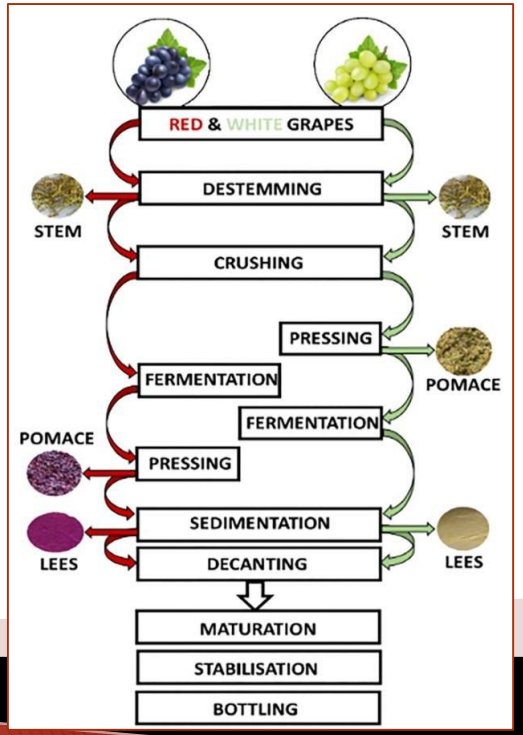
FDE 437
FERMENTATION TECHNOLOGY

Wine Production-Part III



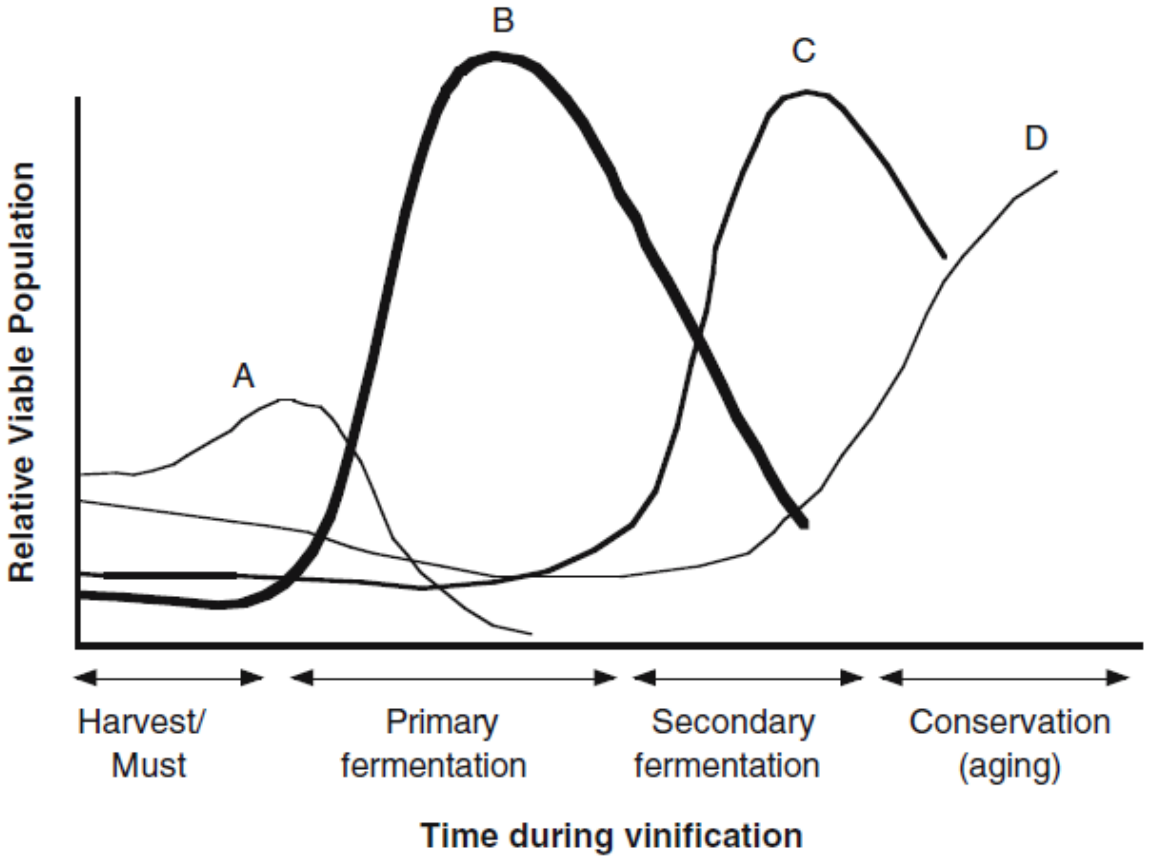
Microbial Ecology During Vinification

- Several genera and species of microorganisms can be found in grape musts and wines at various times during the winemaking process. For instance, *Saccharomyces*, *Brettanomyces*, and *Pediococcus* can be found together in wine.

