COURSE TITLE: FOOD AND INDUSTRIAL MICROBIOLOGY
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Microorganisms and processes involved in the production of industrial alcohol, beer and wine

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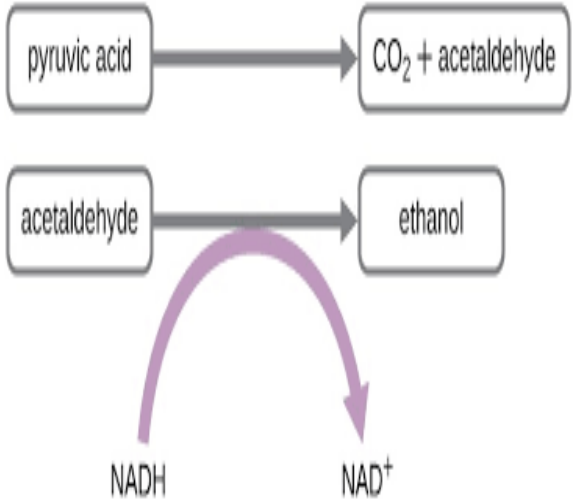
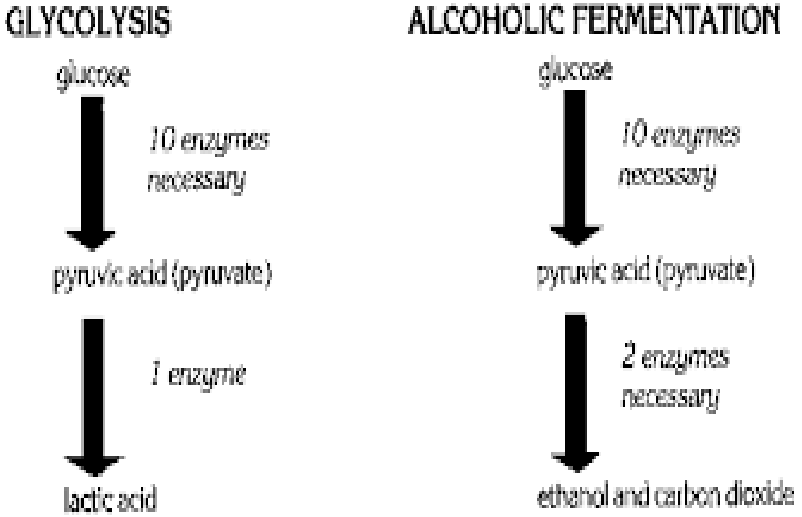




INTRODUCTION

Humans have been producing alcoholic beverages for thousands of years. The production of alcohol in these drinks is based primarily on yeast fermentation. Yeasts are eukaryotic microorganisms that ferment variety of sugars from different sources into the final products of carbon dioxide and alcohol.

Yeasts are the main fermentor and alcohol producer in the production of wine, beer and other alcohol drinks.

The main yeast species used is *Saccharomyces cerevisiae*. It ferments the sugars, coming from different sources, e.g., grapes for wine, barley for beer, to alcohol and carbon dioxide.

Both wild and cultivated strains are used. The species or strains used in the fermentation play an important role in giving the final taste properties of the drink.

ETHANOL FERMENTATION

- Alcohol fermentation --- Chemically Ethanol fermentation is a biological process in which sugars such as glucose, fructose and sucrose are converted into cellular energy and thereby produces ethanol and carbon dioxide as metabolic waste products.
- Yeast perform this conversion in the absence of oxygen, alcoholic fermentation is considered as an anaerobic process.
- In the developing countries, microbial fermentation processes are preferred for the production of alcohol.
- This is mainly because of the cheap raw materials available.

PRODUCTION OF ETHANOL BY FERMENTATION

- Many countries have started production of ethanol by fermentation process.
- The organisms and the raw materials used, along with the production and recovery processes for alcohol are briefly described below……..

Microorganisms:

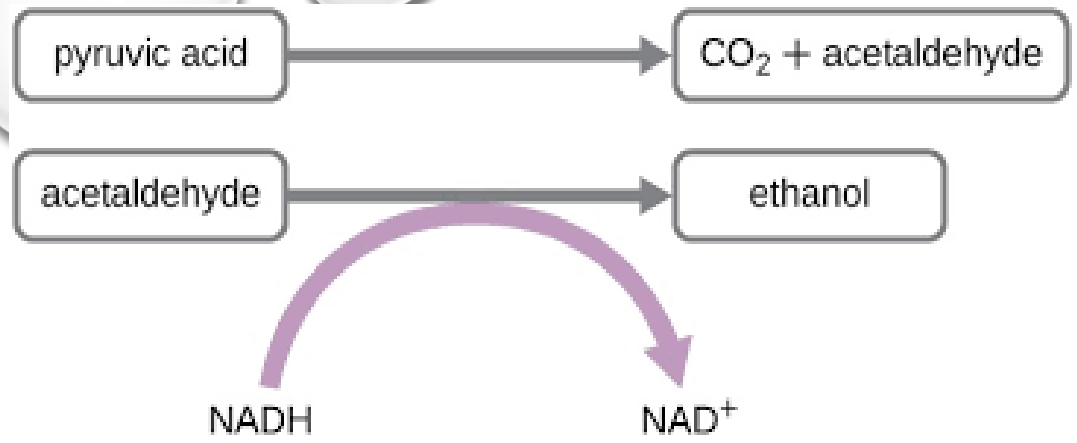
- ✓ Certain yeasts and bacteria are employed for alcohol fermentation.
- ✓ The type of the organism chosen mostly depends on the nature of the substrate used.
- ✓ Among the yeasts, *Saccharomyces cerevisiae* is the most commonly used, while among the bacteria, *Zymomonas mobilis* is the most frequently employed for alcohol production.

