

# FE 305 Food Microbiology

## Microbial Metabolism of Foods

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# Microbial Metabolisms

- Energy and nutrients are essential for the survival, growth and reproduction of microorganisms.
- The ability of bacteria to use the components of a food depends on the ability of those components to pass through the cell wall and the cytoplasmic membrane.
- The molecules of most useful substances in the surrounding medium are too large to pass through the cell wall.
- They must therefore first be broken down to a size that can be taken up by transport systems.
- This is achieved by the hydrolytic enzymes (exoenzymes) that the bacteria synthesise and secrete into the medium.
- Food components (proteins, carbohydrates, lipids, etc.) that have been reduced by these exoenzymes to a size that can cross the membrane are broken down further, to their smallest building blocks, once they are inside the cytoplasm.

# Microbial Metabolisms

- The endoenzymes, which remain inside the cell, catalyse this further breakdown in the cytoplasm (dissimilation, catabolism).
- From these building blocks the microorganism then synthesises the substances it needs — proteins, polysaccharides, lipids, enzymes and so on (assimilation, anabolism).
- These two closely linked biochemical processes together constitute metabolism.
- Metabolism, the integration of the many enzymatic processes involved in the growth and reproduction of microorganisms, comprises all the biochemical changes (anabolic and catabolic) that occur in a living cell.
- Most of the energy released in these chemical reactions is used for microbial activities; the rest is lost as heat.

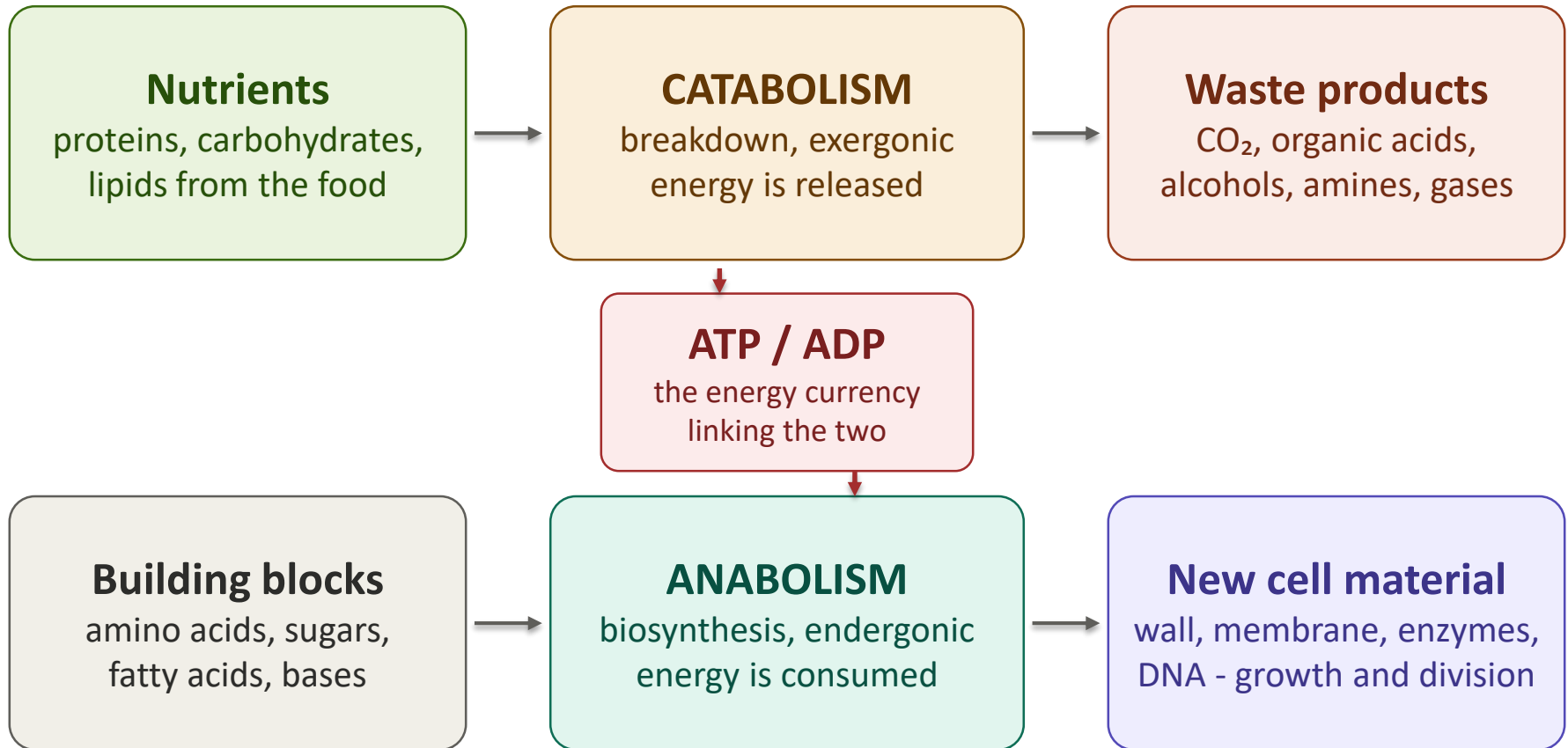
# Microbial Metabolisms

- A good understanding of microbial metabolic reactions is of great practical value.
- Metabolic events can be examined in three groups:
  - digestion:
    - extracellular degradation,
    - intracellular degradation;
  - respiration and fermentation (catabolism);
  - biosynthesis (anabolism).

# Microbial Metabolisms

- Metabolic events are carried out step by step and in sequence through the catalytic action of many enzymes. Even the smallest change in these enzymes can disturb this sequence or change its direction.
- In growing cells, metabolic events are continuous and interrelated.
  - By anabolism the cell renews itself and synthesises the compounds it has consumed and those it needs.
  - By catabolism the energy and the building blocks required for synthesis are made available.
- Metabolism slows down under conditions such as nutrient shortage, desiccation, sporulation, temperatures below the minimum and the action of microbistatic chemicals, and stops almost completely on freezing.
- In catabolic reactions, a considerable amount of energy is released as the nutrients are broken down (exergonic reactions); this energy is captured by ADP (adenosine diphosphate) and stored in the high-energy phosphate bonds of ATP (adenosine triphosphate).
- The energy required for synthesis is supplied from these bonds (endergonic reactions).

# Catabolism and Anabolism



*Catabolism releases energy and traps it in the phosphate bonds of ATP; anabolism spends that ATP to build the cell. In a growing cell the two run continuously and are inseparable.*

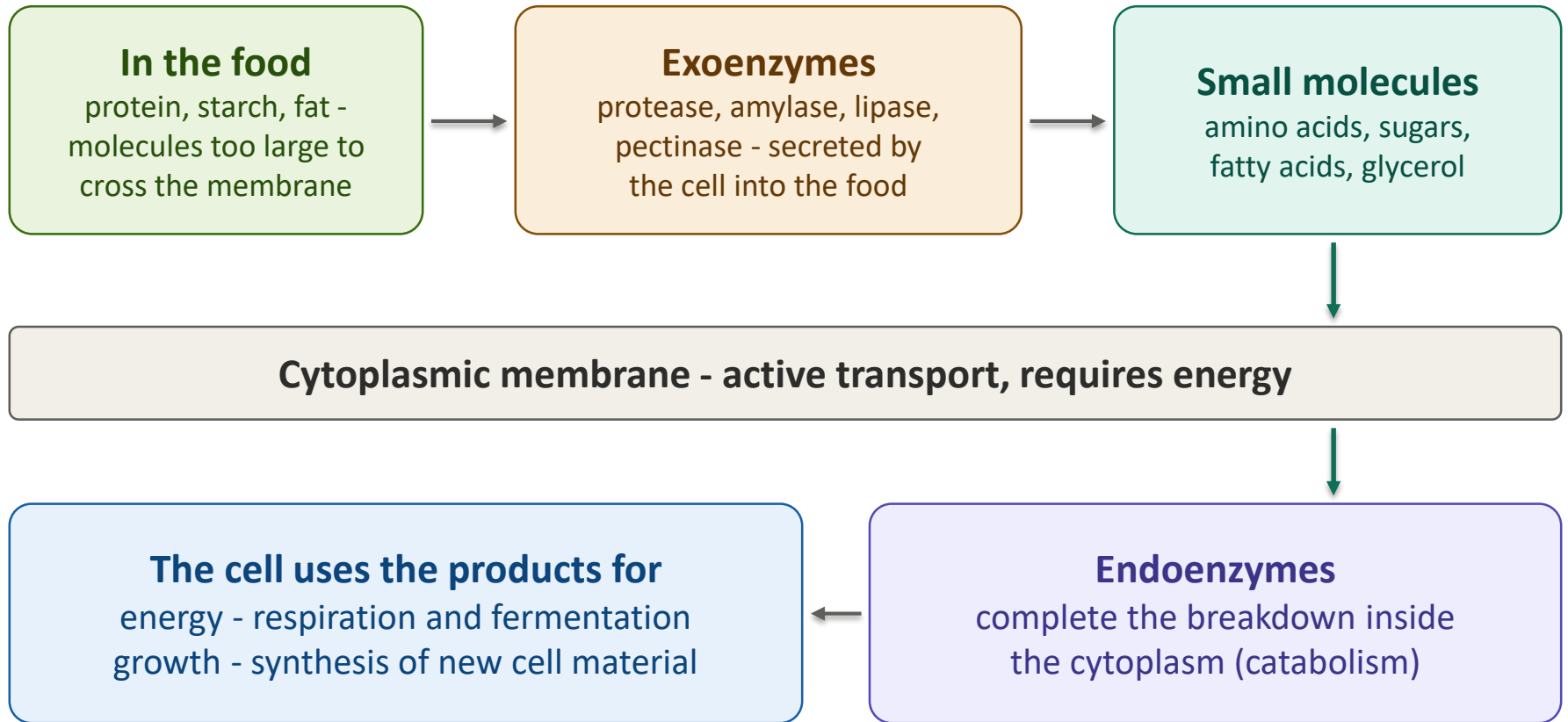
# Extracellular degradation

- Complex molecules are too large to enter the cell through the semi-permeable cytoplasmic membrane.
- Large complex molecules such as proteins and starch are therefore hydrolysed outside the cell to a size that can cross the membrane.
- These reactions are catalysed by extracellular enzymes that the cell synthesises and secretes into the medium.
- The entry of the resulting smaller organic molecules into the cell is not simple diffusion but takes place through carrier-mediated transport, and most of it requires energy (active transport).

