Food Fermentation

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食品發酵(或發酵工業)課題

1) 新產品新資源之開發
2) 高產率菌種之篩選，現有菌種之改良
3) 提高原料利用率，降低加工原料成本
4) 生產技術之改良，縮短發酵週期
5) 自動化，減少人力
6) 改進產物分離與純化技術，並提高產物回收率
7) 廢棄物之有效處理及資源回收，副產物之開發

食品發酵

- 種類

1) 乳品發酵：cheese (乾酪), yogurt (優格), buttermilk, sour cream (酸奶油).
2) 肉品發酵：sausage (香腸)
3) 醋酸發酵
4) 酒精發酵：wine, beer (啤酒), brandy (白蘭地酒).
5) 黃豆品發酵：soy sauce (醬油), miso, tempeh, sufu.
6) 蔬果類發酵：pickle (黃瓜), sauerkraut (泡菜), olive (橄欖).
7) 水產品發酵

Def.: Process in which M.O. alter the flavor and/or rheological properties of food to induce desirable characteristics

Advantages:

- greater nutrient absorption
- adaptation
- variety
- cheaper

(A) Natural fermentation: rely on the M.O. activities which are already naturally existent in food.

(B) Controlled fermentation: Add desirable M.O. to raw materials

Starter culture: M.O. which are added to raw materials to get desirable products.

Advantages:

1. Quicker
2. Cheaper
3. Consistancy

Starter culture requirement

- utilize nutrient
- unable to utilize metabolic end products
- able to bring about desired changes in the conditions we choose
- metabolically stable
- resistant to bacteriophages
- should not produce objectionable characteristics

Vegetable fermentation

- Sauerkraut
- Pickles
- Olives

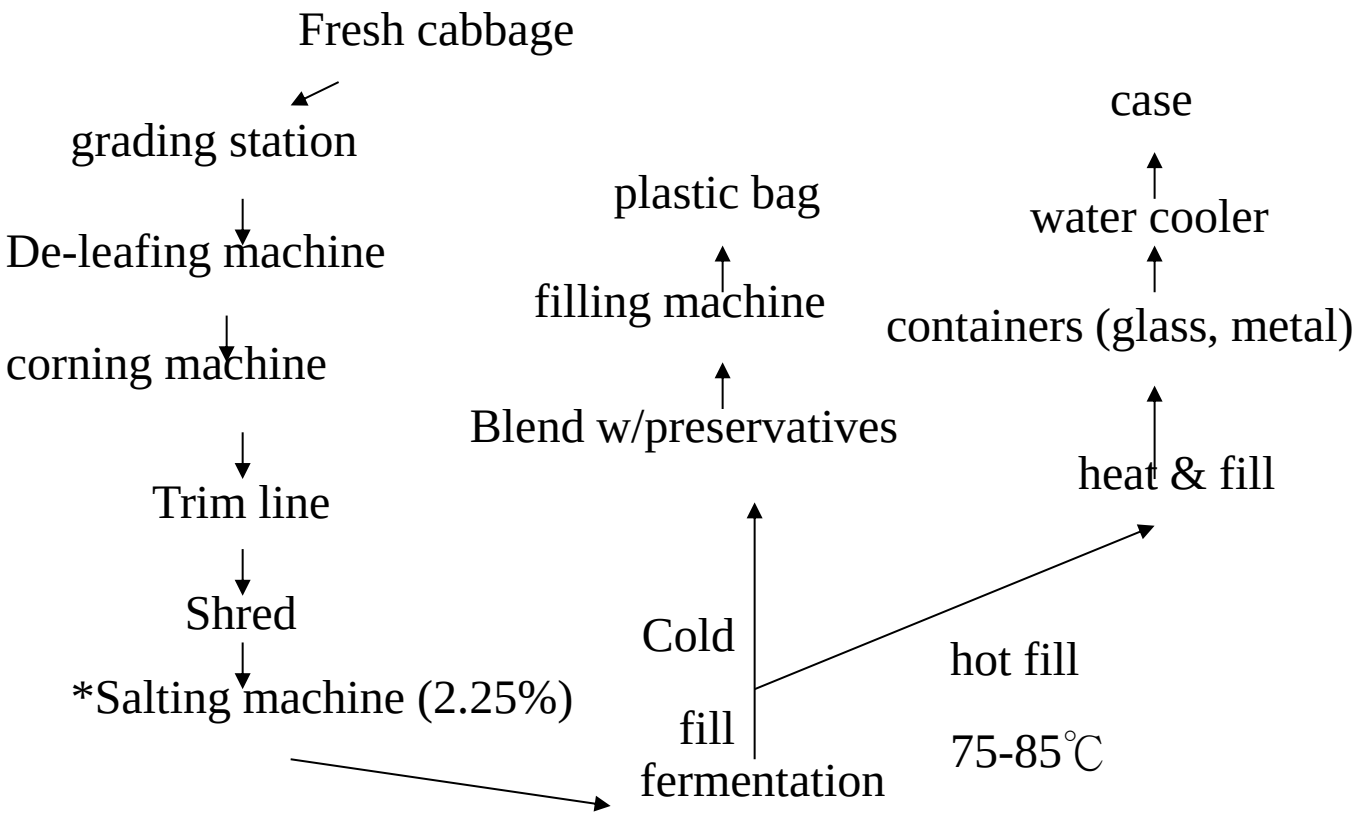
4 basic steps common in each product

1. NaCl brine soln.
2. Immersion
3. Lactic acid bact.
4. Lower pH by production of acid

Product	Salt (%)	Method	Culture	% Acid	pH	Dominant Lact. flora
Olive	5-10	Immersion (can add 3% lactic acid)	*L. plantarum*	0.7-1.0	3.8	Homo or mixed
Pickle	6.6	Immersion -Add NaAC -N2 purge	*L. plantarum* *Pediococcus cerevisiae*	0.6	3.8	Homo
Sauerkraut	2.25	Immersion -N2 purge	**Natural** *Leu. mesenteroides* *L. plantarum* *L. Brevis* *P. cerevisiae* *St. faecalis*	1.75-2.5	3.5	mixied

Sauerkraut

Natural fermentation



Function of salt

- Extract nutrients from plant cell for microbial growth
- Inhibit undesirable microorganisms
- Maintain optimum texture
- Flavor ingredient in product

Salt: 2%< salt <3% (2.25%)

Automatic salting machine: 0.9-1.1 Kg salt/45.5 Kg shredded cabbage

Sauerkraut

- Fermentation

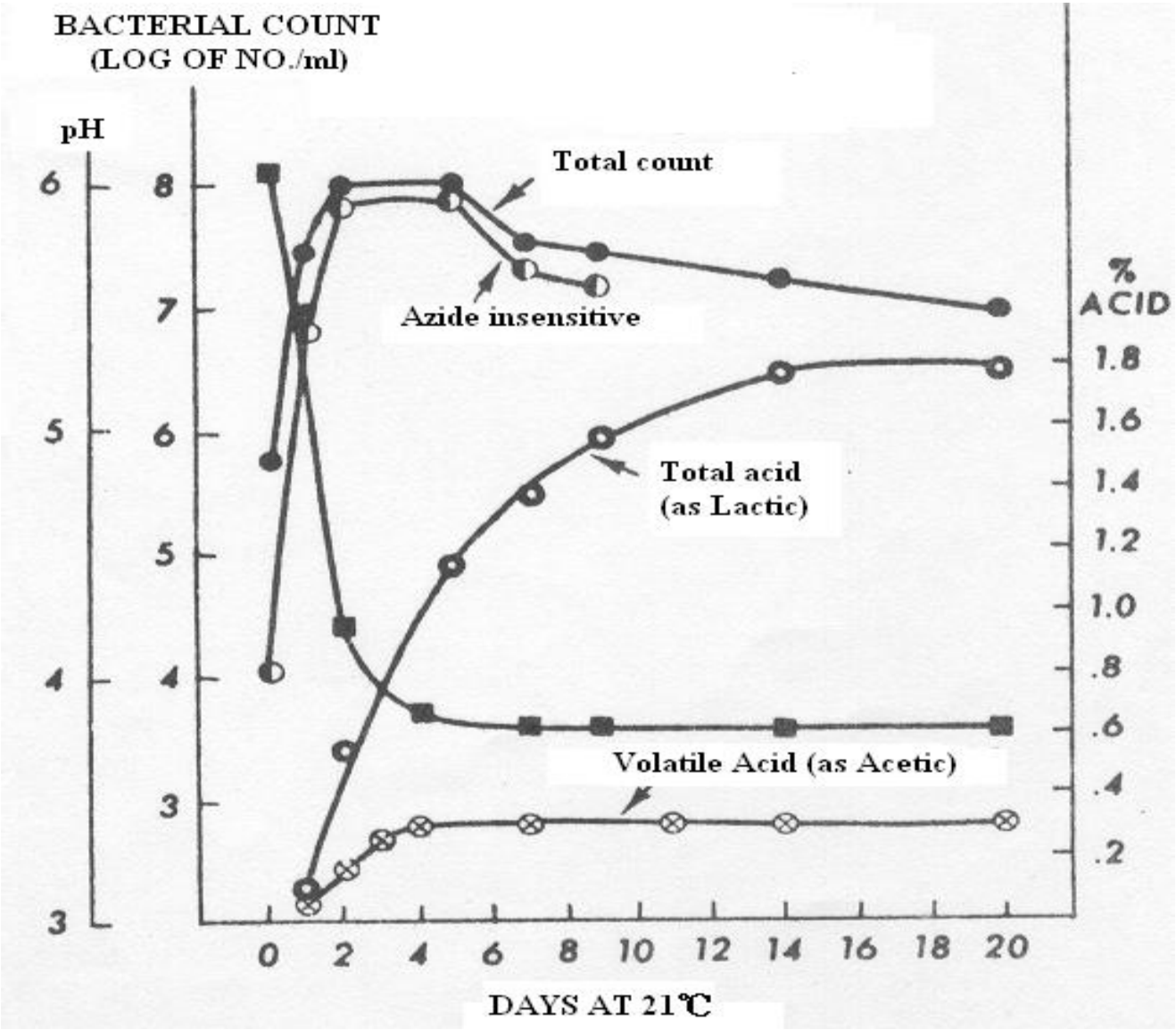
-natural ferment

-controlled by anaerobiosis, salt. & temp.

-original: fresh cabbage contains many G(+) & G(-) bact.

Eg. Coliform, pseudomonas, enterobacter.
acinetobacter, mold & yeast,
lactics only about 0.01-1% population

anaerobic→inhibit pseudomonas, acinetobacter. mold & yeast etc.


BACTERIAL COUNT
(LOG OF NO./ml)
pH
6
5
4
3
8
7
6
5
4
3
Total count
Azide insensitive
Total acid
(as Lactic)
Volatile Acid (as Acetic)
% ACID
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
0
2
4
6
8
10
12
14
16
18
20
DAYS AT 21℃

Sauerkraut

Initiate by *Leuconostoc mesenteroides* (hetero)

- produce lactate, acetate, CO_2, mannitol, esters, EtOH.
  Acid→0.7～1.0% then self-limiting ↑ (Fructose)
- short generation time
- reduced system (CO_2)
- if salt < 2.25%, glucose→accumulate→dextran→slime
  (prefer to use lactose than glucose)

Leuconostoc mesenteroides

Function:

(1).quickly dropdown pH to limit undesirable micro & En. that may soften cabbage

(2).CO_2 replace air & create anaerobic condition to prevent oxidation of ascorbic acid & darkening natural color & cabbage

(3).CO_2 stimulate growth of many lactics

- Acid 1.52% (best at 1.7%)
- Use dextran & mannitol (bitter taste)

---Followed by (1)*Lactobacillus plantarum* (2)*Streptococcus faecalis*

(3)*Pediococcus cerevisiae* (Homo, more acid tolerant); and *L. brevis* (hetero)

If initial salt content greater than 3.5%, or fermentation temperature greater than 30oC, there is homolactics dominant only, without heterolactics function.

good mixture of cultures:

30% hetero cocci

38% hetero rods

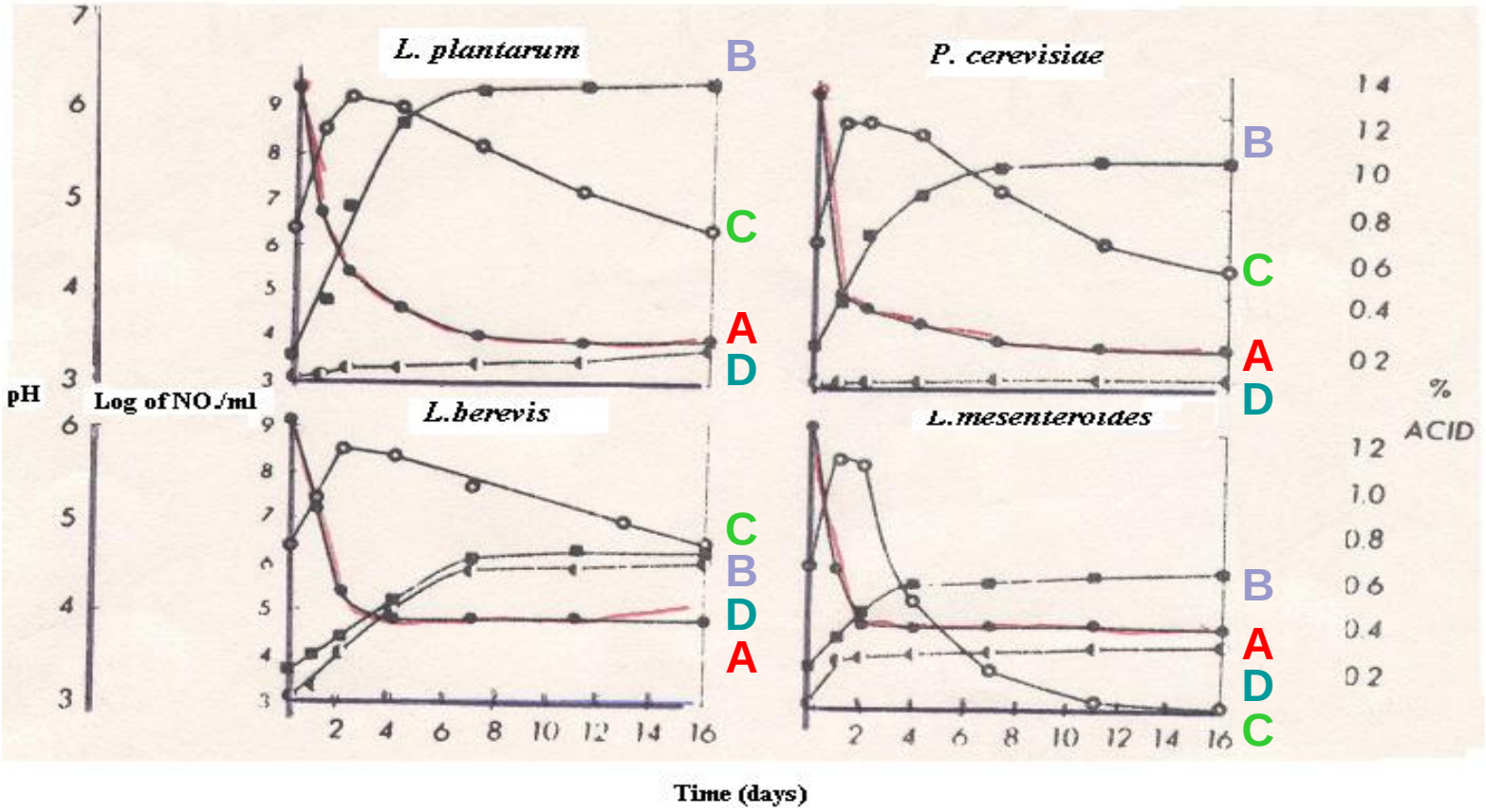
30% homo rods

2% homo cocci

-balance between homo & htero→aromo & flavor profiles

-pure culture sauerkraut still not acceptable yet

Pure culture in filter-sterilized cabbage juice



A. **pH**

B. **Percent non-volatile acidity (lactic acid)**

C. **Viable cell count**

D. **Perecent volatile acidity (acetic acid)**

1. *L. plantarum*
2. *P. cerevisiae*
3. *L. brevis*
4. *L. mesenteroides*

1.If *L. plantarum* or *P. cerevisiae* (homo)

⟶ dull in taste lack body

2.*L. brevis* (hetero) ⟶ product harsh, vinegar-like,

Acetate: lactate =1:1

normal: 1:4~1:6

3.*L. mesonteroides* (hetero) ⟶ do not get enough acidity

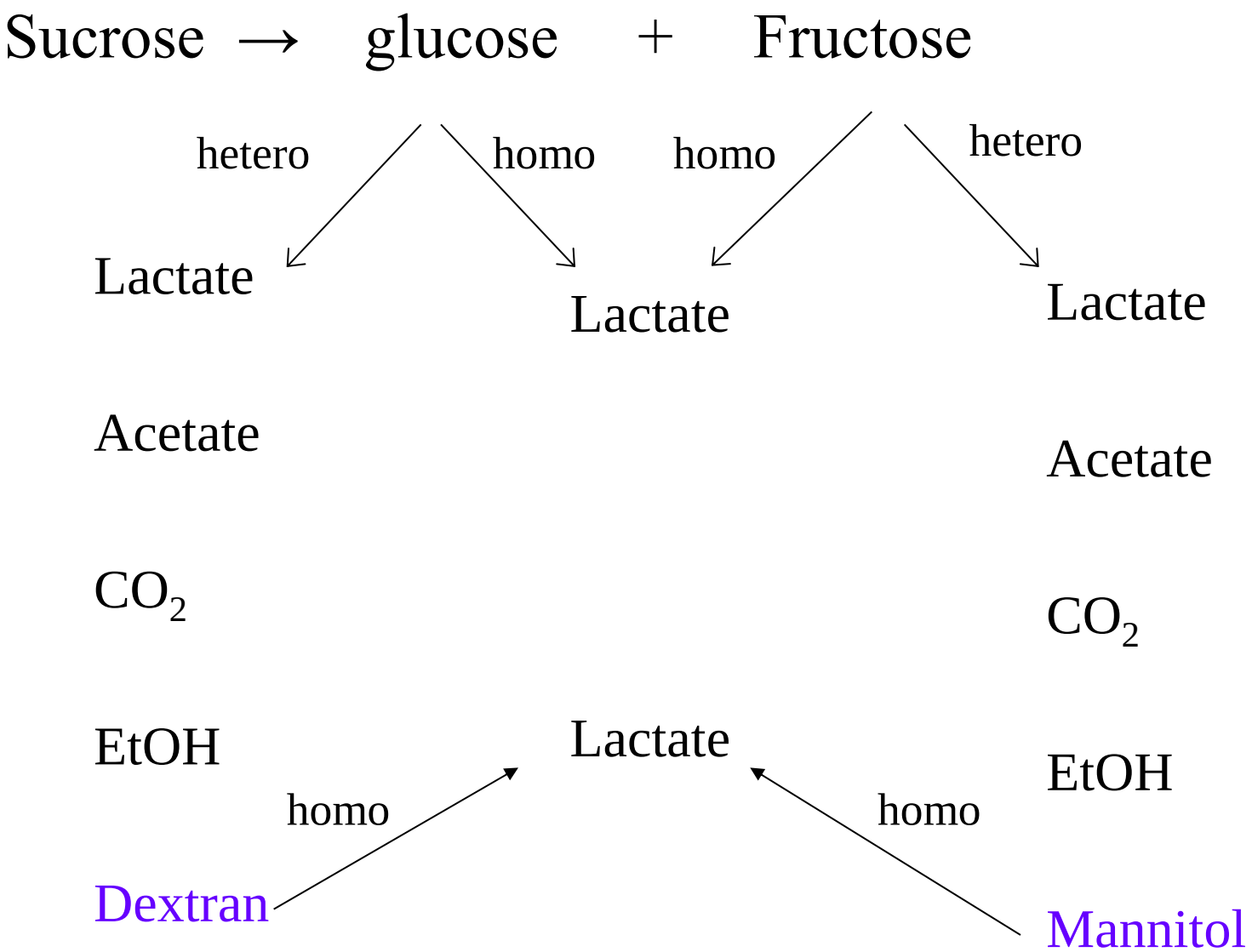
acetate:lactate =1:3

Sauerkraut

Whole fermentation divided by 3 phase:

1. reduction undesirable micro due to acidity, salt, & anaerobic condition
2. heterofermentative dominant early
3. terminated by homolactics

Pathway:


Sucrose → glucose + Fructose
hetero
homo
homo
hetero
Lactate
Acetate
CO_2
EtOH
Lactate
Lactate
Acetate
CO_2
EtOH
homo
homo
Lactate
Dextran
Mannitol

Components of cabbage /sauerkraut

- sulfur compound→masked or altered by fermentation
- glycosides→hydroloyzed→free glucose→acid, alc. Gas
- acetylcholine (from choline by *L. plantarum*)
  functions at nerve endings
- lactic acid→DL. L(+) used in gluconeogenesis
  D(-) excreted or oxidized in liver
  L. mesenteroides D(-), rest DL mix
- Lipid (minor) →glycerol + FFA
  40FFA identified in product

Spoilage of product

1) Abnormal fermentation (wrong microorganisms)

a. Temp.: normal 18-21℃(65℉best)

*if temp. ≤ 10℃, most lactics grow slowly.
sequential micro. not happened. only *L. mesenteroides*. grow

*when Temp↑to 18℃, micro grow rapidly.
production rate↑8x, sequential pattern established

*when temp > 30℃

(1)↑homo growth, hetero~ function↓

(2) loss ascorbic content, texture degradation & flavor browning

*special sauerkraut production

In winter (temp 7.5) ferment, only *L. men* grow

acidity 0.4% (10 day), after winter, temp↑, homo grow 0.8-0.9% (1 month)

total ferment 6 months

b. Salt:

(1)affect texture: 1.8%→soft texture, 2.5%→ fibrous & tough texture

(2)affect micro flora: at 3.5% heterolactics acid product↓90%

homolactics become dominant

2) Film yeast & mold on surface: aerobic condition

-utilize lactate or acetate →pH↑

-if lactics don't ferment all sugar, yeast fermentation of 2nd fermentation will go, gas production

-growth of *Rhodotorula* (yeast) →pink color (pink kraut)
L. brevis in alkaline can also produce red color, but in acid not

After fermentation

- only 1% freshly provided to consumer
- most packed

hot fill method:	heat to 77~82℃, 3min→fill(74-79℃)→cool (38-43℃)	
cold fill (chemical preservatives) :	0.1% Na-benzoate K-metabisulfite	
shell life	plastic bag at 5℃	8~12 months
	Can	18~30 months
	Glass	12 months

Pickle fermentation

Natural fermentation

cucumber

brine (5-8% NaCl) → tank → head → fermentation

Microflora: Coliform (Enterobacter), yeast & lactics.

Controled by [salt], temp.

& type of micro on cucumber & in brine.

Main lactics: **L. plantarum* (most salt-tolerant),

L. brevis, *P. cerevisiae*, *Leu. mesenteroides* not become dominant.

due to high salt

No microbial sequence as sauerkraut.

In exp. at 6-8℃ 4-6% NaCl, *Leu. mesenteroides*

may become dominant. (maybe due to low temp counters the effect of high salt)

Problem

- ”bloating”-due to gas production (coliform & yeast)
- or fail of processing

  if lactics can’t produce acid & drop pH quickly (usually within 2 days~4 days) to suppress undesirable micro., undesirable micro will outgrowth.

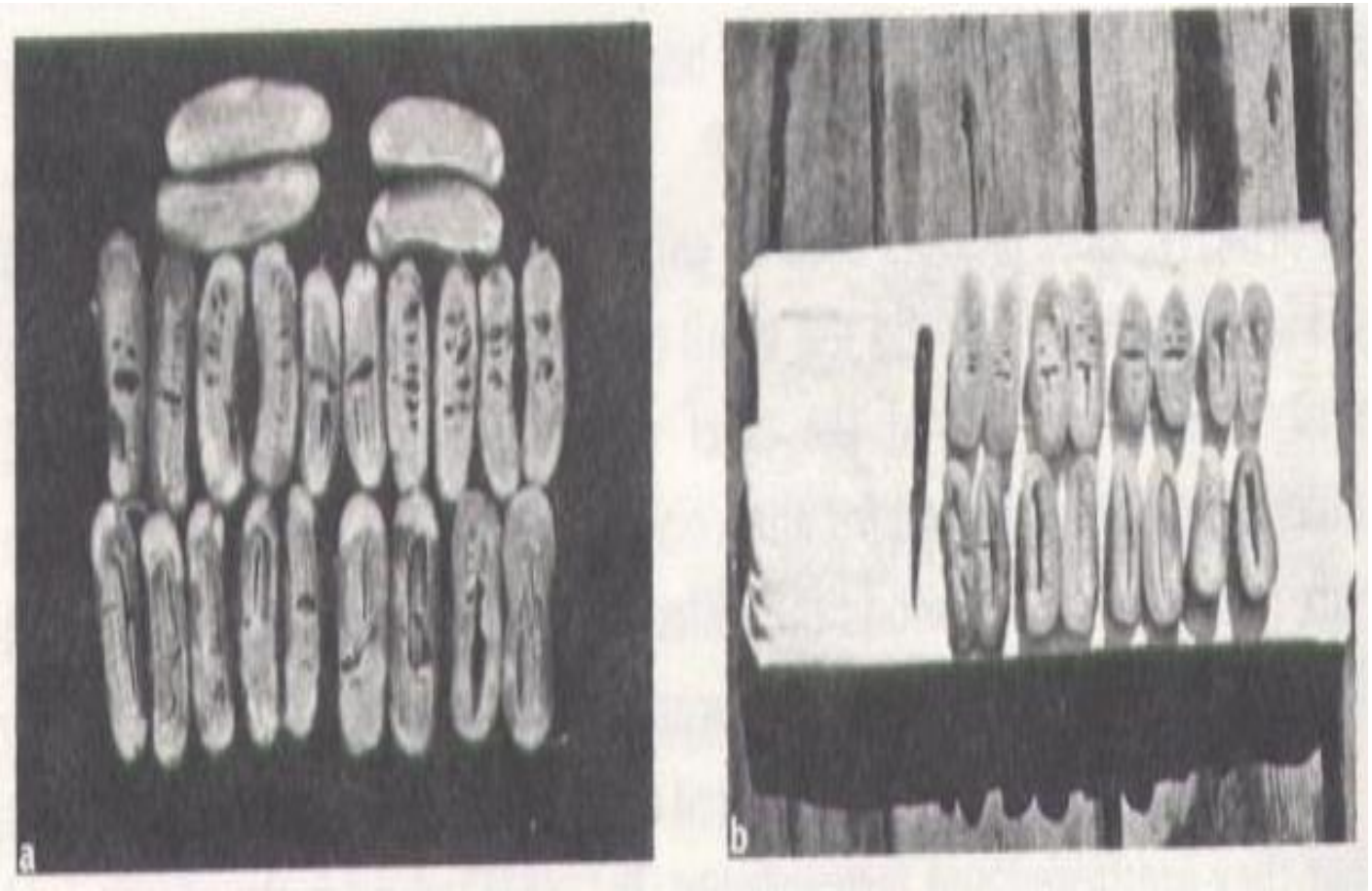


Fig. Gaseous spoilage of pickles. (a) Severe lens and balloon bloaters in partially cured brine-stock; (b) bloaters in small sized brine-stock pickles (about 1 in or less in diameter).

Pickle fermentation

Controlled fermentation

cucumber → thoroughly washed

thoroughly washed → cover head (level of brine above head)

Brine solution (acidify with HAC) → tank → cover head (level of brine above head)

cover head (level of brine above head) → N_2 –purge (remove CO_2)

N_2 –purge (remove CO_2) → add Na-Ac、Ca-Ac (buffering

add Na-Ac、Ca-Ac (buffering → ensure to use of fermentable

ensure to use of fermentable → culture

culture → 3 week- 1 mon.,

culture → activity: 0.5~1.2%,

culture → pH: 3.3~3.5

ensure to use of fermentable → sugar completely by lactics)

ferment (75~85°F)

3 week- 1 mon.,

activity: 0.5~1.2%,

pH: 3.3~3.5

add Na-Ac、Ca-Ac (buffering

ensure to use of fermentable

sugar completely by lactics)

Controlled fermentation

- culture: *L. plantarum* and (or) *P cerevisiae*
  homo~: No gas prodution
  grow well in 6~8%salt & 75-85°F
  P. cerevisiae must not inhibit *L. plantarum*

- buffer calcium salt:
  1.permit lower use of NaCl
  2.texture retention
  3.allow the fermentation to continue to complete & eliminate 2nd ferment

- *CO_2 production not only from $(CHO)_n$ but also from malic acid: *L. plantarum* convert malic acid to lactic acid & CO2 ∴If *L. plantarum* not transfer of (malate → lactate)→ better

Defects

1.”bloater” or “floater”- due to presence of CO_2

source of CO_2

(1)hetero~ lactics→CO_2

gas production bact. →CO_2

yeast→CO_2

(2)homo~ lactics also produce CO_2 by decarboxylation of AA. & org. acid (malate, citrate)

∴decarboxylase (-) strain

(3)respiration of cucumber→CO_2

In lab. unheated cucumber →respiration→CO_2	enough for floater
L. plantarum→small amount CO_2	

2.Softening→due to pectinolytic or cellulolytic en.

