Food Microbiology

Factors That Affect Microbial Growth

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Factors That Affect Microbial Growth

Intrinsic Factors: physical, chemical, and biological properties of foods.

(1) pH—most MO grow best around pH 7.0 but have the following overall ranges:

Bacteria	**4.0—9.0**
Yeasts	**1.5—8.5**
Molds	**1.5—11.0**

(2) Aw—minimum Aw for growth of most MO:

Most spoilage bacteria	0.91
Most spoilage yeasts	0.88
Most spoilage molds	0.80
Halophilic bacteria	0.75
Xerophilic molds	0.65
Osmophilic yeasts	0.60

(3) Oxidation reduction potential (O/R, Eh)—classification of MO as aerobic, anaerobic, microaerophilic, facultative anaerobic.

(4) Nutrient content—from lowest to highest nutrient requirements: molds, yeasts, G(-) bacteria, G(+) bacteria

(5) Antimicrobial agents—naturally occurring or added to food can result in inhibition or selection of MO

(6) Biological structures—natural coverings protect food until damaged. Once damaged MO can enter and initiated spoilage. Packaging can be replaced biological barriers that are destroyed by processing

Extrinsic Factors: properties of the environment that affect both foods and MO

(1) Temperature of storage—will affect microbial flora in food

Organism	range	optimum
Psychrophiles	＜0 to 20℃	15 ℃
Psychrotrophs	0 to 30 ℃	20--30 ℃
Mesophiles	20 to 45 ℃	30--40 ℃
Thermophiles	＞45 ℃	55--65 ℃

(2) Relative humidity—higher temperature means lower relative humidity—store foods so they do not pick up or lose water

(3) Gaseous environment—10% CO_2 or other gases such as ozone help to retard spoilage

(4) Processing—processing foods can change some of the intrinsic factors

Implicit Factors: inherent properties of MO that are modified by influences from intrinsic and extrinsic factors

(1) Specific growth rate—defined under optimal conditions and determined by lag phase, rate of logarithmic growth and total cell numbers.

(2) Symbiosis—one organism causes a change in the growth conditions of another organism. This can be caused by six mechanisms:

(a) availability of nutrients,

(b) change in pH of food,

(c) change in redox potential,

(d) change in Aw of food,

(e) elimination of antimicrobial agents,

(f) damage to food.

(3) Antagonism—one organism kills, injures or inhibits the growth of another organism by five mechanisms:

(a) competitive utilization of nutrients,

(b) change in pH of food,

(c) formation of antimicrobial agents,

(d) change in redox potential,

(e) lysis of bacteria, especially by phage.