Ethanol is used as solvent, chemical intermediate and fuel

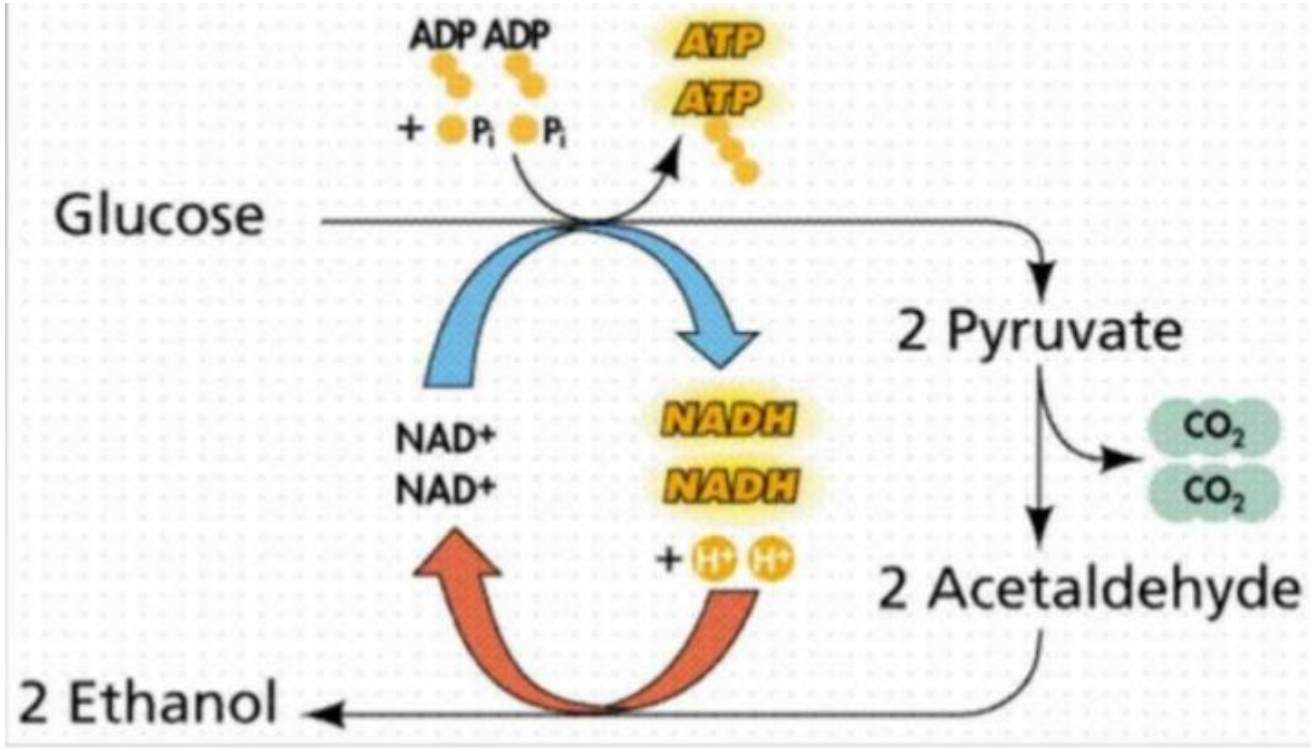
Solvent:- Resins, pharmaceuticals , cosmetics, household cleaning products, industrial solvent

Chemical intermediate :- Petroleum derived chemicals, Butadiene production

Fuel:- Used as a bio fuel, In internal combustion engines



In the first reaction, the enzyme **pyruvate decarboxylase** removes a carboxyl group from pyruvate, releasing CO_2 gas while producing the two-carbon molecule acetaldehyde. The second reaction, catalyzed by the enzyme alcohol dehydrogenase, transfers an electron from NADH to acetaldehyde, producing ethanol and NAD^+. The ethanol fermentation of pyruvate by the yeast ***Saccharomyces cerevisiae*** is used in the production of alcoholic beverages and also makes bread products rise due to CO_2 production.



Overall mechanism

1. Pryvuate is converted to acetaldydehyde, and carbon dioxide is reduced as a byproduct
2. Acetaldehyde is then converted to ethanol, with NAD+ electron carriers produced.

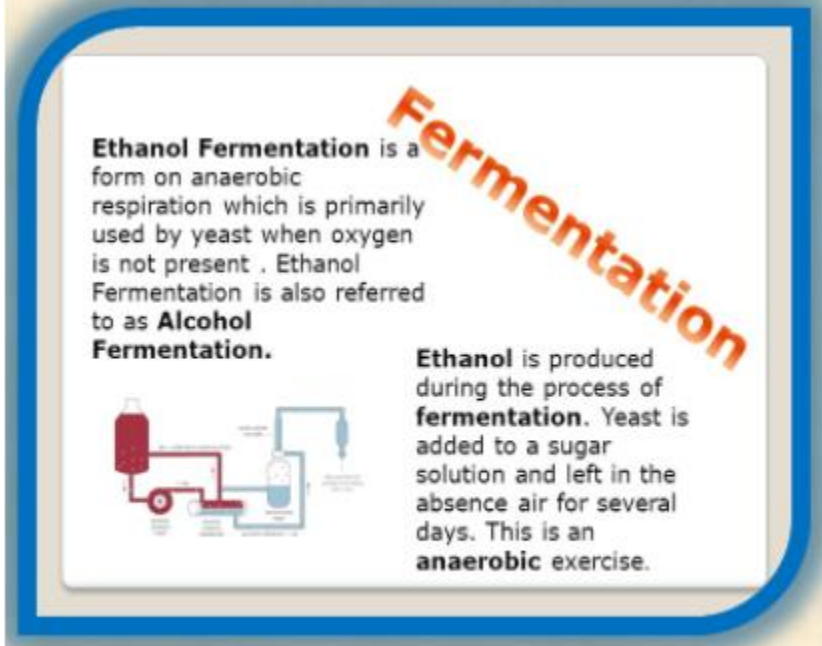
NAD+ then goes back to glycolysis and we have two ATP produced!

