

FE 305 FOOD MICROBIOLOGY

Microbial Growth Characteristics

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Important Bacteria in Foods

- Some of the most important bacterial species that cause spoilage in foods or that cause poisoning and disease are given below. The presence of some of them in foods is particularly undesirable.

Bacillus:

- They are mostly aerobic or facultatively anaerobic, mainly motile, Gram-positive rods that form endospores.
- They are widely found in soil, water, plants and many foods.
- Most are mesophiles, but psychrotrophic and thermophilic species also occur. The thermophilic species are particularly important in the canning industry (for example *Heyndrickxia coagulans*, formerly *Bacillus coagulans*, and *Geobacillus stearothermophilus*, formerly *Bacillus stearothermophilus*) because their spores are highly heat-resistant.
- *B. anthracis* is a pathogenic species that causes anthrax in humans, cattle and sheep.
- *B. cereus* causes food poisoning by producing enterotoxins in the intestine (diarrhoeal type) and the heat-stable emetic toxin cereulide in the food (emetic type).
- Starchy foods such as cooked rice and pasta are the typical vehicles of *B. cereus* poisoning.

Important Bacteria in Foods

Clostridium:

- They are anaerobic, Gram-positive rods; most are motile and they produce heat-resistant endospores.
- They are widespread in nature, in soil, water and the intestinal tracts of humans and animals.
- *C. botulinum* grows in low-acid canned foods and causes the poisoning known as botulism. Its neurotoxin is the most potent biological toxin known.
- Bacteria of this group degrade proteins and carbohydrates and spoil foods by forming butyric acid, acetic acid, ethanol, CO₂ and H₂.
- They cause swelling (blowing, bombage) of canned foods; for example *C. butyricum* and *C. tyrobutyricum*.
- *C. perfringens* causes gas gangrene in contaminated wounds and food poisoning when it grows in meat dishes that are cooled too slowly.

BOTULISM – SYMPTOMS



**Drooping
eyelids**



**Facial
weakness**



**Difficulty
swallowing**



Dry mouth



**Trouble
breathing**



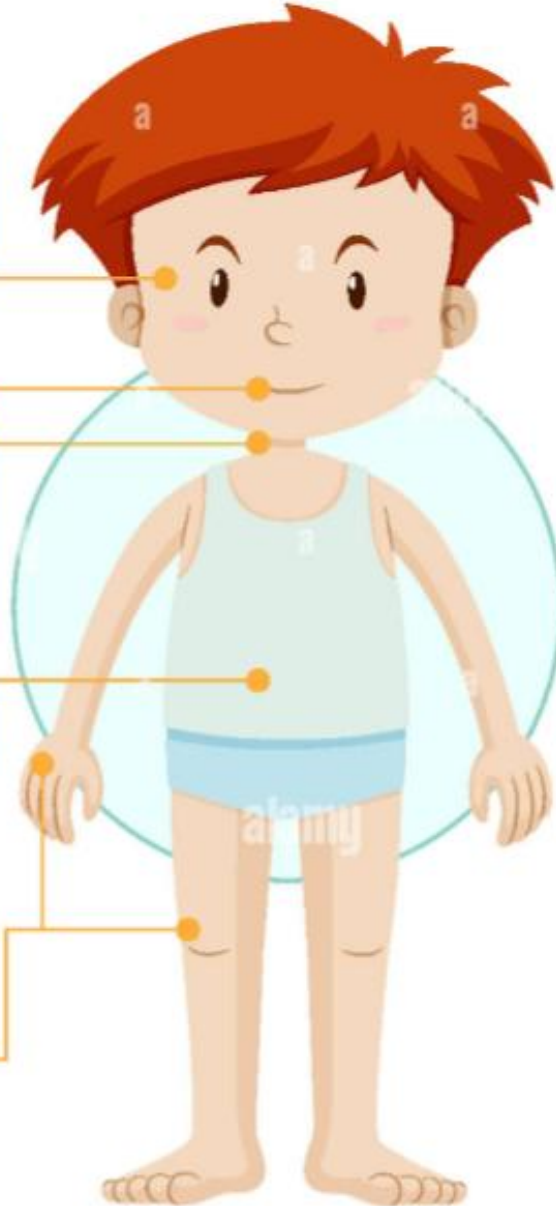
Nausea



Vomiting



Paralysis



Important Bacteria in Foods

Streptococcus:

- They are Gram-positive, mostly facultatively anaerobic cocci that grow in chains.
- They ferment carbohydrates to lactic acid without producing gas.
- Some species cause throat infections, scarlet fever and skin infections in humans and mastitis in animals.
- The former “lactic streptococci” are now placed in the genus *Lactococcus* and are widely used as starters, particularly in dairy products.
 - For example, *Lactococcus lactis* (formerly *Streptococcus lactis*) is used in the manufacture and ripening of many cheeses.
 - *Streptococcus thermophilus* produces yoghurt together with *Lactobacillus delbrueckii* subsp. *bulgaricus*.

Important Bacteria in Foods

Escherichia:

- They are Gram-negative, facultatively anaerobic, motile or non-motile short rods.
- The most important species, *E. coli*, is a natural member of the intestinal flora of humans and animals and from there contaminates water, soil and other environments.
- For this reason the presence of *E. coli* in foods and water is important as an indicator of faecal contamination.
- It occurs frequently in foods and produces acid and gas from lactose. Some strains are pathogenic; Shiga toxin-producing *E. coli* (STEC, e.g. O157:H7) causes haemorrhagic colitis and haemolytic uraemic syndrome (HUS).

Salmonella:

- They are Gram-negative, motile, facultatively anaerobic rods that do not form spores.
- They occur naturally in the intestines of humans and animals and in sewage.
- The genus contains two species, *S. enterica* and *S. bongori*, and more than 2 500 serovars, most of which are pathogenic to humans and cause enteric infection (salmonellosis).

Important Bacteria in Foods

Shigella:

- They are Gram-negative, non-motile, facultatively anaerobic rods.
- They occur in sewage and in the human intestine and cause bacillary dysentery (shigellosis) in humans.
- They produce acid but no gas from glucose and do not ferment lactose.

Staphylococcus:

- They are Gram-positive, aerobic or facultatively anaerobic, non-motile, non-spore-forming cocci arranged in grape-like clusters.
- The most important species are *S. aureus* and *S. epidermidis*; both occur naturally in the skin and nasal flora of humans and animals.
- *S. aureus* causes abscesses and wound infections in humans and mastitis in animals.
- The illness caused by ingesting the heat-stable enterotoxins produced by *S. aureus* in the food is one of the most common food poisonings.

Growing of Microorganisms in Foods

- Microorganisms need nutrients in order to survive and to multiply.
- Foods contain the components required for the vital activities of microorganisms just as they do for humans.
- They are therefore excellent substrates for microbial growth.
- The inner tissues of healthy foods in nature are generally accepted to be sterile.
- The surfaces of foods carry a variable microflora. The number of microorganisms that a food surface can carry depends on the ratio of its surface area to its volume.
 - Fruits and vegetables such as cucumbers and tomatoes have a small surface area relative to their volume.
 - Foods such as lettuce and parsley have a large surface area relative to their volume, which means more sites on which microorganisms can settle and multiply.

Microbial Growth

- Microorganisms are present in the air, water, soil and foods around us.
- Fresh foods, prepared foods and even preserved foods can all become contaminated with microorganisms.
- Uncontrolled and undesirable microbial growth damages foods, causes nutrient losses and leads to heavy economic losses.
- Some microorganisms and the foods they contaminate cause foodborne infection and food poisoning.

Microbial growth in foods is controlled by:

1. preventing contamination;
2. inhibiting the growth of the microorganisms present;
3. inactivating or killing them.

Microbial Growth

- Despite these undesirable effects, microbial activity is essential in the production of many foods, and in those products it is desirable.
 - For example:
 - bread,
 - yogurt
 - cheese,
 - beer,
 - antibiotic,
 - vaccine,
 - vitamin,
 - enzymes, etc.
- are all produced with the help of microorganisms.

Microbial Growth

- All the chemical substances that a microorganism takes up from its environment are its nutrients.
- Energy is released by the oxidation of these substances and is used for the various activities of the cell.
- One of the activities that require energy is growth and multiplication.
- Nutrients/Energy
 - Proliferation;
 - Movement,
 - regulation of activities in the cell, and
 - It is also necessary for its repair.
- Organisms that obtain the energy they need from chemical compounds are called chemotrophs.
 - Chemotrophic microorganisms obtain their energy from organic or inorganic compounds; phototrophs obtain it from light.
 - Examples of organic substrates are carbohydrates, proteins and fats.
 - Examples of inorganic substrates are hydrogen sulphide, ammonia, hydrogen and reduced iron.

Microbial Growth

- For their survival and growth, microorganisms require:
 - carbon (organic compounds, organic acids or CO₂);
 - nitrogen (essential for the synthesis of amino acids and nucleic acids; proteins, peptides, N₂, NO₃⁻ and NH₄⁺ are used as sources);
 - phosphorus, sulphur, magnesium, potassium, calcium and sodium;
 - oxygen and/or carbon dioxide;
 - trace elements such as zinc, iron, manganese and cobalt.
- They obtain the energy they need mainly by oxidising carbohydrates.
 - Glucose is the most widely used energy source.
 - Energy is obtained from glucose by
 - respiration and
 - fermentation.

