

and
ved
n of
ods

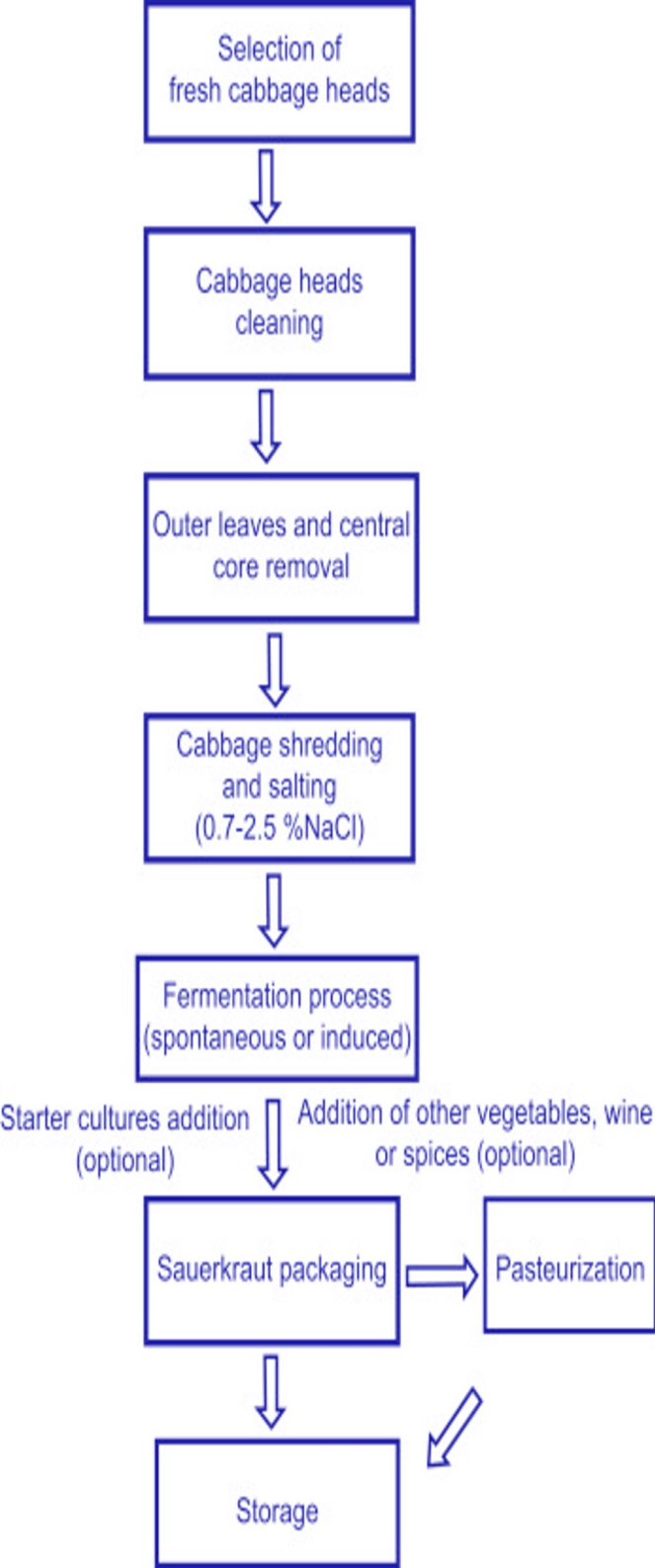


Application and progress of the high throughput biotechnologies into traditional fermented food industries like fermented milk products (yoghurt, cheese), fermented sausages, fermented vegetables (kimchi, sauerkraut), fermented cereals (sourdough) and fermented beans (tempeh, natto) are gaining momentum.

