

Benzoic Acid and the Parabens (苯甲酸及對羥苯甲酸酯類)

- Benzoic acid and its sodium salt along with the esters of *p*-hydroxybenzoic acid (parabens) are permissible in foods $\leq 0.1\%$

Methylparaben
Methyl *p*-Hydroxybenzoate

Propylparaben
Propyl *p*-Hydroxybenzoate

Heptylparaben
n-Heptyl-*p*-Hydroxybenzoate

Introduction

- Chemical food preservatives \rightarrow **prevent or delay the spoilage of foods.**
- The use of food preservatives is regulated by the **Food and Drug Administration (FDA)**. 衛生福利部食品藥物管理署
- The chemical preservatives listed in **Table 13-1** are **generally recognized as safe (GRAS)**.

Benzoic Acid and the Parabens

- antimicrobial activity \rightarrow **undissociated molecule** \rightarrow **most active at the lowest pH**
- The **pK of benzoate is 4.2** \rightarrow **used in high-acid products** (pH<4.0-3.7) \rightarrow **as a mold and yeast inhibitor**
- The **pK for the parabens is around 8.47.** \rightarrow **effective at pH ≤ 8.0**



Benzoic Acid and the Parabens

Mode of action:

- ◆ inhibit the cellular uptake of substrate molecules.
- ◆ The **undissociated form is essential to the antimicrobial activity** of **benzoate** as well as for other **lipophilics** such as **sorbate** and **propionate**.



Sorbic acid

- ◆ **pK = 4.8**
- ◆ primarily effective against **molds and yeasts**
- ◆ also effective against *Staphylococcus aureus*, *salmonellae*, *coliforms*, **psychrotrophic (嗜冷的) spoilage bacteria (especially the pseudomonads)**, and *Vibrio parahaemolyticus*



Sorbic acid (己二烯酸)

- ◆ Sorbic acid is employed as a food preservative, usually as the calcium, sodium, or potassium salt. → are permissible in foods $\leq 0.2\%$.
- ◆ More effective in **acid foods** → used as **fungal inhibitors** → works best \leq **pH 6.0**
- ◆ more effective than sodium benzoate between pH 4.0 and 6.0



Sorbic acid

- ◆ The resistance of the lactic acid bacteria to sorbate → use as a fungistat in products that undergo lactic fermentations.
- ◆ The widest use of **sorbates is as fungistats (抑真菌劑)** in products such as cheeses, bakery products, fruit juices, beverages, salad dressings, and the like.

Sorbic acid

- ◆ use in **meat products** in combination with **nitrites**
- ◆ **no significant differences are found in the organoleptic qualities or in botulinal protection.**
 - **120 ppm NaNO₂ without sorbate**
 - **40 ppm NaNO₂ and 0.26% potassium sorbate** (was proposed by the U.S. Department of Agriculture (USDA) in 1978 but postponed in 1979. ←“chemical”-like **flavors** and producing prickly (刺痛の) mouth sensations

The antimicrobial mechanism of **lipophilic acids** (sorbate, benzoate, and propionate)

- ◆ The membrane gradient represents **electrochemical potential** that the cell employs in the **active transport** of some compounds such as amino acids.
- ◆ After these weak lipophilic acids diffusing across the membrane, **the undissociated molecule ionizes inside the cell** and **lowers intracellular pH**. → a **weakening of the transmembrane gradient** such that amino acid transport is affected adversely

The antimicrobial mechanism of **lipophilic acids** (sorbate, benzoate, and propionate)

- ◆ Involves the **proton motive force** (PMF 質子驅動力).
- ◆ Hydrogen ions (protons) and hydroxyl ions are separated by the cytoplasmic membrane, **hydrogen ions (outside the cell) giving rise to acidic pH** and **hydroxyl ions (inside the cell) giving rise to pH near neutrality.**

Sorbic acid

- ◆ With respect to safety, **sorbic acid is metabolized in the body to CO₂ and H₂O** in the same manner as **fatty acids** normally found in foods.



