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Module
on

Intrinsic And Extrinsic Factors Affecting Microbial Growth In Food

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TEXT

Detailed Report

I. Introduction

Microorganisms like other living things need favourable conditions for growth, which can slightly or largely vary among microorganisms. Some of these conditions, generally considered as factors, influencing the growth of microorganisms are: pH, Nutrition, Moisture, Oxygen, Temperature, and Time.

Some microorganisms have the ability to produce spores when exposed to conditions outside their typical growth range. These organisms cause huge problems to the food industry as the spores are more resistant to the intrinsic and extrinsic factors that are lethal to vegetative cells. Unless a factor or treatment is targeted at the destruction of spores, they can survive in the product, and when the environmental conditions return to suitable levels, the spores are able to germinate and grow. The issue is further compounded by the fact that spores sometimes germinate earlier than would be expected as a result of heat shock if they are exposed to temperatures outside their growth range but less than their lethal limit, and can make the spores more resistant to other factors than normal. It is for this reason that foods which are subject to contamination with spore-forming organisms are often subjected to high temperature processing.

II. Different factors influencing the growth of microorganisms

As listed earlier, the key and formidable factors influencing the growth of microorganisms are: PH, temperature, nutrition, oxygen, moisture and time. Microorganisms respond differently from retard to enhanced growth according to pH value subjected to. Temperature range that is unfavourable can cause death or a retard growth. As moisture helps microorganisms to eliminate waste in solution away from the cell, it also carries substrates in solution into the cell. While some microorganisms grow only in the absence of oxygen, many grows with or without oxygen. And some requires oxygen to grow. All of these parameters mentioned above essentially interplay one another in the growth of microorganisms.

I. Role of intrinsic factors on the microbial growth

The inherent physical, chemical and biological properties of the food, such as pH, redox potential, water activity and the presence of antimicrobial substances have the capacity to either stimulate or retard the growth of micro-organisms. Some intrinsic factors are interlinked with some extrinsic factors. For example, water activity rises with increasing temperature; there is an increase in water activity of 0.03 with a 10 °C rise in temperature.

a) pH (hydrogen ion concentration, relative acidity or alkalinity)

The pH range of a microorganism is defined by a minimum value (at the acidic end of the scale) and a maximum value (at the basic end of the scale). There is a pH optimum for each microorganism at which growth is maximal. Moving away from the pH optimum in either direction slows microbial growth.

The control of intracellular pH is required in order to prevent the denaturation of intracellular proteins. Each organism has a specific requirement and pH tolerance range; some are capable of growth in more acid conditions than others. 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.

The type of microbial growth typically seen in a particular food is partly related to the pH of that product. Fruits are naturally acidic, which inhibits the growth of many bacteria, therefore spoilage of these products is usually with yeasts and moulds. Meat and fish however have a natural pH much nearer neutral and they are therefore susceptible to the growth of pathogenic bacteria. Individual strains of a particular species can acquire acid resistance or acid tolerance compared to the normal pH range for that organism. For example acid-adapted *Salmonella* have been reported that are capable of growth at pH 3.8.

There is a broad distinction between high and low acid foods; with low acid foods being those with a pH above 4.6, and high acid, below this. This is because pH 4.6 is the lower limit for the growth of mesophilic *Clostridium botulinum*. Foods with a pH greater than 4.6 must either be chilled, or if ambient stored, undergo a thermal process to destroy *C. botulinum* spores, or have a sufficiently low water activity to prevent its growth.

Different foods tend to spoil in different ways. For example, carbohydrate-rich foods often undergo acid hydrolysis when they spoil; this usually reduces the pH, and tends to reduce the risk of pathogen growth. This principle is used in the fermentation of dairy and lactic meat fermentations. In contrast, protein-rich foods tend to increase in pH when they spoil, making them possibly less safe, as the pH rise to the zone where more pathogens can grow.

Another useful term relevant to the pH of foods is the pK_a. The term pK_a describes the state of dissociation of an acid. At equilibrium, pK_a is the pH at which the concentrations of dissociated and undissociated acid are equal. Strong acids have a very low pK_a, meaning that they are almost entirely dissociated in solution. For example, the pH (at 25 °C) of a 0.1 M solution of HCl is 1.08 compared to the pH of 0.1 M solution of acetic acid, which is 2.6. This characteristic is extremely important when using acidity as a preservation method for foods. Organic acids are more effective as preservatives in the undissociated state. Lowering the pH of a food increases the effectiveness of an organic acid as a preservative.