§pH and Microbial Growth

pH affect

(1) enzyme function

(2) nutrient transport of MO

Most MO grow best at neutral pH 6.6-7.5

Bacteria	4.0—9.0
Yeasts	1.5—8.5
Molds	1.5—11.0

Temperature, salt, O_2 affect min pH or pH range

temp increase → more acid-tolerant

tolerant amount of salt added →increase pH range

Young cell more sensitive to pH change

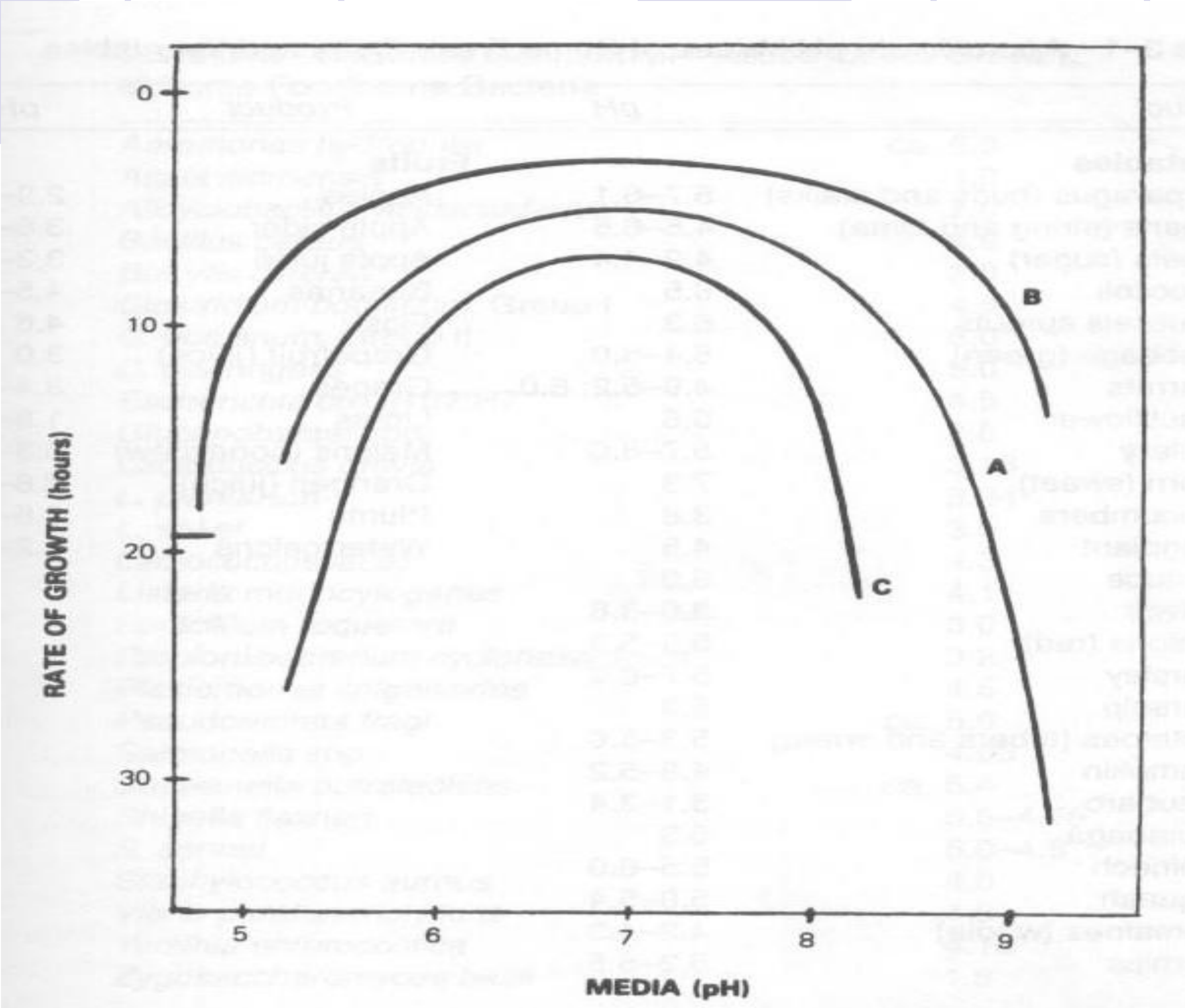

Fig. 3-2 Relationship of pH, NaCl, and Na citrate on the rate of growth of *Alcaligenes faecalis* in 1% peptone:A=1% peptone; B=0.2M NaCl; C=1% peptone + 0.2M Na citrate.

pH and microflora of food:

(a) select microflora

(b) preserves food

(c) alter processing needs

(d) metabolic products→identify MO

(e) methods of changing pH:

fermentation of sugars→acidic

degradation of proteins→basic

Both pH value and acidulant affect MO growth or toxin production

eg.

S. aureus: pH range 4.0-9.8, opt. 6.0-7.0

in milk, pH 4.5 HCl, enterotoxin

pH 4.5 lactate, no toxin

eg.

Salmonella start to grow in pH 4.05 (HCl, citric), but start to grow in pH 5.4-5.5 (acetic, propionic)

Toxicity of adverse pH: undissociated form of acid or base→penetrate into cell→become ionized form→change internal pH→inhibit en. Activity

pH of Food:

(1)buffer capacity—protein food ＞ fruit or vegetable

(2)actual acid content

Foods are categorized by pH

High acid food	＜3.7
Acid food	3.7 ~ 4.6 (4.5)
Medium-acid food	4.6 ~ 5.3
Lower or non acid food	＞5.3

§Aw and Microbial growth

Water need by MO for (a) nutrient transport, (b) enzymatic and chemical reaction, (c) waste removal.

Microorganism	Min Aw
most spoilage bacteria	0.90 ~ 0.91
most spoilage yeasts	0.87 ~ 0.88
most spoilage molds	0.79 ~ 0.80
halophilic	0.75
xerophilic	0.65
osmophilic	0.60 ~ 0.61

G(-) bacteria: most sensitive to Aw.

Aw can be reduced by addition of salt or sugar.

Not all MO have max growth rate near Aw 0.99, osmophilic yeast, xerophilic mold, halophilic bact. different, eg. ***Vibrio costicola*** not grow if Aw＞0.98, ***Penicillium*** sp. Opt. Aw = 0.93 ~ 0.98

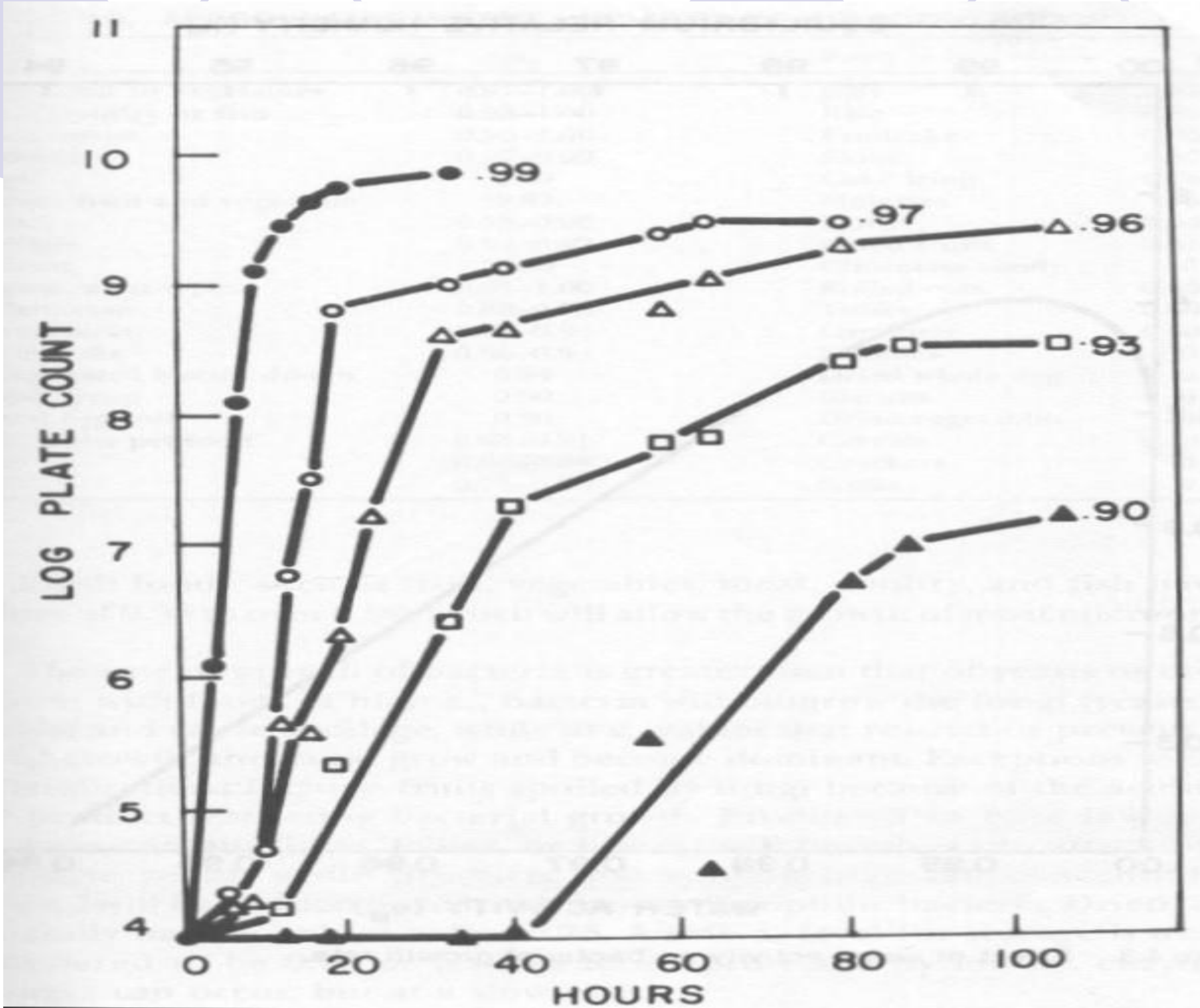

Fig. 4-2 Growth of *Staphylococcus aureus* at various levels of water activity.

