發酵技術學

授課老師：潘崇良、蔡國珍

日 期	內　　容	授課老師
3/06	fermentation history & vegetable fermentation	蔡 國 珍
3/20	Alcoholic beverage production & vinegar production	
4/17	Oriental fermented foods (I) (Soy sauce, miso, tempeh, natto)	
5/01	Oriental fermented foods (II)-Anka fermentation & examination	
05/15	Strain Selection and Improvement	潘 崇 良
05/29	Fermented Dairy Products --Yogurt, cheese	
06/05	Fermented Health Foods, Oligosaccharides addition	
06/26	Phages of LAB,& Bakery Foods-New Development	

成績： 1.二位授課老師自行評分，各佔學期成績的50%。
2. 潘老師授課部份預定以 take home exam. 的型式，於授課結束前一週時將試題發給修課同學，二週後交回評分。

食品發酵

國立台灣海洋大學食品科學系

蔡國珍 博士

發酵食品之歷史

- 發酵食品－遠至4000年前即有. eg.酒. 麵包

- 希臘: 葡萄酒為酒神所製造.
- 啤酒 2500 B.C.已有記載－埃及第四王朝
- 中國古代 Kui (米酒). 2300 B.C.
- 哥倫布發現美洲－印地安人.玉米燒酒
- 3000 B.C. 以青黴菌治病

微生物之發現

- **Anthony van Leeuwenhoek (1632~1723).**顯微鏡觀察到M.O
- **法 L. J. Thenard** 葡萄酒之有微生物 但為自然發生說排斥
- **1859 法 Pasteur**

  否定自然發生說

  微生物學之父 酒精發酵乃yeast而來

  創低溫殺菌 解決葡萄酒酸敗.

  德 Robert Koch gelatin → soild medium

  細菌學之父 for 分離細菌 發現結核菌,赤痢菌

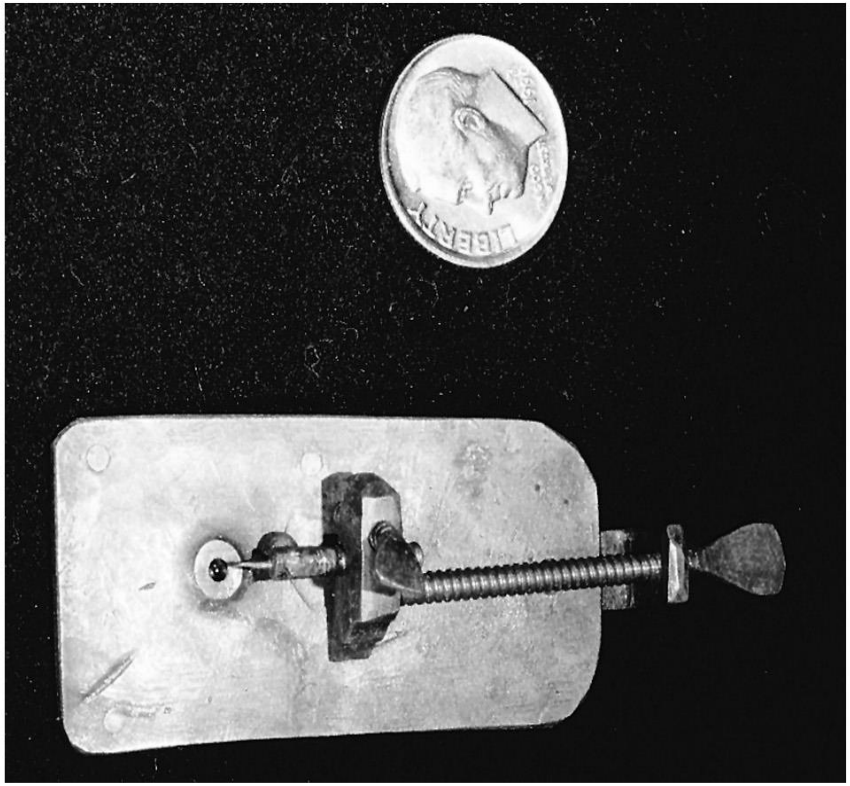
  科克假說 → 仍為確定人類病原菌之準則

圖 1－8

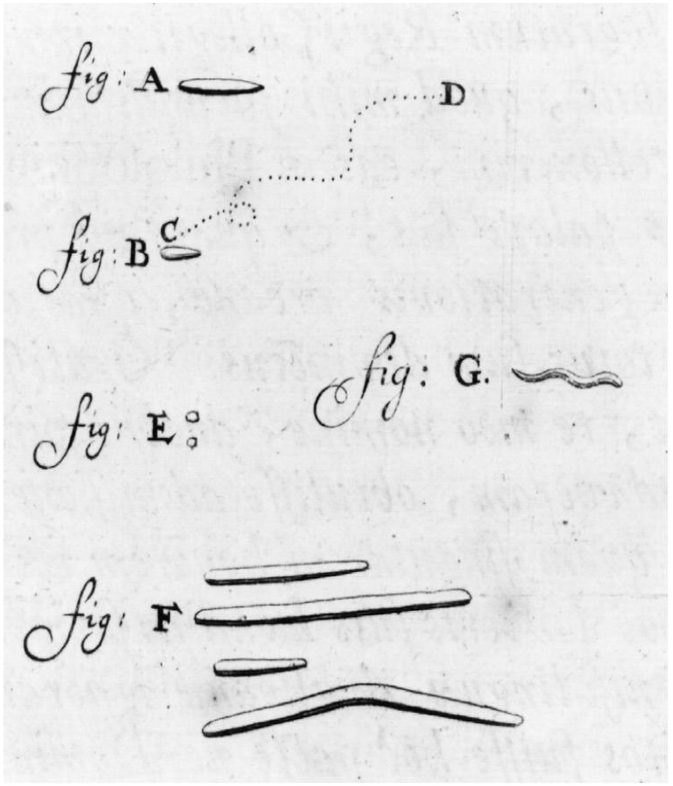
圖 1-8

羅伯特虎克（Robert Hooke）的組合式顯微鏡與他所描繪的軟木塞組織。

圖



a.



b.

圖 1-9

(a) 安東凡雷文虎克（Antony van Leeuwenhoek）所使用的小的掌上型顯微鏡。
(b) 他所描繪的“animalcules”，發表於1684 年。

圖 1 －

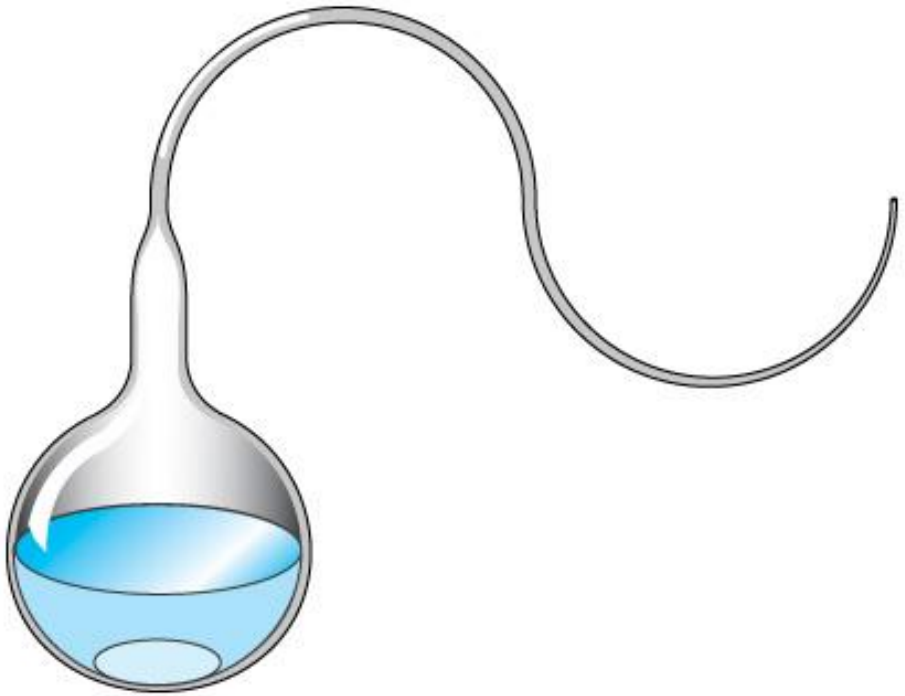


圖1-10

路易斯巴斯德（Louis Pasteur）所使用的鵝頸燒瓶，用以說明在無菌的肉汁中微生物無法自發性形成。

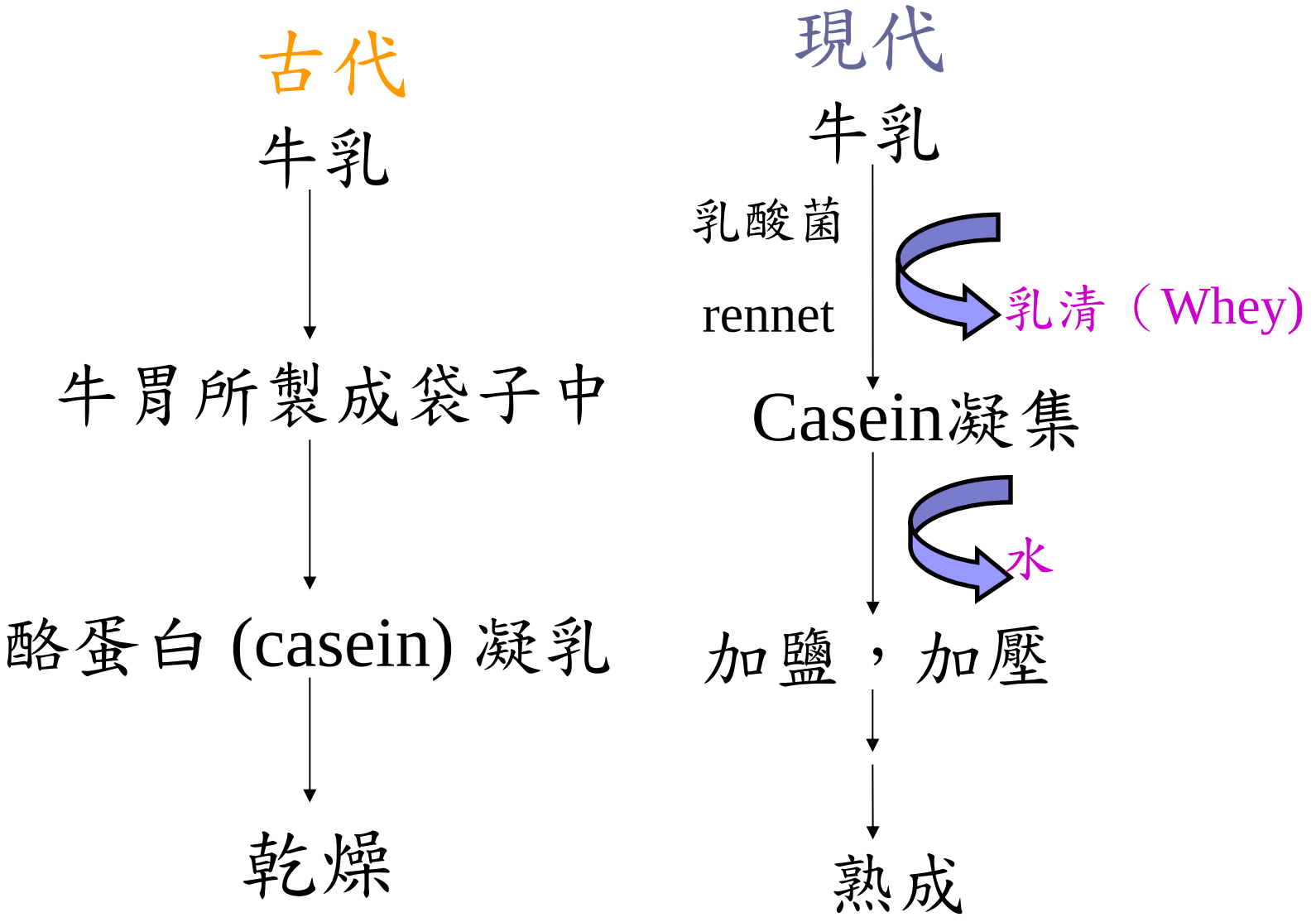
微生物之發現（續）

- **1897 法 Buchner** yeast ＋ 砂 → 磨碎 ＋ 砂糖防腐 → 酒精產生　發酵. 乃酵素而來

- **1929 英 Alexander Fleming**

青黴素 但1941~1943大量生產

乳酪


古代
牛乳
牛胃所製成袋子中
酪蛋白 (casein) 凝乳
乾燥
現代
牛乳
乳酸菌
rennet
乳清（Whey)
Casein凝集
水
加鹽，加壓
熟成

啤酒

古代: 乾燥生大麥 (活的) → 磨碎 (草做的篩子) → 過篩 (加水) →

麵糰 → 靜置，乾燥 (添加物) → 浸入水中發酵

→ 液體置入廣口瓶中

現代: 大麥 → 麥芽 → 磨碎 → 加水糖化

→ 麥汁 (過濾) → 煮沸，冷卻 (啤酒花) → 發酵 (yeast)

→ 貯酒熟成

一、食品發酵

- 乳品發酵
- 酒精發酵 ＞ 溶劑生產:acetone (1915)
- 醋酸發酵
- 麵包發酵
- 蔬果發酵
- 黃豆發酵
- 水產品發酵

二、微生物酵素

- 澱粉酶 amylase
- glucoamylase
- 纖維酶 cellulase
- 果膠酵素 pectinase
- 蛋白酶 protease
- transglutaminase

三、微生物代謝產物及菌體利用

- 有機酸
- 氨基酸：**Glu. Lys. Trp.**
- 色素
- 香味：**diacetyl**
- 多醣類：
- 抗生素及細菌素
- **S. C. P.**
- 植物生長素：**Gibberellins（*Giberella fujikuroi*）**
- **ω—3. fatty acid**

四、特殊機能之應用

- 廢水及廢油之處理
- 開礦
- 生物殺蟲劑
- 生理活性物開發：vit. 類固醇. β- carotene . Hormone . 代用血漿(dextran)
- 燃料及化學物之生產

五、檢驗分析之應用

- 維生素
- 生物感應器
- 突變劑. 致癌劑(物)

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 objectional characteristics

Vegetable fermentation

- Sauerkraut
- Pickles
- Olives

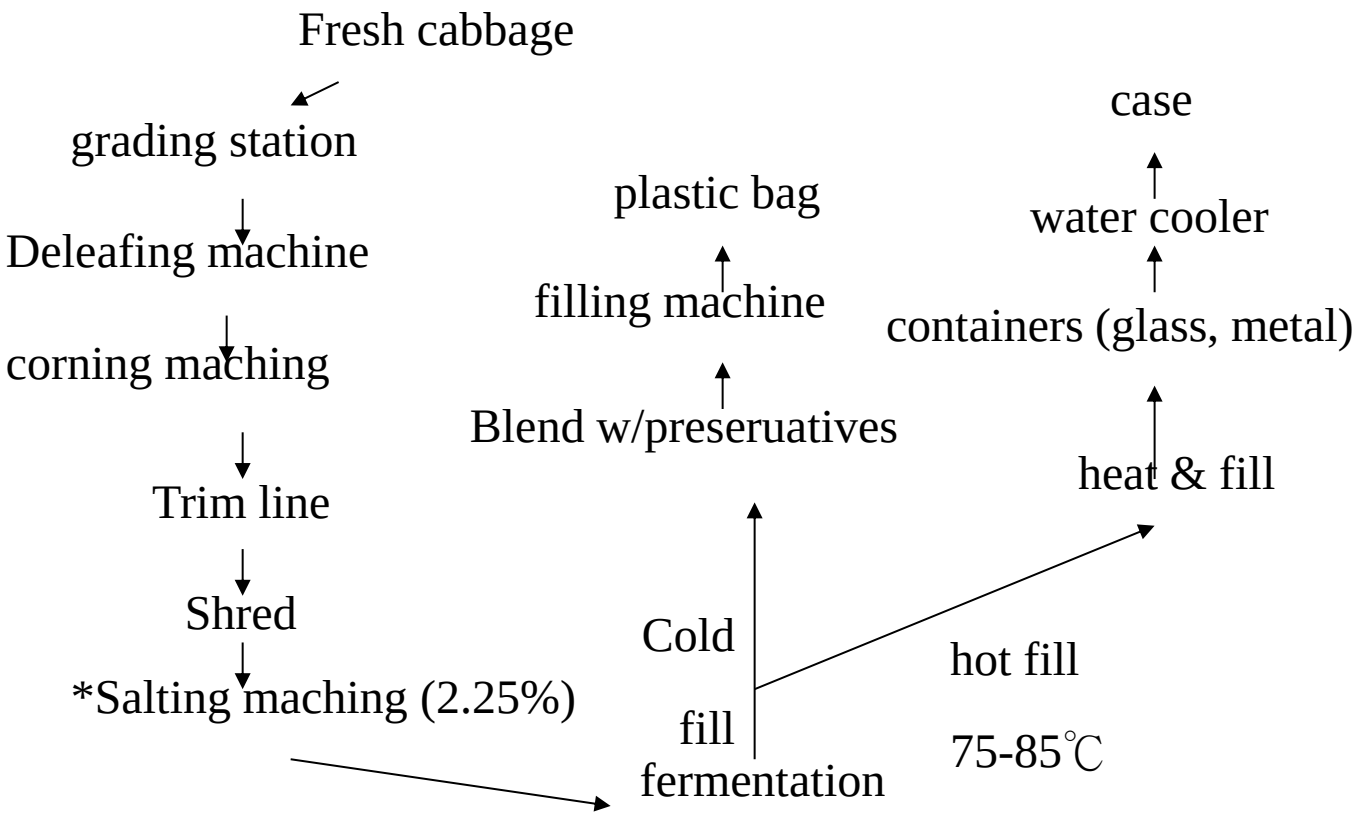
4 basic steps common in each product

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

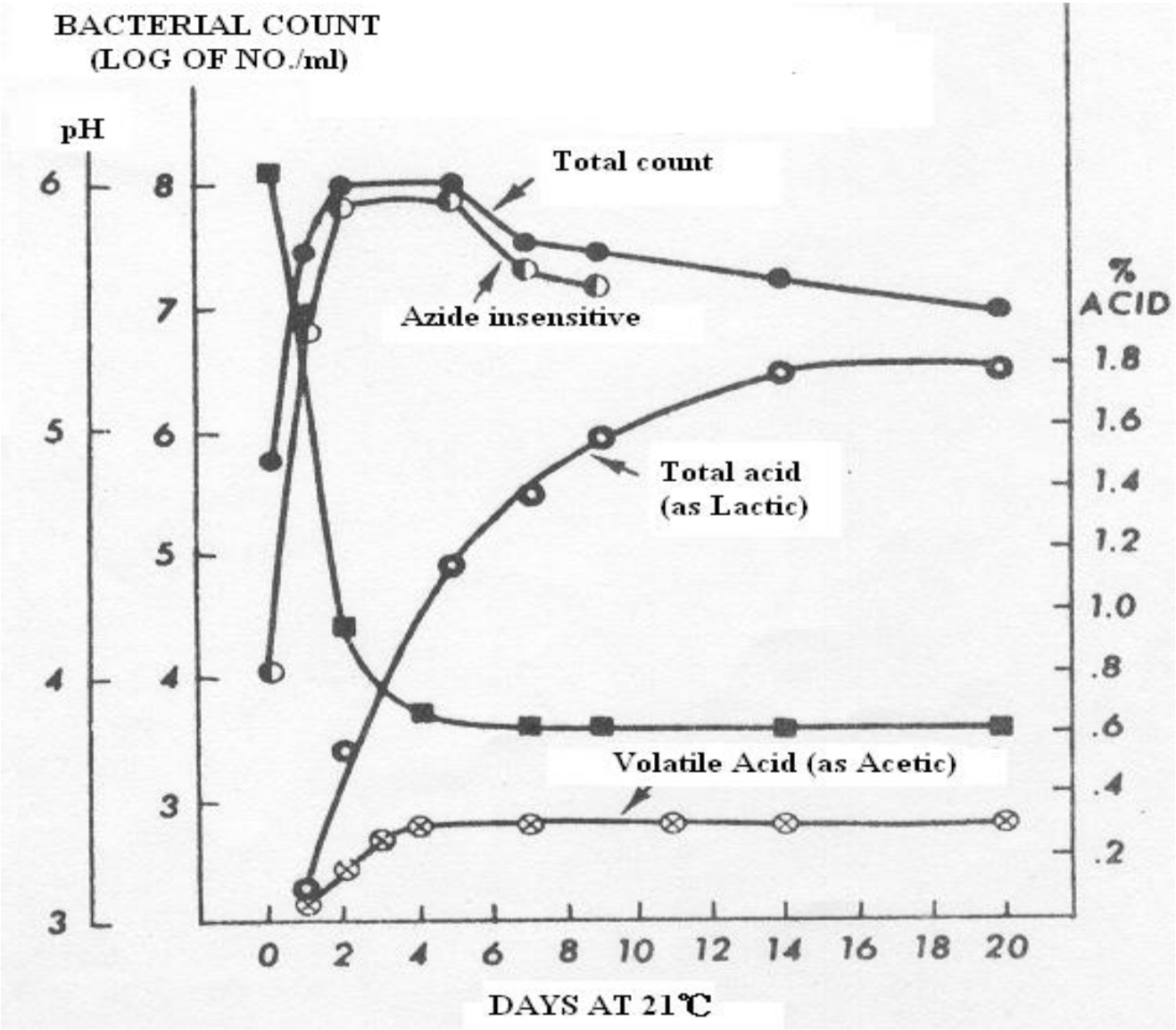
-natural ferment

-controlled by anaerobiosis, salt. & temp.