Another important characteristic of a food to consider when using acidity as a control mechanism is its buffering capacity. The buffering capacity of a food is its ability to resist changes in pH. Foods with a low buffering capacity will change pH quickly in response to acidic or alkaline compounds produced by microorganisms as they grow. Meats, in general, are more buffered than vegetables by virtue of their various proteins.

b) Redox Potential

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 looses 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 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. pH of the food has a bearing on the redox potential and for every unit decrease in the pH the Eh increases by ٥٨ mV. 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.

The redox potential of a food therefore has an impact on microbial growth. Aerobic organisms require a food to have a positive redox potential (an oxidized state) whereas anaerobes require a negative potential (a reduced state) for growth. It should be noted that the presence of oxygen is not an absolute requirement for oxidation reduction reactions as other compounds can accept electrons. Different foods have distinct redox potentials and these influence the type of microbial growth typically seen in that food. Foods of plant origin typically have a redox potential of +300 to 400 mV thereby favoring the growth of aerobic bacteria and moulds. Solid meat typically has a redox value of -200 mV and therefore anaerobic organisms are associated with this food type.

c) Water Activity

Water activity (a_w) is a measure of the amount of freely available water within a food. The a_w of a food can be expressed as the ratio of the water vapour pressure of the food to the water vapour pressure of pure water at the same temperature. Water activity, in practice, is measured as Equilibrium Relative Humidity (ERH) and is given by the formula:

$$\text{Water Activity } (a_w) = \text{ERH} / 100.$$

Water is required for microbial growth; therefore foods with low water activities cannot support the growth of microorganisms. Pathogenic and spoilage bacteria do not grow in food with a water activity of less than 0.85. Many yeasts and moulds however are capable of growth at much lower water activities than this; some can even grow at a_w 0.60. The water activity of a food can be altered from the value typical for a food type in order to prevent microbial growth via the addition of solutes or ions or by freezing or drying. It is as a result of water activity that dry foods such as crackers or dried pasta can have a shelf life of many months and not be spoilt by micro-organisms. Foods such as jams and parmesan cheese (a_w 0.60 - 0.85) will show signs of mould growth over time but no bacterial growth, and foods such as meat and milk (a_w 0.98 - 0.99) are associated with food poisoning causing bacteria. In general, the water activity requirement of microorganisms decreases in the following order: Bacteria > Yeast > Mold. Below ٠,٦٠, 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. 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.

d) Antimicrobials

Certain foods naturally contain antimicrobial substances that will exhibit an inhibitory action on the growth of micro-organisms. Examples are essential oils contained within cloves, garlic, mustard and thyme, lactoferrin in cow's milk and lysozyme in eggs. Similarly, casein as well as free fatty acids found in milk also exhibit antimicrobial activity. The hydroxycinnamic acid derivatives (p-coumaric, feluric, caffeic and chlorogenic acids) found in fruits, vegetables, tea and other plants possess antibacterial and antifungal 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.

It is also known that some types of food processing result in the formation of antimicrobial compounds in the food. The smoking of fish and meat can result in the deposition of antimicrobial substances onto the product surface. Maillard compounds resulting from condensation reactions between sugars and amino acids or peptides upon heating of certain foods can impart some antimicrobial activity. Smoke condensate includes phenol, which is not only an antimicrobial, but also lowers the surface pH. Some processors also lower the surface pH with liquid smoke to achieve an unsliced shelf stable product.

e) Nutrient content

Nutrients are of vital importance to the growth of microorganisms. The nutrients required are basically those that supply energy, carbon and other materials. The famous nutrients which are needed by all living things include nitrogen, carbon, sulphur, phosphorus, potassium, magnesium, oxygen, calcium, iron and additional trace elements

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.

