Effect of external and internal factors on microorganisms growth in foods

FOOD QUALITY AND SAFETY - Prof. Angel Angelov, Assoc. Prof. Velitchka Gotcheva
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Role of microorganisms in foods

Food preservation

Food spoilage

Food pathogens

Microorganisms in foods

Bacteria
- Food diseases
- Food spoilage
- Food fermentations
- Food additives, enzymes

Molds
- Food diseases
- Food spoilage
- Food fermentations
- Food additives, enzymes

Yeasts
- Food spoilage
- Food fermentations
- Food additives, enzymes

Viruses
- Food diseases
- Microbial protein
- Identification of pathogens
- Lysis of starter cultures

Microorganisms in foods

Bacteria		
Acinetobacter	*Erwinia*	*Pediococcus*
Aeromonas	*Escherichia*	*Proteus*
Alcaligenes	*Flavobacterium*	*Pseudomonas*
Arcobacter	*Hafnia*	*Psychrobacter*
Bacillus	*Kocuria*	*Salmonella*
Brochotrix	*Lactococcus*	*Serratia*
Campilobacter	*Lactobacillus*	*Shewanella*
Carnobacterium	*Leuconostoc*	*Shigella*
Citrobacter	*Listeria*	*Staphylococcus*
Clostridium	*Micrococcus*	*Vagococcus*
Corynebacterium	*Moraxella*	*Vibrio*
Enterobacter	*Paenibacillus*	*Weissella*
Enterococcus	*Pantoea*	*Yersinia*

Microorganisms in foods

Molds

Alternaria	*Cladosporium*	*Mucor*
Aspergillus	*Colletotrichum*	*Penicillium*
Aureobasidum	*Fusarium*	*Rhisopus*
Botritis	*Geothrichum*	*Trichotecium*
Byssochlamys	*Monilia*	*Wallemia*
		Xeromyces

Yeasts

Bretannomyces	*Issatchenkia*	*Schizosaccharomyces*
Candida	*Kluyveromyces*	*Torulaspora*
Cryptococcus	*Pichia*	*Trichosporon*
Debaryomyces	*Rhodotorula*	*Zygosaccharomyces*
Hanseniaspora	*Saccharomyces*	

Protozoa

Cryptosporidium parvum	*Cyclospora cayetanensis*	*Entamoeba hystolytica*
Giardia lamblia	*Toxoplasma gondii*	

Historical development

- First data - 10,000 years ago
- 6,000 years ago – cereal products through heat treatment (cooking), cereal fermentation

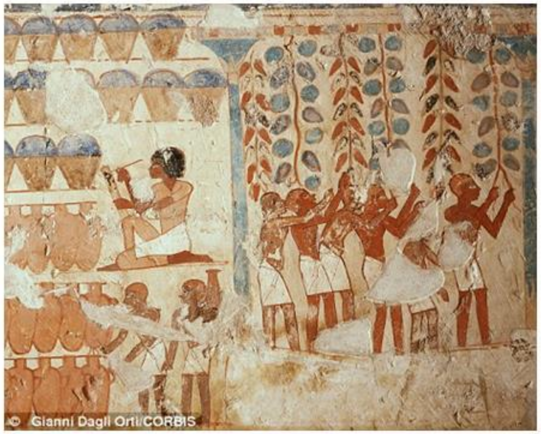
Historical development

- 3,000 г. BC, Sumerians – breeding of domestic animals, dairy products (butter)
- 3,000 - 1,200 г. BC, Jews - salt for food preservation, later adopted in ancient China, Greece and Rome



Historical development

- 3,500 г. BC, Assyria - wine



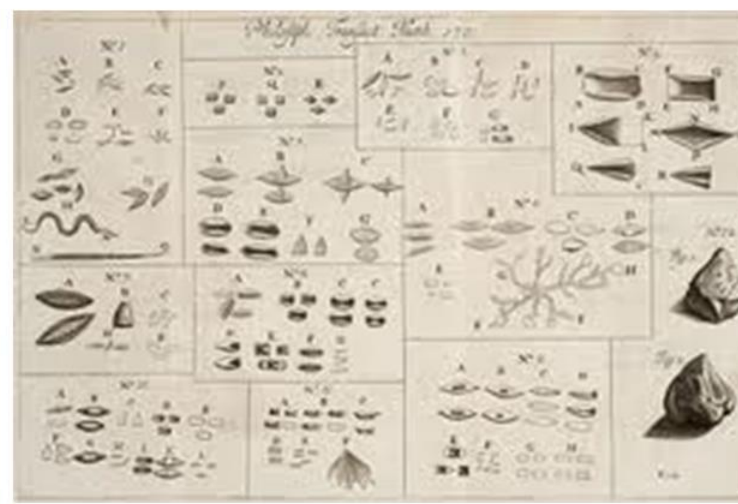
- 1500 г. BC, Babylon and China – fermented sausages

Historical development

- 13th century, Sweden - first legal provisions for mandatory inspection of slaughterhouses

Historical development

- 17th century, Kircher – role of microorganisms for food spoilage
- Antony van Leeuwenhoek, 1680 – observed yeast cells