# Intracellular degradation

- Large organic molecules are broken down further inside the cell.
- These reactions are carried out by intracellular hydrolases.
- The nutrients formed by the action of these intracellular hydrolases are now available to the cell.
- The cell can use them simultaneously for two interrelated vital processes:
  - the synthesis of new cellular components (growth and multiplication), and
  - energy-yielding reactions (respiration and fermentation).

# Extracellular and Intracellular Digestion



*Because the exoenzymes act in the food itself, spoilage is visible long before the cells reach high numbers: softening, sliminess, off-odours and rancidity are the work of these enzymes.*

# Intracellular degradation

- **Respiration and fermentation (catabolism)**
  - The energy stored in the organic molecule is released by respiration and fermentation and is used in cell metabolism.
- **Biosynthesis (anabolism)**
  - These are the complex processes in which more complex biomolecules are built up inside the cell from simple molecules:
  - the synthesis of proteins from amino acids,
  - the synthesis of lipids from fatty acids and glycerol.
  - The programming and execution of these syntheses are directed by the genetic information encoded in the DNA of the chromosome.
  - The end products of these syntheses are the cell wall, the cell membrane, enzyme proteins, chromosomal material, cytoplasmic proteins and the other components necessary for life and reproduction.
  - Biosynthesis cannot take place without energy;
  - it is therefore always coupled to energy-yielding reactions.

# Biosynthesis (anabolism)

- Bacterial metabolism is far more intense than that of animal cells, and the metabolic rate is much higher.
  - A very small bacterial cell can metabolise a large amount of substrate in a short time and increase its weight several times over.
- To match the metabolic activity of a bacterial cell, a human being would have to consume about half a tonne of food per day.
- This very high metabolic activity is due to the small size of the cell, its very high surface-to-volume ratio and its very short generation time.
- A single bacterial cell can give rise to millions of cells within a day.
- Fundamentally, all the changes that take place in a microbial cell are brought about by enzymes, which are biological catalysts.
- Because the production of most foods and products obtained with microorganisms is based on fermentation, the substrates generally targeted in fermentations are carbohydrates.

# Microbial Metabolism

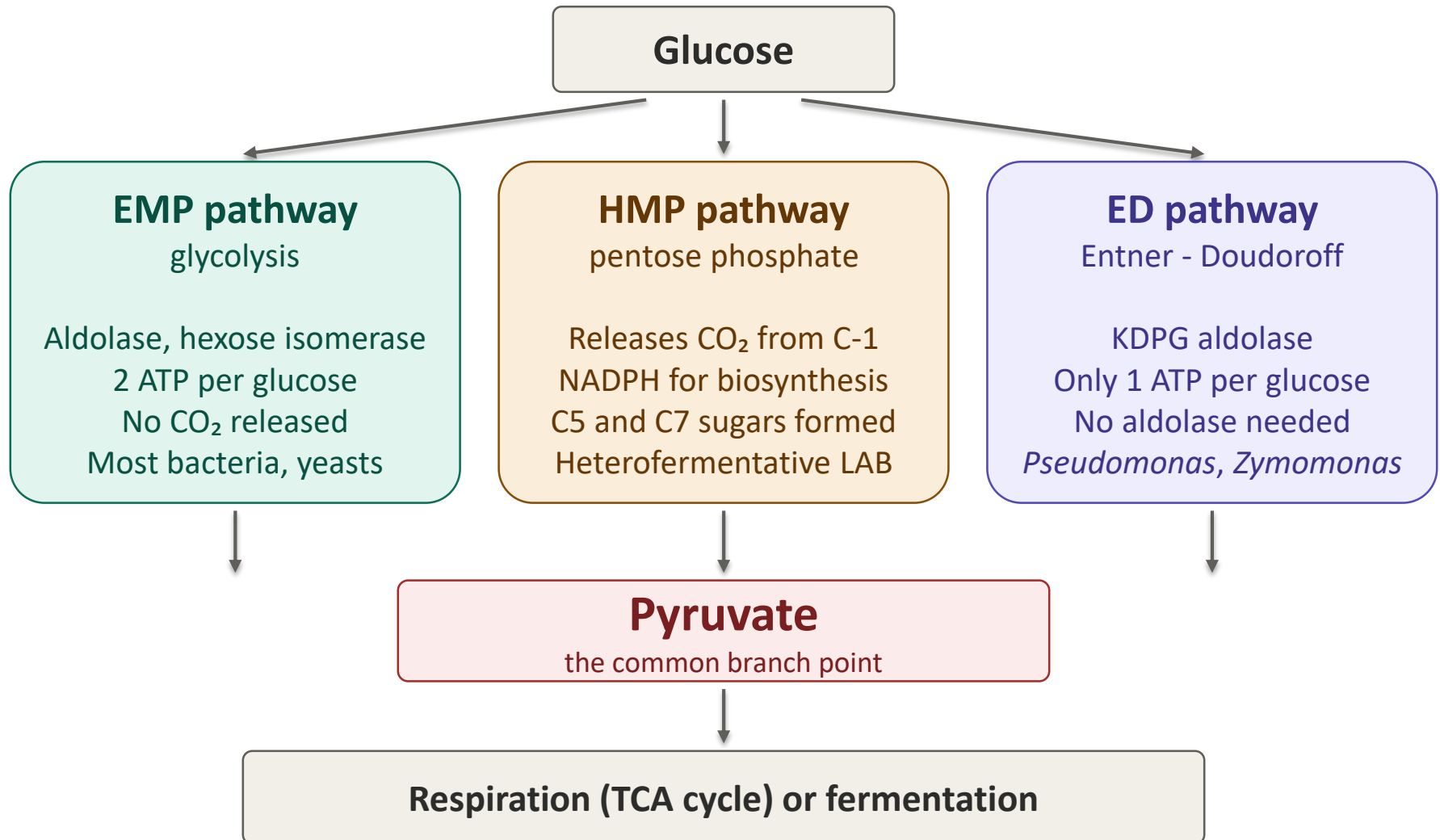
Metabolism in microorganisms is examined under three main headings:

- 1) carbohydrate metabolism,
- 2) lipid metabolism,
- 3) protein and amino acid metabolism.

# Carbohydrate metabolism

- Microorganisms cannot metabolise complex carbohydrates directly.
- Under normal conditions, carbohydrate metabolism is generally understood to mean glucose metabolism.
  - Other sugars are largely converted to glucose or to glycolytic intermediates before entering metabolism.
- Although several pathways exist for the metabolism of monosaccharides, the following four are the most important, and the EMP pathway above all:
  1. breakdown of glucose by the hexose diphosphate route (Embden–Meyerhof–Parnas, EMP, pathway);
  2. breakdown of hexoses by the oxidative pentose phosphate route (hexose monophosphate, HMP, pathway);
  3. breakdown of glucose by the Entner–Doudoroff (ED) pathway;
  4. the tricarboxylic acid (TCA) cycle, also called the Krebs cycle or citric acid cycle.

# Three Routes from Glucose to Pyruvate



# Carbohydrate metabolism

- Carbohydrates are organic compounds composed of carbon (C), hydrogen (H) and oxygen (O), combined in the general proportion  $(\text{CH}_2\text{O})_n$ .
- Carbohydrates serve heterotrophic microorganisms both as an energy source and as a source of carbon skeletons for the synthesis of organic compounds.
- Carbohydrates are classified according to the number of sugar units they contain. Mono- and disaccharides are readily soluble in water and can be crystallised and dialysed, whereas polysaccharides are insoluble in water and are not dialysable.
- **Monosaccharides**
  - Monosaccharides, with the empirical formula  $(\text{CH}_2\text{O})_n$ , are classified according to the number of their carbon atoms:
  - triose ( $\text{C}_3\text{H}_6\text{O}_3$ ): glyceraldehyde;
  - tetrose ( $\text{C}_4\text{H}_8\text{O}_4$ ): erythrose;
  - pentose ( $\text{C}_5\text{H}_{10}\text{O}_5$ ): arabinose, xylose, ribose (rhamnose is a deoxyhexose);
  - hexose ( $\text{C}_6\text{H}_{12}\text{O}_6$ ): galactose, glucose, fructose, mannose.

# Carbohydrate metabolism

## Disaccharides ( $C_{12}H_{22}O_{11}$ )

- Disaccharides are formed by the condensation of two monosaccharide units with the loss of a molecule of water.
  - Lactose: glucose + galactose; maltose: glucose + glucose; sucrose: glucose + fructose.

## Trisaccharides ( $C_{18}H_{32}O_{16}$ )

- They are composed of three monosaccharide units.
  - Raffinose: galactose + glucose + fructose.

## Polysaccharides ( $C_6H_{10}O_5$ )<sub>n</sub>

- These are formed by the condensation of many monosaccharide molecules with the loss of water. The most important homopolysaccharides are dextrin, glycogen, inulin, starch and cellulose; hyaluronic acid is an example of a heteropolysaccharide.

## Alcohols

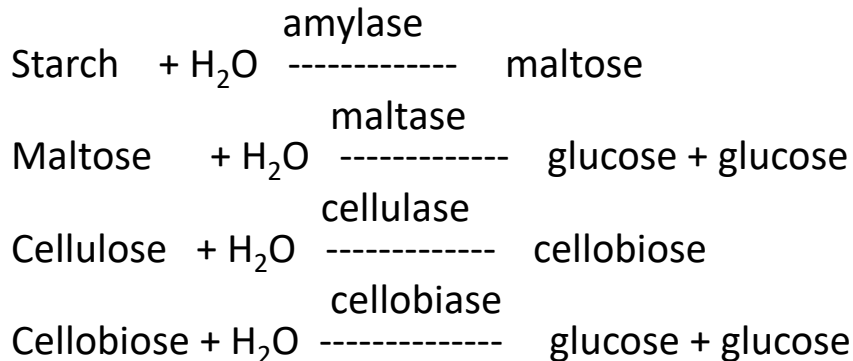
- Adonitol, dulcitol, erythritol, glycerol, inositol, mannitol, sorbitol

# Carbohydrate Decomposition

**A) Breakdown of polysaccharides: polysaccharides are high-molecular-weight polymers consisting of monosaccharide units joined by glycosidic bonds. Breaking these bonds releases the constituent units. This occurs in two main ways:**

**1. Hydrolysis: the glycosidic bond between the monosaccharides is split with the addition of water by the specific enzymes (carbohydrases) of the microorganism, and the polysaccharide is broken down.**

For example:



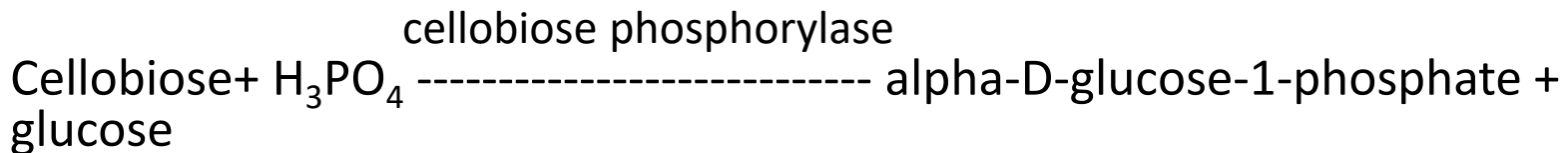
- Hyaluronic acid, a heteropolysaccharide, is hydrolysed by the hyaluronidase produced by many bacteria (streptococci, staphylococci, clostridia, etc.).
- Lactose, a disaccharide, is hydrolysed by the  $\beta$ -galactosidase of *E. coli* and of the lactic acid bacteria.