Microbial Growth Characteristics in Food

1) Competition

- Microorganisms compete with one another for nutrients.
- Competition for a limited supply of nutrients leads to interactions within a population and between different microorganisms,
 - and results in a reduction of the growth rate of some microbial groups.
 - This may slow their growth or inhibit it completely.
- Some species are much better adapted to growth at very low substrate concentrations,
 - whereas others require higher substrate concentrations.
- Foods contain a mixed population of microorganisms.
- Competition among them determines which will outgrow the others and
 - cause its characteristic type of spoilage.

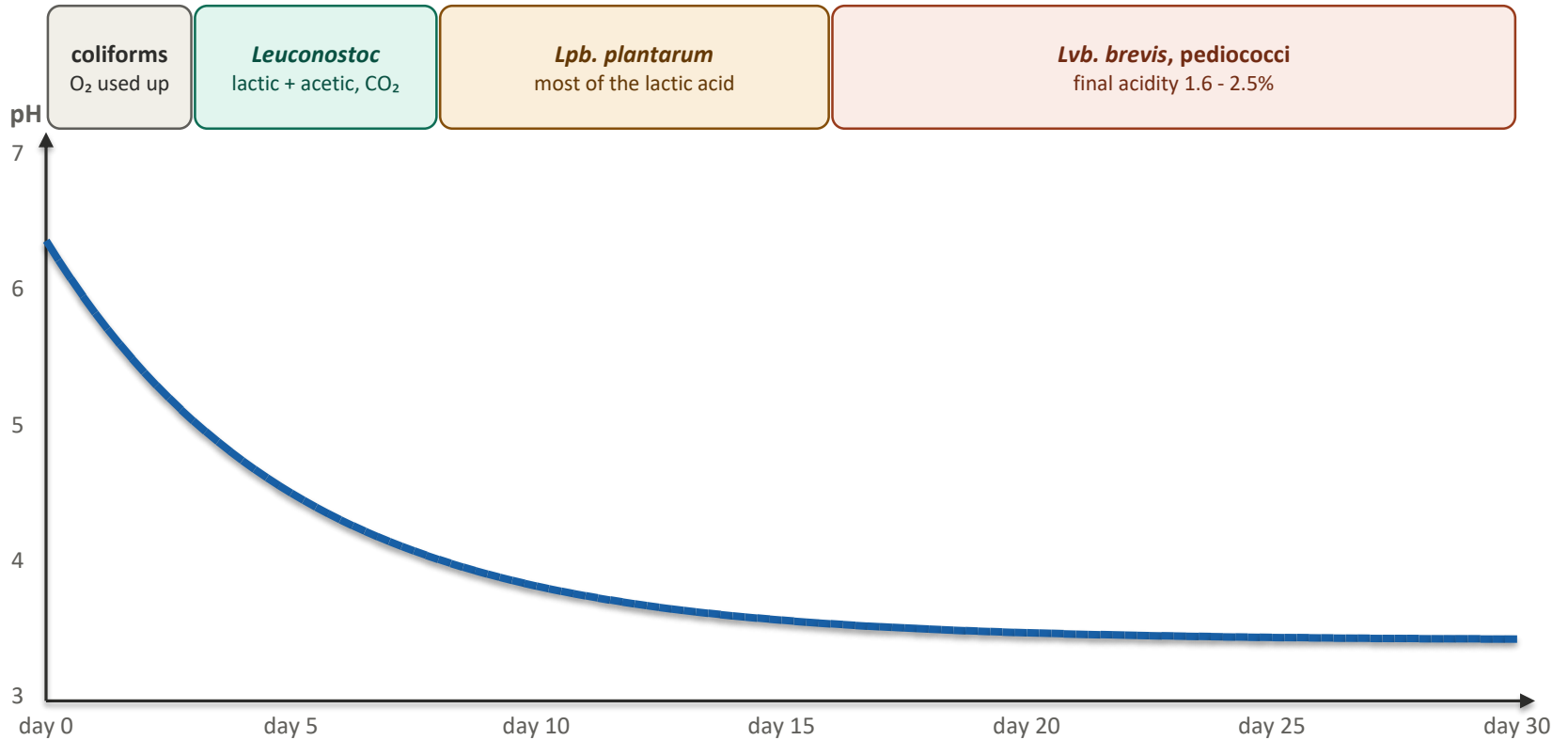
- One or a few types of microorganism will become predominant.
- If the conditions are favourable for all types,
 - foods are spoiled mostly by bacteria rather than by yeasts and moulds,
 - because bacteria have a shorter generation time.
- Yeasts grow faster than moulds.
- Moulds can become predominant only when the conditions are better for them than for yeasts and bacteria (for example at low a_w or low pH).
- Thus, in a mixed population,
 - the intrinsic and extrinsic factors determine which type will predominate and
 - produce the specific changes in the food.
- This is very important in microbial spoilage, food poisoning and bioprocessing.

2) Metabiotic (Sequential) Growth

- Several types of microorganism are present in a food,
 - but only one or a few of them can dominate at a given time.
- Initially one or two types grow best and
 - inhibit the others.
- However, the environment they create may then become favourable for another type,
 - which grows rapidly in its turn.
- This sequential dominance can occur several times during the storage of a food.
- If a food is packaged in a bag containing a little air,
 - aerobes grow first and use up the oxygen;
 - the environment then becomes anaerobic and
 - anaerobes grow.

- Most natural fermentations are based on metabiosis.
- In sauerkraut fermentation,
 - several bacterial species grow in succession,
 - each one creating conditions favourable for the next.
 - The desirable characteristics of the final product depend on this ordered sequence.
 - First, coliforms and other Gram-negative bacteria grow briefly and lower the redox potential, creating conditions favourable for the lactic acid bacteria (LAB);
 - second, the heterofermentative *Leuconostoc mesenteroides* produces lactic and acetic acid and CO₂;
 - third, *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) produces more lactic acid;
 - finally, the acid-tolerant *Levilactobacillus brevis* (formerly *Lactobacillus brevis*) grows.
 - Each species raises the acidity until its own growth is stopped.
 - This succession gives the product its characteristic flavour and texture.
- If the product is not properly protected (kept anaerobic and cool),
 - film yeasts and moulds grow on the surface and use up the acids,
 - so that the product spoils.

Sauerkraut: A Metabiotic Succession



Conditions for a good fermentation

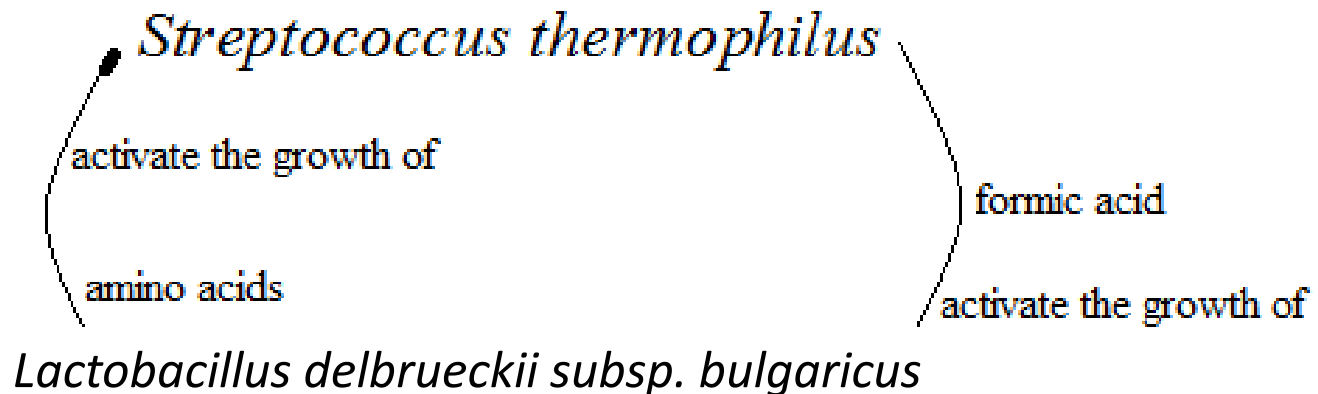
2 - 2.5% salt, 18 - 22 °C, cabbage kept under brine (anaerobic)
exposed to air, film yeasts and moulds use up the acid and the product spoils

Another example of metabiotic growth:

- **raw milk left at room temperature is first fermented**
 - by coliforms, which produce acid;
 - the acid favours the growth of *Lactococcus lactis*,
 - which produces more acid;
 - next the acid-tolerant lactobacilli, such as *Levilactobacillus brevis*, grow
 - and raise the acidity further until they too are inhibited;
 - film yeasts and moulds then grow on the surface and reduce the acidity;
 - finally acid-proteolytic bacteria such as *Enterococcus faecalis* subsp. *liquefaciens* and *Macroccoccus caseolyticus* can grow.