Propionates (丙酸)

- ◆ This acid and its calcium and sodium salts are permitted in breads, cakes, certain cheese, and other foods, primarily as a **mold inhibitor**.
- ◆ permissible in foods $\leq 0.32\%$
- ◆ The **pK is 4.87** → Active in **low-acid foods** (pH 4.6-6.8)



Sulfur Dioxide and Sulfite

- ◆ The sulfites react with various food constituents including **nucleotides, sugars, disulfide bonds**, and others.
- ◆ Also used as an **antioxidant**.
- ◆ SO_2 is **bacteriostatic** against *Acetobacter* spp. and the lactic acid bacteria at low pH, concentrations of 100-200 ppm being effective in fruit juices and beverages. It is **bactericidal at higher concentrations**.
- ◆ SO_2 also show **inhibition on spores of *Clostridium botulinum*** and **on the growth of salmonellae and other Enterobacteriaceae**.



Sulfur Dioxide and Sulfite

(二氧化硫及亞硫酸鹽)

- ◆ Sulfur dioxide (SO_2) and the sodium and potassium salts of sulfite ($=\text{SO}_3$, 亞硫酸鹽), bisulfite ($-\text{HSO}_3$, 重亞硫酸鹽), and metabisulfite ($=\text{S}_2\text{O}_5$, 焦亞硫酸鹽) all act similarly. → in foods **200-300 ppm**
- ◆ Sulfur dioxide is used in its gaseous or liquid form or salts on dried fruits, in lemon juice, molasses, wines, fruit juices, and others.
- ◆ It is **not permitted in meats** or other foods recognizable as **sources of thiamine** (Vitamin B1).



Sulfur Dioxide and Sulfite

- ◆ **Molds** such as *Botrytis* can be controlled on grapes by periodic gassing with SO_2 and bisulfite can be used to **destroy aflatoxins** (黃麴毒素).
 - Both aflatoxins B₁ and B₂ can be reduced in corn.



Sulfur Dioxide and Sulfite

- ♦ The actual mechanism of action of SO_2 is not known.
 - One suggestion is that the **undissociated sulfurous acid or molecular SO_2** is responsible for the antimicrobial activity (Its greater effectiveness at low pH tends to support this).
 - The other suggestion is that the antimicrobial action is due to the **strong reducing power** that allows these compounds to **reduce oxygen tension** to a point below that at which aerobic organisms can grow or by **direct action on some enzyme system**.



Nitrites and Nitrates (亞硝酸及硝酸)

- ♦ Sodium nitrate (NaNO_3) and sodium nitrite (NaNO_2) are used in curing formulas for meats because they
 - **stabilize red meat color,**
 - **inhibit some spoilage and food poisoning organisms,**
 - contribute to **flavor development.**



Sulfur Dioxide and Sulfite

- ♦ SO_2 is also thought to be an **enzyme poison**, inhibiting growth of microorganisms by **inhibiting essential enzymes**.
 - Its **use in the drying of foods to inhibit enzymatic browning** is based on this assumption.
 - Because the sulfites are known to **act on disulfide bonds**, it may be presumed that certain essential enzymes are affected.

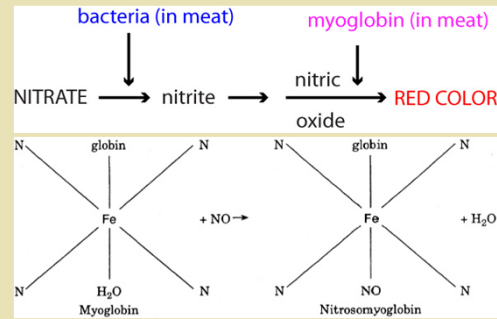


Nitrites and Nitrates

- ♦ Many bacteria can utilize nitrate as an electron acceptor to produce nitrite.
- ♦ The **nitrite ion is more important than the nitrate** in preserved meats.
- ♦ **The nitrite ion is highly reactive** → serving as **both a reducing and an oxidizing agent**.
- ♦ In an acid environment, nitrite ion can be reduced to yield **nitric oxide (NO)** → important for **color fixation in cured meats**.

