จุลินทรีย์ที่ทำให้อาหารเสื่อมเสีย

- ปัจจัยที่มีผลต่อจุลินทรีย์ที่ทำให้อาหารเสื่อมเสีย
- ชนิดและความสำคัญของจุลินทรีย์ที่ทำให้อาหารเสื่อมเสียแบ่งตามกลุ่มอาหาร

โดย

อ. ธนัท อมาตยกุล

FSN 321 Food Microbiology

คณะเทคโนโลยีและนวัตกรรมผลิตภัณฑ์การเกษตร

มหาวิทยาลัยศรีนครินทรวิโรฒ

Spoilage

- According to the Oxford English Dictionary to spoil is to ‘deprive of good or effective qualities’
  - สีเปลี่ยนไป
  - เนื้อสัมผัส
  - กลิ่น
  - รส (ขม/เปรี้ยว)
  - เกิดเมือก
  - เกิดแก๊ส

Microorganism and Food Spoilage

- อาหารที่เน่าเสียจากเชื้อจุลินทรีย์ จะเกิดกลิ่นเหม็นหรือลักษณะต่างๆเปลี่ยนแปลงไปเมื่อมีจำนวนเชื้อจุลินทรีย์อยู่สูงระดับหนึ่ง

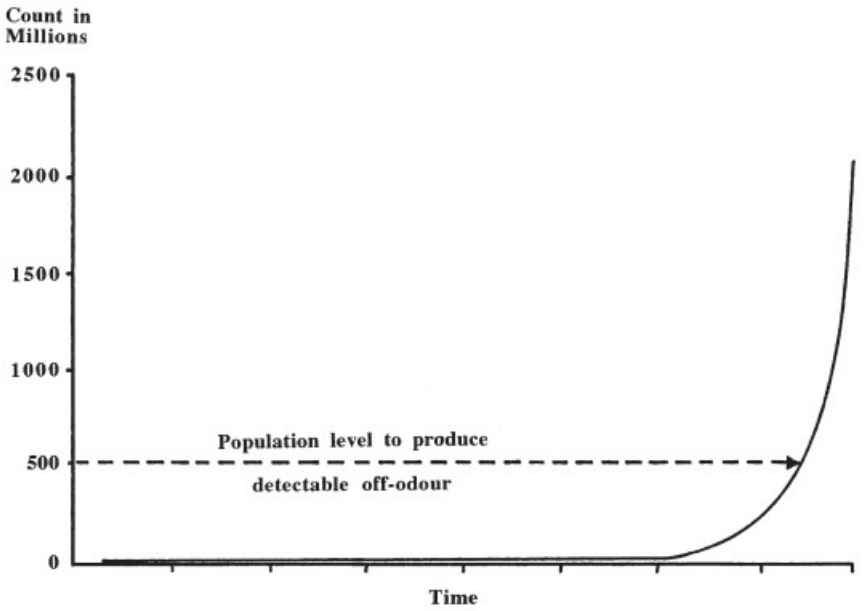

Figure 5.1 *Microbial growth and spoilage*

Adams & Moss (2008). Food Microbiology

$10^{7\text{-}8}$ CFU/g,/mL

Sequence of Food Spoilage

- Microorganism get into food
- Both intrinsic and extrinsic factors favor growth of spoilage microorganism
  - Nutrient (carbohydrate, protein, lipid)
  - A_w
  - pH
  - Oxygen
  - Temperature
- Enough storage time for microorganism to grow

Spoilage microorganism

- Bacteria (shorter generation time) follow by yeast and mold
- This also depends on type of foods
- High initial number of spoilage microorganism speed up spoilage process
- Only predominant microorganisms cause spoilage (mostly the shortest generation time)