# Carbohydrate Decomposition

## 2- Phosphorylation

- Polysaccharide phosphorylases also break down polysaccharides.
- Because this reaction is reversible, it also takes part in the synthesis of carbohydrates.
- Such enzymes occur in *Clostridium*, *Streptococcus*, *Neisseria*, *Chromobacterium* and *Agrobacterium*.
- They catalyse the phosphorolytic cleavage of polysaccharides.

For example:



# Carbohydrate Decomposition

## B) Breakdown of monosaccharides

- Glucose has a central role in the structure and metabolism of the other carbohydrates.
- After glucose has entered the bacterial cell, it is either stored as a reserve polysaccharide or broken down further — aerobically or anaerobically — until the end products are formed.
- The mode of breakdown and the end products formed vary widely between microorganisms, and are used in their identification.
- The end products formed from glucose by heterotrophic microorganisms are:
  - a. organic acids: acetic, butyric, formic, kojic, lactic, propionic, pyruvic and succinic acid, etc.;
  - b. neutral products: acetoin (acetyl methyl carbinol), acetone, butanol, 2,3-butylene glycol, diacetyl, ethanol, isopropanol, n-propanol, etc.;
  - c. gases: hydrogen, carbon dioxide and methane.

# Carbohydrate Decomposition

- Aerobic, facultatively anaerobic and anaerobic microorganisms break glucose down in different ways and form different end products:
  - the breakdown of glucose to the end products is not a single step but a series of reactions;
  - each step is catalysed by a separate, specific enzyme;
  - once the sequence is started, the steps follow one after another.
- During both the aerobic and the anaerobic breakdown of glucose, a key intermediate, pyruvic acid, is formed.
  - The different fermentation routes diverge from this point.
  - The conversion of glucose to pyruvate is itself not a single step but a series of biochemical reactions.
  - These steps can be followed in the Embden–Meyerhof–Parnas scheme.

# Fermentation Products and the Foods They Give

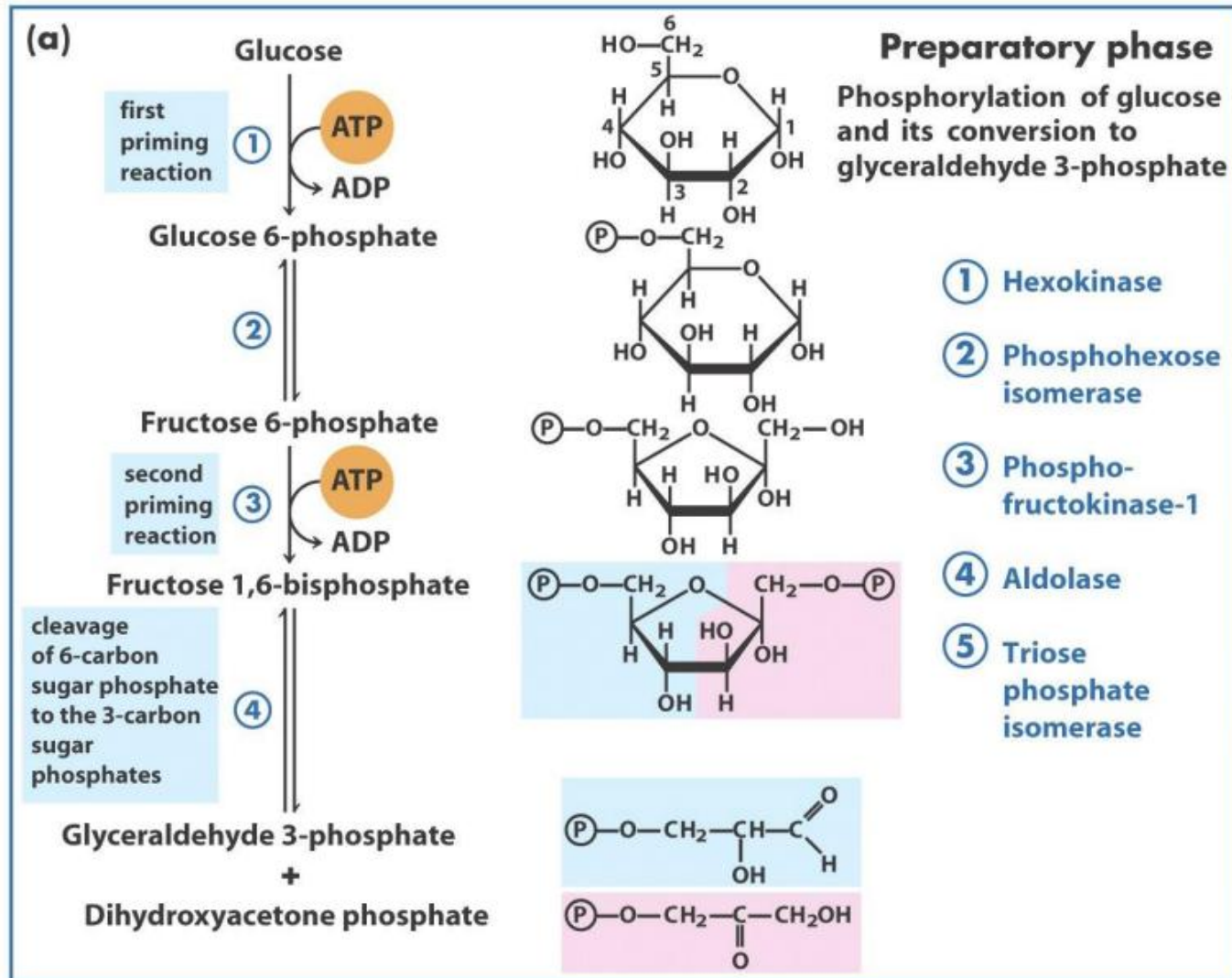
| Pyruvate                         | Main products                                      | Foods and defects                    |
|----------------------------------|----------------------------------------------------|--------------------------------------|
| Homolactic fermentation          | lactic acid                                        | Yoghurt, cheese, pickles, sucuk      |
| Heterolactic fermentation        | lactic acid + CO <sub>2</sub> + ethanol or acetate | Sauerkraut, kefir, sourdough         |
| Alcoholic fermentation           | ethanol + CO <sub>2</sub>                          | Bread, beer, wine                    |
| Propionic acid fermentation      | propionic + acetic acid + CO <sub>2</sub>          | Swiss-type cheeses (the eyes)        |
| Butyric acid fermentation        | butyric acid + CO <sub>2</sub> + H <sub>2</sub>    | Spoilage: blowing of cheese and cans |
| Mixed acid fermentation          | acids, ethanol, CO <sub>2</sub> + H <sub>2</sub>   | Spoilage by coliforms                |
| Oxidative (acetic acid bacteria) | acetic acid from ethanol                           | Vinegar; spoilage of wine and beer   |

*The same reaction is desirable in one product and a defect in another; the difference lies in which organism is allowed to grow.*

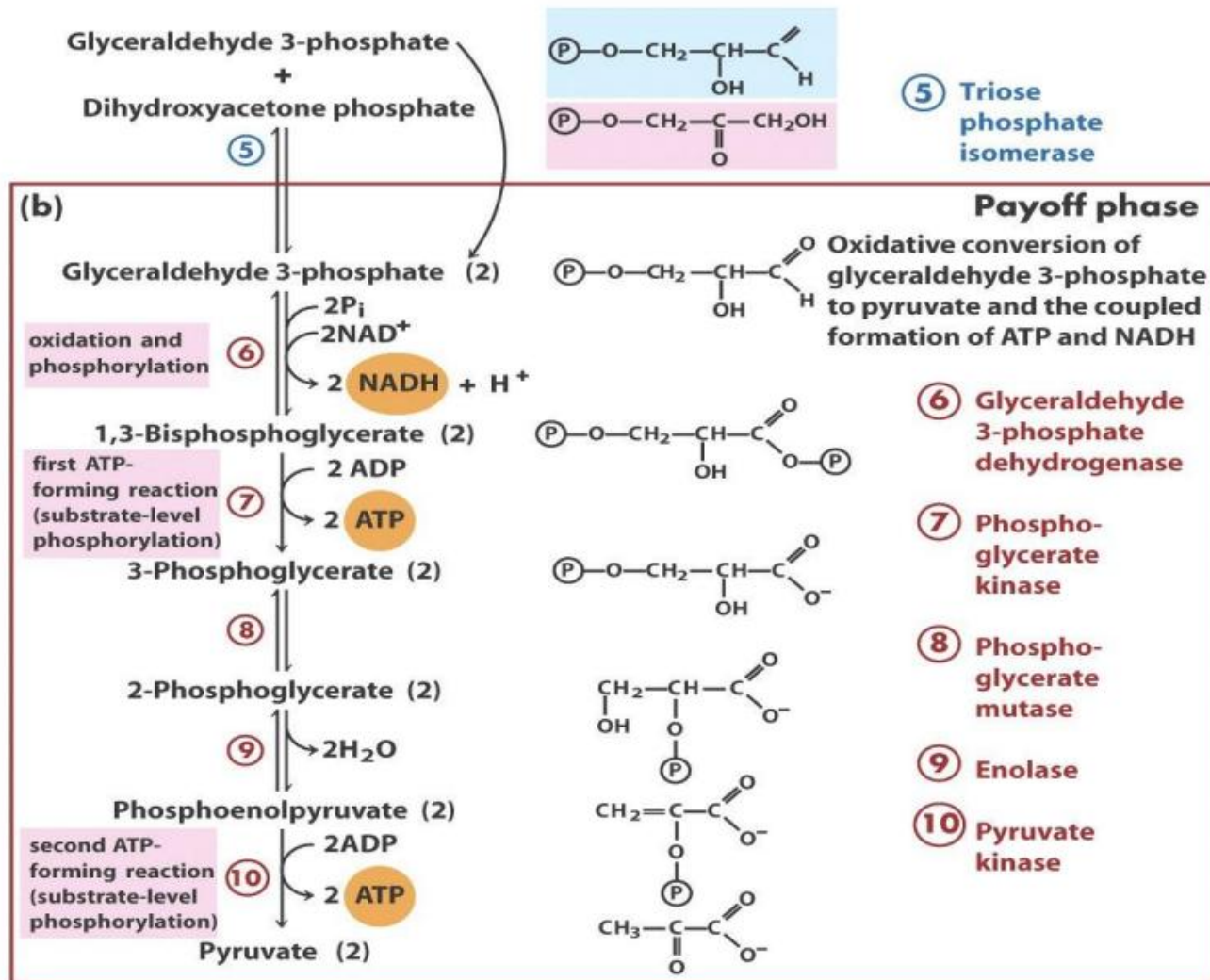
# Embden–Meyerhof–Parnas (EMP) metabolic pathway, glycolysis