Raw material	Fermented food product
Wheat	Bread
Milk	Cheese, yoghurt
Maize	Ogi, Akamu, Kokonte
Cassava	Garri, Foo-foo, Akpu, Lafun
Vegetables	Sauerkraut, pickled cucumbers
Stimulant beverages	Coffee, Tea and Cocoa
Legumes and oil seeds	Soy sauce, Miso, Sufu, Oncom, Idli, Ogili, Dawa dawa, Ugba
Fish	Fish sauce

Fermented Vegetables

- Sauerkraut is produced by fermentation of cabbage, *Brassica oleracea*.
- The cabbage is sliced into thin pieces known as slaw and preserved in salt water or brine containing about 2.5% salt.
- The slaw must be completely immersed in brine to prevent it from darkening.
- Kraut fermentation is initiated by *Leuconostoc mesenteroides*, a heterofermentative lactic acid bacterium (produces lactic acid, acetic acid and CO₂).
- CO₂ creates anaerobic conditions and eliminates organisms which might produce enzymes which can cause softening of slaw and also encourages growth of other lactic acid bacteria.



- Gram negative coliforms and pseudomonads soon disappear, and give way to a rapid proliferation of *Lactobacillus brevis*, which is heterofermentative, and the homofermentative *Lactobacillus plantarum*; *Pediococcus cerevisiae* also occurs.
- Compounds which contribute to the flavor of sauerkraut begin to appear with the increasing growth of the lactics (lactic and acetic acids, ethanol, and volatile compounds such as diacetyl, acetaldehyde, isoamyl alcohol, n-hexanol, ethyl lactate, ethyl butyrate, and isoamyl acetate).
- Besides 2.5% salt, temperature of 15°C be used.
- Higher temperatures cause deterioration of kraut.



HOW TO MAKE SAUERKRAUT

WITH STEP-BY-STEP INSTRUCTIONS



Cucumbers (pickling)

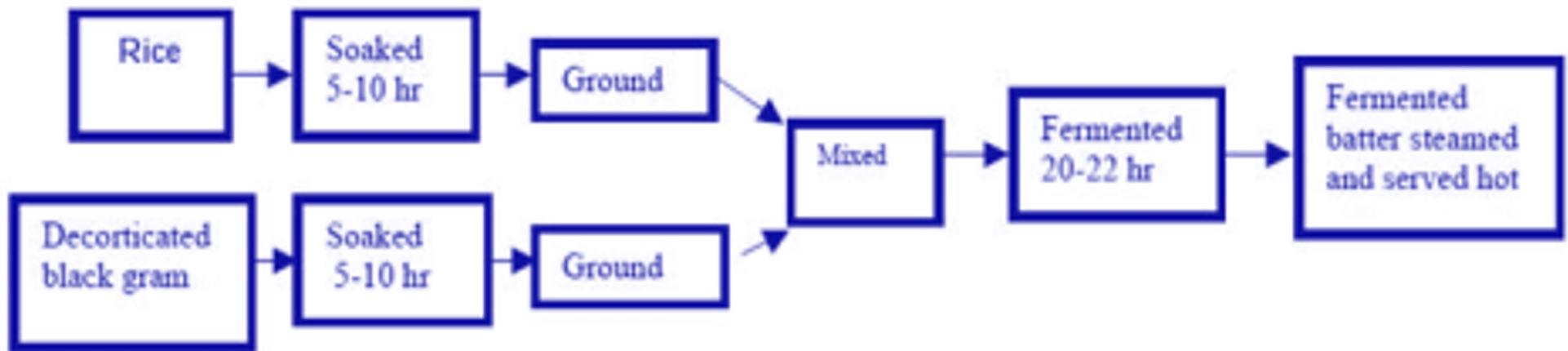
- Cucumbers for pickling are best harvested before they are mature.
- Mature cucumbers are too large, ripen easily and are full of mature seeds.
- Cucumbers may be pickled by dry salting or by brine salting .
- Dry salting is also used for cauliflower, peppers, okra, and carrots. It consists of adding 10 to 12% salt to the water before the cucumbers are placed in the tank. This prevents bruising/other damage to vegetables.
- Brine salting is more widely used. Lower amount of salt is added, between 5 and 8% salt.
- During primary fermentation lasting two or three days, most of the unwanted bacteria disappear allowing the lactics and yeasts to proliferate.
- In final stages, after 10 to 14 days, *Lactobacillus plantarum* and *L. brevis*, followed by *Pediococcus*, are the major organisms.



