

## Chapter 13 Food Preservation with Chemicals and by Biocontrol

Tsuei-Yun Fang

節錄自 Modern Food Microbiology, Jay, J. M., 7th ed.

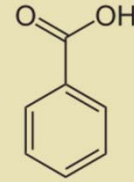


### Introduction

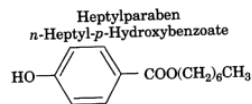
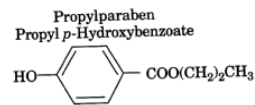
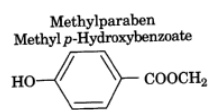
- ◆ Chemical food preservatives → **prevent or delay the spoilage of foods.**
- ◆ The use of food preservatives is regulated by the **Food and Drug Administration (FDA)**. (衛生福利部)食品藥物管理署
- ◆ The chemical preservatives listed in **Table 13-1** are **generally recognized as safe (GRAS)**.



## Benzoic Acid and the Parabens (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  $\leq 0.1\%$



## Benzoic Acid and the Parabens

- Benzoic Acid



Margarine, pickle relishes, apple cider, soft drinks, tomato ketchup, salad dressings



- Parabens



Bakery products, soft drinks, pickles, salad dressings





## Benzoic Acid and the Parabens

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



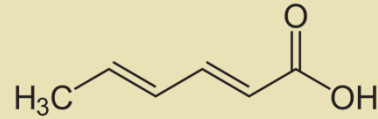
## Benzoic Acid and the Parabens

### 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 (己二烯酸; 山梨酸)



- ◆ 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 (3.7- 4.0 < pH < 4.6) → used as fungal inhibitors → works best  $\leq$  pH 6.0
- ◆ more effective than sodium benzoate between pH 4.0 and 6.0



## 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 假單胞菌屬), and *Vibrio parahaemolyticus* 腸炎弧菌





## 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 botulinal protection.**
  - **120 ppm NaNO<sub>2</sub>**
  - **40 ppm NaNO<sub>2</sub> 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)

- ◆ 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**.

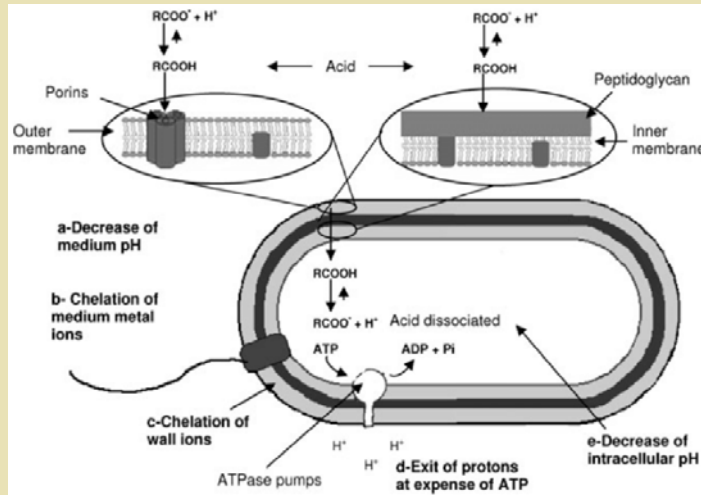


### The antimicrobial mechanism of **lipophilic acids** (sorbate, benzoate, and propionate)

- ◆ 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 organic acids



COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY—Vol. 8, 2009



## Sulfur Dioxide and Sulfite

(二氧化硫及亞硫酸鹽)

- ◆ Sulfur dioxide ( $\text{SO}_2$ ) 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.







## Sulfur Dioxide and Sulfite

- ◆ Because sulfites destroy thiamin, they are **not permitted in meats** or other foods recognizable as **sources of thiamine** (Vitamin B<sub>1</sub>).
- ◆ The sulfites react with various food constituents including **nucleotides, sugars, disulfide bonds,** and others.
- ◆ Also used as an **antioxidant**.
- ◆ SO<sub>2</sub> is **bacteriostatic** against *Acetobacter spp.* (醋酸桿菌屬) 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**.