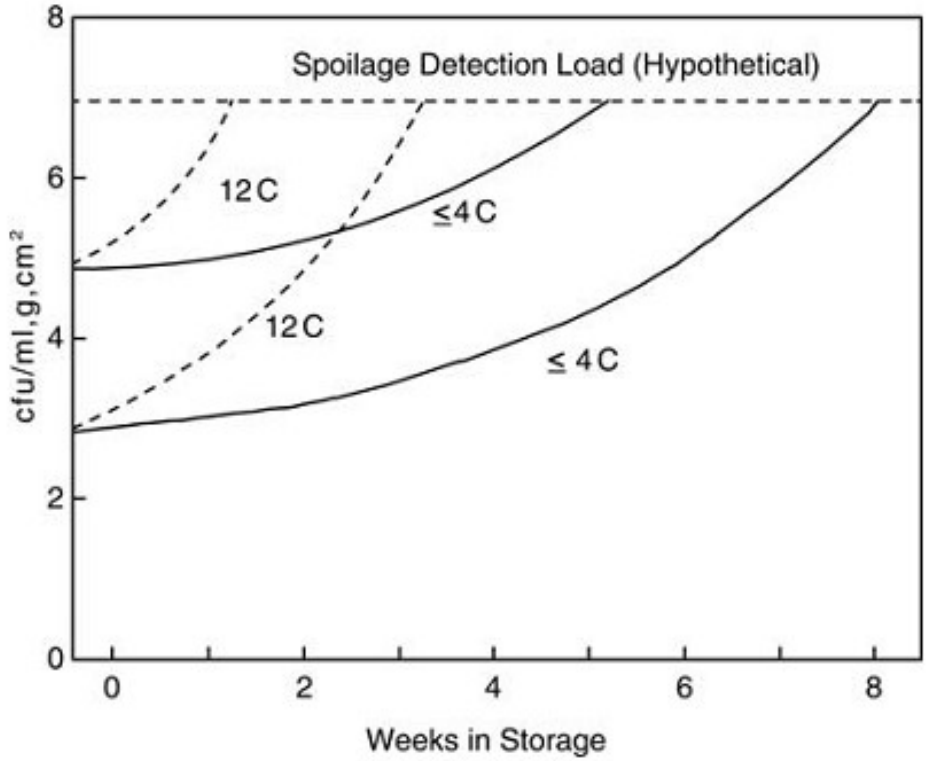

Figure 18.1 Graphical illustration showing the influence of initial bacterial levels and storage temperatures on the shelf life of a refrigerated product.

Ray (2004) Fundamental Food Microbiology

In a study, a beef sample (pH 6.0) was found to initially contain ca. 10^3 bacterial cells/g, with relative levels of *Pseudomonas* spp. 1%, *Acinetobacter* and *Morexella* 11%, *Brochothrix thermosphacta* 13%, and others (*Micrococcus, Staphylococcus, Enterobacteriaceae,* lactic acid bacteria, etc.) 75%.

Following aerobic storage at 2°C for 12 d, the population reached 6 x 10^7 cells/g, with the relative levels of *Pseudomonas* spp. 99% and all others 1%.

What will happen if the beef is kept under anaerobic condition?

Some important spoilage bacteria

Types	Characteristics	Microbial name
Psychrotrophic bacteria - Psychrotrophic Aerobic Spoilage Bacteria - Psychrotrophic Facultative Anaerobic Spoilage Bacteria	the bacterial species capable of growing at 5°C and below, but multiply quite rapidly at 10 to 25°C and even at higher temperatures.	*Pseudomonas fluorescens*, *Pse. fragi*, other *Pseudomonas* species, *Acinetobacter*, *Moraxella*, and *Flavobacterium* *Bro. thermosp hacta, Lactobacillus viridescens, Lab. sake, Lab. curvatus, unidentified Lactobacillus spp., Leuconostoc carnosum, Leu. gelidum, Leu. mesenteroides, unidentified Leuconostoc spp., some Enterococcus spp., Alcaligenes spp., Enterobacter spp., Serratia liquifaciens, some Hafnia and Proteus spp., and Shewanella (previously Alteromonas) putrefaciens*

Some important spoilage bacteria

Types	Characteristics	Microbial name
- Thermoduric Psychrotrophs		*Bacillus coagulans* and *Bac. megaterium*, some strains of *Lab. viridescens*; and anaerobes, such as spores of *Clostridium laramie*, *Clo. estertheticum*, *Clo. algidicarnis*, *Clo. putrefaciens*, and unidentified *Clostridium* spp.
Thermophilic bacteria	the bacteria in this group grow between 40 and 90^{o}C, with optimum growth at 55 to 65^{o}C	Spores or contaminated vegetative of thermophiluc (*Bacillus* sp. and *Clostridium* sp.) can grow in food kept at 60^{o}C for long time (Restaurant)

Some important spoilage bacteria

Types	Characteristics	Microbial name
Aciduric bacteria	Bacteria that can grow relatively rapidly in food at pH 4.6 or below are generally regarded as aciduric (or acidophilic)	Heterofermentative lactic acid bacteria (such as *Lab. fructivorans*, *Lab. fermentum*, and *Leu. mesenteroides*) and homofermentative lactic acid bacteria (such as *Lab. plantarum* and *Ped. acidilactici*)