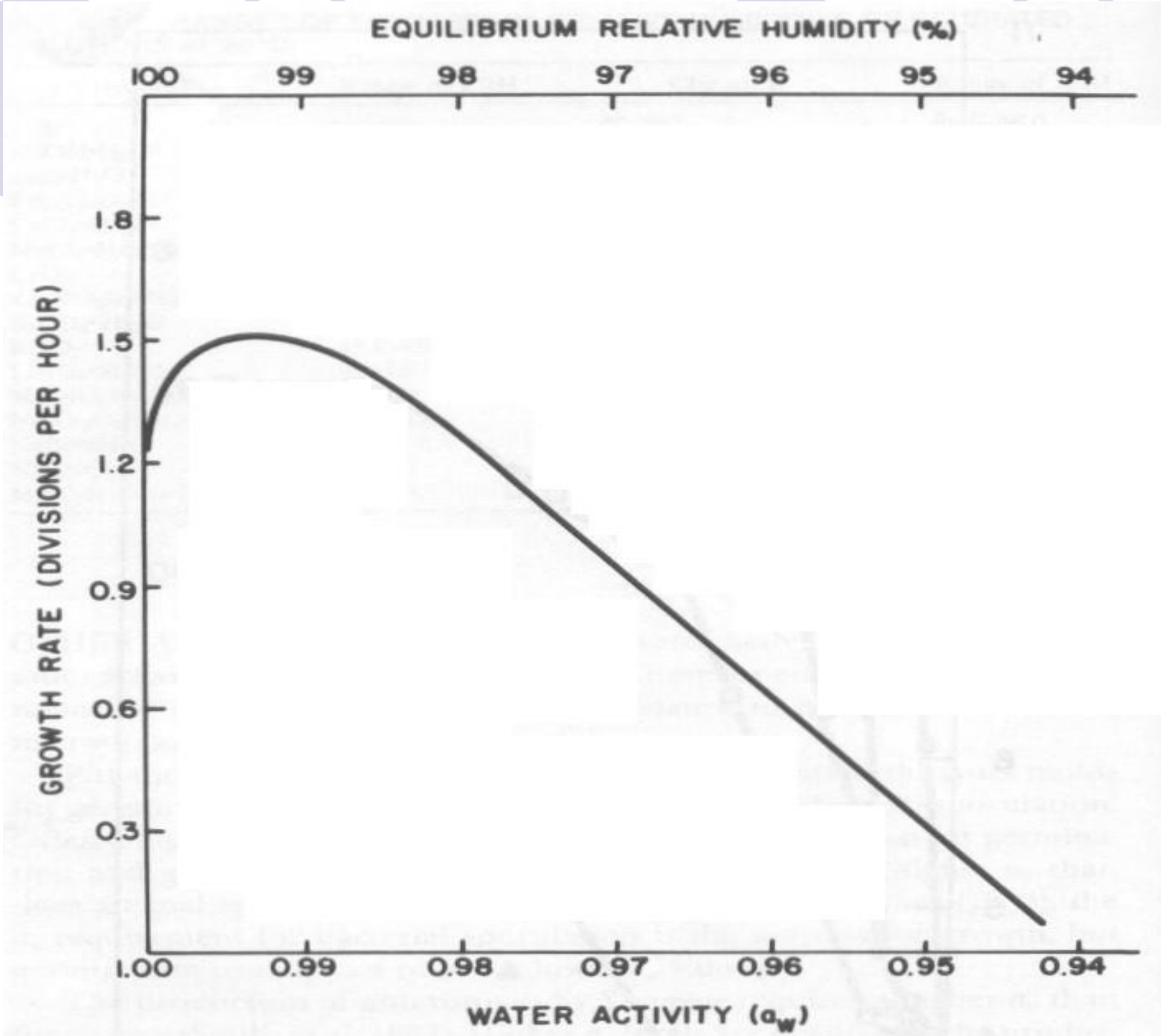

Fig. 4.3. Effect of water activity on bacterial growth rate.

Factors that affect Aw:

(1) Solute—sugar vs. Salt

	Min Aw	
	Glucose	salt
S. rouxii	0.62	0.81
V. parahaemolyticus	0.984	0.948

(2) Temperature—greatest tolerance Aw + opt. temp. for growth

Aspergillus ruber

Aw	℃
0.85	5
0.80	10
0.725	20
0.725	30
0.75	35
0.80	37

(3) pH

S. cerevisiae

pH 1-3 & 5-7	0 to 1 M $NaCl_{(aq)}$
pH 4 ~ 5	3 M $NaCl_{(aq)}$

(4) Oxygen

S. aureus	0.86	O_2
	0.90	no O_2

(5) Nutrients

Aspergillus	0.80	bran
repens	0.72	bran + starch
	0.70	bran + starch + egg albumin

(6) Sporulation

initiate germination at lower Aw than outgrowth

(7) Processing

heat resistance increase as Aw decrease.