- The EMP pathway is the most important route for the anaerobic breakdown of hexoses in the glycolysis and fermentation that occur both in animal tissues and in microorganisms (yeasts and bacteria).
- It is the main route of glucose catabolism in most cells.
- The EMP pathway describes:
  - the conversion of glucose to ethanol and  $\text{CO}_2$  in the alcoholic fermentation carried out by yeast;
  - the conversion of glucose to lactic acid in muscle cells;
- and the reduction of pyruvic acid to lactic acid by the lactate dehydrogenase of some microorganisms, such as the lactic acid bacteria.
- All these reactions proceed under anaerobic conditions, so that the cell is supplied with energy in the absence of free oxygen.

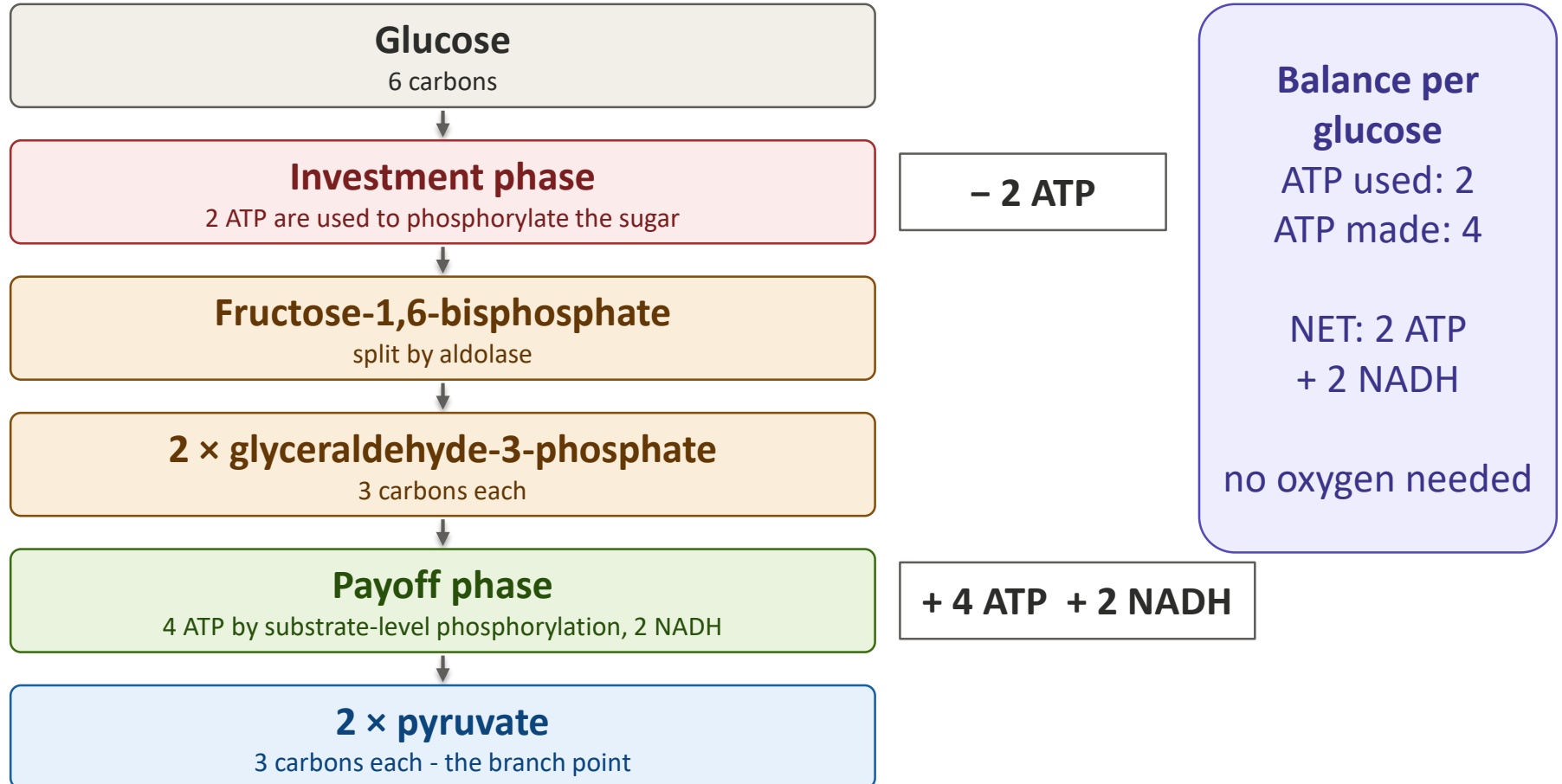
# Embden–Meyerhof–Parnas (EMP) pathway — glycolysis



# Glycolysis



# Glycolysis at a Glance



The 2 NADH must be re-oxidised to  $\text{NAD}^+$ , or glycolysis stops - see the next slide

# Hexose Monophosphate (HMP) or Oxidative Pentose Phosphate Shunt

- The breakdown of carbohydrates by this pathway does not itself yield much ATP. However, the NADPH formed provides reducing power, and its electrons can also be passed to oxygen so that ATP is generated.
- The HMP pathway supplies the precursors needed for the synthesis of nucleic acids, of nucleotide-containing coenzymes, of aromatic amino acids and of some vitamins, as well as the pyridine nucleotides used in biosynthesis.
- If monosaccharides are supplied continuously as nutrients, the HMP pathway is directed towards the formation of pyruvic acid.
- The main distinguishing features of the HMP pathway are:
  1. unlike the EMP pathway, the C-1 carbon of the sugar is released as  $\text{CO}_2$ ;
  2. hexoses are converted to five-carbon sugars for nucleic acid synthesis and to seven-carbon sugars used in the synthesis of aromatic amino acids;
  3. the pathway is particularly important for autotrophic microorganisms, which can convert the  $\text{CO}_2$  they fix into central metabolites through it;
  4. it is an alternative route for the oxidation of glucose and allows energy to be obtained from pentoses.

# Entner Doudoroff (ED) Pathway

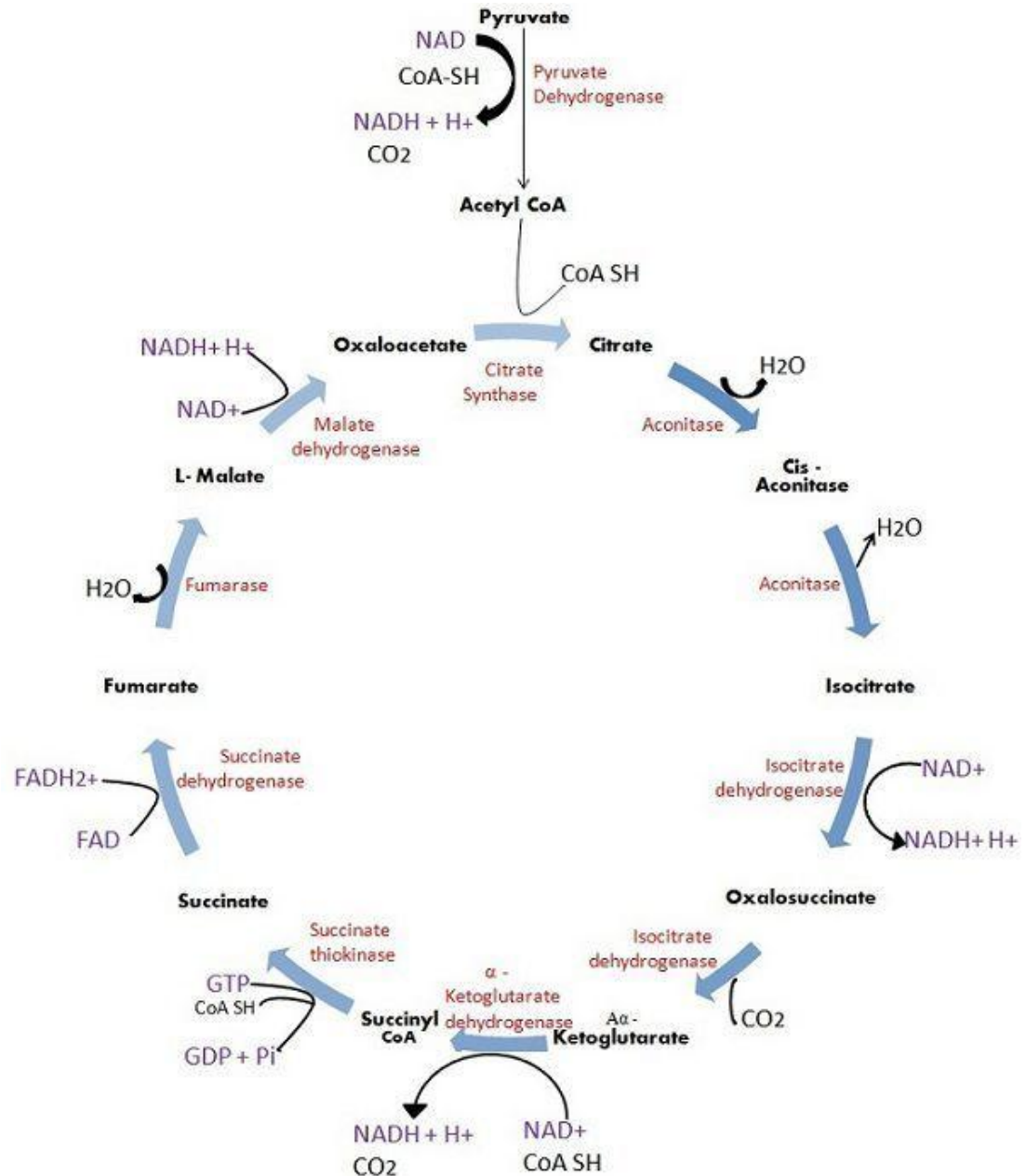
- The ED pathway is another route for the breakdown of glucose.
- The pyruvic acid formed is converted, according to the enzymes present in the organism, to lactic acid, ethanol or another fermentation product.
- This pathway yields only 1 mole of ATP per mole of glucose.
- It is used less widely than the EMP pathway.
- It is characteristic of some Gram-negative bacteria, particularly *Pseudomonas*, *Zymomonas* and *Agrobacterium* species.