MEDIA PREPARATION

After selection of yeast *Saccharomyces cerevisae*, it is then cultured in flask to increase the size of the inoculums

FERMENTATION

Glucose + Yeast = Ethanol + CO_2



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RECOVERY OF ETHANOL

The mass is separated by centrifugation in a centrifuge. Ethanol from fermentation broth can be recovered by distillations. For a concentration above 95%, distillation method is adopted. For 100% alcohol a specific type of distillation known as azeotropic distillation is used.8 For this an azeotrope mixture of Benzene, water, alcohol is first prepared after which the mixture is distilled by gradually increasing the temperature. By this technique, firstly benzene-ethanol-water then releases leaving behind only 100% alcohol.

Raw materials:

There are a large number of raw materials that can serve as substrates for alcohol fermentation.

They may be broadly categorized as sugary materials (e.g. molasses, whey, glucose, sucrose), starchy materials (e.g. wheat, rice, maize, potato) and cellulosic materials (wood, agricultural wastes).

RAW MATERIALS

- Saccharine
- Starch &
- Cellulose materials

Pretreatment of raw materials:

Most of the raw materials of alcohol fermentation require some degree of pretreatment.

The actual process depends on the chemical composition of the raw material.

In general, the sugary raw materials require mild or no pretreatment while the cellulosic materials need extensive pretreatment.

This is because the cellulosic substances have to be subjected to acidic or enzyme hydrolysis to release monosaccharide units that are needed for alcohol production.

PRODUCTION PROCESS OF ETHANOL

Ethanol production can be carried out in three stages-

- preparation of nutrient solution and inoculum,
- fermentation and recovery.
- Preparation of nutrient solution (media) :

• The most commonly used raw materials are molasses, whey, grains, potatoes and wood wastes.

• When molasses is used for fermentation, it is diluted with water so that the sugar concentration is in the range of 10-18%. A concentration higher than this is detrimental to the yeast.

• When starchy materials are used, they have to be first hydrolysed by pre-treatment for use as nutrients.

Sugar cane

Crushing and dissolving

Aqueous sugar solution (starch & sucrose)

Yeast enzyme (*invertase*), anaerobic conditions, 37°C

Aqueous sugar solution (glucose)

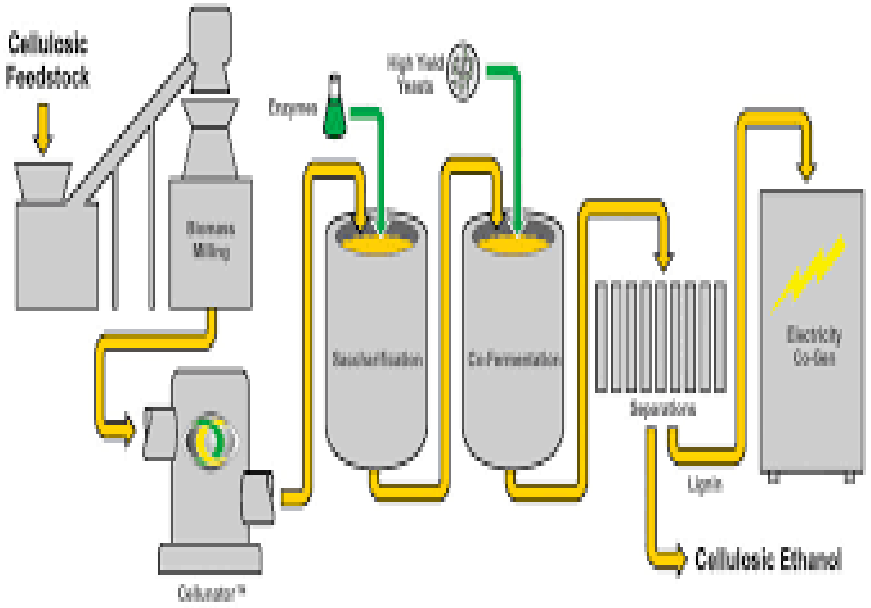
Yeast enzyme (*zymase*), same conditions

