food contains different vitamins, minerals and other growth factors and their composition and

content may vary.

Fresh plant and animal food contain vitamin B complex 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 cause's 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.

Water is another very important component for food nutrients. The water requirement of organisms will depend on the type of organisms. Generally moulds have the lowest requirement, followed by gram-negative bacteria, yeasts, and gram-positive bacteria.

5. Inhibitory Substances:

Inhibitory substances are present in the food as its own origin, or added purposely by preventing or inhibiting the growth of organisms. The stability of certain 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. 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 are some of the best studied examples. Milk contains several antimicrobial substances, including lactoferrin, conglutinin and the lactoperoxidase system.

Milk casein and some fatty acids have been shown to be antimicrobial property against some organisms. Lactoferrin is an iron-binding glycoprotein that is inhibitory to a number of foodborne bacteria and its use as a microbial blocking agent on beef carcasses. Eggs contain lysozyme; ovotransferrin and conalbumin have shown some antimicrobial properties.

6. Biological Structures:

The natural covering of some foods provides excellent protection against the entry and subsequent damage by spoilage organisms. The inner part of healthy tissues of living plants and animals are sterile and contains less microbial count. The protective covering of food such as the skin of eggs, the skin on poultry, rind on fruits and vegetables, shell on nuts and artificial coating helps to protect its inner structures from microbial contamination and spoilage.

Extrinsic Parameters:

The extrinsic parameters are substrate independent and in this case the storage environment that affect both the food and their microorganisms.

The main extrinsic parameters influence the foods are:

1. Relative Humidity (RH):

The relative humidity of the storage environment is important extrinsic parameter both from the standpoint of a_w within foods and the growth of microorganisms at the surfaces. When foods with low a_w contents are placed in high RH environments, the foods takes up more moisture until equilibrium has been established.

Similarly 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. Generally, if the

temperature high then the RH low and vice versa.

2. Atmospheric Gases:

Like O_2 , Carbon dioxide (CO_2) is also most important atmospheric gas that is used to control microorganisms in foods. Modified atmosphere packaged (MAP) foods are make use of this types of gases. Ozone (O_3) is the other atmospheric gas that has high antimicrobial properties; it has strong oxidizing property hence it should not use for fat rich food as it will undergo auto-oxidation. It has been noticed that ozone extend the shelf life of many foods and it has shown to be effective against a variety of microorganisms.

3. Temperature:

Microorganisms can grow over a wide range of temperatures. The lowest temperature at which a microorganism has been reported to grow is -34°C ; the highest is somewhere in excess of 100°C . But some spore producing bacteria such as *Bacillus stearothermophilus*, *Clostridium tetani* and *Clostridium perfringens* can grow above 100°C .

Based on the temperature range microorganisms are classified as three groups –

- i. Psychrophiles (Psychrotrophs), those organisms are grown between the temperature ranges of 2°C to 20-30°C.
- ii. Mesophiles, the organism preferably grow at the temperature between 20°C and 45°C and
- iii. Thermophiles, the organisms grow better in range of 55°C-65°C.

The most important psychrotrophs include *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 temperature and cause spoilage at 5-7°C of meats, fish, poultry, eggs, and other foods normally held at this temperature. Mesophilic species and strains are known bacteria among all genera and may be found on food held at refrigerator

Like bacteria fungi are also able to grow over wide ranges of temperature. Many moulds are able to grow at refrigerator temperatures, especially some strains of *Aspergillus*, *Cladosporium*, and *Thamnidium*, which may be found growing on eggs, sides of beef and fruits. Yeasts prefer psychrotrophic and mesophilic temperature ranges but generally not within the thermophilic range.

4. Other Microbial Flora:

Microorganisms present in the food can undergo various types of negative interactions. These kinds of interaction cause inhibition of some microorganisms as they are undergoing competitions and antibiosis. Some organisms especially moulds can produce various types of secondary metabolites such as antibiotics that are toxic to many bacteria. Some foodborne organisms produce substances that are either inhibitory or lethal to others; these include bacteriocins, hydrogen peroxide and organic acids.