Factors affecting microbial growth in food

RNDr. Jarmila Pazlarová, PhD.
Factors affecting microbial growth in food

- Intrinsic Factors
- Environmental Factors
- Implicit Factors
- Processing Factors
Intrinsic Factors

• Nutrients
• pH and buffering capacity
• Redox potential
• Water activity
• Antimicrobial constituents
• Antimicrobial structures
Nutrient content

- The concentration of key nutrients can, to some extent, determine the rate of microbial growth.
- The relationship between the two, known as the Monod equation, is mathematically identical to the Michaelis-Menten equation of enzyme kinetics.
- It reflects the dependence of microbial growth on rate-limiting enzyme reaction.
Monod equation

\[ \mu = \mu_m S / (S + K_s) \]

- \( \mu \) specific growth rate
- \( \mu_m \) maximum specific growth rate
- \( S \) concentration of limiting nutrient
- \( K_s \) saturation constant
pH and buffering capacity

- As measured with the glass electrode, pH is equal to the negative logarithm of the hydrogen ion activity.

- For aqueous solutions pH 7 corresponds to neutrality, pH values below 7 are acidic and those above 7 indicate an alkaline environment.

- In general bacteria grow fastest in the pH range 6.0-8.0, yeasts 4.5-6.0 and filamentous fungi 3.5-10.0.
Redox Potential - $E_h$

An oxidation-reduction potential of environment
- Difference of platinum electrode potential placed into given environment and potential of standard hydrogen electrode.

oxidace

oxidated matter + $n$ elektrons

reduced matter

redukce

Reduction of redox potentialu
- Addition of reducting compounds
- Growth of aerobic microorganisms
- Vakuum package, etc.
Water activity - $a_w$

**Water activity**

- Is defined as the ratio of the partial pressure of water in the atmosphere in equilibrium with the substrate $p$ ($p$), compared with the partial pressure of the atmosphere in equilibrium with pure water at the same temperature ($p_0$).

$$a_w = \frac{p}{p_0}$$

- Amount of water available for microorganisms to metabolize

- Optimal value for majority of microorganisms $a_w > 0.98$
Water activity - $a_w$
Lowered Water Activity ($a_w$)

Lowering $a_w$ is one of the earliest methods of food preservation used by Man - (partial) drying, addition of salt or sugar

Still part of the modern diet - e.g. jams, hams, hard cheeses, pasta etc.

Many modern bakery goods, snack foods and cereals are preserved using the ancient methodology combined with modern processing techniques

Removing water by (partial) drying)

$a_w$ can be lowered by

Addition of solutes (preserving)

Foods can be classified by their $a_w$ value:

- Fresh foods
  - ≥ 0.98
- “Wet foods”
  - 0.86 – 1.00
- Intermediate Moisture Foods (IMF)
  - 0.60 – 0.85
- “Dried foods”
  - < 0.60

Bacteria can grow down to $a_w = 0.85$ (S. aureus and some salmonellae) and moulds to $a_w = 0.62$

IMF contain 15 – 30% moisture and include dried fruits, jams, and some cakes and sauces
**a_w - Microbial response**

Microbial response to lowered $a_w$

- Accumulation of Compatible Solutes
- Modification of Membrane Lipid Composition

Compatible solutes (CS) are accumulated intracellularly

Typical CS accumulated by food-associated microbes include:

- Betaine and derivatives
- Peptides, amino acids (*e.g.* glutamate, proline)
- Carnitine
- Trehalose, sucrose
- Mannitol, glycerol
- $K^+$

There is no correlation between the ability to withstand low $a_w$ and the type of CS accumulated

Bacteria tend to accumulate N-compounds, moulds mainly sugars/sugar alcohols
Lowered $a_w$ - Microbial response