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

Eg. Coliform, pseudomonas, enterobacter.
acinetobacter, mold & yeast,
lactics only abount 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

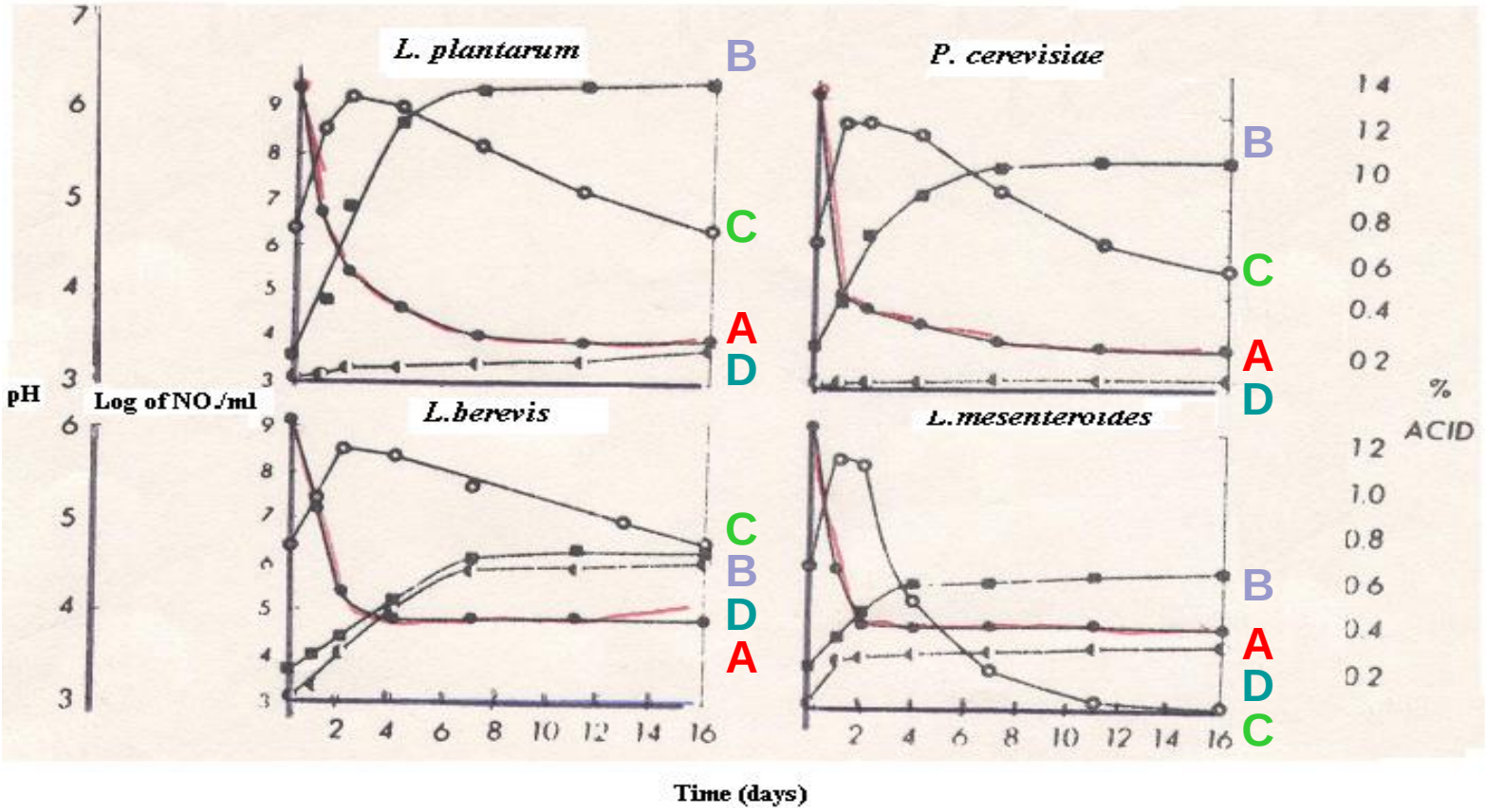
30% homo rods

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

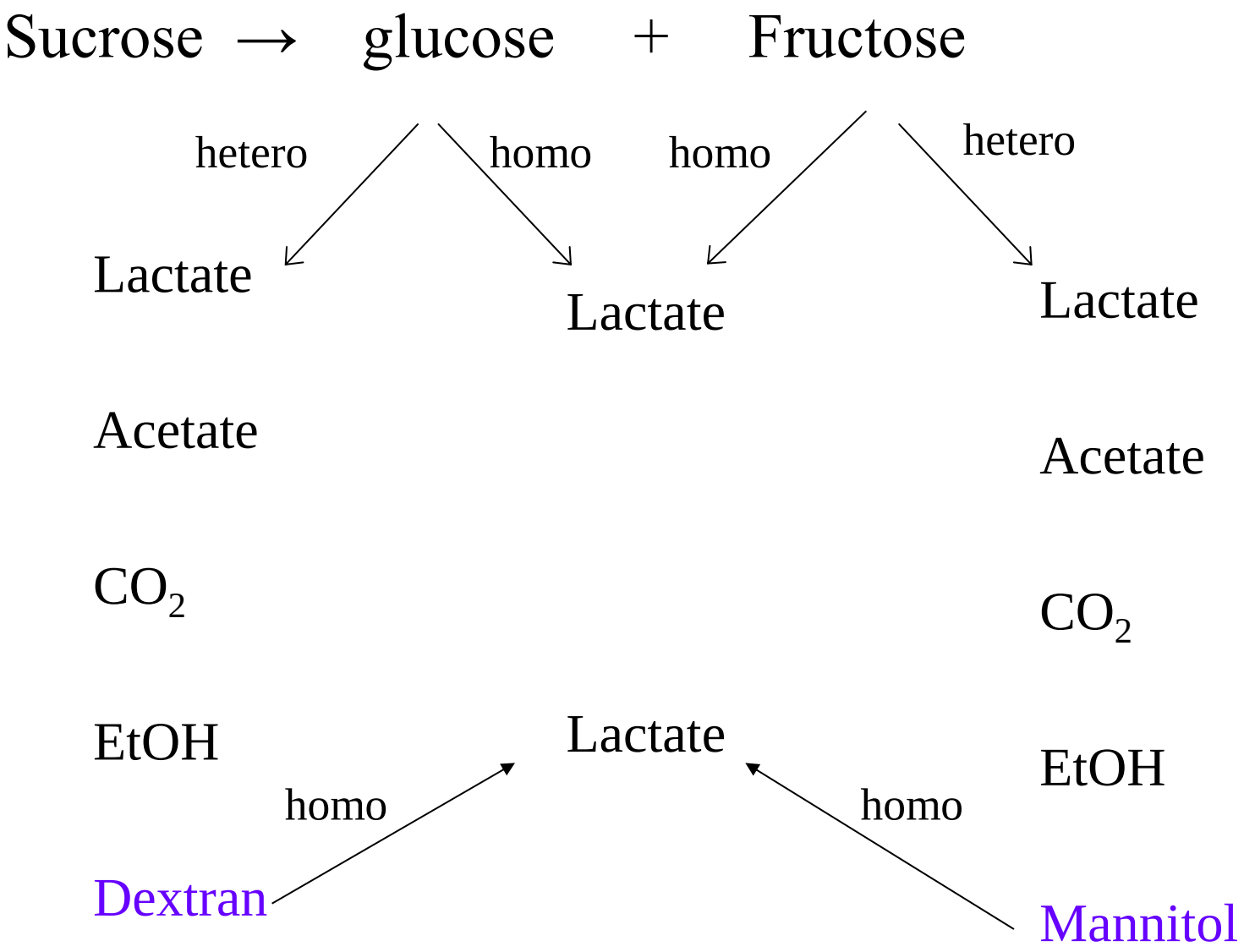
acetate:lactate =1:3

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
Lactate
Lactate
Acetate
Acetate
CO_2
CO_2
EtOH
EtOH
Lactate
homo
homo
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. mesenterokides 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.

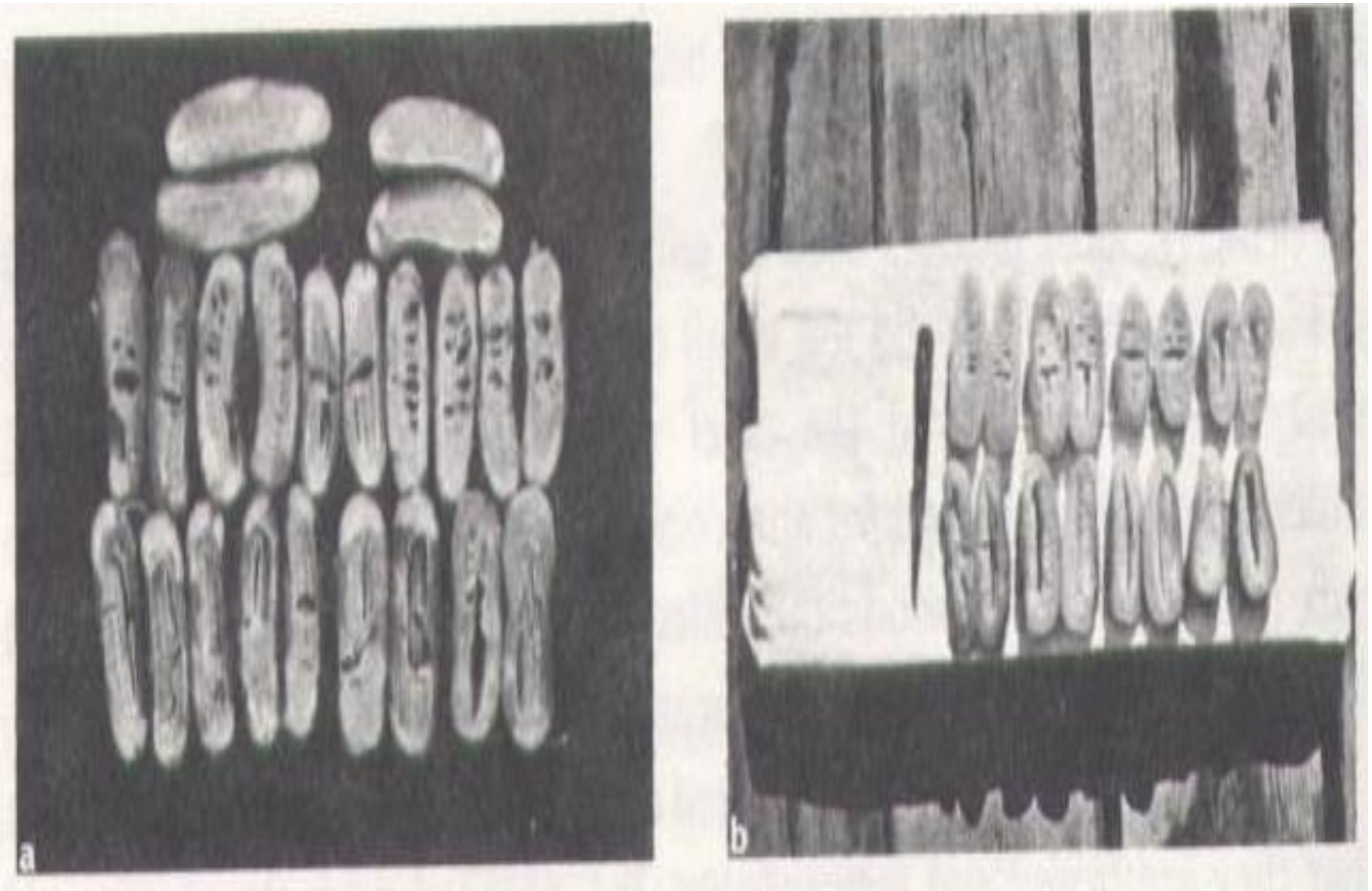


Fig. Gaseous spoilage of pickles. (a) Severe lens and balloon bloaters in partially cured brine-stock; (b) bloaters in small sized brine-stock pickles (about 1 in or less in diameter).

Pickle fermentation

Controlled fermentation

cucumber → thoroughly washed

thoroughly washed → tank

Brine solution (acidify with HAC) → tank

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

ferment (75~85°F)

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

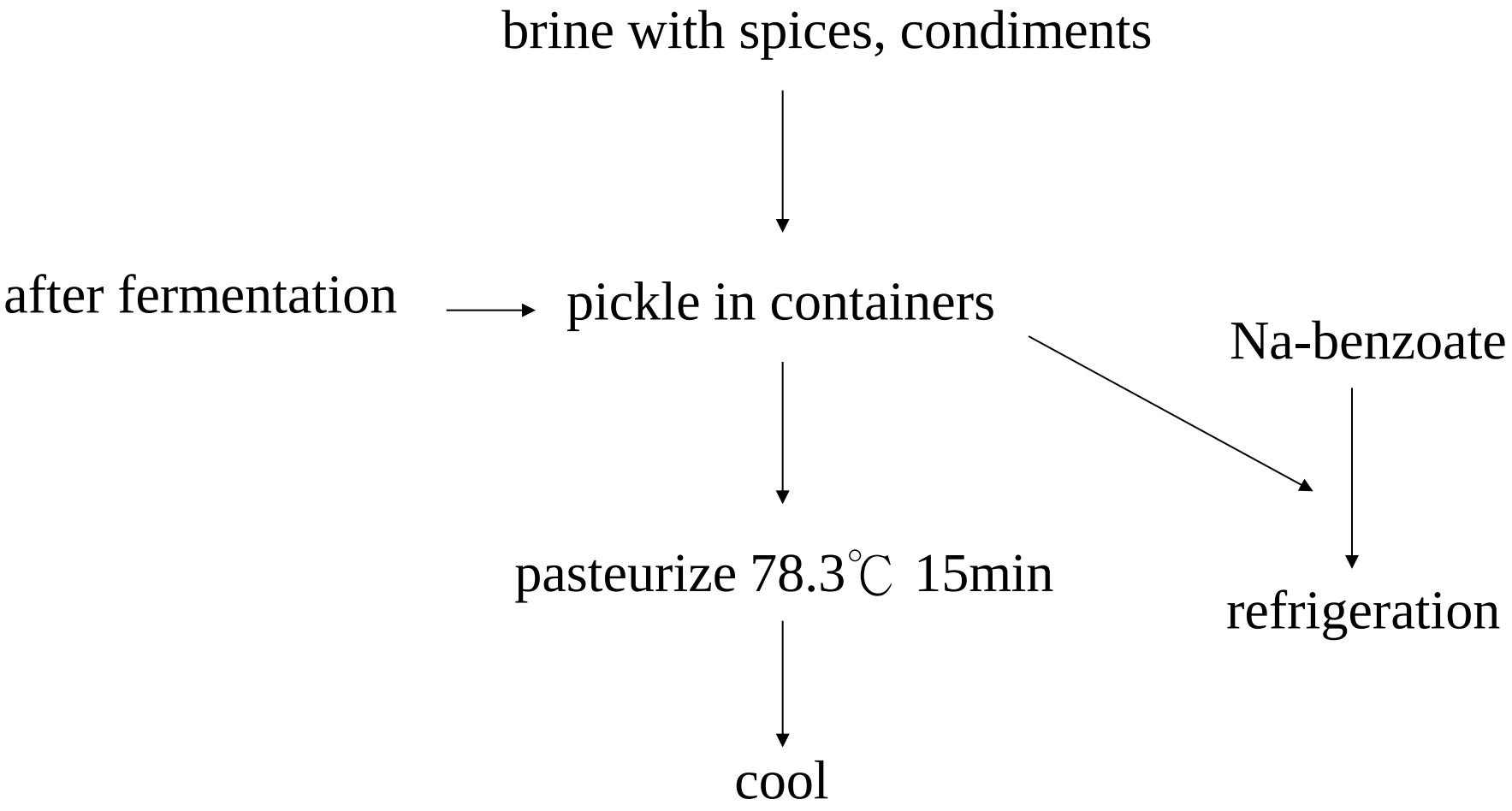
- *mold grow & secrete softening en. to cucumber flower which adhering to cucumber on which they developed.

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

⇒problem of disposal of brines

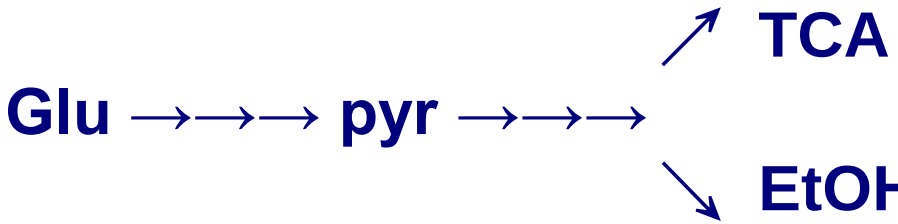
⇒recycle the brine by heating to inactivate en. & M.O. followed by precipitation

Preservation



酒精發酵

- 酵母為主 ***Saccharomyces cervisiae***
  Saccharomyces uvarum
- **O_2 : sugar oxidation → CO_2 + H_2O**
  Respiration for yeast multiplication
- **Without O_2 : fermentation . produce mainly EtOH**
- **可利用糖 : glucose , maltose , maltotriose .**
- 澱粉質澱粉(穀類),需經糖化作用(酵素或黴菌作用)後,酵母菌方能進行酒精發酵.



酒之分類(依製程)

- 釀造酒：紹興、紅露、啤酒、葡萄酒……
- 蒸餾酒：高梁、米酒、白蘭地、威士忌
- 再製酒：參茸酒、虎骨酒、各種藥酒(浸泡酒)

釀酒原料

- 殼類：米、麥(大麥、小麥、燕麥...)、玉米、小米→屬澱粉質原料
- 水果：葡萄、鳳梨、草莓、水蜜桃...→屬糖質原料
- 糖漿：玉米糖漿、楓糖漿...