Historical development

- Nicolas Appert, 1810 – preservation of meat through heat treatment

Nicolas Appert

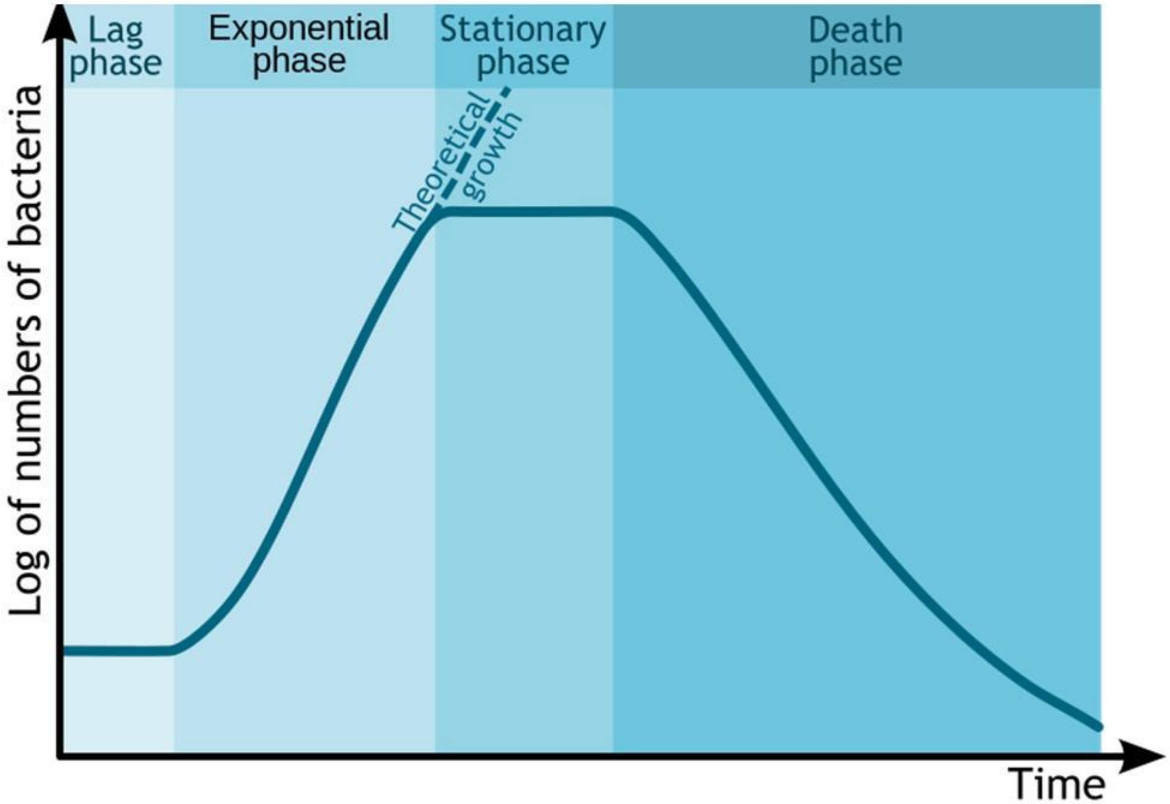
Bouteille de stérilisation

Historical development

- 1857-76, Pasteur - MO (yeasts and bacteria) are responsible for the chemical changes during production and for spoilage of foods and beverages, for acidification of milk; heat treatment to destroy unwanted microorganisms in wine and beer - "**pasteurization**"

Growth of MO in food

- Growth of microorganisms – exponential curve



Growth of MO in food

- Growth rate varies depending on the specific microorganism and food
- Growth inside foods or on their surface

Nutrition of microorganisms

Food components	Microorganisms
C-sources	
Glucose	All MO, concentration in foods is not significant
Galactose	Certain MO, concentration in foods is not significant
Lactose	High concentration in dairy matrices, used by a limited number of MO – lactic acid bacteria, coliforms
Fats	Only MO with lipase activity - *Pseudomonas fluorescens*
Proteins	Only MO with proteolitic activity - *Pseudomonas fluorescens*
Aminoacids	All microorganisms
Citrate	Only certain MO - *Klebsiella spp, Lactococcus lactis*
N- sources	
Aminoacids	All microorganisms
Amonia	All microorganisms
Urea	Only MO with urease activity - *Proteus*
Proteins	Only MO with proteolitic activity - *Pseudomonas fluorescens*
Minerals	All microorganisms – essential for growth
Microelements	All microorganisms – essential for growth
Vitamins	All microorganisms – essential for growth
Purines and pyrimidines	All microorganisms – essential for growth

Death factors for microorganisms

- Nutrient depletion
- pH changes in cell environment
- Accumulation of inhibitory metabolites
- Lack of oxygen (aerobic MO)
- Combinations of the above factors

Active antimicrobial agents

- Heat - high temperature, steam
- Chemical agents - disinfectants, preservatives, antibiotics
- Extreme pH
- Radiation - ultraviolet light, ionizing radiation, microwaves
- Low water activity (a_w) (drying, salting, other agents)