- Lowered $a_w$
  - Cell senses change via two-component regulatory system
    - Influx of $K^+$ ions (universal compatible solute)
      - Altered gene expression
        - Altered gene products
          - Accumulation of CS
          - Altered membrane lipids
            - Restoration and maintenance of cell enzyme and membrane function
Antimicrobial Barriers and Constituents

- **Physical barrier** to infection: skin. Shell, husk or rind of the product. Usually composed from **macromolecules** relatively **resistant to degradation** and provides unhospitable environment for microorganisms by having a low water activity, a shortage of readily available nutrients and often **antimicrobial compounds** such as **short chain fatty acids** (on animal skin) or **essential oils** (on plant surfaces).
Effect of antimicrobial substances

Some substances present in environment display negative effect on microorganisms, based on their specific composition (antimicrobial).

**Microbistatic**
- compounds stop division of microorganism

**Microbicidal**
- compounds killing microorganisms

Effect of concentration (stimulatory effect)
Types of antimicrobial effects

Compounds damaging **structure of cell** or its function (cell wall, cytoplasmatic membrane, ribosomes)

Compounds affecting **microbial enzymes** (oxidative agents, chelating agents, heavy metals, antimetabolites)

Compounds reacting with **DNA** (chemical mutagens - alkylating or deaminating agents, cytostatics)
Presence of antibacterial compounds (biocides) in food

- Some foods contain natural antimicrobial compounds (spices, mineral oils, garlic, mustard, honey)

- Raw cow milk contains lactoferrin, lactoperoxidase system, lysozyme, kasein

- Eggs contain lysozyme, conalbumin, ovotransferrin, avidin
Antimicrobial enzymes

They are abundantly spread in nature and are very important in defensive reactions between living microorganisms.

**Hydrolases** - degrade basic structures of the cell walls (peptidoglycan)

**Oxidoreduktases** - produce reactive molecules, that degrade vital cell proteins.
Antimicrobial enzymes

Bacteriolytic
1. **N-acetylhexosaminidases** catalyze splitting of glucosidic bonds of saccharides in peptidoglycan
2. **N-acetylmuramyl-L-alaninamidases** catalyze splitting between saccharidic peptidic part of peptidoglycan
3. **Endopeptidases** hydrolyze peptidic bonds of peptidoglycan
4. **Others** - chitinases, β-glucanase
Antimicrobial enzymes

Oxidoreductases

**Glucosoxidases** are produced by some molds, princip of cytotoxicity lies in the formation of hydrogen peroxide (glucose oxidation on gluconic acid and \( \text{H}_2\text{O}_2 \)).

**Lactoperoxidases** occur in milk and saliva (thiocyanate oxidation on hyperthiocyanate).

**Lactoferrin** is a glycoprotein forming complexes with Fe ions.
Environmental factors

- Relative humidity
- Temperature
- Gaseous atmosphere
Relative humidity

• Relative humidity and water activity are interrelated, thus relative humidity is essential a measure of the water activity of the gas phase.

• When food commodity having a low water activity are stored in an atmosphere of high relative humidity - water will transfer from the gas phase to the food.
Temperature

Is one of the most important environmental factors, controlling the rate of cell division (multiplication) of microorganisms.

- We recognize 3 basic temperatures:
  - minimal temperature
  - optimal temperature
  - maximal temperature
Division of bacteria according to their relation to temperature

Microorganisms are divided into groups according to their demands on optimal temperature for division and metabolisms.

- Psychrophilic bacteria (12-15 °C)
- Psychrotrophic bacteria (25-30 °C)
- Mezophilic bacteria (30-40 °C)
- Thermophilic bacteria (50-70 °C)
Microorganisms belong to groups according to optimal temperature for division and metabolism:

- **Psychrophilic bacteria** (12-15 °C)
- **Psychrotrophic bacteria** (25-30 °C)
- **Mesophilic bacteria** (30-40 °C)
- **Thermophilic bacteria** (50-70 °C)
Effect of high temperatures

Killing effect of high temperatures (lethal temperature)