## Sulfur Dioxide and Sulfite

- ◆ SO<sub>2</sub> also show **inhibition on spores of *Clostridium botulinum*** (肉毒桿菌) and **on the growth of salmonellae** (沙門氏菌) and other **Enterobacteriaceae** (腸桿菌科).
- ◆ **Molds** such as *Botrytis* (葡萄孢菌屬) can be controlled on grapes by periodic gassing with SO<sub>2</sub> and bisulfite can be used to **destroy aflatoxins** (黃麴毒素).
  - Both aflatoxins B<sub>1</sub> and B<sub>2</sub> can be reduced in corn.





## Sulfur Dioxide and Sulfite

- ◆ The actual mechanism of action of  $\text{SO}_2$  is not known.
  - One suggestion is that the **undissociated sulfurous acid** (亞硫酸  $\text{H}_2\text{SO}_3$ ) or **molecular  $\text{SO}_2$**  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**.




## Sulfur Dioxide and Sulfite

- $\text{SO}_2$  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 (亞硝酸及硝酸)

- ◆ Sodium nitrate (NaNO<sub>3</sub>) and sodium nitrite (NaNO<sub>2</sub>) are used in curing formulas for meats because they
  - stabilize red meat color,
  - inhibit some spoilage and food poisoning organisms,
  - contribute to flavor development.



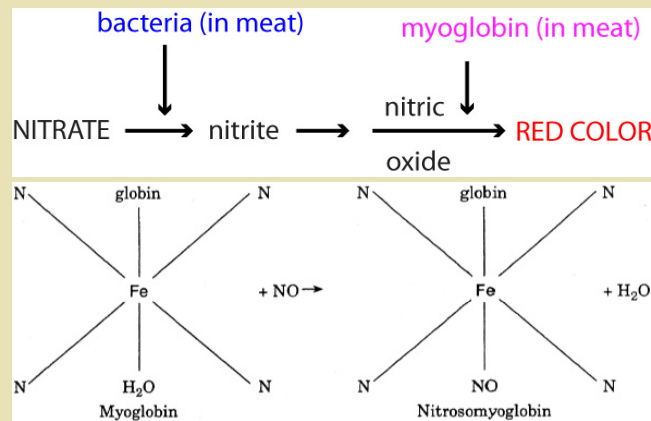



## Nitrites and Nitrates

- ◆ The **nitrite ion** is more important than the **nitrate** in preserved meats.
- ◆ Many bacteria can utilize nitrate as an electron acceptor to produce nitrite.
- ◆ 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

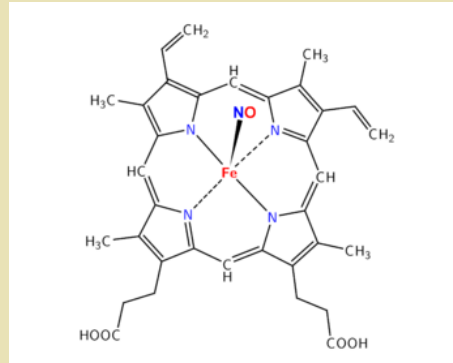
- ◆ **Nitric oxide reacts with myoglobin** (肌紅蛋白) under reducing conditions to produce the desirable **red pigment nitrosomyoglobin** (亞硝化肌紅蛋白).





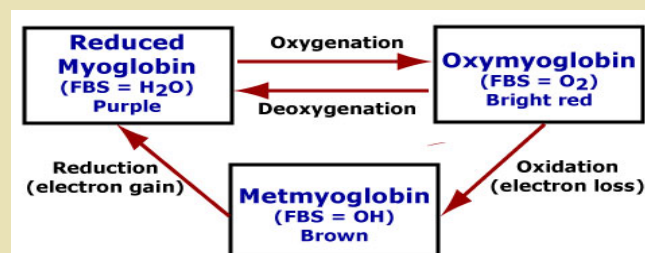
## Nitrites and Nitrates

- ◆ Nitroso-



## Nitrites and Nitrates

- ◆ When the meat pigment exists in the form of **oxymyoglobin** (氧合肌紅蛋白), this compound can be oxidized to **metmyoglobin** (高鐵肌紅蛋白)(brown color).







## 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* (梭菌屬)
  - Against *Staphylococcus aureus* at high concentrations.
- ◆ It is **ineffective against Enterobacteriaceae** (including the salmonellae) and the **lactic acid bacteria**.