(8) Microbial metabolism—increase Aw locally or decrease


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REGAL 康寶
SKIPPY
康寶
草莓果醬
Strawberry Jam
New

鷹牌®煉奶


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EAGLE BRAND
鷹牌煉奶
淨重:397公克


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鷹牌煉奶


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Intermediate moisture foods (IMF)

- Food with an Aw between 0.60-0.85

Dried fruits	0.60-0.75
sugars, syrups	0.60-0.75
some candies	0.60-0.65
cereals(some)	0.65-0.75
Honey	0.75
Jams	0.80-0.91
Sweetened condensed milk	0.83

- Now, IMF not only defined as Aw 0.60-0.85, but also by use of glycerol, glycol, sorbitol, sucrose, etc as humectant
- By use of sorbate and benzoate as fungistant

§Oxidation/Reduction and Microbial Growth

Classification of MO:

(1) Strict aerobe—use O_2 as e- acceptor in respiration

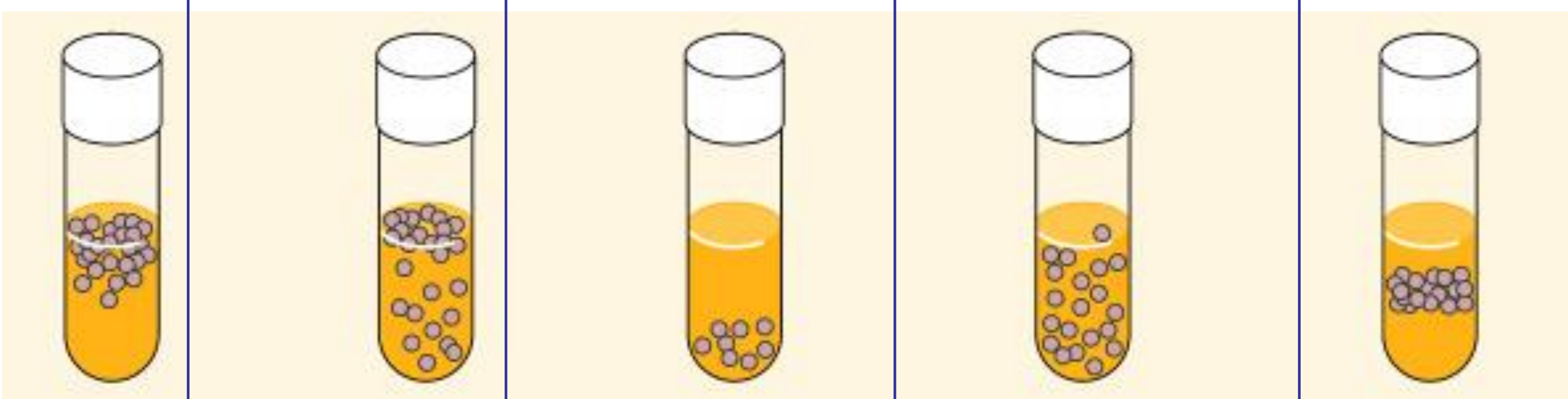
(2) Facultative anaerobe—use O_2 as aerobe, but can use NO_2, SO_4 etc. in absence of O_2, organic acids + wastes

(3) Obligate anaerobe—grow in absence of O_2, reducing agents, eg. Sulfite, thioglycolate, cysteine added to media

The Requirements for Growth: Chemical Requirements

- Oxygen (O_2)

obligate aerobes	Faultative anaerobes	Obligate anaerobes	Aerotolerant anaerobes	Microaerophiles



Most molds and yeasts are aerobes, a few are facultative. Some molds can grow at low O/R

P. roqueforti—blue cheese

Byssochlamys fulva—canned fruit

As MO grow, O/R decreased

O/R or Eh dependent on pH of substrate: Eh tends to more negative under progressively alkaline condition