- Winemaking is a complex microbial process involving yeasts and bacteria. They are both naturally present on grape skins, but are also found in barrels, tanks and the equipment used during vinification.

- Even with this wide diversity of microorganisms, vinification commonly involves a sequential development of microorganisms.
  A. In general, non-*Saccharomyces* yeasts will be the first group to dominate during vinification,
  B. followed by *Saccharomyces* that normally completes alcoholic fermentation
  C. After the primary fermentation is finished, malolactic fermentation may be induced by *Oenococcus* or other lactic acid bacteria.
  D. During the aging of wines, several different yeasts and bacteria may grow, many of which bring about spoilage.

Microbial Ecology During Vinification



A: Non-*Saccharomyces* yeasts
B: *Saccharomyces*
C: *Oenococcus oeni*
D: Spoilage yeasts and/or bacteria

- Wine fermentation is complex microbial process in which various microorganisms coexist sequentially and interact influencing the predominance and persistence of fermenting yeasts and the analytical and aroma profiles of wine.

- During the early stages of alcoholic fermentation, the indigenous non-*Saccharomyces* yeasts naturally present in grape musts (e.g. *Hanseniaspora guilliermondii*, *Hanseniaspora uvarum*, *Candida stellata*, *Kluyveromyces marxianus*, *Torulaspora delbrueckii*) predominate.

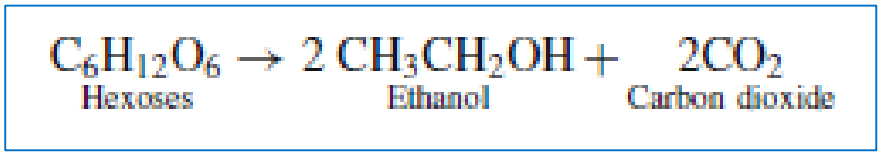
- Suprisingly, *Saccharomyces cerevisiae*, the fermentative yeast responsible for primary alcoholic fermentation, occur in extremely low numbers on the surface of grapes. However, non-*Saccharomyces* yeasts started to die off by mid fermentation when the ethanol concentration reaches 4-5% (v/v), and *S. cerevisiae* dominates and completes the fermentation.

- After the primary fermentation is finished, secondary malolactic fermentation is usually conducted by *Oenococcus oeni* or other lactic acid bacteria.

- During the aging of wines, several different yeasts and bacteria may grow resulting in spoilage of the final product.

Biochemistry of Alcoholic Fermentation

- Alcoholic fermentation is the anaerobic transformation of sugars, mainly glucose and fructose, into ethanol and carbon dioxide.
- This process is carried out by yeast (generally by *Saccharomyces cerevisiae*) and also by some bacteria such as *Zymomonas mobilis*.
- The alcoholic fermentation process can be summarised by this overall reaction.