Fermented Foods from Cereals And Beans

Idli

Idli is a popular fermented breakfast and hospital food which has been eaten in South India for many years. Prepared from rice grains and seeds of leguminous mung grain, *Phaeseolus mungo*, or from black gram (udad dhal), *Vigna mungo*. When the material contains Bengal grain, *Circer orientium*, the product is known as khaman. It has a spongy texture and a pleasant sour taste due to lactic acid. Embellished with flavoring ingredients such as cashew nuts, pepper and ginger.





Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

- The seeds of the black gram are soaked in water for 1-3 hours to soften them and to facilitate decortication, after which the seeds are mixed and pounded with rice (Rice to black gram; 3:1).
- Mixture is allowed to ferment overnight (20-22 h).
- Organisms involved in the acidification are *Streptococcus faecalis*, and *Pediococcus* spp.
- Leavening is brought about by *Leuconostoc mesenteroides*, although the yeasts, *Torulopsis candida* and *Trichosporon pulluloma* have also been found in traditional Idli.
- Fermented batter is steamed and served hot. Idli is highly nutritious, being rich in nicotinic acid, thiamine, riboflavin, and methionine.





Production of Beer

1. **Barley beers can be divided into two broad groups:** top-fermented beers and bottom fermented beers.
2. This distinction is based on whether the yeast remains at the top of brew (top-fermented beers) or sediments to the bottom (bottom-fermented beers) at the end of the fermentation.
3. **Raw Materials for Brewing:** barley, malt, adjuncts, yeasts, hops, and water.
4. **Brewer's yeasts:** Yeasts produce alcohol from sugars under anaerobic conditions, but not all yeasts are necessarily suitable for brewing. Brewing yeasts besides producing alcohol, are able to produce from wort sugars and proteins in a balanced proportion of esters, acids, higher alcohols, and ketones which contribute to the peculiar flavor of beer.



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

Brewery Processes

The processes involved in the conversion of barley malt to beer may be divided into following:

- 1. Malting**
- 2. Cleaning and milling of the malt**
- 3. Mashing**
- 4. Mash operation**
- 5. Wort boiling treatment**
- 6. Fermentation**
- 7. Storage or lagering**
- 8. Packaging**



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

Malting

The purpose of malting is to develop amylases and proteases in the grain.

These enzymes are produced by germinated barley to enable it to break down carbohydrates and proteins in the grain to nourish germinated seedling before its photosynthetic systems are developed enough to support the plant.

Cleaning and milling of malt: Purpose of milling is to expose particles of the malt to the hydrolytic effects of malt enzymes during mashing. Finer the particles, greater the extract from the malt.

Mashing

Mashing is the central part of brewing.

Determines the nature of the wort, hence the nature of the nutrients available to the yeasts and therefore the type of beer produced.



Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

Purpose of mashing is to extract as much as possible the soluble portion of malt and to enzymatically hydrolyze insoluble portions of malt and adjuncts.

Aqueous solution resulting from mashing is known as wort.

Wort is boiled for 1-1½ hours in a stainless steel kettle. When corn syrup or sucrose is used as an adjunct it is added at beginning of boiling.

Hops are also added, some before and some at the end of the boiling.

Hops are the dried cone-shaped female flower of hop-plant *Humulus lupulus*.

The importance of hops in brewing lies in its resins which provide the precursors of the bitter principles in beer and the essential (volatile) oils which provide the hop aroma.



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

Purpose of boiling:

- To concentrate the wort
- To sterilize the wort
- To inactivate any enzymes
- To extract soluble materials from the hops
- To precipitate protein, which forms large flocs because of heat denaturation and complexing with tannins extracted from hops and malt husks. Unprecipitated proteins form hazes in beer, but too little protein leads to poor foam head formation.
- To develop color in beer; some of color in beer comes from malting but bulk develops during wort boiling. Color is formed by several chemical reactions including caramelization of sugars, oxidation of phenolic compounds, and reactions between amino acids and reducing sugars.
- Removal of volatile compounds: Fatty acids which could lead to rancidity in the beer are removed.