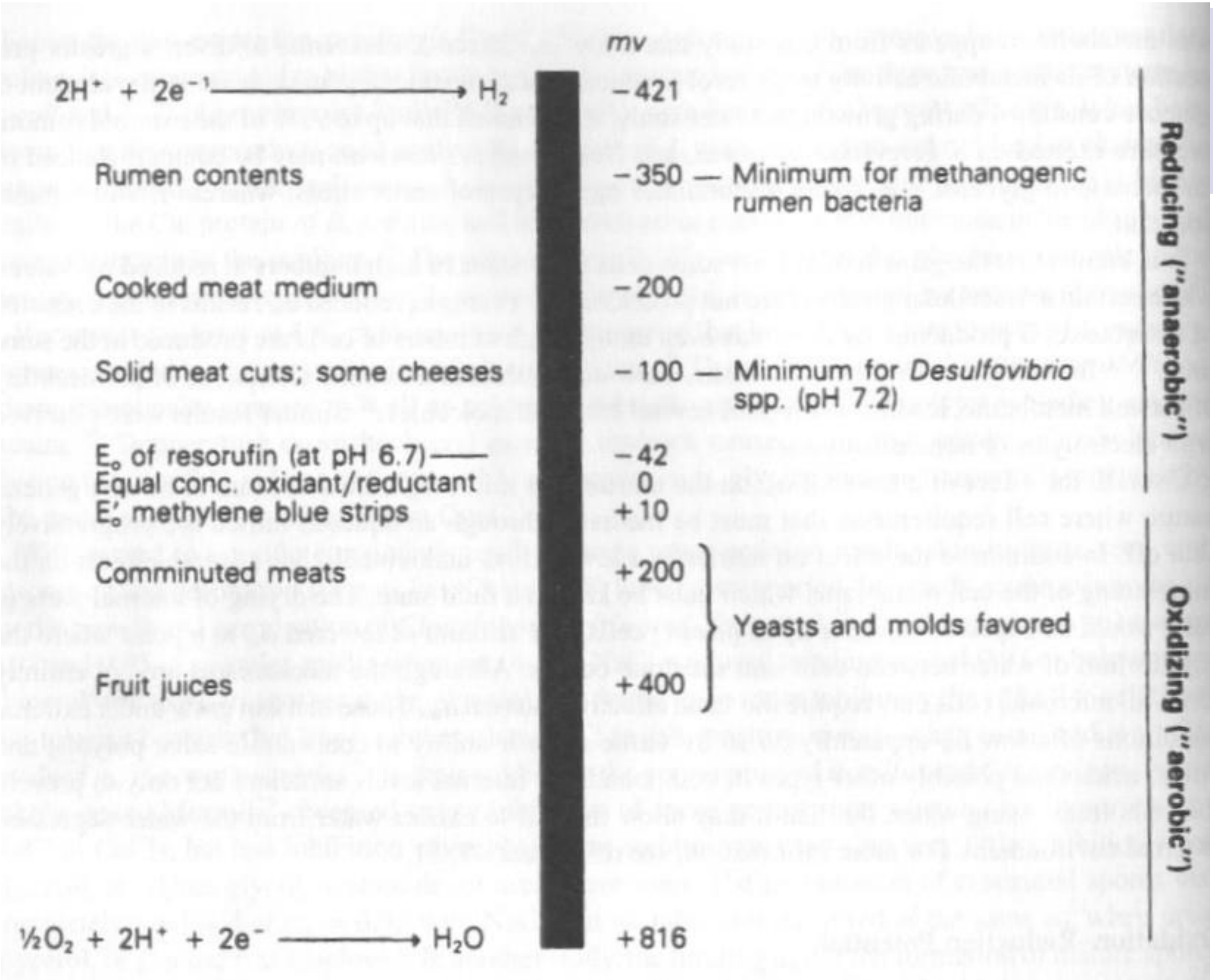


Fig. 3-3 Schematic representation of oxidation-reduction potentials relative to the growth of certain microorganisms.

§Nutrients and microbial growth

MO requires:

1. Water
2. Energy
3. Nitrogen
4. Vitamin & growth factors
5. Minerals

Least requirement to highest:

Molds, Yeast, G(-), G(+)

Energy

sugar, alcohol, amino acids, fats, starch, cellulose, organic acids, pectines, proteins

Nitrogen

amino acids, proteins, peptides & polypeptides, nucleotides, nitrate, ammonium etc.

Minerals

Na, K, Ca, Mg, P, S, Fe, Cu, Mn, Zn, Co

Vitamins

1. Usually B-vitamin required
2. G(+) bacteria = least synthetic
3. Pathogens = more fastidious than non- pathogen
4. Process can reduce vitamin content
5. Heat labile: thiamin, folate, pantothenate, vit.C
6. Light sensitive: riboflavin

§Inhibitory substances naturally found in foods

Animal products

1. Egg white
   - (1) lysozyme lyse cell wall of G(+)
   - (2) ovomucoid inhibit enzyme
   - (3) conalbumin chelate Fe, Cu, Zn
   - (4) avidin bind biotin
   - (5) riboflavin chelate cation

2.Milk

(1) leucocyte phagocized bact., esp. in mastitic milk

(2) Lactoperoxidase (LP) inactivate cell if thiocyanate and peroxide present

SCN^{-}+O2---(LP)----$SCNO^{-}$, $SCNO^{-2}$

(3) antibiotics

3. Meat, poultry, fish—lysozyme, antibiotics, some polypeptide and some polyamine combine with cell walls & disrupt cell function

Plant products

1. Fruit, vegetable, legumes—enzyme inhibitor, essential oil (disrupt enzyme & damage membrane), organic acids (pH effect), hydroxycinnamic acid derivatives, anthocyanin pigments (chelate metals or change redox potential), tannins (interfere with en. activity & alter cell permeability), phenolic compounds (leakage of cell constituents)

2. Spices—essential oils (eugenol(丁香酚)in cloves(丁香), cinnamon; allicin (大蒜素)in garlic; cinnamic acid(肉桂酸) in cinnamon; thymol(百里香酚)in sage and oregano(牛至) disrupt enzymes and damage membranes

Natural barrier problems

1. Eggs—cuticle (protein-like film on shell)—wash disrupt
2. Plant tissues-bruising-insect or bird damage
3. Grains—seed coat
4. Spices—oil & inhibitors

§Gases

1. Controlled atmosphere storage (CA)
2. CO_2 (fruits, vegetables, meats)
3. N_2 (meats)
4. Ethylene oxide (spices)
5. Propylene oxide (spices, meats, nut)
6. Sulfur dioxide (fruit juices, wines)
7. Ozone (water)

CO_2 effect:

$$CO_2 + H_2O \rightarrow H_2CO_3$$

$$H_2CO_3 \rightarrow HCO_3^- + H^+$$

$$HCO_3^- \rightarrow CO_3^- + H^+$$

inhibitory effect increase with temp decrease

G(-) more sensitive

§Temperature & microbial growth

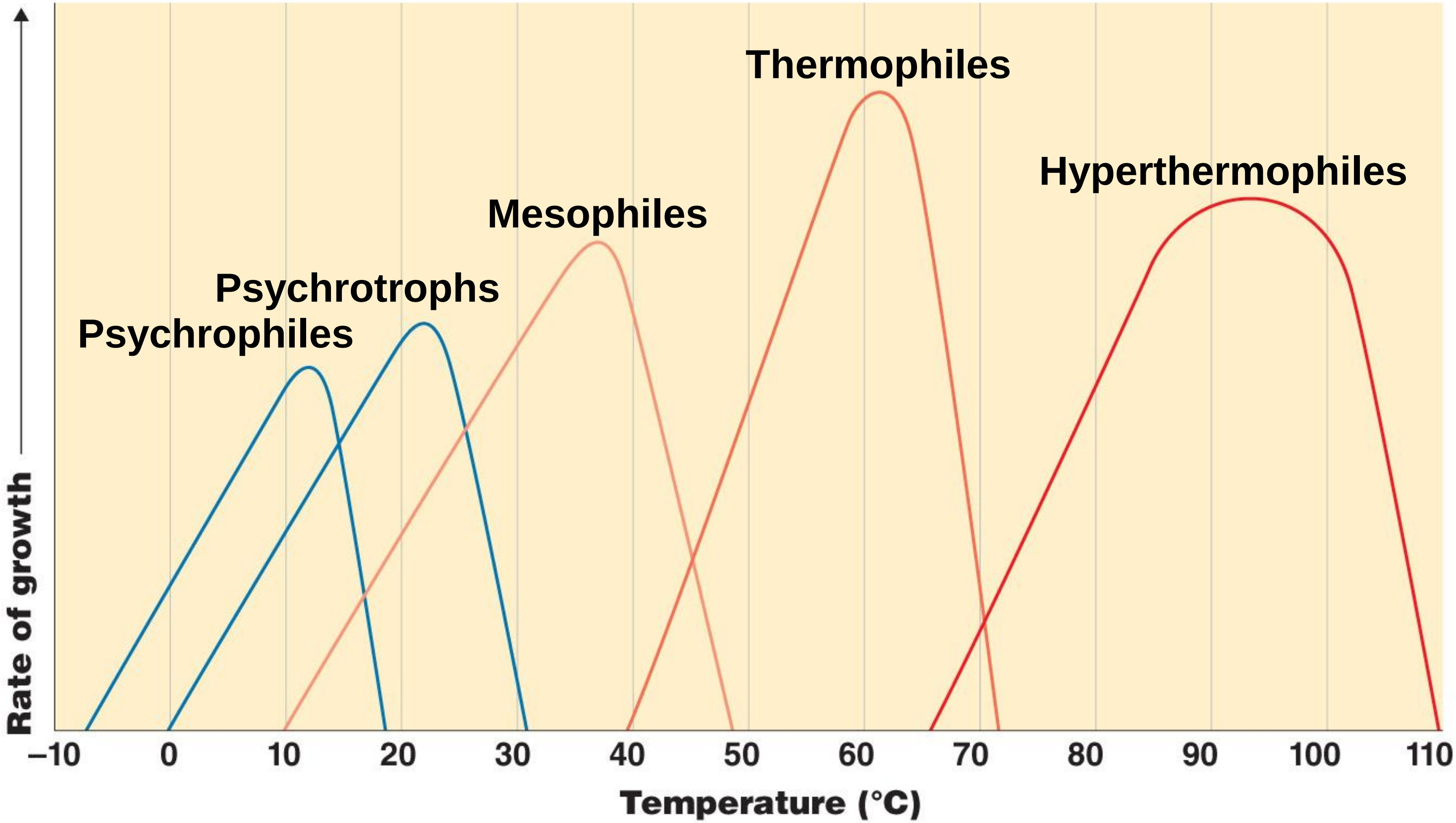
Key environmental factor affecting microbial growth

Range (-10 to 90°C)

	Temperature (°C)		
	Min	**Opt.**	**Max**
psychrophile	**-15**	**10**	**20**
psychrotroph	**-5**	**25**	**35**
mesophile	**10**	**30-40**	**45**
thermophile	**40**	**45-60**	**60-90**
obligate	**40**	**55-65**	**70-90**
facultative	**35**	**45-55**	**60-80**

Temp. retard or prevent microbial growth (refrigeration, freezing), or destroy MO (heat)

Figure 6.1 Typical growth rates of different types of microorganisms in response to temperature.



Psychrotrophs

1. Spoilage bact.—*Pseudomonas*, *Moraxella*, *Acinetobacter*, *Flavobacterium*, *Micrococcus*

2. Pathogenic bact.—*Yersinia enterocolitica*, *C. botulinum* type E, *B. cereus*, *A. hydrophila*, *Listeria monocytogenes*

3. Toxigenic &/or spoilage molds—*Mucor*, *Rhizopus*, *Penicillium*, *Aspergillus*, *Cladosporium* etc.

4. Spoilage yeasts—*Debaryomyces*, *Candida*, *Pichia*, *Sacchromyces* etc.

5. Generation time: 5-30 h depend on genus, species, strain

Mesophiles

1. Most spoilage or pathogenic bact., molds and yeasts

2. Generation time: 30 min or less for bact.

Thermophiles

1. Mainly G(+) bact., species of *Clostridium*, *Bacillus*, *Lactobacillus*, *Streptococcus*, etc.

2. Generation time: less than 30 min or sometimes greater (depend on temp.)

Figure 6.2 Food preservation temperatures.