15% Ethanol solution

Carbon dioxide

Fractional distillation

96% - 98% Ethanol solution



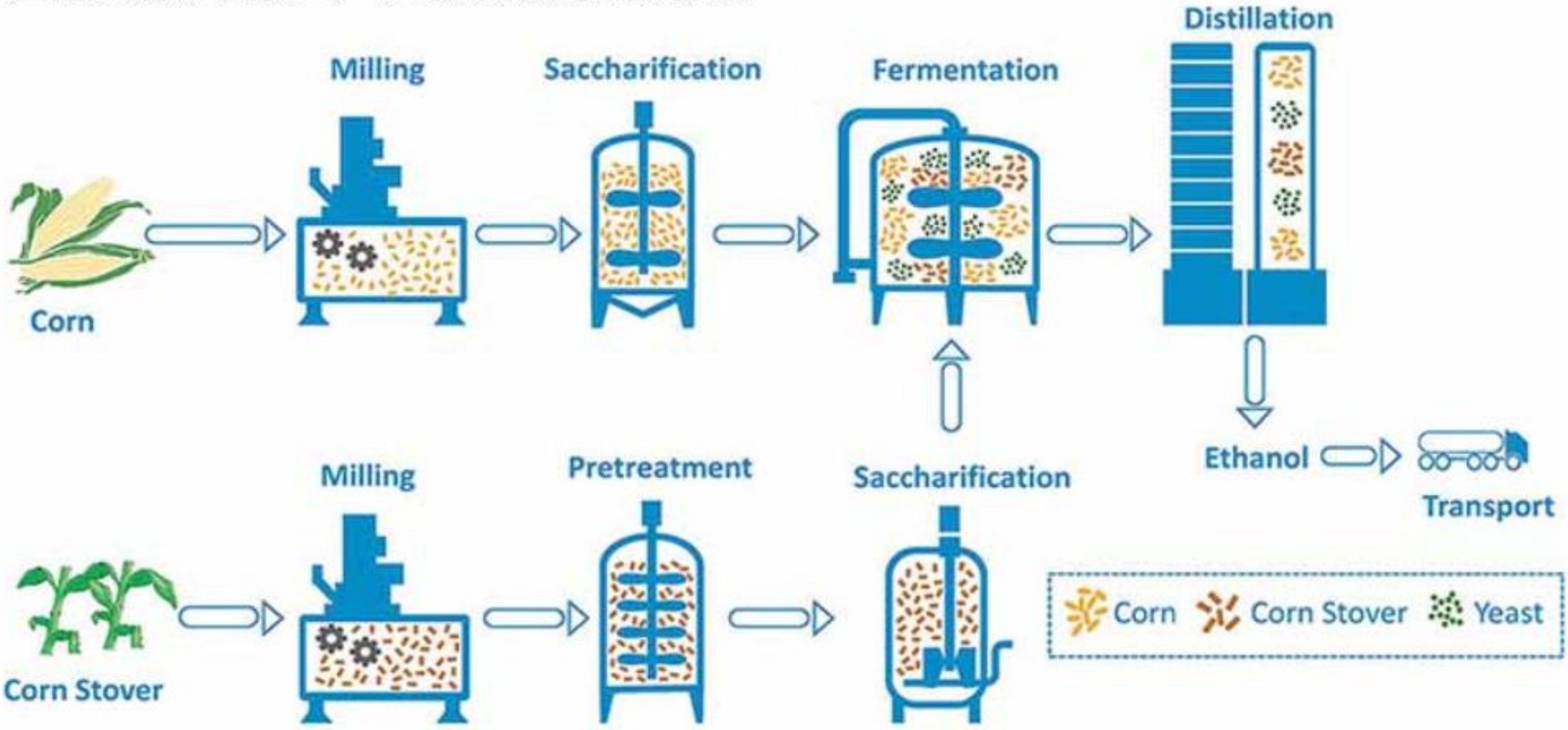
- This may be done by barley malt, dilute acids or fungal amylases (e.g. Aspergillus sp, Rhizopus sp.) Preparation of inoculum:
- After selection of the desired organism (yeast or bacteria) and its isolation in pure form, the inoculum is prepared under aseptic conditions.
- For this purpose, the organisms are first cultured in flasks under aerobic conditions to increase the size of the inoculum which can be used for inoculation. Fermentation proper:
- Originally batch fermentation was adopted. Now, continuous fermentation is used.
- It has been possible to increase alcohol production by 10-12 fold by continuous fermentation compared to batch fermentation.