Source: bacteria, mold & cucumber

"slippery" (skin become soft) pickle

"mushy" (deeper layer soft) pickle

Bacillus, *Achromobacter*, *Aeromonas*, *Enterobacter*, *Erwinia*→pectinolytic action pH 5.0-5.5 in 5~8% salt

yeast *Rhodotorula*,*Saccharomyces*→polygalacturonase but when [NaCl] >5%, growth were inhibited

Some film yeast *Debaryomyces*, *Candida*, *Endomycopsis* & *Zygosaccharomyces* grow on surface & utilize lactic acid → ↑pH → undesirable Micro growth

Many molds-*Alternaria*, *Aspergillus*, *Cladosporium*, produce pectinase (pectinesterase, polygalacturonase, pectin-trans-eliminase)

Endogenous enzyme* in cucumber
located in seed cavity
↑20x during maturation

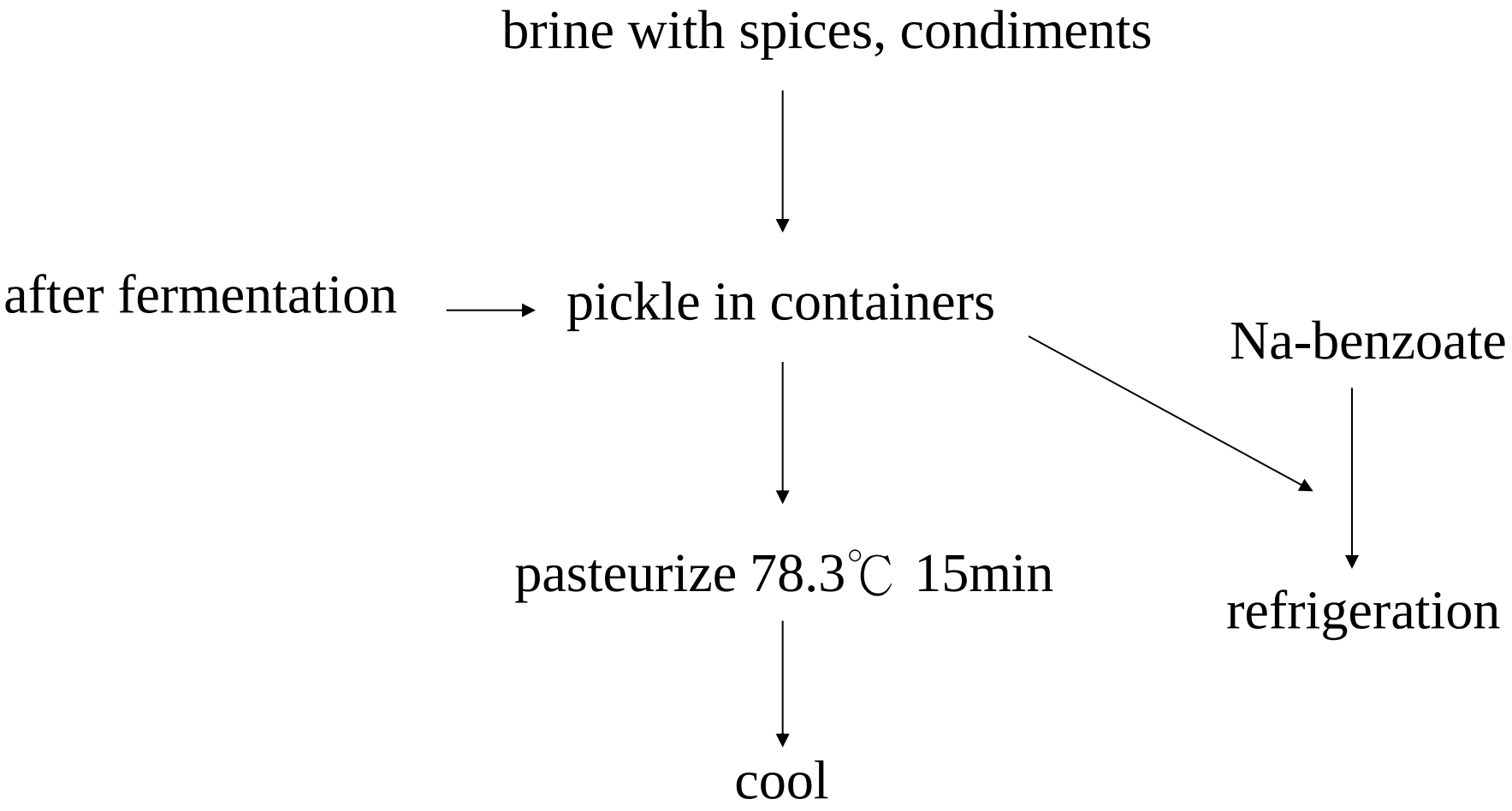
- *mold grow & secrete softening en. to cucumber flower which adhering to cucumber on which they developed.

∴reduced by replacing original cover brine with new brine after 36h. in controlled fermentation

⇒problem of disposal of brines

⇒recycle the brine by heating to inactivate en. & M.O. followed by precipitation

Preservation



Olive

Fresh composition of Olive pulp

H_2O	50-75%
lipid	6-30%
Reducing sugar	2-6%
Non-R.S.	0.1-0.3%
Crude protein	1-3%
fiber	1-4%
ash	0.6-1%

Major sugar: Glu, Fru, Suc ($\Rightarrow$decrease)
minor: xylose rhamnose mannitol 0.5~1% of fresh pulp

phenolic compd: 1~3%, expressed as tannic acid

oleuropein-bitter compd.

org. acid & salt: 0.5~1%

In fermentation, various types:

Storage	no lye treatment
Sicilian type	
Spanish type	lye treatment
Greek type	high salt brine too strong for lactics to ferment acetic fermentation by acid-forming, salt-tolerant yeast

Spanish-type fermentation

(1) 0.9~1.25% (w/v) NaOH for 10-14 h to break down oleuropein (break down product inhibit growth of lactics)

caution: lye damage

fruit lye-treated during early harvest prone to “blister”:

lye solution too warm→gaseous fermentation start

(Coliform) →gas production →skin separated from flesh

If skin remain intake, gas accumulated underneath→”fish-eye” spoilage

∴Cool the lye & olive

lye penetrate 1/2~3/4 from skin to pit except Barouni olive, which need to pit to prevent discoloration problem due to presence of leuco-anthocyanin in the fruit

(2) remove lye by washing & leaching with H_2O extended for 24 h with 3~6 h changed water (about 10 changes, now, about 3~4 changes)

if washing duration too short →lye in pickle

if washing too frequent →nutrient loss
(sugar loss)

(3) brine

add lactic acid to neutralize alkalinity but most add excess to lower pH to 4.5-5.0, brine conc. depend on variety of olive, eg Sevillano easy salt shrived (>5%) than Manzanilla∴1st add 15~25° salt. (4~5%) then gradually increase to 30-32° (7~8%), Manzanilla directly add 40°.

due to loss of sugar during lye & washing, sugar may be added.

(4) Fermentation

Microflora different between low salt fermentation of *Sevillano* and high salt fermentation of Manzanilla

For all varieties of high salt ferment only *L. plantarum* be assured in common

For low salt ferment:

Leu. mesenteroides & *Streptococcus faecalis* always found

L. mesenteroides disappear within 3~4 weeks

L. plantarum: dominate end of 2nd stage & final

L. brevis: found at end of 2nd stage &↑during final stage

Pedicocci-rare

Control of Spanish-type fermentation

-Commonly use 75-86°F at present

1930 Cruess recommend 70-75°F for↑acid production w/o impairing quality

1943 Vaughn max acid Production for

L. mesenteroides	(86°F)
L. plantarum	
L. brevis	93.2°F

in nature, wait for elimination of undesirable M.O. then incubated for ferment.

It takes 1~3 weeks.

If

(1) Acidify initial cover brine

(2) use starter

(3) add sugar

Can accelerate fermentation

Now (1) (2) (3) plus (4) anaerobe = succeed

Spoilage

- gassy deterioration
- malodorous fermentation
- tissue softening
  1. Gas accumulation
  2. malodorous fermentation
- “Zapatera” spoilage
- softening spoilage

Tissue softening

(1) Gas accumulation ⇒separation of skin from flesh

⇒gas pocket

main bact.: Coliform

others:

Bacillus polymyxa, B. macerans	Also cause tissue softening
Aeromonas liquefaciens	
Saccharomyces	
Hansenula→gas forming, no pectinolytic	

(2) malodorous fermentation

- butyric acid fermentation

  -anaerobic, spore-forming butyric acid bact.

  eg. *Clostridium butyricum*

  -happened at low salt fermentation (Sevillano), never happened at high salt

  esp. 5% NaCl, pH ≥4.5 easy happen.

- Hydrogen sulfide fermentation

  -H_2S formation

  -if Fe^{2+}→Black

  Zn^{2+}→white

  -*Desulfovibrio aestuarii* (halophilic)

  control by reduce pH below 5.5

  Salt conc. have no effect due to high tolerance of salt

"Zapatera" spoilage

-may be found in all olive growing area

-start from "cheesy" to faecal-like odor

-happened at the product whose pH around 4.5 (pH not reach 4.5, or a little less)

-At beginning of spoilage, pH↑, acidity↓

-acidic constituent different

Normal: acetic, lactic, succinic acid

Zapater: acetic, lactic, succinic, formic, propionic, butyric, valeric, isovaleric, caproic, caprylic acid

∴⇒may be related w/ *Clostridium*

(*C. befermentans*)

(*C. sporogenes*)

but *Cl. propionicum* not found (produce proionic acid)

∴other bact. involved.

Propionibacterium (*P. pentosaceum*)

(*P. zeae*)

use lactate→propionic acid⇒"cheesy" odor

-control by direct lactic ferment until pH 4 (or 3.8) or below

Softening spoilage

-mechanical (physical, by frosting)
-lye soln
-microbial
G(+) bact.: *Bacillus*, *Clostridium*
G(-) bact.
mold: *Fusarium*, *Geotrichum*, *Penicillium*, *Paecilomyces*
Yeast: oxidative pink yeast *Rhodotorula*
fermenting yeast *Saccharomyces*

opt. pH for bact. pectinolytic en: 8.0-8.5$\Rightarrow$∴bact not main reason

main function from mold & yeast, controlled by anaerobic condition

Beer

Ingredients: malt, adjuncts, water, hops, yeast…others

west Germany: only malt + water + hops + yeast

malt: from barley which is sprouted

adjunct: unmalted cereals-corn, rice, wheat

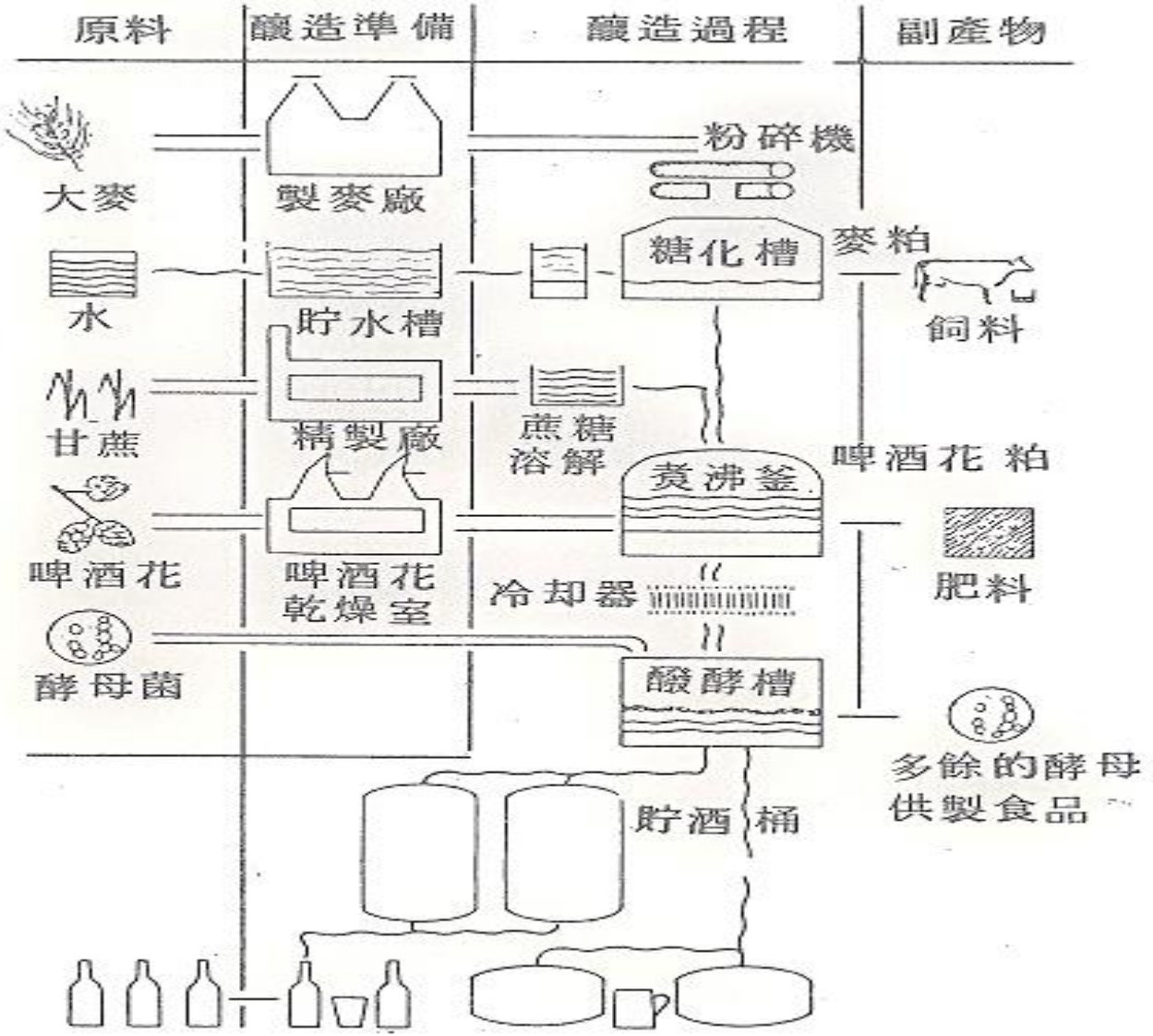


圖 1.1 啤酒釀造之程序圖示

Process

- Malting (製麥)
- Mashing
- Kettle boil & Hop addition (麥汁煮沸)
- Wort clarification, cooling & aeration
- Fermentation
- Aging (後熟)

Malting

Purpose: (1) enzyme formation
(2)flavor (aroma) & color improvement
(3)除去不良物(phenol compounds；可使beer呈澀味)

(a)steeping 浸麥→大麥吸水→產生en & 高釀造抽出物(Brewer's extract)

麥粒→浸水6-8小時→週期性灑水 or 間斷浸水
→42-46% H_2O content (13-16℃)
勿長期浸泡水中

(b) germination發芽

白點麥粒→生長→en→分解大分子，有利Brewer's extract製得

Modification轉變	Poorly modified	不良
	Under modified	不足
	Well modified	良好
	Over modified	過度
Temp 19℃ RH: 95-100%		

Gebberellic acid-胚所產生Hormone，送到糊粉層(Aleurone layer)，促進α-amylase, limit dextrinase, endo-β-1,4, 1,3-glucanase & endoprotease生產

∴可噴GA (0.05~0.25 mg/kg malt，由真菌 *Gibberella fujiburoi*生產)

β-amylase & carboxypeptidase不受GA作用

於未發芽時為latent,發芽↑activation.

germination時，Glucanase分解cell wall之β-glucan，使prot可被protease作用

∴for well modified malt	**75%β-glucan→degrade**
	40% protein→soluble
	5~10% starch→degrade

β-glucanase & protease can't work well at 65℃ in the mashing

傳統製麥室，深度僅20 cm，人為定期攪拌，今自動通風製麥設備，深度1.5 m

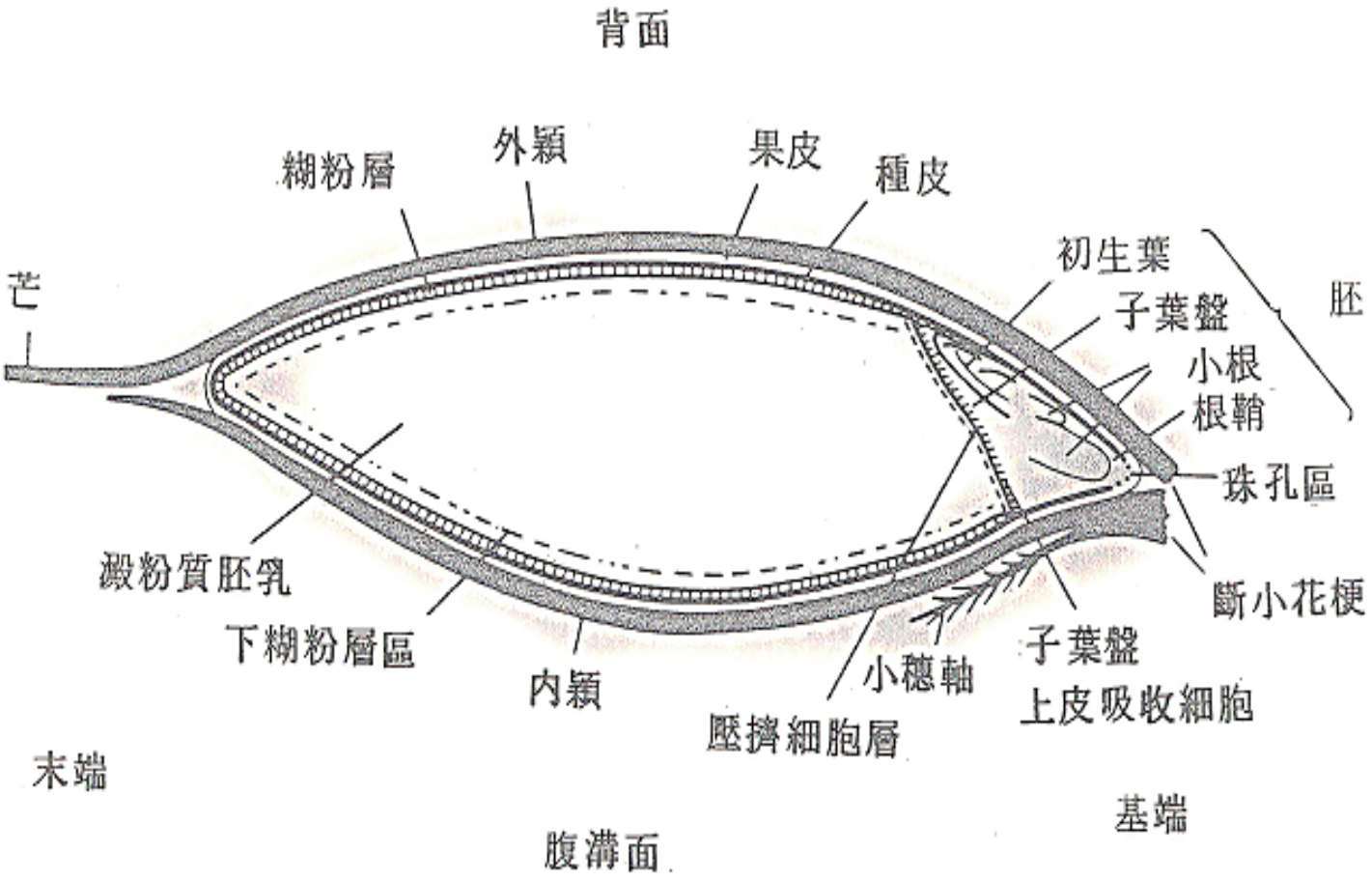


圖 2.2 大麥穀粒的縱切面構造圖

(c) kilning焙乾

Function:(1)stop growth & enzyme function
(2)reduce H2O% to 3~5% that favor for storage
(3)flavor & color

initial air temp 50~70℃→麥芽30-35℃，about 10 hrs，品溫60~70℃，此時 H2O% 44%→5-8%， final 80℃，也有100℃，2 hrs (ale.)

at initial, temp 30-35℃，protease & amylase active→Maillard rxn→color intensity for beer

高溫烤焙過長(70-80℃)，會破壞dimetyl sulphide (DMS)生成力，
100℃焙烤者→beer無DMS
低溫者→DMS高

in general, DMS (20-80μg/l) in beer乃重要風味物
S-methylmethioine or Dimethylsulphoxide

yeast ↓

DMS

problem:天然氣 as heating source→NO_2 + R-NH_2

form malt

↓

nitrosamine

∴heating source: low NO_2 gas, oil.

Mashing

Ground malt + H_2O

Ground:適當，太細→過濾困難，太大→抽出物少，可加Adjunct

Starch —(α&β-amylase)→ small molecular.
Glucose, maltose, maltotriose, maltotetrose, & dextrin

75%可發酵性糖，留有非發酵性糖(dextrin)

pH=5.4	starch en. 5.3-5.4 protease 4.6-5.0
If pH<4.5 amylase↓，滯流麥醪；if pH太高→酚抽出物高，澀味	

糖化期間，90-95% starch become soluble
35-40% protein become soluble
而可溶proteins (α-amino nitrogen)之60%以上在製麥時已存在，mashing時溶出而已。

Temp: from 50℃ to 78℃ (inactivate en.) or 65℃一種temp.

Method: Infusion 浸出法 & Decoction 煮法

Infusion: 65℃單一溫度，H_2O : malt =2.5 : 1

Decoction: Temp variant, H_2O : malt = 4.0-5.0 : 1

35-50℃: protease

50-65℃: amylase 35℃→54℃→65℃→70~78℃

煮沸1/3，加入原醪，使增溫

Double-mash system:煮沸副原料→加入malt 醪，使↑Temp.

Temp-programmed system:

麥芽 + Adjunct + H_2O —△→steam injection.↑temp

(1) 50℃ keep 40 min；(2) 65℃, 35 min；(3) 65℃, 45 min；(4) 75℃, 20 min

糖化→過濾→麥汁(wort)

↓

麥粕(spent grains)

20~22% total solid.
11~21% crude prot. (dry weight)
18~19% crude fiber
6~7% ether extract
4~4.5% ash

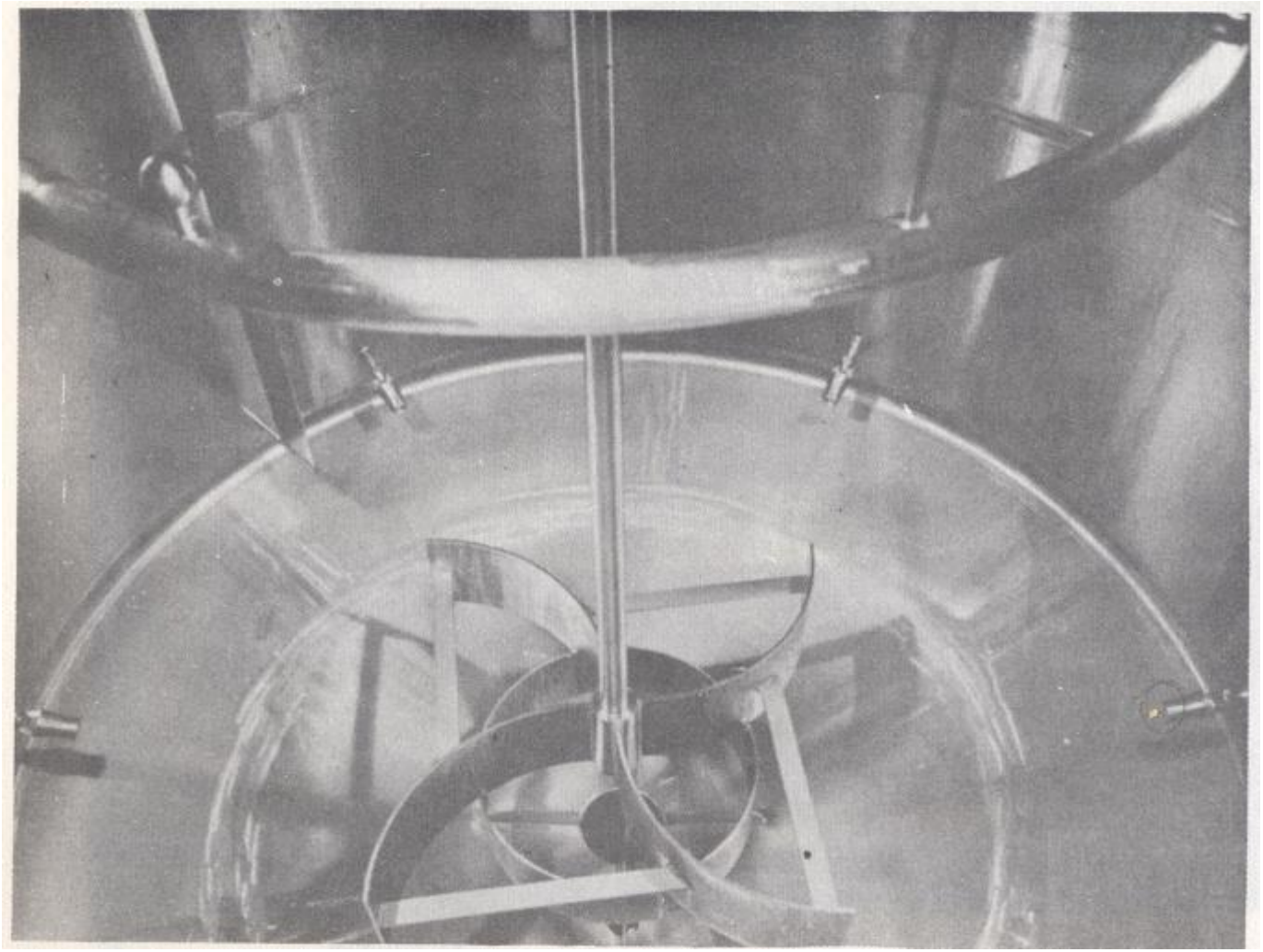
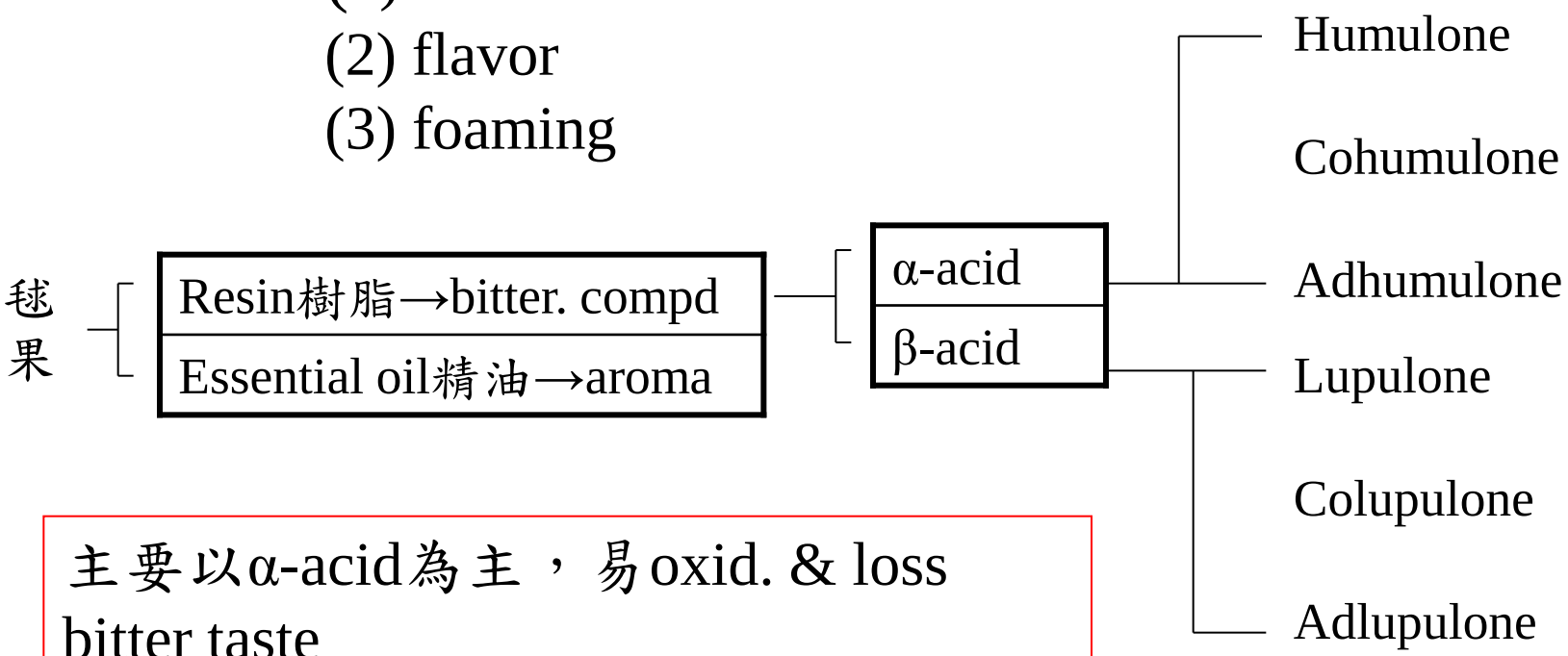


圖 5.13 穀物煮沸釜內部，使用直接蒸氣噴嘴

啤酒花 Hop (*Humulus lupulus*)

function: (1) bacteriostatic effect
(2) flavor
(3) foaming



主要以α-acid為主，易oxid. & loss bitter taste

∴(1)aroid contact w/air
(2)low temp storage 4-10℃
(3)extraction

hop extract:早期以二氯乙烯和二氯甲烷萃取→產品黑色，
solvent residues，德國禁用，近年CO2(l) extract,
orange color, high α-acid

α-acid &β-acid 之H_2O & wort 溶解性不佳

α-acid:(1) oxidation & polymerization in storage

(2) isomerization during boiling

β-acid: oxidation but no isomerization during boiling

α-acid —isomerization→ storage bitter taste, important for brewing

α-acid
β-acid
Hexane
soluble

—oxidation→ part of product become hexane-insoluble

Kettle boil & Hop addition

wort + Hop→boil for 1~2h 若time↑bitter↓

若high pressure (150℃)，98 sec.

效果 = 100℃，90 min.

Function:

1.stop en. Rxn

2.wort sterilization

3.flocculant precipitate-“hot break” hot trub

protein + tannins + metal + hop resin→ppt

4.eliminate undesirable aroma

5.color development→maillard rxn. Polyphenol oxidation, caramerization

6.hops are extracted (α,β-acid, polyphenols, essential oil,α-acid→isomerization

7.partial concentration, 5~15% volume↓

在煮沸前，亦有加糖→high gravity brewing method

高濃度(alc)之beer $\xrightarrow{\text{dilute}}$ normal beer

or 加入carrageenan or Furcellarin，或其抽出物Irish moss (為帶 “-”之polysaccharide)有助於prot. & polyphenol之沉澱.

啤酒花利用率

(1)低conc.之wort，有較高Hop利用率(萃取物多)

∴低conc. Wort + Hop (多量)-boil ＼
高conc. Wort + Hop (少量)-boil ／ mix

(2)高M. wt prot%高之wort→hop extract↓

∴添加prot % low之Adjunct→↑利用率

(3)yeast可吸附Hop，If culture medium w/o hop，之yeast吸較多Hop

(4)fermentation時，foaming，含大量苦味物，若除去泡沫，lose bitter compd.

∴closed system keep foam.