## The Perigo effect/factor (皮瑞果因子)

- ◆ Produced from **heating of the culture medium with nitrite** → 10 times more inhibitory to botulism than nitrite alone
- ◆ The Perigo factor does not form in filter-sterilized media. It develops in canned meat only when nitrite is present during heating. Once formed, it is **not affected greatly by pH change**.
- ◆ Questionable in cured meats

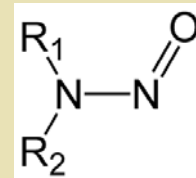
## Nitrites and Nitrates



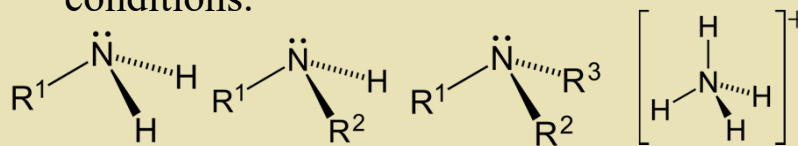
- ◆ The **antibotulinal activity of nitrite in cured meats** is of greater public health importance than the facts of color and flavor development.
  - For **color and flavor development**, initial nitrite levels as low as **15-50 ppm** are enough for various meat products.
  - The **antibotulinal effect 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

- ◆ In an effort to reduce the potential hazard of nitrosamine formation in bacon, the USDA in 1978 reduced the input NO<sub>2</sub> 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.



## Nitrite-Sorbate and Other Nitrite Combinations

- ◆ 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)
- ◆ **Chelating Agent (螯合劑):** EDTA (500 ppm) and 8-hydroxyquinoline (200 ppm) have been evaluated as a **nitrite-sparing agent (亞硝酸鹽節約劑)**.



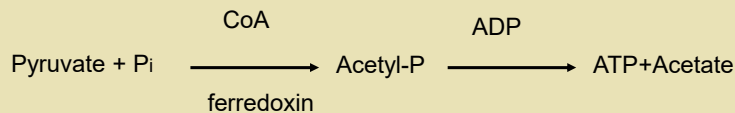
## Mode of Action

- ◆ 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* (産孢梭菌) and *C. botulinum* is inhibited by **nitric oxide** → accumulation of pyruvic acid in the medium.



## Mode of Action

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.



Electrons are transferred first to **ferredoxin** and from ferredoxin to  $\text{H}^+$  to form  $\text{H}_2$  in a reaction catalyzed by **hydrogenase**. **Ferredoxin and hydrogenase** are **iron-sulfur** (nonheme) proteins or enzymes.



## 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

- ◆ 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
  - the prevention of germination and growth of spores





## 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 media or canned meats → produce **Perigo effect/factor** or Perigo inhibitor. It 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.





## 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 exists as undissociated nitrous acid at low pH values. The maximum undissociated state and consequent **greatest antibacterial activity of nitrous acid (nitrite) are between pH 4.5 and 5.5**.



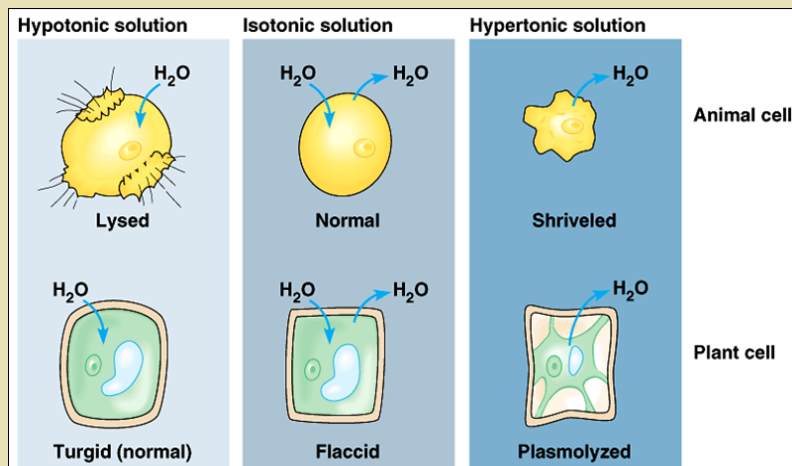
## NaCl and Sugars

- ◆ 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.

## NaCl and Sugars

- ◆ 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.