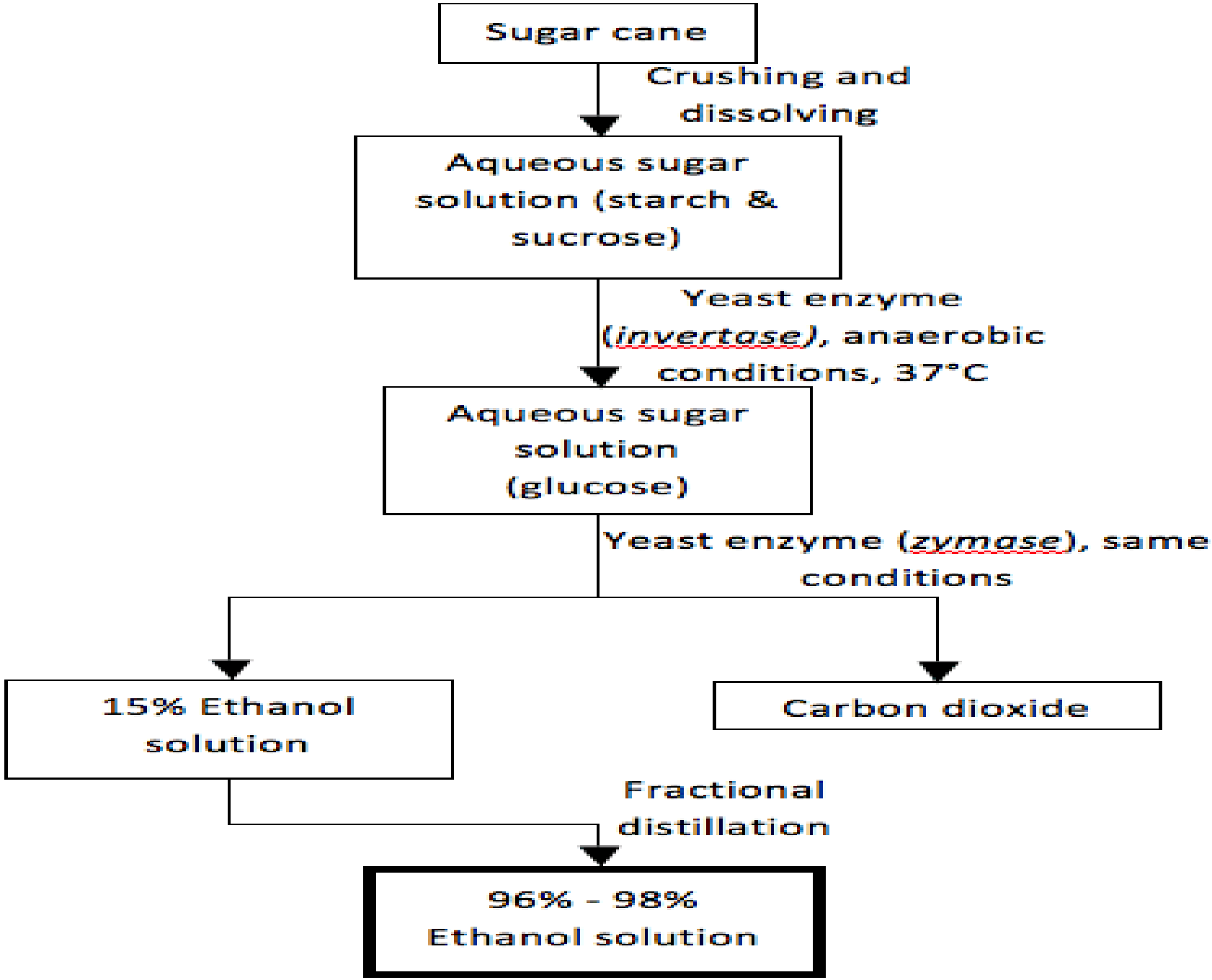
- Industrial production of alcohol is carried out in huge fermenters up to a size of 1,25,000 gallons.
- The ideal pH is around 4.0-4.5. the initial temperature is kept between 21-26˚C.
- Ethanol gets evaporated at temperature above 27˚C.
- Aeration is initially required for good growth of the organisms.
- Later, anaerobic conditions are created by withdrawal of oxygen coupled with production of carbon dioxide.
- It takes about 2-3 days for the fermentation to be completed.
- As the fermentation is complete, the fermentation broth contains ethanol in the range of 6-9% by volume. This represents about 90-95% conversion of substrate to ethanol.

Recovery of ethanol:

- The cell mass is separated by centrifugation or sedimentation.
- Ethanol from fermentation broth can be recovered by successive distillations. For a concentration above 95%, special techniques of distillation have to be adopted.
- Separation for a mixture of liquids
- It relies on differences in B.P of component liquids to be separated
- The mixture to be separated is added to the distilled pot & is heated
- Low B.P will vaporize first. this vapors passes into distilling head & then into condenser.
- With in the condenser the vapor is cooled & it liquefies.
- The resulting liquid is then collected in receiving flask
- For a preparation of absolute (100%) alcohol, an azeotropic mixture of benzene, water and alcohol is first prepared. This mixture is then distilled by gradually increasing the temperature.
- By this technique, it is possible to first remove benzene- ethanol-water mixture, and then ethanol-benzene mixture. Thus, absolute alcohol is left out.