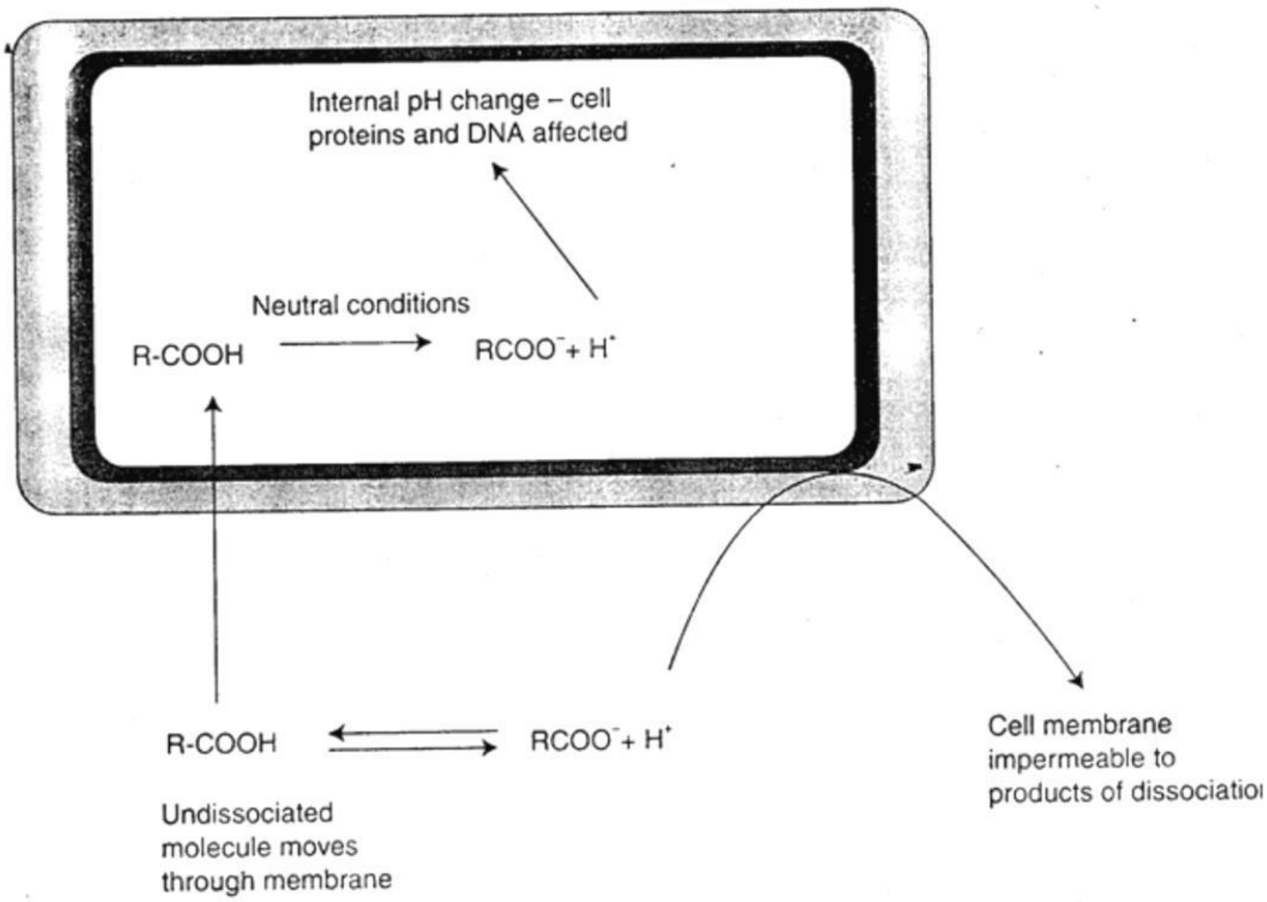
Active antimicrobial agents

- Freezing
- Cooling, cold shock
- Reduced reduction potential (Eh) (low oxygen)
- Ultrasonic sound
- Hydrostatic pressure (100-1000 MPa)
- Competitive microflora (fermentations)

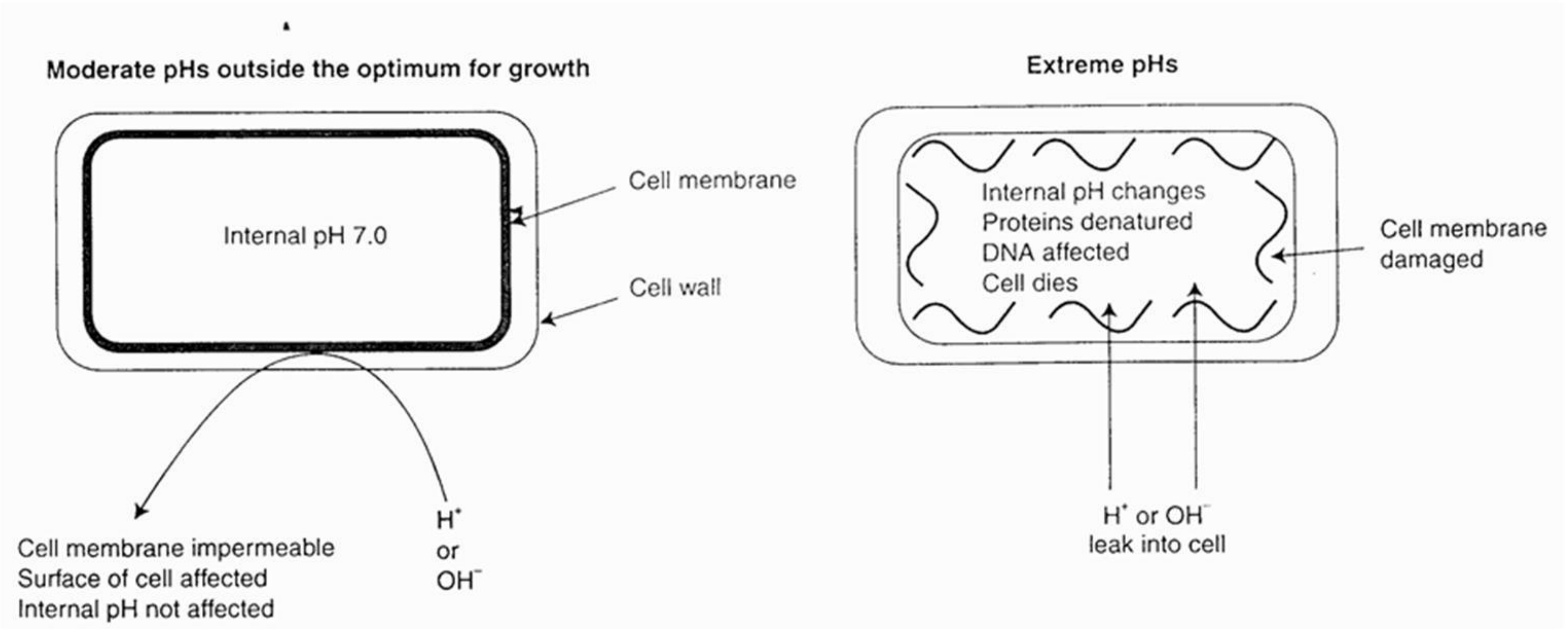
Mechanisms of microorganisms death

- Structure of the cell wall
- Cell membranes
- Cellular proteins, including enzymes
- RNA and DNA
- Mechanisms of synthesis of cell wall and cell growth