台湾啤酒
TAIWAN BEER
台湾啤酒
TAIWAN BEER
TAIWAN
BEER
台湾啤酒
TAIWAN BEER
TAIWAN BEER
台湾啤酒
生
DRAFT
台湾啤酒
TAIWAN BEER

台灣啤酒
Taiwan Beer
（酒精含量4.5%）

清涼有勁！　　營養保健！

品質優異！　　享譽國際！

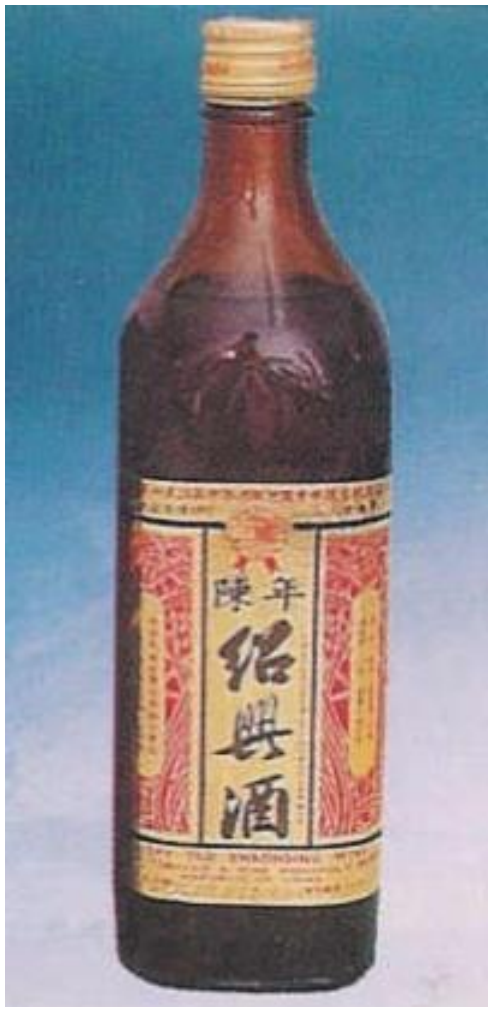
使用優質大麥芽、精選啤酒花為主要原料、配以省產上等蓬萊米及純淨的釀造水，採傳統釀製技術精釀而成，口味清爽可口，風味獨特。含豐富的礦物質及維生素B及人體所需之氨基酸，極具營養價值。並含多量的二氧化碳，是極佳的清涼飲料。


玉泉清酒

玉泉清酒
jade Spring Sake
（酒精含量15.5%）

八十七年新研發的產品，仿傚日式清酒以蓬萊白米精碾至七成米心，並利用優良之米麴糖化菌及特選酵母菌於精密程式控制下，採低溫發酵精釀而成，酒色清亮。本風味精香，口感醇和柔順，尤適合冰涼飲用，或調配紅葡萄等果汁，並加冰塊，風味絕佳，特別適合夏天飲用。飲後口齒留香，飄然舒暢，是穀類釀造酒愛用者的新寵。


紹興酒
SHAOHSING WINE
紹興酒
SHAOHSING WINE


陳年
紹興酒

紹興酒
Shaohsing Wine
（酒精含量16%）

原產於我國江南，歷史悠久，享譽中外，以浙江省為最。本局紹興酒釀造方法乃以科學化管理，使用精白圓糯米，加添選至大陸優良紹興酒麴中所分離出的麴菌及酵母，予以培養製成米麴、麥麴及酒母，以低溫糖化發酵精釀而成。經壓榨、澄清、殺菌後盛裝於陶質酒甕內密封陳貯後包裝上市。民國七十一年起，本局以現代化的工業技術，使用大桶方式進行貯酒試驗，成功可行之後，部份紹興酒於是進入大桶貯存的新紀元。本局釀製紹興酒所用之菌種乃於民國四十年從取至大陸紹興酒麴中分離所得，經小規模試驗後，民國四十一年於埔里酒廠生產上市，五十年起銷量扶搖直上，取代早期台灣特級清酒的地位，七十九年產量達最高峰。


黄酒

黃　酒
yellow Wine
(酒精含量15.5%)

以上等蓬萊米與小麥為原料，採用類似日本清酒釀造方式改良釀造，經大桶、裝甕長期貯藏後調裝上市，口味較紹興酒清淡醇和。質醇味厚，芬芳澄澈，色如琥珀，故曰黃酒。

葡萄酒類	酒精含量	含糖量	原料	特性
金香白葡萄酒	10.5%	2%	金香葡萄	濃香醇和
特級紅葡萄	10.5%	1%	黑后紅葡萄	酒色紅
特級玫瑰紅酒	10.5%	8%	金香+黑后	甜醇可口
玉泉紅葡萄酒	12%	2%	卡本-內蘇維翁葡萄	酒色悅目 果香迷人 酒質醇厚 酸甜適口
葡萄蜜酒	10.5%	8%	金香+龍眼花蜜	蜜香甜醇
葡萄淡酒	2.0%	12%	金香+龍眼花蜜	純果汁酒 香甜爽口

（二）蒸餾酒

Whisky

Brandy

白酒

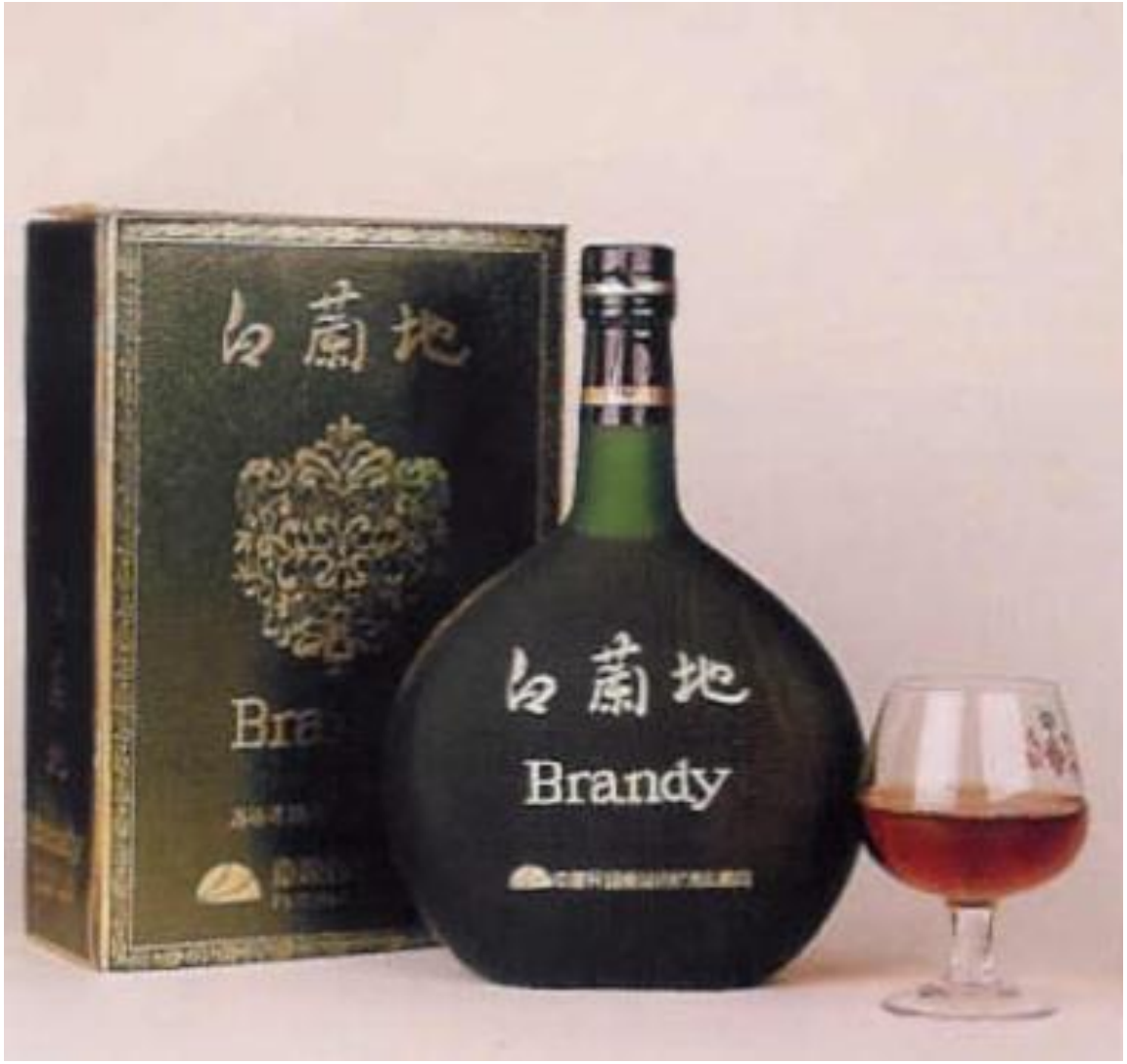
米酒（料理米酒）

高粱酒


威士忌
威士忌

威士忌
Whisky
(酒精含量41%)

威士忌係採用大麥經發芽產製之純大麥芽，粉碎、糖化製成麥汁，發酵經過二次蒸餾，貯存於橡木桶熟成再調製而成。酒色如琥珀，入口濃郁，清沁爽口，具大專芽及橡木桶獨特之香味，回味濃馥香醇。0.6公升裝，0.2公升裝。


白蘭地
白蘭地
Brandy

Brandy

係以新鮮的白葡萄為原料，經過壓榨、發酵，再以新式蒸餾設備多次蒸餾之後，取其精華段的酒液裝入橡木桶內陳熟，儲陳數年始裝瓶應市。其以磨砂綠色扁平玻璃瓶包裝，造型美觀，酒精度41%。尤以需經橡木桶長時間的儲陳，因此能萃取橡木之精華，加以熟陳作用，故酒質醇厚、氣味芬芳、風味特殊，飲之令人心曠神怡。


20°
米酒

米酒
Michiu
(酒精含量20%)

俗稱紅標米酒，以蓬萊米為原料，採用改良式阿米洛法釀造而成之米酒，調合精製食用酒精而成。為無色透明液體，酒質清純具有特殊香醇風味。歷史悠久、價廉物美、消費遍及各階層，是家喻戶曉、不可或缺的生活必需品。早期直接飲用者眾多，近年來生活改善，多以烹飪用為主。民間婦女坐月子的麻油雞、家常菜、燒酒雞浸泡補葯酒等普遍選用。0.6公升裝。

釀酒理論(Ⅰ)

- 凡原料為澱粉質原料者，所含澱粉需先轉化成小分子糖(糖化作用)，方能為酵母菌所用，發酵產生酒精。

  例如：紹興、紅露酒等，均利用黴菌進行此種轉化。啤酒則利用麥芽本身酵素進行轉化。

- 若原料為小分子糖類原料，則不需進行上述操作，直接以酵母菌進行酒精發酵。

釀酒理論(Ⅱ)

- 酒精發酵

  酵母菌在無氧條件下，將小分子糖代謝，產生酒精與二氧化碳。

- 發酵速度受溫度影響，溫度越高，速度越快，但一般以不超過30℃為宜。

- 原料糖濃度越高，產生酒精越高，但一般糖含量不超過30%。

釀酒理論(Ⅲ)

- 酒，除了酒精度外，風味口感為決定其品質重要因素，剛酒精發酵完之酒，風味差，釀造酒一般需經貯酒，以達風味熟成及酒液澄清的目的，貯酒一般在低溫進行。

釀酒理論(IV)

- 蒸餾酒由酒精發酵完的酒經蒸餾而得，藉由控制蒸餾時截取點，可得不同酒精度之產品。
- 部份蒸餾酒經橡木桶貯酒操作。

米　酒

- 蒸餾酒一種，東方特有酒之一。
- 利用蒸煮將米中澱粉糊化(煮成飯)，再利用黴菌將澱粉切成小分子糖(糖化)，再由酵母菌將糖轉化成酒精，然後利用蒸餾，將酒精及風味成份蒸餾出來。

米酒釀造

(1)在來法

米（蓬萊米）$\xrightarrow{\text{蒸煮}}$ 飯 → 冷卻至35℃（水量含約38%） $\xrightarrow{\text{麴粉（1%）}}$ 混合均勻 →

中間留孔洞，以利通氣 → 室溫（< 30℃, 36h）→ 加水 (原料2.5倍)

→ 發酵（室溫） $\xrightarrow{\text{8～9天}}$ 11～12% Alc. → 蒸餾

(2)阿米洛法

米加水(1：3) → 加酸調至pH4.0～4.5 $\xrightarrow{\text{蒸煮}}$ 液狀（稠狀） →

降溫至55～60℃ $\xrightarrow{\text{糖化菌（Rhizopus, } 1\times10^{8}\text{）}}$ 4h → 降溫至35℃

$\xrightarrow{\text{yeast}}$ 5～6天 → 9～10% Alc → 蒸餾

(3)酒母法

米 $\xrightarrow{\text{蒸煮}}$ 飯 $\longrightarrow$ 降溫至55～60℃ $\xrightarrow{\text{麴}}$ 4h $\longrightarrow$

加水降溫至35℃ $\longrightarrow$ yeast $\longrightarrow$ 5～6天 $\longrightarrow$ 9～10% Alc

$\longrightarrow$ 蒸餾

麴

- 穀類原料上，接種黴菌，使之生長，再將之乾燥(便於保存)粉碎或製型。

- 市售酒麴除黴菌外，尚加入酵母，黴菌作用為將穀物中澱粉分解成小分子糖，酵母菌再將小分子糖發酵成酒精。

水果酒

- 水果中糖份多為小分子糖，可直接(或)加酵母菌發酵。
- 香蕉含大量澱粉，可先加酵素水解，再加酵母菌。

- 以葡萄酒為代表
- 分為自然發酵與控制式發酵
- 產品受原料水果品質影響大，葡萄甜度高，酸度低者較好

（一）自然發酵

- 利用原料所含的酵母菌進行發酵(不外加菌)。
- 菌種類多，產物風味物質多，較佳。
- 起始菌低，所需發酵時間較長。
- 地域、氣候等環境影響大。

（二）控制式發酵

- 人為添加有用菌進行發酵。
- 菌種較少，風味不如自然發酵者。
- 起始菌較高，所需發酵時間短。
- 品質穩定。

- 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*)

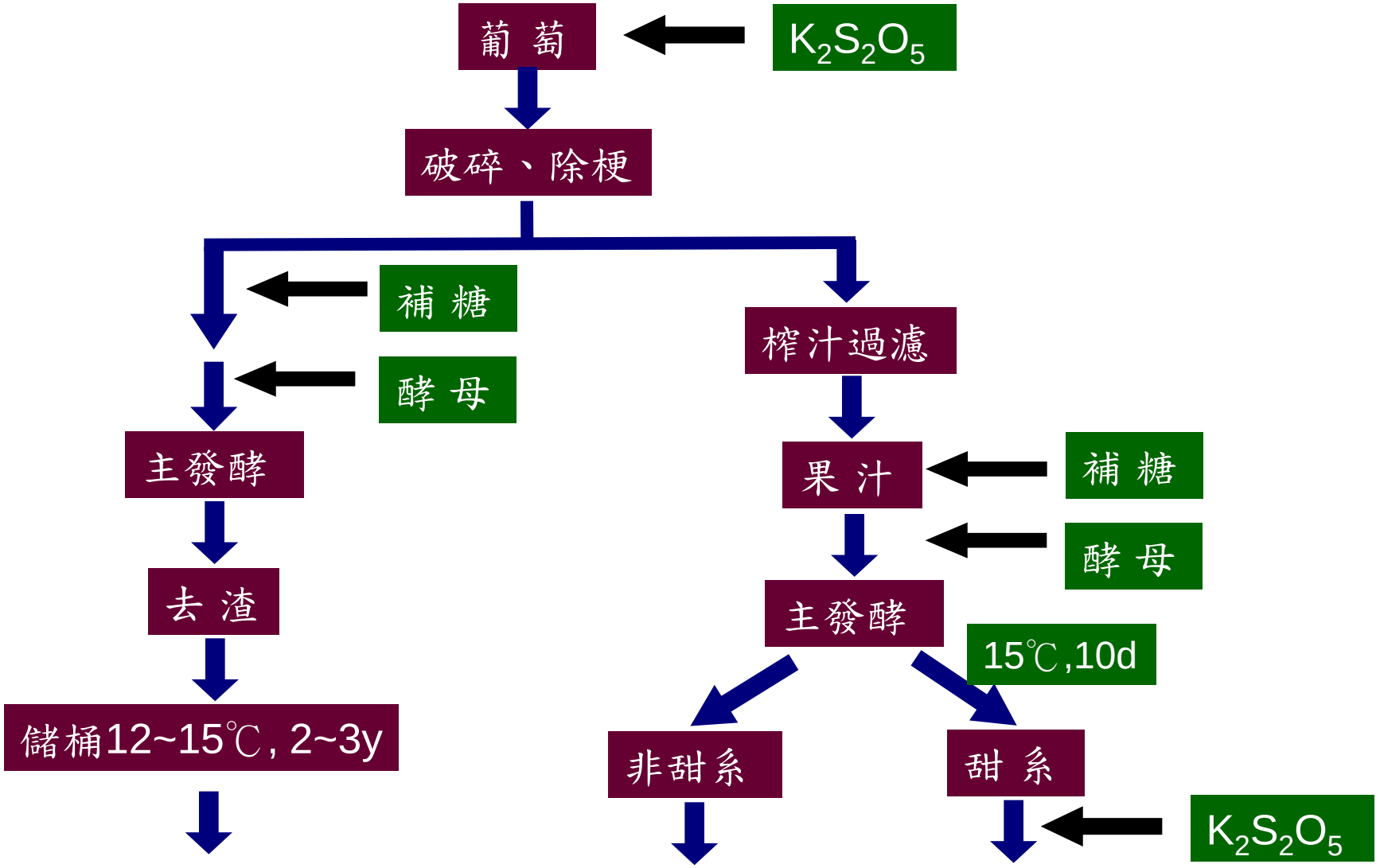
S. rouxii & *S. bailii* (osmopbilic, strong fermentation of Fru).

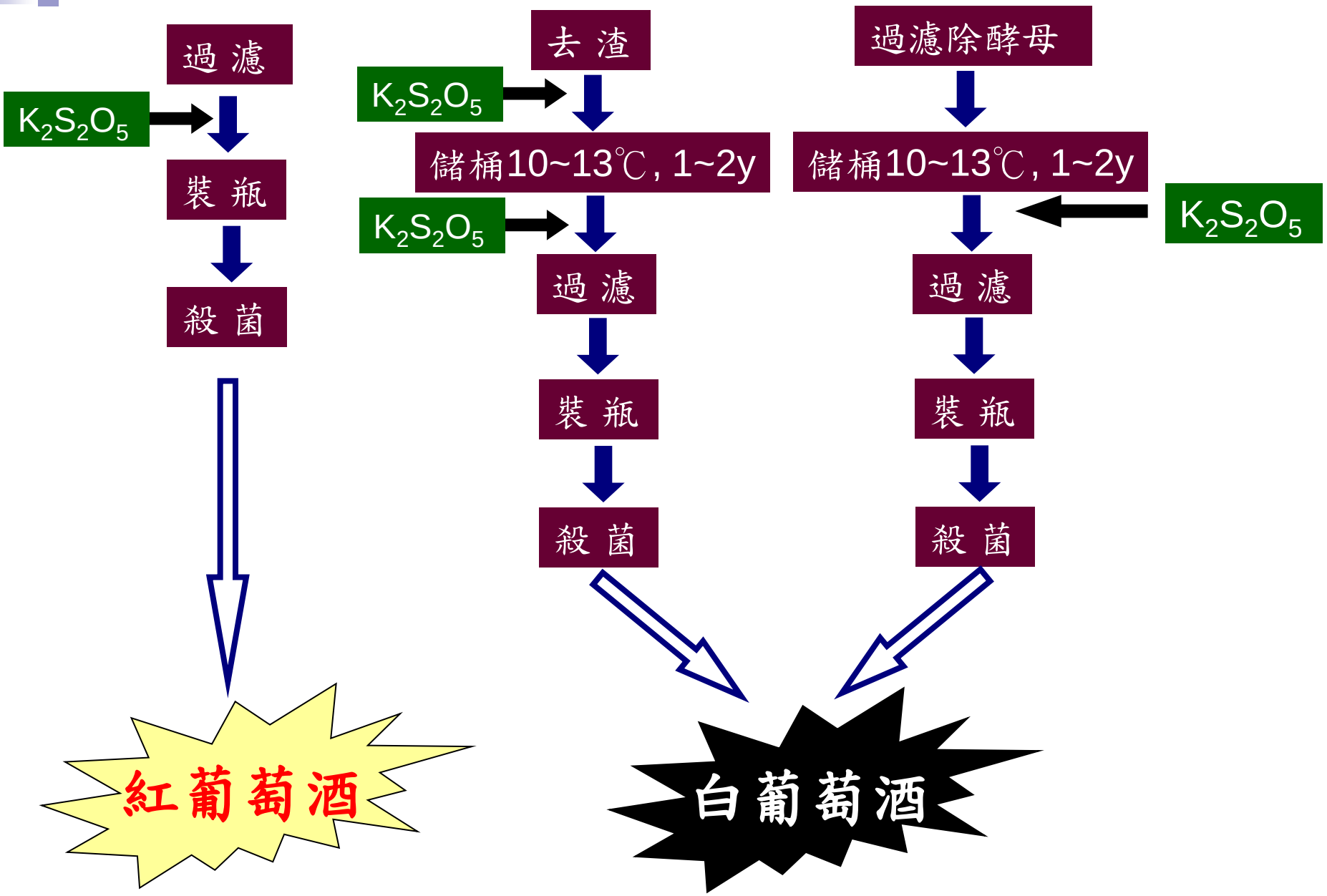
Pentose (arabinose & rhamnose) 1 g/l juice not fermentable by yeast

Wine 葡萄酒

- 紅葡萄酒：紅_黑葡萄連皮發酵.
- 白葡萄酒：綠葡萄不連皮發酵.
- 糖分：20～25%
- $K_2S_2O_5$：抑制雜菌,防止褐變.<100ppm

葡萄酒製程


葡 萄
$K_2S_2O_5$
破碎、除梗
補 糖
酵 母
主發酵
去 渣
儲桶12~15℃, 2~3y
榨汁過濾
果 汁
補 糖
酵 母
主發酵
15℃,10d
非甜系
甜 系
$K_2S_2O_5$


過濾
$K_2S_2O_5$
裝瓶
殺菌
去渣
$K_2S_2O_5$
儲桶10~13℃, 1~2y
$K_2S_2O_5$
過濾
裝瓶
殺菌
過濾除酵母
儲桶10~13℃, 1~2y
$K_2S_2O_5$
過濾
裝瓶
殺菌
紅葡萄酒
白葡萄酒

發酵溫度

15-35℃ 雖Temp↑,速率加快,但最終酒精濃度較低 EtOH效應增加,副產物↑(glycerol , acetoin , acetaldehyde.) fusel oil↑ (higher alcohol)

∴一般採低溫發酵,尤其white wine,避免褐變, 溫度較低, red wine,為有利色素溶出,溫度較高.

糖濃度

濃度增加,酒精度↑但>300g/l→osmotic effect

→↓yeast growth & fermentation.

→alcohol production↓

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.