3) Symbiotic Growth

- In symbiotic growth, two or more microorganisms help one another while growing in a food.
- One type produces a metabolite that the second type needs but cannot make itself.
- In turn, the second organism produces a nutrient that allows the first one to grow better.
- Such synergism occurs between *Lactiplantibacillus plantarum* (formerly *Lactobacillus arabinosus*) and *Enterococcus faecalis* in a minimal medium in which neither organism can grow alone.
- *E. faecalis* requires folic acid, which is produced by *Lpb. plantarum*, which in turn requires phenylalanine, produced by *E. faecalis*.



4) Synergistic Growth

- When two types of microorganism grow together,
 - they may bring about changes that neither could produce alone.
- When *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* grow separately in milk, each produces only about 8–10 ppm acetaldehyde,
 - the characteristic flavour compound of yoghurt.
- When they grow together they produce 30 ppm or more.
- In another example, *Pseudomonas syncyanea* growing alone in milk produces only a light brownish colour, and *Lactococcus lactis* produces no colour change at all.
 - When the two bacteria grow together,
 - a bright blue colour develops.

5) Commensalism

- Microorganisms may not affect one another directly,
 - but one organism uses a substrate produced by another.
 - For example, cellulose-hydrolysing microorganisms release glucose, which is then used by organisms that cannot hydrolyse cellulose.
- The anaerobic thermophile *Acetivibrio thermocellus* (formerly *Clostridium thermocellum*) produces a cellulosome complex that hydrolyses cellulosic substrates.
- Other cellulolytic bacteria are *Acetivibrio cellulolyticus*, *Bacteroides cellulosolvens*, *Clostridium cellulovorans* and *Ruminococcus flavefaciens*.
- One population benefits from the growth factors excreted by another, while the latter is unaffected.

- Vinegar production is another example of commensalism.
 - In vinegar production, yeasts (*Saccharomyces cerevisiae*) first produce ethanol from sugar, and
 - *Acetobacter aceti* and *Gluconobacter oxydans* then oxidise the ethanol to acetic acid.
- The final product, vinegar, owes its character to this acetic acid.

6) Antagonistic Growth

- Microorganisms can also affect each other adversely.
- Sometimes one organism kills the other.
- Such antagonism occurs between bacterial strains,
 - between bacteria and yeasts, between yeasts and moulds and between bacteria and moulds.
 - It is caused by the production of one or more antimicrobial compounds by one or more strains in the mixed population.
- Some Gram-positive bacteria produce antimicrobial peptides called bacteriocins,
 - for example *Lactococcus lactis* subsp. *lactis* produces nisin, which kills many Gram-positive bacteria; Gram-negative bacteria are protected by their outer membrane unless it is damaged.
- Antagonism is important in the control of spoilage and pathogenic microorganisms in foods (biopreservation).
- The production of lactic acid by LAB, or of sulphuric acid by *Acidithiobacillus thiooxidans*, inhibits other, non-acidophilic microorganisms.

7) Predation

- Predation is the growth of one bacterium at the expense of other microorganisms.
- *Bdellovibrio bacteriovorus* and similar predatory bacteria attach to Gram-negative bacteria,
 - penetrate the cell wall and multiply within the periplasmic space.
- They grow on the cellular components of the host cell.
- The contents of the host are partly degraded and used.
- *Myxococcus* can lyse susceptible strains at a distance with the aid of exoenzymes,
- and obtains its nutrients from the material released by the lysed cells.
- These predators differ from viruses in that
 - they do not depend directly on the metabolic machinery of the host cell.

Factors Affecting Microbial Growth in Foods

- Many factors affect the growth of microorganisms in foods.
- The effect of these factors depends on:
 - the characteristics of the food and of the environment in which it is kept,
 - the process applied to the food, and
 - the type of microorganism concerned.
- The factors affecting microbial growth are also the basis of the methods used to protect foods from microbial activity.
- By combining them, the “hurdles” used in food preservation are created.

Factors Affecting Microbial Growth in Foods

Intrinsic factors	Extrinsic factors	Implicit factors	Processing factors
Nutrient content pH and buffering capacity Oxidation-reduction potential Water activity (moisture) Antimicrobial constituents Biological structure	Relative humidity Storage temperature Gaseous atmosphere	Microbial alteration Synergism Metabiotic Antagonism Commensalism Growth rate	Physical treatments Use of chemicals Changes in pH Contamination

Factors Affecting Microbial Growth in Foods

1. Intrinsic (internal) factors:

- a. nutrient content;
- b. acidity and hydrogen ion activity (pH) and buffering capacity;
- c. oxidation–reduction potential (Eh);
- d. water activity (a_w);
- e. antimicrobial substances naturally present in the food, or preservatives added to it;
- f. the biological structure of the food.

2. Extrinsic (environmental) factors:

- a. relative humidity;
- b. composition and concentration of the gases in the atmosphere;
- c. storage temperature and time;
- d. radiation.

Factors Affecting Microbial Growth

Intrinsic factors

- pH and buffering capacity
- Water activity (a_w)
- Redox potential (Eh)
- Nutrient content
- Natural antimicrobials
- Biological structure

Extrinsic factors

- Storage temperature
- Relative humidity
- Gas atmosphere
- Storage time
- Radiation

Implicit factors

- Competition
- Metabiosis (sequential)
- Symbiosis and synergism
- Antagonism
- Commensalism
- Predation

Which microorganism becomes dominant
and how fast it grows in the food

Processing factors act on all three
heat, chilling, drying, acidification, preservatives

Water Activity

- The survival of microorganisms and their metabolic activities depend on the water available in the environment. Water activity (a_w) is a measure of the free water available to the microorganism; it is defined as the ratio of the vapour pressure of the food to that of pure water at the same temperature.
- The highest value of water activity is 1.00 (pure water) and the lowest is 0.00.
- Free water can be removed from a food by drying or freeze-drying.
 - For this reason the water activity of fresh foods is high (fresh fruit, $a_w \approx 0.97$),
 - whereas that of dried foods is low (dried fruit, $a_w \approx 0.60$).
- Besides drying, water activity can be lowered by adding solutes such as salt (NaCl) or sugar.
- In this way microbial growth can be delayed or stopped.
 - This principle is used in jams, brines and salted or dried meat products.

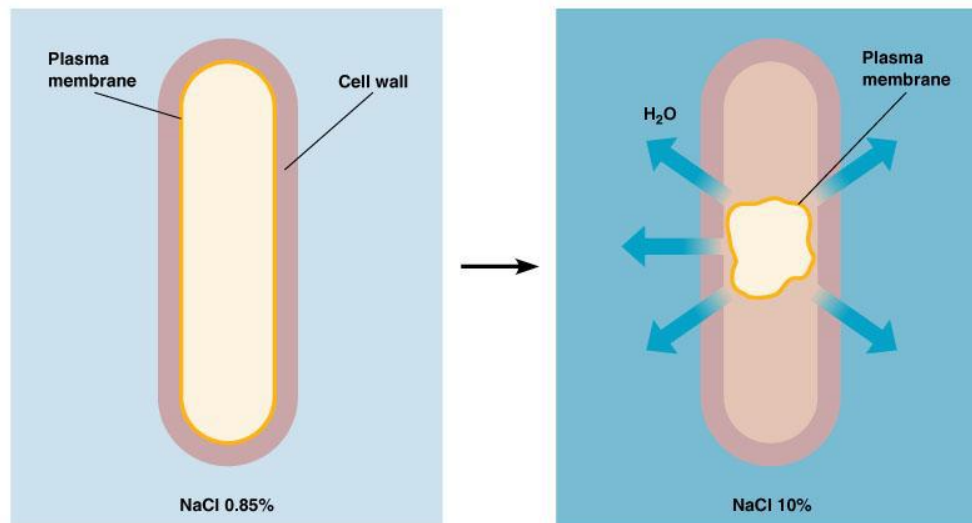
Water Activity

- Some water-binding substances, such as NaCl, also have a direct inhibitory effect on microorganisms.
- The minimum a_w for growth is modified by other factors:
- at their optimum temperature, microorganisms tolerate lower a_w values better;
- aerobic microorganisms can grow at lower a_w values in the presence of oxygen;
- in the presence of an inhibitor, the a_w range over which microorganisms can grow becomes narrower.
- In an environment of high salt concentration, water moves out of the cell;
 - the high salt concentration disturbs bacterial metabolism;
 - if this is not compensated, plasmolysis follows,
 - with dehydration and shrinkage of the cytoplasm and
 - damage to the cell membrane.