Ethanol Production



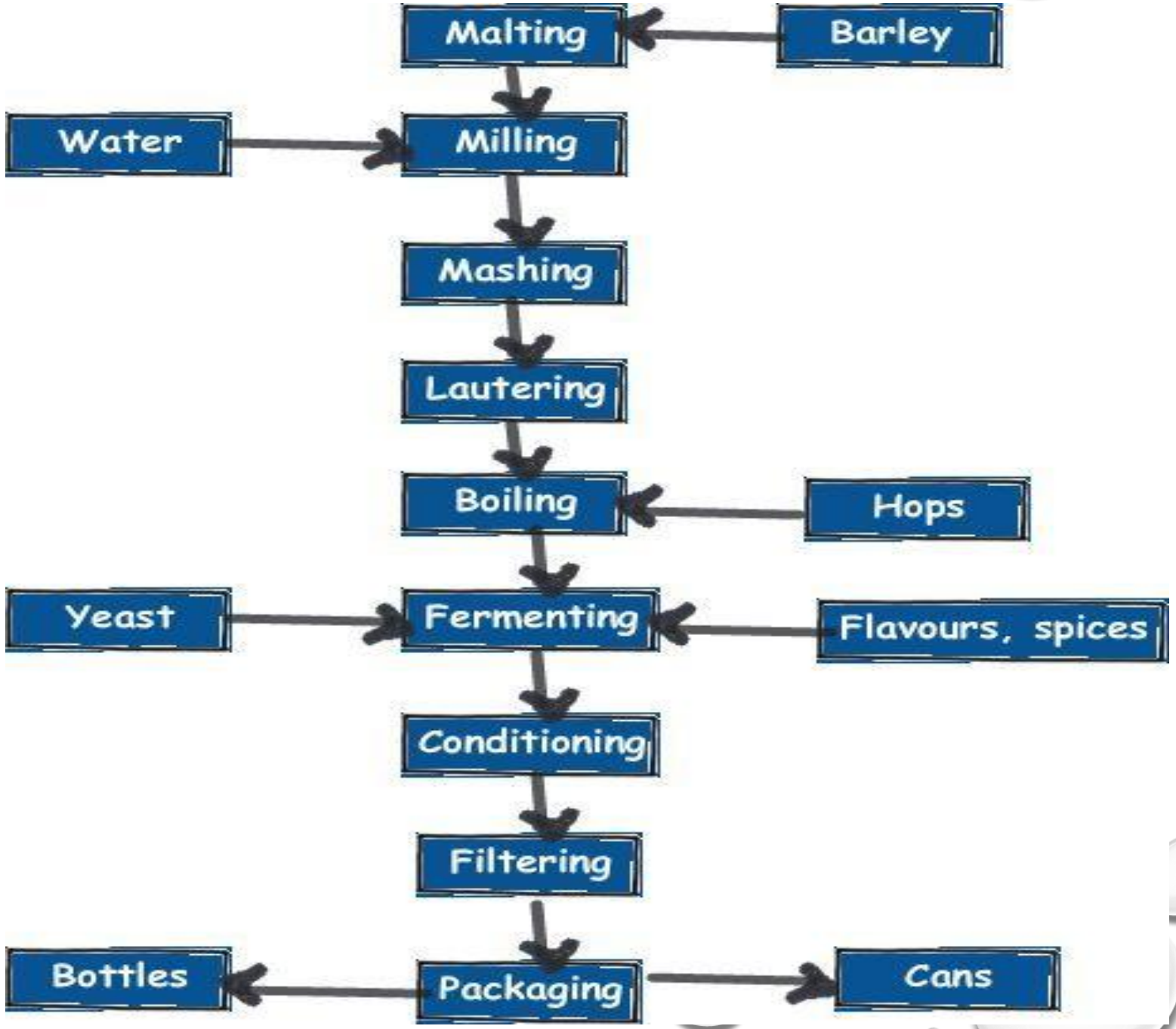

Sugar cane
Crushing and dissolving
Aqueous sugar solution (starch & sucrose)
Yeast enzyme (invertase), anaerobic conditions, 37°C
Aqueous sugar solution (glucose)
Yeast enzyme (zymase), same conditions
15% Ethanol solution
Carbon dioxide
Fractional distillation
96% - 98% Ethanol solution

Beer Production

Beer is the most consumed alcoholic beverage in the world. It is made most often of malted barley and malted wheat. Sometimes a mixture of starch sources can be used, such as rice. Unmalted maize can be added to the barley or wheat to lower cost. Potatoes, millet and other foods high in starch are used in different places in the world as the primary carbohydrate source.

The process of making beer is called brewing. It includes breaking the starch in the grains into a sugary liquid, called wort, and fermenting the sugars in the wort into alcohol and carbon dioxide by yeasts. Two main species are used in the fermentation process: *Saccharomyces cerevisiae* (top-fermenting, since it forms foam on top of the wort) and *Saccharomyces uvarum* (bottom-fermenting).

Beer Production



Beer Production

Brewing in the brewery by a brewer makes the beer

The execution of Beer Production is highly precise and sophisticated process. It requires a series of steps, to make a good brew. It has to go through the phases of Malting, Milling, Mashing, Lautering, Boiling, Fermenting, Conditioning, Filtering, and Packaging.

1. **Malting:** The conversion from carbohydrates to dextrin and maltose takes place. The grain used as the raw material is usually barley. Barley as a cereal can be preserved for a long time after harvesting and it is the malted barley that gives Beer its characteristic color and taste.

2. **Milling:** The malt is then mixed with water to complete the conversion of starches in the grain to sugar. After that the grain is milled to create the proper consistency to the malt.

3. **Mashing:** This process converts the starches released during the malting stage, into sugars that can be fermented.

4. **Lautering:** The liquid containing the sugar extracted during mashing is now separated from the grains. It is then generally termed as wort.

5. **Boiling and Hopping**: Boiling the wort, ensures its sterility, and thus prevents a lot of infections. Hops are added during this stage of boiling. Hops are also used to add flavor and aroma to balance the sweetness of the malt.

6. **Fermenting:** The yeast is now added and the Beer is fermented. The yeast breaks down the sugars extracted from the malt to form alcohol and CO2.

7. **Conditioning:** Fermented Beer contains suspended particles, lacks sufficient carbonation, lacks taste and aroma, and less stable. Conditioning reduces the levels of these undesirable compounds to produce a more finished product.

4. **Lautering:** The liquid containing the sugar extracted during mashing is now separated from the grains. It is then generally termed as wort.

8. **Filtering:** Filtration helps to remove excess of the yeast and any solids, like hops or grain particles, remaining in the Beer. Filtering is the process which produces the clear, bright and stable Beer.

9. **Packaging:** Packaging is putting the beer into the bottles, cans or some other high volume vessels. One of the most important things in packaging is to exclude oxygen away from the Beer.

These are the basic steps and the style of brewing may vary little. These can be customized to improve the taste of your beer. I will follow up this post with the type of machinery, equipment's, weather conditions, for the Beer making process.