# Tricarboxylic Acid (TCA) Cycle

- The citric acid cycle is also known as the tricarboxylic acid cycle or the Krebs cycle.
- It is the cycle in which the acetyl groups of acetyl-CoA — the common end product of carbohydrate, fat and protein catabolism — are oxidised during cellular respiration. In aerobic cells the fuel molecules are finally oxidised to CO<sub>2</sub>.
- In each turn of the cycle, a two-carbon acetyl group enters as acetyl-CoA and two molecules of CO<sub>2</sub> are released.
- The cycle begins with oxaloacetic acid and, after eight successive reaction steps, regenerates oxaloacetic acid.

# TCA cycle

- Each turn of the cycle yields one molecule of GTP (or ATP) by substrate-level phosphorylation, but its four oxidation steps supply most of the electron flow to the respiratory chain.
  - In each turn, 3 moles of  $\text{NAD}^+$  and 1 mole of FAD are reduced.
  - The 3 moles of NADH and 1 mole of  $\text{FADH}_2$  formed are re-oxidised in the respiratory chain.
  - Classically, 2 electrons from NADH were taken to yield 3 ATP and from  $\text{FADH}_2$  2 ATP.



# Total ATP production from glucose

## ■ Total **ATP** Yield

**02 ATP** - **glycolysis** (substrate-level phosphorylation)

**04 ATP** - converted from 2 NADH - **glycolysis**

**06 ATP** - converted from 2 NADH - **grooming phase**

**02 ATP** - **Krebs cycle** (substrate-level phosphorylation)

**18 ATP** - converted from 6 NADH - **Krebs cycle**

**04 ATP** - converted from 2  $\text{FADH}_2$  - **Krebs cycle**

**36 ATP** - **TOTAL**

# Respiration or Fermentation? The Fate of Pyruvate

## Pyruvate + NADH

from glycolysis

### Oxygen present: RESPIRATION

pyruvate  $\rightarrow$  acetyl-CoA + CO<sub>2</sub>

TCA cycle: more NADH and FADH<sub>2</sub>

electron transport chain, O<sub>2</sub> = final acceptor

about 30 - 32 ATP per glucose

### No oxygen: FERMENTATION

NADH reduces pyruvate itself

LAB: pyruvate  $\rightarrow$  lactic acid

yeast: pyruvate  $\rightarrow$  ethanol + CO<sub>2</sub>

NAD<sup>+</sup> regenerated - only 2 ATP per glucose

## Why this matters in food

Fermenters get 15 times less energy from each glucose, so they must break down far more sugar - and produce large amounts of acid or alcohol. This is the basis of yoghurt, cheese, pickles, bread, beer and wine. Yeast uses sugar more economically when air is present (the Pasteur effect).

# Lipid Metabolisms

Lipids are essential components of cell membranes.

Their energy value is higher than that of carbohydrates and proteins.

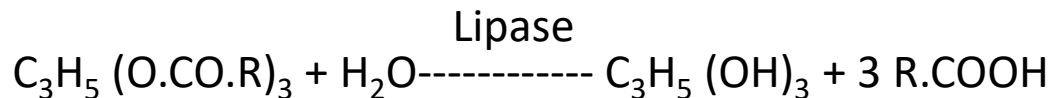
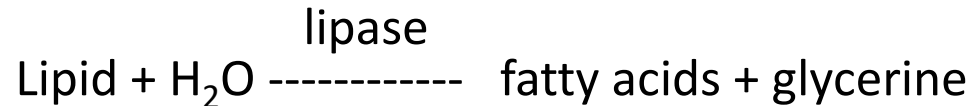
- About 90% of food lipids are triacylglycerols: glycerol esterified with three fatty acids.
- The central route of lipid catabolism is the  $\beta$ -oxidation of fatty acids.
- Lipids occur in microbial cells as components of the cell wall (especially in Gram-negative bacteria), of the cytoplasmic membrane and, intracellularly, as reserve material.
- In mycobacteria, large amounts of waxy lipids (mycolic acids) are present in the cell wall.
- Lipids generally occur as
  - fats and oils (glycerol esters of fatty acids),
  - waxes (esters of fatty acids with long-chain alcohols) and
  - complex lipids (phospholipids, lipopolysaccharides, lipoproteins, etc.).

# Lipid Metabolisms

- Lipids are organic compounds composed mainly of carbon (C), hydrogen (H) and oxygen (O); they may also contain phosphorus (P), nitrogen (N) and sulphur (S).

## Dissociation of Lipids

- Among the hydrolytic enzymes of microorganisms, the lipases break fats down into their constituents. Hydrolysis of a lipid yields fatty acids and glycerol.



- The lipopolysaccharides of Gram-negative bacteria and the ribitol or glycerol teichoic acids of Gram-positive bacteria are important as surface macromolecules that determine the antigenic properties of these microorganisms.

# Protein and Amino Acid Metabolisms

- Proteins are complex organic compounds and form the main structures of the cell.
- Proteins contain about 50% carbon (C), 25% oxygen (O), 16% nitrogen (N) and 7% hydrogen (H). Some also contain up to 1% sulphur (S), and some contain phosphorus, iron, zinc or copper.
- Cells contain a great variety of proteins differing in structure and in function.
- Because of their high molecular weight (5 000 to over a million), proteins form colloidal solutions and are not dialysable.
- They are broken down into their constituent amino acids by proteolytic enzymes or by acid hydrolysis.
- Amino acids are joined to one another by covalent peptide bonds, formed between the carboxyl group of one amino acid and the  $\alpha$ -amino group of the next.
- A polypeptide chain may contain hundreds of amino acids. Some proteins consist of a single polypeptide chain and others of several.

# Nitrogen Metabolisms

- According to their structure, proteins are divided into two main groups.
  1. Simple proteins: on hydrolysis they yield only amino acids and no other organic or inorganic products.
  2. Conjugated proteins: on hydrolysis they yield, in addition to amino acids, other organic or inorganic components (nucleoproteins, lipoproteins, phosphoproteins, flavoproteins, glycoproteins, etc.).
- Since proteins are built up from amino acids joined to one another, breaking these bonds brings about the hydrolysis of the protein.



- Proteinases and peptidases are mostly extracellular enzymes; they hydrolyse the large protein molecules outside the cell and break them down into small units (peptides and amino acids).

# Food Spoilage

1. spoilage caused by microorganisms (bacteria, moulds, yeasts),
2. damage caused by insects and rodents,
3. the action of the food's own enzymes,
4. chemical reactions (oxidation, browning, etc.),
5. physical factors such as freezing, freezer burn and drying.

According to their stability, foods are divided into three groups:

1. stable (non-perishable) foods,
2. semi-perishable foods,
3. perishable foods.

# Microbial Spoilage in Foods

- According to their activity, the microorganisms in a food can be divided into three groups:
  1. saprophytic microorganisms causing spoilage,
  2. pathogenic microorganisms causing foodborne infection and intoxication,
  3. microorganisms used in the production of fermented foods.

## Microorganisms Used in the Production of Fermented Foods

### Microorganisms

Lactic acid bacteria

*Saccharomyces cerevisiae*

*Saccharomyces pastorianus*

*S. cerevisiae* and *Acetobacter aceti*

*Penicillium camemberti*

*P. roqueforti*

### Products

Pickle, Cheese, yoghurt

Wine, beer, bread

Beer

Vinegar

Camembert cheese

Roquefort cheese

# Microbial Spoilage in Foods

- Some properties arising from the natural structure of foods prevent microbial growth, and the processes applied to the raw material and the storage conditions provided can prevent or delay spoilage by these organisms.
- The undesirable changes in colour, taste, odour, appearance and texture that result from the growth of microorganisms in a food are called microbial spoilage.
  - Spoilage is a change in the appearance, odour and/or taste of a food that makes it unacceptable to the consumer.
- The spoilage of a food indicates that microorganisms have grown and reached high numbers during the supply of the raw material, transport, processing or storage.
- Most foods provide a suitable environment for microbial growth, including the growth of human pathogens.

# Microbial Spoilage in Foods

- Foods are generally spoiled by the microorganisms that contaminate them.
- Their susceptibility to microbial growth differs according to their nutrient composition and water content.
- Stable, semi-perishable and perishable foods have different and limited shelf lives.
- Spoilage is caused by a wide variety of microorganisms, and spoilage organisms may also be potential pathogens. The control of microorganisms in foods has two aims:
  1. to delay or prevent microbial spoilage, and
  2. to prevent diseases transmitted to humans through food.

# Microbial Spoilage in Foods

Microorganisms that spoil foods use them as sources of nutrients and energy. Their hydrolytic exoenzymes play the main part in food spoilage.