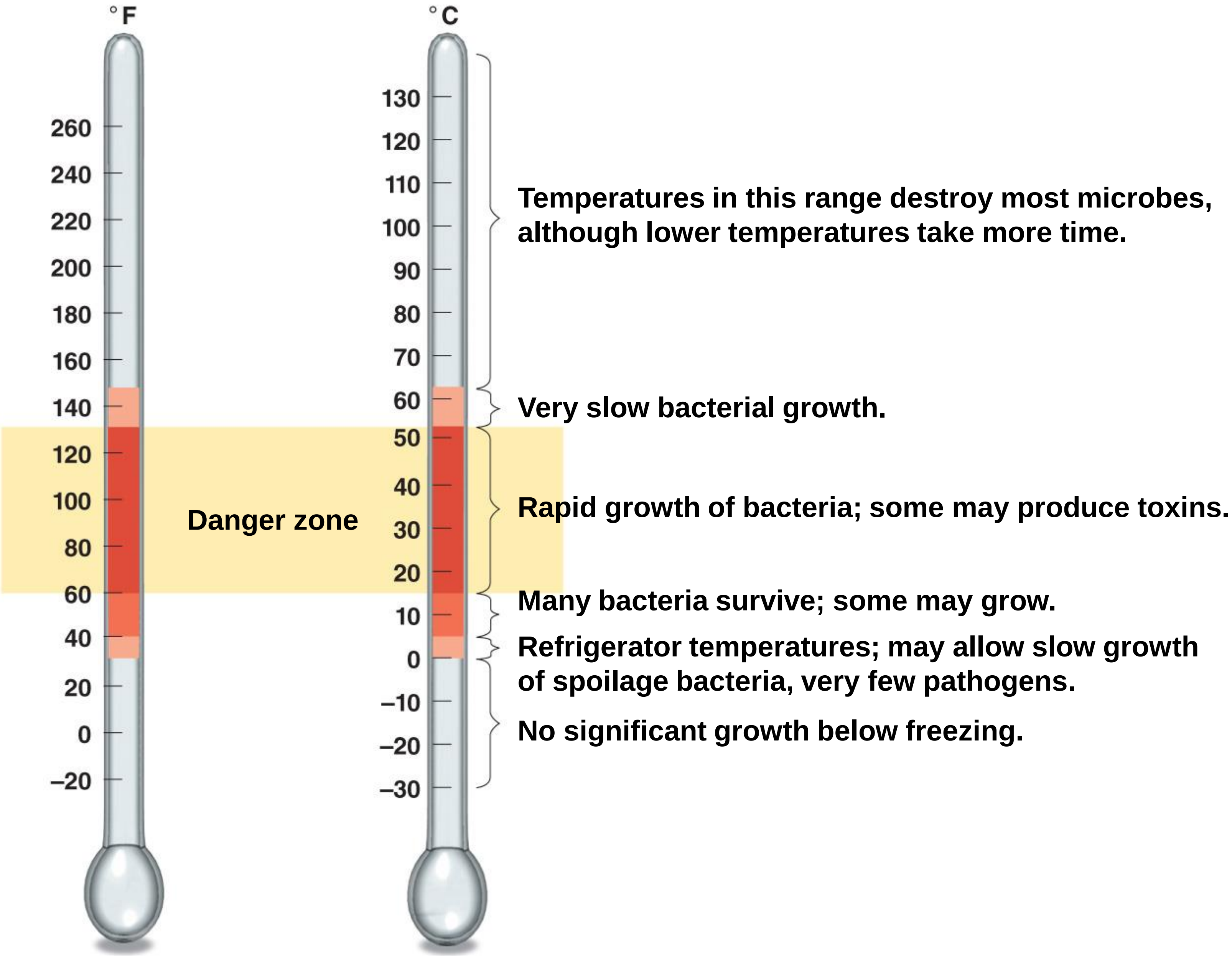
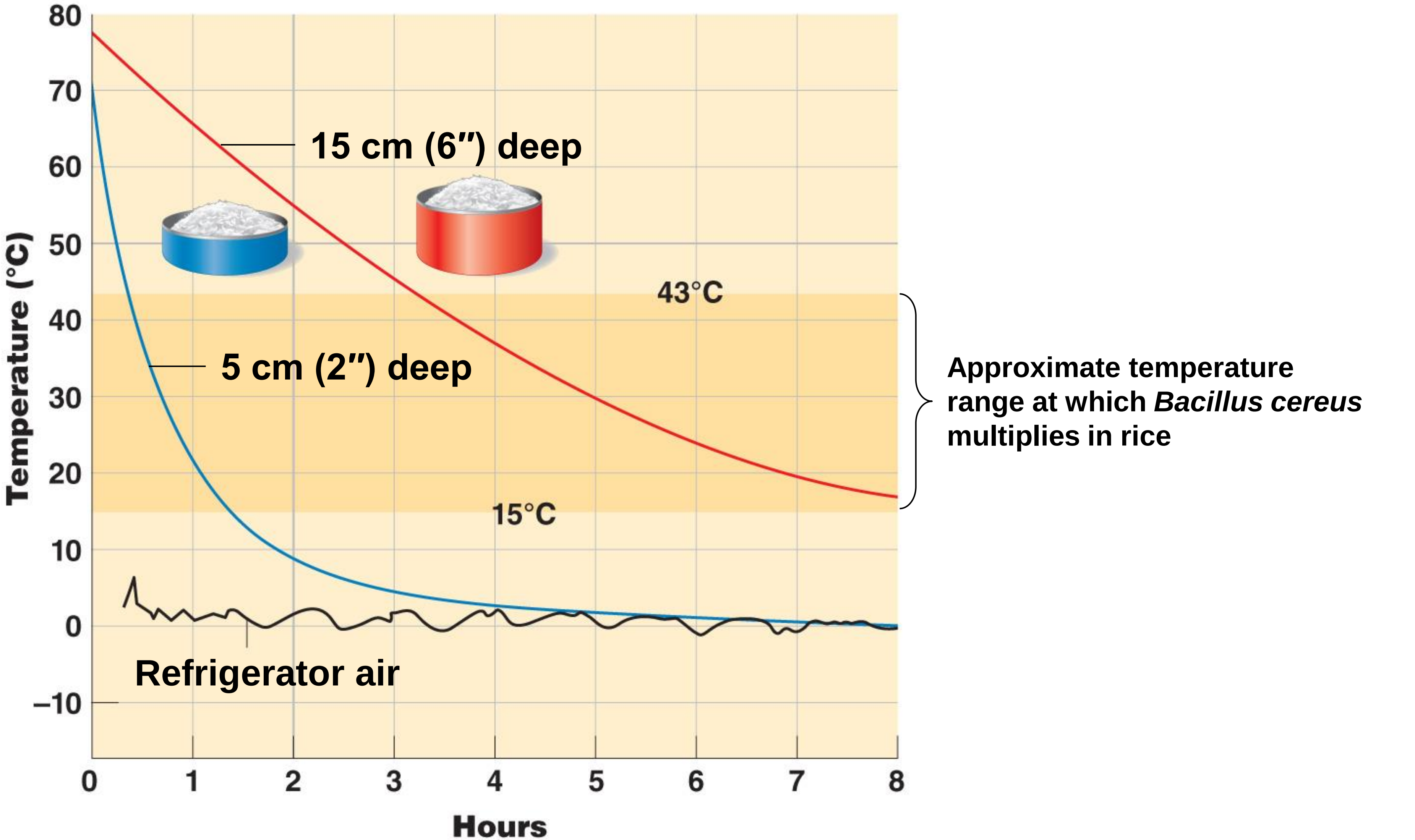


Figure 6.3 The effect of the amount of food on its cooling rate in a refrigerator and its chance of spoilage.



