FACTORS AFFECTING THE GROWTH OF MICRO-ORGANISMS IN FOODS
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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. Growth is possible over a wider range of temperatures than that of toxin production. For example, *Aspergillus flavus* can grow from 10 - 12 °C to 43 - 48 °C, whereas aflatoxins are produced from 13 - 15 °C up to 37 °C. The minimum water activity (a_w) for *A. flavus* is 0.78 - 0.80, but aflatoxins are produced above a_w 0.82 - 0.83.

Some organisms have the ability to produce spores when exposed to conditions outside their typical growth range. These organisms pose difficulties for 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 destruction of the 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 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.

INTRINSIC FACTORS

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.
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**pH**

The intracellular pH of any organism must be maintained above the pH limit that is critical for that organism. 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. Most micro-organisms grow best at neutral pH (7.0). Yeasts and moulds are typically tolerant of more acidic conditions than bacteria but several species of bacteria will grow down to pH 3.0. These species are typically those that produce acid during their metabolism such as the acetic or lactic acid bacteria. Bacterial pathogens are usually unable to grow below pH 4.0. 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.

**Redox Potential**

Also known as the oxidation-reduction potential or Eh, the redox potential of a food has an impact on microbial growth. Aerobic organisms require a
food to have a positive redox potential (an oxidised 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 favouring 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.

**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. Equilibrium relative humidity values can be converted to $a_w$ by dividing by 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.

**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 cows’ milk and lysozyme in eggs. Some plants release isothiocyanates which have antibacterial and antifungal effects.
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EXTRINSIC FACTORS

The characteristics of the environment in which the food is maintained, such as the temperature, atmosphere and relative humidity can affect the properties of the food as well as the potential for the growth of micro-organisms.

Temperature

As temperature influences enzymic reactions it has an important role in promoting or preventing microbial growth. Micro-organisms can be categorised into one of four groups depending on their optimum growth temperature and the temperature range at which they will grow.

- 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.
MICRO-FACTS

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. 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.

Relative Humidity

The relative humidity in which a food is stored can have an influence on the water activity of that product and an influence on the growth of micro-organisms on the surface of a product. If the growth of micro-organisms in a food is controlled by the water activity of a product then it is very important that the food be stored under relative humidity conditions which will not allow the uptake of moisture from the air, and therefore an increase in water activity. Packaging can be used to limit the migration of moisture into the product.

HURDLE CONCEPT

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.
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in their presence. Therefore mild but reliable preservation can be achieved. Combinations of water activity, temperature and pH can be used. For example, *Listeria monocytogenes* is inhibited by 15% salt, pH 4.1 or a temperature at 0 °C or below. Whereas if a product has a combination of 5% salt and a pH of 5.5, then a temperature of 14.1 °C is sufficient.
Micro-Facts
The Working Companion for Food Microbiologists
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