(5)In fermentation，pH↓，low Temp. & alc formation→prot-tannin ppt. (called cold break，可吸附苦味物)

Wort clarification, cooling & aeration

- 麥汁澄清，冷卻，通氣
- 迅速冷卻10-15℃
- 通氣，增加dissolved oxygen (8-10 mg/l)

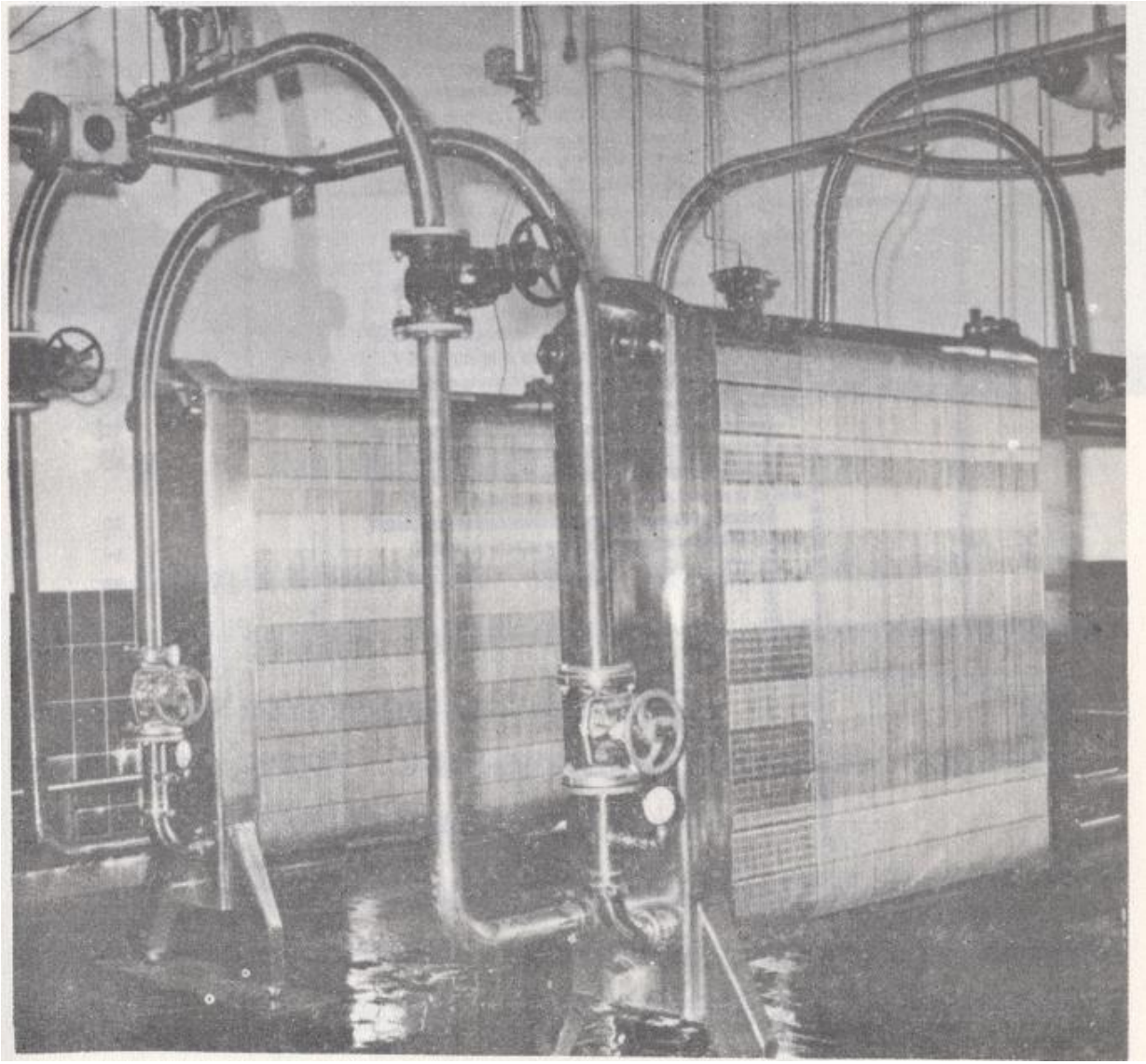


圖 7.7 板式冷卻器 (Plate type cooler)

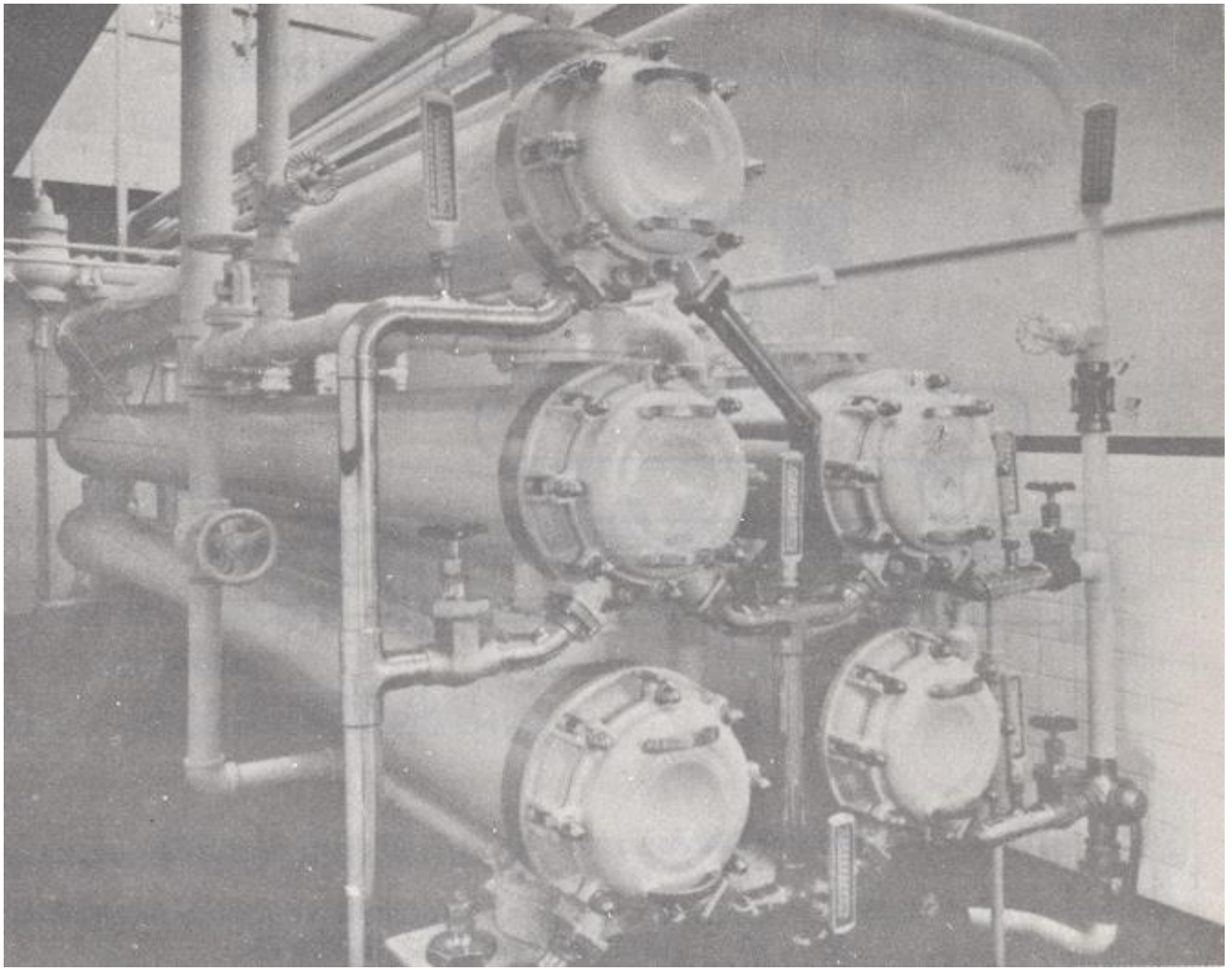


圖 7.9 管殼式麥汁冷卻器 (Shell and tube wort cooler)

Fermentation

2 types of beer

Ale- *S. cerevisiae*

Lager- *S. carlsbergenesis* (uvarum)

不同性質：

(1) Ale yeast-Top yeast

Ferment時，表層厚層酵母泡沫(yeast head)→刮除，供下批之cultured yeast

Lager yeast- bottom yeast

(2) Ale fermentation temp: 12-18℃

Lager 8-12℃

S. cerevisiae opt. 40℃, low temp (<5℃) not grow

S. uvarum opt. 30-33℃, low temp (0℃) still grow

(3) *S. uvarum*可利用melibiose (gal-glu)

*S. cerevisiae*不能

wort cooling後→fermentor，迅速接菌，以免雜 菌污染，一天後，O_2用盡，可微通氣，或換至另一槽

initial yeast: 10^7/ml, after ferment Ale 2x

Ale約3天　　Lager 4~5x

Lager 7~10天→除yeast, 行2°fermentation

0~4℃低溫貯酒

before: 1 month

now: 1~2 wks

fermentation, 酒醪比重↓

∵放熱∴要cooling system

“green beer”剛ferment完之啤酒

“Flocculation of yeast”因種不同而影響澄清速度及程度，需中度者，受[EtOH]而促進，可發酵性糖而抑制

醱酵桶-開放式及密閉式

↙ ↘

需保留1%可利用糖　　CO_2可回收，今多採用，節省時間

連續式：48 h finish，僅少數用

雖投資低，但complex & high tech.不夠彈性，易污染

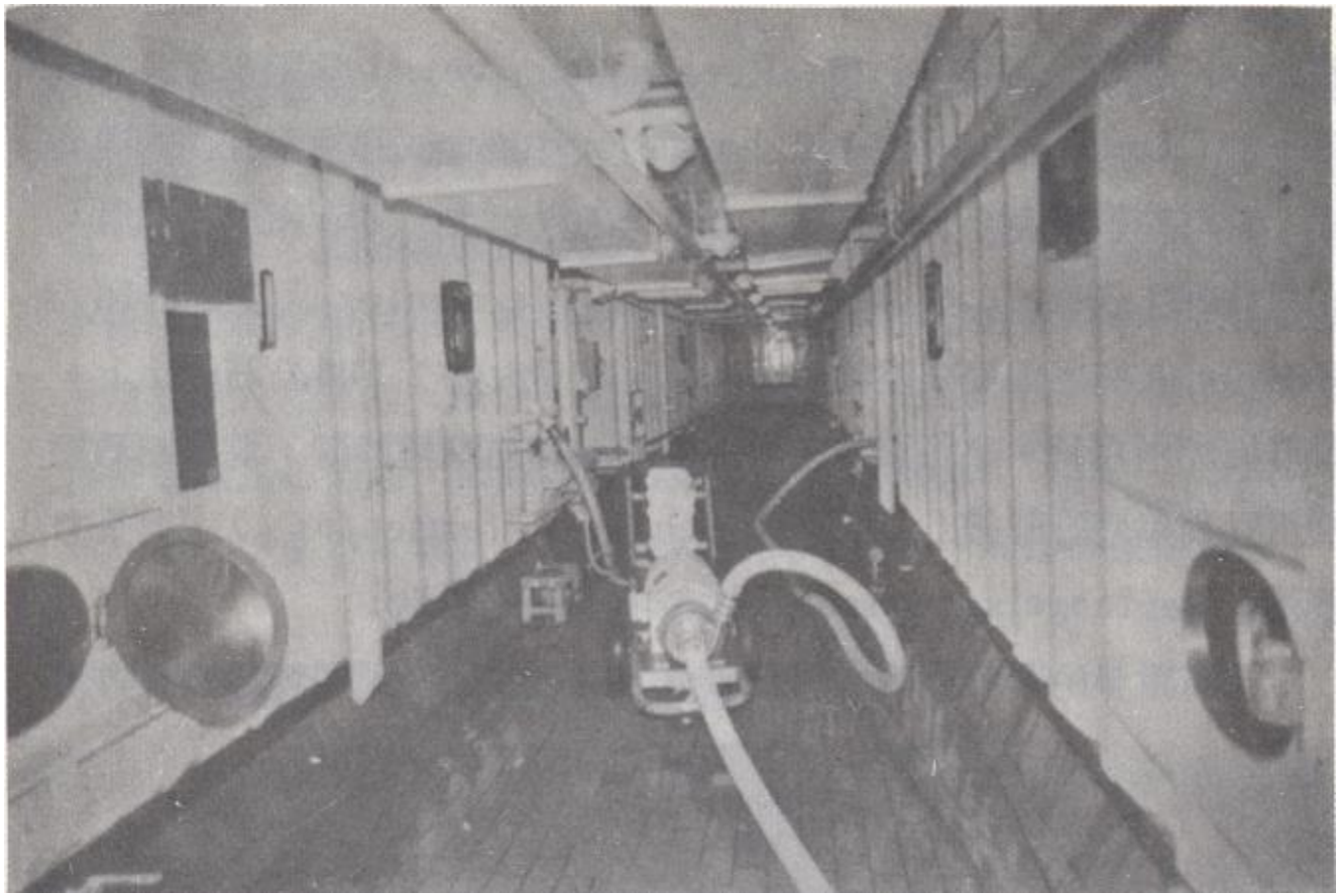


圖 8.7 長方形槽 (Rectangular vessel)

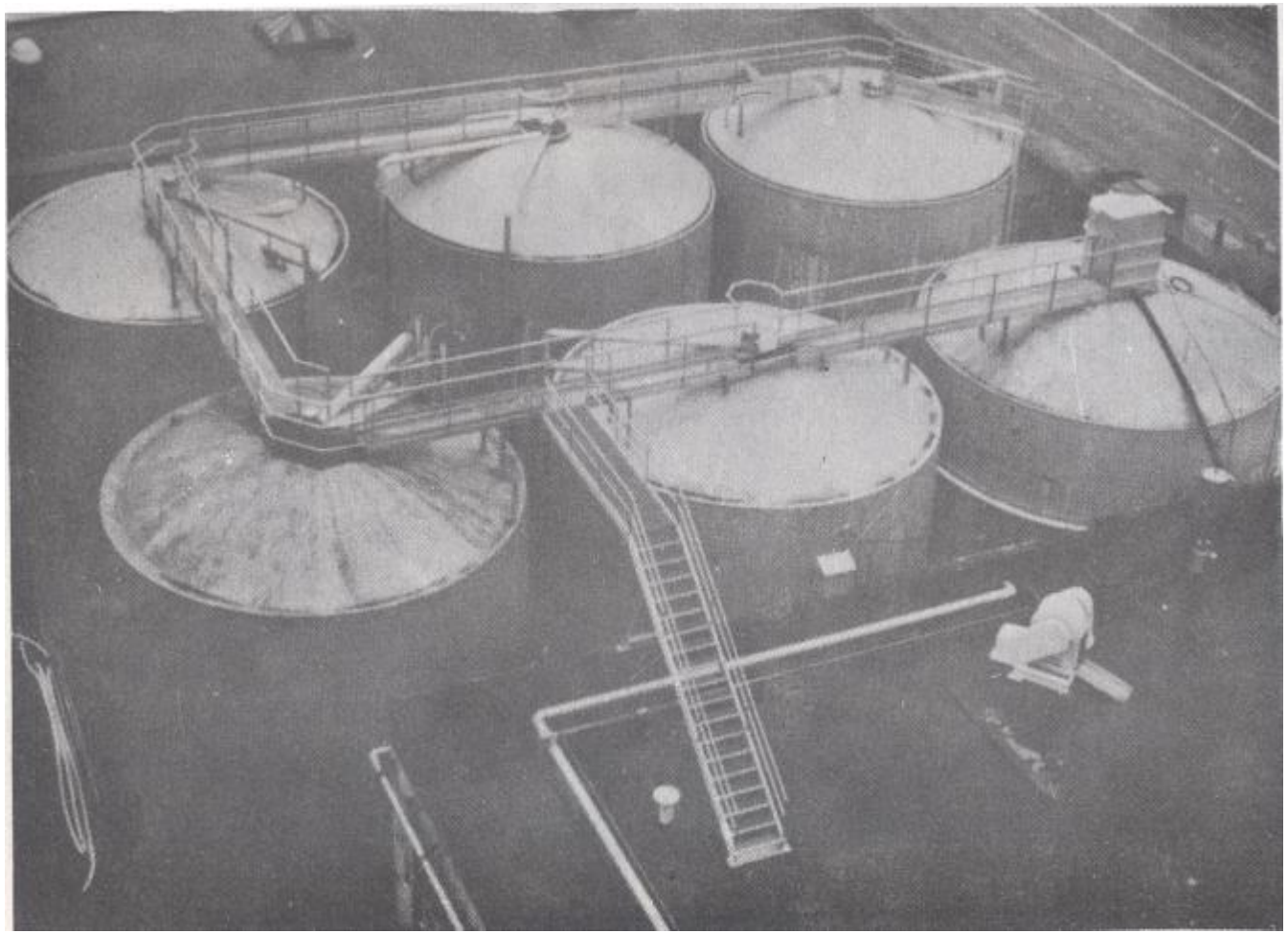


圖 8.8 單一槽 (Uni-tank)

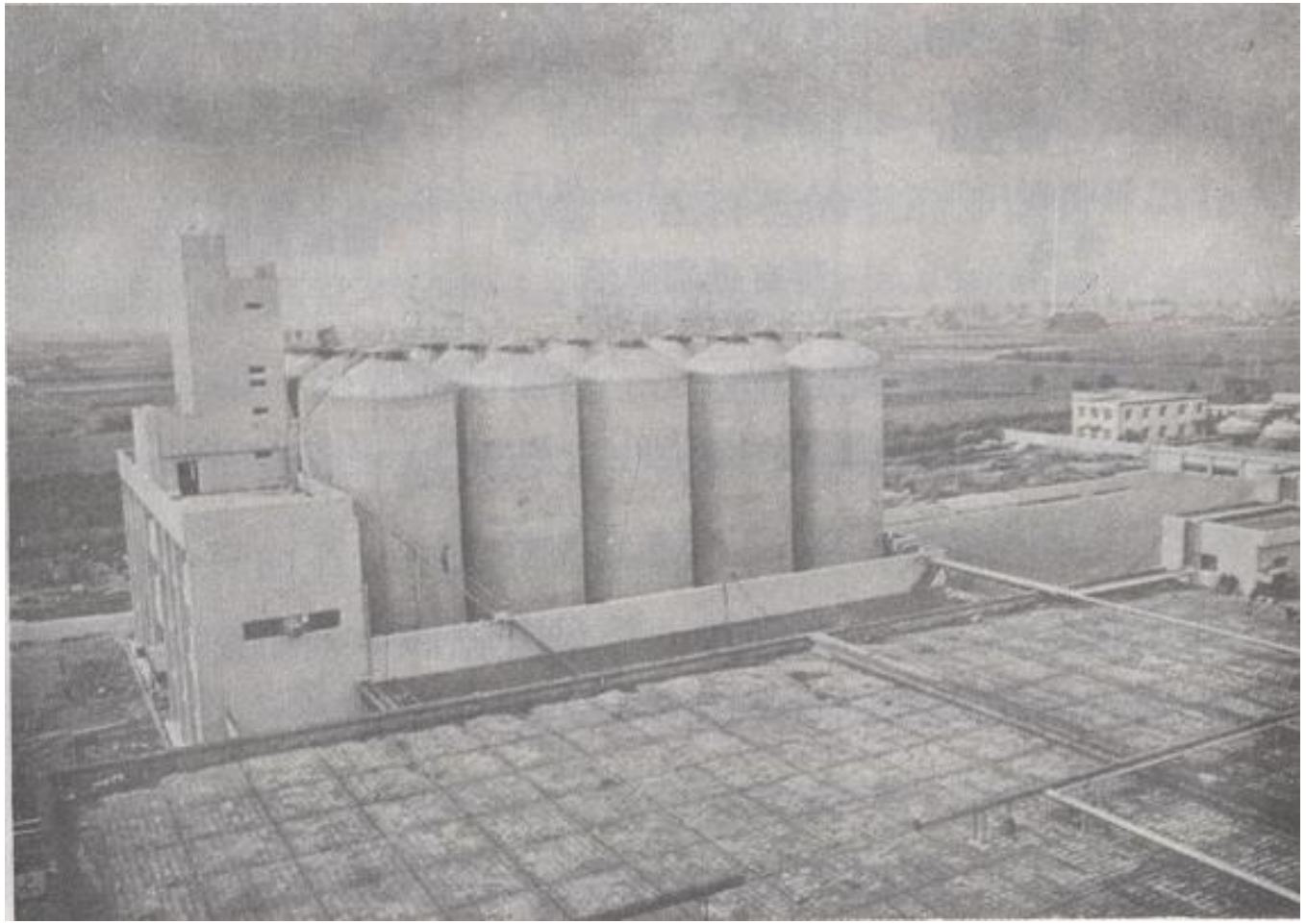


圖 8.9 成功啤酒廠之戶外圖筒錐底醱酵槽 (總容量 5,640 公石)

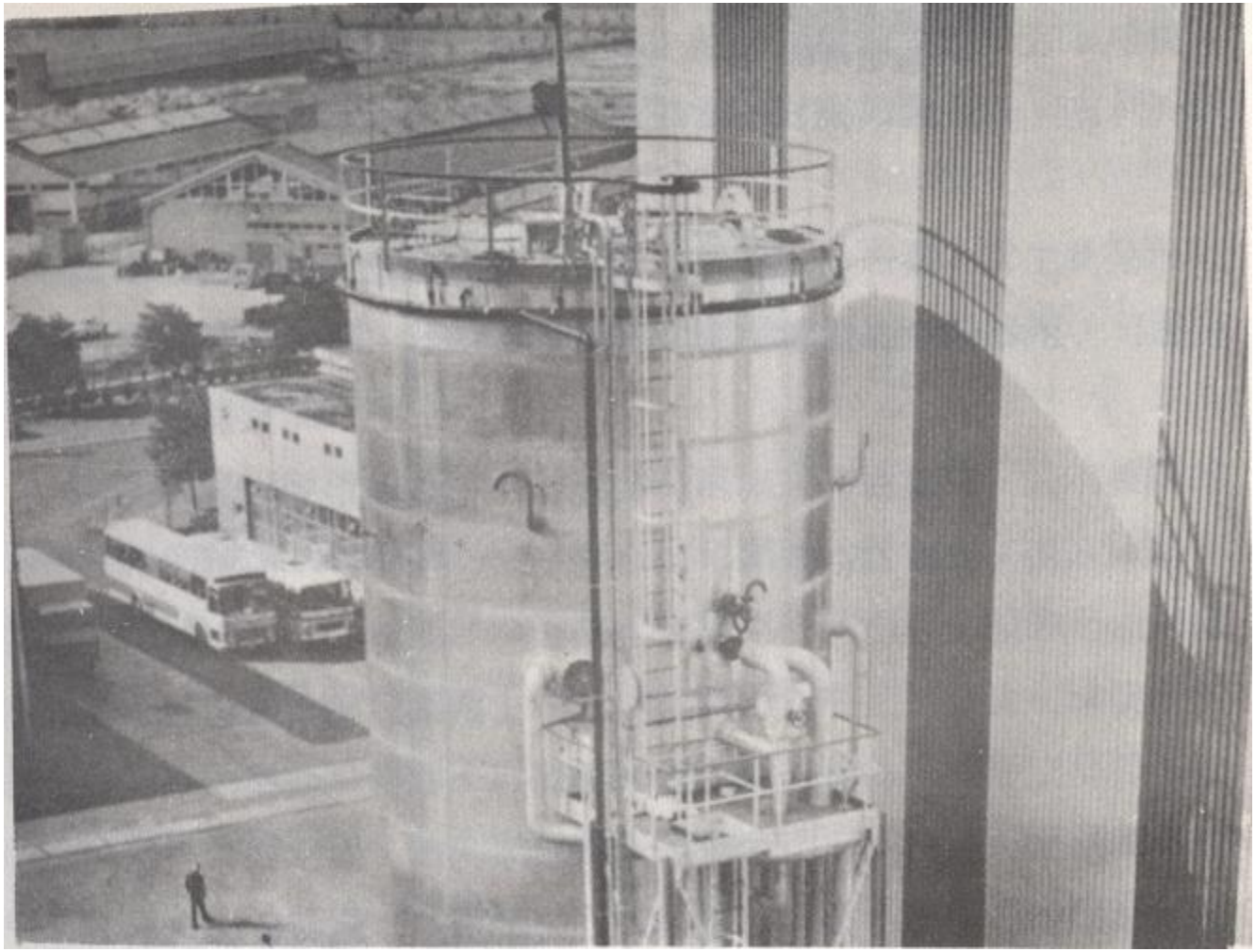


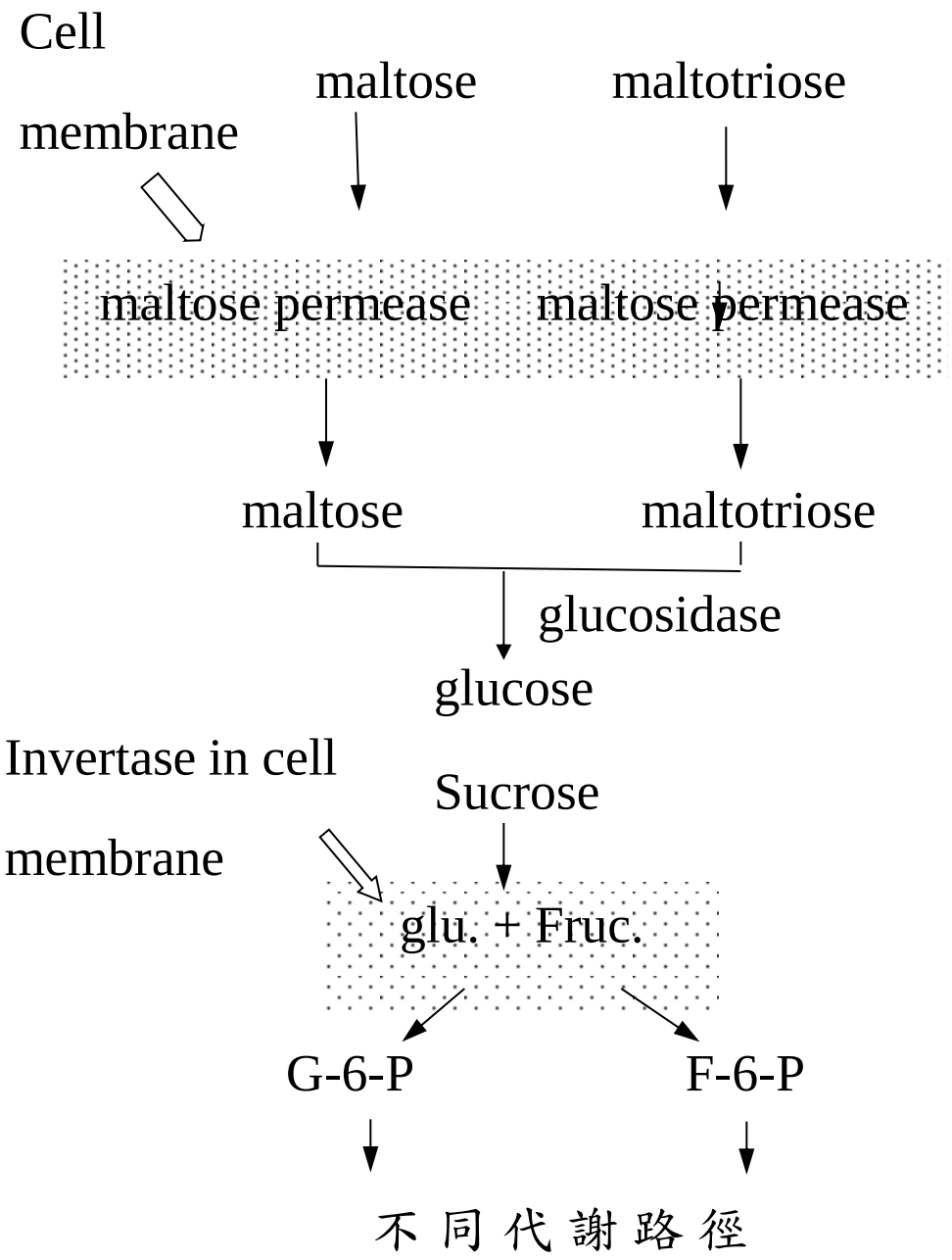
圖 8.9 大型戶外圓筒錐底醱酵槽 (Giant ylindro-conical fermentro)

Yeast metabolism

wort: fermentable sugar: maltose, maltotriose, glucose

adjunct: sucrose

utilization order: glu, Fru, suc. >maltose>maltotriose



permease activity inhibited

by monomer. (glu. Fru)

maltotriose permease.

Inhibited by maltose

∴[maltose] in wort↓

maltotriose permeas

eactivity ↑ 方 利 用

maltotriose

EtOH formation by Embden-Meyerhof pathway

Glu + 2ATP + 2 Pi + 2ADP → 2pyruvate + 4 ATP

↓

acetaldehycle

↓

alcohol

除alc外之產物Acid, ester, ketone, aldehyde

ester: ethyl acetate

雖然EtOH + EtCOOH→ethyl acetate化學合成，但占少數，主要靠yeast

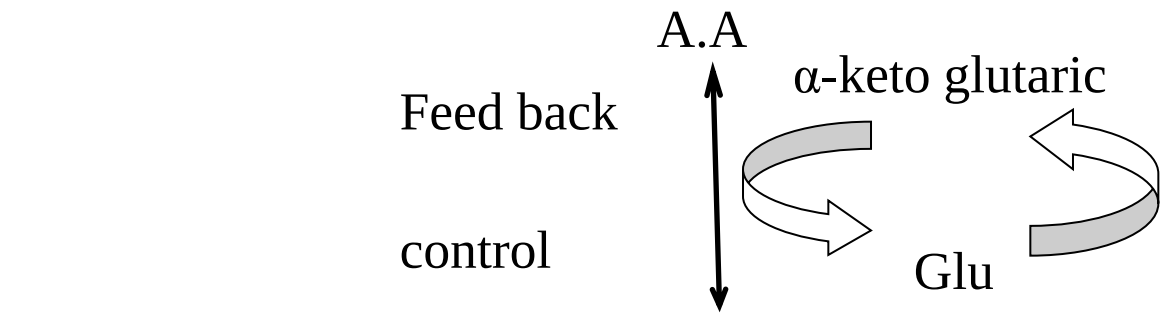
R-CO-S-CoA + R'OH →RCOOR' + CoA-SH

∴菌種占決定因素

N化合物：多以A.A.方式吸收，藉permease進入cell內

ferment beginning, Arg, Asp, Asn, glu, Gln, Lys, Ser & Thr.可迅速吸收，其餘慢，需等前述A.A.少時，才進入

故在cell內，yeast要合成其它A.A.以製造prot.