Water Activity

Classification by osmolarity

- **Halotolerant**
 - Grows without salt but tolerates high salt concentrations
- **Halophile**
 - Requires salt for growth
- **Extreme halophile**
 - Requires a high salt concentration ($> 4 \text{ M NaCl}$)
- **Osmophile / xerophile**
 - Xerophiles are moulds that grow at low water activity (dry environments)
 - Osmophiles are yeasts that grow at high sugar concentrations



A _w	Growing of microorganisms in Relevant Value	Food Example
0.95	<i>E. coli</i> , <i>Clostridium perfringens</i> , Many saprophytic microorganisms some yeasts	Perishable foods (fresh and canned fruit, vegetables, meat, fish), milk, cooked sausage, bread, foods containing 4% sucrose or 7% NaCl
0.91	<i>Salmonella</i> spp., <i>C. botulinum</i> , <i>Lactobacillus</i> spp., some moulds	Some cheeses, cured meats, juice concentrates, foods containing 55% sucrose or 12% NaCl
0.87	Most of the Yeast	Fermented sausages, some cakes, hard cheeses, margarine, foods containing 65% sucrose or 15% NaCl
0.80	Most of the molds <i>Saccharomyces</i> spp., <i>Staphylococcus aureus</i>	Most juice concentrates, condensed milk, syrup, flour, high-sugar cakes, some cured meats
0.75	Most halophilic bacteria Mycotoxigenic <i>Aspergillus</i> spp.	Jam, marmalade, fruit candies, marzipan, some biscuits, some cured meats
0.65	Some molds	Oatmeal at 10% moisture, jelly, molasses, nuts
0.60	Osmophilic yeasts and xerophilic moulds	Dried fruits (15–20% moisture), caramel, marshmallows, honey
0.50	No growth	Pasta (12% moisture), spices (10% moisture)
0.40		Egg powder (5% moisture)
0.30		Biscuits, crackers, dried bread 3-5% moisture
0.20		Milk powder (2–3% moisture), dried soups

Water Activity and Microbial Growth

Water activity	Lowest a_w allowing growth	Typical foods
1.00 - 0.95	Most Gram-negative bacteria, some yeasts	Fresh meat, fish, milk, fruit, vegetables
0.95 - 0.91	Most Gram-positive bacteria, <i>Salmonella</i> , <i>C. botulinum</i>	Cured meat, some cheeses, juice concentrates
0.91 - 0.87	Most yeasts, <i>Lactobacillus</i>	Fermented sausage, hard cheese, margarine
0.87 - 0.80	Most moulds, <i>Staphylococcus aureus</i>	Condensed milk, syrup, flour, cured meat
0.80 - 0.75	Halophilic bacteria	Jam, marmalade, marzipan
0.75 - 0.61	Xerophilic moulds, osmophilic yeasts	Dried fruit, honey, caramel, nuts
below 0.61	No microbial growth	Milk powder, dried soup, biscuits

Drying, salting and sugaring all work by lowering a_w ; the organism with the lowest a_w limit determines how the food finally spoils.

Acidity (pH)

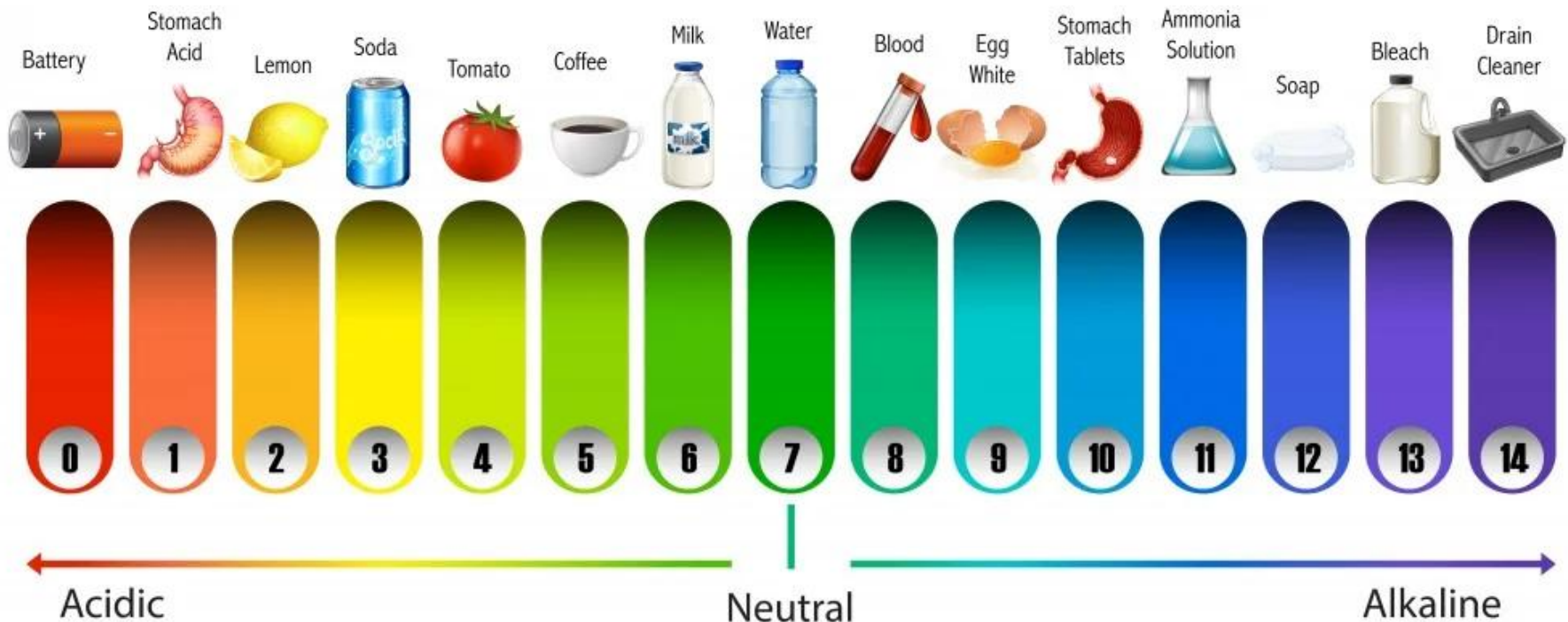
- The acidity of a food is expressed as pH and as titratable acidity. The pH indicates the H^+ ion concentration of the medium and is scaled from 0 to 14.
- The pH has a major effect on the growth of microorganisms.
- Most microorganisms, and especially the pathogens, grow over a fairly narrow pH range, and even a small change in pH may stop their growth.
- Microorganisms generally grow best at pH values close to neutrality (pH 6.0–8.0).
 - Moulds can grow between about pH 1.5 and 11 and yeasts between pH 1.5 and 8.5, whereas most bacteria grow only between about pH 4.0 and 9.0.
- Bacteria are therefore more sensitive to acidity than yeasts and moulds.
- From the point of view of microbiological safety, foods with a pH below 4.6 are regarded as safe with respect to *Clostridium botulinum*, because this organism cannot grow or produce toxin below that value.

Microorganism	Minimum pH	Optimum pH	Maximum pH
Bacteria	4.0 – 4.5	6.5 – 7.5	9.0
Yeast	1.5 – 3.5	4.0 – 6.5	8.0 – 8.5
Mold	1.5 – 3.5	4.5 – 6.8	9.0 – 11.0

Acidity (pH)

- Foods can be stored for longer if they are acidified and their pH is lowered. A low pH affects microorganisms in two ways:
 - it prevents the growth of microorganisms;
 - and it makes microorganisms more sensitive to heat.
- Canned foods with a pH above 4.6 (low-acid foods) must be sterilised at temperatures above 100 °C, whereas for foods with a pH below 4.6 (acid foods) a pasteurisation treatment below 100 °C is sufficient.
- **The acidity of a food may have three origins:**
 - it may be a natural constituent of the food;
 - it may be produced by the growth of microorganisms in the food, either deliberately (fermentation) or accidentally;
 - or it may come from organic acids added deliberately to the food.

Acidity (pH)