Food

- Nutrient (sugar first)
  - Carbohydrate (polysaccharides, trisaccharides,
  - disaccharides, monosaccharides, and sugar alcohols)
  - Protein (proteins, peptides), NPN compounds (amino acids, urea, creatine, trimethylamine oxide)
  - Lipid (triglycerides, phospholipids, fatty acids, sterols)
- The metabolism depends on microorganism and condition
- Processing (cutting, grinding, heat treatment, etc.) affect growth of microorganism

Table 18.1 Some End Products from Microbial Metabolism of Food Nutrients

Food Nutrient	End Products
Carbohydrates	CO_2, H_2, H_2O_2, lactate, acetate, formate, succinate, butyrate, isobutyrate, isovalerate, ethanol, propanol, butanol, isobutanol, diacetyl, acetoin, butanediol, dextran, levans
Proteinaceous and NPN compounds	CO_2, H_2, NH_3, H_2S, amines, keto-acids, mercaptans, organic disulfides, putrescine, cadaverine, skatole
Lipids	Fatty acids, glycerol, hydroperoxides, carbonyl compounds (aldehydes, ketones), nitrogenous bases

Under specific conditions, some microorganisms can also synthesize (anabolism) **polymeric compounds** as end products, such as dextran (polymer of glucose) production by *Leu. mesenteroides* while metabolizing sucrose. Some microorganisms can also **secrete extracellular enzymes** to break down large molecular nutrients (polymers) in a food (such as breakdown of starch by amylase produced by some molds). Finally, some microorganisms can synthesize **pigments** while growing in a food (such as *Micrococcus luteus* producing a yellow pigment)

Sequential growth

- In milk
- First, *Lactococcus* grow and reduce pH from 6.5 to 5.0
- Then, other bacteria start to grow such as aciduric *bacillus* sp. By using protein and increase pH to 5.8
- Enhance the growth of *Pseudomonas* sp. Which preferably use NPN and increase pH further.

ชนิดและความสำคัญของเชื้อจุลินทรีย์ที่ทำให้อาหารเสื่อมเสียแบ่งตามกลุ่ม

- เนื้อ
- ปลา
- นม
- ผัก
- เครื่องดื่ม
- ธัญพืช
- มายองเนส
- ……

http://www.phtnet.org/article/images/a39_1.jpg

Spoilage microorganism in raw meat

- เชื้อจุลินทรีย์ที่มักพบในอาหารที่าจากเนื้อสัตว์ประกอบด้วย

  Pseudomonas, Acinetobacter, Moraxella, Shewanella, Alcaligenes, Aeromonas, Escherichia, Enterobacter, Serratia, Hafnia, Proteus, Brochothrix, Micrococcus, Enterococcus, Lactobacillus, Leuconostoc, Carnobacterium, and *Clostridium,* as well as yeasts and molds

- เชื้อตัวไหนจะเจริญเติบโตเด่นขึ้นมาขึ้นกับสภาวะต่างๆที่ทำให้เชื้อนั้นๆเจริญเติบโตเด่นขึ้นมา

- Postrigor Meat
  - Rich NPN, low carbohydrate, low pH (5.5), high water activity (0.97)

- Most studies dealing with the spoilage of meats have been done with beef, ork, lamb, veal, and similar meats, are presumed to spoil in a similar way.

Table 4–10 Meat and Meat Products: Approximate Percentage Chemical Composition

Meats	*Water*	*Carbohydrates*	*Proteins*	*Fat*	*Ash*
Beef, hamburger	55.0	0	16.0	28.0	0.8
Beef, round	69.0	0	19.5	11.0	1.0
Bologna	62.4	3.6	14.8	15.9	3.3
Chicken (broiler)	71.2	0	20.2	7.2	1.1
Frankfurters	60.0	2.7	14.2	20.5	2.7
Lamb	66.3	0	17.1	14.8	0.9
Liver (beef)	69.7	6.0	19.7	3.2	1.4
Pork, medium	42.0	0	11.9	45.0	0.6
Turkey, medium fat	58.3	0	20.1	20.2	1.0

Nutrient and moisture contents are adequate to support the growth of all organisms

- Raw meat is usually kept under refrigerated condition (aerobic).
- This enhance growth of psychrotrophic bacteria.
- Under aerobic condition, *Pseudomonas* sp. First use glucose, then amino acids causing acids and off flavor.
- Other bacteria involved include *Acinetobactor* and *Morexella* (prefer to use amino acids and produce off flavor)

- Raw meat kept under anaerobic condition =>
- Vacuum pack, MAP
- Psychrotrophic facultative anaerobe or anaerobe
- *Lactobacillus curvatus* and *Lab. sake* metabolize glucose to produce lactic acid and the amino acids leucine and valine to isovaleric and isobutyric acids.
- Some bacteria metabolite cysteine to H_2S affecting both odor and color.