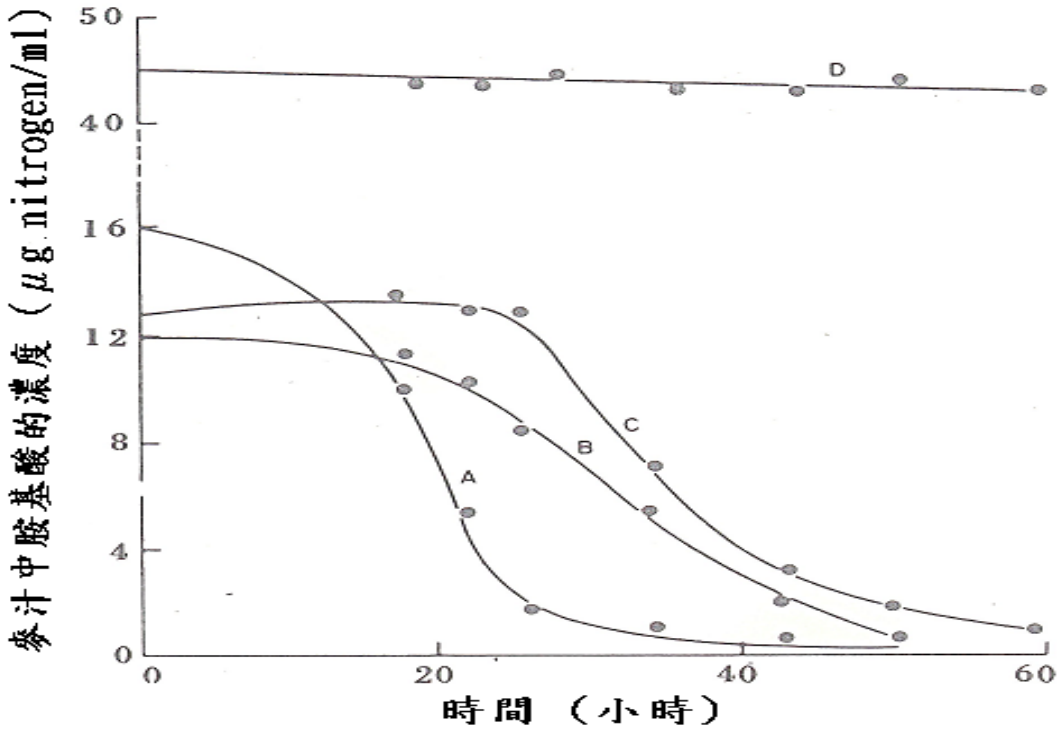
Pyruvic acid→acetyl CoA→α-keto acid

↓

aldehyde + CO_2

↓

alcohol



A：立即吸收，包括arginine, asparagine, aspartic acid, glutamic acid , glutamine, lysine, serine and threonine.

B：緩慢吸收，包括histidine, isoleucine, leucine, methionine and valine.

C：後期吸收，包括alanine, ammonia, glycine, phenylalanine, tyrosine and tryptophan.

D：不被吸收，包括proline 和 hydroxyproline.

圖 8.13 酵母菌對胺基酸的吸收速度

後熟(aging)及處理技術

- Carbonation
- 風味熟成
- 添加著色及風味物
- 防止非生物性混濁
- 澄清

aging時yeast 0.25~2.0 x 106 cell/ ml

若無可發酵性糖，可加糖漿(sucrose, inverted sugar, or starch hydrolysatc)，稱之 “Priming sugar”，也可加glycoamylase分解dextrin or 加aroma type Hop

sodium metabisulphite (防止lactic bact.污染)

澄清劑(fining)

Aginate or carrgaeenan “-”, 可吸 “+” prot

Isinglass “+”可與 “-” prot結合

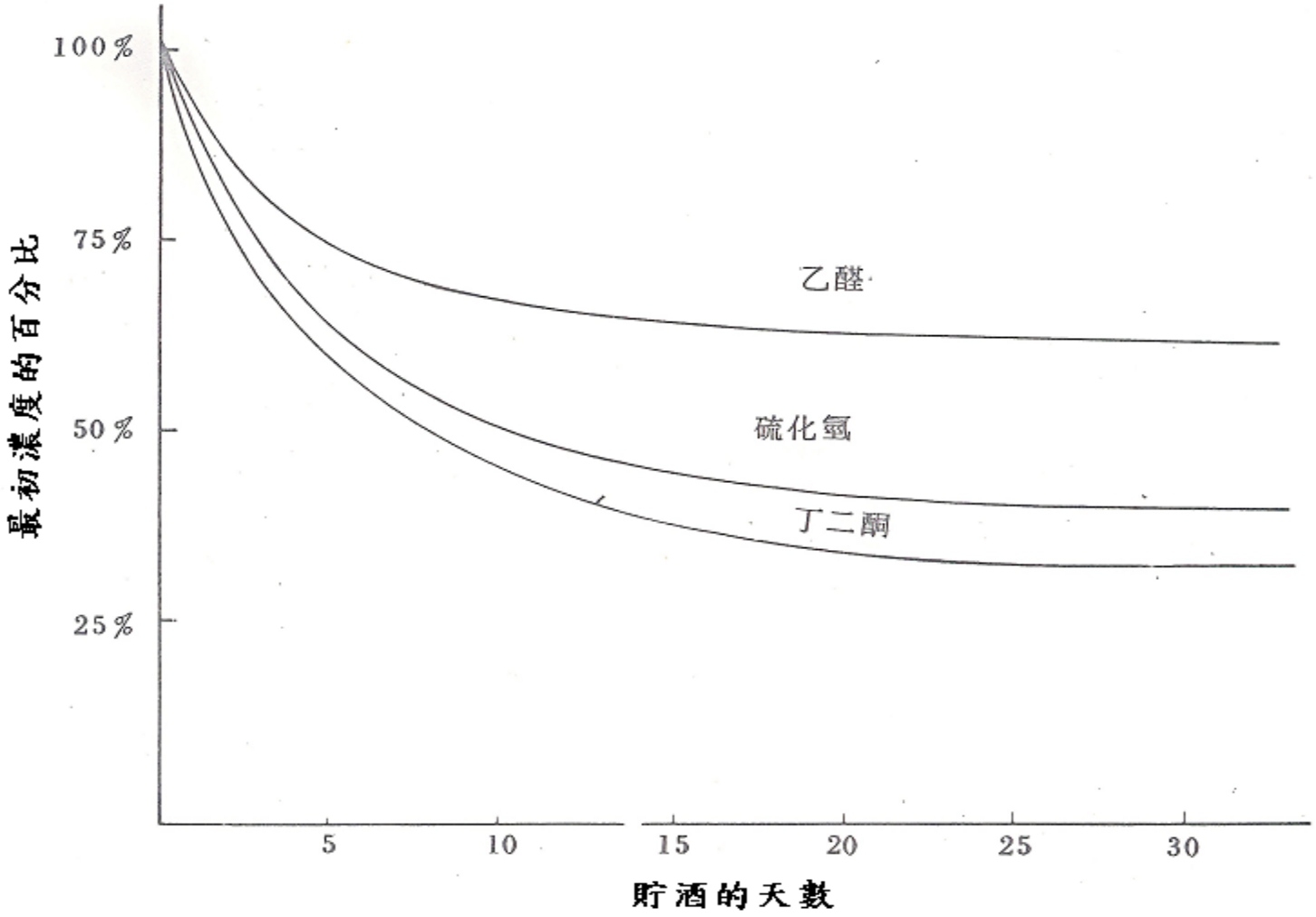
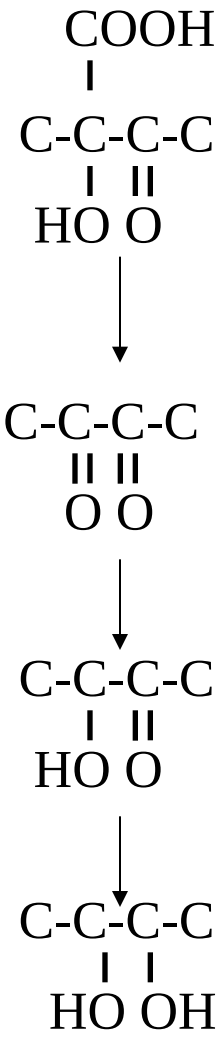
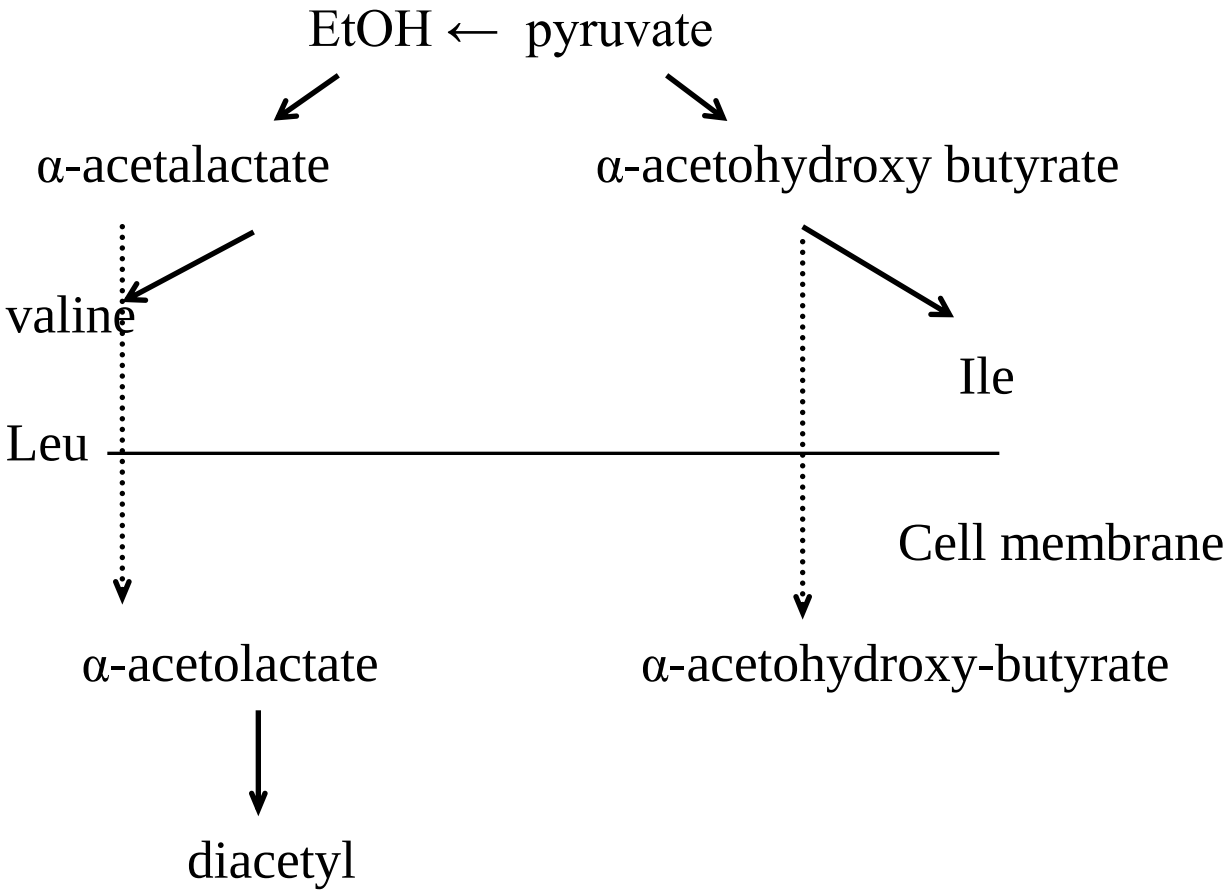


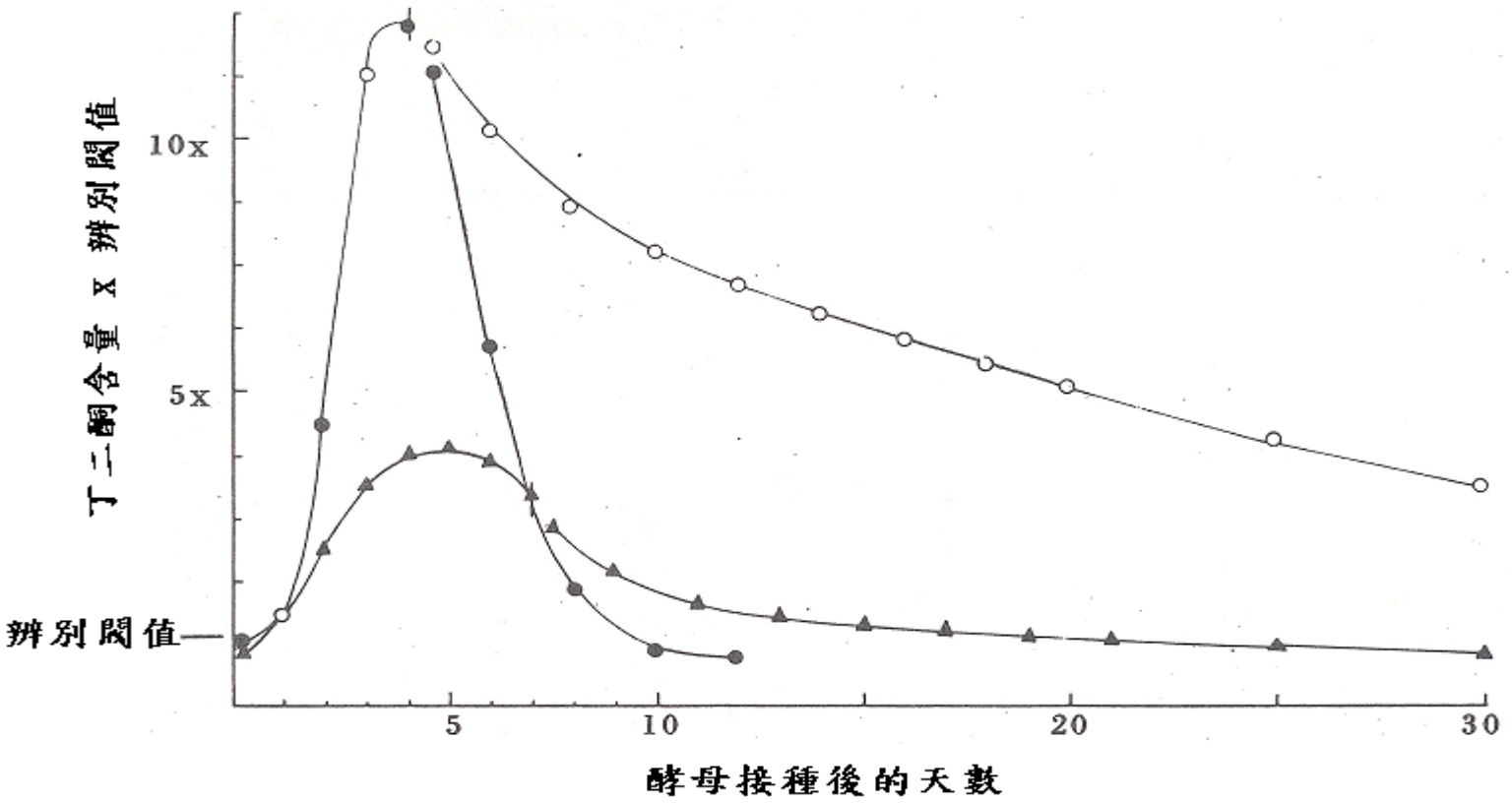
圖 9.2 貯酒期間風味化合物之減少情形

diacetyl由bact & yeast產生





- If wort 含氮量過高、快速ferment & 太早除去yeast→diacetyl↑
- Diacetyl 可因yeast繼續作用，成為acetoin及butane-2,3-di ol無味
- 熟成常以diacetyl含量為指標，0.15 mg/l以下，高溫有助diacetyl還原
- ∴ferment完，升溫，促diacetyl還原(稱diacetyl rest)，待含量至0.15 ppm以下，再低溫貯酒



●—● 醱酵 (8℃接種，最高溫度14℃)，14℃下貯酒

○—○ 醱酵 (8℃接種，最高溫度14℃)，0 ℃下貯酒

▲—▲ 醱酵 (4.5℃接種，最高溫度8.5℃)，0℃下貯酒

圖 9.3 醱酵和貯酒時丁二酮 (Diacetyl) 之生成與還原

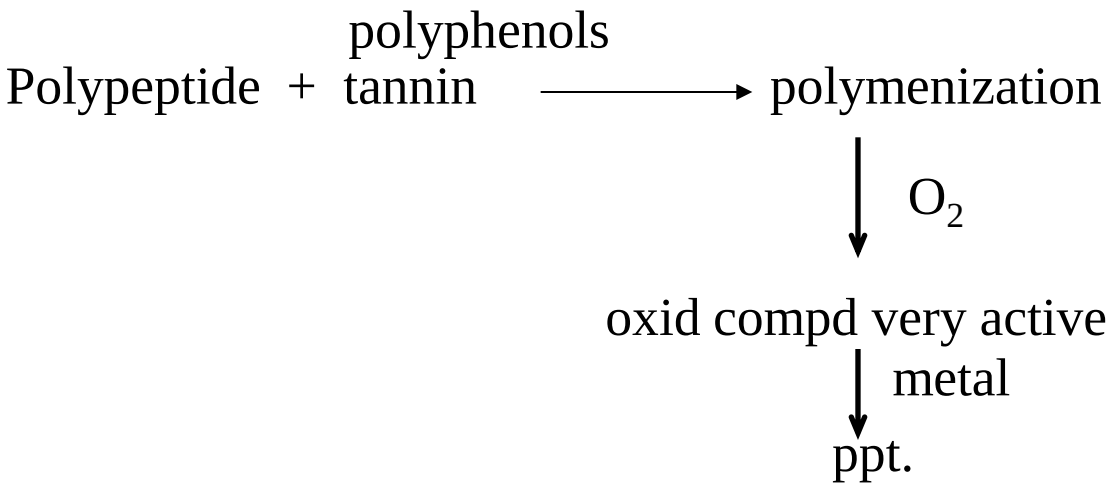
混濁(Haze) 預防

原因

(1) Ca^{2+}- calcium oxalate

(2) (CHO)n-β-glucan

(3) prot Hordein (醇溶prot)之分解物(polypeptide)



metal: Ti, Sn, Pb, Cu, Fe

conc> 1 ppm即可促進混濁(haze)

避免

1. H_2O raw material免於重金屬污染
2. 原料適當，wort N_{total}勿太高
3. 低polyphenol-不易達到
4. proper mashing & boiling→hot break or hot trub
5. mashing促使protease function 45-55℃
6. remove hot trub & cold trub
7. HCHO處理麥芽→↓polyphenol
8. active growth of yeast: yeast表面可吸附protein-polyphenol
9. Papain
10. 蛋白質吸著劑(absorbent)如Silica gel.除去prot.
11. 加polyphenol吸著劑Nylon 66 or PVPP (polyvinylpolypyrrolidine)，在過濾前加，與beer 混合→過濾
12. ↓dissoved O_2 in beer
13. 低溫storage

foam formation

- foam retention持續
- Lacing泡沫沾邊
- Glycoprotein-泡沫促進劑，來自麥芽
- Polypeptide→hydrophobic→interface between liq & gas., alginate, cellulose ester→bind small Gum peptide→起泡劑
- 脂肪性物質及精油→抑制劑

裝瓶、殺菌

- 裝瓶後殺菌60℃，20 min or先殺菌(71-79℃, 10-60 s)再裝瓶

- sparkling wine: Champagne, Sparkling Burgundy
- still wine: no CO_2 inside, eg. Claret, Rhine, Sauterne
- Dry wine: 10~13% EtOH, little or no unfermented sugar
- Sweet wine: have sugar left
- fortified wine: contain added brandy or distilled wine spirits (19~21% EtOH) eg. Port, Sherry, Maderira
- Table wine: low alcohol (8~15%), little or no sugar
- Dessert wine: fortified, sweet wines (19~20% EtOH) eg. Muscatel, Port.

Wine & Brandy

Wine: Europe 78% of World production

Grape juice: H_2O, carbohydrate (glu, Fru, pentose, pectin)

nitrogen compounds (protein & protein split products)

acids (tartaric & malic acids)

minerals

tannin, pigments, vitamins, enzymes.

fermentable sugar: glu & Fru. 120-250 g/l juice

generally glu : Fru = 1 : 1 (0.80~1.07)

most yeast use glu, Fru, mannose, & most prefer glu (glucophilic, eg. *S. cerevisiae*)

S. rouxii & *S. bailii* (osmopbilic, strong fermentation of Fru).

Pentose (arabinose & rhamnose) 1 g/l juice not fermentable by yeast

N-compd.

protein & degraded product: 0.2-1.4 g/l juice enough for yeast fermentation

But, if grapes infected with *Botrytis cinerea* ("Noble rot"貴腐黴), N-compd. may be reduced to 50% of original⇒Add N-source (ammonium phosphate or ammonium sulfate) for yeast fermentation special raw material for high quality wine.

Sauterne Wine

pH

3.0~3.9　5~15 g/l as tartaric acid

most pathogenic & spoilage bact. can't survive.

only acetic & lactic bact. grow.

Yeast ferement well pH 3~6 But pH affect the by-product.

eg. pH↑→glycerol↑

pH↑→↓lag phase of growth & fermentation activity↑

Temp

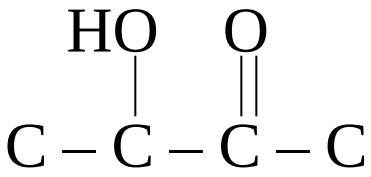
Temp↑→rate of fermentation↑. But high temp may kill yeast & favor growth
of thermophilic bact. Also, loss of alcohol & aroma↑

S. cerevisiae Max temp: 40~45℃

Min temp: ~0℃

15~35℃:Temp↑

- fermentation faster, also faster in cession
- Yeast count & alc% at the end of fermentation lower
- EtOH effect↑
- by-product↑ glycerine, acetoin
  Acetaldehyde,
  Butanediol-2,3
- Pyruvic acid & 2-ketoglutaric acid↑
- Fusel oil (higher alcohol)↑(at 20℃ max)



Generally wine fermentation vary 10~30℃
German white wine 18-20℃
California white wine 10-16℃
Red wine 22-30℃ for color extraction

Advantages of low temp. fermentation

-fresh & more fruity character
-more EtOH production
-smaller loss of EtOH
-reduction of bacterial infections
-less volatile acidity

Sugar concentration

-higher (>300 g/l)→osmotic effect→↓yeast growth & fermentation

-alcohol productivity↓when sugar↑

-inhibition effect of higher sugar↑with EtOH↑

-*B. cinerea* infected grapes must inhibit growth of yeast not only due to decreasing of N-compd. but also to higher sugar conc.

CO_2

-higher CO_2→affect yeast growth

-7.2 atm at 15℃→yeast growth stop but sugar fermentation up to 14 atm

30 atm∴kill yeast

-high CO_2 pressure fermentation to inhibit growth of yeast but have fermentation activity⇒save sugar⇒higher EtOH%

higher residual sugar.

-lactic acid bact. Not inhibited at high CO_2 pressure volatile acid↑

-CO_2 effect↑with EtOH & lower pH

SO_2

biological effect: bact. & mold more sensitive than yeast (100 ppm), acetic acid: 20 ppm.

free SO_2 = SO_2 (H_2O)

~C-S-C~ + SO_2 (H_2O)

protein

→ ~C-SH + ~C-S-SO_3H

interfere with enzyme or protein in fungi or bact.

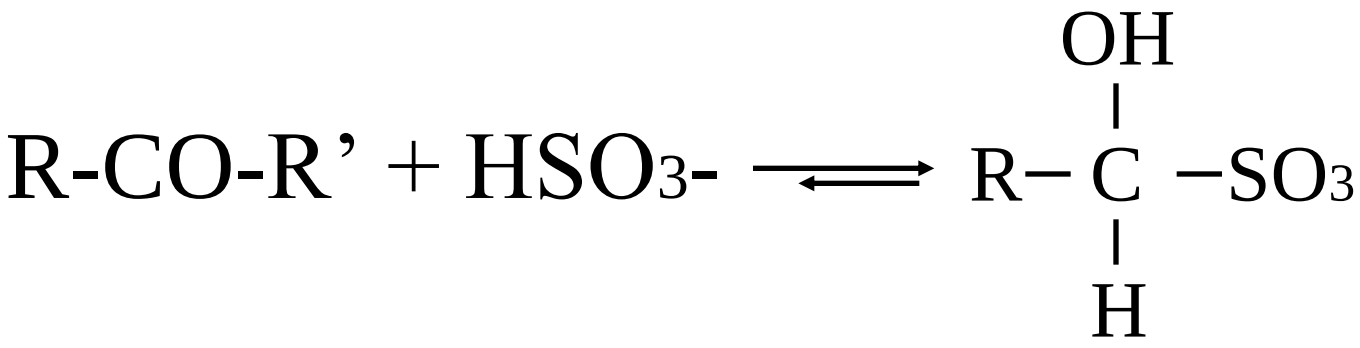
- yeast can tolerate 300 ppm (table wine < 100ppm) wine making, 100~200 ppm $K_2S_2O_5$ added
- SO_2 inhibit lactic bact to prevent malo-lactic fermentation
- Suppress *Kloeckera apiculata*
  Candida pulcherrima
  Which produce high volatile acid & pyruvic & 2 KG⇒↓quality
- Delay onset of fermentation

100 ppm delay by 3 days
200 ppm delay by 20 days

Only "free SO_2" inhibit yeast

chemical effect

SO_2 + aldehyde→sulfonate, reduce undesirable organoleptic properties



In wine pH (3.0~3.8) favor→rxn

health concern in humans

- harmless to normal people at legal levels in wine (350 ppm) most table wine < 100 ppm
- asthmatics, esp. steroid-deficient sensitive to SO_2
- persons deficient in sulfite oxidase (convert $SO_2 \rightarrow$ sulfate) sensitive to both free & bound SO_2

SO_2↑ aldehyde production by yeast

at beginning, yeast utilize (CHO)n for growth, which result in accumulation of large amounts of aldehyde. When yeast stop growth, they convert these aldehyde and NADH to alcohol.

$$H^+ + NADH + CH_3CHO \xleftrightarrow{\text{Alcohol DH}} NAD^+ + CH_3CH_2OH$$

SO_2 bind with CH_3CHO & prevent its conversion.

$\Rightarrow$aldehyde↑

SO_2-compd addition depend on medium composition.

Botrytis infected grape must need higher amount of SO_2 due to higher keto-acid presence.

yeast can also produce SO_2 by reduction of sulfate in must

strain dependent

Saccharomyces yeast in wine making⇒produce 3~12 mg/L

Some *Saccharomyces* not used in wine making⇒1300 mg/L

yeast

-Naturally exist on grapes by caring of insects

-vary with climate,

only 1~10% are desirable *Saccharomyces* in must.

After press, its %↑

Table 9.11 the Natural Yeast Flora of Must

Start of the Fermentation	Main Fermentation	End of the Fermentation
[1]*Kloeckera apiculata*	[1]*Saccharomyces cerevisiae*	[1]*Saccharomyces cerevisiae*
[1]*Metschnikowia pulcherrima*	[1]*Saccharomyces uvarum*	*Saccharomyces bayanus*
Kloeckera corticis	*Saccharomyces bayanus*	
Candida krusei	*Saccharomyces chevalieri*	
Candida vini	*Saccharomyces delbrueckii*	
Hansenula anomala	*Saccharomyces fermentati*	
Hansenula subpelliculosa	*Saccharomyces florentinus*	
Pichia fermentans	[2]*Saccharomyces rosei*	
	Saccharomuces rouxii	
	Kl veromyces veronae	

Source: Modified from Benda (196 A).

Yeast species were isolated from grape musts of *Fra iconia* during the course of the fermentation.

[1]Predominant species during the different stages of the fermentation.

[2]*S. rosei* tolerates relatively small concentrations of ethanol and appears between the start of the fermentation and the main fermentation.

Saccharomyces cerevisiae

8~17% by vol.

- high EtOH production (18~20% by vol)
- cold resistance→cold fermenting yeast
- EtOH tolerance 8~12% by vol normal fermentation
- SO_2 resistant
- Osmotolerance 30% [sugar]→10~13% EtOH

∴become dominant due to EtOH production & tolerate EtOH.