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

Fermentation

- Cooled wort is pumped or allowed to flow by gravity into fermentation tanks and yeast is inoculated or pitched in' at a rate of $7-15 \times 10^6$ yeast cells/ml, usually collected from a previous brew.
- Progress of fermentation is followed by wort specific gravity.
- During fermentation the gravity of wort gradually decreases because yeasts are using up the extract. However, alcohol is also being formed.
- As alcohol has a lower gravity than wort, reading of special hydrometer (known as a saccharometer) is even lower.
- °Brix is used in sugar industry, whereas Balling (United States) and °Plato (continental Europe) are used in brewing industry.



Centurion
UNIVERSITY
*Shaping Lives...
Empowering Communities...*

Lagering

1. During lagering, secondary fermentation occurs.
2. Yeasts are sometimes added to induce this secondary fermentation, utilizing some sugars in green beer.
3. Secondary fermentation saturates beer with CO₂.

Packaging:

1. Beer is transferred to pressure tanks from where it is distributed to cans, bottles and other containers.
2. Beer is not allowed to come in contact with oxygen during this operation; it is also not allowed to lose CO₂ or become contaminated with microorganisms.
3. To achieve these objectives, beer is added to tanks under a CO₂ atmosphere, bottled under counter pressure of CO₂ and all equipment cleaned and disinfected regularly.



Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

Beer defect:

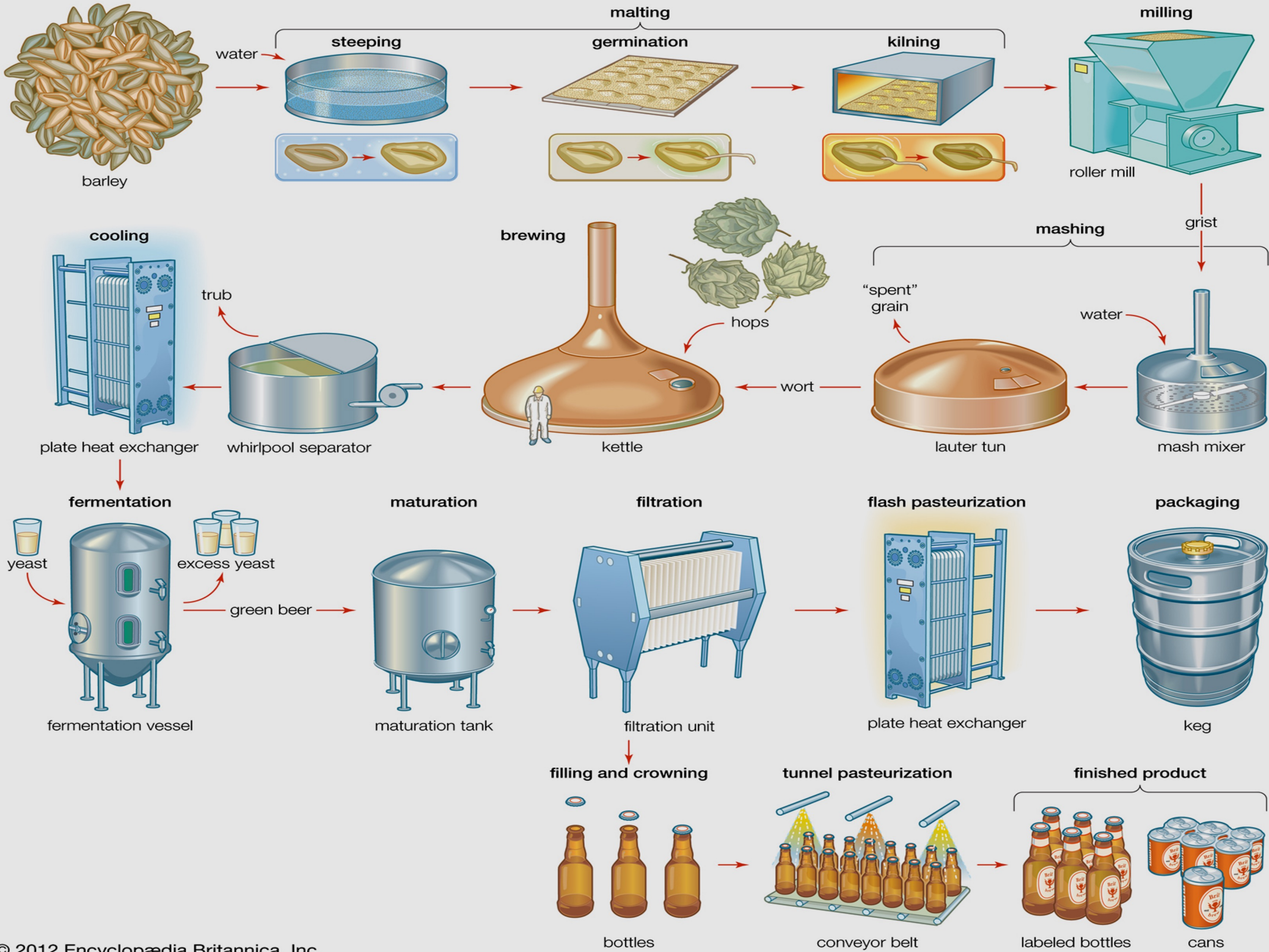
- Presence of haze or turbidity, which can be of biological or physicochemical origin.
- Biological turbidities are caused by spoilage organisms and arise because of poor brewery hygiene (i.e. poorly washed pipes) and poor pasteurization.
- Spoilage organisms in beer must be able to survive following stringent conditions found in beer: low pH, antiseptic substances in hops, pasteurization of beer, and anaerobic conditions.
- Wild or unwanted yeasts which have been identified in beer spoilage including *Kloeckera*, *Hansenula*, *Brettanomyces*, but *Saccharomyces* spp appear to be commonest, particularly in top-fermented beers.
- Among the bacteria, *Acetobacter*, *Lactobacillus* and *Streptococcus* are the most important.



Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

- ***Acetobacter*** is an acetic acid bacterium and produces acetic acid from alcohol thereby giving rise to sourness in beer.
- ***Lactobacillus pastorianus*** is the typical beer spoiling lactobacilli, in top-fermented beers, where it produces sourness and a silky type of turbidity.
- ***Streptococcus damnosus* (*Pediococcus damnosus*, *Pediococcus cerevisiae*)** is known as “beer sarcina” and gives rise to sarcina sickness of beer which is characterized by a honey-like odor.





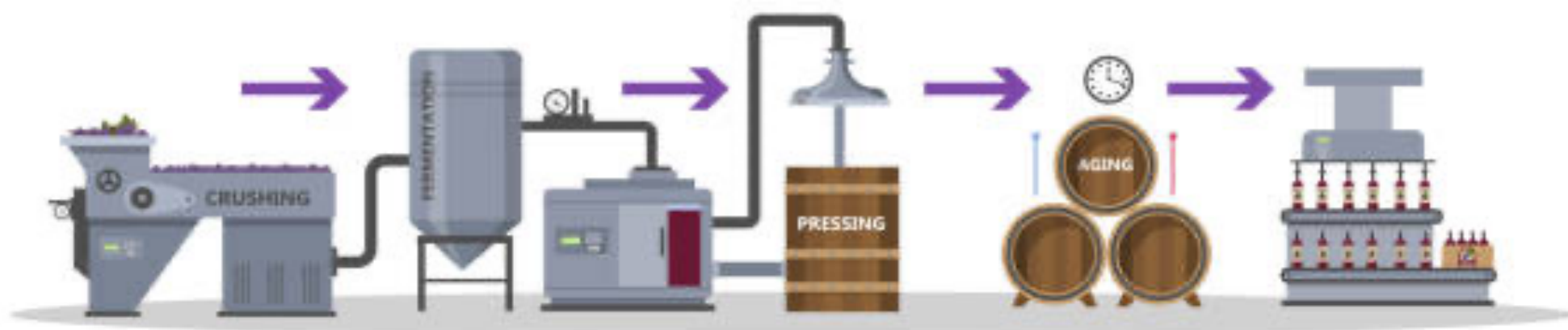
Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

Wines

- Wine is defined as a product of the normal alcoholic fermentation of the juice of sound ripe grapes.
- Nevertheless any fruit with a good proportion of sugar may be used for wine production.
- If the term is not qualified then it is regarded as being derived from grapes, *Vitis vinifera*.
- Production of wine is simpler than beer in that no need exists for malting since sugars are already present in fruit juice being used.