Introduction

Wine is made from grapes or other fruit. The grapes are first cleaned of leaves and stems and the fruit is crushed into must that is ready for fermentation. The yeasts used for the fermentation grow a film on the fruit or in the environment. These wild strains play an important role in the final properties of the drink. However, cultivated strains of *Saccharomyces cerevisiae* are often added to improve the consistency of the final product. There are hundreds of commercially available yeast strains for wine fermentation.

Wine is an alcoholic beverage made from fermented fruit juice.
Grape wine is produced by fermenting crushed grapes using various types of yeast.

- Types of wines – Red wine – White wine
- Classification of wines – Sparkling wine – Desert wine – Ice wine – Fortified wine – Table wine

Wine Production

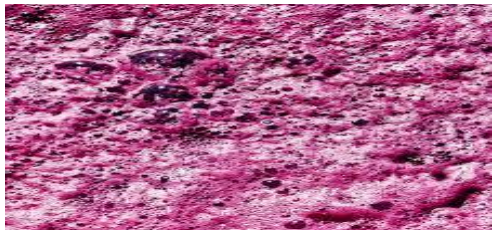
1. Viticulture -- Factors which inflence grape's flavor:
•climate of the vineyard's region •drainage around the vines •humidity of the region •sun exposure. •soil quality

2. Harvesting -- Grapes are picked up by hand or mechanically. Decision of harvest informed by level of sugar and acid. weather forecasts

3. Stemming/Crushing Stemming is the separation of the stems and grapes (which are sends to the press) Crushing: A horizontal press squeezes the broken grapes, separating the fresh juice (must) from the skins (marc) After crushing starts the fermentation process.

4. Fermentation -- Sugar and acids that naturally react with wild yeasts, Vineyard adding their own yeasts. Fermentation can take from 10to 30 days to convert natural sugar to alcohol.

5. Draining -- Liquid wine is drained from the vat without being pressed and go into barrels (free-run wine). The remaining pulp retains about 20% of the wine.

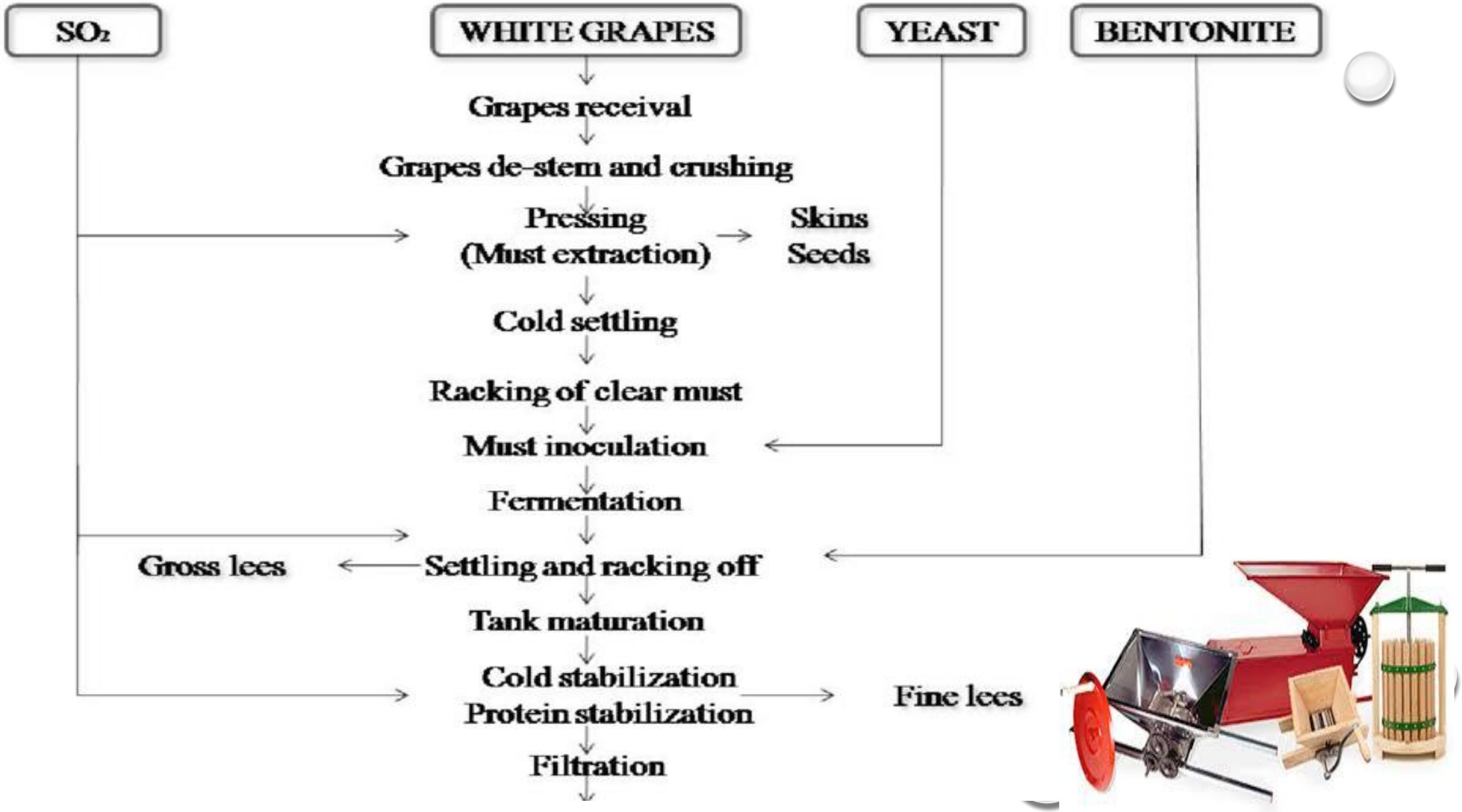
6. Pressing -- The remaining pulp, after draining, is pressed to squeeze out the press wine.