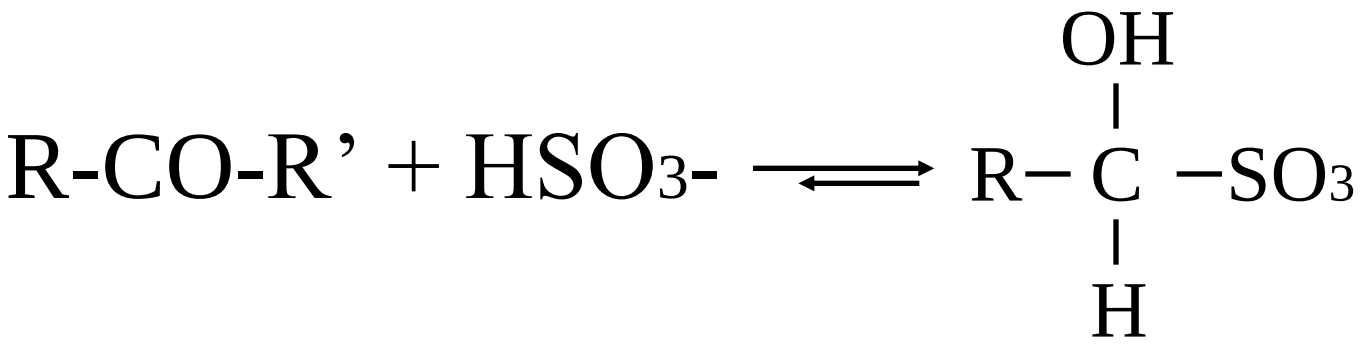
- yeast can tolerate 300 ppm (table wine < 100ppm) wine making, 100~200 ppm $K_2S_2O_5$ added
- SO_2 inhibit lactic bact to prevent malo-lactic fermentation
- Suppress *Kloeckera apiculata*
  Candida pulcherrima
  Which produce high volatile acid & pyruvic & 2 KG⇒↓quality
- Delay onset of fermentation

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

SO2↑ 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↑

Sauterne wine

葡萄 生長時受 *Botrytis cinerea* (貴腐黴)污染，H_2O % 降低, N -compd. %↓,相對的 $(CH_2O)_n$ ↑.由此原料而製得之紅葡萄酒稱之, 價格高.

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 cererisiae

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 %)

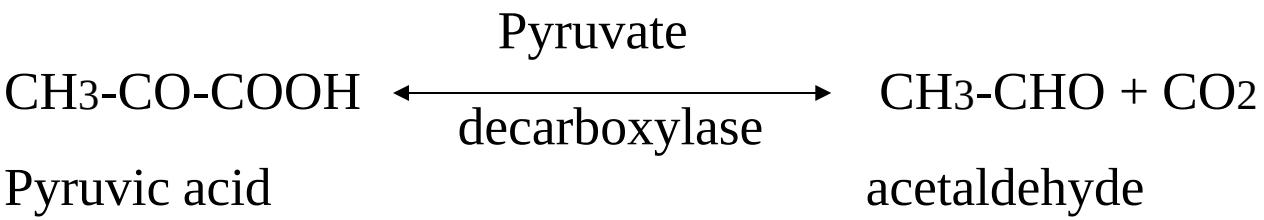
Schizosaccharomyces pombe

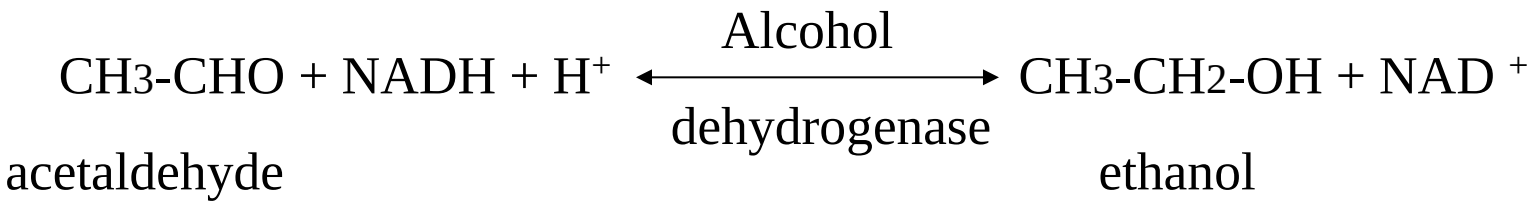
- not belong to yeast flora of must & wine.
  Except in western part of Sicily
- cell division, not budding
- EtOH production similar as *S. cerevisiae*
- degrade 1-malic acid→EtOH & CO_2
- tolerate high SO_2
- volatile acid & higher alcohol low
- Tolerate acetic acid & low pH
  at pH 2.8 → *S. cerevisiae*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

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

白蘭地

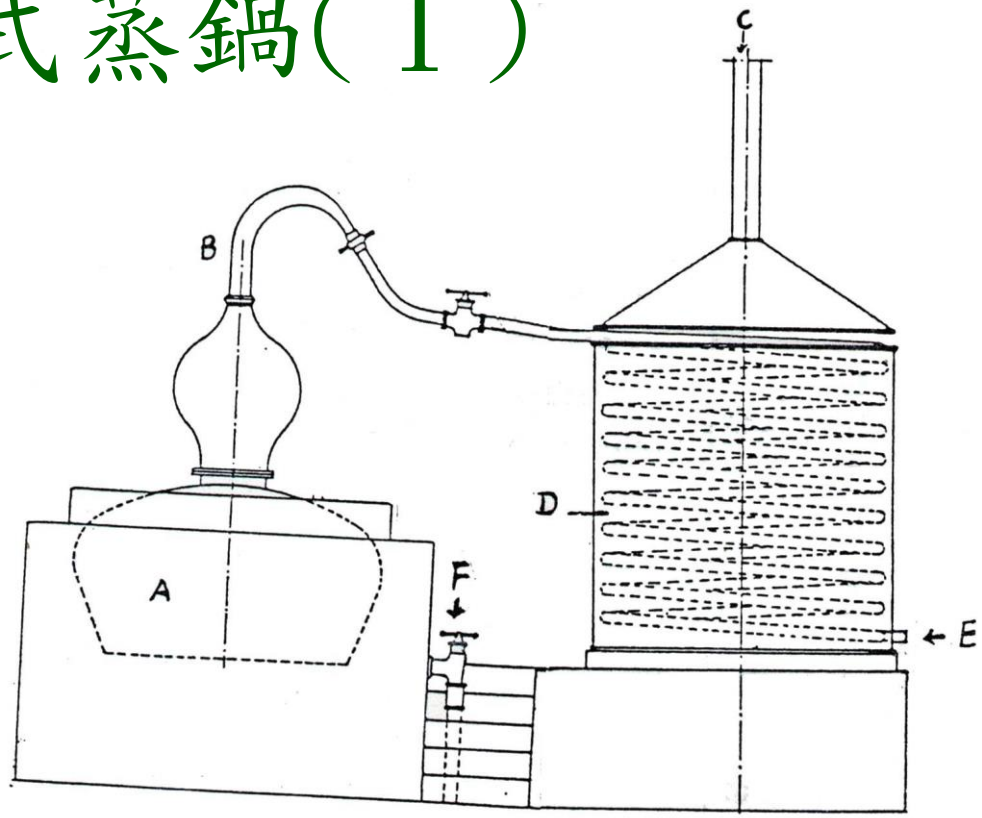
- 水果酒蒸餾而得。
- 蒸餾技術影響。
- 蒸餾後貯存於橡木桶。

蒸餾

- 銅鍋複式蒸餾或柱狀連續式蒸餾。

- 法國Cognac銅鍋複式蒸餾
  Armagnac半連續柱狀蒸餾機(5～6個板數)
  美國：連續式蒸餾(30～40板數)

銅鍋複式蒸鍋(Ⅰ)



A: Pot; B: Goose neck; C: Cooling water inlet; D: Cooling water tank; E: Distillte outlet; F: Drain valve

銅鍋複式蒸餾(Ⅱ)

- **初蒸**：主要以回收酒精為主，以及濃縮其它成份。體積收率約原酒之1/4～1/3。

- **複蒸**：酒液截分，以選取品質好的部份。分酒頭、酒心、及後段三部份。

  酒頭：占初蒸液體積1～2%，一般混入原酒再蒸。

  酒心：取酒精度75～60%部份，為所要部份。

  後段：混入原酒或初蒸液中蒸餾。

香檳

- 白葡萄酒發酵完，裝瓶，補糖，瓶口封緊，低溫貯存，酒液中酵母菌再繼續發酵所加入的糖，產生二氧化碳與酒精

啤酒 (Beer)

- 原料：大麥芽(malt).副原料(adjuncts).H_2O.啤酒花(Hop).酵母

- 副原料：含澱粉之穀類,依地區而不同

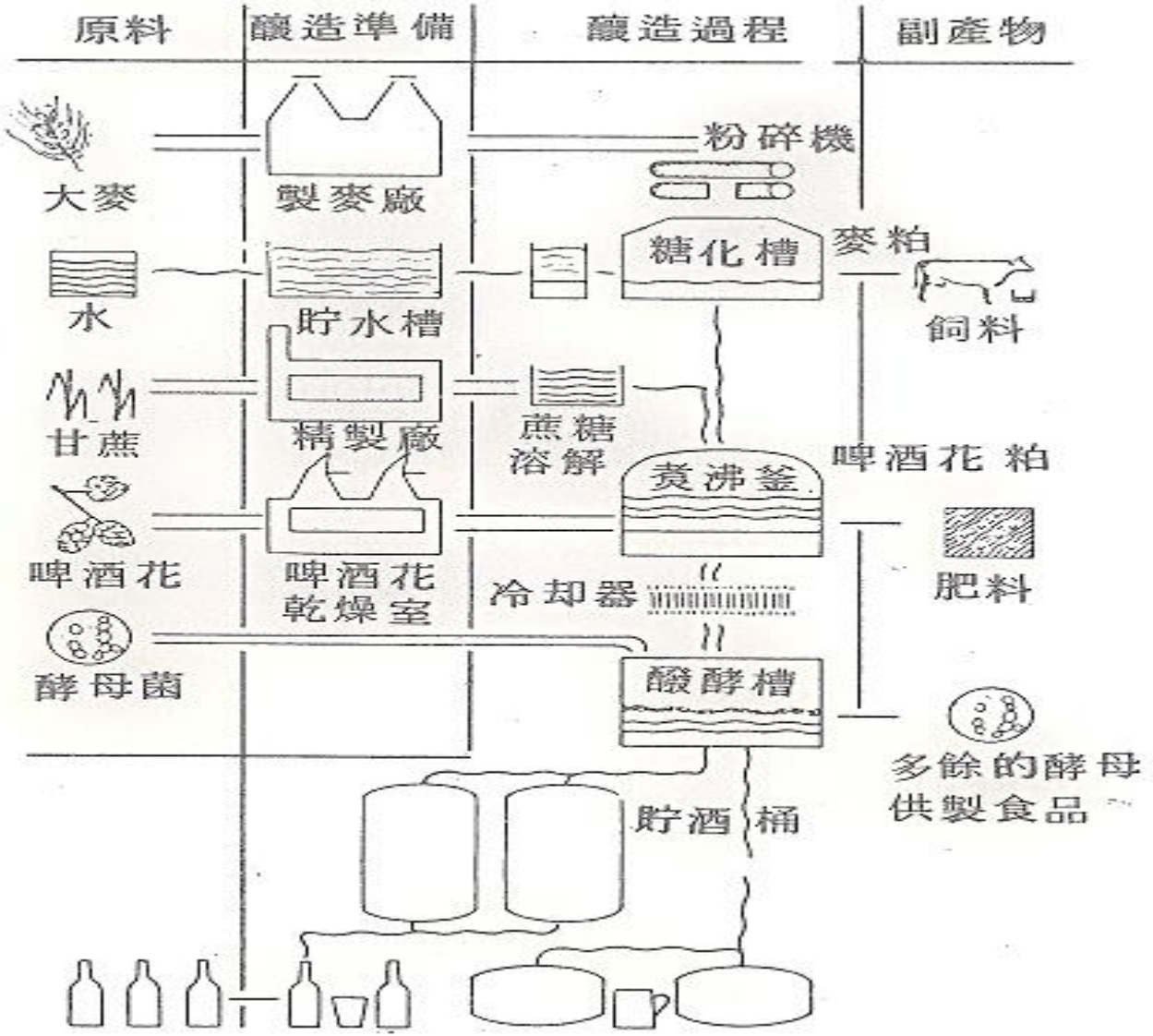


圖 1.1 啤酒釀造之程序圖示

■ 製程：

A.製麥

目的：①生成酵素及作用

②色澤風味產生

麥粒→浸水6～8h(13～16℃)→週期性洒水→42-46% H_2O含量,發芽→培乾(Kilning)

種子發芽

- Gebberellic acid-胚所產生Hormone，送到糊粉層(Aleurone layer)，使種子中α,β-amylase, limit dextrinase, protease, carboxypeptidase 活性增加，分解其中澱粉及蛋白質，以供種子細胞生長

- 發芽時大麥中:

  75% β- glucan→ 分解

  40% protein → 可溶

  5-31% starch →分解

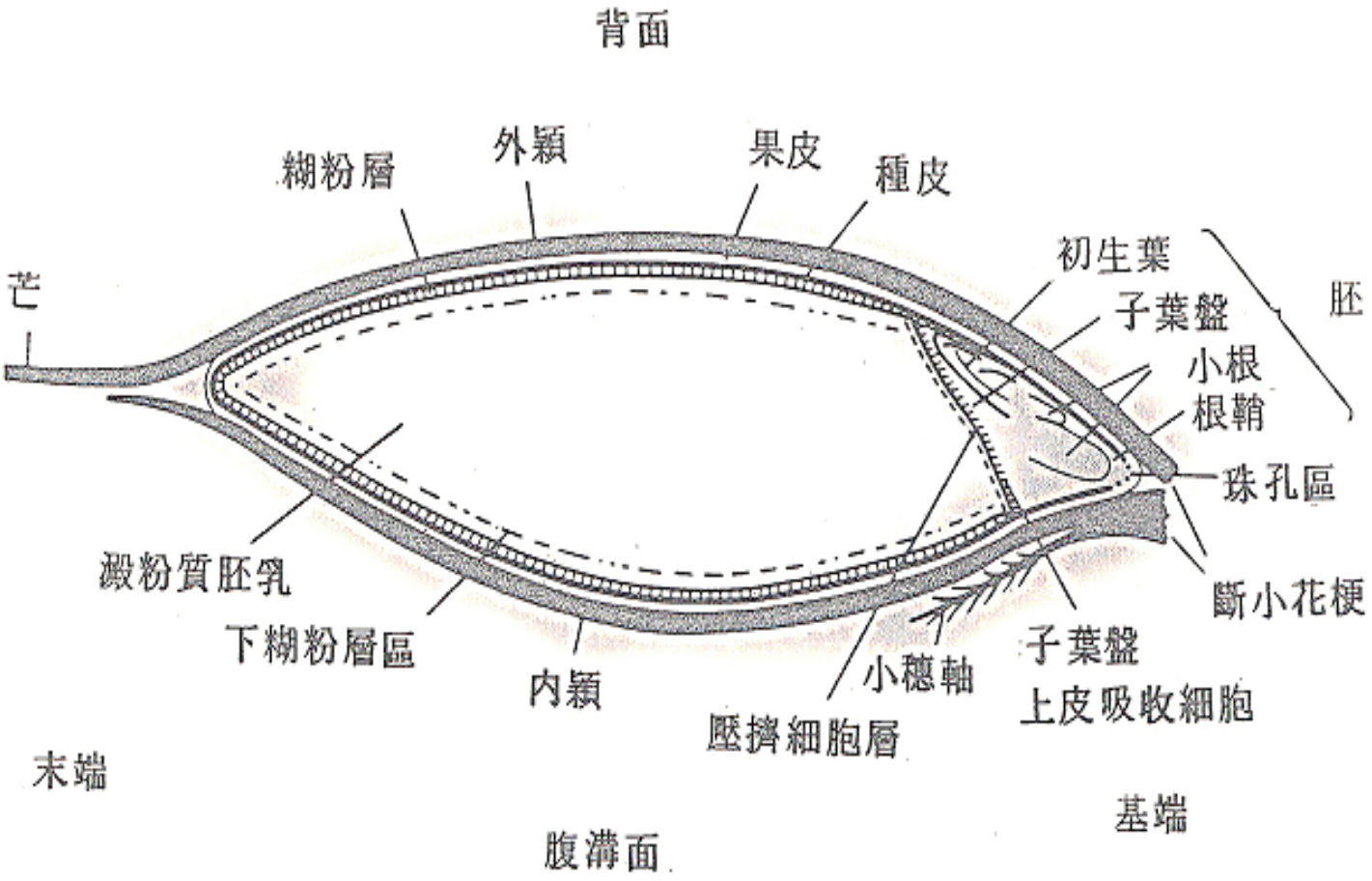


圖 2.2 大麥穀粒的縱切面構造圖

B. 糖化 (mashing)　麥芽粉碎 + H_2O ⟶

- 主要使90～95% starch 分解,成為可溶性.可在此加副原料(澱粉質)

starch → glucose, maltose, maltotriose, maltetrose, dextrin.

- pH : 5.4 (opt pH for amylase 5.3-5.4, protease 4.6-5.0)
- 溫度:有定溫(65℃), 或有多段升溫者
  35℃→54℃→65℃→70℃→78℃

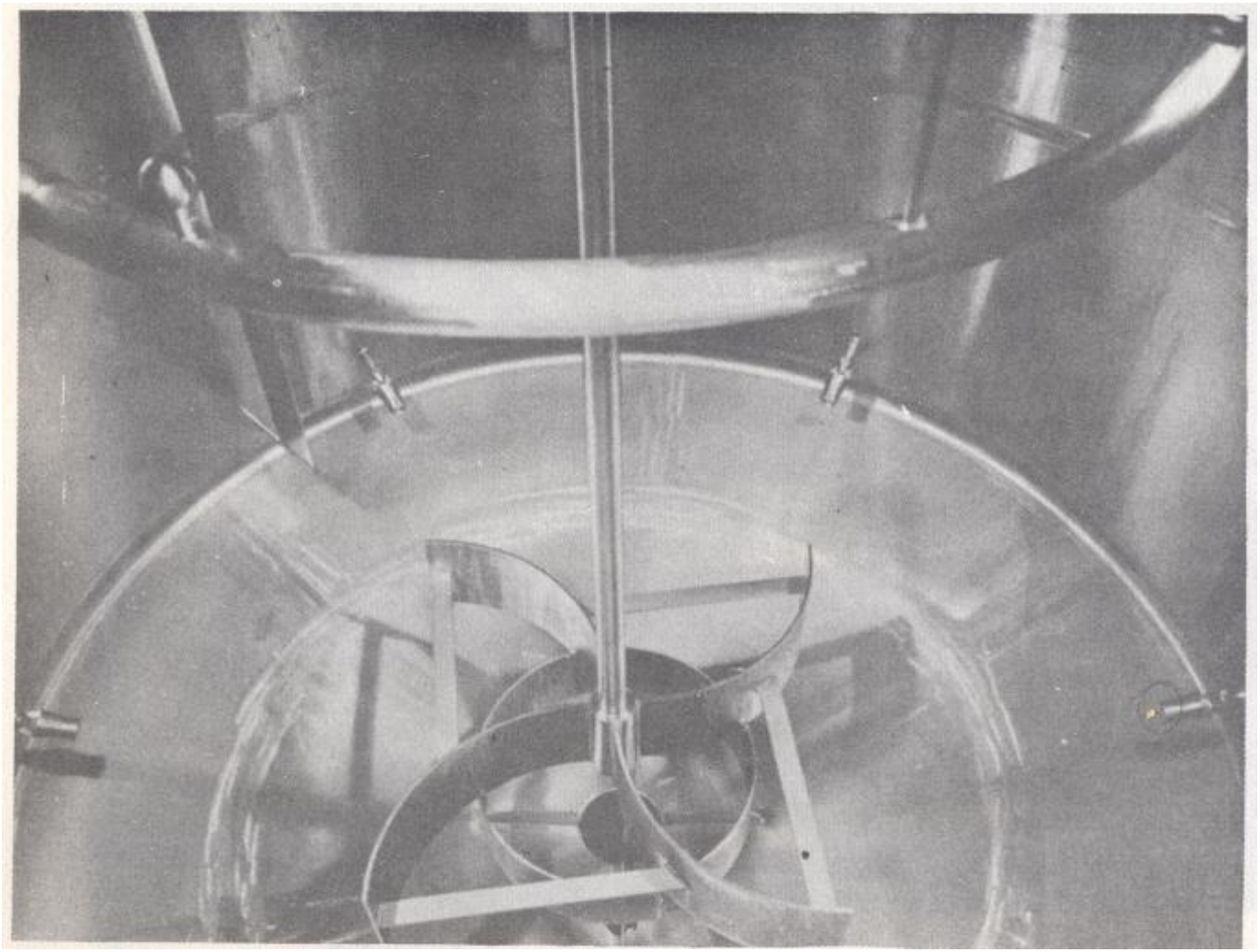


圖 5.13 穀物煮沸釜內部，使用直接蒸氣噴嘴

C. 過濾→麥汁(wort)

↓

麥粕(spent grain)

D. 添加啤酒花及麥汁煮沸

wort + Hop → boil for 1～2h

功能: (1) 終止酵素反應
(2) 麥汁殺菌
(3) 促使熱渣(hot trub ,or hot break)產生
(4) 去除麥汁中的青澀味
(5) 褐變反應
(6) 萃取啤酒花中的苦味物質及風味物質,可在煮沸前加副原料(糖類)