- Heterofermentative *Leuconostoc carnosum* and *Leu. gelidum* produce CO_2 and lactic acid, causing accumulation of gas and liquid in the package.
- *Shewanella putrefacience*, which can grow under both aerobic and anaerobic conditions, metabolizes amino acids (particularly cysteine) to produce methylsulfides and H_2S in large quantities. Along with offensive odors, they adversely affect the normal color of meats. H_2S oxidizes myoglobin to a form of metmyoglobin, causing a green discoloration.
- Facultative anaerobic *Enterobacter, Serratia, Proteus*, and *Hafnia* species metabolize amino acids while growing in meat to produce amines, ammonia, methylsulfides, and mercaptans, and cause putrefaction.

- Psychrotrophic *Clostridium* spp., such as *Clostridium laramie*, have been found to cause spoilage associated with proteolysis and loss of texture of meat, accumulation of liquid in the bag, and offensive odor, with an H_2S smell predominating
- เนื้อบดมักจะเสื่อมเสียเร็วกว่าเนื้อที่ตัดแบ่งขาย เนื่องจากมีพื้นที่ผิวสัมผัสมากกว่า
-

Spoilage microorganism in processed meat

- Low-heat-processed cured and uncured meats, such as roast, ham, bacon
- Core temp at least 70^{o}C
- Only spore of *Bacillus* sp. and *Clostridium* sp. and some extremely thermoduric vegetative species (*Lab. viridescens*, some *Enterococcus*, *Micrococcus* inside the product) can survive.
- Also, contamination prior to packing from equipment, personnel, water, and air
- Vacuum packed products can be spoiled by Psychrotrophic facultative anaerobic and anaerobic bacteria (psychrotrophic *Lactobacillus* spp. (such as homofermentative *Lab. sake, Lab. curvatus,* heterofermentative *Lab. viridescens*) and *Leuconostoc* spp. (such as *Leu. carnosum, Leu. gelidum,* and *Leu. mesenteroides*) causing gas, liquid due to acid production, color change from brown to pink and off odor.

Table 5–1 Genera of Bacteria and Fungi Most Frequently Found on Processed Meats

Bacteria			*Fungi*	
Genus	*Gram Reaction*	*Relative Prevalence*	*Genus*	*Relative Prevalence*
Acinetobacter	–	X	**Yeasts**	
Aeromonas	–	X	*Candida*	X
Alcaligenes	–	X	*Debaryomyces*	XX
Bacillus	+	X	*Saccharomyces*	X
Brochothrix	+	X	*Trichosporon*	X
Carnobacterium	+	X	Yarrowia	X
Corynebacterium	+	X	**Molds**	
Enterobacter	–	X	*Alternaria*	X
Enterococcus	+	X	*Aspergillus*	XX
Hafnia	+	X	*Botrytis*	X
Kocuria	+	X	*Cladosporium*	X
Kurthia	+	X	*Fusarium*	X
Lactobacillus	+	XX	*Geotrichum*	X
Lactococcus	+	X	*Monilia*	X
Leuconostoc	+	X	*Mucor*	X
Listeria	+	X	*Penicillium*	XX
Microbacterium	+	X	*Rhizopus*	X
Micrococcus	+	X	*Scopulariopsis*	X
Moraxella	–	X	*Thamnidium*	X
Paenibacillus	+	X		
Pediococcus	+	X		
Pseudomonas	–	XX		

Spoilage of Process Meat

Spoilage of these products is generally of three types: sliminess, souring, and greening.

- ***Slimy spoilage***
  - occurs on the outside of casings, especially of frankfurters
  - Yeasts, lactic acid bacteria of the genera *Lactobacillus*, *Enterococcus*, *Weissella*, and *B. thermosphacta*, may be isolated from the slimy material.
- ***Souring***
  - generally takes place underneath the casing of these meats and results from the growth of lactobacilli, enterococci, and related organisms.
  - The souring results from the utilization of lactose and other sugars by the organisms and the production of acids.