Crushing of Grapes:

- Selected ripe grapes are crushed to release the juice which is known as “must”, after stalks which support the fruits have been removed.
- Stalks contain tannins which would give wine a harsh taste if left in the must.
- Skin contains most of materials which give wine its aroma and color.
- Grape juice has an acidity of 0.60-0.65% and a pH of 3.0-4.0 due mainly to malic and tartaric acids with a little citric acid.

Fermentation:

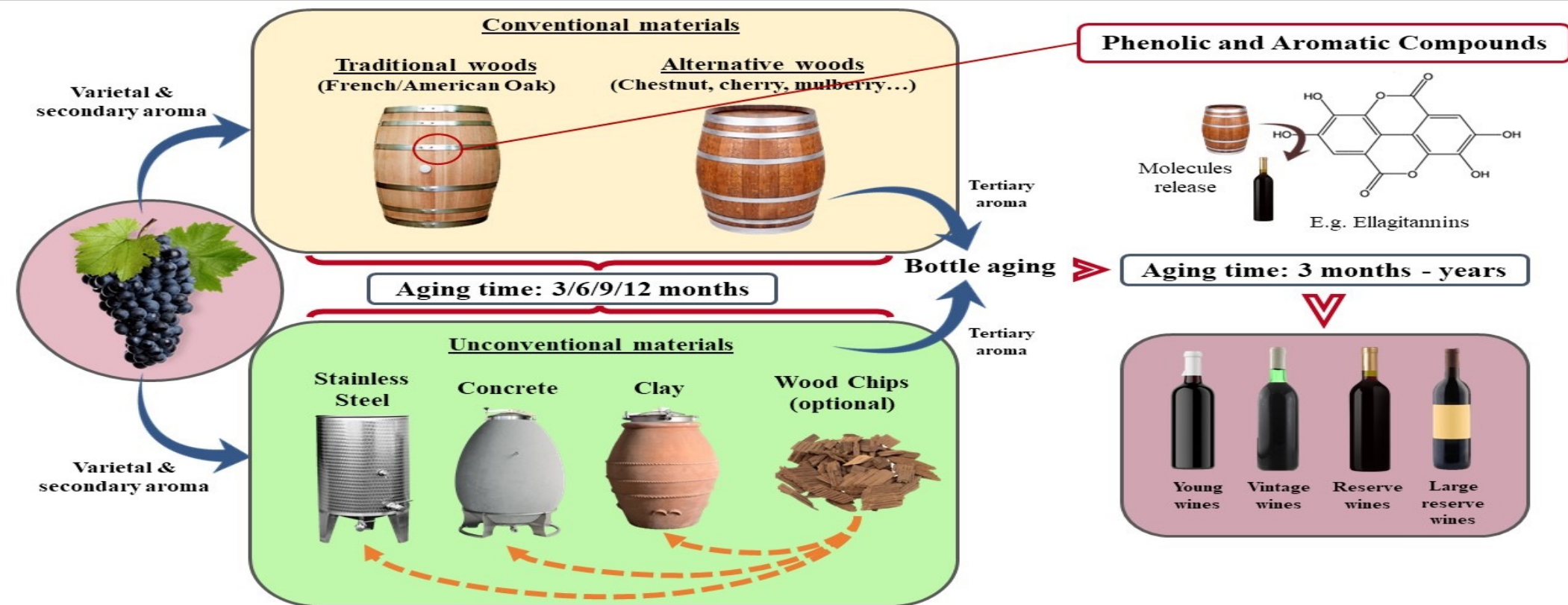
- Yeast used: Grapes themselves harbor natural flora of microorganisms (the bloom) which in previous times brought about fermentation and contribute to special characters of various wines.
- Yeasts are then inoculated into the must.
- *Saccaromyces cerevisiae* var. *ellipsoideus* (synonyms: *Sacch. cerevisiae*, *Sacch. ellipsoideus*, *Sacch. vini*.).
- Wine yeasts have the following characteristics: (a) growth at the relatively high acidity (i.e., low pH) of grape juice; (b) resistance to high alcohol content (higher than 10%); (c) resistance to sulfite