E. 麥汁冷卻、澄清、過濾

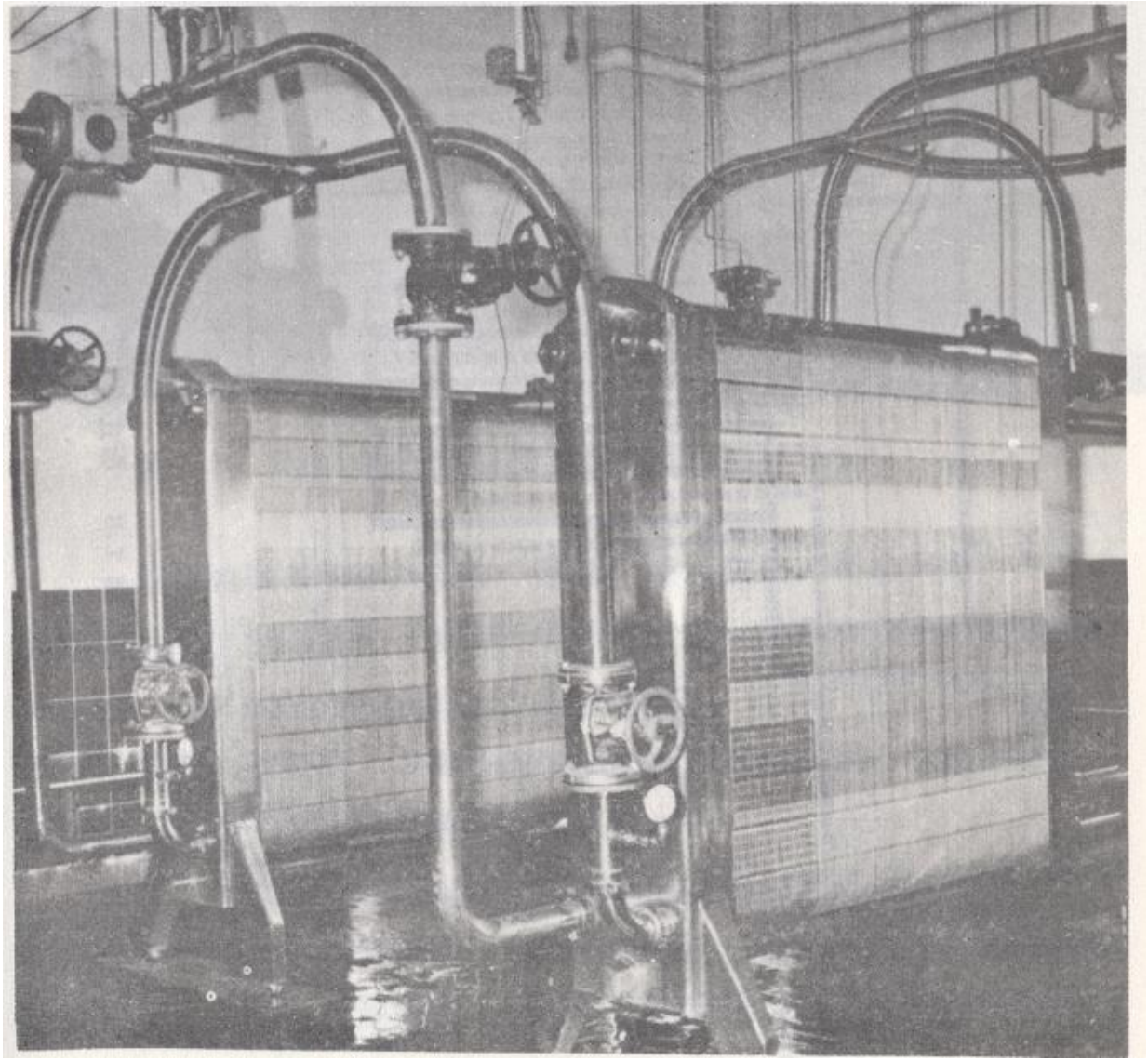


圖 7.7 板式冷卻器 (Plate type cooler)

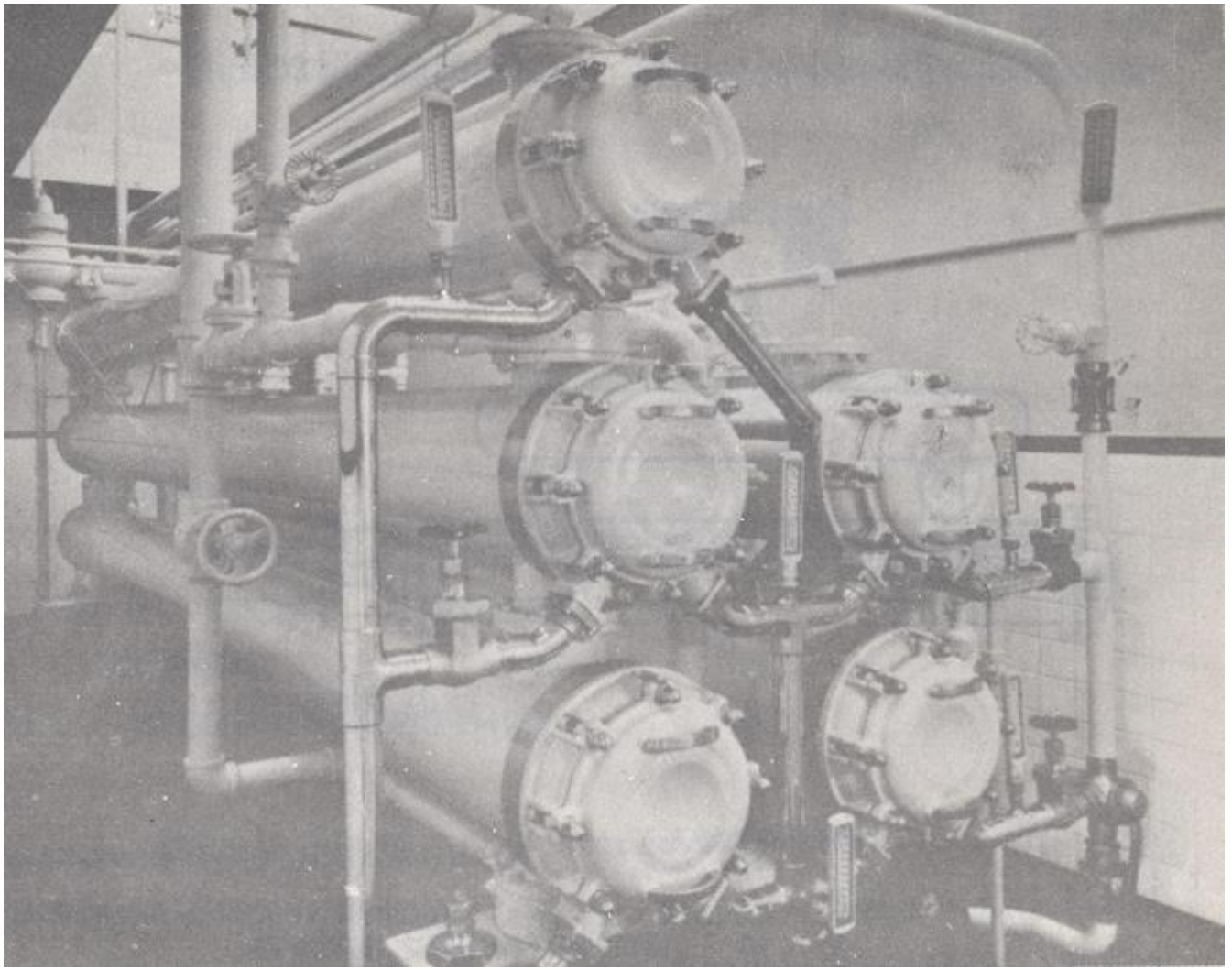


圖 7.9 管殼式麥汁冷卻器 (Shell and tube wort cooler)

F. 發酵：所用菌不同,啤酒分**頂面發酵啤酒(Ale)**；**底部發酵啤酒(Lager)**

- **Ale**： ***Saccharomyces cerevisiae***
  opt 40℃, < 5℃不長
- **Lager**： ***S. uvarum*** **opt 30-33℃ 0℃仍可生長**
- **Temp : Ale : 12-18℃ 3d**
  Lager : 8-12℃ 7-10d

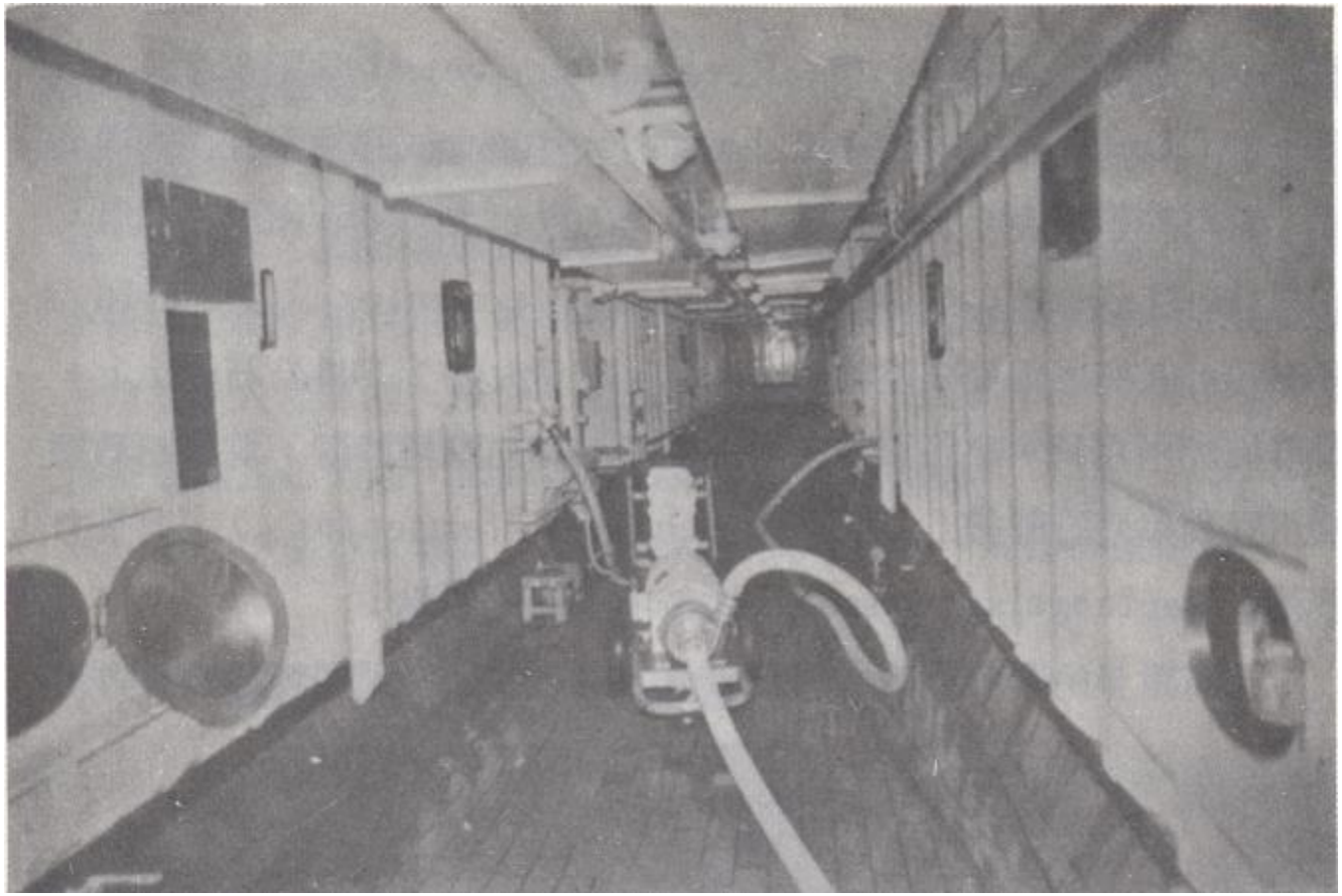


圖 8.7 長方形槽 (Rectangular vessel)

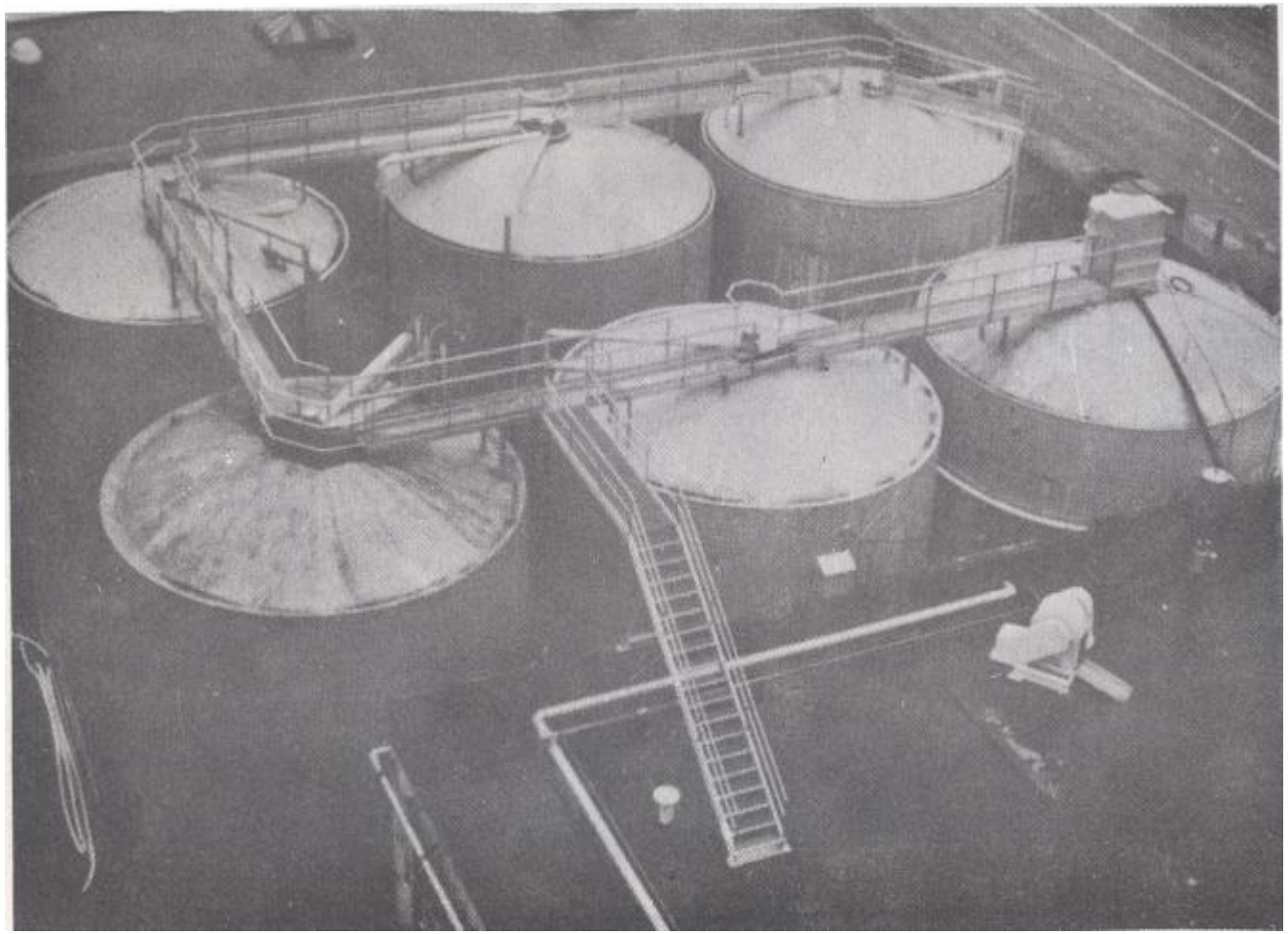


圖 8.8 單一槽 (Uni-tank)

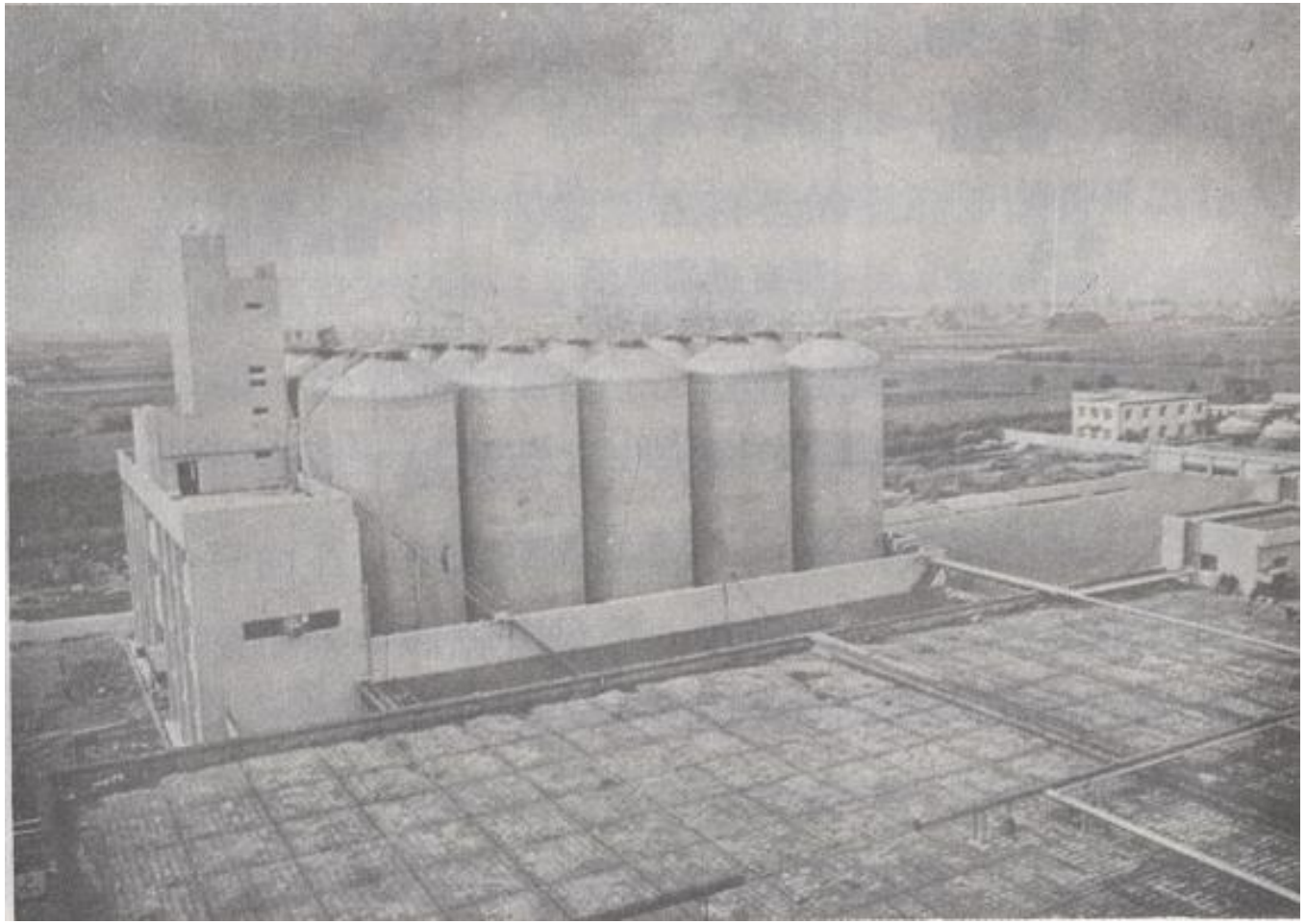


圖 8.9 成功啤酒廠之戶外圖筒錐底醱酵槽 (總容量 5,640 公石)