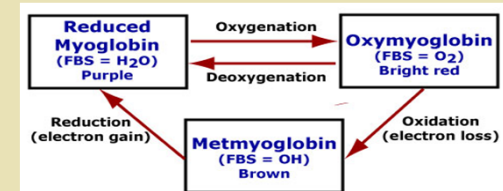
Nitrites and Nitrates

- Nitric oxide reacts with myoglobin (肌紅蛋白) under reducing conditions to produce the desirable **red pigment nitrosomyoglobin** (亞硝化肌紅蛋白).



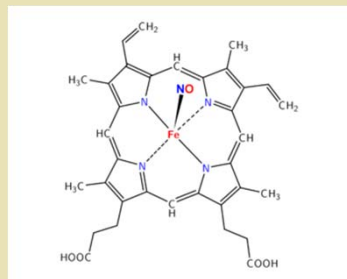
Nitrites and Nitrates

- When the meat pigment exists in the form of **oxymyoglobin** (氧合肌紅蛋白), this compound is first oxidized to **metmyoglobin** (高鐵肌紅蛋白)(brown color).
- Nitric oxide can reduce **metmyoglobin** to yield **nitrosomyoglobin**.



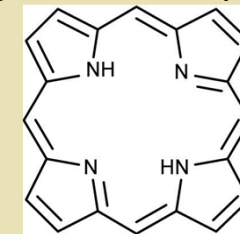
Nitrites and Nitrates

- Nitroso-



Nitrites and Nitrates

- Nitric oxide can react with other porphyrin(卟啉,紫質)-containing compounds such as catalase, peroxidases, cytochromes, and others → some of its antibacterial effects against aerobes may be due to this action.





Nitrites and Nitrates

- ◆ The **antibacterial effect of NO₂ increases as pH is lowered** within the acid range ← increase in the **undissociated HNO₂**.
- ◆ The cooked cured meat pigment is **dinitrosyl ferrohemochrome (DNFH)**. It forms when globin in nitrosomyoglobin is replaced with a second NO group.



The Perigo Factor (皮瑞果因子)

- ◆ Produced from **heating of the culture medium with nitrite** → 10 times more inhibitory to botulism than nitrite alone
- ◆ **Heating to at least 100°C is necessary for its development**, although some activity develops in meats when heated to as low as 70°C.
- ◆ Questionable in cured meats



Nitrites and Nitrates

Organisms Affected

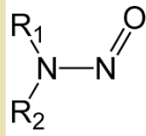
- ◆ Although the greatest concern relative to nitrite inhibition is *Clostridium botulinum*, the compound is antimicrobial for other organisms.
 - Against other clostridia
 - Against *Staphylococcus aureus* at high concentrations.
- ◆ It is **ineffective against Enterobacteriaceae** (including the salmonellae) and the **lactic acid bacteria**.



Nitrites and Nitrates

- ◆ The **antibotulinal activity of nitrite in cured meats** is of greater public health importance than the facts of color and flavor development.
- ◆ **For color and flavor development**, initial nitrite levels as low as **15-50 ppm** are enough for various meat products.
- ◆ The **antibotulinal effect requires at least 120 ppm** for bacon, comminuted cured ham, and canned, shelf-stable luncheon meat.

Nitrosamines (亞硝酸胺)



- ◆ When nitrite reacts with secondary amines, **nitrosamines** are formed, and many are known to be **carcinogenic**.
- ◆ Tertiary amines and quaternary ammonium compounds also yield nitrosamines with nitrite under acidic conditions.

Nitrite-Sorbate and Other Nitrite Combinations

- ◆ EDTA at 500 ppm and chelate, 8-hydroxyquinoline at 200 ppm have been evaluated as a nitrite-sparing agent.
- ◆ Potassium sorbate significantly decreased toxin production by types A and B spores in pork slurries when NaCl was increased or pH and storage temperature were reduced.