- is the lowest temperature, at which under certain time are all microorganisms killed (70 °C/10 min)
- denaturation of proteins, enzyme inactivation, DNA and cytoplasmatic membrane disruption
- is dependent on: species of microorganisms
  its physiologic status
  cell concentration
  environment character
Thermoresistance

Degree of microorganisms resistance depends on:

- fysiologic status of bacteria
- their genetic properties
- amount of bacteria
- water content in substrate
- quantity of protective compounds (lipids, proteins, saccharides)
Appertization

Processes where the only organisms that survive processing are non-pathogenic and incapable of developing within the product under normal conditions of storage.

- Appertized products have a long shelf-life even when stored at ambient temperature.
Pasteurisation

Properly done pasteurisation assure:

- devitalisation of pathogen microorganisms *(M. tuberculosis)*
- devitalisation of substantial portion of saprophytic microflora (vegetative cells)
- conservation of original fysical, chemical, nutritive and sensoric qualities

**PASTERISED FOOD CONTAIN MICROORGANISMS!**
There are 4 main types of heat treatment:

- Low Temperature Holding (LTH) 63°C for 30 minutes
- High Temperature Short Time (HTST) 72°C for 15 seconds
- Ultra High Temperature (UHT) 135°C for 1 second
- “Sterilised” >100°C for 20 – 40 minutes

LTH has been superceded by HTST pasteurisation nowadays (shelf-life of 10 – 20 days) and membrane filtration is also being explored to produce quasi-sterile milk.

UHT milk is essentially sterile and there is no problem with Clostridium botulinum as it is rare in milk and the oxygen levels are too high and the heat treatment sufficient.

Pasteurisation is monitored by enzyme assay of alkaline phosphatase.

Gram-negative psychrotrophs are easily killed by pasteurisation, but some “thermoduric” Gram-positives (e.g. B. cereus) do survive.

Spoilage of milk is usually by Gram-negative rods (Pseudomonas, Alcaligenes, Acinetobacter and Psychrobacter), which are post-pasteurisation contaminants.
Sterilisation

• One-time application of temperature higher than 100 °C

• Sterilisation is defined as combination of temperature and time

• Sterilisation of food survive certain spores (genus Bacillus and Clostridium)

• Practical (commercial) sterility x absolute sterility

STERILISED FOOD MAY CONTAIN SPORES!
Quantifying the thermal death of microorganisms

- When the temperature is increased above the maximum for growth, cells are injured and killed as key cellular components are destroyed.
- The generally accepted view is that thermal death is a first order process, it means, the rate of death depends upon the number of viable cells present.

\[
d\frac{N}{dt} = -cN
\]

- \(d\frac{N}{dt}\) - is the rate of death,
- \(N\) — the number of present viable cells
- \(C\) — proportionality constant
D value

- As the temperature is increased so the D value decreases. This is an exponential process over the range of temperatures used in the heat processing of food so that plotting log D against temperature gives a straight line.

- From this is possible to derive another important parameter in heat processing, $z$: the temperature change which results in a tenfold (1 log) change in D.
Thus after an organism is reduced by 1 D, only 10% of the original organisms remain. The population number has been reduced by one decimal place in the counting scheme. Generally, each lot of a sterilization-resistant organism is given a unique D-value.

When referring to D values it is proper to give the temperature as a subscript to the D.