Important hydrolytic exoenzymes produced by microorganisms

| Enzyme    | Influenced component              |
|-----------|-----------------------------------|
| Pectinase | Pectin (Fruits and vegetables)    |
| Cellulase | Cellulose (Fruits and vegetables) |
| Protease  | Protein (meat, fish etc.)         |
| Amylase   | Starch (Starchy foods)            |
| Lipase    | Fats and oils (fatty foods)       |

## Proteolytic degradation

- In proteolytic degradation, the proteins of the food are hydrolysed to peptides and amino acids. This is desirable in cheese ripening, but in many foods it produces malodorous compounds.
- Especially under anaerobic conditions, malodorous compounds such as H<sub>2</sub>S, ammonia, mercaptans, indole and skatole are formed (putrefaction).

# Microbial Spoilage in Foods

## Carbohydrate breakdown

- Di-, tri- and polysaccharides can be used by microorganisms only after hydrolysis to simple sugars.
- Under aerobic conditions glucose is oxidised to  $\text{CO}_2$  and water, whereas under anaerobic conditions many different products can be formed:
  1. ethanol and  $\text{CO}_2$  by yeasts;
  2. lactic acid by homofermentative lactic acid bacteria (LAB);
  3. lactic acid together with other organic acids, alcohols and  $\text{CO}_2$  by heterofermentative LAB;
  4. organic acids, alcohols and gases by coliform bacteria;
  5. propionic acid, acetic acid and  $\text{CO}_2$  by propionic acid bacteria;
  6. butyric acid, acetic acid,  $\text{CO}_2$  and  $\text{H}_2$  by anaerobic butyric acid bacteria.

# Microbial Spoilage in Foods

## Degradation of organic acids

- Acids such as acetic and lactic acid are oxidised to  $\text{CO}_2$  and water by some aerobic microorganisms (film or oxidative yeasts and moulds), which raises the pH and allows other spoilage organisms to grow.

## Lipolytic degradation

- The fats of a food are hydrolysed to glycerol and free fatty acids by microbial lipases. The glycerol and fatty acids are then further degraded, ultimately to  $\text{CO}_2$  and water; the free fatty acids cause hydrolytic rancidity.

## Pectolytic degradation

- The pectin of plant cell walls consists of polymers of galacturonic acid, part of which is methyl-esterified.
- Microbial pectinesterase hydrolyses the methyl ester bonds and converts pectin into pectic acid and methanol.
- Polygalacturonase then hydrolyses the bonds between the galacturonic acid units of pectic acid, releasing D-galacturonic acid; the plant tissue softens (soft rot).

# Spoilage According to the Substrate Attacked

| Proteins                                                                                                                          | Carbohydrates                                                                                                       | Lipids                                                                                                           | Pectin                                                                                                            |
|-----------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|
| Protease, peptidase                                                                                                               | Amylase, invertase                                                                                                  | Lipase                                                                                                           | Pectinesterase, polygalacturonase                                                                                 |
| ↓                                                                                                                                 | ↓                                                                                                                   | ↓                                                                                                                | ↓                                                                                                                 |
| proteolysis                                                                                                                       | fermentation                                                                                                        | lipolysis                                                                                                        | pectolysis                                                                                                        |
| H <sub>2</sub> S, ammonia, mercaptans<br>indole, skatole, amines<br>putrefaction of meat and fish<br>desirable in cheese ripening | Acids, alcohols, CO <sub>2</sub><br>souring, gas, blowing<br>ropiness from dextrans<br>desirable in fermented foods | Free fatty acids, glycerol<br>hydrolytic rancidity<br>soapy and goaty off-flavours<br>desirable in mould cheeses | Methanol, galacturonic acid<br>soft rot of fruit and vegetables<br>loss of texture<br>used in juice clarification |

*Which substrate is attacked first depends on the organism and on the composition of the food: Pseudomonas attacks protein and fat in chilled meat, lactic acid bacteria attack sugars, and Pectobacterium attacks pectin in vegetables.*

# Microbial Spoilage in Foods

- These forms of spoilage are closely related to the water activity ( $a_w$ ) of the food.
- Water activity is a measure of the water available for metabolic processes.
  - Non-perishable foods have a low water activity.
  - Perishable and semi-perishable foods have a relatively high water activity.
- Fresh foods are spoiled by a variety of bacteria and fungi, and each type of fresh food is degraded by its own characteristic microorganisms.
  - For example, among the enteric bacteria, many of which are potentially pathogenic:
    - *Salmonella*
    - *Shigella*      —▶ It is rarely seen in fruit and vegetable spoilage.
    - *Escherichia*      It is very common in meat spoilage.
- This contamination occurs when the intestinal contents leak and contaminate the carcass during slaughter.

# Microbial Spoilage in Foods

- The dominant microorganisms in dairy products are the lactic acid bacteria.
- *Pseudomonas* species are closely associated with the spoilage of fresh chilled foods, since they occur both in soil and on animals.
- The growth rate of a microorganism during the log phase depends on
  - the temperature,
  - the nutrient composition of the food and
  - the other growth conditions.
- Once the microbial population reaches a certain level (generally about  $10^7$  cfu/g), the signs of spoilage begin to appear.
- During the earlier part of the exponential phase the population density is still low and no effect is noticeable.
- For most of the period of microbial growth in a food, therefore, there is no visible or easily detectable change in quality.

# Indicators of Microbial Spoilage in Foods

- If foods are not protected and are not consumed within a short time, they become contaminated and spoiled by microorganisms.
- Spoilage produces undesirable changes in the quality characteristics of the food (colour, taste, texture and odour).
- Pathogens and spoilage organisms can contaminate foods because of inadequate hygiene and sanitation at various stages between the field and the consumer.
- Besides threatening human health, these microorganisms cause spoilage during storage and therefore economic losses.
- It is not practicable to isolate every microorganism in order to find out whether a food has been produced hygienically and whether pathogens and spoilage organisms are present.
- For this reason certain groups of microorganisms and their metabolites are used as indicators.
- In other words, the microorganisms that show whether the production conditions were hygienic and whether the processes applied were carried out correctly are called indicator microorganisms.

# Indicators of Microbial Spoilage in Foods

- An indicator microorganism, or its metabolite, should have the following properties:
  1. it should be easy and quick to detect and to enumerate;
  2. its growth should not be suppressed by other microorganisms and it should be readily distinguishable;
  3. it should be able to occur together with the pathogen whose presence it indicates;
  4. there should be a parallel relationship between the number of the indicator and the number of the pathogen;
  5. its growth requirements and growth rate should be similar to those of the pathogen concerned;
  6. it should be at least as resistant and as persistent as the pathogen;
  7. it should not be a normal member of the natural flora of the food examined;
  8. it should not grow or die off rapidly at the storage temperature;
  9. in a high-quality fresh product its numbers should be low and its metabolites absent;
  10. when spoilage occurs, the indicator organism or its metabolite should be the main agent of that spoilage;
  11. the presence and number of the indicator should be inversely related to the quality of the food.

# Additional Criteria for Fecal Indicators

1. Ideally, a faecal indicator should occur only in the intestinal environment.
2. It should be present in very high numbers in faeces.
3. It should be reasonably resistant in the external environment.
4. It should be readily detectable even when present in low numbers.

# Indicators of Microbial Spoilage in Foods

- The type and composition of the food, the production method, the packaging and the storage conditions (time–temperature, temperature fluctuation, etc.) all affect microbial spoilage.
- In order to reduce losses caused by spoilage, the shelf life and the current microbiological quality of foods must be determined.
- The indicators used for this purpose are the typical spoilage microorganisms of the food, or their metabolic products, present at certain levels.
- An increase in the number of spoilage organisms causes loss of quality and shortens the shelf life of the product.
  - *Pseudomonas* species and other psychrotrophic bacteria can be used as indicators in protein-rich chilled foods such as meat, poultry, fish and milk.
  - Yeasts and lactic acid bacteria are the main spoilage organisms of fruit juices with a high sugar content and a low pH.
  - The total yeast and mould count is an important spoilage indicator for foods whose composition does not allow bacteria to grow.

# Microbial Spoilage Indicators

| Foods            | Spoilage Indicators                         | Foods                            | Spoilage Indicators                                        |
|------------------|---------------------------------------------|----------------------------------|------------------------------------------------------------|
| Raw milk         | Psychrotrophic bacteria, total viable count | Fresh meat (without package)     | Psychrotrophic aerobic bacteria                            |
| Pasteurized milk | Psychrotrophic bacteria, total viable count | Fresh meat (with package)        | Psychrotrophic lactic acid bacteria and Enterobacteriaceae |
| Butter           | Lipolytic microorganisms                    | Processed meats (vacuum package) | Psychrotrophic lactic acid bacteria and Enterobacteriaceae |
| Cream Cheese     | Psychrotrophic bacteria                     | Sea foods (fresh)                | Psychrotrophic bacteria and <i>Shewanella</i>              |
| Drinks           | Acetic acid bacteria, moulds and yeasts     | Salad dressings                  | <i>Lactobacillus</i> spp., yeasts                          |

# Microbial Spoilage Indicators

| Foods                         | Microorganisms                                                                         |
|-------------------------------|----------------------------------------------------------------------------------------|
| Fresh meat, minced            | <i>Pseudomonas fragi</i> , <i>P. fluorescens</i>                                       |
| Fish and Fish products        | <i>Shewanella putrefaciens</i> , <i>P. fragi</i>                                       |
| Raw milk                      | <i>Lactococcus lactis</i> , psychrotrophic<br><i>Pseudomonas</i>                       |
| Milk                          | <i>Bacillus cereus</i>                                                                 |
| Butter                        | <i>Shewanella putrefaciens</i> (formerly<br><i>Pseudomonas putrefaciens</i> )          |
| Fresh fruit juice             | <i>Acetobacter</i> , <i>Gluconobacter</i>                                              |
| Fruit juice concentrate       | Osmophilic yeasts                                                                      |
| Dough                         | <i>Bacillus</i> spp.                                                                   |
| Beer                          | <i>Pediococcus</i> , <i>Lactobacillus</i>                                              |
| Wine                          | <i>Pediococcus</i> , <i>Brettanomyces</i> , <i>Penicillium</i> ,<br><i>Aspergillus</i> |
| Mayonnaise and salad dressing | <i>Lactobacillus</i> , <i>Z. bailii</i>                                                |
| Sugar (refined)               | <i>Leuconostoc mesenteroides</i>                                                       |