Nitrite-Sorbate and Other Nitrite Combinations

- ◆ In an effort to reduce the potential hazard of nitrosamine formation in bacon, the USDA in 1978 reduced the input NO₂ level for bacon to **120 ppm** and set a **10-ppb** maximum level for nitrosamines.
- ◆ A proposal to allow the use of **40 ppm nitrite in combination with 0.26% potassium sorbate** for bacon was made in 1978 but rescinded a year later when taste panel studies revealed undesirable effects.
- ◆ Many studies have shown that **0.26% sorbate** in combination with **40 or 80 ppm nitrite** is effective in preventing botulinal toxin production. (Table 13-2)

Mode of Action

- ◆ Nitrite inhibits *C. botulinum* by reacting with **iron-sulfur enzymes** such as ferredoxin and thus **preventing the synthesis of ATP from pyruvate**.
 - The phosphoroclastic system of *C. sporogenes* and *C. botulinum* is inhibited by nitric oxide → accumulation of pyruvic acid in the medium.
 - The phosphoroclastic reaction involves the breakdown of **pyruvate** with **inorganic phosphate and coenzyme A** to yield **acetyl phosphate**. In the presence of ADP, ATP is synthesized from acetyl phosphate with **acetate** as the other product.

Mode of Action

Phosphoroclastic reaction

In the breakdown of pyruvate, electrons are transferred first to ferredoxin and from ferredoxin to H⁺ to form H₂ in a reaction catalyzed by **hydrogenase**. **Ferredoxin and hydrogenase** are iron-sulfur (nonheme) proteins or enzymes.

$$\text{Pyruvate} + \text{P}_i \xrightarrow[\text{ferredoxin}]{\text{CoA}} \text{Acetyl-P} \xrightarrow[\text{ADP}]{\text{ferredoxin}} \text{ATP} + \text{Acetate}$$

Summary of Nitrite Effects

- ◆ When added to processed meats, nitrite has definite **antibotulinal effects**. It also forms desirable product **color** and enhances **flavor** in cured meat products.
- ◆ **The antibotulinal effect:**
 - **inhibition of vegetative cell growth and**
 - **the prevention of germination and growth of spores**

Mode of Action

- ◆ **Nitric oxide reacted with iron—sulfur complexes** to form iron-nitrosyl complexes. → destruction of iron-sulfur enzymes such as ferredoxin.
- ◆ The resistance of the lactic acid bacteria to nitrite inhibition ← **lack ferredoxin**

Summary of Nitrite Effects

- ◆ Clostridia other than *C. botulinum* are affected in a similar manner. Whereas **low initial levels of nitrite are adequate for color and flavor development**, considerably **higher levels are necessary for the antimicrobial effects**.
- ◆ **When nitrite is heated in certain laboratory media** → produce **Perigo effect/factor** or Perigo inhibitor. It **does not form in filter-sterilized media**.
 - It develops in **canned meats** only when nitrite is present during heating. Once formed, the Perigo factor is not affected greatly by pH changes.



Summary of Nitrite Effects

- ◆ Measurable levels of nitrite decrease considerably during heating in meats and during postprocessing storage—more at higher storage temperatures than at lower.
- ◆ The antibotulinal activity of nitrite is interdependent with pH, salt content, temperature of incubation, and numbers of botulinal spores. Heat-injured spores are more susceptible to inhibition than uninjured.



NaCl and Sugars

- ◆ At high concentrations, salt exerts a drying effect on both food and microorganisms.
- ◆ **0.85-0.90%** salt produces an **isotonic** condition for nonmarine microorganisms.
- ◆ When microbial cells are suspended in a 5% saline solution → the cell is **plasmolysis** → growth inhibition and possibly death.
- ◆ When high concentrations of salt are added to fresh meats → both the microbial cells and those of the meat undergo plasmolysis (shrinkage), → drying of the meat + inhibition or death of microbial cells. ← Enough salt must be used to effect **hypertonic** conditions.
- ◆ The inhibitory effects of salt are **not dependent on pH**. Most nonmarine bacteria can be inhibited by **20% or less of NaCl**, whereas some molds generally tolerate higher levels.