§Symbiosis—cooperative growth between MO

1.nutrient availability

Soy sauce fermentation: (*Aspergillus oryzae, A. soyae)*

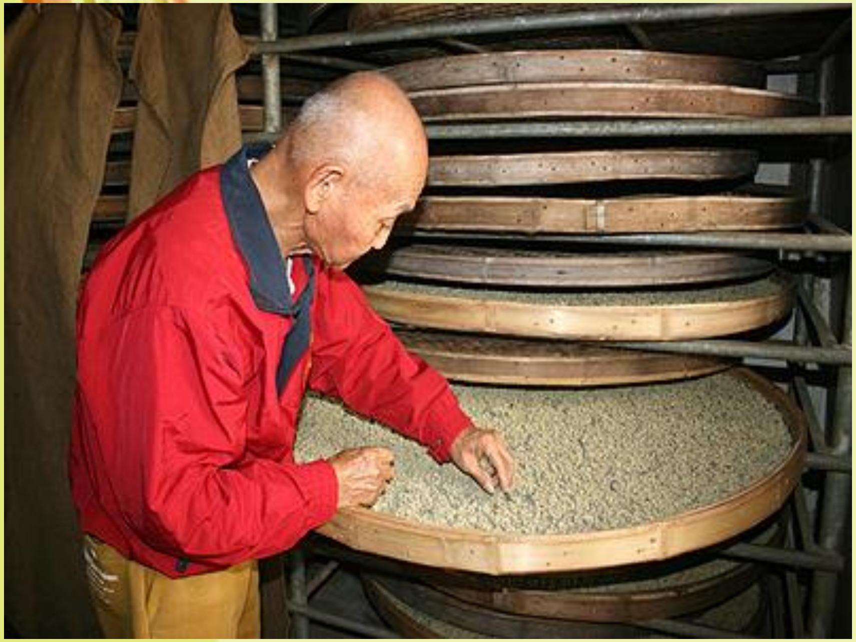
1.mold produce amylase & protease to degrade starch (from wheat) & protein (from soybean)

2.yeast ferment sugars→CO_2 + EtOH (*Saccharomyces rouxii)*

3.lactic bacteria use vitamins produced by molds & yeast and sugar→lactic acid (*Pediococcus halophilus, P. soyae)*

Microorganism involved in production of soy sauce

- *Aspergillus soyae, A. oryzae..*
- Lactic acid bacteria, yeasts



http://easy.sina.com.tw/info/stores/storeinfo_id-3840.html

Soy sauce

Steamed polished rice or wheat bran plus soybean flour inoculated with *Aspergillus oryzae* (80%) (mixed strains)

A. Soyae (20%)

Inoculated 3-5 days at 30°C

Seed (tane) koji

0.1-0.2%

Soybeans

Soaked in water for 12-15hr

↑ 2-2.5X wt.

Steamed or boiled* until soft ; cooled

10-13 psi, 1hr

Wheat

170-180°C Roasted

Crushed (cracked)

50% — Mixed — 50%

↓

Defatted soybean spray w/ H_2O to 130% of soybean wt. →steam 45 min

Mixed

Spread on trays or in fermentors ca.5 cm deep, placed in koji room

Incubated at 25-30°C, 2-3 days

Koji mixed with salt (17-19% final) water to form mash (moromi)

Lactic acid fermentation (*Pediococcus halophilus* or *P.soyae*) followed by yeast fermentation (*Saccharomyces rouxii*, *Torulopsis* sp.) for 2 months to 1 year or more

Pressed

Press cake → **Animal feed**

Liquid → **Pasteurized** → **Bottled** → **Soy sauce**

Fig. Flow diagram for manufacture of soy sauce (Koikuchi type).

Yogurt:

Streptococcus thermophilus, Lactobacillus bulgaricus

1:1 ratio = desirable, not all strain compatable

L. bulgaricus→11 amino acids→stimulate *S. thermophilus*

S. thermophilus→stimulatory factors (pyruvate, formic acid) for *L. bulgaricus*

（三）yogurt

Milk , Low fat milk ,
Skim milk , Nonfat dry milk

↓

standardize yogurt mix

milk fat	**1~2%**		**1~2%**
MSNF	**10.5%**	**or**	**12.5%**
Stabilizer	**0.7%**		

↓

Pasteurize at 95°C, 30 min

↓

Homogenize at 60°C, 1500 psi

↓

Cool to 43^{o}C

Starter culture

Mix in holding vat

Package in containers

Incubate at 43^{o}C till pH 4.5

Set type

culture vat,at 43^{o}C till pH 4.5

Cool to 25^{o}C

package in container

Stirred type

2.pH change

--mold on cheese surface→inc. pH & Aw→*S. aureus* grow

--sausage fermentation: *Pediococcus* lower pH below 5.0 →mold grow as spoilage MO

3.change redox

--*Pseudomonas* grow in fresh vacuum pkg meat and use oxygen →lactics, *E. coli*, etc. become dominant

--local oxygen tension reduced→change microflora of food

4.change Aw

--xeropilic mold on dried food→H_2O for less xerotolerant molds

--mold on cheese surfece (the same as pH)

5.elimination of antimicrobial agents

--some *Penicillium* use sorbic acid

--some *Debaryomyces* use nitrite

--oxidation of ethanol by *Acetobacter*

6.damage to structure

--molds→degrade outside of plant tissue (cellulose & pectin)→other mold & bacterium entry

§Antagonism: one microorganism injures, inhibits or kills another

1.competition for nutrients

--pseudomonas outcompete *Bacillus* when growing in refrigerated milk, meat. Fish, etc.

--bacteria outcompete molds & yeasts in fresh animal products

2.change in pH

--production acids→prevent many G(-) bacteria

3.formation of antimicrobial agents

--CO_2, H_2O_2, ethanol. NH_3, organic acids

--nisin-*Streptococcus* spp., lacticin B from *Lactobacillus acidophilus*

4.Redox change

--*S. aureus* growth = limit Micrococcus spp.

--anaerobe lower Eh of food more than aerobe

5.Bacterial lysis

--bacteriophage

Protective:

- Present no health risk
- Provide beneficial effects in the product
- Have no negative impact on sensory properties
- Serve as “indicators” under abuse conditions

Lactic acid bacteria:

Produce antibiotics, H_2O_2, pH↓, diacetyl, nutrient depletion, bacteriocins or bacteriocin-like factor. →Inhibit other bacteria esp. pathogens. = **Lactic antagonism (乳酸菌拮抗作用)**

GRAS: generally recognized as safe