S. bayanus

Synonyms:

- *S. oviformis, S. pastorianus, S. beticus*
  S. cheriensis
- strong represented in Italian region
- less frequently found in France
- may produce EtOH 10%~17.6%~19%
- found at end of fermentation

S. uvarum

- typical microflora in grape
- occur during main fermentation,
  suppressed in final phase by *S. cerevisiae*
  S. bayanus

S. rosei

- typical yeast flora in grape must
- found at beginning of main fermentation, small percentage
- EtOH formation (7 ~ 11 % by V) most 6 ~ 8 %
- low volatile acid & ethyl acetate production

weak EtOH fermentation

S. rouxii

- -Osmophilic yeasts

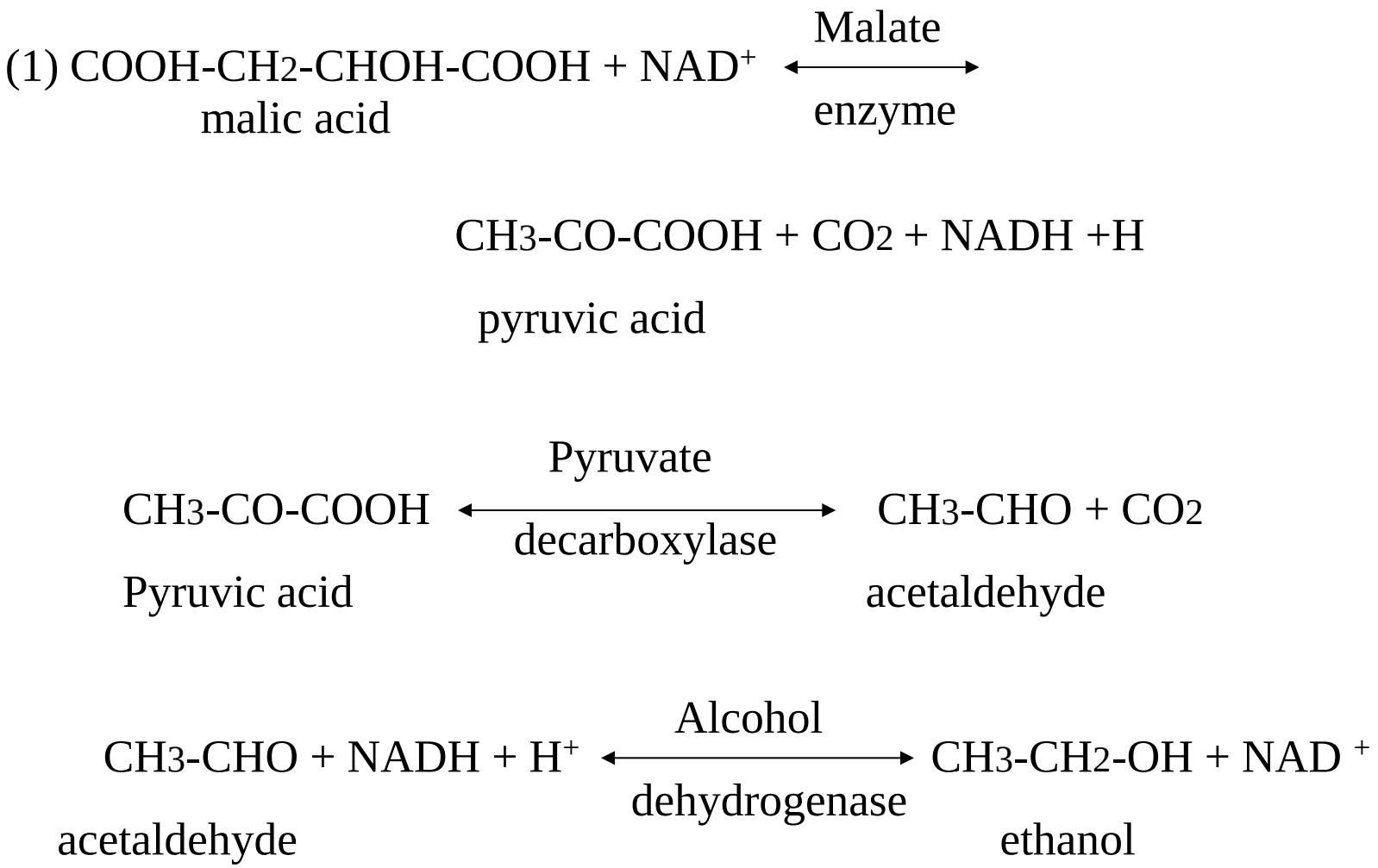
  isolated from *Botrytis* infected grape must
- -EtOH (13 ~ 14 %)

Schizosaccharomyces pombe

- not belong to yeast flora of must & wine.
  Except in western part of Sicily
- cell division, not budding
- EtOH production similar as *S. cerevisiae*
- degrade 1-malic acid→EtOH & CO_2
- tolerate high SO_2
- volatile acid & higher alcohol low
- Tolerate acetic acid & low pH
  at pH 2.8 → *S. cerevisiae* inhibited & fermentation ceased at pH 2.5 . but *Schizosaccharomyces* active.

10 ~ 12 % (w/o O_2)

13.2~15.1% (O_2)


(1) COOH-CH2-CHOH-COOH + NAD+
malic acid
Malate
enzyme
CH3-CO-COOH + CO2 + NADH +H
pyruvic acid
CH3-CO-COOH
Pyruvic acid
Pyruvate
decarboxylase
CH3-CHO + CO2
acetaldehyde
CH3-CHO + NADH + H+
acetaldehyde
Alcohol
dehydrogenase
CH3-CH2-OH + NAD +
ethanol

(2) $COOH\text{-}CH_2CHOH\text{-}COOH + NAD^+$ $\xleftrightarrow[\text{dehydrogenase}]{\text{Malate}}$ $COOH\text{-}CH_2\text{-}CO\text{-}COOH + NADH + H^+$

malic acid — Oxalacetic acid

$COOH\text{-}CH_2\text{-}CO\text{-}COOH$ $\xleftrightarrow[\text{decarboxylase}]{\text{Oxalacetate}}$ $CH_3\text{-}CO\text{-}COOH + CO_2$

Oxalacetic acid — pyruvic acid

$CH_3\text{-}CO\text{-}COOH$ $\xleftrightarrow[\text{decarboxylase}]{\text{pyruvate}}$ $CH_3\text{-}CHO + CO_2$

Pyruvic acid — acetaldehyde

$CH_3\text{-}CH_2\text{-}OH + NAD$ $\xleftrightarrow[\text{dehydrogenase}]{\text{alcohol}}$ $CH_3\text{-}CHO + NADH + H^+$

acetaldehyde — ethanol

Fermentation rate of malic acid ≒ 1/10 of sugar fermentation

- try to↓acid of wine by this species

exp. mix of *S. cerevisiae* & *Shizosaccharomyces pombe*

growth of latter is inhibited, esp. in low temp.

exp. 2 stage fermentation

▲*S. cerevisiae* ferment 1st, than add *Shizo* -

or *Shizosaccharomyces* ferment 1st to rapid reduction of malic acid, remove yeast, than add *S. cerevisiae*

Pure culture yeasts

-Differences among strains of *Saccharomyces* will not be great, because aroma of grape overlay those of fermentation by product

-Aroma of natural fermentation more complex

pure culture yeast

- Different Acetaldehyde, Pyruvate, α-KG ⟶ production → bind w/SO_2 ↓ recommended SO_2 vary
- various amount of SO_2 produced
- H_2S production
- Acid reduction

  } undesirable
- temp. medium composition & product characterization

Winemaking made simple

White table wine

Crush and destem

Must — Add: Sulfur dioxid (Pectic enzymes)

Drain or press, clarify,and ferment

Juice — Add: Yeas
(Tartaric acid)
(Antifoaming agents)

Clarify and stabilize

wine — Add: Bentonite
(sulfur dioxide)

Settle, clarify, and filter

Age

Add:
(Sulfur dioxide)

Stabilize, adjust, filter,and bottle

Bottled wine — Add: Sulfur dioxide
(Citric acid
(Sugar)
(Sorbic acid)
(Ascorbic acid)

Finished wine

Red table wine

Crush, destem, and ferment

Must — Add: Yeast
(Sulfur dioxide)
(Malolactic bacteria)

Press and finish fermentation

wine

Clarify, fine, stabilize, and filter

Add:
Protein fining agents
Sulfur dioxide

Age

Add:
(Sulfur dioxide)

Stabilize, adjust, filter and bottle

Bottled wine — Add: Sulfur dioxide
(Citric acid
(Sugar)
(Sorbic acid)
(Ascorbic acid)

Finished wine

Processing-handout

spoilage problems

1.Acetic acid bact.-strict aerobe.

fresh grape juice 10^{2-4} cells/ml

Acetobacter spp. *Gluconobacter oxydans*

$$EtOH \xleftrightarrow{O_2} \text{acetic acid}$$

control by : temp (opt 30-35℃)

EtOH (limit 12-15%)

pH (> 3.2-3.0)

SO_2 (sensitive)

2.Lactic Acid bact.

-↑ acidity of wine

-bitter & off-odors (diacetyl)

-volatile acid ↑ (due to use up unfermentable sugar)

pentose

-ferment organic acid (tartaric acid) → aroma

citric acid change

-control by temp, pH, EtOH, SO_2

- opt pH for lactic bact. 4.3 ~ 4.8

  lower pH limit for sugar & malic acid metabolism 3.0 ~ 4.0

- opt temp. 25℃

:

EtOH (V%)	inhibit growth (%)	Inhibit malo-lactic fermentation (%)
6	0~22	6-11
10	18-40	13-30

but some still grow at 20 % EtOH

Lactobacillus trichodes- not ferment malic acid

-lactics quite sensitive to SO_2 (free)

cocci more sensitive to rod

growth, sugar & malic acid metabolisms inhibited

0~8 ppm free SO_2 or 95 ~ 135 ppm bound inhibit

malo – lactic fermentation

-monitor lactics by presence of D-lactic acid

-butanediol-2,3→2-butanol

indication f bact. Spoilage

Lac. brevis

3.Killer (K) yeasts

-toxin excreted, which is lethal for " sensitive " strains

-glycoprotein, which adheres to the cytoplasmic membrane→↓synthesis of macromolecular

outpouring of ATP & K+ etc.

inhibition of active transport system for amino acid

-At 5 % [glu], opt pH = 4.5 for toxin production.

-independent of N – nutrients for the yeast.

-resist heating to 40℃ for 1h

-could detect K effect when cells at 2% total population

-One of the most frequent factors which prevent growth of a selected yeast strain in a must

-*Saccharomyces*, *Deloaryomyces*, *Torulopsis*, *Candida* & *Pichia* spp.

Vinegar

§Definition:

In Germany:

min 5~ max 15.5 g water-free acetic acid/100g

these vinegar may be from:

(1) fermentation vinegar

(2) dilution of HAC with H_2O

(3) blend fermentation vinegar with HAC

In U.S.A & many other European countries

Vinegar = fermentation vinegar only

British: fermented Alcohol→vinegar

USA: Extended to synthetic alcohol

To prevent alcohol abuse & to avoid the payment of duty, the alcohol is usually denatured to some special formula.

eg. SDA 29: 1 gallon of ethyl acetate to every 100 gallons of 190°proof ethanol

$(C_6H_{10}O_5)_n$ carbohydrate source

from grain, fruit root crops

$$(C_6H_{10}O_5)_n \xrightarrow[\text{acid}]{\text{en.}} C_6H_{12}O_6 + C_{12}H_{22}O_{11}$$

Fermentable mono- & di-saccharides

$$C_6H_{12}O_6 \xrightarrow[\text{anaerobic}]{\text{yeast}} C_6H_5OH + CO_2$$

EtOH

$$C_2H_5OH + O_2 \xrightarrow[\text{air}]{\text{acetic acid bact.}} CH_3COOH + H_2O$$

Acetic acid (HAC)

1g glucose ⟶ 0.51g EtOH ⟶ 0.67g HAC

§use

▲non-food use:

-diluted acetic acid + nitric acid→dissolve minerals

-production of the pigment ceruse or white lead

▲food use

-pickling

-salad & marinades dressing

-preservative

-not only by pH effect, also by organic acid

eg *Salmonella* growth at pH 5.4 by using HAC

as acidulant

but growth may occurred

down to pH 4.05 if HCl was used

0.1% un-dissociated acid inhibit most food poisoning & spore-forming bacteria

0.3% prevent growth of mycotoxigenic molds.

reason:

unionized lipophilic compd→penetrate CM. & disrupt CM transport process →dissociating within cell to ↑acidity

∴Effective pH range is indicated by pKa (4.75)

▲ **Medical use: healthy food**

§Vinegar fermentation

raw materials

- any non-toxic materials that furnishes a juice or solution containing fermentable sugars. Theoretically 1g glu→0.67g HAC. In practice 2% w/v sugar→1% w/v HAC
- Name of vinegar from name of raw material
- Need inexpensive

§raw materials preparation

molasses, corn syrup & honey---easy storage

diluted (10~15% w/v sugar) before use

acidity to pH 4.5-5.0 w/ H_2SO_4 & pasteurized to reduce infection

fruit→extract juice

starch→ hydrolyze to sugar by acid or en. or mold

***Asp. oryzae* rice vinegar**

§Alcoholic fermentation

- *S. cerevisiae* most often used.

  sometimes *Schizosaccharomyces pombe* is used with molasses fermentation

- *Kluyveromyces fragilis* or *Candida pseudotropicalis* is used if whey is the substrate

  vinegar processing, alcoholic fermentation & acetous fermentation well separated each other

- Although natural alcoholic fermentation work, most inoculate large active yeast & ferment at 30℃ 48-72h →centrifuge to remove yeast→mix with a portion of raw vinegar

  "backslopping"

§Acetification

$C_2H_5OH + O_2 \rightarrow CH_3COOH + H_2O$

46 g **60 g**

當9.2% v/v Alc.
約1% alc→1% HAC

exothermic rxn 8.4 mj/1l EtOH

limitation of solubility of air in solution

⇒O_2-availability is the rate limiting factor in acetification

(Normal solubility of O_2 in H_2O = 8.1 ppm at 25℃)

§Acetic acid Bacteria

-film on top during wine or alcohol fermentation

1935 proposal

-Asai (1968) → *Acetobacter* →isolated from vinegar

→ *Gluconobacter* →isolated from fruit

Gluconobacter: produce large gluconic acid from glucose inability to form film in liq.

Poor growth in EtOH-containing substrate

-Leifson (1954)

→ *Acetobacter* based on morphology週邊毛

→ *Acetomonas* 極鞭毛(3~8)

∴Acetobacter: lactaphilic, grow well on lactate can use

ammonium salt as sole N-source

poorly on glucose

oxidize lactate & acetate to $CO_2 + H_2O$

best C source: lactate

EtOH

glycerol

Gluconobacter/Acetomonals (glycolytic)

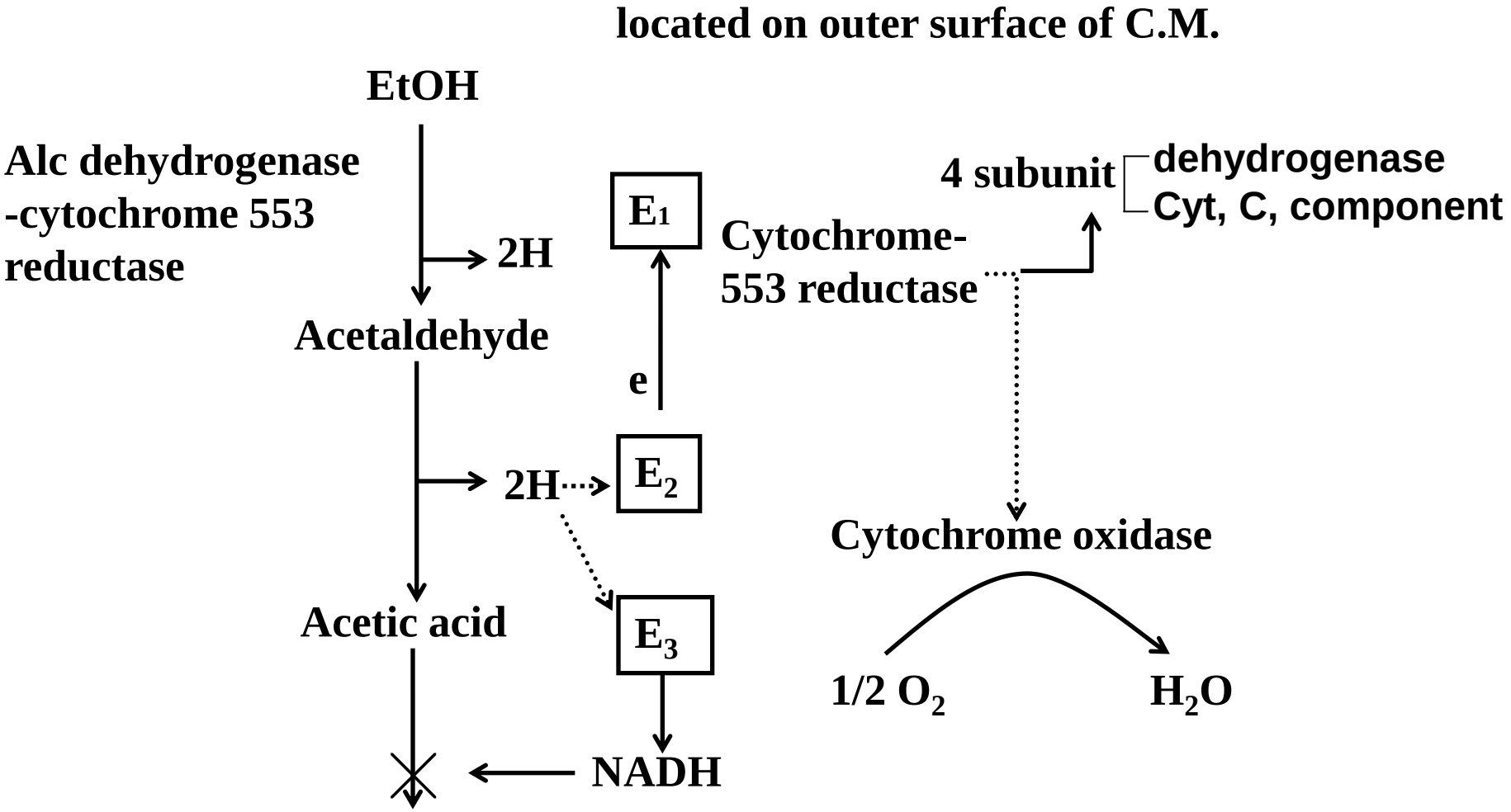
Nutritionally fastidious

grow well in glucose & other sugar alcohol

not oxidize lactate & HAC to $CO_2 + H_2O$

growth factor: nicotinic acid, pantothenate,

β-amino benzoate



Nakayama's scheme for EtOH oxidation

From EtOH→→HAC. Entire system works as a tightly organized multi-enzyme complex

§Acetification process

1. “let-alone” process
2. “Shallow tray” for surface acetification

   Acetifying liquid is passed at constant rate through a series of trays in sequences using methods that minimize disturbance to bact. film. very slow, need large land & personnel.

3. Orleans process
4. Quick vinegar process- Frings type generator
5. Submerged acetification- Frings Acetator

50-100 gal half-filled
1/3 siphoned off recharged
24-27^{o}C give finest quality
Clean every 6-8 years
Slow
Last 25 years

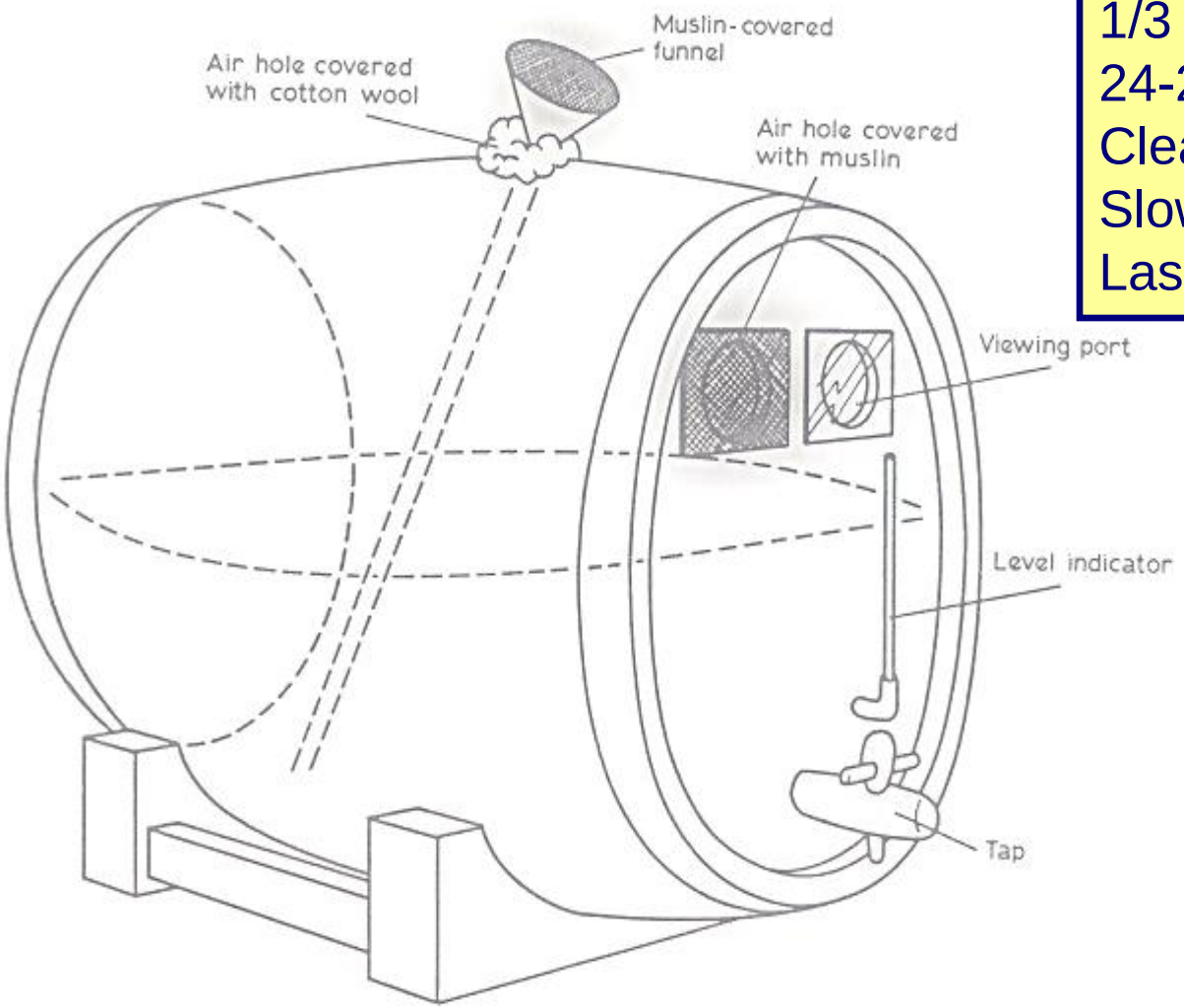


Fig. Acetification by the Orleans process.

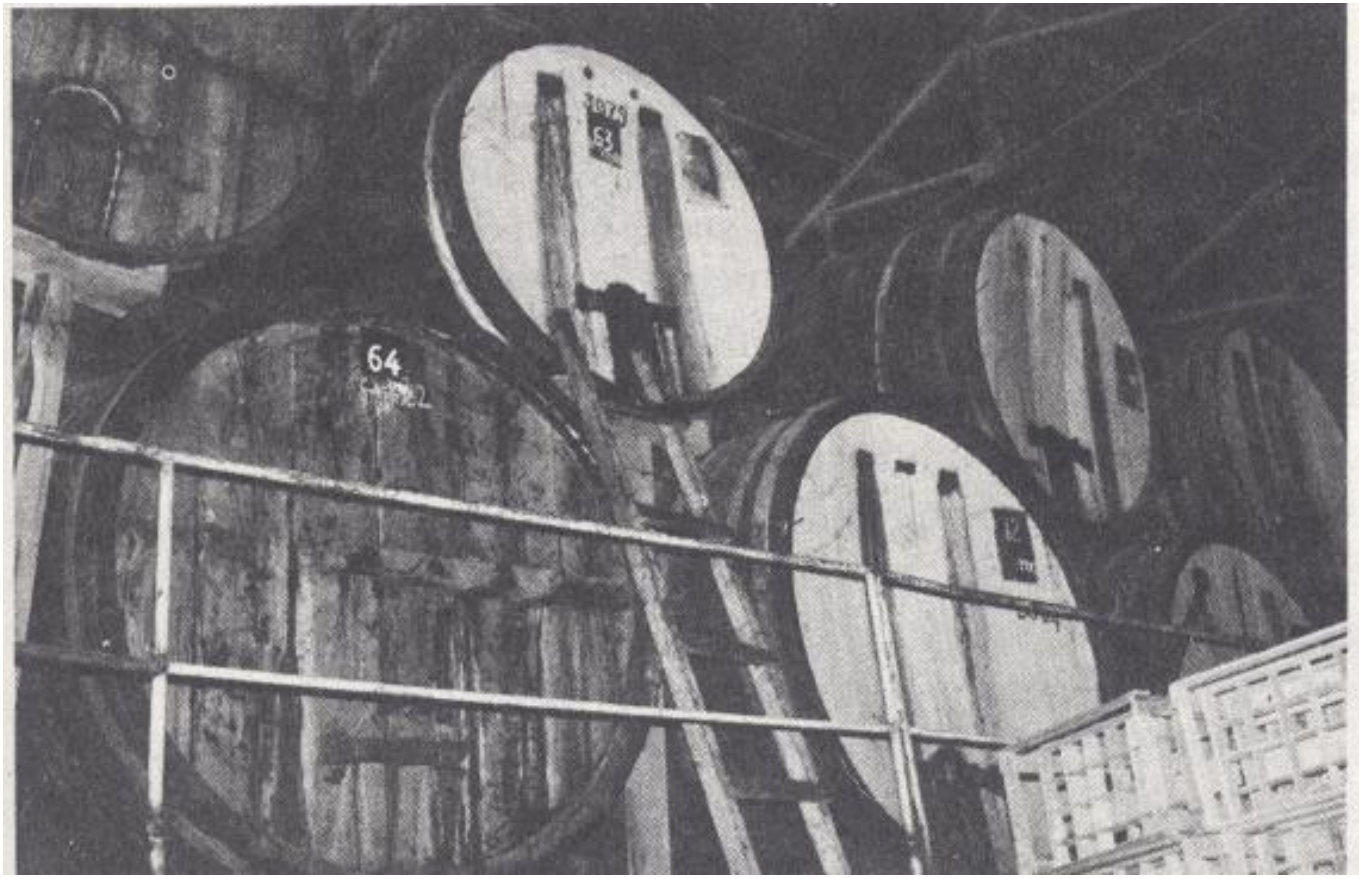
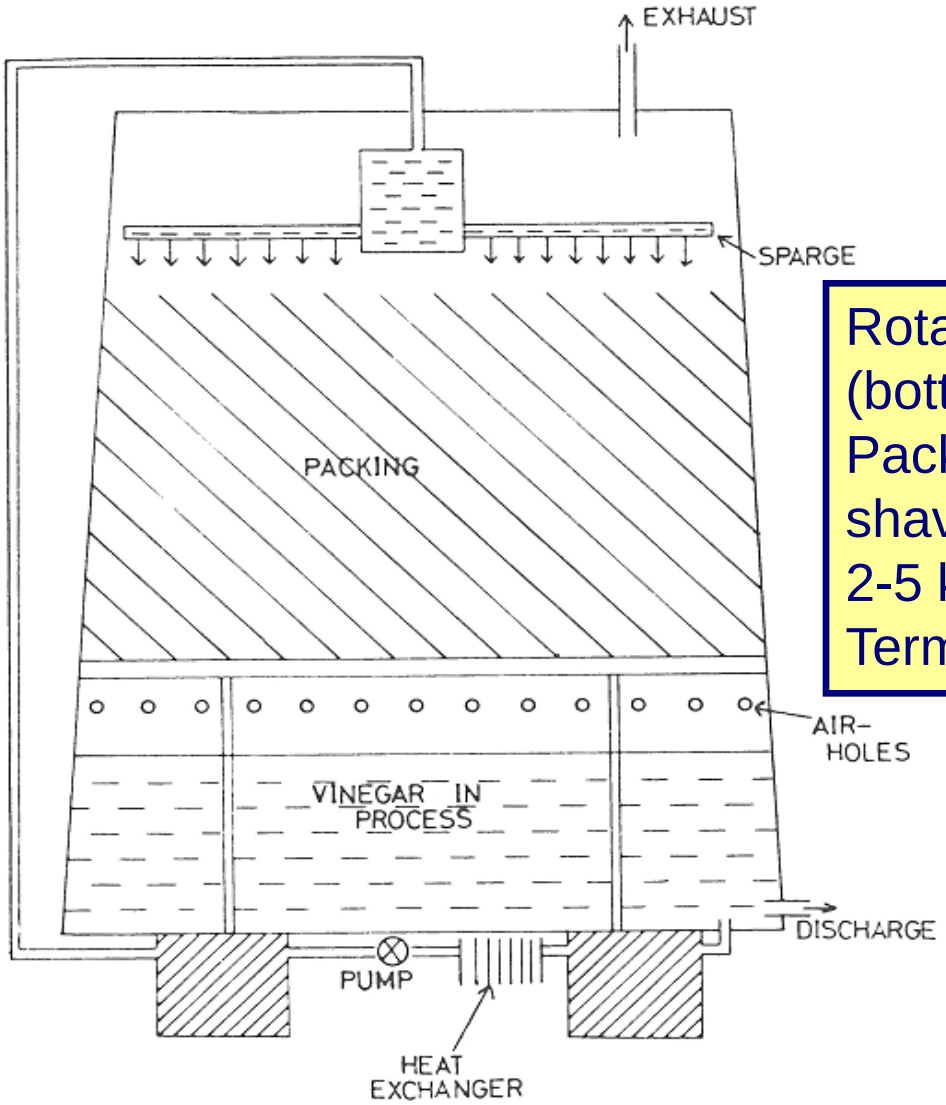
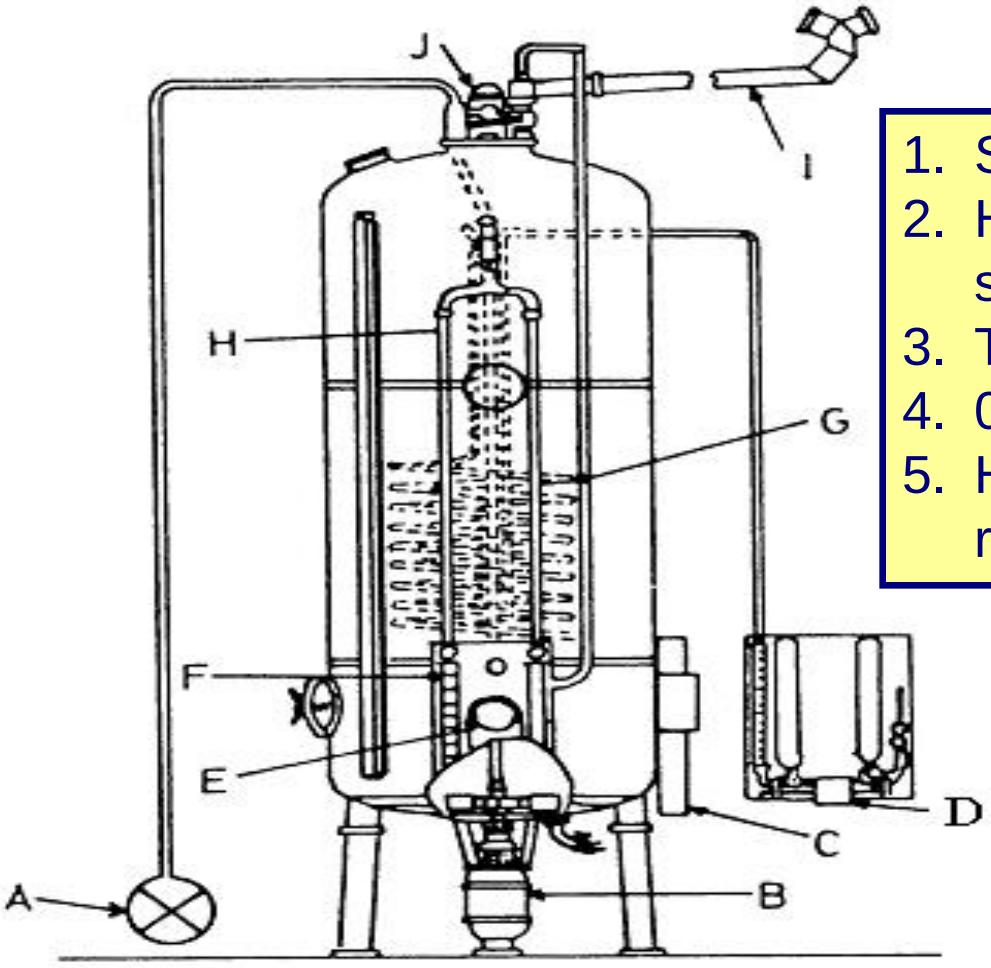


Fig. A vinegar brewery in Turkey using the Orleans process.