Summary of Nitrite Effects

- ◆ Lactic acid bacteria are relatively resistant to nitrite.
- ◆ **Endospores remain viable in the presence of the antibotulinal effect** and will germinate when transferred to nitrite-free media.
- ◆ **Nitrite has a pK of 3.29** and, consequently, exists as undissociated nitrous acid at low pH values. The maximum undissociated state and consequent greatest antibacterial activity of nitrous acid are between **pH 4.5 and 5.5**.



NaCl and Sugars

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NaCl and Sugars

- ◆ Organisms that can grow in the presence of and **require high concentrations of salt** are referred to as **halophiles**; those that can withstand but not grow in high concentrations are referred to as **halodurics**.
- ◆ Sugars, such as sucrose, exert their preserving effect in essentially the same manner as salt. One of the main differences is in **relative concentrations**.
 - It generally **requires about six times more sucrose than NaCl** to effect the same degree of inhibition.
- ◆ The most common uses of sugars as preserving agents are in the making of **fruit preserves, candies, condensed milk**, and the like. → **high concentrations of sugar makes water unavailable to microorganisms.**



Indirect Antimicrobials

- ◆ The compounds and products in this section are multifunctional food additives.
- ◆ They are added to foods primarily for effects other than antimicrobial.
 - Antioxidants
 - Flavoring agents (including spices and essential oils)
 - Phosphates
 - Medium-chain fatty acids and esters



NaCl and Sugars

- ◆ Microorganisms differ in their response to **hypertonic concentrations of sugars**, with yeasts and molds being less susceptible than bacteria.
 - Some yeasts and molds can grow in the presence of as much as 60% sucrose, whereas much lower levels inhibit most bacteria.
- ◆ Organisms that are able to grow in high concentrations of sugars are designated **osmophiles (嗜高渗微生物)**; **osmoduric** microorganisms are those that are unable to grow but are able to withstand high levels of sugars.



Antioxidants

- ◆ Although used in foods primarily to prevent the autooxidation of lipids, many phenol antioxidants (Table 13-8) have been shown to possess antimicrobial activity against a wide range of microorganisms.
- ◆ These compounds have been evaluated extensively as **nitrite-sparing agents** in processed meats and in combination with other inhibitors.



Flavoring Agents

- ◆ Impart aromas and flavors to foods
- ◆ more **antifungal** than antibacterial.
- ◆ The nonlactic, Gram-positive bacteria are the most sensitive, and the lactic acid bacteria are rather resistant.
- ◆ The **essential oils** and **spices** have received the most attention by food microbiologists
- ◆ One of the most effective flavoring agents is **diacetyl**, which imparts the **aroma of butter**. It is more effective against Gram-negative bacteria and fungi than against Gram-positive bacteria.



Phosphates

- ◆ Added to processed meats to increase their **water holding capacity**.
- ◆ Food-grade phosphates range from one P (e.g. trisodium phosphate, TSP) to at least 13 (sodium polyphosphate).
- ◆ They possess antibotulinal activity, especially when combined with nitrites.
- ◆ Filter-sterilized phosphate preparations were more inhibitory than autoclaved in one study.
- ◆ Active growth of cells was necessary for its bactericidal effect.



Flavoring Agents

- ◆ Diacetyl inhibits **arginine utilization** by reacting with arginine-binding proteins of Gram-negative bacteria.
- ◆ Many spices possess significant antimicrobial activity. Their antimicrobial activities are due to specific chemicals or essential oils.
- ◆ At least 20 spices or their extracts against most food-poisoning organisms, including mycotoxigenic fungi.



Medium-Chain Fatty Acids and Esters

- ◆ Acetic, propionic, and sorbic acids are short-chain fatty acids used primarily as preservatives.
- ◆ Medium-chain fatty acids are employed primarily as **surface-active** or **emulsifying agents**.
- ◆ The antimicrobial activity of the medium-chain fatty acids is best known from **soaps**, which are salts of fatty acids. Those most commonly employed are composed of **12-16 carbons**.