Effect of organic acids on microbial cells



Effect of pH on microbial cells



Effect of moderate and extreme pH on microbial cells

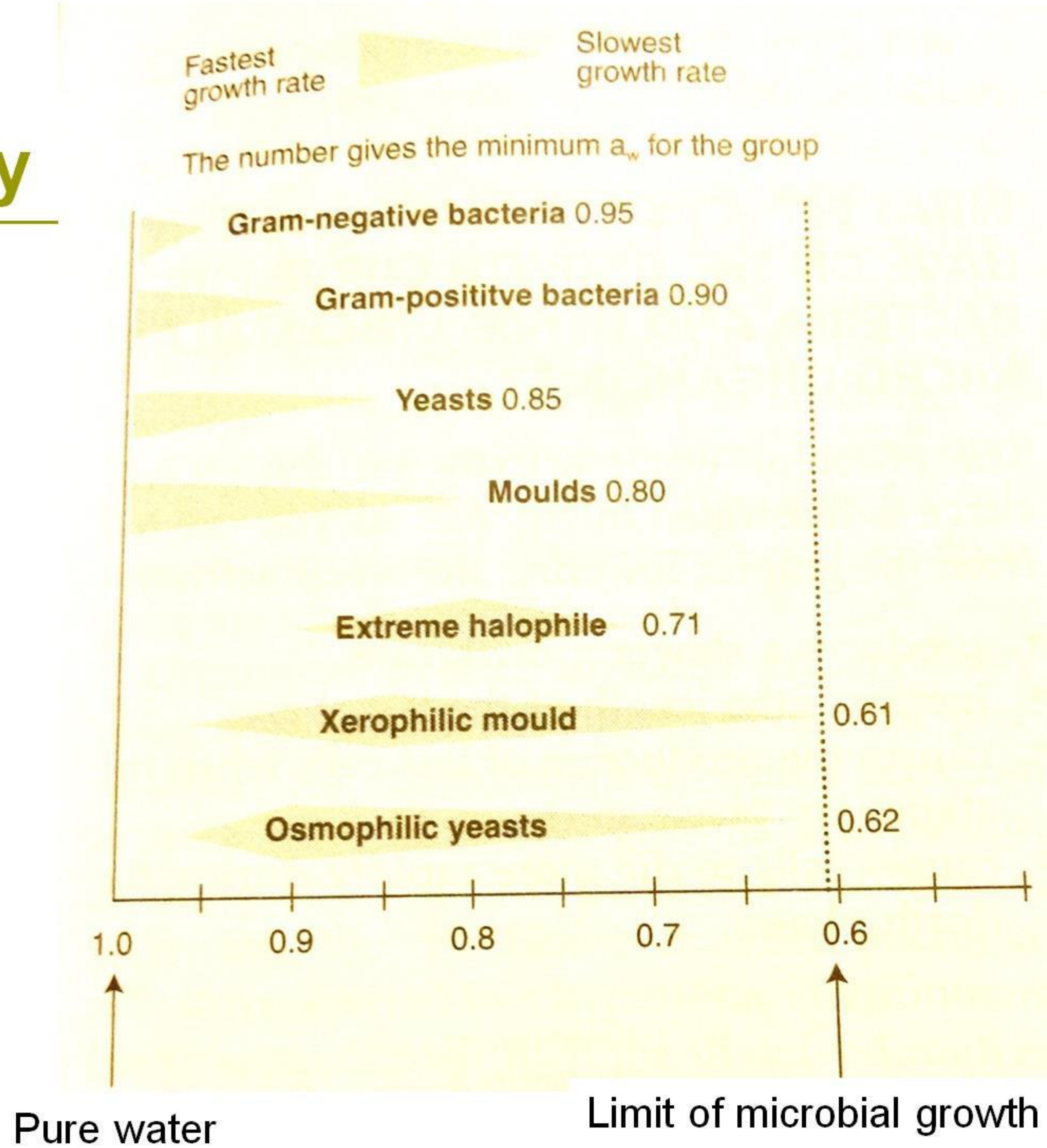
Water activity (a_w)

- Cells of living organisms – more than 75% water, bacterial spores – 15% (dormant state, metabolism is not active)
- Water activity (a_w)– the amount of water available for microbial growth

$$a_w = \frac{\text{Vapour pressure of a substance or solution}}{\text{Vapour pressure of water at the same temperature}}$$

- Water content ≠ water activity
- For each MO – specific a_w interval for growth

Water activity



Water activity (a_w)

Substance	a_w
Distilled Water	1 [4]
Tap water	0.99
Raw meats	0.99[4]
Milk	0.97
Juice	0.97
Salami	.87[4]
Cooked bacon	< 0.85
Saturated NaCl solution	0.75
Point at which cereal loses crunch	0.65
Dried fruits	0.60[4]
Typical indoor air	0.5 - 0.7
Honey	0.5 - 0.7
Dried fruit	0.5 - 0.6

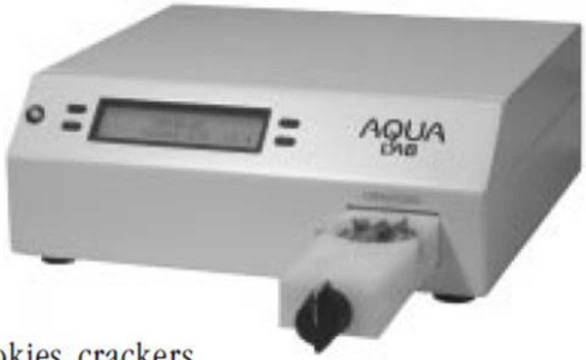
Microorganism Inhibited	a_w
Clostridium botulinum A, B	.97
Clostridium botulinum E	.97
Pseudomonas fluorescens	.97
Clostridium perfringens	.95
Escherichia coli	.95
Salmonella	.95
Vibrio cholerae	.95
Bacillus cereus	.93
Listeria monocytogenes	.92
Bacillus subtilis	.91
Staphylococcus aureus	.86[5]
Most molds	.80[5]
No microbial proliferation	.50

Water activity (a_w)

a_w	Microorganisms generally inhibited by a_w at this point	Examples of foods within this range of water activity.
0.950	*Pseudomonas, Escherichia, Proteus, Shigella, Klebsiella, Bacillus, Clostridium perfringens*, some yeasts	Highly perishable foods (fresh and canned fruits, vegetables, meat, fish) and milk; cooked sausages and breads; foods containing up to 4oz (w/w) sucrose or 7% NaCl
0.910	*Salmonella, Vibrio parahaemolyticus, C. botulinum, Serratia, Lactobacillus, Pediococcus, some molds, Rhodotorula, Pichia*	Some cheese (Cheddar, Swiss, Muenster, Provolone), cured meat (ham), some fruit juice concentrates foods containing 55% (w/w) sucrose or 12% NaCl
0.870	Many yeasts *(Candida, Torulopsis, Hansenula), Micrococcus*	Fermented sausage (salami), sponge cakes, dry cheeses, margarine; foods containing 65% (w/w) sucrose (saturated) or 15% NaCl
0.800	Most molds (mycotoxigenic penicillia), *Staphylococcus aureus*, most *Saccharomyces (baillii) spp., Debaryomyces*	Most fruit juice concentrates, sweetened condensed milk, chocolate syrup, maple and fruit syrups, flour, rice, pulses containing 15-17% moisture; fruit cake; country style ham, fondants, high-sugar cakes
0.750	Most halophilic bacteria, mycotoxigenic aspergilli	Jam, marmalade, marzipan, glacé fruits some marshmallows