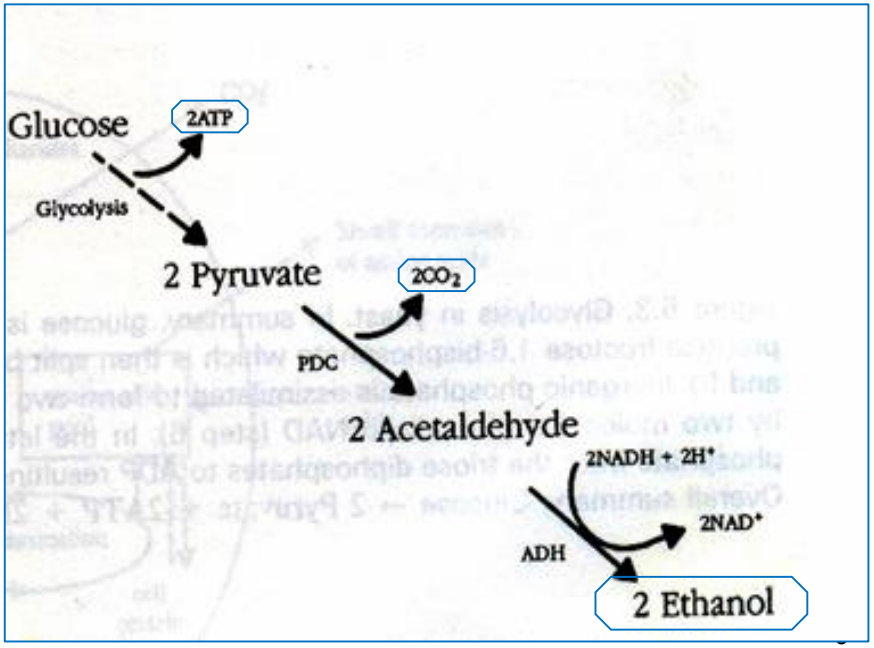


- However, alcoholic fermentation is a much more complex process.
- At the same time as this overall reaction proceeds, a lot of other biochemical, chemical and physicochemical processes take place, making it possible to turn the grape juice into wine.
- Besides ethanol, several other compounds are produced throughout alcoholic fermentation such as higher alcohols, esters, glycerol, succinic acid, diacetyl, acetoin and 2,3-butanediol.
- Simultaneously, some compounds of grape juice are also transformed by yeast metabolism. Without the production of these other substances, wine would have little organoleptic interest.

Biochemistry of Alcoholic Fermentation

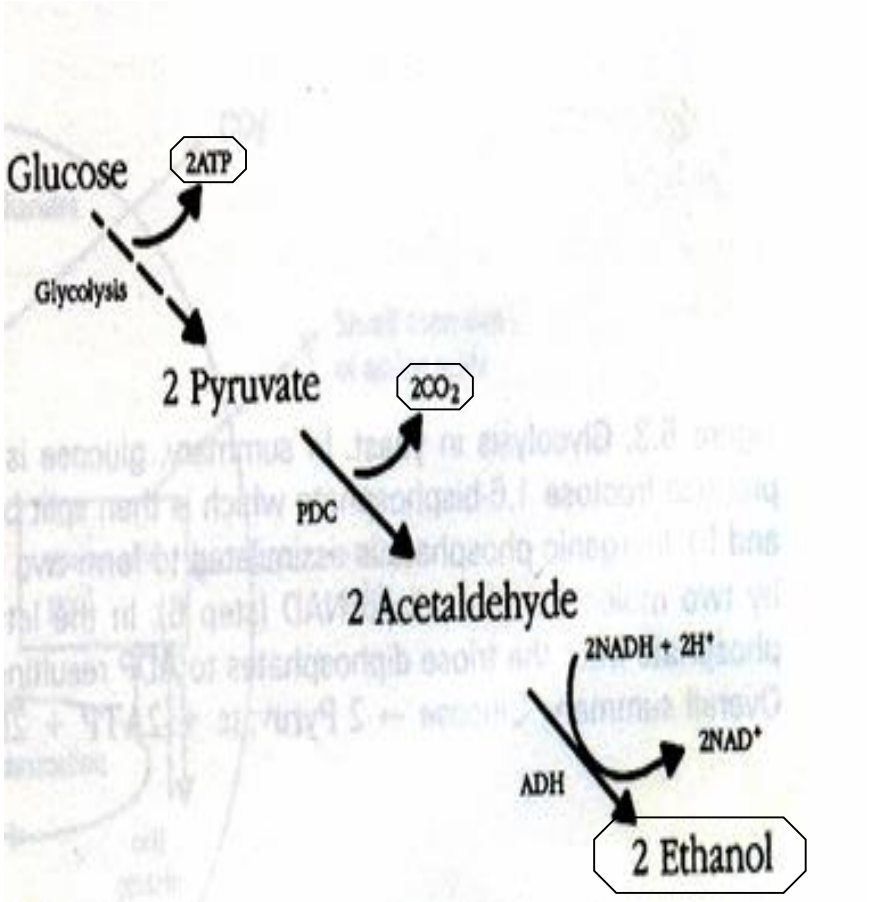
The three main reactions that take place during the conversion of sugar in grape must to ethyl alcohol by wine yeasts are;

1. **Glycolysis, or breakdown of sugar**,
2. **Decarboxylation of pyruvic acid**, and
3. **Reduction of acetaldehyde**



Alcoholic Fermentation

- Pyruvate is decarboxylated to acetaldehyde by pyruvate decarboxylase generating CO_2.
- Acetaldehyde is then reduced to ethanol by alcohol dehydrogenase.
- NADH is reoxidized to NAD^+ by transferring the hydrogen to acetaldehyde.
- End products of alcoholic fermentation:
  - 2 mol ethanol
  - 2 mol CO_2
  - 2 mol ATP per mole of glucose.