## Hypotonic, Isotonic, and Hypertonic



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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 requires about **six times more sucrose** than NaCl to effect the same degree of inhibition.



## NaCl and Sugars

- ◆ The most common uses of sugars as preserving agents are in the making of **fruit preserves (fruit+sugar), candies, condensed milk, and the like.** → **high concentrations of sugar** makes **water unavailable** to microorganisms





## 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 60% sucrose, whereas much lower levels inhibit most bacteria.
- ◆ Organisms that **are able to grow in high concentrations of sugars** are designated **osmophiles** (嗜高滲透壓生物); **osmoduric** (耐高滲透壓) microorganisms are those that are **unable to grow but are able to withstand high levels of sugars**.



## 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**





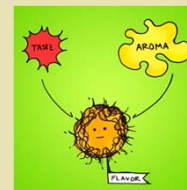
## Antioxidants

- ◆ 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 調味劑

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



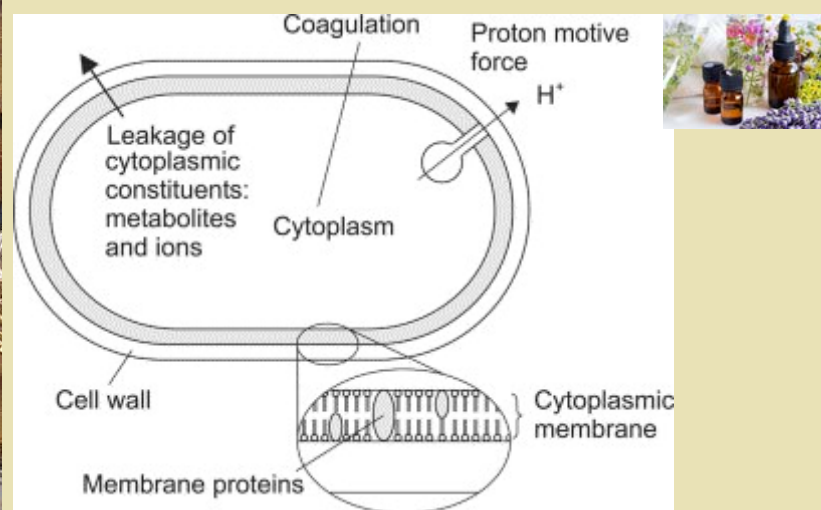


## Flavoring Agents

- ◆ **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**.



## Sites of action for essential oils



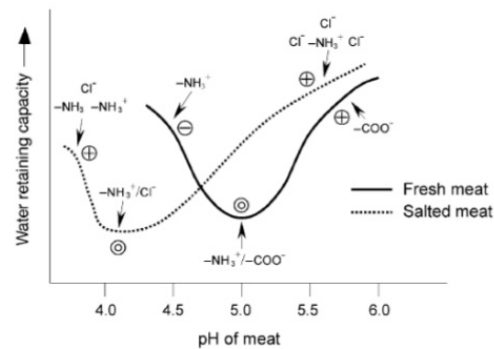
International Journal of Food Microbiology 94, 223-253

## 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 P (sodium polyphosphate).
- ◆ They possess antibotulinal activity, especially when combined with nitrites.
- ◆ Filter-sterilized phosphate preparations were more inhibitory than autoclaved ones.
- ◆ Active growth of cells was necessary for its bactericidal effect.

Salt - Effect on Water Holding Capacity



Source: Xiong YL (2004). Muscle proteins. In Yada RY (Ed.), Proteins in food processing. Cambridge, England: Woodhead Publishing.

- ◆ Phosphate are basics and raise the pH of meat
- ◆ Phosphate are anions (-) and may create a chloride effects on charges



## Medium-Chain Fatty Acids and Esters

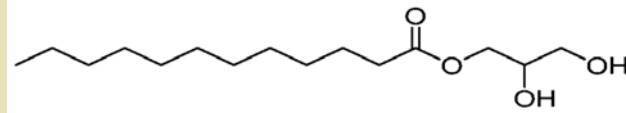
- ◆ Acetic, propionic, and sorbic acids are **short-chain fatty acids** used primarily as preservatives.
- ◆ **Medium-chain fatty acids** are employed primarily as **surface-active** or **emulsifying agents**.
- ◆ The antimicrobial activity of the medium-chain fatty acids is best known from **soaps**,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