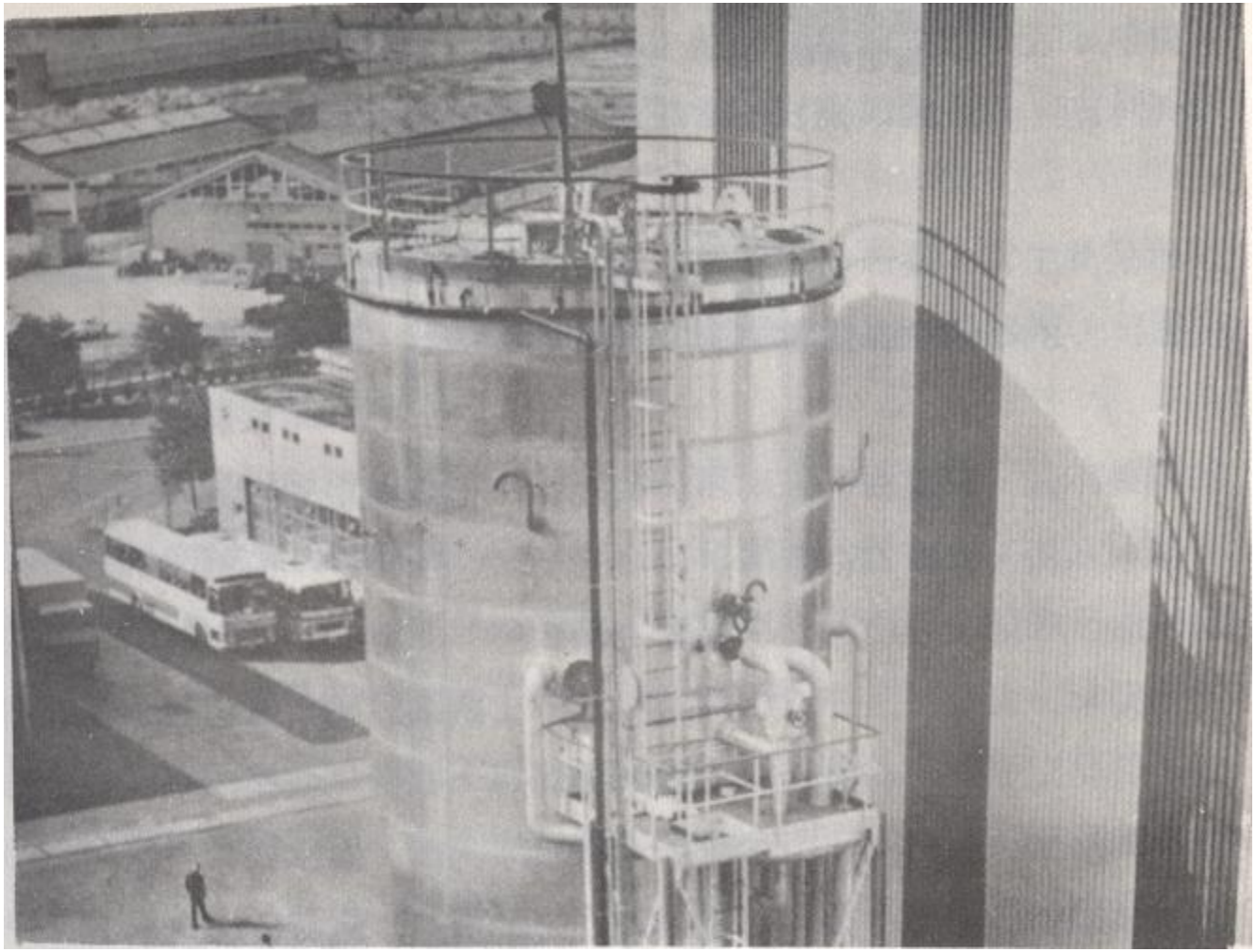


圖 8.9 大型戶外圓筒錐底醱酵槽 (Giant ylindro-conical fermentro)

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

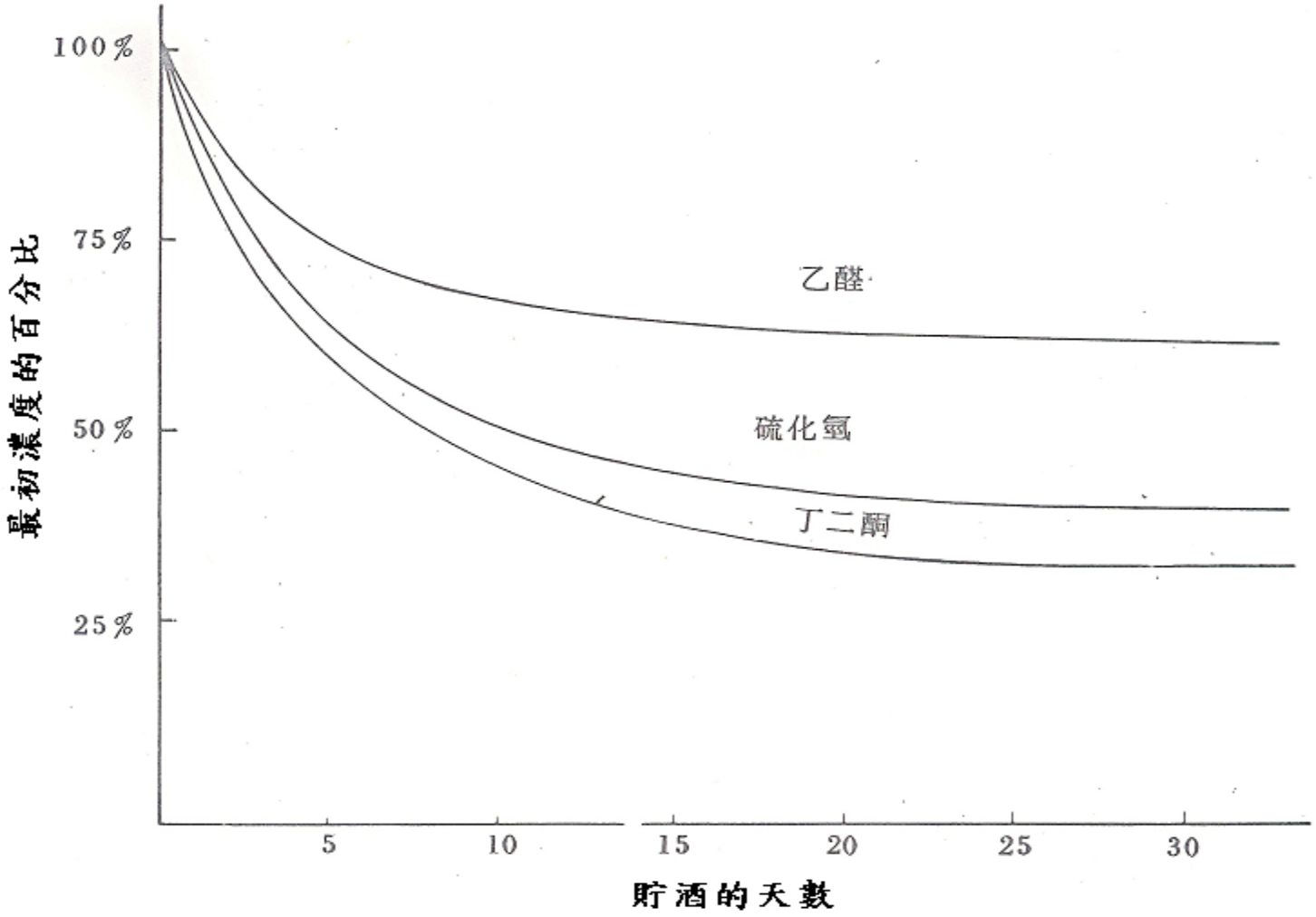
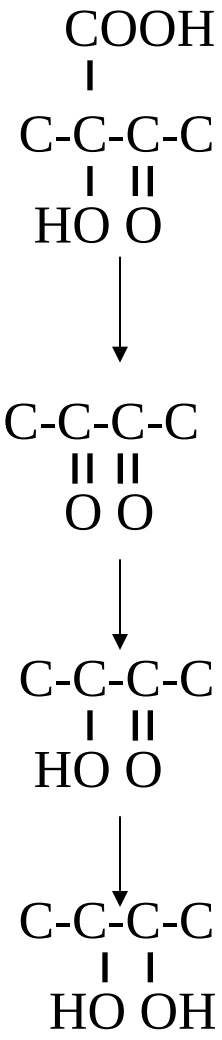
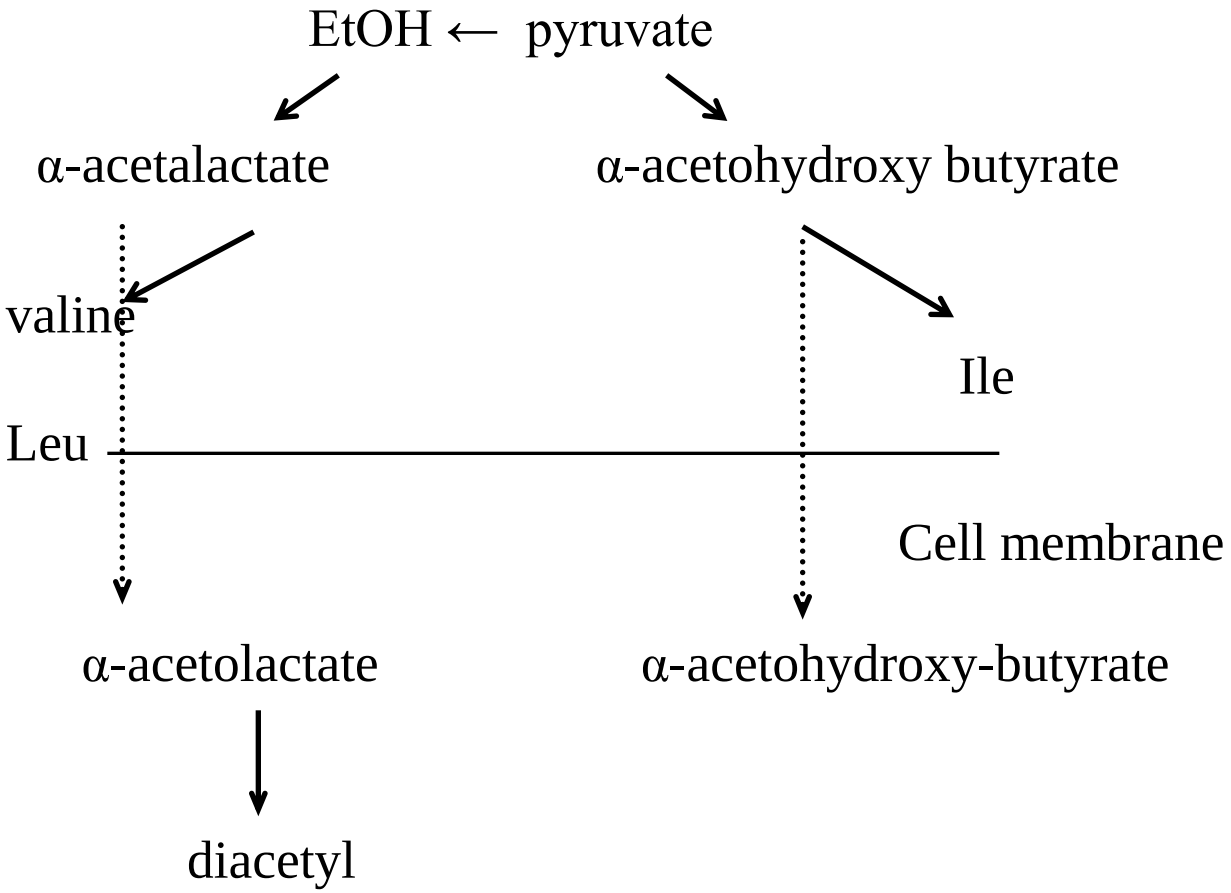


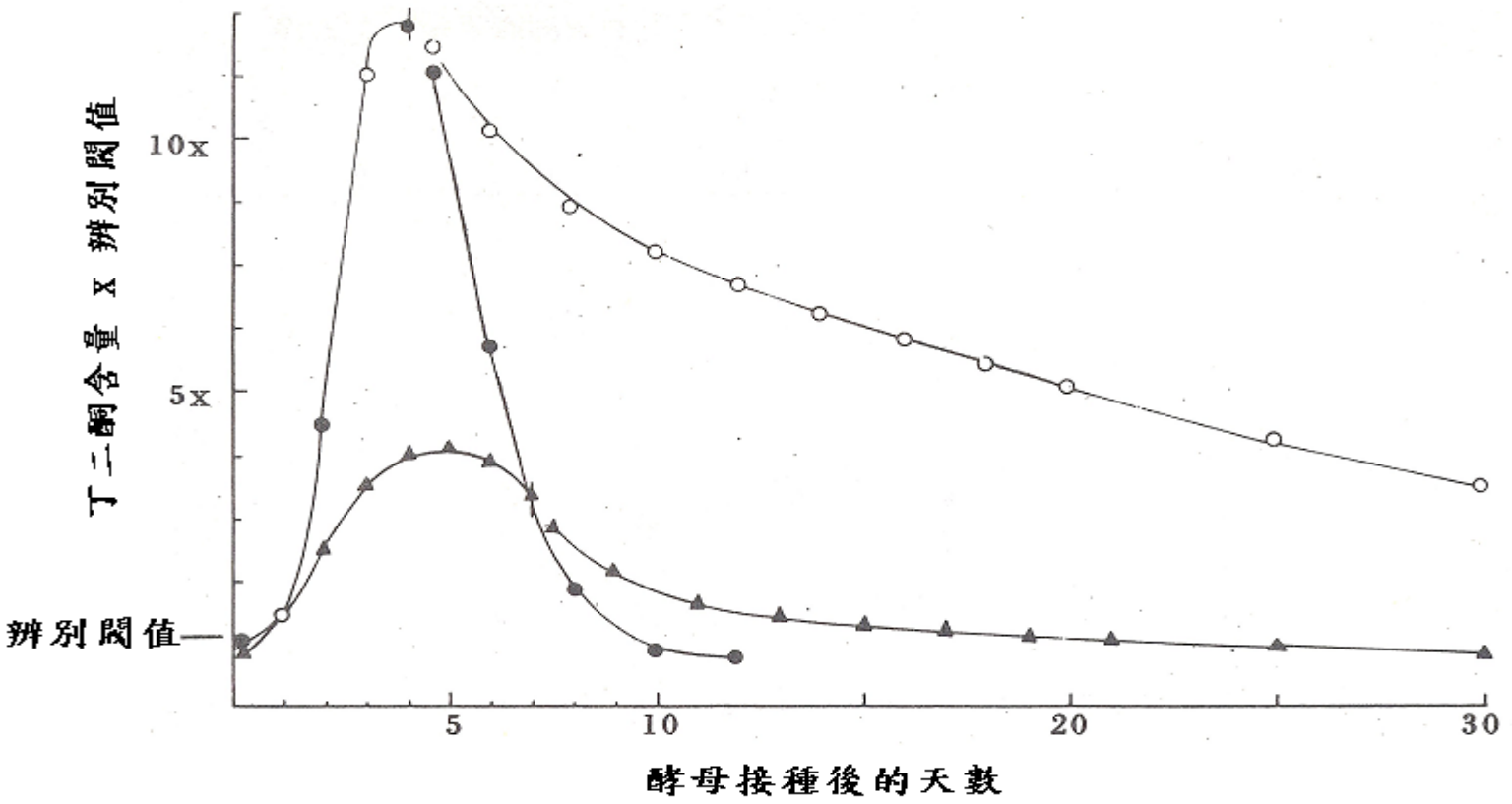
圖 9.2 貯酒期間風味化合物之減少情形

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

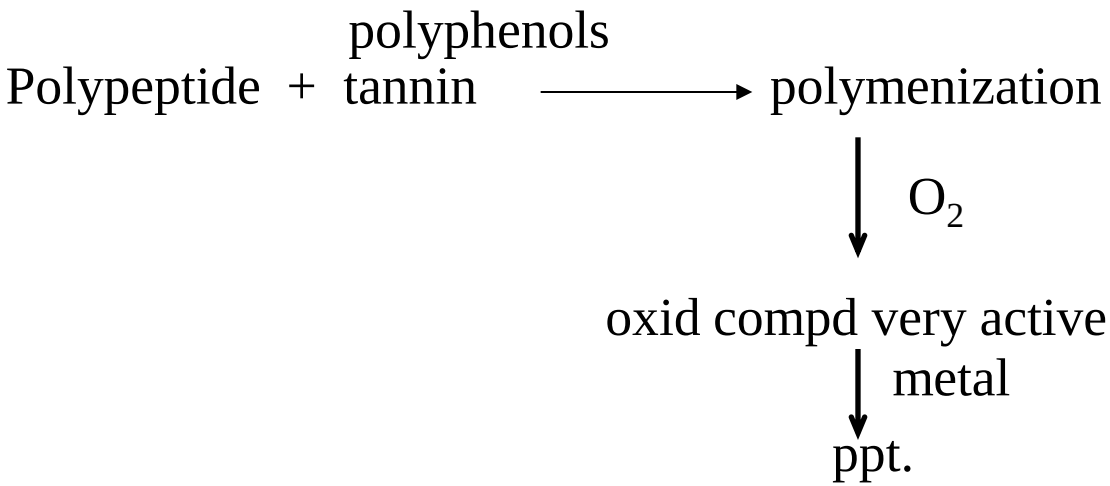
混濁(Haze) 預防

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

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

§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

3.Orleans process

- 50-100 gal 橡木桶裝一半體積原料
- 發酵完放出1/3體積產品以原料補充之
- 於24-27°C發效所得產品品質最好
- 每 6-8 清洗一次
- 發酵速度緩慢
- 橡木桶可用 25 年

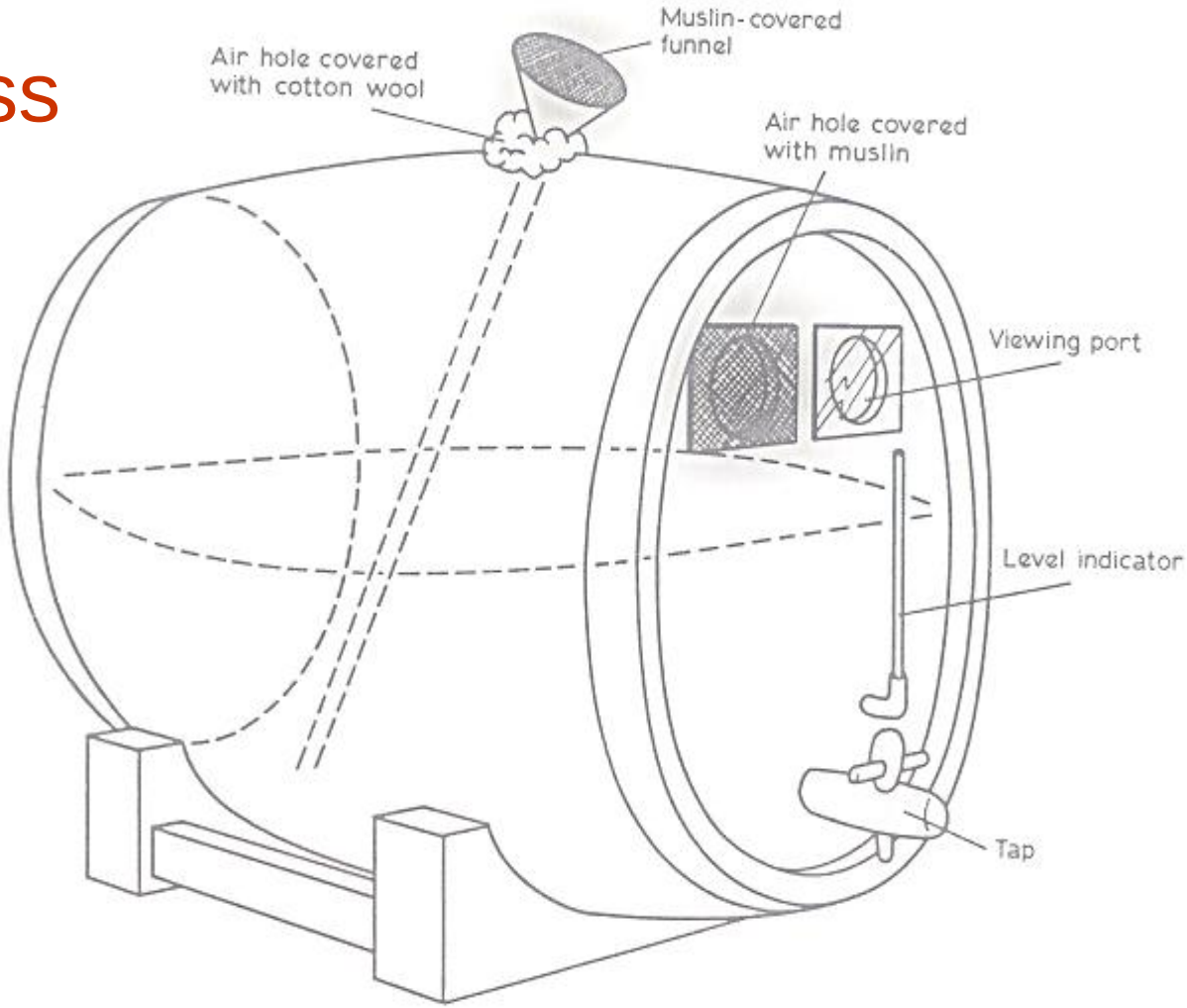


Fig. Acetification by the Orleans process.