f) Biological structure

Plant and animal derived foods, especially in the raw state, have biological structures that may prevent the entry and growth of pathogenic microorganisms. Examples of such physical barriers include testa of seeds, skin of fruits and vegetables, shell of nuts, animal hide, egg cuticle, shell, and membranes. Plant and animal foods may have pathogenic microorganisms attached to the surface or trapped within surface folds or crevices. Intact biological structures thus can be important in preventing entry and subsequent growth of microorganisms. Several factors may influence penetration of these barriers. The maturity of plant foods will influence the effectiveness of the protective barriers. Physical damage due to handling during harvest, transport, or storage, as well as invasion of insects can allow the penetration of microorganisms. During the preparation of foods, processes such as slicing, chopping, grinding, and shucking will destroy the physical barriers. Thus, the interior of the food can become contaminated and growth can occur depending on the intrinsic properties of the food. For example, Salmonella spp. have been shown to grow on the interior of portions of cut cantaloupe, watermelon, honeydew melons, and tomatoes, given sufficient time and temperature. Fruits are an example of the potential of pathogenic microorganisms to penetrate intact barriers. After harvest, pathogens will survive but usually not grow on the outer surface of fresh fruits and vegetables. Growth on intact surfaces is not common because foodborne pathogens do not produce the enzymes necessary to break down the protective outer barriers on most produce. This outer barrier restricts the availability of nutrients and moisture. Survival of foodborne pathogens on produce is significantly enhanced once the protective epidermal barrier has been broken either by physical damage, such as punctures or bruising, or by degradation by plant pathogens (bacteria or fungi). These conditions can also promote the multiplication of pathogens, especially at higher temperatures. Infiltration of fruit was predicted and described by Bartz and Showalter (1981) based on the general gas law, which states that any change in pressure of an ideal gas in a closed container of constant volume is directly proportional to a change in temperature of the gas. In their work, Bartz and Showalter describe a tomato; However, any fruit, such as an apple, can be considered a container that is not completely closed. As the container or fruit cools, the decrease in internal gas pressure results in a partial vacuum inside the fruit, which then results in an influx from the external environment. For example, an influx of pathogens from the fruit surface or cooling water could occur as a result of an increase in external pressure due to immersing warm fruit in cool water. Internalization of bacteria into fruits and vegetables could also occur due to breaks in the tissues or through morphological structures in the fruit itself, such as the calyx or stem scar. Although infiltration was considered a possible scenario, the panel concluded that there is insufficient epidemiological evidence to require refrigeration of intact fruit.

The egg is another good example of an effective biological structure that, when intact, will prevent external microbial contamination of the perishable yolk; Contamination is possible, however, through transovarian infection. For the interior of an egg to become contaminated by microorganisms on the surface, there must be penetration of the shell and its membranes. In addition, the egg white contains antimicrobial factors. When there are cracks through the inner membrane of the egg, microorganisms penetrate into the egg. Factors such as temperature of storage, relative humidity, age of eggs, and level of surface contamination will influence internalization. For example, conditions such as high humidity and wet and dirty shells, along with a drop in the storage temperature will increase the likelihood for entry of bacteria. If eggs are washed, the wash water should be 12 °C (22 °F) higher than the temperature of the eggs to prevent microbial penetration. After washing, the eggs should be dried and then cooled. The Food and Drug Administration (FDA) published a final rule that applies to shell eggs that have not be processed to destroy all live Salmonella before distribution to the consumer. The rule mandates that eggs should be kept dry and chilled below 7.2 °C (٤٥ °F) to prevent growth of Salmonella Enteritidis. Heating of food as well as other types of processing will break down protective biological structures and alter such factors as pH and aw . These changes could potentially allow the growth of microbial pathogens.

II. Role of Extrinsic factors in defining the microbial growth and characteristics.

The characteristics of the environment in which the food is maintained, which includes temperature, atmosphere and relative humidity, can affect the properties of the food as well as the growth of microorganisms. Some Extrinsic factors are highlighted and explained under:

a) Temperature

Principally, microorganisms thrive best within a specific range of temperature. The temperatures outside those ranges and on the extremes could mean retarded growth or death. While some species grows well in low temperature, some are good with high temperature. In practice, they are classified into three cardinal groups as Psychrophiles, mesophiles and thermophiles based on optimum growth temperature. In food microbiology mesophilic and psychrotrophic organisms are of greatest importance.

- Thermophiles have optimum growth ca. 55 °C and a growth range of 30 - 75 °C.
- Mesophiles have optimum growth ca. 35 °C and a growth range of 10 - 45 °C
- Psychrotrophs have optimum growth ca. 20 - 30 °C and a growth range of 0 - 40 °C

- Psychrophiles have optimum growth ca. 15 °C and a growth range of -5 - 20 °C

At temperatures higher than an organism's optimum growth range, cells die rapidly. Lower temperatures still result in cell death but at a slower rate. Temperature can therefore be used to eliminate or control the growth of micro-organisms. Heat treatments (pasteurisation or sterilisation) eliminate contaminating micro-organisms via the application of heat for a specific time period (time and temperatures used being dependent upon the target organism). Refrigeration of a food can prevent spoilage by controlling the growth of thermophilic or mesophilic organisms. Most pathogens are capable of growth at refrigeration temperatures and therefore cannot be controlled via refrigeration alone. Some, for example Listeria, can grow at very low temperatures.

b) Atmosphere

As all micro-organisms have specific requirements for oxygen and carbon dioxide, by altering the atmosphere within a food package the growth of micro-organisms can be controlled. Oxygen being part of the surrounding, plays a great role in the growth of microorganisms. While some microbes use oxygen to propagate energy (obligate aerobes), some cannot survive the presence of oxygen (obligate anaerobes). And some can live with or without oxygen (facultative anaerobes).