- Acidic medium;
 - moulds, yeasts, lactobacilli and acetic acid bacteria

- Neutral medium;
 - most of the pathogenic bacteria

- Alkali medium;
 - *Vibrio cholerae* and many soil bacteria

Approximate pH Values of Some Foods

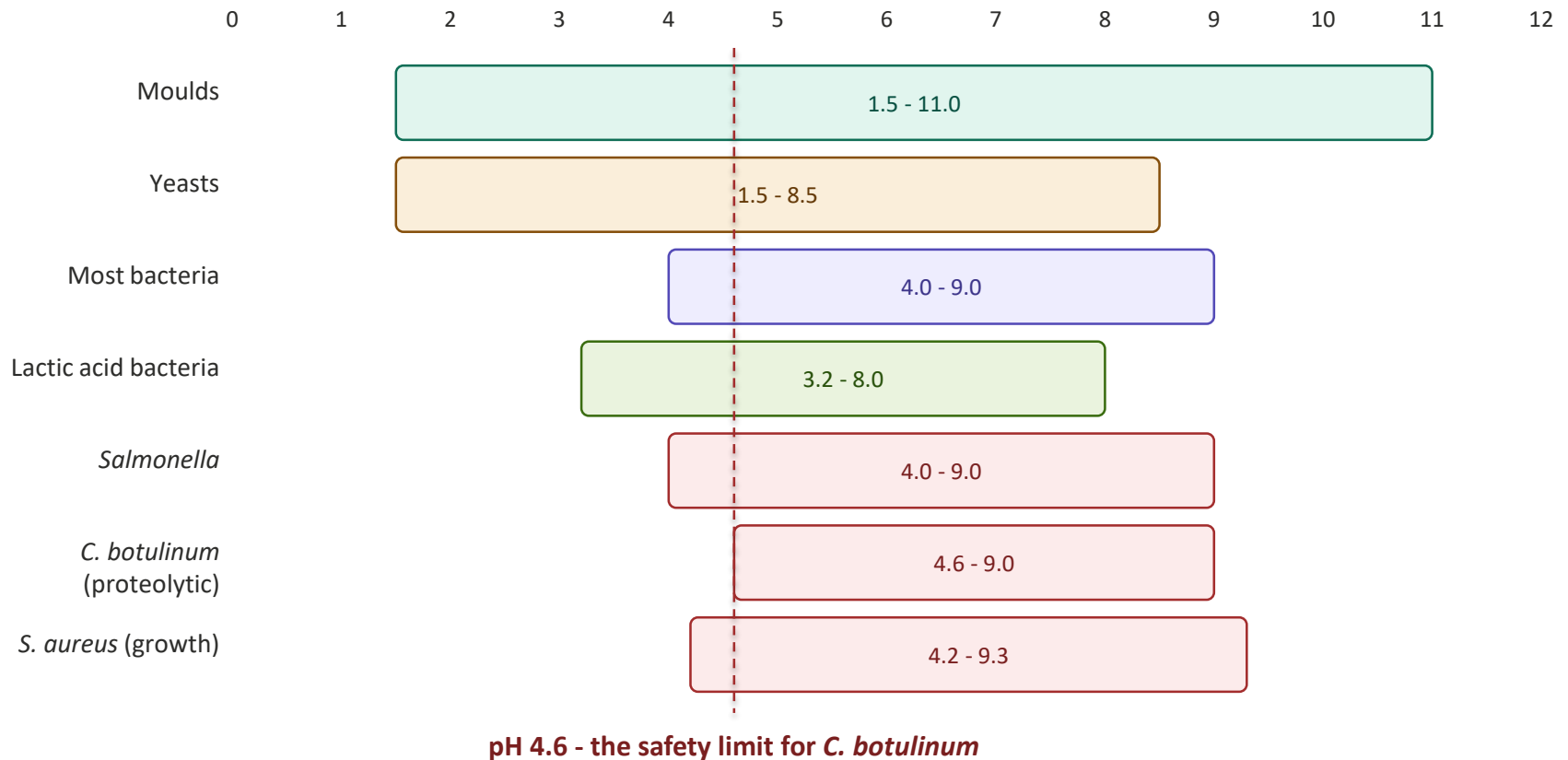
Highly Alkaline	Moderately Alkaline	Mildly Alkaline	Neutral/ Mildly Acidic	Moderately Acidic	Highly Acidic
pH 9.5 alkaline water Himalayan salt Grasses Cucumber Kale Kelp Spinach Parsley Broccoli Sprouts (soy, alfalfa etc) Sea Vegetables (Kelp) Green drinks All Sprouted Beans/ Sprouts	Avocado Beetroot Capsicum/Pepper Cabbage Celery Collard/Spring Greens Endive Garlic Ginger Green Beans Lettuce Mustard Greens Okra Onion Radish Red Onion Rocket/Arugula Tomato Lemon Lime Butter Beans Soy Beans White Haricot Beans Chia/Salba Quinoa	Artichokes Asparagus Brussels Sprouts Cauliflower Carrot Chives Courgette/Zucchini Leeks New Baby Potatoes Peas Rhubarb Swede Watercress Grapefruit Coconut Buckwheat Quinoa Spelt Lentils Tofu Other Beans & Legumes Goat & Almond Milk Most Herbs & Spices Avocado Oil Coconut Oil Flax Oil/ Udo's Oil	Black Beans Chickpeas/Garbanzos Kidney Beans Seitan Cantaloupe Currants Fresh Dates Nectarine Plum Sweet Cherry Watermelon Amaranth Millet Oats/Oatmeal Spelt Soybeans Rice/Soy/Hemp Protein Freshwater Wild Fish Rice & Soy Milk Brazil Nuts Pecan Nuts Hazel Nuts Sunflower Oil Grapeseed Oil	Fresh, Natural Juice Ketchup Mayonnaise Butter Apple Apricot Banana Blackberry Blueberry Cranberry Grapes Mango Mangosteen Orange Peach Papaya Pineapple Strawberry Brown Rice Oats Rye Bread Wheat Wholemeal Bread Wild Rice Wholemeal Pasta Ocean Fish	Alcohol Coffee & Black Tea Fruit Juice (Sweetened) Cocoa Honey Jam Jelly Mustard Miso Rice Syrup Soy Sauce Vinegar Yeast Dried Fruit Beef Chicken Eggs Farmed Fish Pork Shellfish Cheese Dairy Artificial Sweeteners Syrup Mushroom

← CAN BE INCLUDED IN YOUR 20% ACID →

Acidity (pH)

- Because the pH of most fruits is below 4.5, their microbial spoilage is caused mainly by yeasts and moulds, which can grow below pH 3.5.
 - This is well below the minimum pH at which most spoilage and pathogenic bacteria can grow.
- In fermented products such as cheese, fermented sausage and pickles, lactic acid bacteria produce lactic acid and lower the pH.
 - In this way the growth of many undesirable bacteria is prevented.
- Products such as fruit juices, soft drinks, vinegar and wine keep for a long time because their pH is below the value at which bacteria can grow.

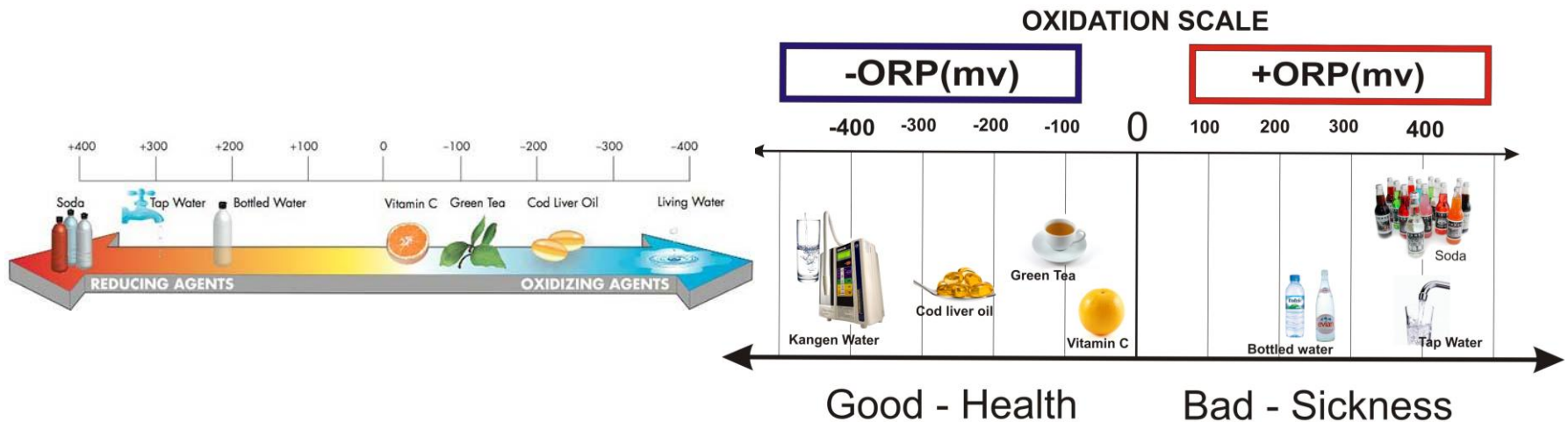
pH Ranges for Microbial Growth



Foods below pH 4.6 (acid foods) need only a pasteurisation treatment; foods above pH 4.6 (low-acid foods) must be sterilised above 100 °C.

Oxidation-Reduction Potential

- The oxidation–reduction potential (Eh) is a measure of the tendency of a chemical or biochemical system to become oxidised (to lose electrons) or reduced (to gain electrons).
- It is also called the redox potential and is denoted by O/R or Eh.
 - A positive value indicates an oxidised state and a negative value a reduced state. The Eh of milk is about +150 mV and that of the interior of cheese about –250 mV.
- The redox potential determines which microorganisms can grow with respect to their oxygen relationships.
 - Aerobic microorganisms need a high (positive) Eh to grow,
 - whereas anaerobic microorganisms require low (negative) Eh values.
- The Eh values of foods vary between about +500 mV and –500 mV.
- The Eh of fruits and vegetables is between +300 and +400 mV.
 - This means that aerobic bacteria and moulds can grow easily and spoil fruits and vegetables.
- The interior of a large piece of fresh meat has an Eh of about –200 mV, whereas that of minced meat is about +200 mV; mincing incorporates air and therefore raises the redox potential.
- The Eh values measured in canned foods range between –20 mV and –440 mV, which explains why anaerobic microorganisms can develop in them.



Antimicrobial Substances

- Substances that kill microorganisms (microbicidal), prevent their multiplication (microbistatic) or inhibit their activity are called antimicrobial agents.
 - Substances that kill bacteria are called bactericidal and those that stop them from multiplying are called bacteriostatic.
 - Substances that kill fungi (yeasts and moulds) are called fungicidal and those that stop their growth fungistatic.
- Antimicrobial substances are important for preventing undesirable microbial growth during food production.
- They may be naturally present in the food or added to it from outside.
- For an antimicrobial substance to have the desired effect, the amount used must be adjusted carefully and the pH, water activity and other components of the food must be taken into account.
- When antimicrobial substances are added to foods, the levels laid down in the relevant food regulations must be observed.