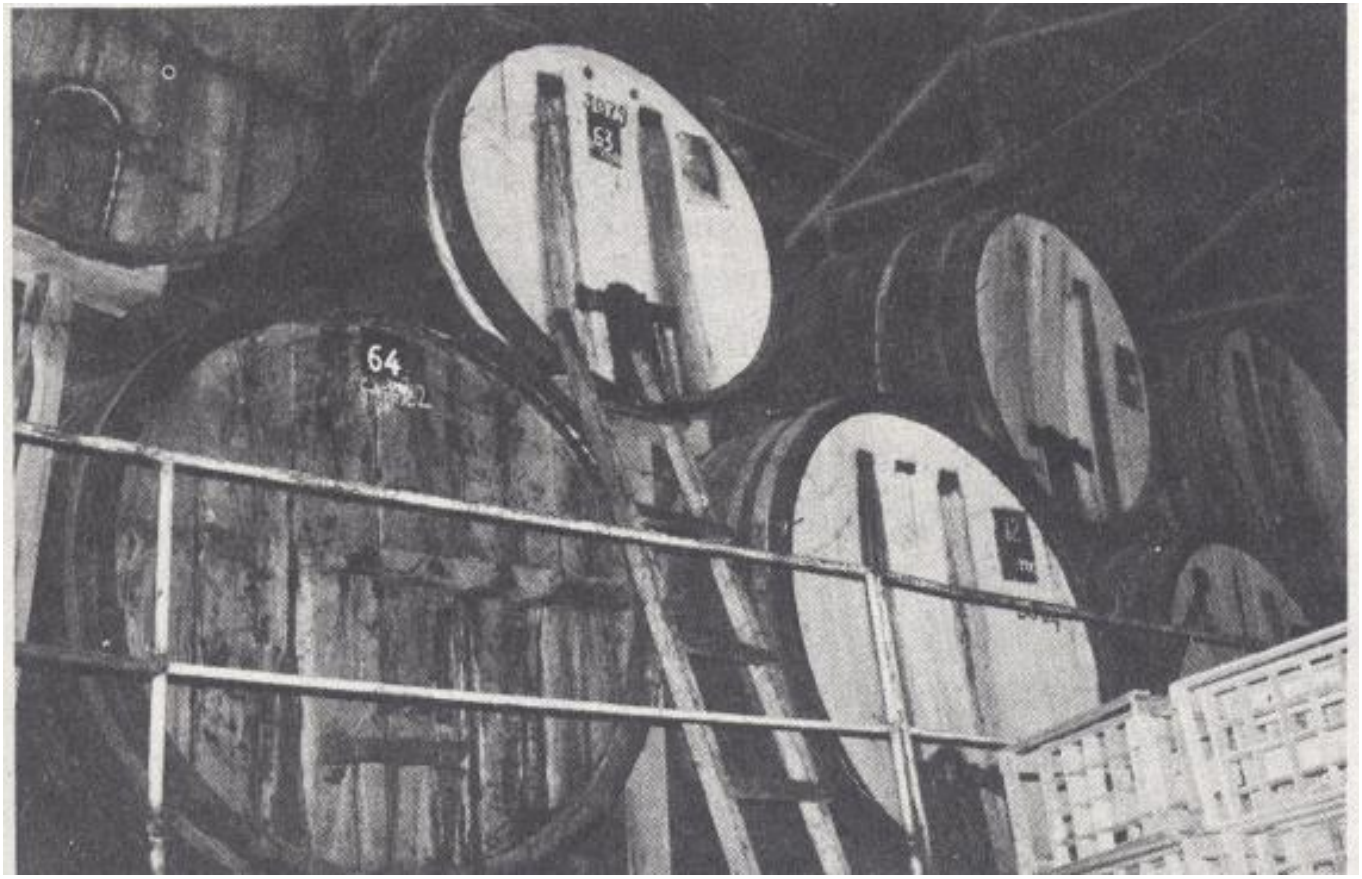


Fig. A vinegar brewery in Turkey using the Orleans process.

4.Quick vinegar process- Frings type generator

- 上部旋轉棒噴出液溫度為 28°C，底部為35°C
- 槽體填充螺旋木片
- 每立方米體積每天生產2-5 kg醋酸
- 酒精剩 0.3% 時終止

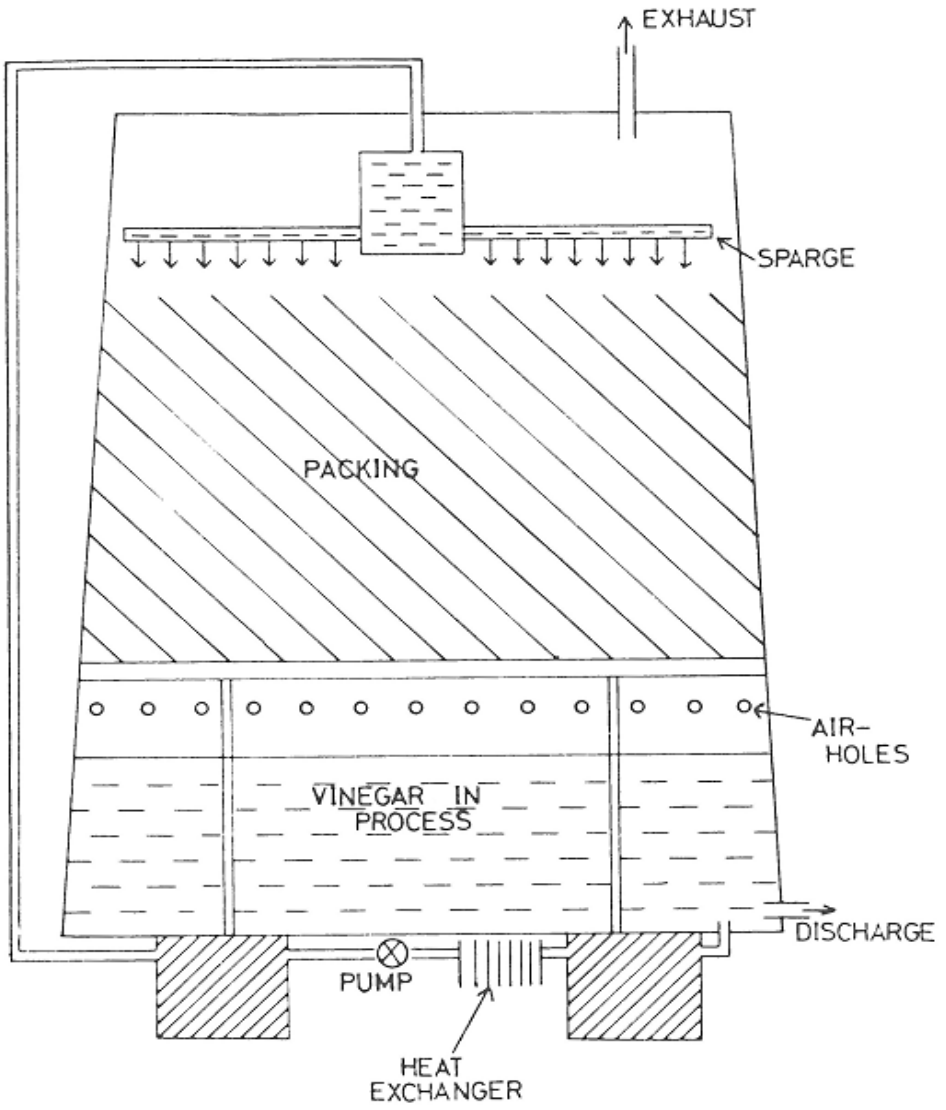
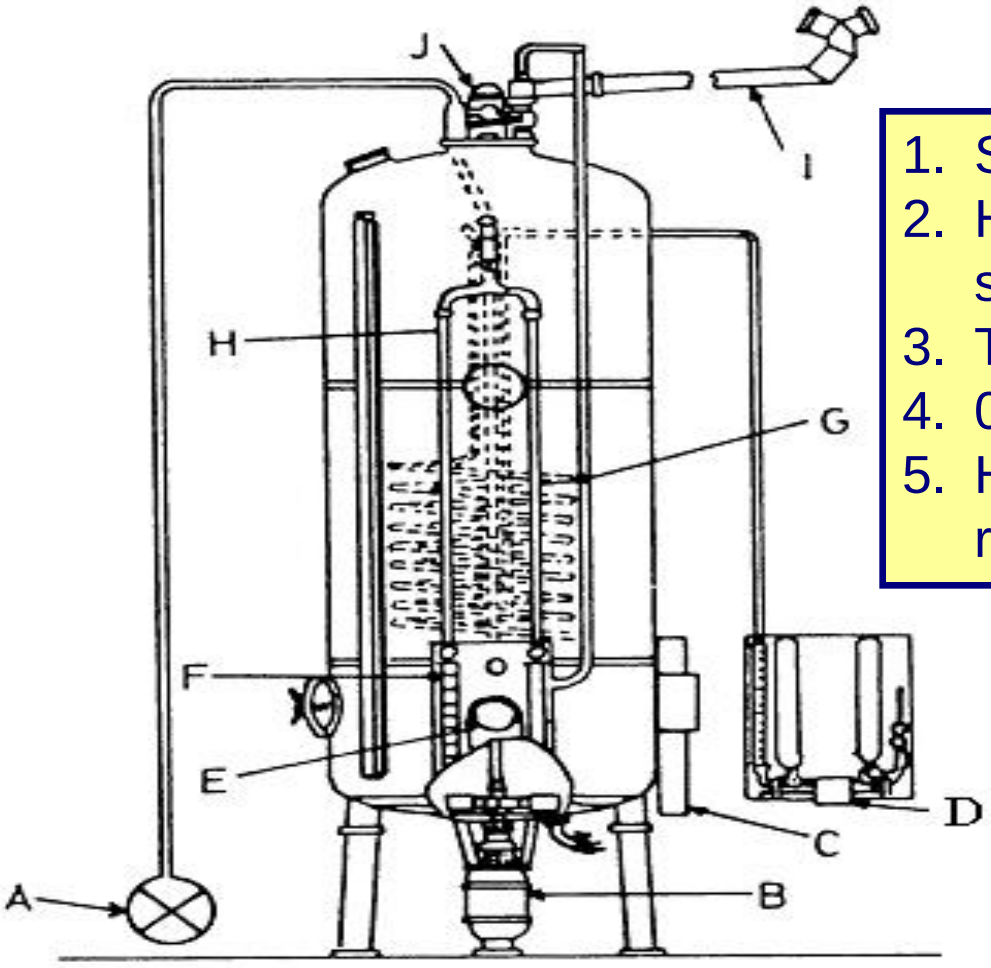


Fig. The quick vinegar process.

- 若 *Acetobacter xylinum* 生長 →產生纖維素(cellulose) → 造成阻塞(clog packing)
- 若基質酒精濃度 < 4~5 % ,會延遲醋酸化速度
- 發酵液酒精濃度 剩0.3 % 時即需終止反應以避免醋酸菌將醋酸轉變成CO25
- 貯存期間酯類風味物質增加

5.浸漬式醋酸發酵槽- Frings Acetator

- 半連續式　24~48 h
- 高效率供應O2
- 溫度 30ºC
- 酒精剩0.1~0.3% 時終止
- 抽出一半產品，以一半體積原料補充



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

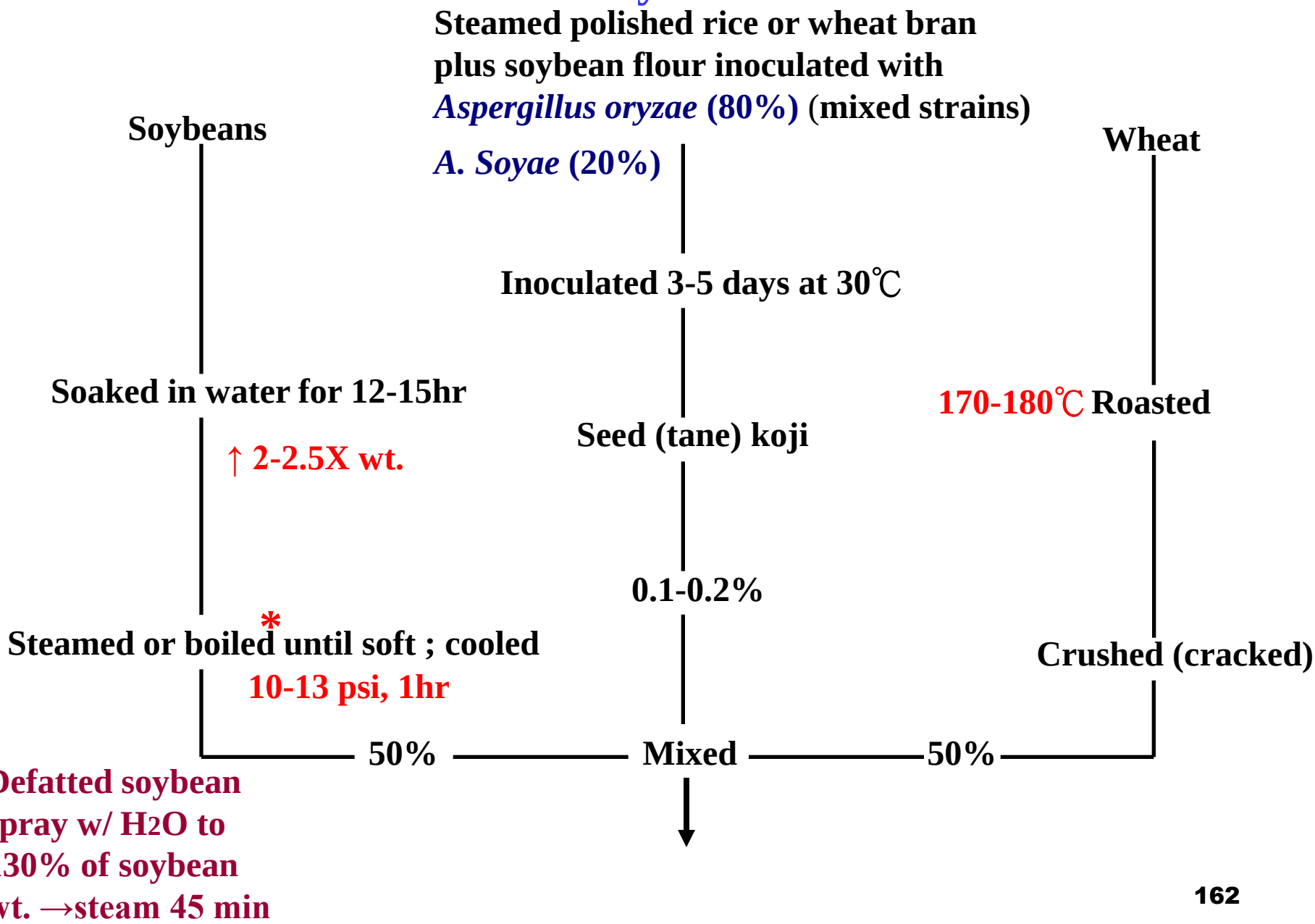
  or by mixing in a hydrate forming fluid

  (trichlorofluoromethane)

§Standards

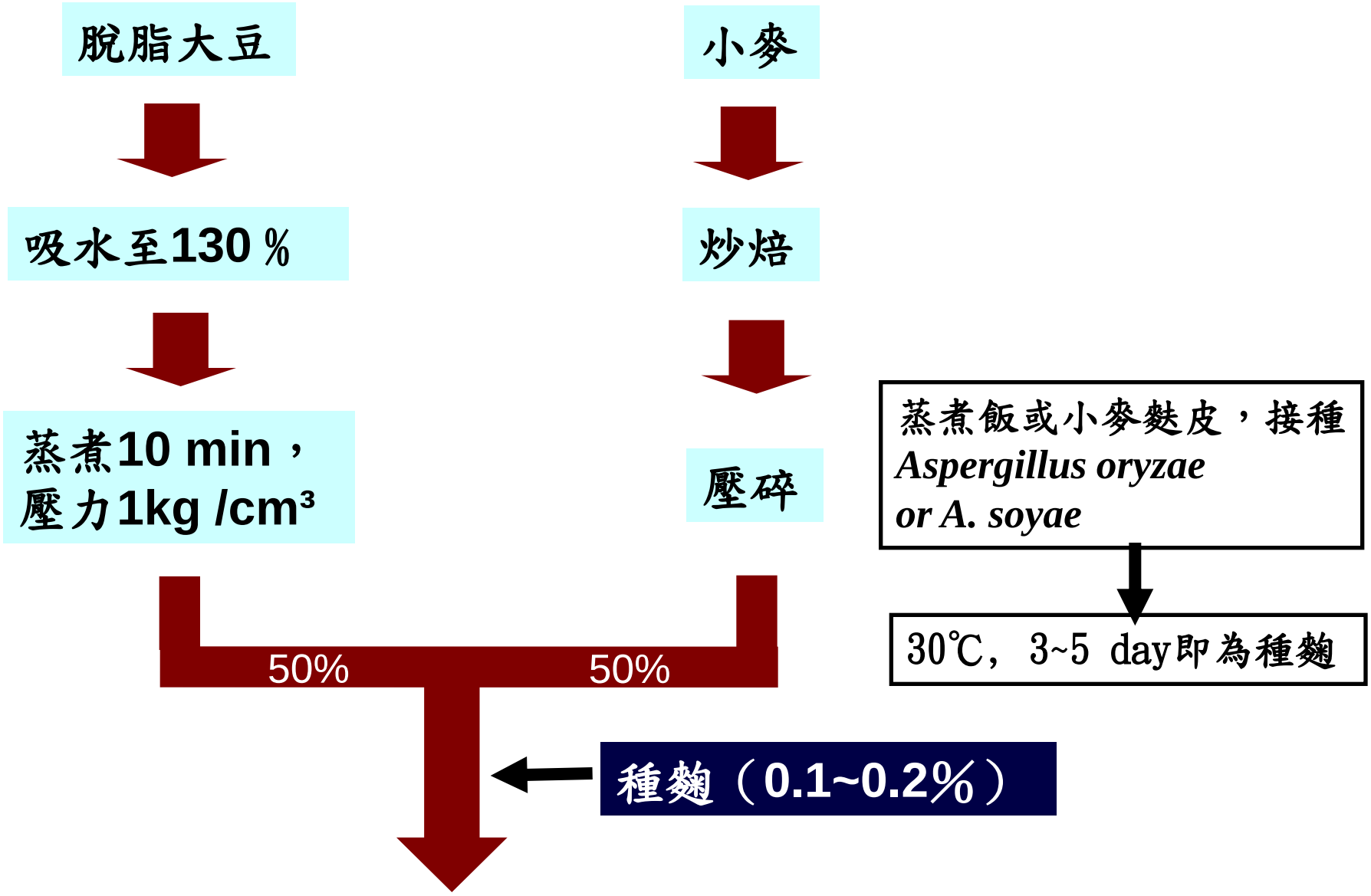
- HAC content: min level 4% W/A in U.S.A,
  UK bottle normally contain 5%

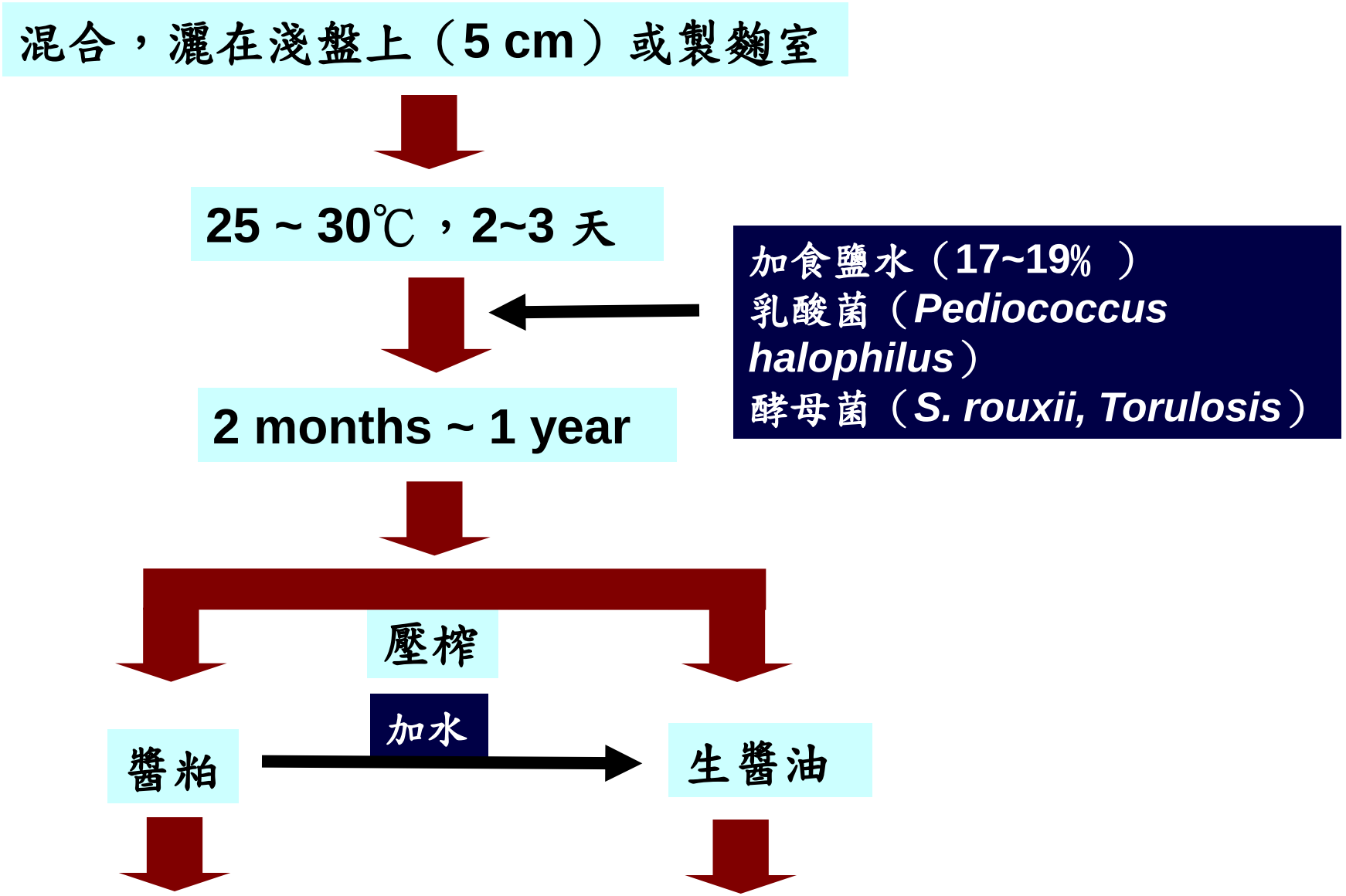
Soy sauce


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

醬　油

- 原料：soybean，wheet，seed koji (麴)
- koji：黴菌在穀類上生長繁殖，含有大量酵素（protease，amylase）
- Koji之製程：