Rotating sparges are 28 (top)-35^{o}C (bottom)
Packed with coiled wood chip shaving
2-5 kg HAC/M^{3} packing volume /day
Terminated 0.3% ETOH

Fig. The quick vinegar process.



1. Semi-continuously 24-48 h
2. High efficient method of supplying air
3. Temperature 30oC
4. 0.1-0.3 ETOH stop running
5. Half of content pump off & replace with fresh material

Fig. The Frings Acetator A, charging pump; B, aerator and motor; C, Alkograph; D, cooling water valve; E, thermostat controlling D; F, rotameter; G, cooling coil; H, air line; I, air exhaust line; J, defoamer.

§Processing of vinegar

- in full, closed vat of wood or stainless steel for 1 year
- cloudiness

orleans process: less problem, without treatment

quick vinegar process: plate & frame filter press using diatomaceous earth filter aid

submerged acetification: Add fining agent Ising lass, casein, gelatin, bentonite clay most common

***Anguillula aceti*-vinegar eel**

Cause non-bacterial cloudiness

found in quick vinegar generator & Orleans process

not in submerged acetification

removed by pasteurization & filtration

- haze (ppt) formation

  due to non-acid-resistant materials, esp. iron & copper

- hot filled or pasteurized in bottle (60℃, 30 min) or chemical preservative (SO_2)

- concentration process

  [HAC] 10~15% by fermentation

  difficultly concentrated by fractional distillation process

  may use freezing concentration

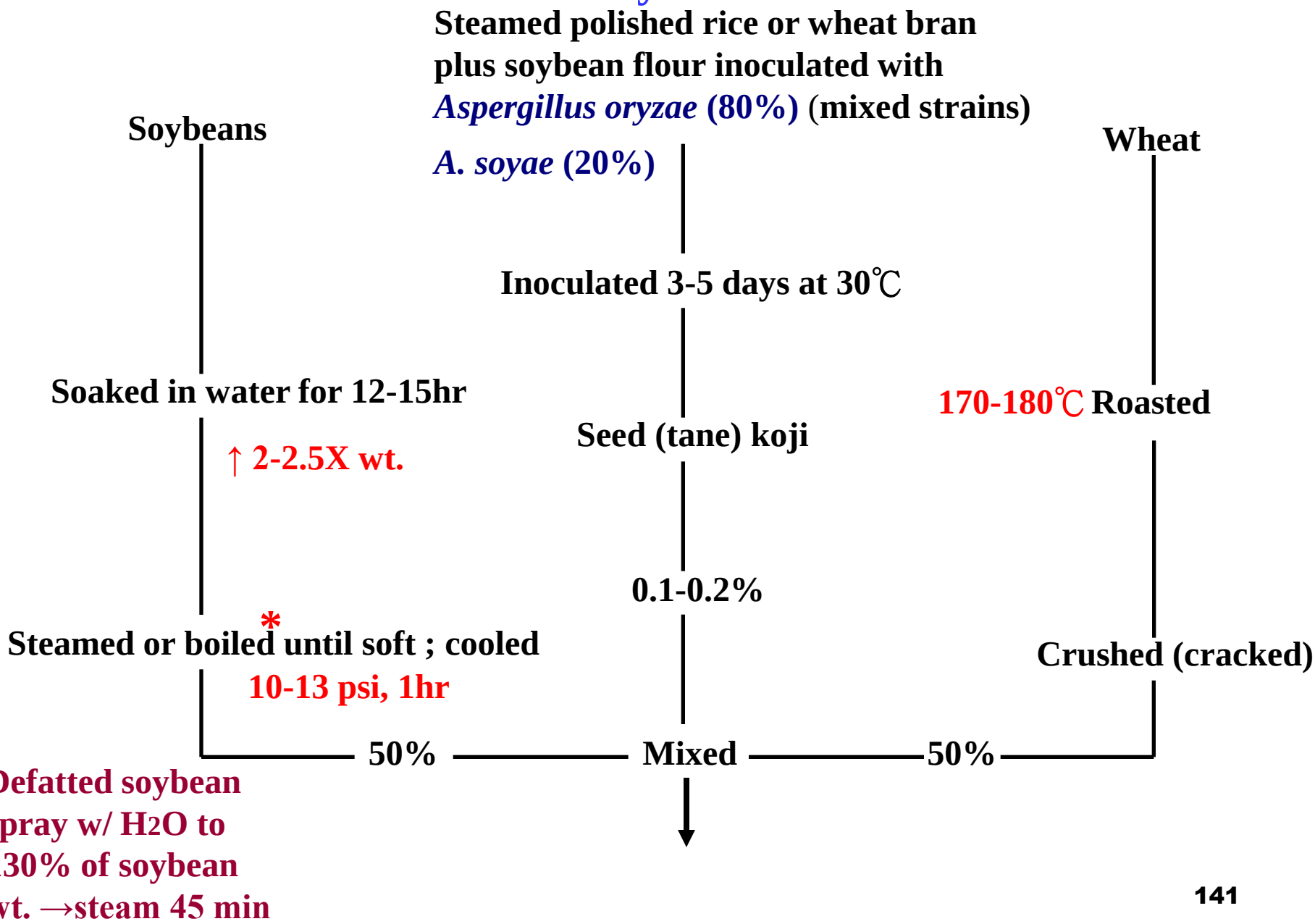
  or by mixing in a hydrate forming fluid

  (trichlorofluoromethane)

§Standards

- HAC content: min level 4% W/A in U.S.A,
  UK bottle normally contain 5%

Soy sauce


Steamed polished rice or wheat bran
plus soybean flour inoculated with
Aspergillus oryzae (80%) (mixed strains)
A. soyae (20%)
Soybeans
Wheat
Inoculated 3-5 days at 30℃
Soaked in water for 12-15hr
↑ 2-2.5X wt.
170-180℃ Roasted
Seed (tane) koji
0.1-0.2%
Steamed or boiled* until soft ; cooled
10-13 psi, 1hr
Crushed (cracked)
50%
Mixed
50%
Defatted soybean
spray w/ H2O to
130% of soybean
wt. →steam 45 min

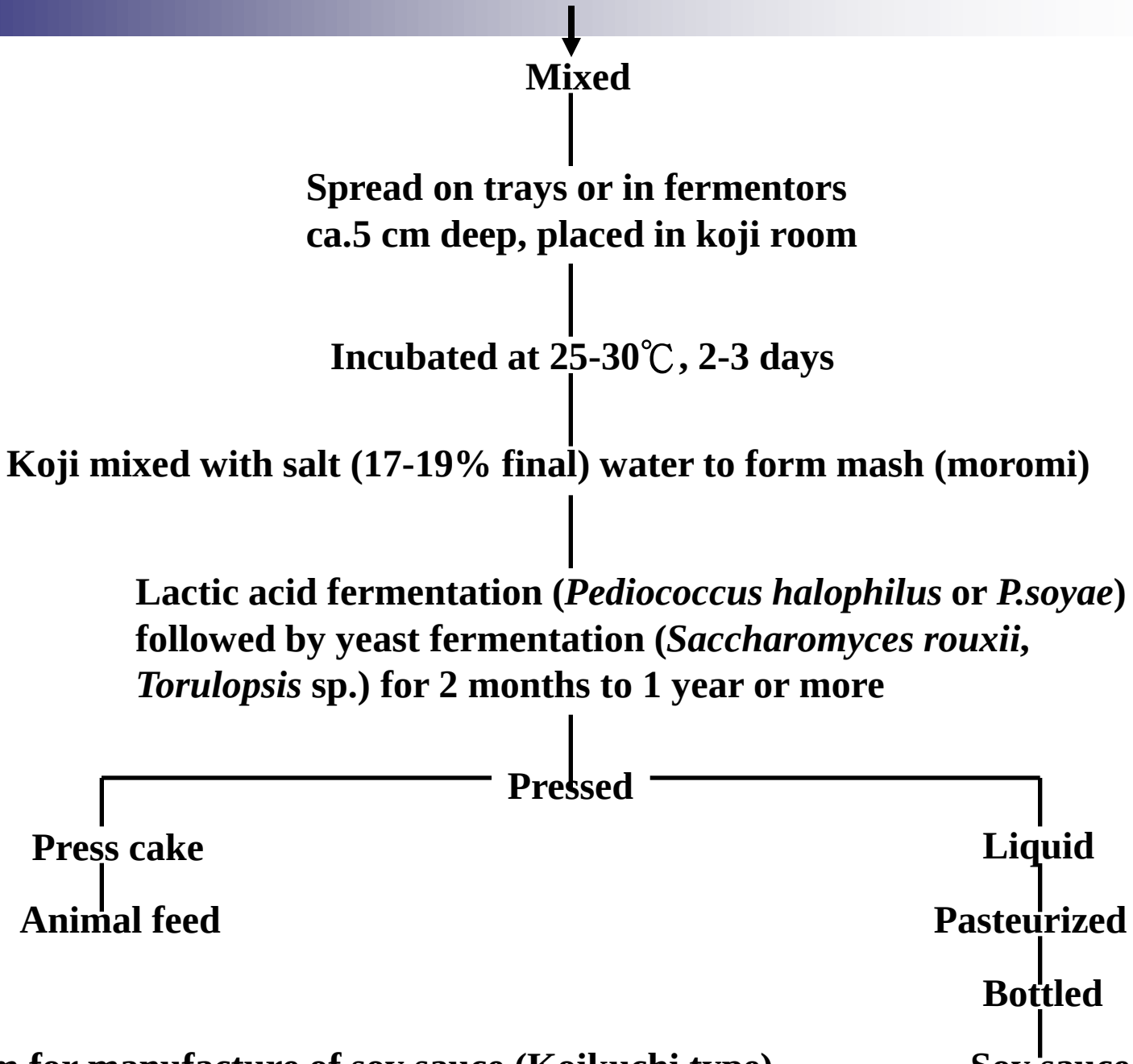


Fig. Flow diagram for manufacture of soy sauce (Koikuchi type).

* 近年來，使用高溫短時間 (HTST) 蒸煮，可提高蛋白利用率

(110~150℃, 15~20S, 消化率94%)

(120℃, 40~60min,消化率73%)

§Microbiology in Koji

Aspergillus oryzae (or *A. soyae*)

- procuce extracellular protease & protease & amylase breakdown polysaccharide & protein give substrate for bact. & yeast
- lipase→rancidity→problem

  if use defatted soybean→better
- temp. for greater en. (<30℃) production lower

  than for opt. growth temp. (30~33℃)
- -H_2O% 27~37% for en production

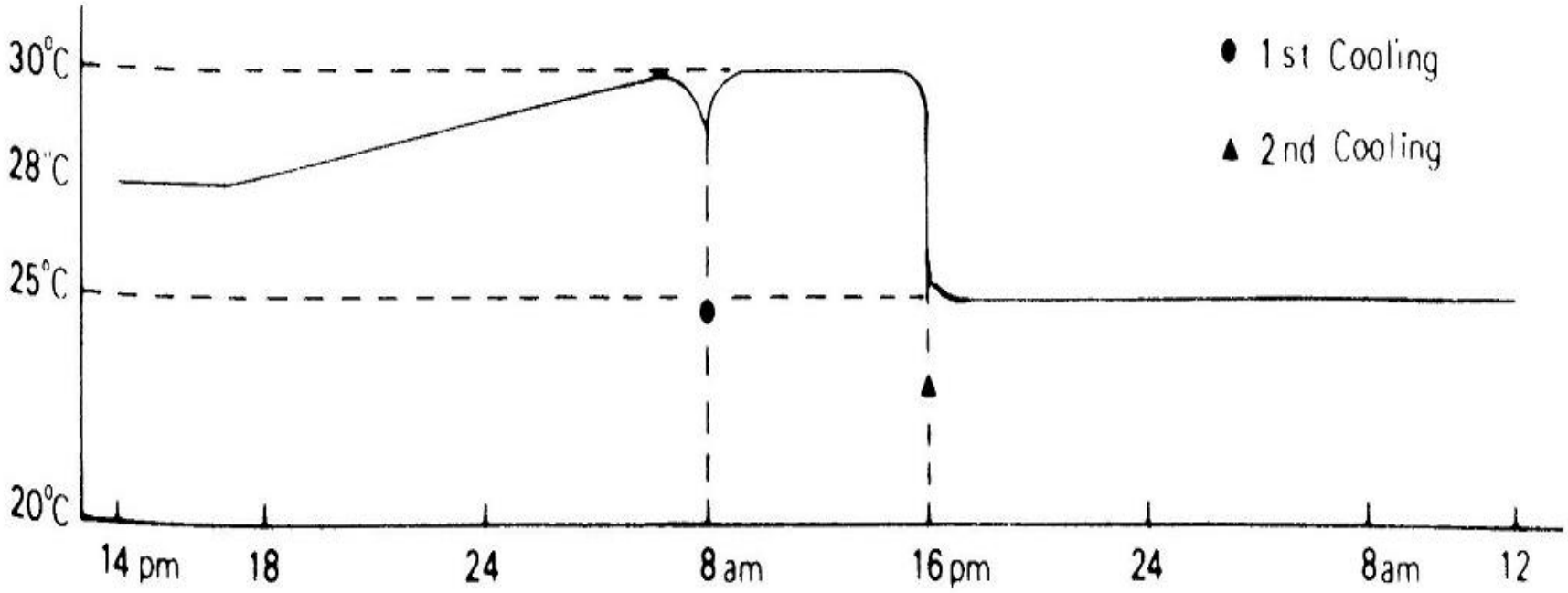


Fig. Preferable temperature change of materials during 3-day shoyu-koji cultivation.

§Moromi (mash)醬醪

- brine fermentation

- Koji + equal volume of salt soln
  = moromi recently use 1:1.1~1.2 ratio

- [NaCl]: 17~19%
  If < 16%⇒undesirable putrefactive bact.
  If > 23%⇒retard growth of desirable bact. & yeast

- -mycelium of mold is killed

(1) en. Function
(2) more A.A released
(3) balance between lactic fermentation & yeast fermentation
(4) good volatile flavor profiles

- Traditionally natural fermentation
  - Lactic acid fermentation (*Pediococcus halophilus*, *P. soysae* hetero~) go first→acid drop down 2 pH within first 10 D start from 10^2~10^3/ml→4 month 10^8~10^9/ml stop growth if pH < 5.0
  - After pH dropdown, *S. rouxii*, *Torulopsis* dominant
    S. rouxii can't grow in high salt except pH 4-5.
    Produce EtOH & many flavor (greatest contribution to sensory quality)
    Not ferment gal, sucrose or lactose
    In high salt, aerobic, convert 50% glucose→glycerol

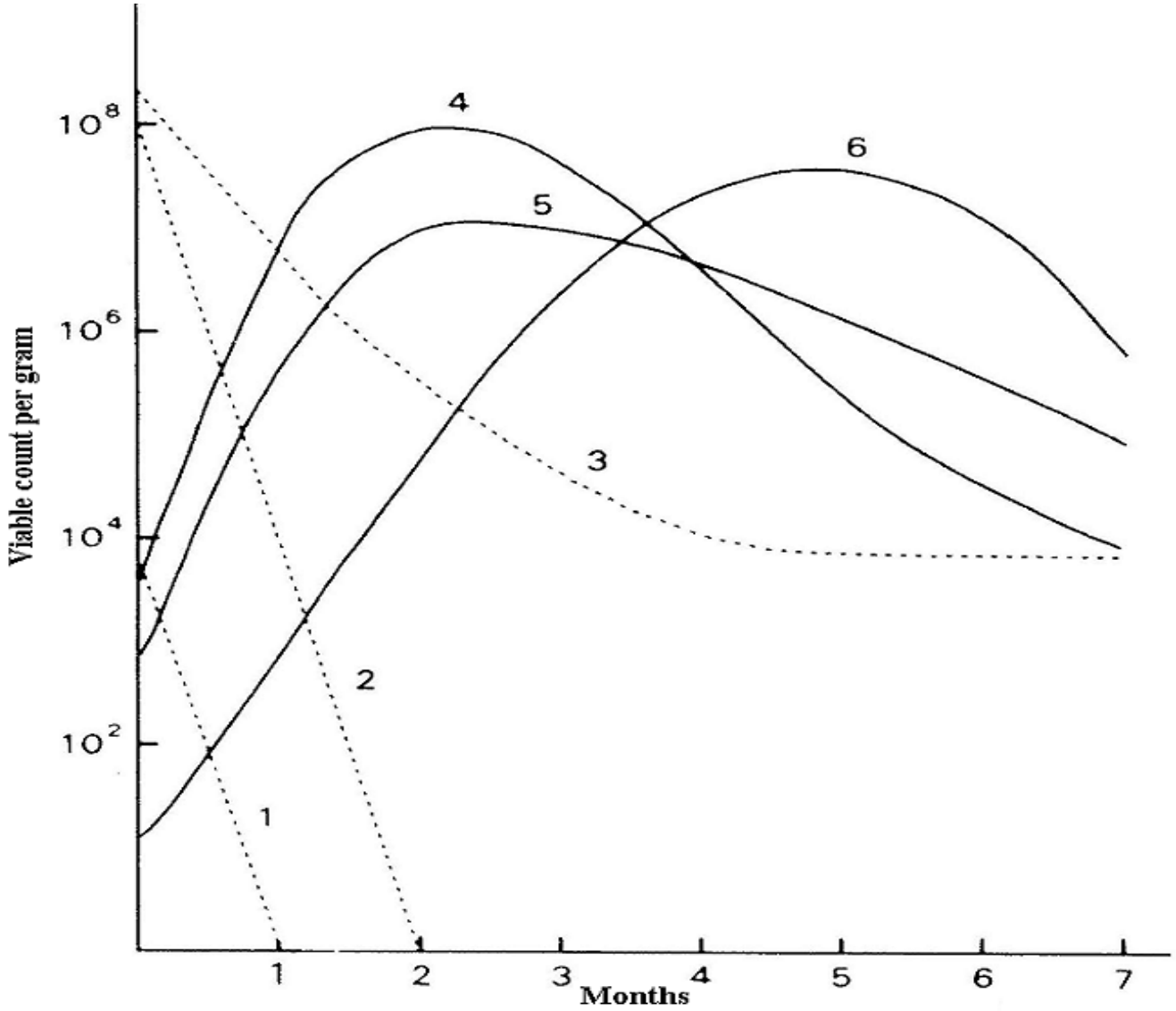


Fig. Microflora changes in shoyu mash fermentation. 1, wild yeasts; 2, *Micrococcus* spp.; 3, *Bacillus* spp.; 4, lactobacilli; 5, *Saccharomyces rouxii*; 6, *Torulopsis* spp.

- controlled fermentation by adding *P. soysae* & *S. rouxii* together or separately

- good product when temp. of moromi controlled

  as start 15℃ 1 month

  28℃ 4 month

  15℃ 1 month

冷醬醪作用

<1>防止peptidase (esp. 25-30℃失活 glutaminase)失活

<2>抑制乳酸發酵，防止pH迅速降低而使protease失活

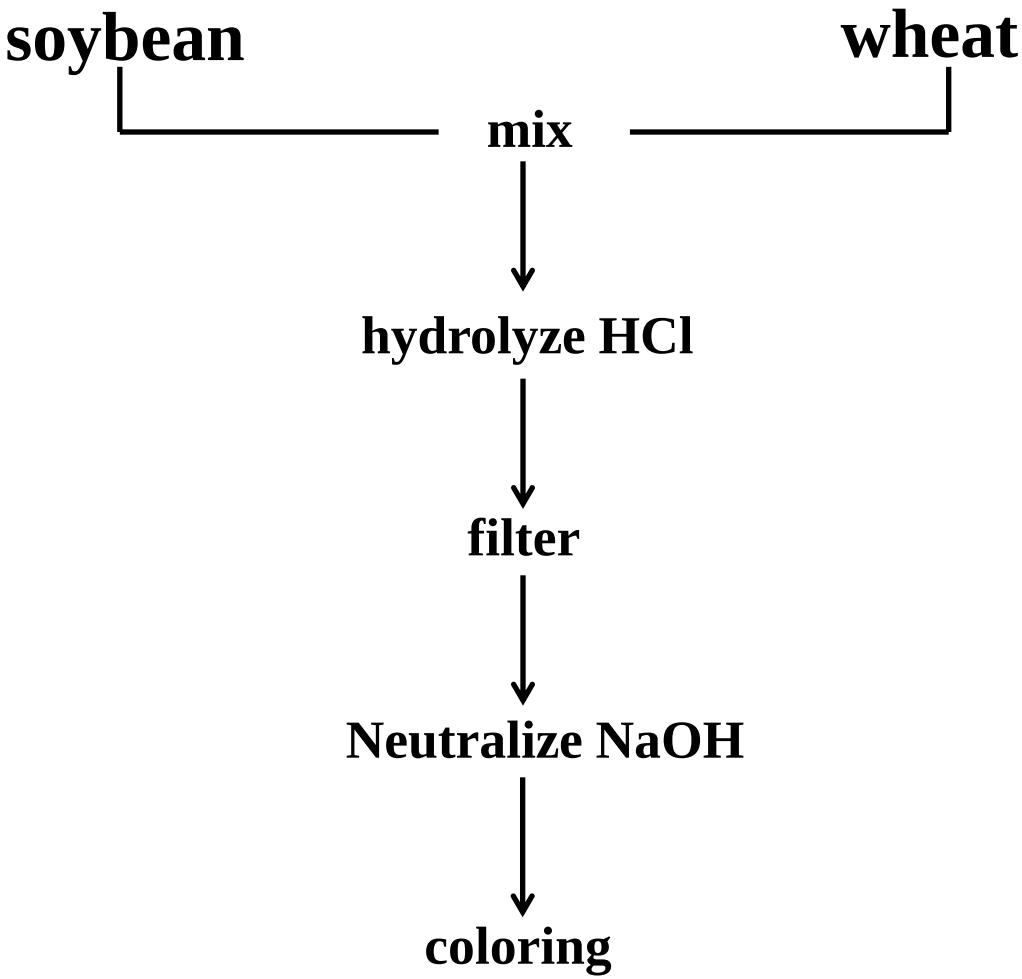
<3>防止前段yeast產生太多EtOH, if > 2%, protease失活

- final pH 4.6~4.8, 80~90% total protein soluble

total N in soy sauce 1.5~1.8%

Glycerol	0.4~0.5% (1.5%)
NaCl	18%
Lactic acid	1%
Glucose	1~5%
EtOH	2~3%

§Chemical process



§Pasteurization

70~80℃→filter→bottle (may add 50μg/ml butyl-p-hydroxybenzoate or 200μg/ml Na benzoate)

Vegetable Protein Hydrolysate (VPH)(I)

- VPH is used as the base for the formulation of various salty sauce products, such as shrimp sauce, oyster sauce, bar-B-Q sauce.
- Soybean is most frequently as the raw material and HCl is used to degrade soy proteins to produce VPH.
- Enzyme can also be used to degrade soybean. However, its cost is higher.

Vegetable Protein Hydrolysate (VPH)(II)

- Even the defatted soy flake is used for VPH production, it contains 5-10% of oil.
- During hydrolysis by HCl, the triacylglyceride in defatted soy flake is degraded into glycerol and free fatty acids.
- The glycerol can be further reacted with HCl to produce 3-monochloropropanediol (MCPD), 2,3-dichloropropanol
- MCPD, a potent carcinogen, cause cancer in rat (1 g/kg body weight ingestion), toxic to kidney and respiratory system
- Limit: 10 ppb in 1994 Europe Union

Miso

Miso-Japan, Taiwan, China, Korea, Philippines

Soybean paste (yellowish-white to dark brown)

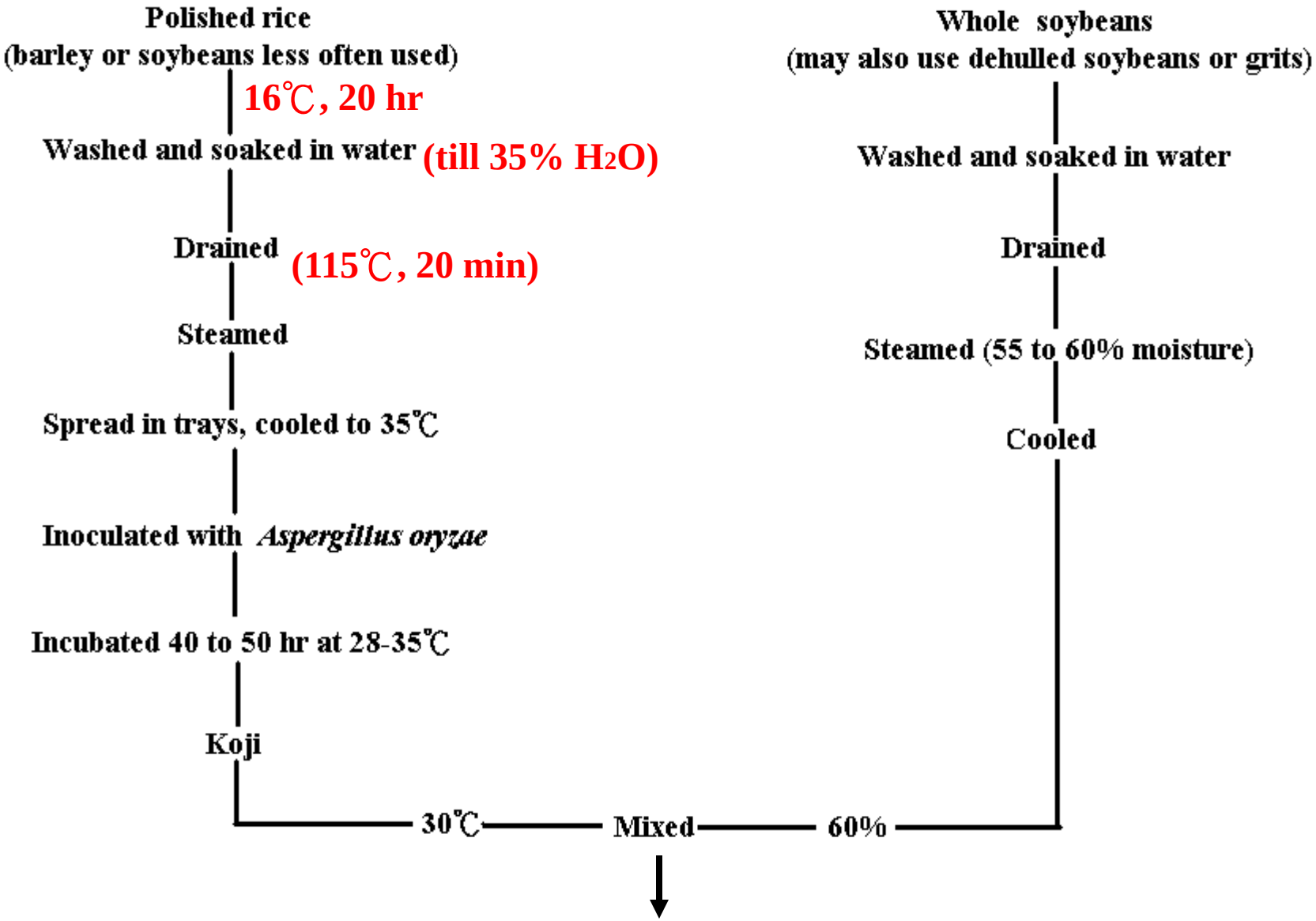
3 major type in Japan

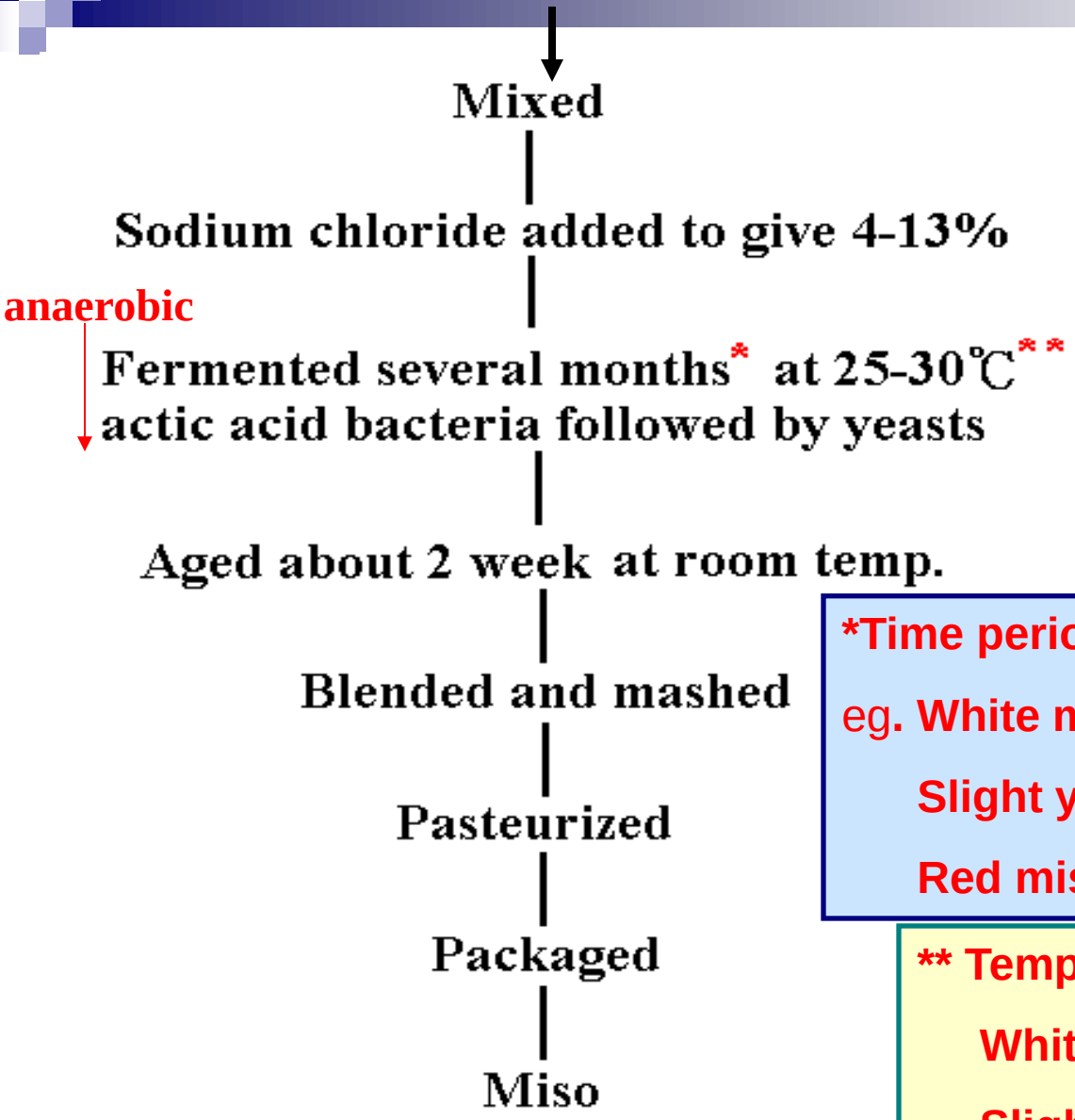
Rice miso (rice + soybean + salt)-80%

Barley miso (barley + soybean + salt)

Soybean miso (soybean + salt)

Miso


Polished rice
(barley or soybeans less often used)
16℃, 20 hr
Washed and soaked in water (till 35% H_2O)
Drained
(115℃, 20 min)
Steamed
Spread in trays, cooled to 35℃
Inoculated with *Aspergillus oryzae*
Incubated 40 to 50 hr at 28-35℃
Koji
30℃
Whole soybeans
(may also use dehulled soybeans or grits)
Washed and soaked in water
Drained
Steamed (55 to 60% moisture)
Cooled
60%
Mixed



Pediococcus halopilus

Streptococcus faecalis

S. rouxii

Torolopsis

***Time period dependent on types of miso**

eg. White miso 1wk (2-4 day)

Slight yellow miso 30 day

Red miso 60 day

**** Temp also various:**

White miso 50℃

Slightly yellow miso 30-35℃

Red miso 30-35℃

Fig. Flow diagram for manufacture of miso.