# Indicators of Microbial Spoilage in Foods

- In addition to the microorganisms themselves, some of the metabolites they produce also serve as spoilage indicators.
- The heat-stable lipases and proteinases produced by psychrotrophic bacteria growing in raw milk are important indicators for the shelf life of UHT milk.
- Ethanol produced by yeasts in fruit products, and lactic acid produced by bacteria in canned vegetables, act as spoilage indicators for those foods.

| Foods                     | Metabolites                                       |
|---------------------------|---------------------------------------------------|
| Meat and meat products    | Cadaverine, putrescine, indole, H <sub>2</sub> S  |
| Fish and seafood          | Cadaverine, putrescine, histamine, trimethylamine |
| Milk and dairy products   | Proteinase, lipase                                |
| Apple juice               | Fumaric acid, ethanol                             |
| Grape                     | Acetoin, ethanol                                  |
| Frozen fruit concentrates | Diacetyl                                          |
| Canned vegetables         | Lactic acid                                       |
| Cereals                   | Ergosterol                                        |

# Indicator Microorganisms

- Indicator microorganisms are used to judge whether production in the food industry has been carried out according to the rules.
- They give sufficient information about the raw material, the production technology and the application of good manufacturing practice (GMP).
  - In short, indicator microorganisms are indicators of quality.
- Indicator microorganisms and pathogens must not be confused with one another.
- The groups counted in order to form an opinion about food quality are groups such as the total viable count, total yeasts and moulds, total coliforms and faecal coliforms.
  - Even if pathogenic bacteria (such as *Staphylococcus aureus*) are present among them, they are recorded simply as part of the total count according to the method used.
  - Conversely, a microorganism regarded as beneficial because it is used in the production of one food (for example *Penicillium roqueforti* in Roquefort cheese) will, if it contaminates another food (for example Cheddar cheese), cause that product to be rejected, because the total yeast and mould count used as an indicator will then exceed the standard.

# Indicator Microorganisms

- Not every microorganism is suitable for use as an indicator.
- In recent years it has been emphasised that, in addition to microorganisms, products of microbial growth such as lactic acid, diacetyl and ethanol can also be used as microbial indicators.
- Different branches of the food industry use indicator microorganisms specific to their own operations.
  - For example, the number of lipolytic microorganisms is an important quality criterion in a butter plant, whereas lipolytic bacteria are of no importance in the fruit juice industry.
  - Likewise, faecal indicator bacteria are an important quality criterion for many foods, but it is unnecessary to look for them in canned vegetables.
- Food businesses may define their own indicator microorganisms, starting from the raw material, within the framework of their own quality programmes.
- In addition, indicator microorganisms and limits are laid down by the official control authorities.

# Properties of Indicator Microorganisms

- Total viable count, total yeasts and moulds, total osmophilic and osmotolerant yeasts, xerophilic moulds, total proteolytic bacteria and total coliforms are among the indicators used to assess the quality of different foods.
  - As a general principle an indicator microorganism should not itself be pathogenic; *Clostridium perfringens*, used as a faecal indicator, is an exception.
- Since a largely linear relationship was found between pathogenic microorganisms and faecal contamination, organisms of faecal origin came to be used as indices of sanitation in water.

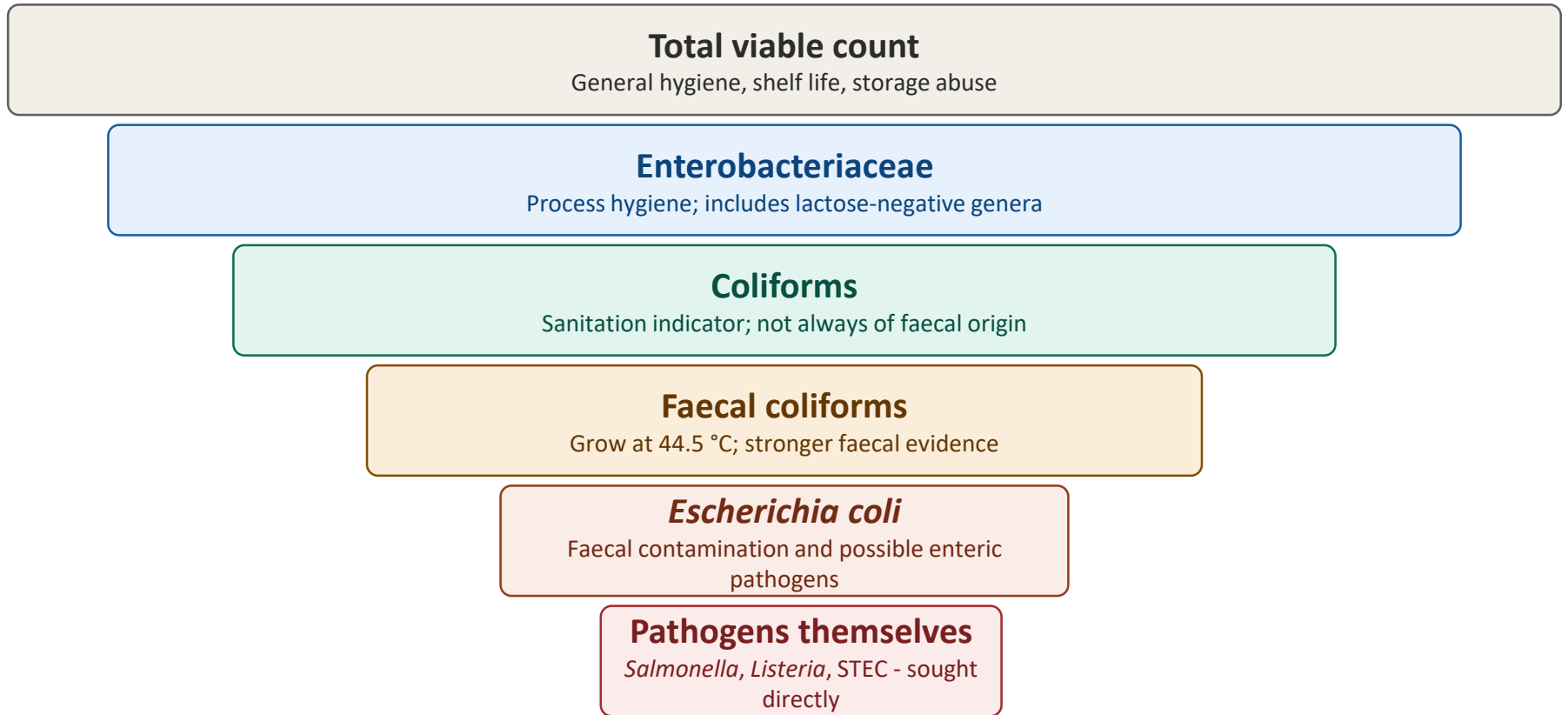
# Indicator Microorganisms

- In addition to the general criteria given above, a microorganism used as an indicator of faecal contamination should have the following properties:
  - it should be of intestinal origin;
  - it should be able to survive outside the intestine;
  - it should be easy and reliable to detect, even when present in low numbers;
  - it should be present in high numbers in faeces, so that it remains detectable after dilution.
- Indicator microorganisms can be examined under two headings:
  - pathogen (safety) indicators,
  - spoilage (quality) indicators.

# Indicator Pathogen Microorganisms

- Pathogenic microorganisms are known by their ability to cause disease; indicators are used to signal their possible presence.
- The main pathogen indicators are:
  - coliforms,
  - enterococci,
  - faecal coliforms and *E. coli*,
  - the total viable count,
  - and other safety indicators (Enterobacteriaceae, coliphages).

# The Hierarchy of Indicator Organisms



**Broad and easy to count**

**Narrow, specific, slower and more expensive**

*An indicator is never a substitute for the pathogen: absence of *E. coli* does not prove that a food is free from enteric pathogens, and *Listeria* is not detected by any of the faecal indicators.*

# Coliform Bacteria

- Coliforms are Gram-negative, non-spore-forming bacteria that are inactivated by pasteurisation and that produce gas from lactose.
- They were first used as indicators of the safety of water, and later as indicators of possible faecal contamination in other foods and of sanitation in the food industry.
  - They are Gram-negative, non-spore-forming, aerobic or facultatively anaerobic rods of the family Enterobacteriaceae that produce acid and gas from lactose within 48 h at 35 °C. On EMB and Endo agar they form colonies with a metallic sheen. The genera included are *Escherichia*, *Enterobacter*, *Klebsiella* and *Citrobacter*.
  - The bacteria formerly called the coli-aerogenes group are *E. coli* and *Klebsiella aerogenes* (formerly *Enterobacter aerogenes*). *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Citrobacter freundii* are other important species.
- Because they occur in the intestinal tract of humans and animals they were accepted as the best indicators of faecal contamination, but it has been shown that not all coliforms are of faecal origin.
  - *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Citrobacter freundii* are widespread in the environment.
- The presence of coliforms in a food is therefore not conclusive evidence of faecal contamination or of the presence of enteric pathogens; they are better used as indicators of sanitation.
  - The presence of coliforms in raw milk indicates inadequate hygiene during milking, transport, storage and processing.
  - In frozen vegetables, where *Enterobacter* strains occur naturally, coliforms are significant only as indicators of sanitation during processing.
- These bacteria can be differentiated using the IMViC tests.

# Coliform Bacteria

## a) Characteristics

- The term coliform covers a group of species: *E. coli*, *Klebsiella aerogenes*, *Klebsiella pneumoniae* and *Citrobacter freundii*,
  - and sometimes also *Serratia marcescens*.
- They are grouped together because of their shared characteristics.
  - They belong to the family Enterobacteriaceae; they are Gram-negative, non-spore-forming rods, aerobic or facultatively anaerobic, relatively resistant to many disinfectants, ferment lactose with the production of acid and gas within 48 h at 35 °C, are often motile and grow in foods between pH 4.0 and 7.5.
- They occur in the faeces of humans, animals and birds.
- The sources of the coliforms that contaminate foods are the intestinal tract of humans and animals, raw materials, equipment, workers, water, air, dust, additives and packaging materials.

# Effects of Coliforms on Foods

## b) Effects of coliforms on foods

- They are undesirable in foods because of their spoilage potential.
- They ferment carbohydrates to acids and gas.
- They hydrolyse proteins and produce off-flavours,
  - described as unclean or barny.
- *Enterobacter* and *Klebsiella* cause ropiness and sliminess in foods.
- High numbers of coliforms are undesirable because of their metabolic end products and of the cellular components (endotoxins) released from lysed cells.