Spoilage of Process Meat

- ***Greening*** *:* Two types of *greening* occur on stored and processed red meats
  - One caused by **H_2O_2** and the other by **H_2S**
  - Upon exposure to air, H_2O_2 forms and reacts with nitrosohemochrome to produce a greenish oxidized porphyrin.
  - H_2O_2 may accumulate when heating if nitrite destroys catalase, and the peroxide reacts with meat pigments to form choleglobin, which is green.
  - *Weissella viridescens* is the most common organism in this type of greening, but leuconostocs, *Enterococcus faecium*, and *Enterococcus faecalis* are capable of producing greening of products.
  - H_2S reacts with myoglobin to form sulphmyoglobin: This type of greening does not usually occur when meat pH is below 6.0.
  - The organism responsible in one study was thought to be *Pseudomonas mephitica*

Spoilage of fish

- ปลาจะเสื่อมเสียได้จากเอ็นไซม์โปรติเอส ออกซิเดชั่นของ free fatty acid, และการเจริญเติบโตของเชื้อจุลินทรีย์
- การเสื่อมเสียจากเชื้อจุลินทรีย์ขึ้นกับหลายปัจจัย ประกอบด้วย the microbial types, their level, fish environment, fish types, methods used for harvest, and subsequent handling
- โดยปกติเนื้อปลาจะมีโปรตีน อะมิโนแอซิด และ NPN ในปริมาณสูงและมี carbohydrate ในปริมาณต่ำ รวมทั้งมี pH ประมาณ 6.0
-

Gram-negative aerobic rods, such as *Pseudomonas* spp., *Acinetobacter, Moraxella,* and *Flavobacterium*, and facultative anaerobic rods, such as *Shewanella, Alcaligenes, Vibrio,* and coliforms, are the major spoilage bacteria.

However, because of the relatively shorter generation time, spoilage by psychrotrophic *Pseudomonas* spp. predominates under aerobic storage at both refrigerated and slightly higher temperature.

In fish stored under vacuum or CO_2, lactic acid bacteria (including *Enterococcus*) can become predominant

- Off odor come from metabolism of NPN compounds giving NH_3, trimethylamine (fishy odor), histamine, putrescine, cadaverine, indoles, H_2S, mercaptans, dimethyl sulfide
- Bacterial growth is also associated with slime production, discoloration of gills and eyes (in whole fish), and loss of muscle texture (soft due to proteolysis).

Spoilage of fruits

- Fresh fruits are high in carbohydrates (generally 10% or more), very low in proteins (<1.0%), and have a pH 4.5 or below.
- Mold, yeast and aciduric bacteria (lactic acid bacteria, Acetobactor, Gluconobactor)
- Rot by different types of molds from genera *Penicillium, Aspergillus, Alternaria, Botrytis, Rhizopus*,
- Yeasts from genera *Saccharomyces, Candida, Torulopsis*, and *Hansenula* are associated with fermentation of some fruits,

Spoilage by Fungal Agents

- **Gray mold rot :** This condition is caused by ***Botrytis cinerea***, which produces a gray mycelium. This type of spoilage is favored by high humidity and warm temperatures.
  - **The vegetables affected are asparagus, onions, garlic, beans (green, lima, andwax), carrots, parsnips, celery, tomatoes, endives, globe artichokes, lettuce, rhubarb, cabbage, Brussels sprouts, cauliflower, broccoli, radish, rutabagas, turnips, cucumbers, pumpkin, squash, peppers, and sweet potatoes**
- **Sour rot** (oospora rot, watery soft rot) : This condition of vegetables is caused by ***Geotrichum candidum*** and other organisms.
  - **The vegetables affected are asparagus, onions, garlic, beans, carrots, parsnips, parsley, endives, globe artichokes, lettuce, cabbage, sprouts, cauliflower, broccoli, radishes, rutabagas, turnips, and tomatoes.**
- **Rhizopus soft rot :** This condition is caused by *Rhizopus stolonifer* and other species that make vegetables soft and mushy.
- **Blue mold rot :** is a post-harvest disease of apples and pears that is caused by *Penicillium expansum*

SPOILAGE OF FRUITS

- On the basis of nutrient content, these products would appear to be capable of supporting the growth of bacteria, yeasts, and molds.
- However, the pH of fruits is below the level that generally favors bacterial growth.
- The wider pH growth range of molds and yeasts suits them as spoilage agents of fruits.
- Many yeasts are capable of attacking the sugars found in fruits and bringing about fermentation with the production of alcohol and carbon dioxide.
- It is not clear whether some molds are dependent on the initial action of yeasts in the process of fruit and vegetable spoilage. Many molds are capable of utilizing alcohols as sources of energy.

Table 6–10 Log_{10} Aerobic Plate Counts (per Gram) of RTU Vegetables Held at 4°C[a]

Product	*Day 0*	*Day 4*
Chopped lettuce	4.85	5.63
Salad mix	5.35	6.05
Cauliflower florets	4.82	5.45
Sliced celery	5.67	6.59
Coleslaw mix	5.14	6.95
Carrot sticks	5.13	6.27
Broccoli florets	5.58	6.59
Green peppers	5.99	7.22

[a]The products had a 7-day recommended shelf life.