For example, a hypothetical organism is reduced by 90% after exposure to temperatures of 60 degrees Celsius for 2 minutes, Thus the D-value would be written as

\[ D_{60}^\circ F = 2 \text{ minutes} \]

D-value determination is often carried out to measure a disinfectant's efficiency to reduce the number of microbes, present in a given environment.
Thermal Resistance

• **D-Value:**
  – Time at a given temperature needed to reduce a microbial population by 90%

• **Z-Value:**
  – Change in temperature needed to change D-value by 90%
Z - value

- **Z-value** of an organism is the temperature, in degrees Celsius or Fahrenheit, that is required for the organism to move (decrease) one log cycle.
- It is the reciprocal of the slope resulting from the plot of the logarithm of the D-value versus the temperature at which the D-value was obtained.
- It may be simplified as the temperature required for one log reduction in the D-value.
- While the D-value gives us the time needed at a certain temperature to kill an organism, the z-value relates the resistance of an organism to differing temperatures.
Z - value

- So, the z-value allows us to calculate a thermal process of equivalency, if we have one D-value and the z-value.
- So, if it takes an increase of 10°C to move the curve one log, then our z-value is 10.
- So then, if we have a D-value of 4.5 minutes at 65°C, we can calculate D-values for 65°C by reducing the time by 1 log.
- So, our new D-value for 65°C is 0.45 minutes. This means that each 10°C increase in temperature will reduce our D-value by 1 log. Conversely, a 10°C decrease in temperature will increase our D-value by 1 log.
- So, the D-value for a temperature of 55°C would be 45 minutes.
Microbial heat resistance

- *Salmonella* sp.  \( D_{65} \) 0.02 – 0.25
- *Staphylococcus aureus*  \( D_{65} \) 0.2 – 2.0
- *Escherichia coli*  \( D_{65} \) 0.1
- *Listeria monocytogenes*  \( D_{60} \) 5.0 – 8.3
- *Campylobacter jejuni*  \( D_{55} \) 1.1
- Yeasts and molds  \( D_{65} \) 0.5 – 3.0
Thermal Death Time Curves for 2 *Enterobacter sakazakii* Strains Heated at 58°C

- Thermal Death Time, D = 30.5 sec
- Thermal Death Time, D = 591.9 sec
Comparison of $D_{58^\circ C}$-Values for Different Enterobacteriaceae

- E. sakazakii 607
- E. coli O157:H7
- E. sakazaki N&F-pooled
- K. pneuomoniae
- Salmonella Hartford
- E. coli
- E. aerogenes
- E. sakazakii 51329
Gaseous atmosphere

- Oxygen forms 21% of the earth atmosphere.
- Effect of carbon dioxide is not uniform.

- Anaerobic microorganisms
- Aerobic microorganisms
- Facultatively anaerobic microorganism
- Microaerobic microscopic organisms
Factors affecting microbial growth in food

Implicit factors

• Implicit factors:
• Specific growth rate / $\mu$
• Synergism
• Antagonism
• Commensalism
Factors affecting microbial growth in food

**Processing factors**

- Processing factors:
  - Slicing
  - Washing
  - Packing
  - Irradiation
  - Pasteurization
Radiance and radiation

Electromagnetic waves of different lengths display different effect on microorganisms

• **Infrared** – no direct lethal effect (heat!)
• **Visible light** – positive and negative effect on some activity of cells
• **Ultraviolet** – strong mutagenic and lethal effects (low penetration)
• **Ionic radiation** – strong mutagenic and lethal effects (high penetration)
Microwave radiation

The microwave region of the e.m. spectrum occupies frequencies between $10^9$ Hz up to $10^{12}$ Hz and so has a relatively low quantum energy.

**Microwaves act indirectly** on microorganisms through the generation of heat.

**When a food containing water** is placed in a microwave field, the dipolar water molecules align themselves with the field. As the field reverses its polarity 2 or $5 \times 10^9$ times each second, depending on the frequency used, the water molecules are continually oscillating.

**This kinetic energy** is transmitted to neighbouring molecules leading to a rapid rise in temperature throughout the product.
Microwave radiation

The principal problem associated with the domestic use of microwave is non-uniform heating of foods, due to the presence of cold spots in the oven, and the non-uniform dielectric properties of food.

These can lead to cold spots in some microwaved foods and concern over the risks associated with consumption of inadequately heated meals.