Similar to soy sauce except

(1) Koji from rice or barley only or other carbohydrate materials

(2) Miso→solid food, no filtration

Note: defatted soybean not suitable

Various cereal used, ratio of bean to cereal, salt content, length of fermentation, addition of other ingredient⇒different types of miso

Composition of various types of miso

	H_2O%	Protein%	Reducing sugar%	Fat%	NaCl%
White miso	44	8	33	2	5
Edo sweet miso	46	10	20	4	6
Salty light yellow miso	49	11	13	5	12
Salty red miso	50	12	14	6	13
Salty barley miso	48	12	11	5	12
Soybean miso	47	19	2	10	10

H_2O%: 44-50%

Prot%: 8~19%

Fat%: 2~10%

} ↑with soybean used

NaCl: most > 10% ∴keep at Tm, also limited as protein food

§Modification

- Okada: replacement of salt by EtOH→EtOH inactivate peptidase & inhibit M.O. growth

- In koji: total & free A.A. levels & color intensity↑α [NaCl]↓, but patterns of free A.A. & bound A.A. no difference

- Shien & Beuchat

  R. oligosporus instead of *A. oryzae*, & peanut instead of soybean⇒*S. rouxii* #↑, sol. N & F.A.A↑, FFA no difference.

§Tempeh

Soybeans

↓

Soaked in H_2O, seed coat removed

↓

Drained, cooked

↓

Drained, cooled

↓

Inoculated with *Rhizopus oligosporus*

↓

Packed into trays

↓

Incubated 20-24 h at 30-38℃

- main dish & meat substitute, low-cost protein not only used as flavoring agents
- only one oriental fermented product extensively investigated by the scientist in the West
- fermentation rather simple & quick

§Microbiology & Biochemistry

isolates from natural fermentation

25/40 *R. oligosporus* Saito

15/40
- *R. stolonifer*
- *R. arrhizus*
- *R. oryzae*
- *R. formosaensis*

- principal $(CHO)_2$ of soybean: stachyose, raffinose, sucrose not used as source of carbon.

- use glu, fru, gal, maltose, xylose

- various vegetable oils be used as energy source

  ∴strong lipase activity, use fatty acids of soybean as energy source

- highly proteolytic.

  2 proteolytic enzymes: opt pH 3.0 & opt 5.5

  both: max activity at 50-55℃

  stable at pH 3~6

  denature pH < 2 or >7

- one amylase activity, no pectinase

- antibacterial agent production & fast growth
  →reduce bacterial contamination
  purposely inoculated w/

E. coli	***B. mycoides***
Pseudomonas pyocyanea	***Proteus* sp.**

- De-hulling, soaking, washing, cooking, fermenting→loss of solid in soybean. totally 24.5~48.3%
  ∴ try to reduce water amount used in soaking, cooking.
  ⇒less mold development, unpleasant odor & poor flavor
  ⇒H_2O-soluble & heat stable mold inhibitor in soybean inhibit formation of proteolytic en.

  conclusion⇒soaking & cooking in excess water is essential

§Tempeh product

- perishable

  preservation:

  ▲ blanch the sliced tempeh (inactivate mold & en) & freeze

  ▲ hot air dry 93℃ 90-120 min⇒↓soluble solids

  ▲ sealed in a can & -29℃ for 10 weeks

  ⇒No significant change

exp:

- components change:
  pH↑due to protein degradation

- soluble solids 13→68% after 6 hr
  soluble N 0.5→2.0% ⇒free A.A.↑
  but total N constant

- ether-extractable substances↓⇒use lipid as energy source

- free A.A.↑but A.A. composition not change

- niacin, riboflavin, pantothenic acid & vitamin B_6↑but thiamin no significantly change

- lipids in tempeh more resistant to autoxidation than in control soybean eg. peroxide value: 1.1 vs 18.3~201.9

exp:

Tempeh at 37℃ for 5 months

peroxide value 6→12

control: 6→426

corn oil w/ 50% tempeh show higher antioxidant potential than oil w/ 25% tempeh 0.01% α-tocopherol, or 0.03% α-tocopherol

compound responsible for antioxidant activity unknown

- mycelial infiltration + enzymatic activity
  ⇒softness of soybean after fermentation

- Nutrition:

PER Tempeh similar as unfermented

Tempeh of frying 3min⇒↓PER

steam 2h no effect

antimicrobial agent-polypeptides having high $(CHO)_n$ content

esp. active against G(+) { anaerobe or microaerophilic

→delay time for gas forming, which frequently occurred due to stachyose, raffinose in soybean.

活性紅麴產品之研發

紅麴之製造過程

台大潘子明教授提供

紅麴生產步驟

Rice → Washing → Leaching → Steaming →
大米 洗淨 瀝乾 蒸米
(或山藥)

Inoculuming →Culture → Red mold rice
接種 培養 紅麴米成品

台大潘子明教授提供

培養於培養皿中之紅麴菌

培養於試管中之紅麴菌

顯微鏡下之紅麴菌

台大潘子明教授提供

常見的紅麴菌

- *Monascus pilosus*
- *Monascus purpureus* (2種健康食品菌株)
- *Monascus ruber*
- *Monascus floridanus*
- *Monascus albus*
- *Monascus sanguineus*
- *Monascus kaoliang* (以上7種BCRC存有)
- *Monascus anka* (被分類至*M. purpureus*)
- *Monascus pallens*

紅麴菌分類原則

依有性世代之形態分類

閉囊果與分生子孢子顏色	無色	子囊孢子 3.5- 4 × 2.5- 3 μm 不產生溶解性色素		*M. pallens*
		子囊孢子＞5 μm 產生紅色或桔色溶解性色素	子囊孢子 5- 7× 3- 3.5 μm	*M. pilosus*
			子囊孢子 6- 7 × 4- 4.5 μm	*M. purpureus*
	具重要色素特徵	子囊孢子 3.4- 4.5 × 2.5- 3 μm		*M. floridanus*
		子囊孢子 ＞ 5 μm	不具溶解性色素或褐色	*M. ruber*
			產生亮紅色溶解性色素	*M. sanguineus*

酒廠紅麴菌的分類

菌叢顏色	monacolin	澱粉水解酵素	供試菌株	典型菌種名
1.深紅色	無	中等--強	41	紫色紅麴
2.白色	無	中等--強	9	發白紅麴
1.粉紅色	無或有	弱—中等	11	叢毛紅麴
2.紅褐色	無或有	弱	29	巴克紅麴
3.煙灰色	無或有	弱	20	煙色紅麴

各地紅麴菌相關產品及主要菌種

產地	產品名稱	主要菌種
臺灣	紅露酒、紅豆腐乳	*M. anka*
	紅糟、紅糟泡菜、叉燒肉	*M. purpureus*
	高粱酒用麴子	*M. kaoliang*
香港	紅豆腐乳	*M. serorubescence*
中國大陸	紅糟、老紅酒、紅糟肉、	*M. purpureus*
	紅糟鰻、紅糟蛋、紅糟泡菜	*M. pilosus*
	叉燒肉、蘇武醬鴨等	*M . pubigerus*
	高粱酒用麴子(奉天)	*M. albidus*
	高粱酒用麴子(遼陽)	
	醬豆腐(上海)	
日本(沖繩)	紅豆腐糕	*M. anka*
		M. purpureus

三、紅麴製造流程

現代紅麴製造流程

- 原料處理
- 接種
- 翻拌
- 頭水
- 次水
- 完水
- 後熟
- 乾燥

紅麴之製造—接種後之管理

- 第1天：接種　接種後品溫降至 33±1℃，用紗布包妥，置於恆溫箱中，控制箱溫為 35±1℃，俟品溫升高達 40℃，攤開，將蒸飯集中於麴盤的中央。

- 第2天：翻拌　菌絲急速繁殖，為防止品溫過高，適時給予翻拌，且視繁殖情形，將蒸飯厚度逐漸改薄，以控制保持紅麴菌的最適繁殖條件（即品溫在34±1℃，濕度在 85±1%）。

紅麴之製造—接種後之管理

- 第3天：頭水　紅麴菌急速繁殖，米粒中水分，除一部分溫度上升被蒸發外，大部分被繁殖所消耗，故米粒變乾燥。為使紅麴菌順利繁殖須施行浸水，給予適當水分。將麴盤內的半製品取出，浸於無菌水中30分鐘，浸畢用紗布濾水30分鐘，使水分保持50±2%。頭水後仍將半製品盛於麴盤內，並放入恆溫箱培養。

- 第4天：次水　因此時期半製品變紅色，繁殖最為旺盛，水分被紅麴繁殖及蒸發所消耗，故須施行第二次浸水，此項操作稱為次水。將半製品用紗布包妥，浸於無菌水中20秒鐘，次水後水分約為 47±2%。

紅麴之製造—接種後之管理

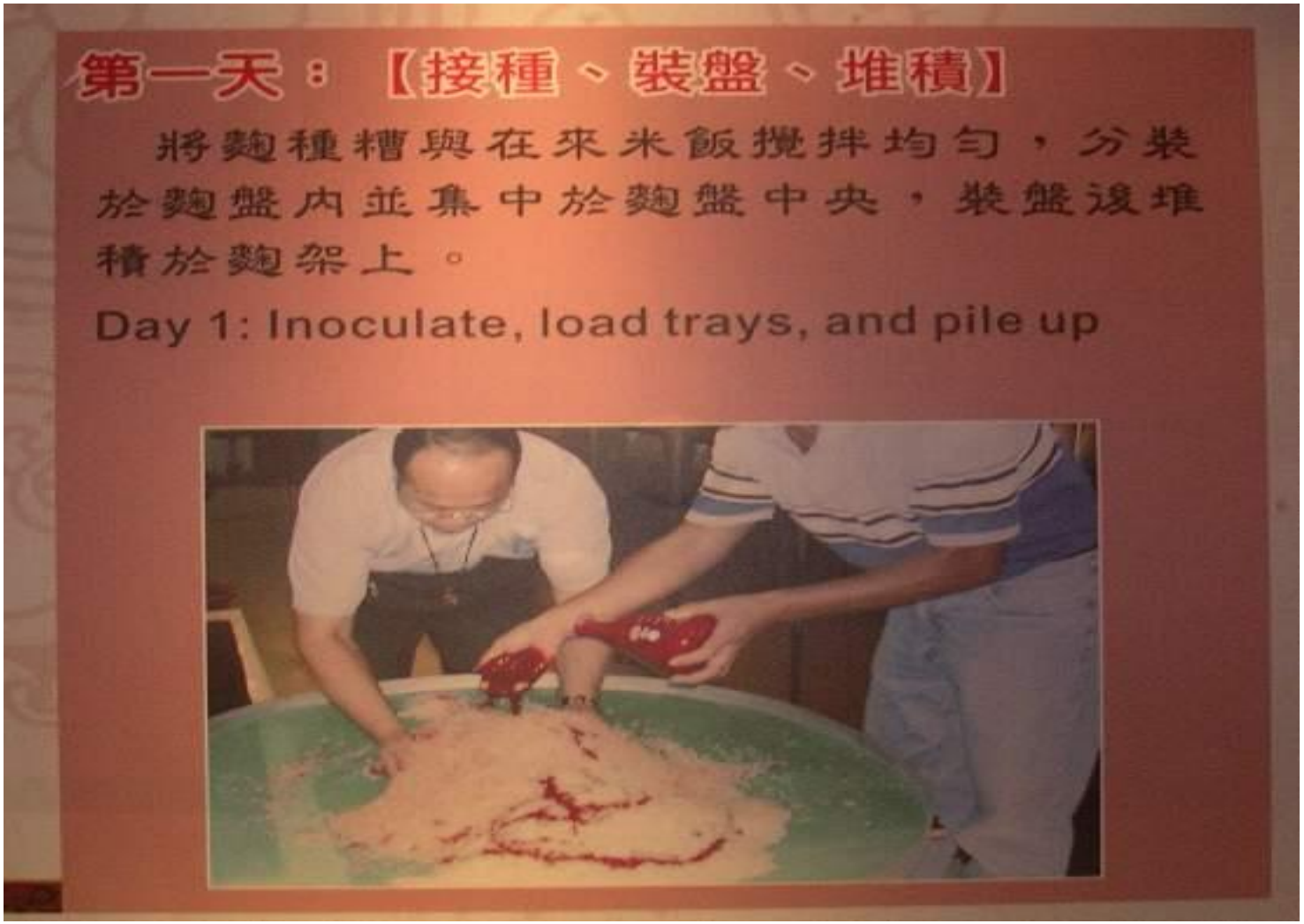
- 第5天：完水　為調節半製品適當水分，俾促進繁殖與菌絲的滲透，須施行第三次灑水，此項操作稱為完水。品溫須控制不超過40℃，完水後水分含量約為48±2%。

- 第6天：後熟　完水後，漸進入後熟階段。此時須給予適當翻拌，約10小時翻拌一次。品溫須控制在30±1℃，以促進繁殖。

- 第7天：乾燥　將紅麴在45℃下進行乾燥22小時。

- 第8天：成品　乾燥後的紅麴為成品，其水分含量約為 10±1%。

第一天：接種、裝盤、堆積

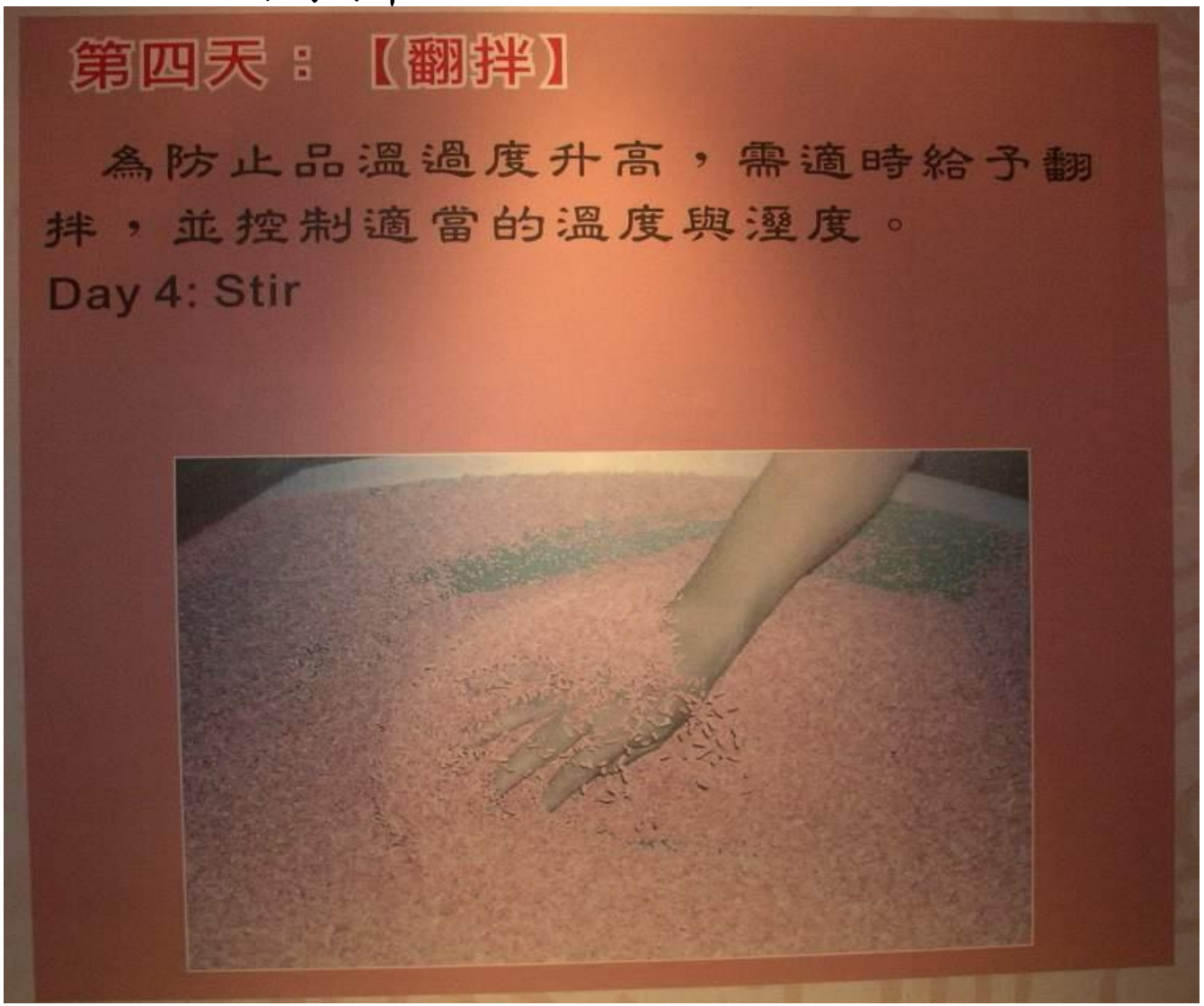

第一天：【接種、裝盤、堆積】
將麴種糟與在來米飯攪拌均勻，分裝於麴盤內並集中於麴盤中央，裝盤後堆積於麴架上。
Day 1: Inoculate, load trays, and pile up

第二天：翻拌

第三天：頭水


第三天：【頭水】
飯粒上會出現淺紅色的紅麴菌斑，手抓麴料有乾燥感覺時，實行第一次浸水，以補充水分，使紅麴菌順利繁殖。此項操作稱為「頭水」。
Day 3: First water immersion

第四天：翻拌



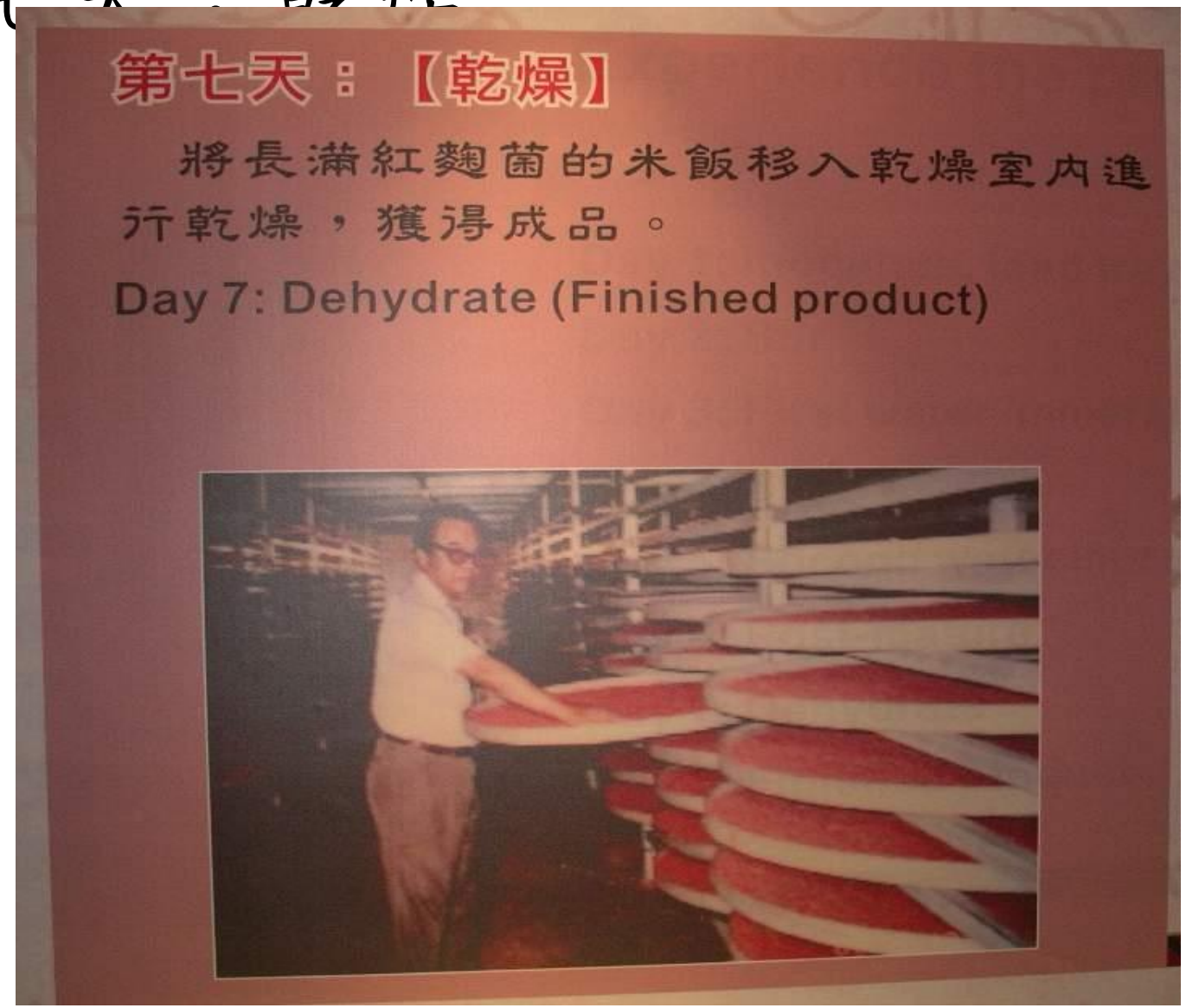
第五天：次水


第五天：【次水】
此時期紅麴半製品顏色變紅最為顯著，紅麴菌繁殖非常旺盛，水分被紅麴菌繁殖及蒸發所消耗，需實行第二次浸水，以補充水分。此項操作稱為「次水」。
Day 5: Second water immersion

第六天：後熟


第六天：【後熟】
次水後，進入後熟階段。需適時給予翻拌並控制溫度，使紅麴菌繼續繁殖至長滿整個米飯。
Day 6: Post-mature

第七天：乾燥


第七天：【乾燥】
將長滿紅麴菌的米飯移入乾燥室內進行乾燥，獲得成品。
Day 7: Dehydrate (Finished product)

紅麴成品

紅麴所產生的高價經濟產物

- 菌體外水解酵素
- 一級代謝產物（不飽和脂肪酸、醇及酯類化合物）
- 二級代謝產物
  - 色素（紅色、橘色和黃色等）
  - 抗腐敗菌物質（monascidin）
  - 膽固醇合成抑制劑（monacolin）
  - 降血壓物質（GABA）
  - 天然抗氧化劑，如類黃酮（flavonoids）
  - 降血糖及其他尚待鑑定的生理活性物質

紅麴菌生產的二級代謝產物

- 天然抗氧化劑：

  紅麴的抗氧化能力,於1999年被 Aniya 等提出,得知其抗氧化能力的成分為dimerumic acid

- 降血糖物質：

  1988年玉田英明,發現兔子在進食添加0.2-0.3%紅麴培養物的飼料,一小時後血糖量比對照組下降了19-29%,不過有效成分尚待進一步分析

紅麴菌生產的二級代謝產物

- 色素：

  紅麴色素的研究很廣，目前已知有八種化學結構被確定出來，可分為

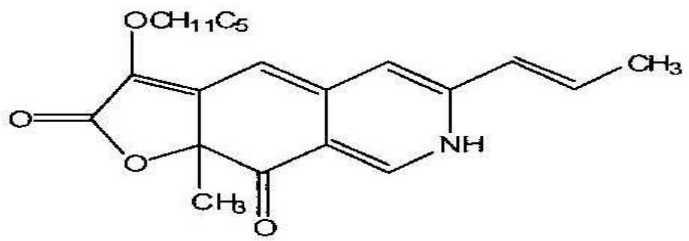
  - 紅色素(monascorubramine 及rubropunctamine)
  - 橘色素(monascorubrin, rubropunctatin)
  - 黃色素(ankaflavin、monascin 、yellowⅡ 、xanthomonascin)。

- 抗腐敗菌物質：

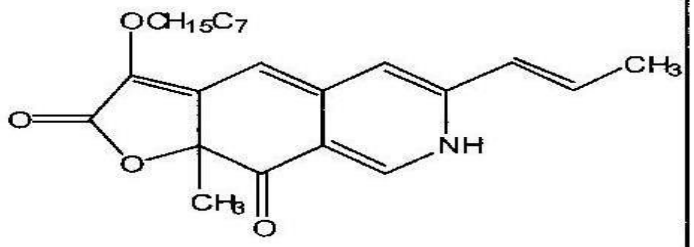
  M. purpureus 於1977年被 Wong 及 Bau等報導具有抗菌效果,經學者研究 monascidin A為主要抗菌成分

紅麴色素結構1

Red pigment

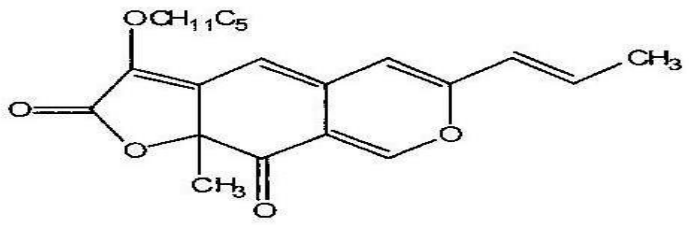


Rubropunctamine(M=353)

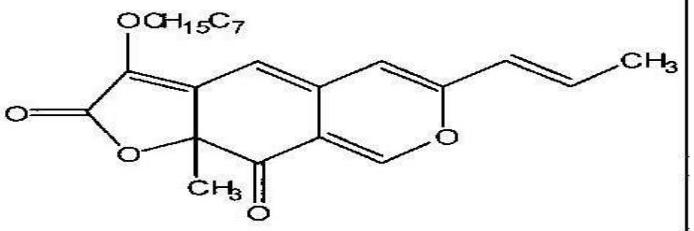


Monascorubramine(M=381)

Orange pigment



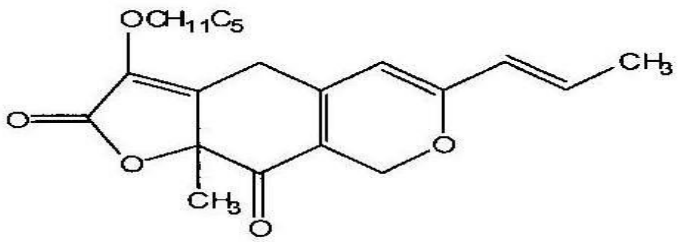
Rubropunctatine(M=354)



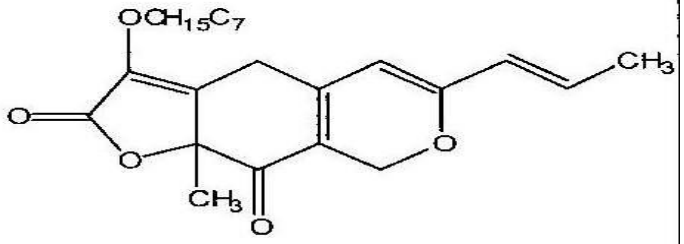
Monascorubrine(M=382)

紅麴色素結構2

Yellow pigment



Monascine(M=358)



Ankaflavine(M=386)