Antimicrobial Substances

Foods that naturally contain antimicrobial substances, and the antimicrobial substances they contain

Foods	Antimicrobial Substances	Foods	Antimicrobial Substances
Cow's milk	Lactoferrin, conglutinin, lactoperoxidase system	Cinnamon	Cinnamaldehyde and eugenol
Egg	Lysozyme	Mustard	Allyl isothiocyanate
Onion and garlic	Allicin	Sage	Eugenol and thymol
Clove	Eugenol	Fruits, vegetables, tea, molasses	Hydroxycinnamic acids
Citrus fruits (peel)	Citral and limonene		

Antimicrobial Substances

- Antimicrobial substances added to foods, the microorganisms they act on and the foods in which they are used

Antimicrobial substance	Microorganisms to which it is effective	Used Food
Sulphur dioxide (SO ₂) and sulphites	Moulds, yeasts and bacteria	Dried fruits, syrups, purees
Nitrite and nitrate	<i>Clostridium botulinum</i> , <i>Clostridium butyricum</i> , <i>Clostridium sporogenes</i>	Meat and meat products, cured fish
Sorbic acid	Moulds and yeasts	Cheese, cereals, jams, marmalades, sauces, margarine, ketchup, dried fruit, meat and fish products, pickles and brine
Benzoic acid	Moulds and yeasts	Bread, pastry, biscuits, wafers, cake creams, soft drinks, fruit juices, table olives, sauces, cocoa products, ketchup, margarine
Propionic acid	Moulds and <i>Bacillus subtilis</i> (rope in bread)	Bread, bakery products and cheese
Acetic acid	Yeasts and bacteria	Ketchup, pickles, bread, bakery products

Nutrients

- Foods consist of six groups of nutrients: carbohydrates, lipids, proteins, vitamins, minerals and water. Microorganisms need all or some of these nutrients in order to grow.
- The nutrients that different microorganisms need or can use vary from species to species.
- Which nutrients a microorganism can use is determined mainly by the enzymes it possesses.
- With these enzymes, microorganisms break large nutrient molecules down to a size that can be taken into the cell, and then use them according to their needs; as a result they produce a variety of compounds called metabolites.
- Carbohydrates are the most important energy source for the microorganisms growing in foods. Small molecules such as glucose are taken directly into the cell, whereas large molecules such as starch and cellulose can be used only by organisms possessing amylase or cellulase.

Nutrients

- The proteins of a food cannot be used directly by microorganisms; they must first be broken down into amino acids and small peptides.
 - Microorganisms that possess proteases and peptidases hydrolyse proteins to amino acids and use them.
- Microorganisms possessing such enzymes are called proteolytic microorganisms.
- Fats (lipids) can be hydrolysed and used by microorganisms that possess lipase; these are called lipolytic microorganisms.
- Fresh and unprocessed foods in particular are rich in vitamins and easily meet the needs of microorganisms. In addition, many microorganisms synthesise the vitamins they need themselves.
- The degradation and use of the nutrients of a food by microorganisms is what we call microbiological spoilage.

Nutrients

- Minimum nutrient requirement:
moulds > yeasts > Gram(-) bacteria > Gram(+) bacteria
- In general, aerobic microorganisms are more strongly lipolytic, and lipolytic organisms are usually proteolytic as well.
- Gram-positive bacteria generally have a low biosynthetic capacity and are nutritionally fastidious.
- Gram-negative bacteria and moulds can synthesise most of their nutritional requirements themselves.
- Growth factors: substances that some microorganisms need for growth but cannot synthesise, and that must therefore be supplied ready-made from outside.

Protective Biological Structures

- The peel and waxy layers of fruits and vegetables and the hard shells of nuts are natural barriers against microorganisms.
- Damage to these protective structures increases the entry of microorganisms and the damage they cause.
- Fruits picked in such a way that the stalk is pulled out are susceptible to stem-end rot.
- These protective structures can be damaged by insects as well as during harvesting.

Nutrients

- The microflora of a food is mixed, and its members affect one another.
- Some microorganisms inhibit or kill others with the metabolites they produce.
- This phenomenon is called antagonism; the most common form is lactic antagonism. LAB act on other microorganisms through lactic acid and other organic acids, diacetyl and hydrogen peroxide.
 - Antibiotics: subtilin produced by *Bacillus subtilis*, and natamycin (pimaricin) produced by *Streptomyces natalensis*.
 - Bacteriocins: antimicrobial peptides produced by bacteria, for example nisin produced by *Lactococcus lactis*.
 - Other inhibitors: propionic acid produced by propionic acid bacteria and ethanol produced by yeasts.

Structure of Foods

- The outer coverings of some foods and the layers that make them up prevent microorganisms from reaching the nutrients inside.
- Examples are the waxy cuticle of fruits and of the egg shell and the hard, tightly woven shells of oilseeds such as hazelnut and walnut.
- Likewise, the skin and scales of fish and the mucus layer covering them provide protection against microorganisms.
- Injury or cracking of these coverings gives microorganisms an easy route to the nutrients and allows them to grow and spoil the food.

Factors Affecting Microbial Growth in Foods

Extrinsic (environmental) factors:

- a. relative humidity;
 - b. composition and concentration of the gases in the atmosphere;
 - c. storage temperature and time;
 - d. radiation.
- The extrinsic factors are the characteristics of the place where the food is kept or stored. They have an important influence on microbial growth and can in time change intrinsic factors such as a_w and Eh and alter the physical properties of the food.

Temperature and Time

- One of the most important requirements of microorganisms for growth is a suitable temperature.
- Above the temperature range in which they grow, microorganisms may be killed; below it, they usually only stop multiplying and survive.
 - Low temperatures therefore cannot be relied on to kill microorganisms.
- According to the temperature range in which they grow, microorganisms are divided into three main classes:
 - **psychrophilic (cold-loving),**
 - **mesophilic (moderate-temperature) and**
 - **thermophilic (heat-loving); the psychrotrophs, which grow in the cold but have a mesophilic optimum, are an important additional group.**

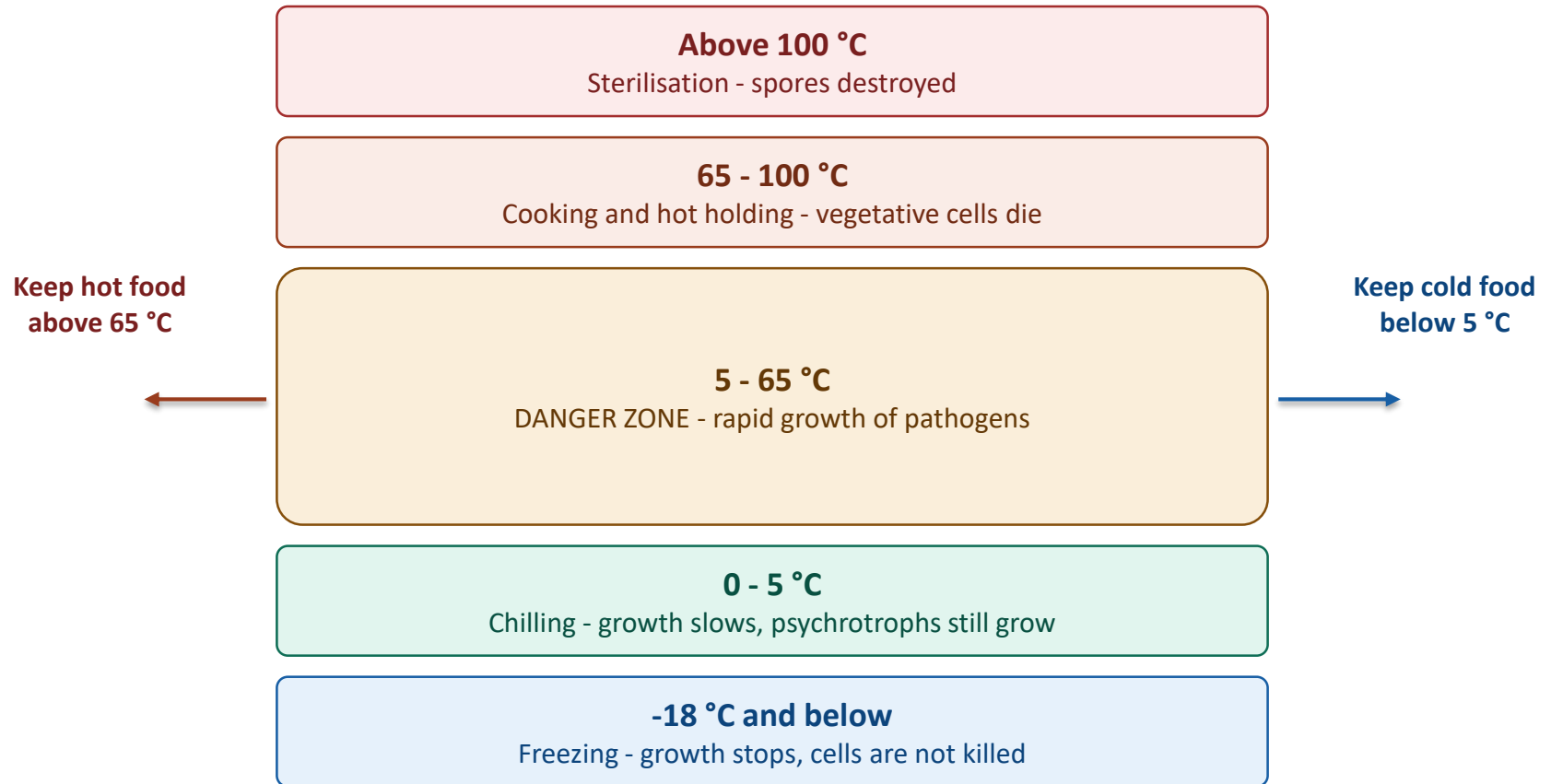
Microorganism Grup	Growth temperature (°C)		
	Minimum	Optimum	Maximum
Psychrophilic	0	15	20
Mesophilic	5 - 15	25 - 45	45 - 50
Thermophilic	35- 45	45 - 65	60 - 90

Temperature and Time

- Since most of the pathogens and spoilage organisms are mesophilic, storage temperatures below 5 °C and above 65 °C are regarded as safe; the range between them is called the danger zone.
- When a storage temperature is chosen, it must also be ensured that the quality characteristics of the food are not adversely affected (for example, chilling injury).
- The optimum storage temperature differs from food to food.