Oxygen forms toxic by-products within the cells of microbes. These by-products such as Super Oxide, Hydrogen Peroxide and hydroxyl radicals have a great interference with the chemical activities in the cell. What happens is that the obligate aerobes and facultative anaerobes produce enzymes that detoxify these oxygen by-products. The most prominent enzymes are catalase which breaks down hydrogen peroxide (H2O2) and Superoxide Dismutase which breaks down Superoxide.

Oxygen is one of the most important gases which comes in contact with food, thereby influencing the redox potential and finally the microbial growth. The inhibitory effect of CO ٢ on the growth of microorganisms is applied in modified atmosphere packaging of foods. The storage of foods in atmosphere containing 10% of CO2 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 CO2 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 CO2 levels and dominates the spoilage microflora of carbonated beverages. Some microorganisms are killed by prolonged exposure to CO2 but usually its effect is bacteriostatic. Also, the presence of CO2 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.

Vacuum packing food removes available oxygen and thereby prevents the growth of aerobic organisms; it does however still allow the growth of anaerobes such as C. botulinum. Modified atmosphere packaging (MAP) allows the food producer to select the atmosphere within the package using varying combinations of oxygen, carbon dioxide and nitrogen, depending upon the product type and target micro-organisms. The majority of MAP foods have varying combinations of carbon dioxide and nitrogen.

c) Relative Humidity

There is a direct link between Relative humidity and water activity. When foods with low a_w are stored in environment of high humidity, water will transfer from the gas phase to the food and thus increasing a_w of the food leading to spoilage by the viable flora. There is a relationship between temperature and humidity. 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.

There are some other factors, which have impact on growth. Among them one is time:

III. Impact of time on microbial growth

Microorganism needs time to grow and multiply. Under favourable condition, cell division may occur over 20 to 30 minutes (Julie and Susan 2014). According to Thomas et al. (1994), the time gap for the formation of two cells from one is called a generation, and the time required for this to happen is called the generation time. In food industries, time is used as great tool in mapping out the product's shelf life as it relates to microbial growth.

Hurdle concept in the context of microbial growth

Hurdle technology is a method of ensuring that pathogens in food products can be eliminated or controlled. This means the food products will be safe for consumption, and their shelf life will be extended. Hurdle technology usually works by combining more than one approach. These approaches can be thought of as "hurdles" the pathogen has to overcome if it is to remain active in the food. The right combination of hurdles can ensure all pathogens are eliminated or rendered harmless in the final product.

Hurdle technology has been defined by Leistner (2000) as an intelligent combination of hurdles which secures the microbial safety and stability as well as the organoleptic and nutritional quality and the economic viability of food products. The organoleptic quality of the food refers to its sensory properties that is its look, taste, smell and texture.

Examples of hurdles in a food system are high temperature during processing, low temperature during storage, increasing the acidity, lowering the water activity or redox potential, or the presence of preservatives. According to the type of pathogens and how risky they are, the intensity of the hurdles can be adjusted individually to meet consumer preferences in an economical way, without compromising the safety of the product.

There is an increasing demand from consumers for minimal processing of the food that they eat. Therefore the hurdle concept has increasingly been used to preserve foods. The hurdle concept controls food safety and spoilage by ensuring that a number of factors are in place that prevent the growth of micro-organisms rather than one single controlling factor at a level beyond the range of the target organism. The benefit of hurdle technology is that the controlling factors can be at sub-optimal limits for the micro-organisms concerned rather than at lethal limits, and that when used in combination they can control microbial growth, the concept being that by placing small barriers against micro-organism growth by the various intrinsic and extrinsic influences, the micro-organisms are unable to overcome all the small 'hurdles' and are unable to grow. Also as cells are injured by the conditions of one hurdle, they become more sensitive to the other hurdles and are killed.

IV. Interplay between the two broad categories of factors. viz, Intrinsic and extrinsic factors

Although each of the major factors listed above plays an important role, the interplay between the factors ultimately determines whether a microorganism will grow in a given food. Often, the results of such interplay are unpredictable, as poorly understood synergism or antagonism may occur. Advantage is taken of this interplay with regard to preventing the outgrowth of *C. botulinum*. Food with a pH of 5.0 (within the range for *C. botulinum*) and a_w **of ٠،٩٣٥ (above the minimum for** *C. botulinum*) may not support the growth of this bacterium. Certain processed cheese spreads take advantage of this fact and are therefore shelf stable at room temperature even though each individual factor would permit the outgrowth of *C. botulinum*.

Therefore, predictions about whether or not a particular microorganism will grow in a food can, in general, only be made through experimentation. Also, many microorganisms do not need to multiply in food to cause disease.