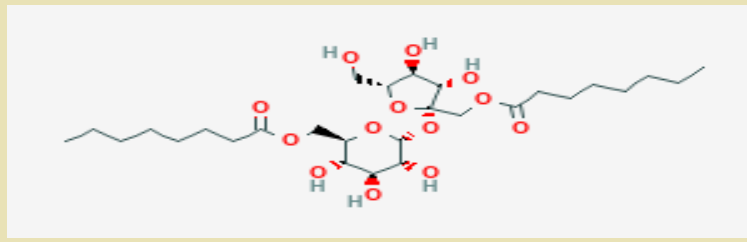
## Medium-Chain Fatty Acids and Esters

- ◆ 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.

## Medium-Chain Fatty Acids and Esters



- ◆ **Monolaurin** (glyceryl monolaurate單月桂酸甘油酯) is the most effective of the glycerol monoesters, and **sucrose dicaprylate** is the most effective of the sucrose diesters.



## Preservative system

- ◆ Using **combinations of chemicals**
- ◆ Consist of three compounds—**monolaurin/EDTA/butylated hydroxyanisole (BHA)**, for example.
  - Although EDTA (可以螯著多種金屬離子) 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

- ◆ Acetic and lactic acids are widely employed as preservatives ← **produced by lactic acid bacteria**. Products include pickles, sauerkraut (德國酸菜), and fermented milks.

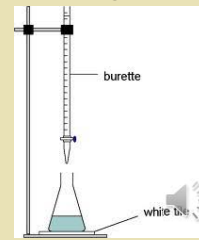


- ◆ The antimicrobial effects of organic acids such as propionic and lactic is due to both the **depression of pH** below the growth range and **metabolic inhibition** by the **undissociated acid molecules**.



## Acetic and Lactic Acids

- ◆ 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**.
- ◆ 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








## Acetic and Lactic Acids

- ◆ **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  $5 \times 10^6$  *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.**



## Salts of Acetic and Lactic Acids

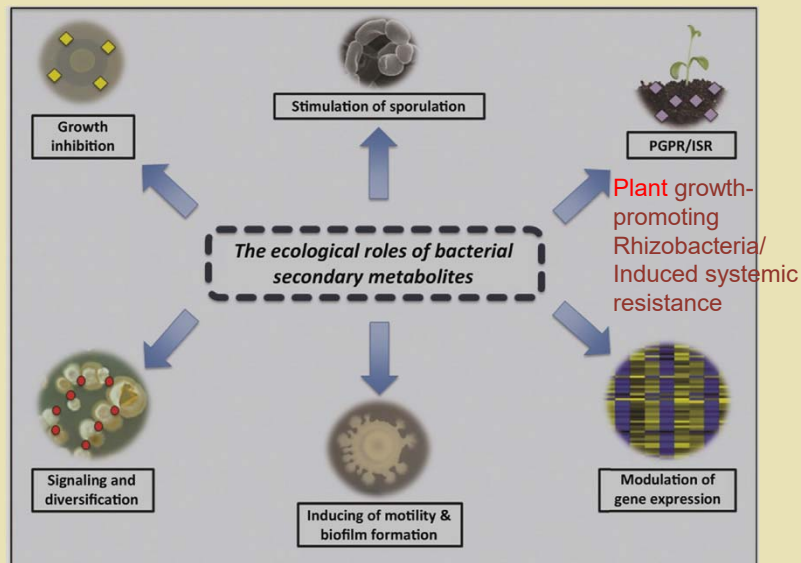
- ◆ The sodium and potassium salts of acetic and lactic acids are widely used in food.
- ◆ **Sodium diacetate** → used in bread and cakes  
→ prevent moldiness  
- ◆ Extend shelf life of processed meats 
- ◆ Against psychrotrophic pathogens (*Listeria monocytogenes* 李斯特菌)
- ◆ Control growth of *Clostridium perfringens* (產氣莢膜梭菌)





## Antibiotics

- ♦ **Antibiotics** are **secondary metabolites** produced by microorganisms that **inhibit or kill a wide spectrum of other microorganisms**.
  - Primary metabolites are considered essential to microorganisms for proper growth.
  - Secondary metabolites have no apparent significance to the growth or metabolism and are usually formed during the stationary phase of growth.
- ♦ Most antibiotics are **produced by molds and bacteria** of the genus *Streptomyces* (鏈絲菌屬), and a few by *Bacillus* (芽孢桿菌屬) and *Paenibacillus* (類芽孢桿菌屬).
- ♦ Many of the clinically useful agents are synthetic products.