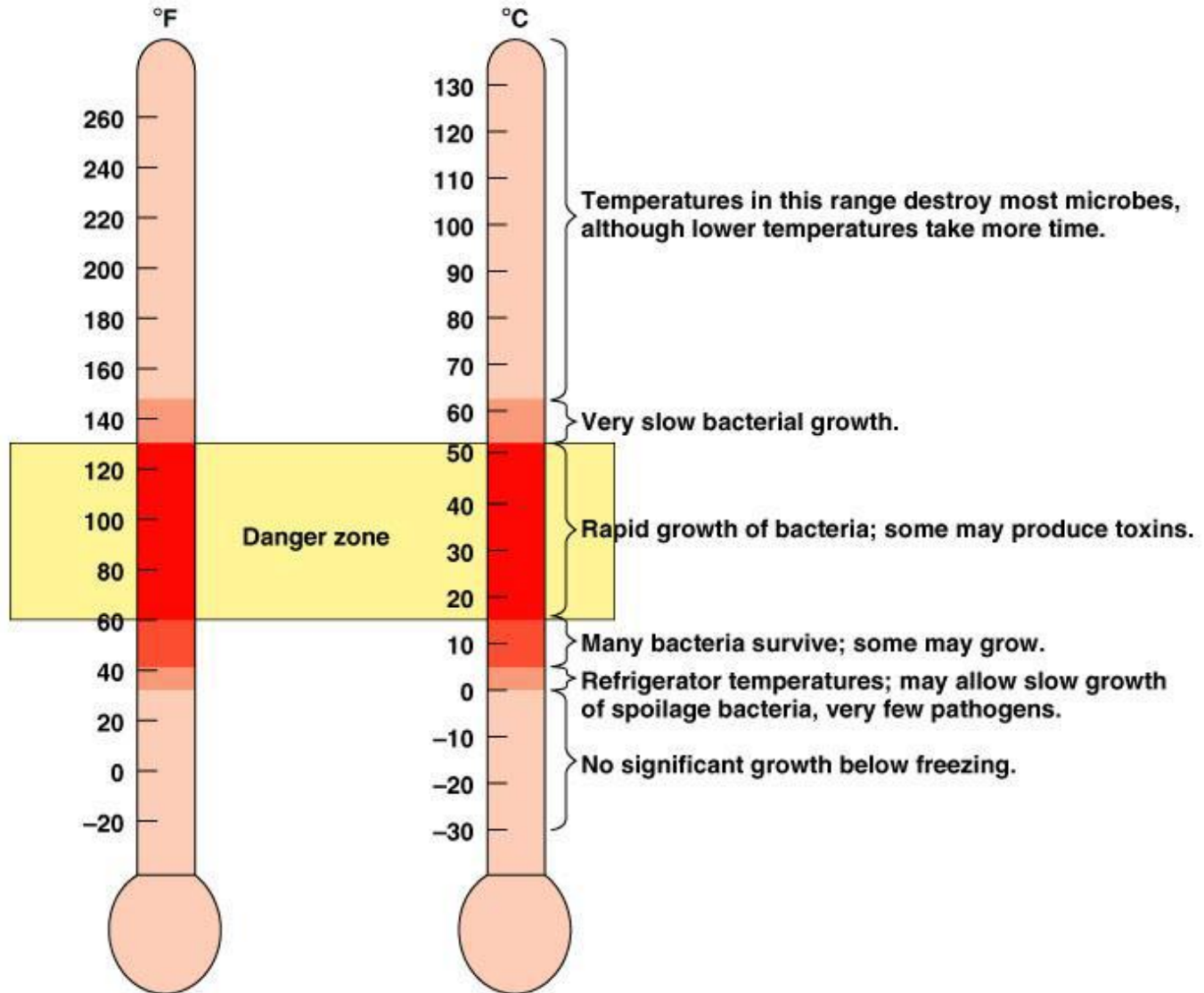
Foods	Storage Temperature (°C)	Foods	Storage Temperature (°C)
Pumpkin	+10	Fresh meat	0 - (+1)
Banana	+15	Fish	-1 - (+2)
Potato	+10	Cheese	+1 - (+4)
Beans	+4	Milk powder	+7 - (+21)
Vegetable oil	+21	Butter	+4

The Temperature Danger Zone



Most foodborne pathogens are mesophilic, so the 5-65 °C range is where they multiply fastest; time spent in this zone must be kept to a minimum.

Temperature and Time



Relative Humidity

- The relative humidity of a food store is important both for the microorganisms that can grow on the surface of the food and for the water activity of the food itself.
- Water molecules always move from the place where they are more abundant to the place where they are less abundant. If the food is drier than the surrounding air, moisture passes from the air into the food. As the moisture content of the food rises, its a_w also rises, microorganisms grow and the food spoils.
- If the food is more moist than the surrounding air, moisture passes from the food into the air. In fruits and vegetables this causes undesirable wilting, shrinkage and softening.
- To prevent microbiological spoilage and quality loss during storage, every food must therefore be stored at the relative humidity appropriate to its moisture content.

Relative Humidity

Recommended storage relative humidity and moisture content of some foods

Food	Storage Relative Humidity (%)	Moisture content of food (%)	Food	Storage Relative Humidity (%)	Moisture content of food (%)
Fresh meat	88-92	62-77	Cucumber	90-95	96
Fresh chicken meat	90-95	74	Lettuce	95	95
Fish	90-95	60-80	Mandarin	85-90	87
Cheese	65-70	30-60	Garlic (dry)	65-70	61
Candies	50	6-10	Dried fruit	50-60	14-26

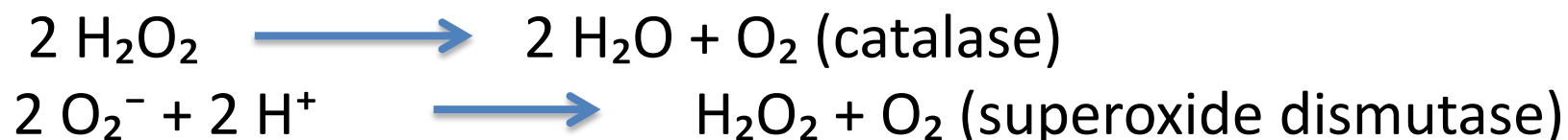
Gases

- Gases have an important effect on microbial growth. The gases in the storage room and in the package are particularly effective against the microorganisms growing on the surface of the food.
- Aerobic microorganisms need oxygen in order to grow, whereas oxygen is toxic to obligate anaerobes.
- There are also microaerophilic bacteria, as well as moulds and yeasts, that grow at very low oxygen concentrations.
- Carbon dioxide, which is present in air at about 0.04%, inhibits the growth of aerobic microorganisms when its concentration is raised. Spoilage is delayed in this way, particularly in the storage of fruits and vegetables.

Gases

- The normal atmosphere contains fixed proportions of O₂ (about 21%), CO₂ (about 0.04%), N₂ (about 78%) and other gases. The proportions of these gases affect microbial growth.
- Storage under an atmosphere in which the O₂ and CO₂ levels are continuously regulated is called “controlled atmosphere” storage, whereas adjusting the O₂, CO₂ and N₂ levels inside a package at the time of packaging is called “modified atmosphere” packaging.
- In general, Gram-negative bacteria are more sensitive to CO₂ than Gram-positive bacteria.
- Ozone (O₃) is widely used in the disinfection of drinking water and of processing water.

Oxygen Requirement



- **Obligate aerobes: oxygen is required for growth**
 - *Bacillus subtilis*,
 - *Mycobacterium tuberculosis*,
 - *Bacillus anthracis*,
 - *Corynebacterium diphtheriae*
- **Microaerophilic bacteria: grow at 1–4% O₂ and 5–10% CO₂. They need oxygen for their metabolism but cannot survive at the atmospheric concentration (21%).**
 - *Brucella abortus*
 - *Campylobacter jejuni*

Oxygen Requirements: The Tube Test

Thioglycolate broth: oxygen diffuses from the surface, so the top is aerobic and the bottom is anaerobic.