Water activity (a_w)

0.650	Xerophilic molds *(Aspergillus chevalieri, A. candidus, Wallemia sebi), Saccharomyces bisporus*	Rolled oats containing ~10% moisture, grained nougats, fudge marshmallows, jelly, molasses, raw cane sugar, some dried fruits, nuts
0.600	Osmophilic yeasts *(Saccharomvces rouxii),* few molds *(Aspergillus echinulatus, Monascus bisporus)*	Dried fruits containing 15-20% moisture; some toffees and caramels, honey
0.500		Noodles, spaghetti, etc. containing ~12% moisture; spices containing ~10% moisture
0.400	No microbial proliferation	Whole egg powder containing ~5% moisture
0.300		Cookies, crackers, bread crusts, etc. containing 3-5% moisture
0.030		Whole milk powder containing 2-3% moisture; dried vegetables containing ~5% moisture; corn flakes containing ~5% moisture; dehydrated soups; some cookies, crackers

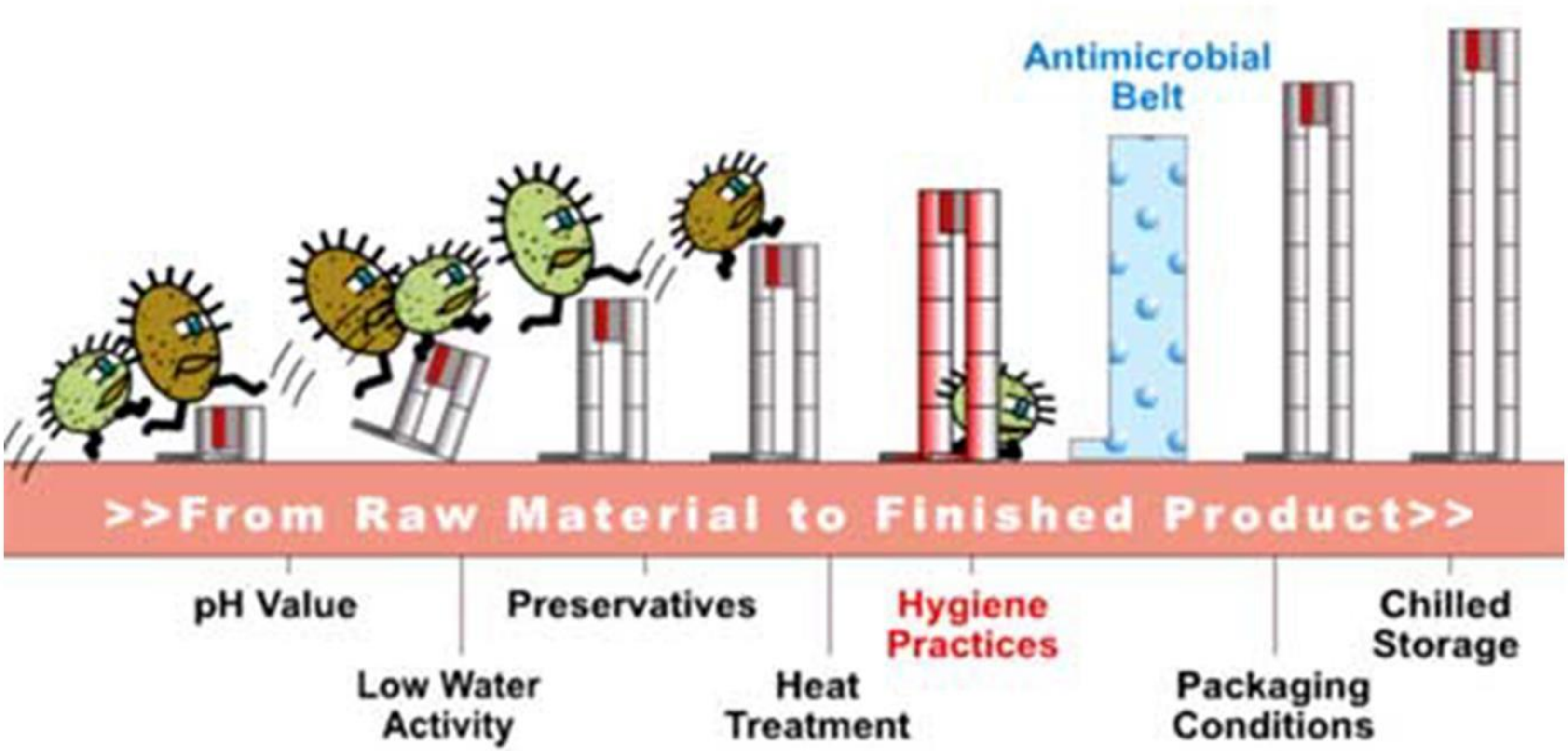


The hurdle concept

- Safe foods – the use of a single preservative method requires extreme parameter values (high salt/sugar concentrations, high acidity, high concentrations of preservatives, severe heat treatment, etc.) – altered characteristics of the product

- **Hurdle technology** – combination of different inhibiting factors at lower levels → effective control and preserved food quality