Medium-Chain Fatty Acids and Esters

- ◆ In general, fatty acids are effective primarily against gram-positive bacteria and yeasts.
- ◆ The **monoesters of glycerol** and the **diesters of sucrose** are more antimicrobial than the corresponding free fatty acids and better than sorbic acid and the parabens as antimicrobials.
- ◆ **Monolaurin** is the most effective of the glycerol monoesters, and **sucrose dicaprylate** is the most effective of the sucrose diesters.



Acetic and Lactic Acids

- ◆ Acetic and lactic acids are widely employed as preservatives ← **produced by lactic acid bacteria**. Products include pickles, sauerkraut, and fermented milks.
- ◆ **The antimicrobial effects of organic acids** such as propionic and lactic is due to both the **depression of pH below the growth range** and **metabolic inhibition by the undissociated acid molecules**.
- ◆ In determining the quantity of organic acids in foods, **titratable acidity** is of more value than pH alone, because the latter is a measure of hydrogen-ion concentration and **organic acids do not ionize completely**.



Preservative system

- ◆ Using combinations of chemicals
- ◆ Consist of three compounds— monolaurin/EDTA/BHA, for example.
 - Although EDTA possesses little antimicrobial activity by itself, it makes gram-negative bacteria more susceptible by rupturing the outer membrane and thus enhances the effect of fatty acids or fatty acid esters.
 - Antioxidant BHA would exert effects against bacteria and molds and also serve as an antioxidant
 - By use of such a system, **the development of resistant strains could be minimized** and the **pH of a food could become less important** relative to the effectiveness of the inhibitory system.



Acetic and Lactic Acids

- ◆ In measuring titratable acidity, **the amount of acid that is capable of reacting with a known amount of base is determined**. → ? volume of 0.1N alkali reacted/100g or 100 ml of original material
- ◆ **The bactericidal effect of acetic acid** : When two species of *Salmonella* were added to an oil-and-vinegar-based salad dressing, the initial inoculum of 5×10^6 *S. enteritidis* could not be detected after 5 min nor could *S. typhimurium* be detected after 10 min.
- ◆ Organic acids are employed to **wash and sanitize animal carcasses** after slaughter to **reduce their carriage of pathogens** and to **increase product shelf life**.



Antibiotics

- ◆ **Antibiotics are secondary metabolites** (which are **not** formed during the exponential growth phase and have no apparent significance to the growth or metabolism) produced by microorganisms that **inhibit or kill a wide spectrum of other microorganisms**.
- ◆ Most antibiotics are produced by molds and bacteria of the genus *Streptomyces*, and a few by *Bacillus* and *Paenibacillus* spp.
- ◆ Many of the clinically useful agents are synthetic products.



Monensin (孟寧素)

- ◆ Approved by FDA as a **cattle additive** in the 1970, and it is used primarily to improve feed efficiency in ruminants.
- ◆ Inhibits Gram-positive bacteria
- ◆ Like nisin, monensin is an ionophore (離子載體, a lipid-soluble molecule that transports ions across a cell membrane) → destroys selective permeability of cell membranes.



Antibiotics (Table 13-9)

- ◆ The general view in USA is that the benefits to be gained by using antibiotics in foods do not outweigh the risks.
- ◆ **Several key considerations on the use of antibiotics as food preservatives** are summarized as follows:
 - The antibiotic agent **should kill, not inhibit**, the flora and should ideally decompose into innocuous products or be destroyed on cooking for products that require cooking.
 - The antibiotic **should not be inactivated** by food components or products of microbial metabolism.
 - The antibiotic **should not readily stimulate the appearance of resistant strains**.
 - The antibiotic should not be used in foods if used therapeutically or as an animal feed additive.



Natamycin (納他黴素)

- ◆ Used as a **food preservative** with the consideration of the following facts:
 - It does **not affect bacteria**,
 - it stimulates an unusually **low level of resistance** among fungi,
 - it is **rarely involved in cross-resistance** among other antifungal polyenes,
 - DNA transfer between fungi is less than bacteria.



Natamycin

- ◆ Quite effective against yeasts and molds but not bacteria.
- ◆ Isolated from *Streptomyces natalensis*.
- ◆ Natamycin appears to act in the same manner as other polyene antibiotics—by **binding to membrane sterols** and inducing **distortion of selective membrane permeability**.
 - Because bacteria do not possess membrane sterols, their lack of sensitivity to this agent is thus explained.