Obligate aerobe

Pseudomonas, B. subtilis

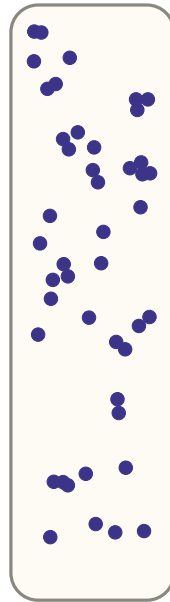
catalase +, SOD +



Obligate anaerobe

Clostridium

no catalase, no SOD



Facultative anaerobe

E. coli, Salmonella, yeasts

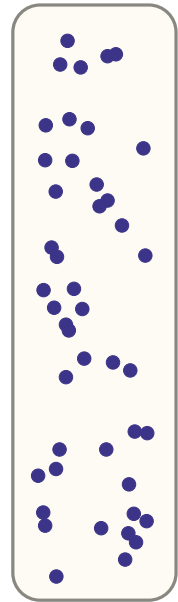
catalase +, SOD +



Microaerophile

Campylobacter

low levels



Aerotolerant

lactic acid bacteria

SOD +, catalase -

Catalase and superoxide dismutase (SOD) destroy the toxic by-products of oxygen

Facultative anaerobic bacteria

- They use aerobic respiration in the presence of oxygen
- and fermentation or anaerobic respiration in its absence.
- Facultative anaerobes use oxygen when it is available, but continue to grow without it.
 - *Salmonella*,
 - *Shigella*,
 - *Streptococcus*,
 - *Escherichia coli*
 - *Klebsiella*,
 - *Proteus*

Anaerobic bacteria

- They cannot use oxygen and are killed in its presence.
- They lack catalase and superoxide dismutase.
 - Obligate anaerobes
 - *Clostridium*,
 - *Fusobacterium*,
 - *Bacteroides*,
 - *Peptococcus*,
 - *Peptostreptococcus*

Gases

- During packaging, the air inside the pack can be removed (vacuum packaging) or replaced with a suitable gas mixture (modified atmosphere packaging, MAP).
- In this way microbial growth is slowed down and the shelf life of the food is extended. The gases used in modified atmosphere packaging are nitrogen (N_2), carbon dioxide (CO_2), carbon monoxide (CO) and oxygen (O_2), in proportions suited to the food.



Radiation (Ionization Radiation)

- Microorganisms are affected by radiation of certain wavelengths. Besides the ionising gamma and beta rays, ultraviolet light is used in the disinfection of foods and surfaces.
- Gamma and beta rays penetrate into the food but leave no significant radioactive residue. Because they do not raise the temperature of the food appreciably, irradiation is also called cold sterilisation.
- Ultraviolet light reaches the earth from the sun but can also be produced by special lamps. Since it does not penetrate into materials, it is used for the inactivation of microorganisms on surfaces.
- Irradiated foods must carry the Radura symbol on the package.

Radiation (Ionization Radiation)

The Ministry of Agriculture and Forestry has laid down the conditions for food irradiation in the Food Irradiation Regulation:

1. Irradiation cannot replace good manufacturing, good agricultural or good hygiene practices and is applied only when:

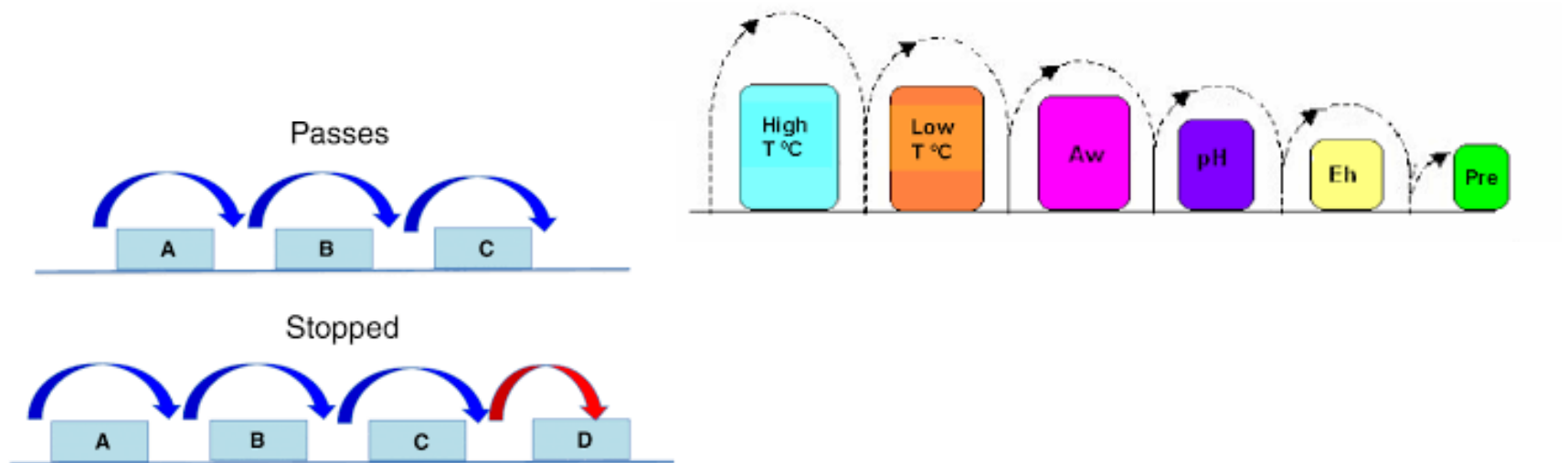
- a. there is a technological need;
- b. it presents no health hazard and is carried out under the recommended conditions;
- c. it is in the interest of the consumer.

2. Food irradiation is used only for the following purposes:

- a. preventing foodborne disease by inactivating pathogenic microorganisms;
- b. preventing spoilage by inactivating spoilage microorganisms and delaying decay;
- c. reducing losses caused by sprouting, germination and ripening;
- d. disinfestation of plants and plant products.

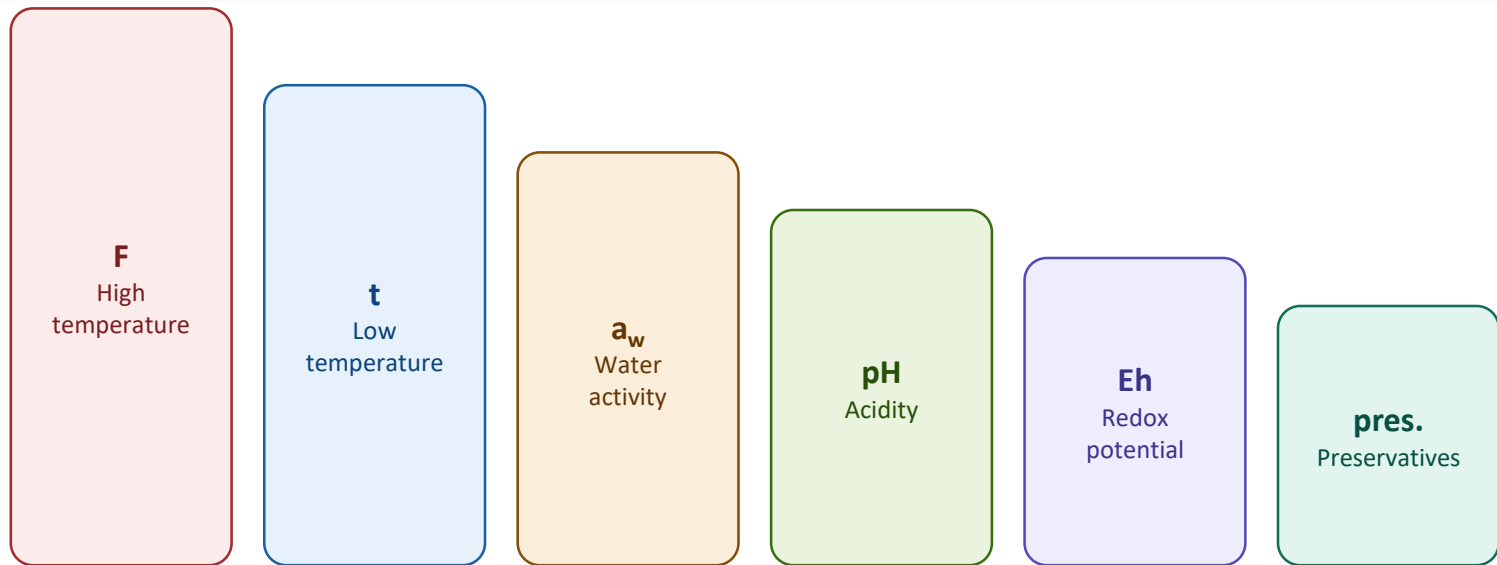
The Concept of Hurdle (Combination of Factors)

- The hurdle concept refers to the combined use of several factors, each applied at a sub-lethal level, to control spoilage and the growth of pathogenic microorganisms in foods.
 - Hurdle technology, combined preservation and combined techniques are terms used for the same concept.



The Hurdle Concept

Microorganism



Each hurdle on its own is sub-lethal; together they prevent growth

The organism must clear every hurdle in turn. Because each factor is applied only at a mild level, the sensory and nutritional quality of the food is preserved far better than with one severe treatment.