# Effects of Coliforms on Foods

## c) Significance in foods

- Coliforms are among the most useful indicators.
- They occur in many raw foods and ingredients in high numbers.
- Their numbers may increase in refrigerated foods, because some species grow at low temperature.
- Their presence in heat-processed products indicates post-process contamination.
- Coliform cells injured during processing may not be recovered on selective media,
  - but they can repair themselves and grow during processing or storage.
- They may therefore be detected only in low numbers even when pathogens are present in the food.
- Coliform counts can indicate the effectiveness of a process.
- Their presence in raw foods in high numbers (for example  $10^3$  cfu/g or ml) indicates:
  - improper handling,
  - inadequate sanitation and
  - the possible presence of pathogens.

# Fecal Coliforms

- Faecal coliforms are those species of the coliform group
  - that are specifically of intestinal and faecal origin.
- The group consists mainly of *E. coli* and some *Klebsiella* strains.
- Faecal coliforms grow both at 35 °C and at 44.5 °C,
  - whereas non-faecal coliforms cannot grow at 44.5 °C.
- They may occur in foods of animal origin as a result of contamination from faeces, soil and water.
- Faecal coliforms are easily destroyed by heat and
- may also die during freezing and frozen storage.
- Contamination of a food with faecal coliforms indicates a risk that other enteric pathogens may also be present.

# Fecal Coliforms

## b) Significance in foods

- The presence of faecal coliforms in processed foods indicates:
  - inadequate sanitation,
  - possible faecal contamination and
  - the possible presence of enteric pathogens.
- Their presence in raw foods in high numbers (for example  $10^3$  cfu/g or ml) indicates:
  - faecal contamination,
  - improper handling,
  - inadequate sanitation and
  - the possible presence of enteric pathogens.
- Faecal coliforms are widely used as indicators for foods of marine origin and for foods that are extensively handled.

# Fecal Contamination Indicator

- The most important group of indicator microorganisms are the bacteria used as indices of faecal contamination.
  - Their presence shows that the food has been contaminated, directly or indirectly, with sewage or faeces at one or more stages between the raw material and distribution.
- Faecal coliforms, enterococci and *Clostridium perfringens* are the typical indices of faecal contamination.
- *E. coli* is now commonly used instead of faecal coliforms. Of these organisms, the enterococci are considered the better indicator of faecal contamination in water.
- The following points must be considered when a faecal index organism is chosen:
  1. it should be found only in faeces;
  2. it should be present in faeces in high numbers;
  3. it should be resistant to adverse environmental conditions;
  4. it should be detectable relatively easily and reliably in foods, even in very low numbers.

# Fecal Contamination Indicator

- Coliforms, faecal coliforms, *E. coli*, Enterobacteriaceae, *Enterococcus*, *Bifidobacterium*, *Pseudomonas*, *Clostridium*, *Staphylococcus*, coliphages and aerobic plate counts are all used as indicator tests.
- The presence of these bacteria in the material examined indicates a lack of hygiene.
  - The question is whether this lack of hygiene originates from the raw material or from the operating conditions.
- For example, the droppings of birds on raw materials cannot easily be prevented under field conditions, so a degree of faecal contamination is natural in many spices.
- Conversely, if udder hygiene and milking conditions are controlled, contamination of raw milk with animal faeces can be prevented completely; there should then be no faecal coliforms in the raw milk.
- In a product such as cheese made from pasteurised milk, all coliforms are destroyed by pasteurisation.
- The presence of faecal coliforms in such products is therefore due to post-pasteurisation contamination, most often because employees do not observe basic hygiene rules after using the toilet.

# *E. coli*

## a) Characteristics

- *E. coli* conforms to the general characteristics described for the coliform group.
- Biochemically it can be differentiated from the other coliforms by
  - the IMViC tests (indole production from tryptophan, the methyl red test, the Voges–Proskauer reaction and citrate utilisation);
  - and by tests for H<sub>2</sub>S production, ornithine decarboxylase and motility.
- *E. coli* is used as an indicator of faecal contamination and of the possible presence of enteric pathogens in foods.
- Most strains are non-pathogenic and occur naturally in the gastrointestinal tract of humans, animals and birds; some strains, however, are pathogenic (STEC, ETEC, EPEC, EIEC, EAEC).
- The absence of *E. coli* from a food does not guarantee the absence of enteric pathogens.
- Raw foods of animal origin usually contain small numbers of *E. coli*,
  - because of their close association with the animal environment and because carcasses are contaminated from faecal material, hides or feathers during slaughter.

# *E. coli*

## **b) Significance in foods**

- *E. coli* may die off faster than some enteric pathogens in dried, frozen and low-pH products.
- Some enteric pathogens, such as *Listeria monocytogenes* and *Yersinia enterocolitica*, grow at low temperatures (0–2 °C)
  - at which *E. coli* cannot grow.
- *E. coli* cells are injured by processing treatments to a greater extent than some enteric pathogens
  - and may then not be detected on selective media.
- Heat-processed foods should be free from *E. coli*;
  - its presence indicates recontamination.
- The presence of *E. coli* in a heat-processed food indicates:
  - failure of the process,
  - post-process contamination from equipment, employees or contact with raw foods, and
  - the possible presence of enteric pathogens.

# ***Enterobacteriaceae***

- Some enteric pathogens do not ferment lactose; *Salmonella* is an example.
- Instead of counting only the coliforms or faecal coliforms, the whole family Enterobacteriaceae can therefore be enumerated as an indicator.
- This family includes genera that are enteric pathogens (*Escherichia*, *Salmonella*, *Shigella*, *Yersinia*) and genera that are not (*Edwardsiella*, *Pectobacterium*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Hafnia*, *Proteus*, *Morganella*, *Serratia*).
- Counting the whole group is a better indicator of the level of sanitation, of possible faecal contamination and of the possible presence of enteric pathogens.
- The enumeration method uses a selective
- agar medium containing glucose instead of lactose (violet red bile glucose agar, VRBG).
- Many species of the Enterobacteriaceae are not of faecal origin;
- many occur naturally in the environment and on plants.
- In heat-processed and ready-to-eat foods, their presence in high numbers indicates
  - inadequate sanitation, possible faecal contamination and the possible presence of enteric pathogens.

# ***Enterococcus***

The genus *Enterococcus* now contains more than 50 recognised species.

## **a) Characteristics**

- The species commonly used as indicators are
  - *E. faecalis* var. *faecalis*,
  - *E. faecalis* var. *zymogenes*,
  - *E. faecalis* var. *liquefaciens* and
  - *E. faecium*.
- Enterococci were formerly classified among the Lancefield group D streptococci. They are Gram-positive, non-spore-forming, non-motile cocci or coccobacilli occurring in pairs or short chains, catalase-negative and facultatively anaerobic.
- They grow between 10 and 50 °C.
- Some require several vitamins and amino acids for growth.
- Some survive pasteurisation temperatures (they are thermotolerant).
- They are more resistant than coliforms to refrigeration, freezing, drying, low pH and salt.

# ***Enterococcus***

- Compared with *E. coli*, enterococci are more resistant to adverse environmental conditions (freezing, drying, low pH, etc.); although they occur in lower numbers in human faeces, this resistance makes them valuable indicators.
- Because of this resistance, enterococci are used as indicators of faecal contamination and inadequate sanitation in frozen foods.
- High numbers of enterococci, which also resist pasteurisation, in a pasteurised food indicate that the microbial load of the raw material was high.

# ***Enterococcus***

- The sources of enterococci are the faeces of warm-blooded and cold-blooded animals, plants and birds.
- Many occur on equipment, in processing environments, on raw foods and in sewage and water, especially in polluted water and mud.
- Enterococci differ from coliforms in that they
  - are salt-tolerant (they grow in the presence of 6.5% NaCl and 40% bile salts) and
  - are relatively resistant to freezing.
- Certain species (*E. faecalis* and *E. faecium*) are also relatively heat-resistant and may survive pasteurisation.
- Because of their salt tolerance and their resistance to freezing and to low pH, enterococci are used as indicator bacteria in certain foods.

## **b) Significance in foods**

- Their presence in foods indicates faecal contamination, inadequate sanitation and
- the possible presence of pathogens.
- The correlation of enterococci with enteric pathogens is weaker than that of coliforms.
- Their ability to survive pasteurisation and to persist in dried, frozen, refrigerated and low-pH foods nevertheless makes them useful indicators for such products.
- Some strains are opportunistic pathogens and may be associated with foodborne gastroenteritis; antibiotic-resistant strains (VRE) are also of concern.

# Total Viable Count

- **Total count of viable microorganisms:**
  - aerobic viable count,
  - anaerobic viable count,
  - psychrotrophic viable count,
  - mesophilic viable count,
  - thermophilic viable count.
  - It cannot be used for fermented foods.
- **Yeast-mold count**
- The total count of a food gives information about:
  - the suitability of the transport and storage conditions,
  - the adequacy of sanitation in the plant,
  - the shelf life of the product,
  - the onset and extent of microbial spoilage,
  - and the deterioration occurring during the thawing of frozen foods.
- Most human and animal pathogens are mesophilic. The detection of high numbers of mesophilic bacteria in a food therefore shows that the food was produced or stored under conditions that would also allow these pathogens to multiply.

# Other Food Safety Indicators

- *Staphylococcus*: found in the mouth, nose, on the hands and on the skin of humans. Both pathogenic and non-pathogenic species occur. Counts above  $10^6$  cfu/g or ml are regarded as a health risk, because enterotoxin may then have been produced.
- *Pseudomonas aeruginosa*: found in the human intestinal tract, but in lower numbers than the other faecal indicators.
- *Clostridium*: found in the human intestinal tract but also widespread in soil.
- Mesophilic spore count: a high count in chilled or dried foods increases the likelihood of pathogenic spore formers such as *Clostridium perfringens*, *C. botulinum* and *Bacillus cereus*.
- Thermophilic spore count: in the canning industry it is used as an index of the efficiency of the washing and cleaning of the vegetables to be canned.
- *Bacteroides*: present in high numbers in faecal material. It is a strict anaerobe and is difficult to determine by routine methods.
- *Geotrichum candidum*: known as the machinery mould. It is destroyed by the heat treatments applied to foods, so the presence of its hyphae in processed foods indicates contamination from equipment and inadequate sanitation (Howard mould count).
- *Kurthia zopfii*: can grow on meat at temperatures above refrigeration temperature; its presence indicates storage above the recommended temperature.