It has led to more explicit instruction on microwaveable foods.
UV radiation

UV radiation has wavelengths below 450 nm and a quantum energy of 3-5 eV ($10^{12}$).

The quanta contain energy sufficient to excite electrons in molecules from their ground state into higher energy orbitals making the molecules more reactive.

Chemical reactions thus induced in microorganisms can cause the failure of critical metabolic processes leading to injury or death.
UV radiation

The greatest lethality of UV radiation is shown by wavelengths around 260 nm which correspond to a strong absorption by nucleic acid bases.

The photo chemical dimerization of thymine.

Generally, the resistance to UV irradiation follows the pattern: Gram-negatives < Gram-positives = yeasts < bacterial spores < mould spores < < viruses
Hydrostatic pressure

Majority of microorganisms multiply under normal atmospheric pressure.

Pressure increase at 10-20 MPa delay multiplication and 30-40 MPa completely stops it.

Some bacteria easily grow even at 60 MPa – barophilic x barotolerant (deep see)

High hydrostatic pressure acts primarily on non-covalent linkages, such as ionic bonds, hydrogen bonds and hydrophobic interactions.
Hydrostatic pressure

Vegetative bacteria and fungi can be reduced by at least one log cycle by 400 MPa applied for 5 min.

Bacterial endospores are more resistant to hydrostatic pressure, tolerating pressures as high as 1200 MPa.

At present, commercial application of high-pressure technology has been limited to acidic products. (Juices, jams)
Ultrasound

Sound waves with frequency higher than 20 kHz, lethal effect on bacteria is effective only at high intensity.

Kavitative ultrasound

In subsequence of vibration an intensive pulsation of cell membranes and cytoplasm occurs (lethal effect)

Sensitive to ultrasound are rods and fibrils, cocci are more resistant.
Mechanic effects

High mechanic resistance of microorganisms is caused by a rigid cell wall and small size.

Destruction of cells is caused by:
• repeated slow freezing and thawing (enzymes resistance)
• high pressure treatment
• mixing with abrazive materials (glass beads)
**Hurdle Technology**

**Hurdle technology** is the use of (synergistic) combinations of preservation factors to enhance the preservative effect and give better organoleptic and nutritional qualities to foods.

Driving force to its development is the public demand for more “natural” wholesome foods that taste/smell better and have improved nutritional quality.

For example, if a limiting $a_w$ of 0.85 or pH 4.6 alone will prevent growth of a food-borne pathogen, similar protection might be given by a combined $a_w$ of 0.92 and pH 5.2.

Chilling will further enhance the synergism.

Each preservative factor is called a “hurdle”.

\[ a_w \quad \text{pH} \quad \text{Temp} \]
The sizes of hurdles can be varied in different combinations to suit different food types.
The individual preservative factors of Hurdle Technology should target different homeostatic mechanisms in the food-associated microbes:

- low $a_w$ places demands on energy supply to accumulate CS
- low pH places demands on energy supply to pump out $H^+$
- low temperature demands energy to maintain membrane homeostasis

Because of the overlaps in the stress responses (e.g. via the RpoS system) this will drain the cell of energy, slow growth rate and even stop growth, leading eventually to cell death.
Target points of AMC on bacterial cell

- **Cell wall synthesis**
  - β-Lactams
  - Glycopeptides
  - Lantibiotics (Type B)

- **DNA gyrase**
  - Fluoroquinolones
  - Nalidixic acid
  - Novobiocin

- **RNA polymerase**
  - Rifampicin

- **Protein synthesis** (50S subunit)
  - Macrolides
  - Clindamycin
  - Chloramphenicol
  - Streptogramins

- **Protein synthesis** (30S subunit)
  - Aminoglycosides
  - Tetracyclines
  - Nitrofuranes

- **Nucleotide synthesis**
  - Trimethoprim
  - Sulfonamides

- **Cytoplasmic membrane**
  - Polymyxin B
  - Colistin
  - Lantibiotics (Type A)