Trends in Microbiology  
25, p280–292





## Antibiotics (Table 13-9)

- ◆ 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**.



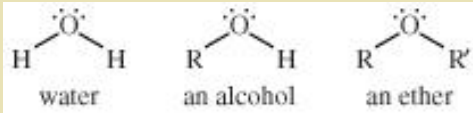
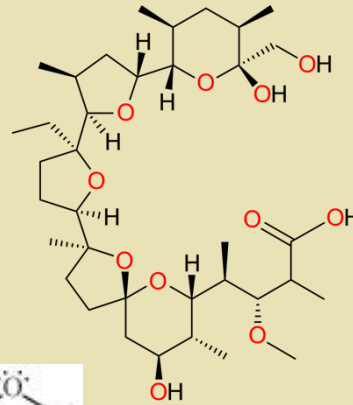
## Monensin (孟寧素)

- ◆ Approved by FDA as a **cattle additive**, 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**.





Monensin is a **polyether** antibiotic isolated from *Streptomyces cinnamomensis*.



## Natamycin (納塔黴素)

- ◆ Used as a **food preservative** with the consideration of the following facts:
  - **Against yeasts and molds** but not bacteria
  - Stimulates an unusually **low level of resistance** among fungi,
  - **Rarely involved in cross-resistance** among other antifungal polyenes
  - **DNA transfer** between fungi is **less** than bacteria.



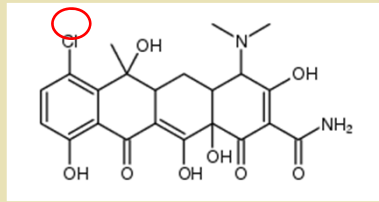






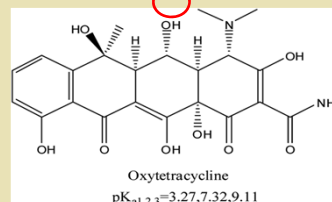
## Tetracyclines (四環黴素)

- ◆ used to treat **bacterial infections**
- ◆ produced by the *Streptomyces*
- ◆ **protein synthesis inhibitor** by associating with the **30S ribosomal subunit**.



Chlortetracycline (CTC)

氯四環素；金黴素



Oxytetracycline (OTC)

土黴素；氧四環素



## Tetracyclines (四環黴素)

- ◆ Chlortetracycline (CTC) and oxytetracycline (OTC) delay bacterial spoilage of seafoods, poultry, red meats, vegetables, raw milk, and other foods.

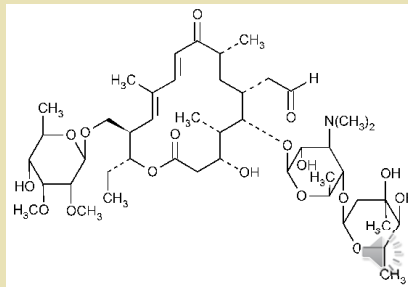
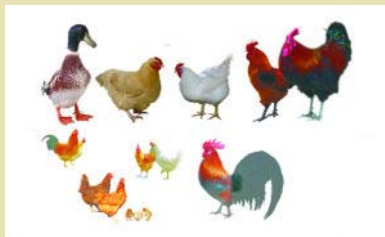


- ◆ CTC is generally more effective than OTC.
- ◆ The tetracyclines are both **heat sensitive** and **storage labile** in foods.
- ◆ They are used to **treat diseases** in humans and animals and are used also in **feed supplements**.