The hurdle concept



Thermal death of microorganisms

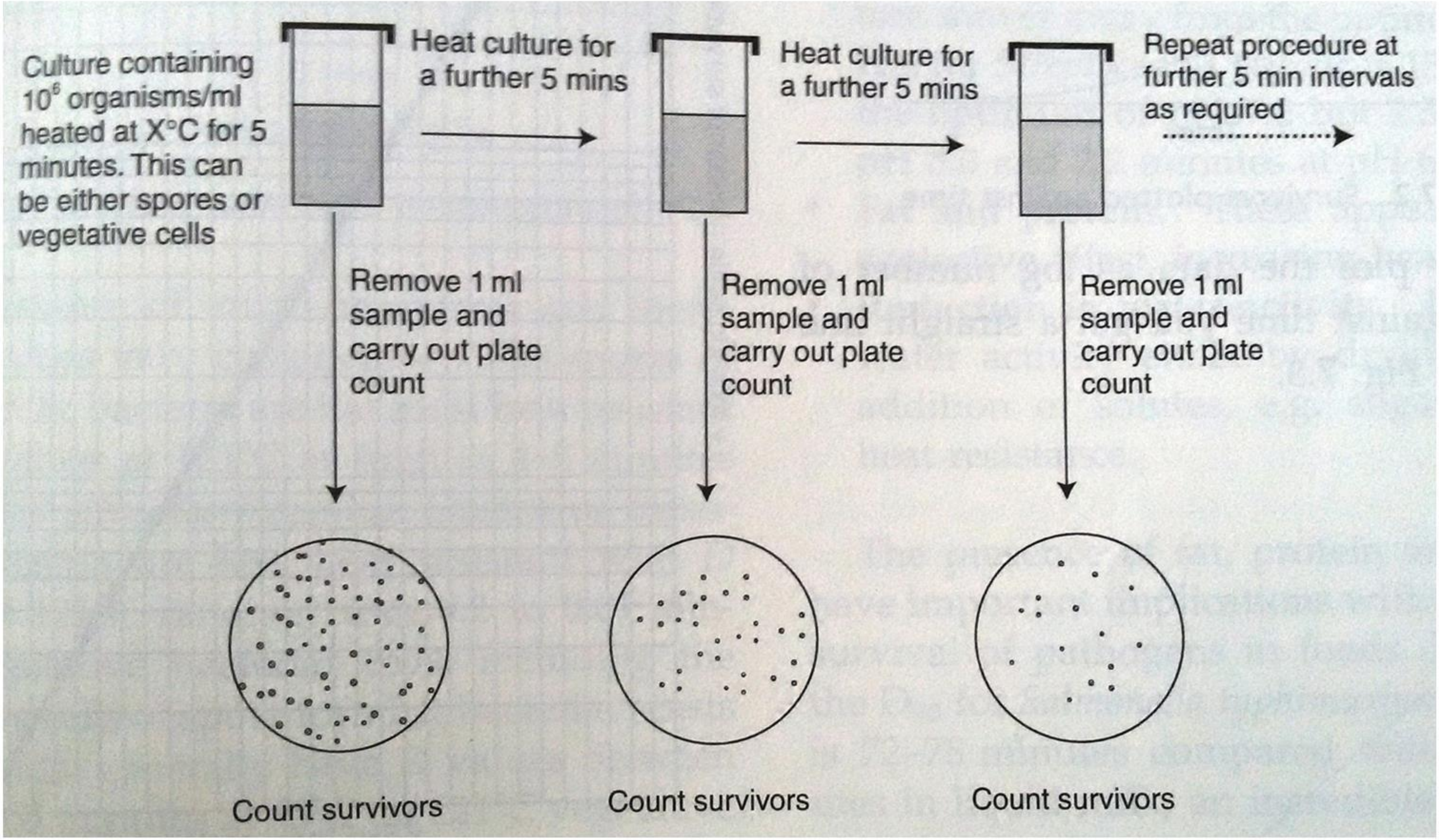
- Experimental determination of heat resistance of microorganisms
  - heating is not instantaneous – time lags in heating and cooling
  - complex and expensive apparatus

Thermal death of microorganisms

Example

- Cell concentration in a product - 10^6 CFU/cm^3 (g)
- Heating up to T > Tmax for MO growth (X^{o}C) for 5 min, sample for analysis
- Plate count to estimate the number of living cells
- Repeat the steps several times