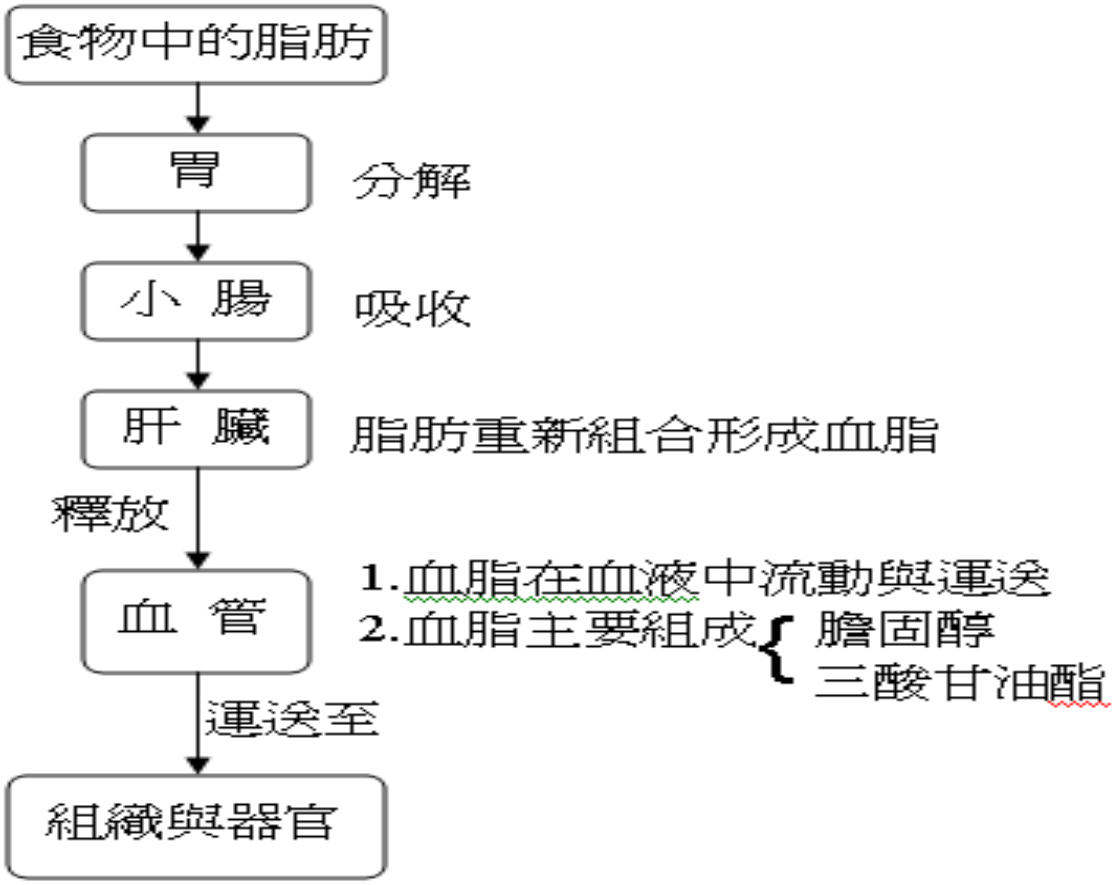
紅麴與膽固醇

- 膽固醇合成抑制劑：

  1979年遠藤教授,率先從分離自泰國發酵食品的粉紅色紅麴菌 Monascus ruber 的培養液中,找到 Monacolin K (Mevinolin),可抑制膽固醇合成過程中關鍵酵素HMG-CoA reductase的活性,而達到降低膽固醇的效果

- 降血壓物質：

  1993年發現添加0.2-0.3%紅麴培養物的飼料,可使患有先天性高血壓症老鼠,血壓由200mmHG降至180 mmHG以下,有效成分為 GABA 及 glucosamine


食物中的脂肪
胃
分解
小 腸
吸收
肝 臟
脂肪重新組合形成血脂
釋放
血 管
1.血脂在血液中流動與運送
2.血脂主要組成
膽固醇
三酸甘油酯
運送至
組織與器官

血脂代謝機制

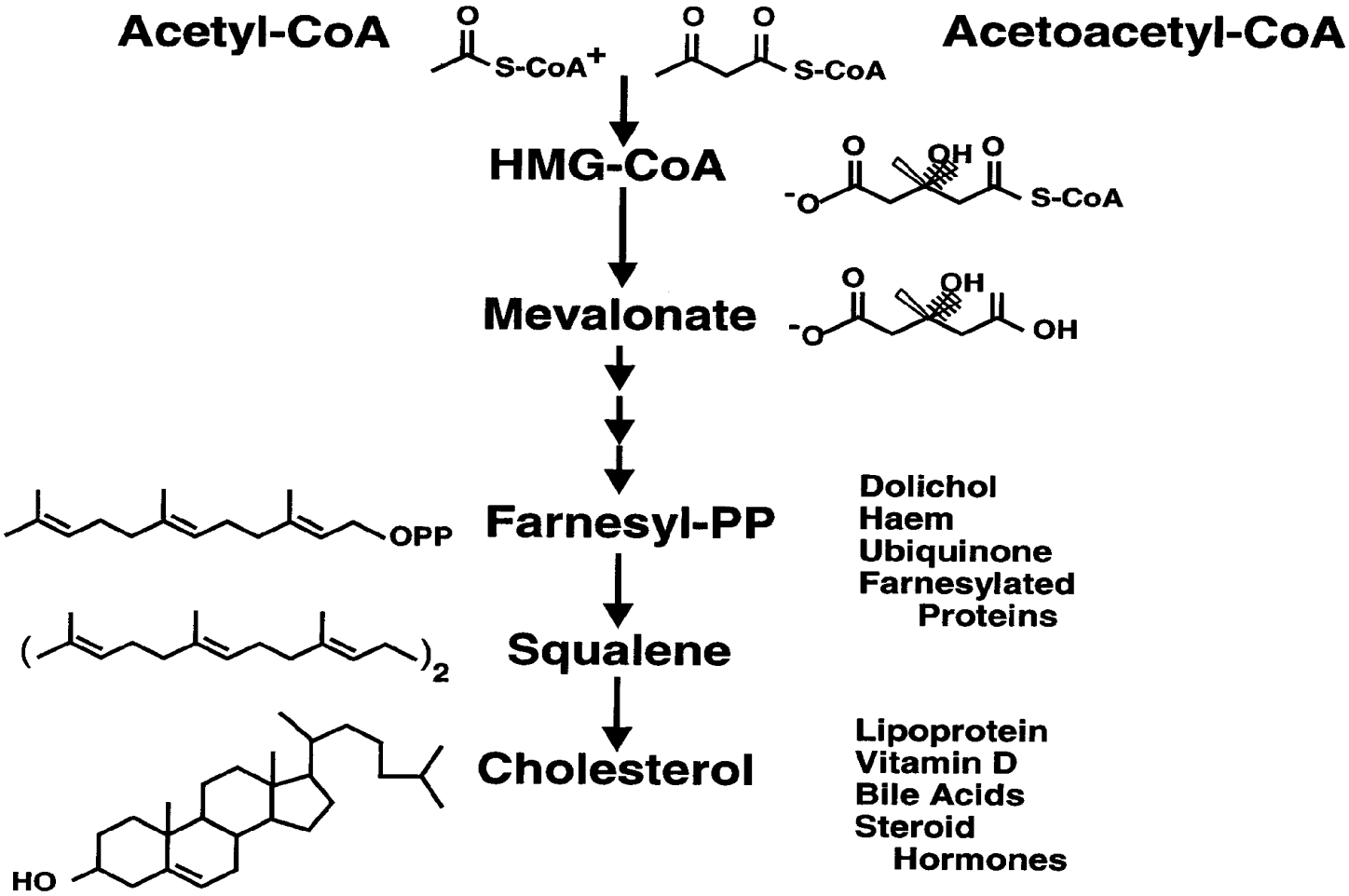


圖1.1 膽固醇之生成

(Istvan et al., 2003)

紅麴降低膽固醇的機制

- 在降低膽固醇的藥物中，目前最有效的是HMG-CoA reductase。
- *Monascus*黴菌屬中提煉出九種能抑制 HMG-CoA reductase 的成分，其中一種成分便是Monacolin K (Stuart *et al*., 1979)
- 現今已有許多monacolin衍生物，均統稱為 statin，可以抑制 HMG-CoA reductase，而使肝臟無法製造膽固醇。

(Wei *et al*., 2003)

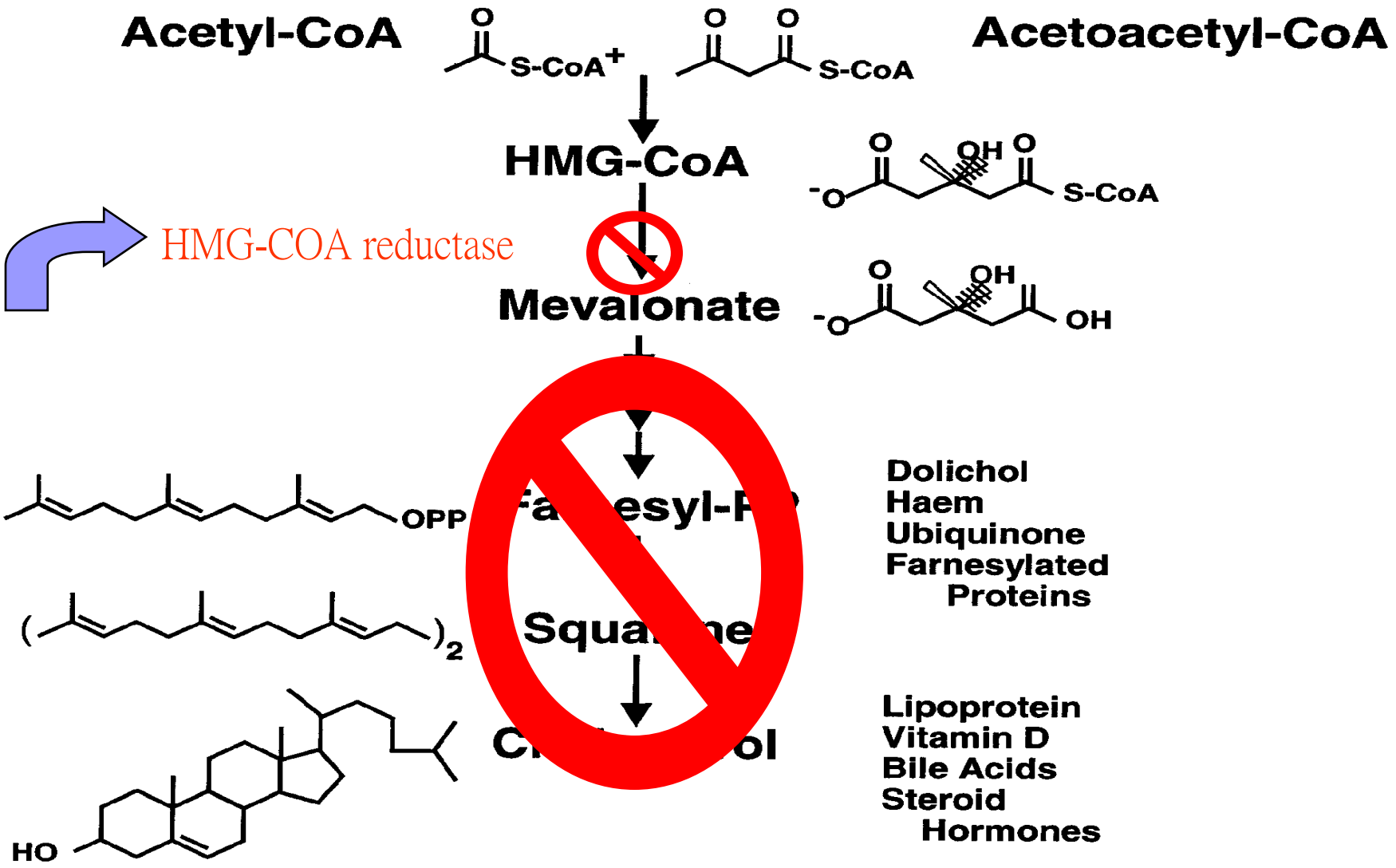
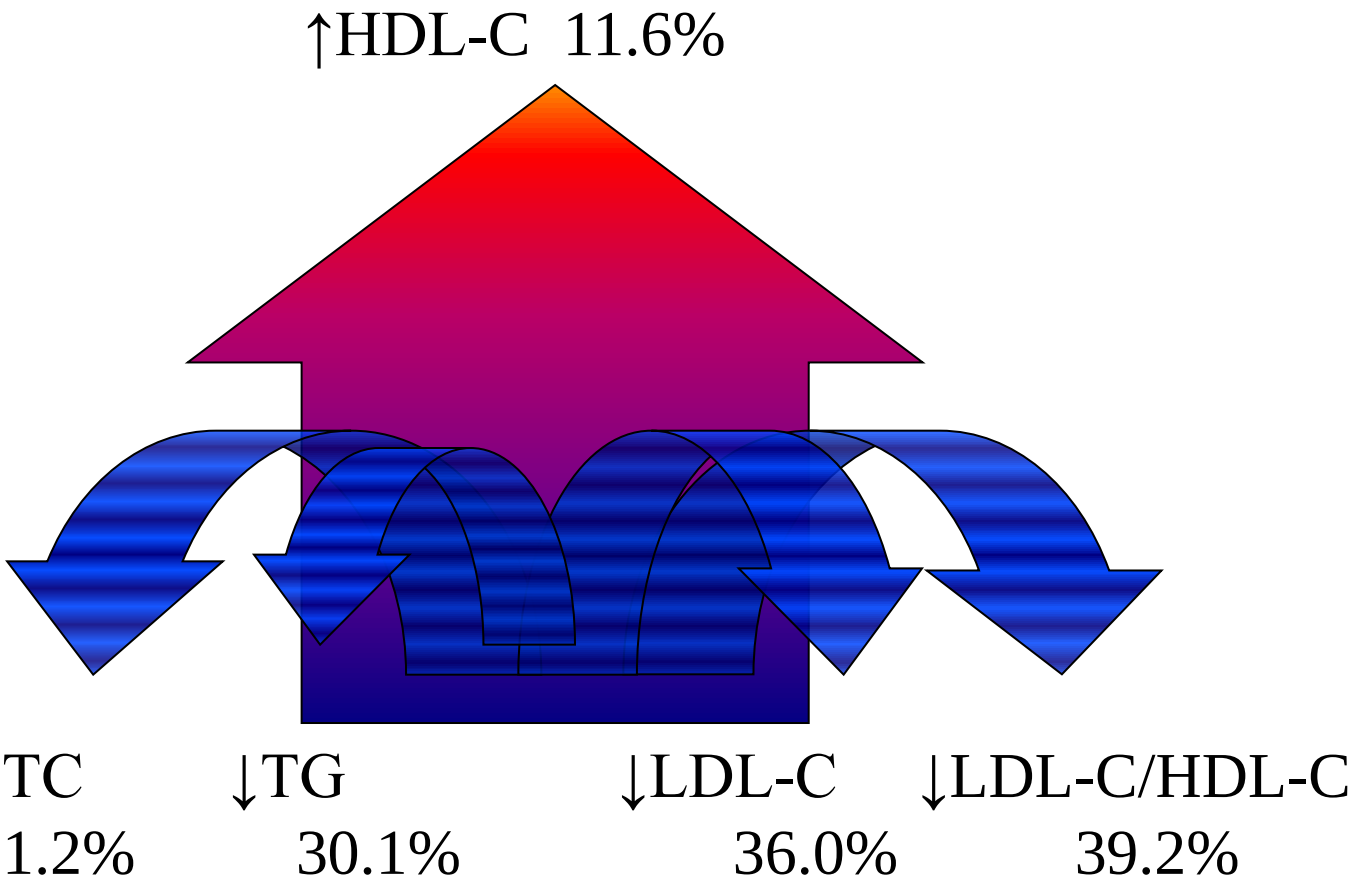


圖1.2 抑制膽固醇

(Istvan et al.,2003)

由 *Monascus purpureus* NTU 568 生產紅麴降膽固醇功效 (4 週, 10.78 mg/天/100 g 體重)



In vivo hypolipidemic effects and safety of low dosage monascus powder in a hamster model of hyperlipidemia, Appl. Microb. & Biotech. (2006) 70: 533-540.

紅麴在預防醫學上之應用
—血脂調節之結論

- 餵食紅麴組 (每天每100克體重餵食10.78毫克)，經四週後血漿中總膽固醇顯著下降 31.2%。
- 餵食紅麴組，經四週後血漿中總三酸甘油酯及低密度脂蛋白膽固醇比高飲食組顯著下降 30.1%及36.0%。
- 高密度脂蛋白膽固醇比高飲食組顯著上升 11.6%
- 餵食紅麴血漿中GOT及GPT無明顯變化。
- 餵食紅麴組之肝切片與控制組無差異。

台大潘子明教授提供

GABA(Gamma-Aminobutyric Acid)

作用機制目前已證實主要為γ-胺基丁酸可透過GABA受體系統 (GABAergic system) 達到控制人體各項生理功能，其作用機制主要包括:

1.會讓血管擴張，降低血管收縮，使血壓降低；

2.會阻斷交感神經系統之末梢神經活性，所以有 降低或延緩神經過度敏感所引起的問題，例如失眠、焦慮、癲癇、阿爾海默茲症...等；

3.可以促進生長激素分泌；

4.人體最重要的抑制性神經傳導物質。

- γ-胺基丁酸是人體分佈最廣，也是最重要的抑制性神經傳導物質，自1950年代科學家就發現人體腦髓、血液及許多器官組織中含豐富的γ-胺基丁酸。
- 根據動物實驗證實，在飼料中添加紅麴培養物的動物試驗中，發現添加的紅麴培養物的飼料，可使患有先天高血壓症老鼠的血壓由 200 mmHg 降至 180 mmHg 以下。

紅麴含GABA之降血壓作用機轉：

1、GABA 主要作用於腎臟交感神經上的GABAB receptor，可抑制 noradrenaline的分泌，而達到降血壓之作用。

2、GABA會降低 renin 之釋出，即降低血漿中 renin 的濃度，而使的 angiotensin II無法形成，則不會引起血壓上升。

3、GABA 對於自發性高血壓之患者可降低其血壓而改善其症狀，但對正常血壓的人則無影響。

4、當投予 GABA 與 control 組對照，排出尿的體積、排出尿的 Na 量、排出尿的Na/creatinine 比，顯示投予 GABA 組呈明顯上升趨勢，因尿中排出水分及鈉離子增加，腎的蓄積水及鈉離子量降低，則不會誘發 noradrenaline 及 renin 的分泌，相對亦較不會引起血壓上升。 (Table 1)

5、當投予 GABA 與 control 組對照，對於心收縮壓作用亦呈現顯著下降之差異。

(Fig. 1)

Table 1. Experiment 1: Time-course data for body weight, urine volume, urinary Na, and Na/creatinine in spontaneously hypertensive rats treated with GABA.

	Age (weeks)	Body weight (g)	Urine volume (ml 24 h^{-1})	Urinary Na (nmol 24 h^{-1})	Na/creatinine (nmol mg^{-1})
Control	8	190.6±1.3	10.1±0.6	2.6±0.1	0.65±0.01
	9	229.5±1.6	10.9±0.8	2.8±0.1	0.60±0.02
	10	263.2±2.3	11.5±0.8	2.8±0.1	0.57±0.02
	11	285.8±3.2	13.9±0.7	3.0±0.1	0.50±0.01
	12	305.9±3.2	15.1±0.7	2.9±0.1	0.43±0.01
GABA	8	190.6±1.4	10.3±0.8	2.6±0.1	0.63±0.02
	9	231.0±1.9	11.7±0.9	2.9±0.1	0.65±0.02
	10	259.8±2.5	12.4±1.3	3.1±0.2	0.64±0.02*
	11	281.8±2.6	14.9±1.0	3.2±0.2	0.55±0.04
	12	300.8±2.9	14.9±1.0	3.2±0.2	0.53±0.02**

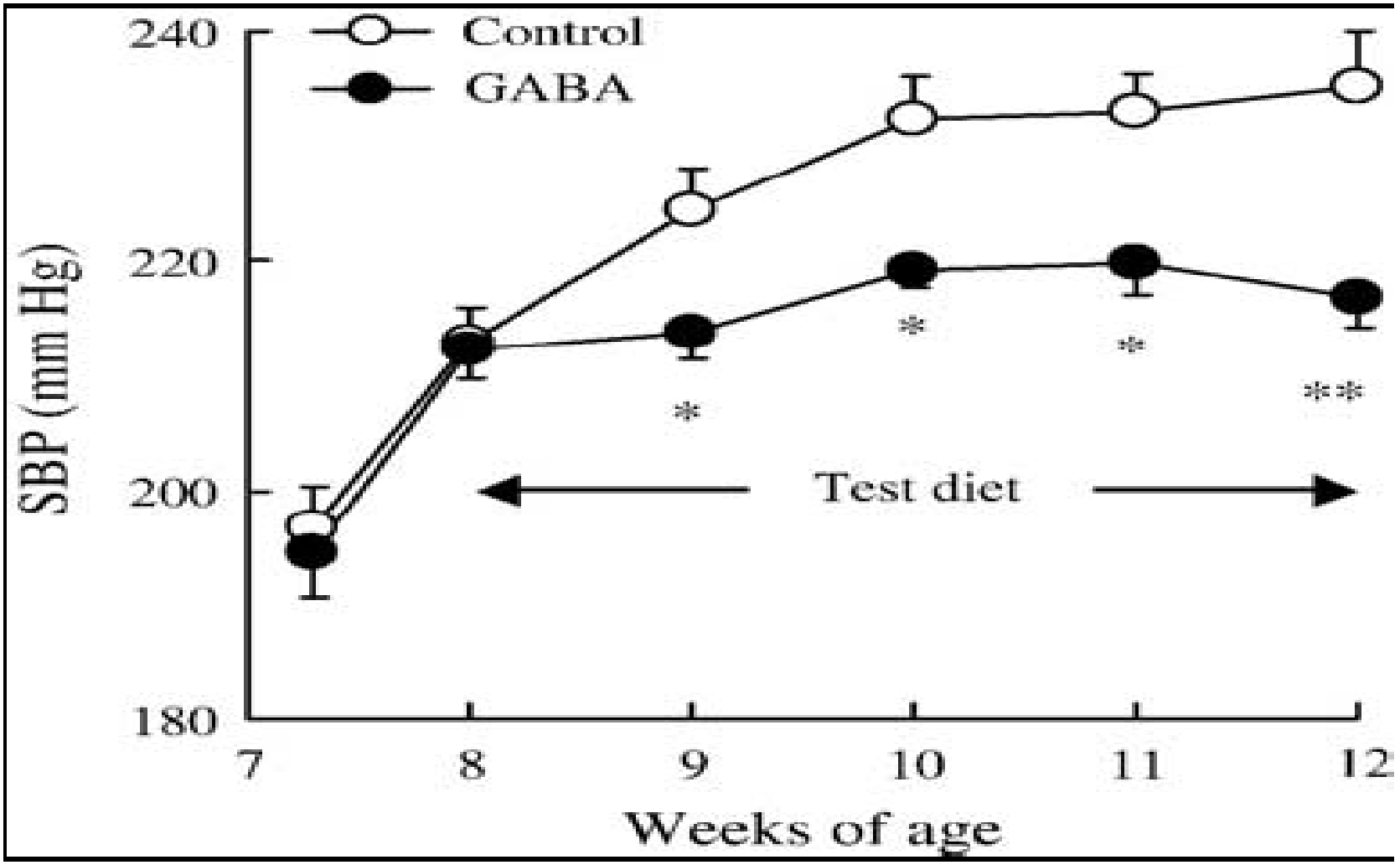
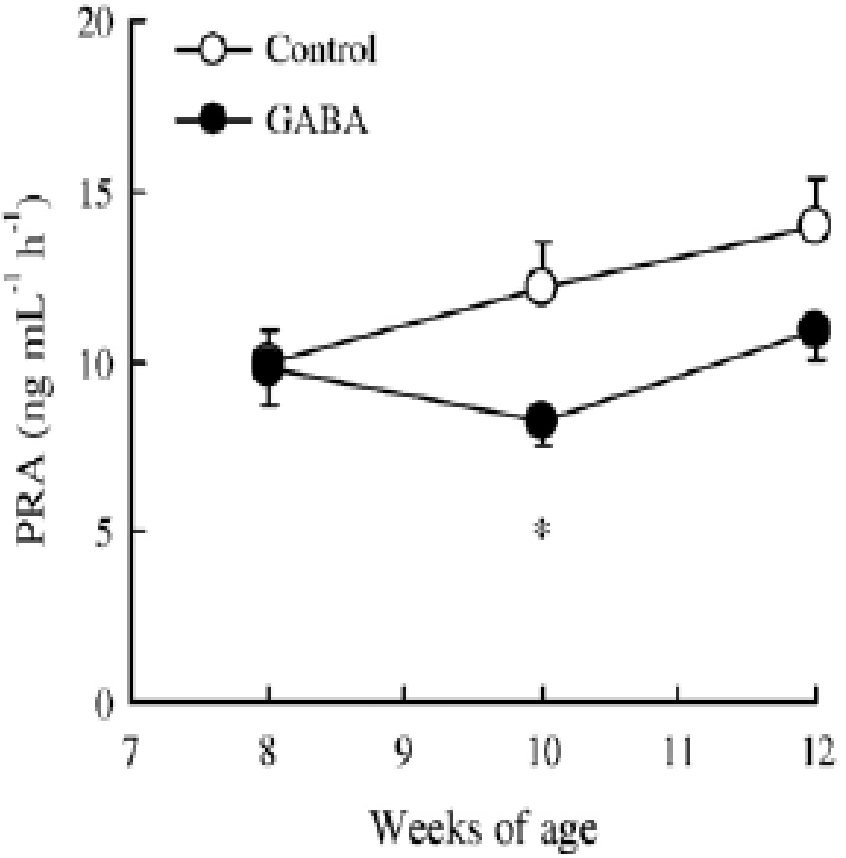


Fig. 1. Experiment 1. Time-course data for systolic blood pressure in spontaneously hypertensive rats treated with GABA. Each data-point represents the mean±S.E.M. for 10 animals. SBP, systolic blood pressure.



當投予 GABA 與 control 組對照，對於血漿中腎素(rennin)之活性亦呈現顯著下降之差異。

Fig. 2. Experiment 1. Time-course data for plasma renin activity in spontaneously hypertensive rats treated with GABA. PRA, plasma rennin activity.

Monascus purpureus NTU 568 生產紅麴抗疲勞功效

- **試驗動物：16 周齡雄 Wistar rat**
- **餵食時間：28 天**
- **餵食劑量： 5 g/kg 體重 (HD)或 1 g/kg 體重 (LD)**
- **試驗項目：**
- **運動：游泳**
- **生化檢驗：**
  - **乳酸 (lactate concentration)**
  - **尿氮 (blood urea nitrogen, BUN)**
  - **紅血球 (hemoglobin concentration)**
  - **血糖 (glucose concentration)**
  - **脂質過氧化 (lipid peroxidation)**

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游泳試驗

組別	體重 (g)		游泳時間 (分鐘)	游泳時間增加 (%)
	試驗前	試驗後		
控制組	427.3±30.1[a]	491.4±33.4[a]	78.0±6.4[a]	-
低劑量組	421.1±32.9[a]	477.1±41.2[a]	104.2±9.6[b]	33.59
高劑量組	435.2±33.3[a]	486.2±40.3[a]	129.4±10.9[c]	65.90

Effect of red mold rice on antifatigue and exercise-related changes in lipid peroxidation in endurance exercise, Appl. Microb. & Biotech. (2006) 70: 247-253.

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乳酸與血糖

組別	乳酸 (mg/dL)		血糖 (mg/dL)	
	游泳前	游泳後 (變化%)	游泳前	游泳後 (變化%)
控制組	29.52±1.44[a]	45.00±0.90[a] (+52.44%)	124.00±13.08[a]	76.67±8.08[a] (-38.17%)
低劑量組	27.72±0.99[a]	31.41±1.80[b] (+13.31%)	120.33± 4.62[a]	111.33±8.50[b] (-7.48%)
高劑量組	27.63±1.17[a]	28.89±1.62[c] (+4.56%)	121.33±10.50[a]	117.67±11.06[b] (-3.02%)

The blood lactates concentrations of low dosage and high dosage groups were significantly lower (decrease 39.13% and 47.88%) than those of the control group.

Effect of red mold rice on antifatigue and exercise-related changes in lipid peroxidation in endurance exercise, Appl. Microb. & Biotech. (2006) 70: 247-253.

尿氮與紅血球

組別	尿態氮 (mg/dL)		紅血球 (g/dL)	
	Before swimming	After swimming (change%)	Before swimming	After swimming (change%)
控制組	16.37±1.02[a]	21.87±0.75[a] (+33.6%)	15.80±0.55[a]	14.20±0.21[a] (-10.1%)
低劑量組	17.26±0.81[ab]	20.33±0.83[b] (+17.8%)	15.64±0.34[a]	13.70±0.55[ab] (-12.4%)
高劑量組	17.74±0.91[b]	20.53±1.09[b] (+15.7%)	15.31±0.38[a]	13.28±0.35[b] (-13.3%)

The blood urea nitrogen concentrations of low dosage and high dosage groups were significantly lower (decrease 15.8% and 17.9%) than those of the control group.

Effect of red mold rice on antifatigue and exercise-related changes in lipid peroxidation in endurance exercise, Appl. Microb. & Biotech. (2006) 70: 247-253.

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紅麴在預防醫學上之應用
──抗疲勞之結論

- 除控制組外，低劑量組與高劑量組每公斤體重各攝食1 g及5 g紅麴米。
- 餵食28天後進行游泳試驗，低劑量組與高劑量組顯著比控制組延長 33.6%及65.9%。
- 游泳前後，乳酸增加量低劑量組與高劑量組顯著比控制組下降 39.13%及47.88%。
- 游泳前後，尿態氮增加量低劑量組與高劑量組顯著比控制組下降 15.8%及17.9%。

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紅麴在預防醫學上之應用
—抗阿茲海默症

- 紅麴之神經保護效果以下列兩種方式確認
  - 行為模式
  - 動物試驗腦部之生化檢查
- 記憶試驗之結果顯示：
  - 在被動迴避試驗中，攝食紅麴組於明室的停留時間會顯著延長
  - 參考記憶之影響方面，餵食紅麴米的阿茲海默症大鼠可顯著降低尋找平台的時間
- 試驗結果證實：每日攝食紅麴米可改善由於輸注類澱粉樣蛋白所引起之記憶損傷

紅麴在預防醫學上之應用
—抗阿茲海默症

- 大腦皮層及海馬迴組織中乙醯膽鹼酶、氧化壓力、發炎反應均會受到攝食紅麴米之抑制，因而改善記憶能力損傷
- 紅麴米在生體外及生體內神經保護效果均優於 lovastatin
- 此結論顯示紅麴米對阿茲海默症之改善效果，除monacolin K 外，紅麴米中其他類型之monacolins、抗氧化劑、抗發炎之代謝物均應有效

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紅麴山藥降血壓功效試驗

- **ACE 抑制活性：紅麴山藥水萃物較紅麴米要好。**
- **單一次餵食：紅麴米顯著降低收縮壓 8 mmHg 與舒張壓 9 mmHg，紅麴山藥顯著降低收縮壓 20 mmHg 與舒張壓 17 mmHg，且持續至八小時後仍有效果。**
- **餵食八週：紅麴米顯著降低收縮壓 20 mmHg 與舒張壓 18 mmHg，紅麴山藥顯著降低收縮壓 26 mmHg 與舒張壓 22 mmHg。**
- **血管切片：餵食八周紅麴山藥、紅麴米及 GABA 組別之血管內壁彈性蛋白 (elastin) 排列較餵食水及餵食發酵山藥組別要整齊。**
- **餵食八周後安全性：均不影響心律、肝功能、腎功能、肌肉功能及體內電解質平衡。**

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