脫脂大豆
吸水至130％
蒸煮10 min，
壓力1kg /cm³
小麥
炒焙
壓碎
50%
50%
種麴（0.1~0.2%）
蒸煮飯或小麥麩皮，接種
Aspergillus oryzae
or A. soyae
30℃，3~5 day即為種麴


混合，灑在淺盤上（5 cm）或製麴室
25 ~ 30℃，2~3 天
加食鹽水（17~19% ）
乳酸菌（Pediococcus halophilus）
酵母菌（S. rouxii, Torulosis）
2 months ~ 1 year
壓榨
加水
醬粕
生醬油

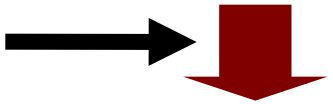
飼料

pasteurized

去渣

防腐劑

butyl-ρ-hydroxybenzoate（50μg / ml）or Na-benzoate（200μg / ml）



裝瓶

- 近年來，溫度控制：

開始	15 ℃	1 mon
	28 ℃	4 mon
	15 ℃	1 mon
品質良好		6 mon

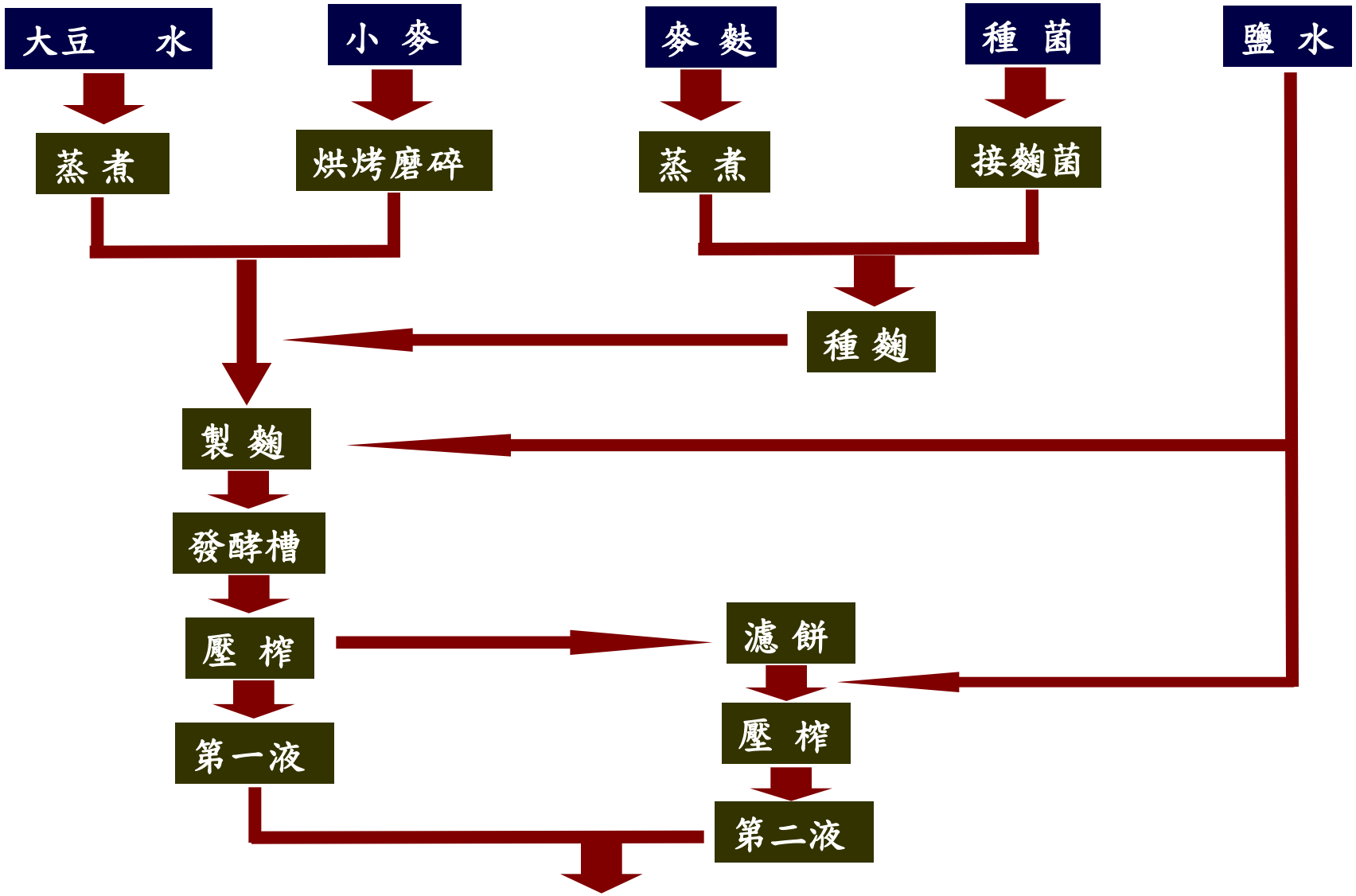
冷醬醪作用

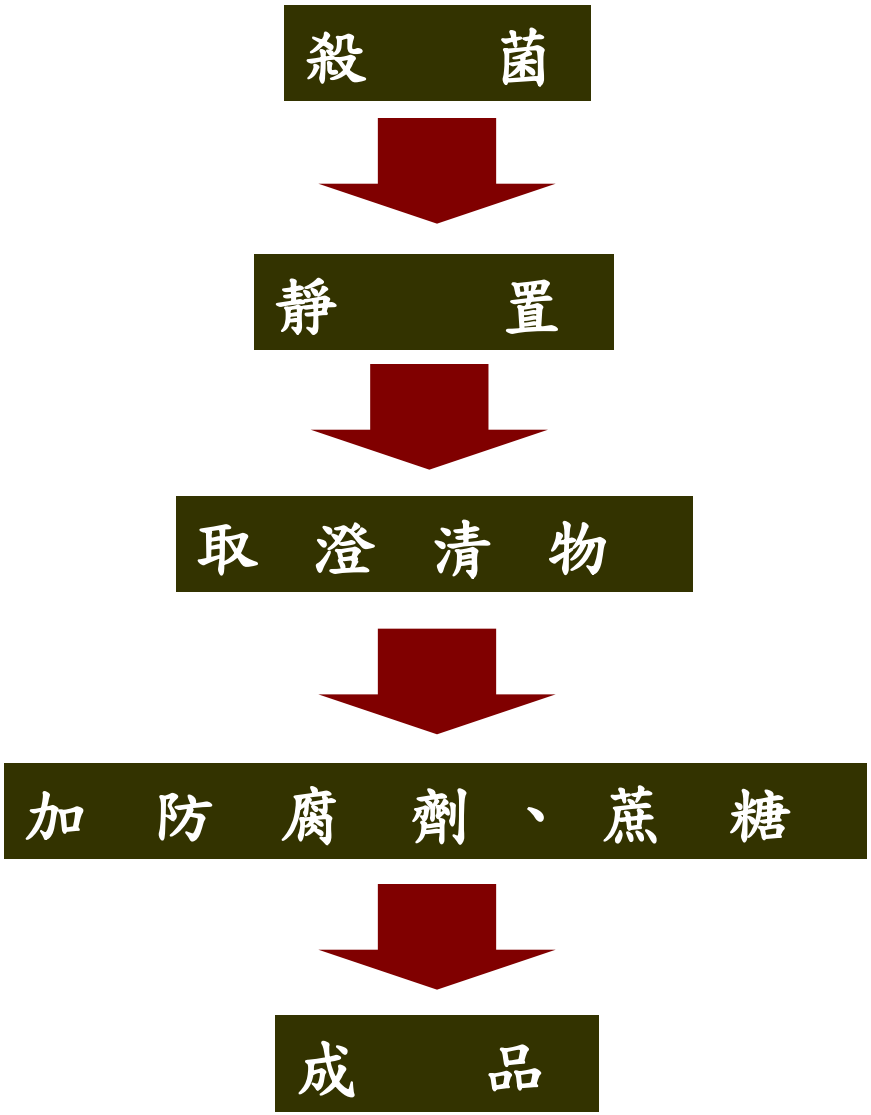
<1>防止peptidase (esp. 25-30℃失活 glutaminase)失活

<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

醬油製造流程




殺　菌
靜　置
取 澄 清 物
加 防 腐 劑 、 蔗 糖
成　品

- 大豆：蛋白質來源 $\xrightarrow{\text{protease}}$ a. a. 或小分子 peptide
- 小麥： 澱粉 $\xrightarrow{\text{amylase}}$ 可溶性糖
- mold：產生protease 及 amylase，分解原料中大分子protein及startch
- 乳酸菌：產酸，降低pH，促使酵母菌作用。風味物
- 酵母菌： 主要風味物產生者. EtOH , ester, glycerol

§Moromi (mash)醬醪

- brine fermentation

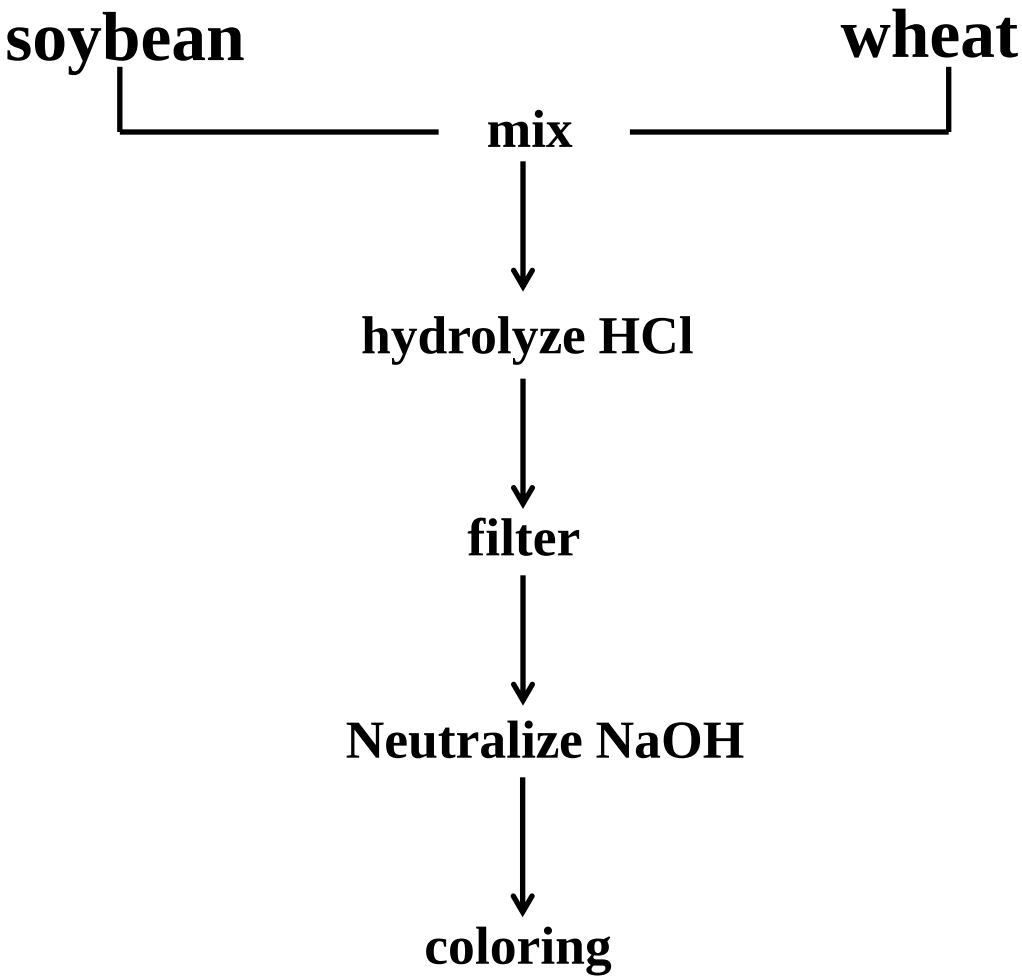
- Koji + equal volume of salt soln
  = moromi recently use 1:1.1~1.2 ratio

- [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

§Chemical process



- **化學醬油：**

  以鹽酸直接水解脫脂大豆，再中和，所得氨基酸液加焦糖色素調色，及食鹽水來調味即成。氮利用率高（90％）

- **醬油色澤深淺：**

與（1）大豆，小麥之比例

（2）小麥炒焙程度，有關。

一般醬油	
pH	4.6 ~ 4.8
總氮	1.5 ~ 1.8％
glycerol	0.4 ~ 0.5％
NaCl	18％

§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

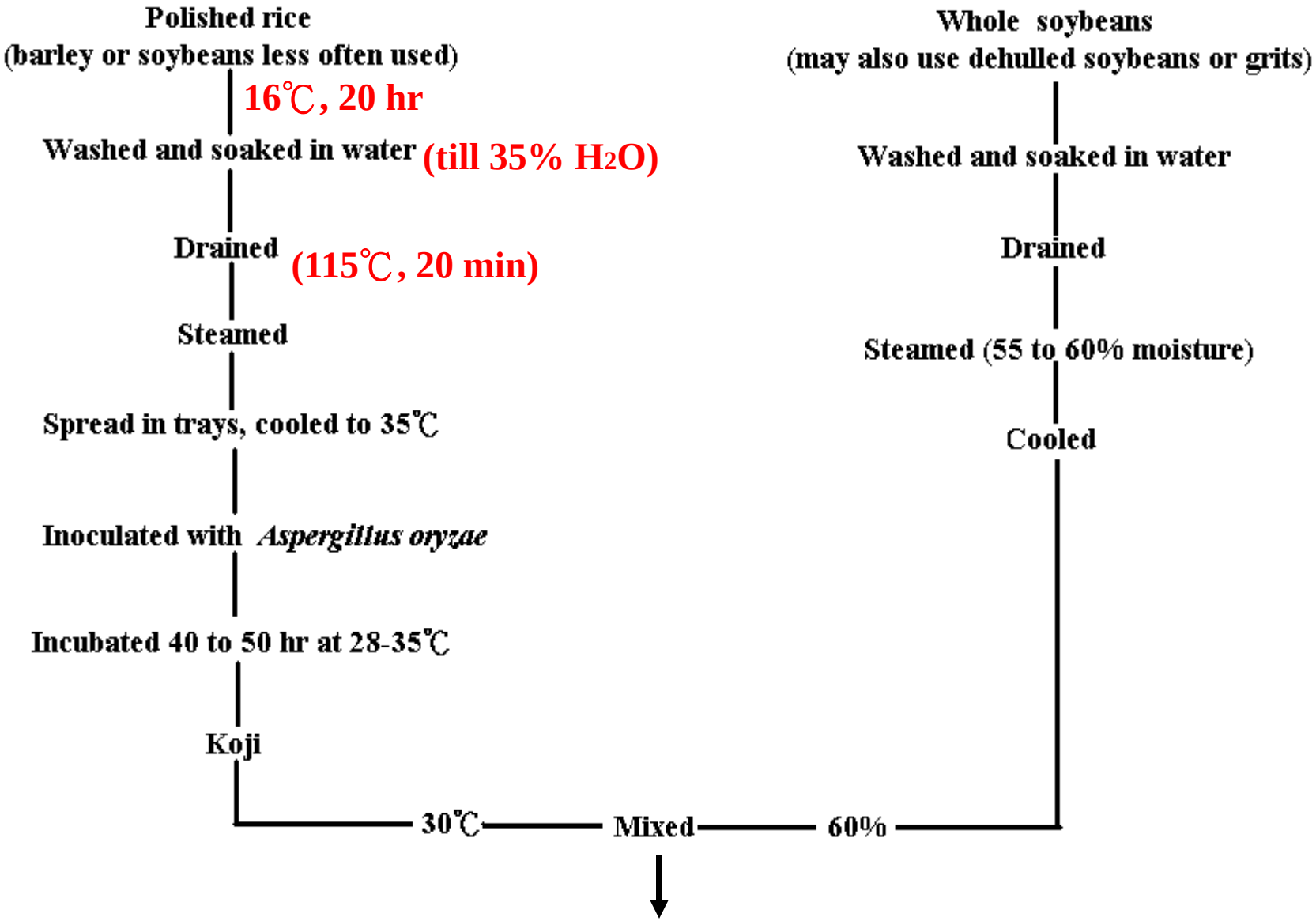
Miso

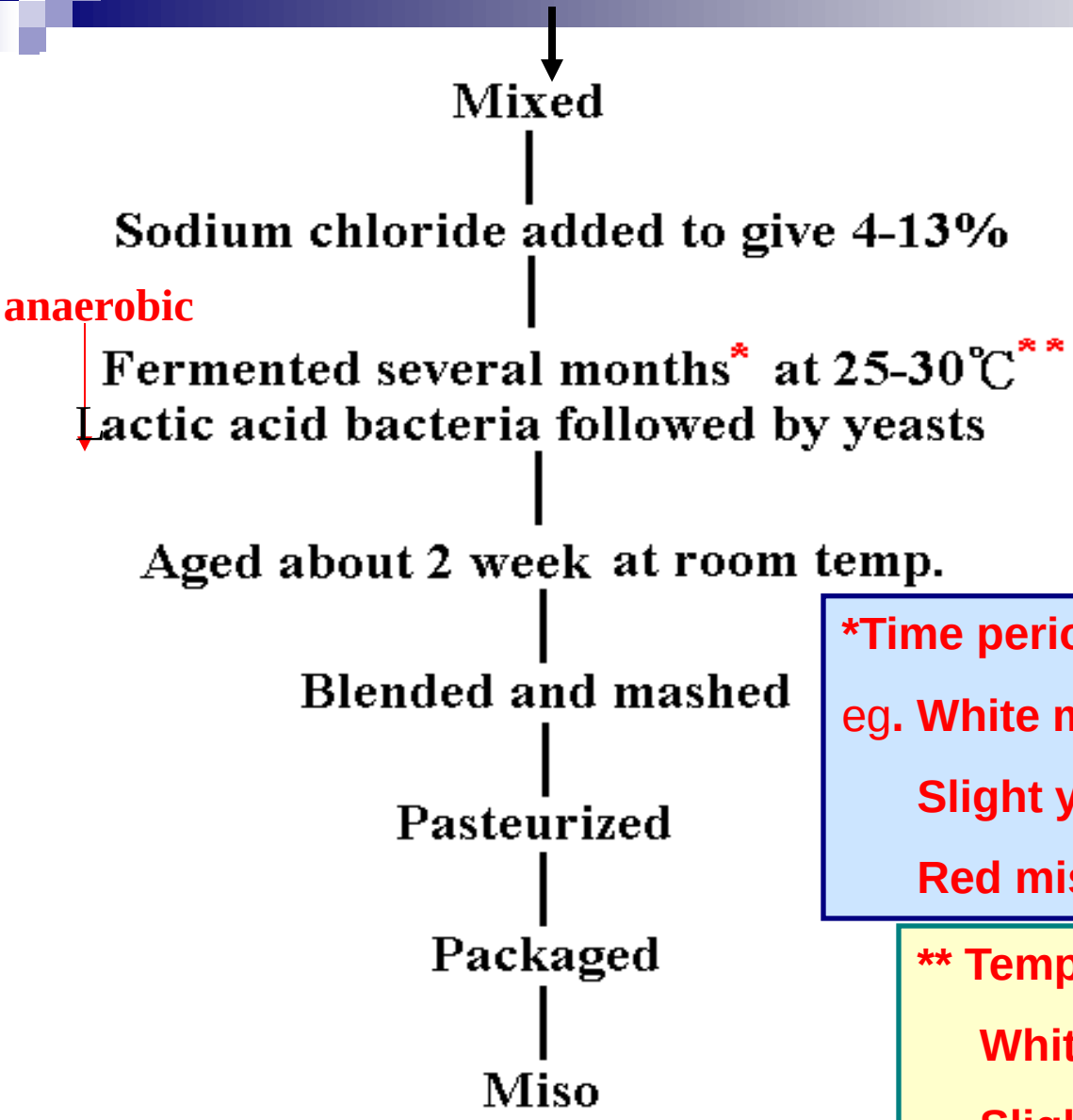
Miso-Japan, Taiwan, China, Korea, Philippines
Soybean paste (yellowish-white to dark brown)

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
Whole soybeans
(may also use dehulled soybeans or grits)
Washed and soaked in water
Drained
Steamed (55 to 60% moisture)
Cooled
30℃
Mixed
60%



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.

- 1000 Kg soybean + 600 Kg rice + 430 Kg
  →3300 Kg light yellow salty miso
  (48% H_2O)

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
Saltry 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.

Thanks for your attention