Thermal death of microorganisms

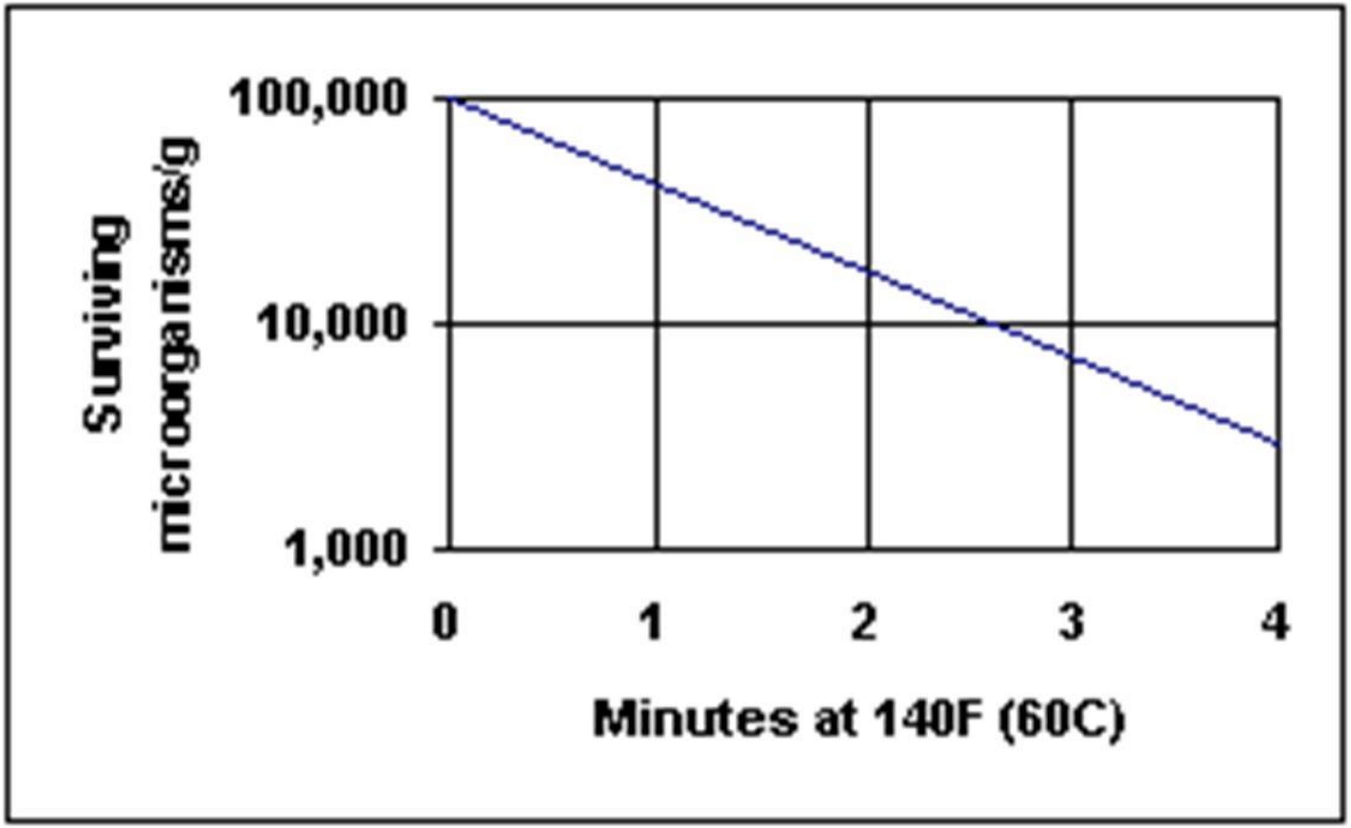


Procedure for determining the number of survivors

Thermal death of microorganisms

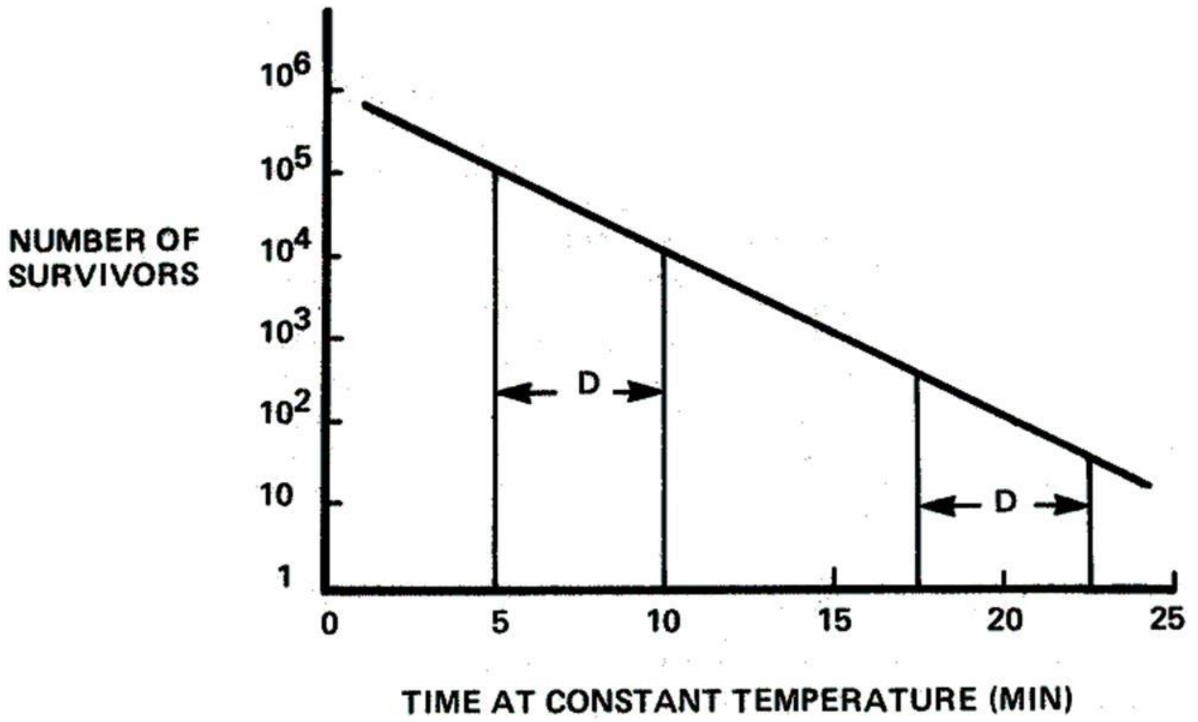
Heationg time, min	Number of killed cells	Number of surviving cells	Log_{10} of surviving cells
0	0	10^6	6
5	900 000	10^5	5
10	990 000	10^4	4
15	999 000	10^3	3
20	999 900	10^2	2
25	999 990	10^1	1
30	999 999	10^0 (1) (1/ml)	0
35	999 999.9	10^{-1} (1/10 ml)	-1
40	999 999.99	10^{-2} (1/100 ml)	-2

Survivor curve



For 1 log cycle 90% of microbial cells are killed.