Control of Fermentation:

- **Temperature:** Heat is released during fermentations. Temperature of a must containing 22% sugar would rise 52°F (11°C) if all the heat were stopped from escaping. If the initial temperature were 60°F (16°C) the temperature would be 100°F (38°C) and fermentation would halt while only 5% alcohol has been accumulated. For this reason, fermentation is cooled and temperature is maintained at around 24°C with cooling coils mounted in fermentor.
- **Yeast nutrition:** Yeasts normally ferment glucose preferentially although some yeasts e.g. *Sacch. elegans* prefer fructose. Most nutrients including macro- and micro-nutrients are usually abundant; occasionally, however, nitrogenous compounds are limiting. Made adequate with small amounts of $(\text{NH}_4)_2 \text{SO}_4$.
- **Oxygen:** Oxygen is required in earlier stage of fermentation when yeast multiplication is occurring. In second stage, when alcohol is produced the growth is anaerobic and this forces yeasts to utilize such intermediate products as acetaldehydes as hydrogen acceptors and hence encourage alcohol production.
- **Flavor development:** Although some flavor materials come from grape most of it come from yeast action and has been shown to be due to alcohols, esters, fatty acids, and carbonyl compounds. Diacetyl, acetoin, fuel oils, volatile esters, and hydrogen sulfide have received special attention.

Ageing and Storage

- Fermentation is usually over in three to five days.
- Pomace formed from grape skins (in red wines) will have risen to top of brew.
- At end of this fermentation, wine is allowed to flow through a perforated bottom if pomace had been allowed.
- When pomace has been separated from wine and fermentation is complete or stopped, next stage is racking.
- Wine is allowed to stand until a major portion of yeast cells and other fine suspended materials have collected at bottom of container as sediment or “lees”.
- It is then “racked”, during which process the clear wine is carefully pumped.





- Wine is then transferred to wooden casks (100-1,000 gallons), barrels (about 50 gallons) or tanks (several thousand gallons).
- Wood allows wine only slow access to oxygen.
- Water and ethanol evaporate slowly leading to air pockets which permit growth of aerobic wine spoilers e.g. acetic acid bacteria and some yeasts.
- Casks are regularly topped up to prevent the pockets.
- In modern tanks made of stainless steel the problem of air pockets is tackled by filling airspace with an inert gas such as carbon dioxide or nitrogen.
- During ageing, desirable changes occur in wine.



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

Clarification

- **Wine is allowed to age in a period ranging from two years to five years, depending on the type of wine.**
- **At end of period, some will have cleared naturally.**
- **For others artificial clarification may be necessary.**
- **Addition of fining agent is often practiced to help clarification.**
- **Fining agents react with tannin, acid, protein or with some added substance to give heavy quick-settling coagulums.**
- **Usual fining agents for wine are gelatin, casein, tannin, egg albumin, and bentonite.**



Packing

- Before packing in bottles the wine from various sources is sometimes blended and then pasteurized.
- In some wineries, the wine is not pasteurized, rather it is sterilized by filtration. In many countries the wine is packaged and distributed in casks.





Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

Wine Defects

- Important cause of wine spoilage is microbial; less important defects are acidity and cloudiness.
- Factors which influence spoilage by bacteria and yeasts include (a) wine composition, specifically sugar, alcohol, and sulfur dioxide content; (b) storage conditions e.g. high temperature and the amount of air space in container (c) extent of initial contamination by microorganism during bottling process.
- When proper hygiene is practiced bacterial spoilage is rare.
- Acetic acid bacteria cause sourness in wine.
- Lactic acid bacteria especially *Leuconostoc*, and sometimes *Lactobacillus* also spoil wines.



Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

- Most prevalent is *Brettanomyces*, slow growing yeasts which grow in wine causing turbidities and off-flavors.
- Other wine spoilage yeasts are *Saccharomyces oviformis*, which may use up residual sugars in a sweet wine and *Saccharomyces bayanus* which may cause turbidity and sedimentation in dry wines with some residual sugar.
- *Pichia membranifaciens* is an aerobic yeast which grows especially in young wines with sufficient oxygen.
- Other defects of wine include cloudiness and acidity.