7.Mixing -- The free-run wine and press wine, always from the same source, are mixedtogether in appropriate ratios to obtain the desired balance.

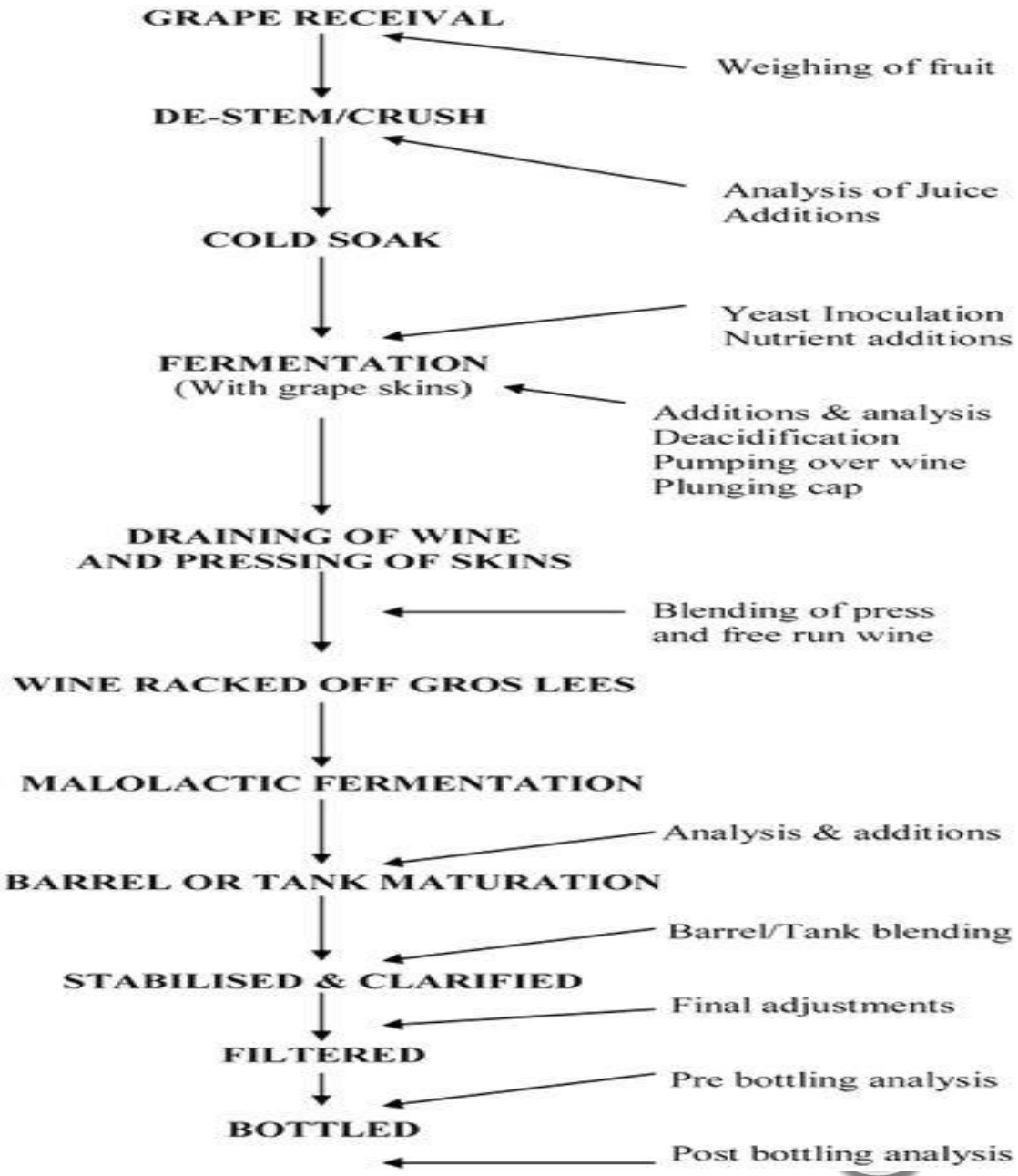
8. Clarification Stabilization of fermentation. Remaining solids are removed.
 Clarification done in numerous ways: 1. Fining 2. Filtration 3. Siphoning the liquid off the top of the fermenting vats after the solids have settled to the bottom 4. Floatation

9. Aging -- The final stage in vinification is aging the wine. At this point, the clarified wine is transferred into either wooden barrels or metal vats in which the wine is allowed to further mature and develop flavors.

10. Bottleing -- A dose of sulfite is added to preserve the wine and prevent unwanted fermentation in the bottle. The wine bottles then are traditionally sealed with a cork,


SO₂
WHITE GRAPES
YEAST
BENTONITE
Grapes receival
Grapes de-stem and crushing
Pressing
(Must extraction)
Skins
Seeds
Cold settling
Racking of clear must
Must inoculation
Fermentation
Gross lees
Settling and racking off
Tank maturation
Cold stabilization
Protein stabilization
Fine lees
Filtration

FLOW DIAGRAM FOR RED WINE MAKING



THANK YOU