(Walker 1998)

Malolactic Fermentation

- Malolactic fermentation (MLF) in wine is a secondary fermentation that usually occurs at the end of alcoholic fermentation by yeasts.
- After alcoholic fermentation, wines frequently undergo the malolactic fermentation (MLF).
- The MLF commences naturally 2-3 weeks after the alcoholic fermentation and lasts about 2-4 weeks.
- Lactic acid bacteria resident in the wine are responsible for the MLF, but many winemakers encourage this reaction by inoculation with cultures of *Oenococcuc oeni*.
- Of the organic acids ordinarily present in grapes, malic acid is particularly important because of its ability to influence acidity and pH. This is because malic acid is a dicarboxylic acid, meaning it contains two carboxylic acid groups and can release or donate two protons.
- Thus, musts containing high concentrations (0.8% to 1.0%) of malic acid may be overly acidic and have a lower than desired pH.
- High malic acid concentrations are especially common in grapes grown <u>in cooler, more northern climates</u>.

Malolactic Fermentation

- One way to reduce the malic acid levels and to "deacidify" the wine is to promote the biological decomposition of malic acid. This deacidification process occurs via the malolactic fermentation pathway that is performed by specific species and strains of lactic acid bacteria.
- These bacteria may be naturally present in wine and may, therefore, initiate and perform the fermentation on their own. It has now become common to add selected malolactic strains, in the form of a starter culture, directly to the must.
- The importance of the malolactic fermentation to wine quality is now well-appreciated.
- Although this fermentation is initiated in some white and rose wines (including sparkling wines), it is especially important and necessary for most red wines.
- It is important to note that in low-acid grapes, the malolactic fermentation is undesirable, since some acidity is desired in wine. Thus, under some circumstances, the presence of naturally occurring malolactic bacteria is unwanted and the source of potential defects.
- In contrast, the malolactic fermentation not only is performed for deacidification, but also to promote flavor stability and balance. Moreover, malolactic bacteria often produce diacetyl from citrate, which may, at the appropriate concentration (generally between 1 mg/L and 4 mg/L), be desirable in some wines.

Malolactic Fermentation

- The main reaction is decarboxylation of L-malic acid to L-lactic acid, giving a decrease in acidity of the wine and an increase in its pH by about 0.3-0.5 unit.

- Wines produced from grapes cultivated in cool climates have a higher concentration of malic acid and lower pH (3.0-3.5), which can mask varietal character. A decrease in acidity by MLF gives the wine a softer, more mellow taste. Also, the growth of malolactic bacteria contributes additional metabolites that can enhance the flavour profile.

- Wines that have not undergone MLF before bottling risk its spontaneous occurrence at some later stage in the bottle. If this happens, the wine becomes gassy and cloudy, and is spoiled. Wines with completed MLF appear to have greater microbiological stability and are less prone to spoilage by other species of bacteria. These wines probably contain less nutrients for microbial growth, and bacteriocin production by *Leuc. oenos* may be a further inhibitory factor.

- Malolactic fermentation is not beneficial to all wines.

- Wines produced from grapes grown in warmer climates tend to be less acid (pH> 3.5) and further reduction in acidity by MLF may be deleterious to sensory balance. Also, MLF increases their pH to values where spoilage bacteria are more likely to grow. However, preventing the natural occurrence of MLF in these wines (as might occur after bottling) is an extra technical burden. Consequently, some winemakers prefer to encourage the MLF and later adjust wine acidity, if necessary.

- Nearly all red wines go through malolactic fermentation, but only a few white wines. For example; white wine Chardonnay. MLF is responsible for Chardonnay's creamy and buttery flowers.

Malolactic Fermentation (MLF)

- Malolactic fermentation (MLF) in wine is by definition the enzymatic conversion of L-malic acid to L-lactic acid, a secondary process which usually follows primary (alcoholic) fermentation of wine but may also occur concurrently.

- This reduction of malic acid to lactic acid is not a true fermentation, but rather an enzymatic reaction performed by lactic acid bacteria (LAB) after their exponential growth phase.

- MLF is mainly performed by *Oenococcus oeni*, a species that can withstand the low pH (<3.5), high ethanol (>10 vol.%) and high SO_2 levels (50 mg/L) found in wine.

- More resistant strains of *Lactobacillus*, *Leuconostoc* and *Pediococcus* can also grow in wine and contribute to MLF; especially if the wine pH exceeds 3.5.

- The most important benefits of MLF are the deacidification of high acid wines mainly produced in cool climates, LAB contribute to wine flavour and aroma complexity and improve microbial stability.