## 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

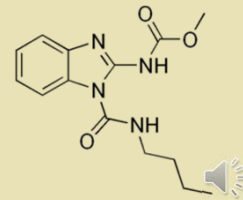
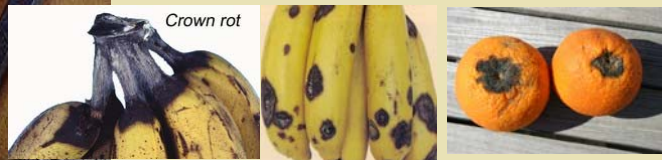
Table 13–10 Some Chemical Agents Employed to Control Fungal Spoilage of Fresh Fruits

Compound	Fruits
<b>Thiabendazole</b>	Apples, pears, citrus fruits, pineapples
<b>Benomyl</b>	Apples, pears, bananas, citrus fruits, mangoes, papayas, peaches, cherries, pineapples
<b>Biphenyl</b>	Citrus fruits
<b>SO<sub>2</sub> fumigation</b>	Grapes
<b>Sodium-<math>\alpha</math>-phenylphenate</b>	Apples, pears, citrus fruits, pineapples



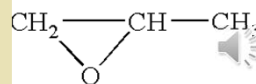
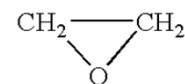
## Antifungal Agents for Fruits

- ◆ **Benomyl** (苯菌靈) 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** (冠腐病) and **anthracnose** (炭疽病) of bananas, and **stem-end rots** (果柄腐爛) of citrus fruits.



## Ethylene and Propylene Oxides (環氧乙烷及環氧丙烷)

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





## Ethylene oxide

- ◆ Its **antimicrobial activity** is presumed to be related to **alkylation**.
  - In the presence of **labile H** atoms, the unstable three-membered ring (三元環) 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  **$-\text{COOH}$ ,  $-\text{NH}_2$ ,  $-\text{SH}$ , and  $-\text{OH}$** .



## Ethylene oxide

- ◆ Affect **endospores of *C. botulinum*** by **alkylation of guanine and adenine components of spore DNA**.
- ◆ Used as a **gaseous sterilant** for flexible (柔軟的) and semirigid (半硬式的) 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 lactic acid bacteria) or it may be effected by **indirect actions or agents** (such as the production of 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

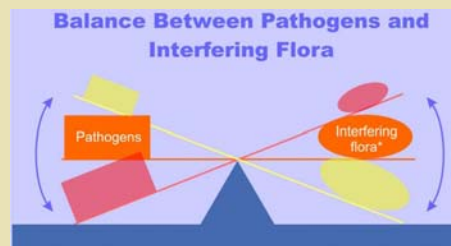
- ◆ The general **nonspecific inhibition or destruction** of one microorganism by other members of **the same habitat** or environment.
- ◆ The mechanisms are not clear, but some observations are worthy of note.
  - The background biota needs to be **larger in a number of viable cells** than the organism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





## Microbial Interference

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



## *Lactic antagonism* (乳酸拮抗作用)

- ◆ 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,  $H_2O_2$ , diacetyl, and bacteriocins
  - pH depression
  - nutrient depletion





### *Lactic antagonism* (乳酸拮抗作用)

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



### Nisin (乳酸鏈球菌素)

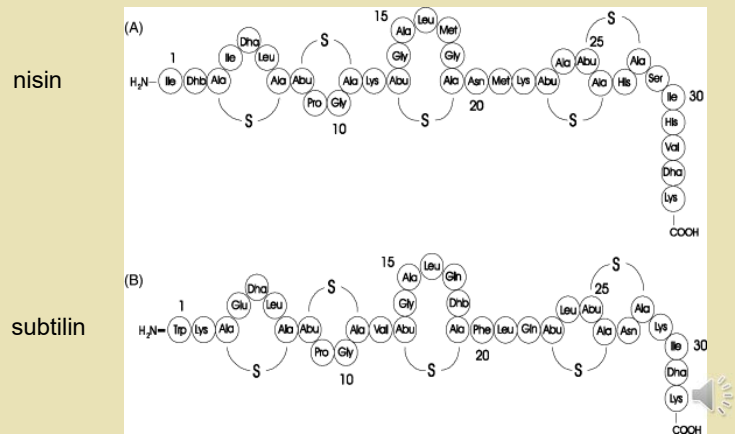
- ◆ Nisin is a **bacteriocin**.
- ◆ Like **antibiotics**, bacteriocins are chemical compounds **produced by microorganisms that inhibit or kill other microorganisms**.
- ◆ Unlike antibiotics, **bacteriocins inhibit or kill generally only closely related species or strains of the same species**.