D-values



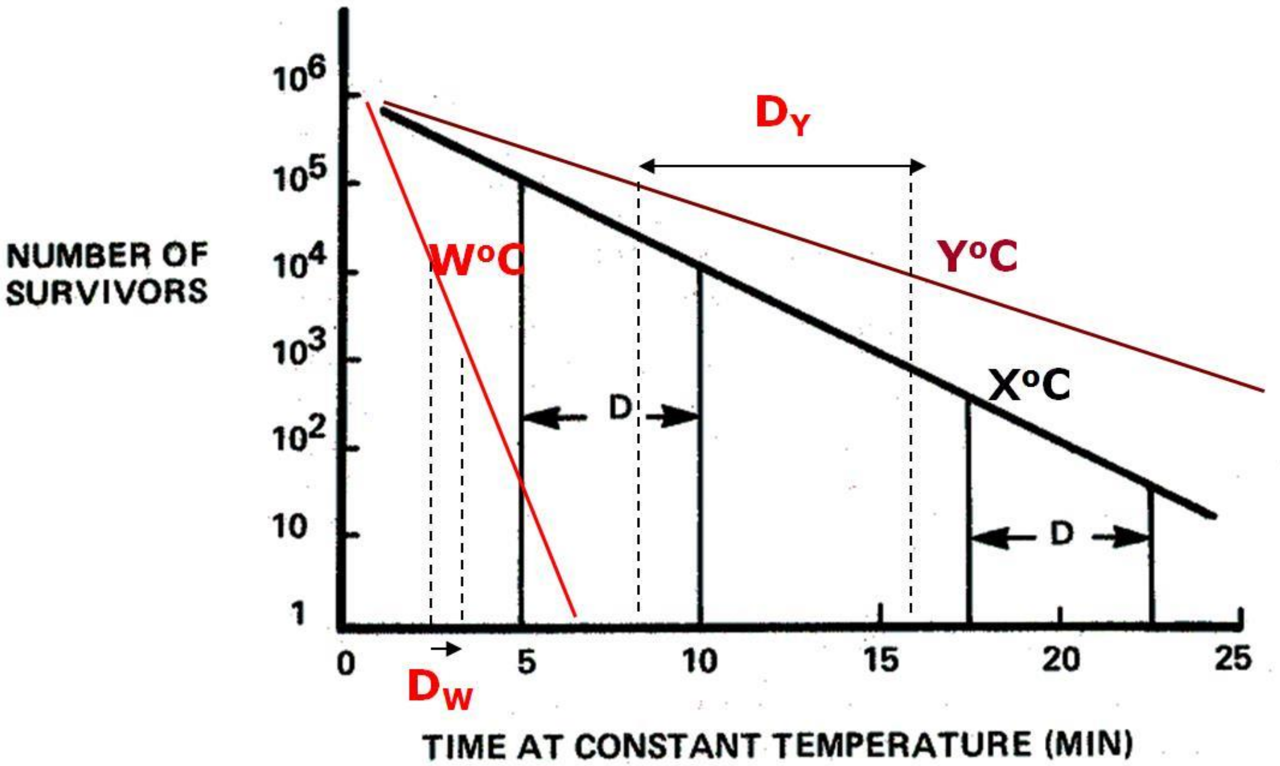
D-value: the time taken for the population to pass through a log cycle

D_{121}: time to kill 90% of a population at 121°C

D-values

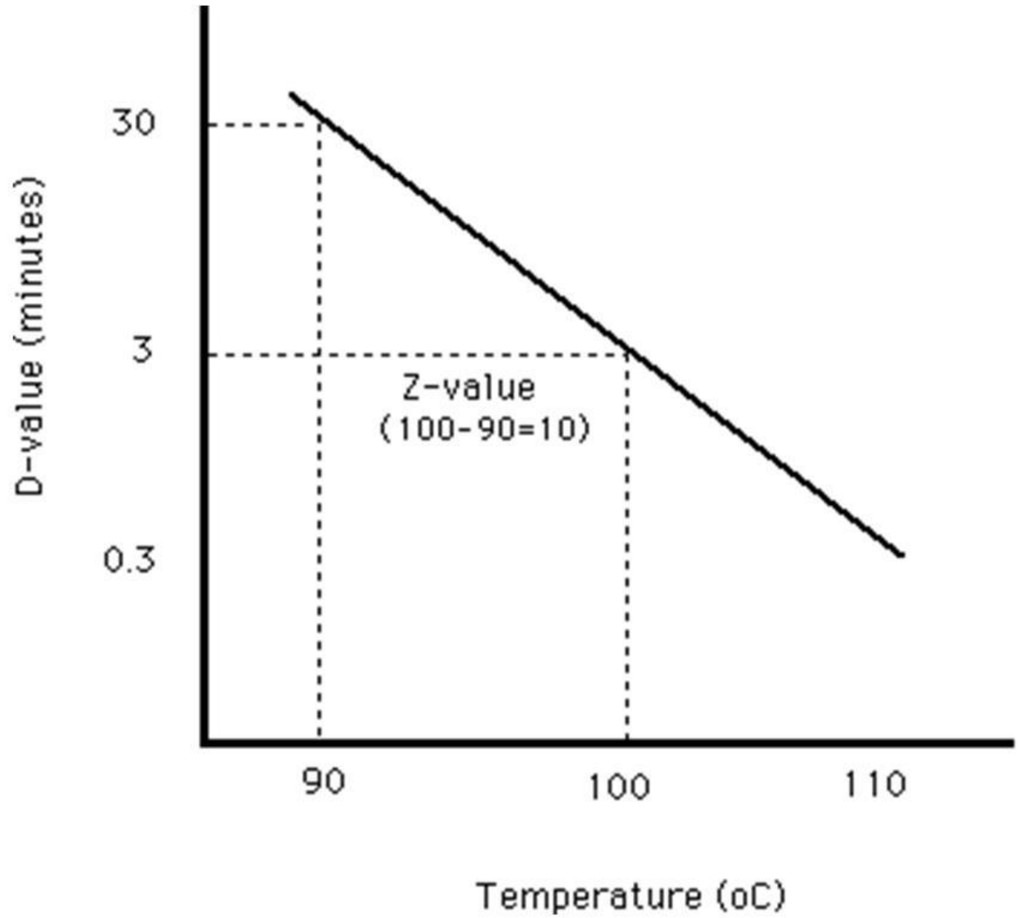
- Comparison of the relative heat resistance of microorganisms
- Calculation of sterilization time
- Spores of thermophilic bacteria: D_{121}= 4 - 5 min for *Bacillus stearothermophilus*
- Spores of thermophilic bacteria: D_{121} = 0.2 -0.01 min
- Vegetative bacteria, yeasts and molds: D_{65} = 0.5 - 3 min

Survivor curve at different temperatures



W°C > X°C > Y°C

z-values



z-value: the change of temperature (°C), required to achieve a 10-fold change of the D-value

z-values - application

- Determination of the equivalent D-values for different temperatures

$z = 10^oC$, $D_{121} = 5$ min;

Increase of T from 121°C to 131°C →

$D_{131} = 0.5$ min;

Decrease of T from 121°C to 111°C →

$D_{111} = 50$ min;

z-values

- Molds, yeasts and vegetative cells of bacteria: $z = 5\text{-}8^{o}C$ (normally 5°C)
- Bacterial spores: $z = 6\text{-}16^{o}C$ (normally 10°C)
- Determination of the equivalent D-values for different temperatures

Factors influencing heat resistance

- Growth phase: cells of stationary phase are more resistant than cells in log phase
- Growth temperature: the higher T of growth is, the more resistant are MO
- pH of the environment: higher heat resistance at pH optimal for growth
- Fat and protein: protective effect
- Water activity: reduction of a_w increases heat resistance