Subtilin (枯草桿菌素)

- ◆ **Structurally similar to nisin** (Fig. 13-6)
- ◆ Produced by *Bacillus subtilis*.
- ◆ Like nisin, it is effective against gram-positive bacteria, is **stable to acid**, and **possesses enough heat resistance to withstand destruction at 121 °C for 30-60 min.**
- ◆ Effective in canned foods at levels of **5-20 ppm** in preventing the outgrowth of germinating endospores



Tetracyclines (四環黴素)

- ◆ Chlortetracycline (CTC) and oxytetracycline (OTC) delay bacterial spoilage of seafoods, poultry, red meats, vegetables, raw milk, and other foods.
- ◆ CTC is generally more effective than OTC.
- ◆ The tetracyclines are both **heat sensitive** and **storage labile** in foods.
- ◆ They are used to treat diseases in humans and animals and are used also in feed supplements.



Tylosin (泰黴素)

- ◆ More inhibitory than nisin or subtilin.
- ◆ Used in animal feeds and also to treat some diseases of poultry
- ◆ Effective against gram-positive bacteria
- ◆ **Inhibits protein synthesis** by associating with **the 50S ribosomal subunit.**



Antifungal Agents for Fruits

- ◆ Table 13-10 (thiabendazole, benomyl, biphenyl, SO₂ fumigation)
- ◆ **Benomyl** is applied uniformly over the entire surface of fruits. It can penetrate the surface of some vegetables and is used worldwide to control crown rot and anthracnose of bananas, and stem-end rots of citrus fruits.



Ethylene oxide

- ◆ Its antimicrobial activity is presumed to be related to **alkylation**.
 - In the presence of labile H atoms, the unstable three-membered ring of ethylene oxide splits.
 - The H atom attaches itself to the oxygen, forming a **hydroxyl ethyl radical**, $\cdot\text{CH}_2\text{CH}_2\text{OH}$
 - $\cdot\text{CH}_2\text{CH}_2\text{OH}$ attaches itself to the position in the organic molecule left vacant by the H atom. The hydroxyl ethyl group blocks reactive groups within microbial proteins, thus resulting in inhibition.
- ◆ Among the groups capable of supplying a labile H atom are —COOH , —NH_3 , —SH , and —OH .



Ethylene and Propylene Oxides (氧化乙烯及氧化丙烯)

- ◆ Exist as gases and are employed as fumigants in the food industry.
- ◆ Applied to dried fruits, nuts, spices, and so forth, primarily as **antifungal compounds**.
- ◆ are **alkylating agent**.



Ethylene oxide

- ◆ Affect endospores of *C. botulinum* by **alkylation of guanine and adenine components of spore DNA** .
- ◆ Used as a **gaseous sterilant** for flexible and semirigid containers for packaging aseptically processed foods.
- ◆ Similarly effective against vegetative cells and endospores.



BIOCONTROL

- ◆ The use of one or more organisms to inhibit or control other organisms.
- ◆ May require a **living organism** (such as phages) or it may be effected by **indirect actions or agents** (such as bacteriocins).
- ◆ Related to the food protection provided by the activities of the lactic acid bacteria, bacteriocins, endolysins (溶菌酶), bacteriophages, and “protective cultures” in general.



Microbial Interference

- ◆ Possible explanations:
 - Competition of nutrients
 - Competition for attachment/adhesion sites
 - Unfavorable alteration of the environment
 - Combinations of these.



Microbial Interference

- ◆ The general **nonspecific inhibition or destruction** of one microorganism by other members of the same habitat or environment.
- ◆ The mechanisms are not clear, but some observations are worthy of note.
 - The background biota needs to be **larger in a number of viable cells** than the organism to be inhibited.
 - The interfering biotas generally **not homogeneous**, and the specific roles that individual species play are unclear.