## Nisin (乳酸鏈球菌素)

- ◆ A polypeptide → structurally related to subtilin.
- ◆ the **most widely used antibiotic for food preservation**



## Nisin (乳酸鏈球菌素)

- ◆ **Desirable properties as a food preservative** are the following:
  - a) **nontoxic**
  - b) produced naturally by *Lactococcus lactis* (乳酸乳球菌) strains
  - c) **heat stable** and **excellent storage stability**
  - d) **destroyed by digestive enzymes**
  - e) **not contribute to off-flavors or off-odors**
  - f) has a **narrow spectrum of antimicrobial activity**





## Nisin (乳酸鏈球菌素)

- ◆ **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**.



## Subtilin (枯草桿菌素)

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

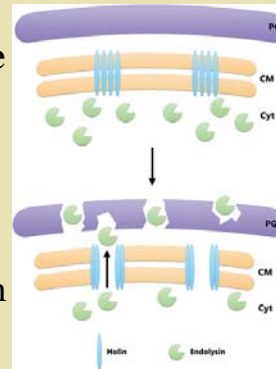






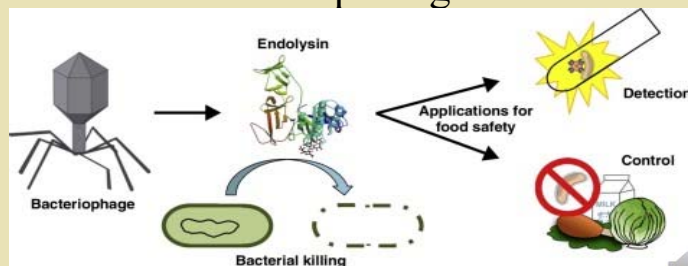
## ENDOLYSINS (内容素)

- ◆ To release newly formed **bacteriophages** from their host cell, two small hydrophobic proteins were used:
  - **Holins (穿孔素)** 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.



## 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.

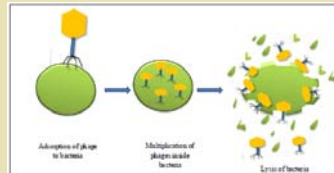


Current Opinion in Biotechnology 2016, 37:76–87

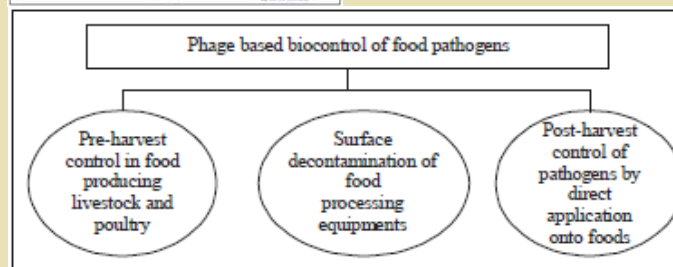


## BACTERIOPHAGES AS BIOCONTROL AGENTS

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

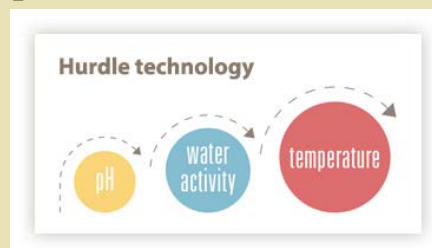


Asian Journal of Animal and Veterinary Advances 10 (11): 708-723, 2015



## THE HURDLE CONCEPT

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



<http://foodingredients.treetop.com/>





- ◆ **愛吃燒烤、香腸，17歲男大腸癌離世**(2015-4-1優活健康網)
  - 香腸和臘肉都有亞硝酸鹽，進入人體會產生致癌物
  - 肉類經高溫燒烤產生致癌物
  - 建議同學們少吃，**要多吃蔬果多運動**
- ◆ **氣喘過敏頻發作？可能是香腸、火腿吃太多！**（華人健康網 2017-11-10）
  - 目前仍不清楚加工肉品造成氣喘惡化的確切原因，但**可能與加工肉品含有大量的亞硝酸鹽、鹽分、脂肪**有關係
  - 攝取加工肉品頻率最高的氣喘患者，症狀惡化的機率比食用頻率最低的患者高出76%