Lactic antagonism (拮抗作用)

- ◆ Lactic acid bacterium inhibits or kills closely related and food-poisoning and food spoilage organisms. → The most effective method used was spraying the lactic organism on food surfaces
- ◆ Precise mechanisms are not clear. The possible factors identified are:
 - the production of antibiotics, H₂O₂, diacetyl, and bacteriocins
 - pH depression
 - nutrient depletion



Lactic antagonism

- ◆ Protective cultures: the microorganisms that can be found in or added to a food product to effect preservation/protection. They should
 - present no health risks
 - provide beneficial effects on the product
 - have no negative impact on sensory properties
 - serve as “indicators” under abuse conditions



Nisin (乳酸鏈球菌素)

- ◆ A polypeptide → structurally related to subtilin (Fig. 13-6).
- ◆ the most widely used antibiotic for food preservation
- ◆ **desirable properties as a food preservative** are the following:
 - a) nontoxic.
 - b) produced naturally by *Lactococcus lactis* strains.
 - c) heat stable and has excellent storage stability.
 - d) destroyed by digestive enzymes.
 - e) does not contribute to off-flavors or off-odors.
 - f) has a narrow spectrum of antimicrobial activity.



Nisin

- ◆ Nisin is a **bacteriocin**.
- ◆ Like **antibiotics**, bacteriocins are chemical compounds **produced by microorganisms that inhibit or other microorganisms**.
- ◆ **Bacteriocins** inhibit or kill generally only **closely related species or strains of the same species**.



Nisin

- ◆ Effective against **Gram-positive** bacteria, primarily **sporeformers**, and ineffective against fungi and gram-negative bacteria.
- ◆ **Mode of action**
 - nisin and subtilin appear to be identical.
 - **react with cytoplasmic membranes and result in pore formation**.
 - The formation of pore causes **the loss of accumulated amino acids and the inhibition of amino acid transport**.



ENDOLYSINS

- ◆ In order to release newly formed bacteriophages from their host cell, two small hydrophobic proteins were used:
 - **Holins** disrupt the cell membrane and form holes through which endolysins can pass.
 - **Endolysins** target bonds in the peptidoglycan, and upon the destruction of this cell barrier, the phage progeny is released.



BACTERIOPHAGES AS BIOCONTROL AGENTS

- ◆ Lytic phages can destroy their specific host cells in foods.
- ◆ The true potential need more research



ENDOLYSINS

- ◆ In addition to their **lysis of bacterial cells from within**, endolysins from Gram-positive bacteria phages also **lyse bacteria exogenously**.
- ◆ Phage endolysin can control some foodborne bacterial pathogens.



THE HURDLE CONCEPT

- ◆ Multiple factors or techniques are employed to effect the control of microorganisms in foods.
- ◆ Barrier technology, combination preservation, and combined methods



天天吃烤香腸 14歲少年大腸癌亡

2014/04/25 12:00 楊依嘉 盧松佑 報導

- ◆ 燒烤類的食物很香，但吃多可能致命。一名14歲國中生，從小愛吃烤香腸，幾乎天天吃，最後血便送醫，發現是大腸癌第四期，雖然開刀治療，四個月後還是不治。
- ◆ 放在鐵架上的香腸，不斷飄出香味，烤香腸是很多人愛吃的小吃，一名14歲的國中生也是從小就愛吃，甚至幾乎天天吃，但他怎麼也沒想到，會吃到血便送醫，發現是大腸癌第四期，開刀治療後還是不幸喪命。
- ◆ 香腸和臘肉都有亞硝酸鹽，進入人體會產生致癌物，沒想到每天吃香腸會這麼嚴重。醫生說，肉類經過高溫燒烤，只要超過150度，兩分鐘，就會產生多環芳香烴化合物，其中的成分「苯基嘍哈」是一級致癌物，而吃燒烤食物對人體的傷害，不輸抽菸產生的致癌物，所以建議民眾還是少吃。應要多吃蔬果多運動，才不會像 這位14歲國中生一樣，生命提早畫下終點。
- ◆ [愛吃燒烤、香腸, 17歲男大腸癌離世 2015.04.01](#)