Best fermentation management practices

WF101: CURRENT ISSUES IN FERMENTATION MANAGEMENT
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Know your grapes

- Brix – potential EtOH production
- YAN – nutritional needs
- TA – acid balance
- pH – antimicrobial control, taste
- Organic acid composition
  - For ex. malic acid contribution to predict pH change in the case of red wine production
Know your grapes

- Condition of the grapes
  - Raisons – Brix measurement will be inaccurate
  - Mold or rot – what is the risk?
  - Uneven ripeness
  - Unripe/over-ripe
Brix

• Why measure?
  • Indication of fruit ripeness
  • Potential ethanol production in wine
    • Yeast vary in their efficiency to convert sugar to alcohol – could differ up to 0.8 % v/v
    • Conversion factor of Brix to EtOH% 0.55-0.64
  • Determine optimal yeast to use for fermentation
  • Follow progress of fermentation
Brix adjustments

- Water back
- Grape concentrate
  - Use Pearson’s Square
    - Vol of water/concentrate = V(D-A)/(C-D)
      - V = vol juice
      - D = desired Brix
      - A = initial Brix
      - C = Brix of water/concentrate
- Saignee
- Remember changing in some cases more than Brix - TA, pH, YAN
  - Retest after any adjustments
Brix adjustments

- Error in calculation
  - Accuracy of Brix determination depends on homogeneity of grapes
    - Influenced by grape ripeness
    - Dehydrated and raisined berries
pH

- Why is knowing the pH important?
  - Microbial stability (pH < 3.7)
  - Effectiveness of SO$_2$
    - Molecular SO$_2$ is the form effective against microorganisms
pH

- Juice pH levels range from 2.8 to 4+
  - *Saccharomyces* optimal pH of 6, but grows well below and above this value
  - pH > 3.5 favors acetic acid bacteria and *Oenococcus* (LAB)
  - pH > 3.6 enables growth of larger diversity of LAB
pH

- Mouth-feel ("flabby") – wine style
- Color of red wine
- High pH ↑ oxidation risk

Fig. 6.19. Changes in the proportion of different forms of anthocyanins according to pH: $pK_a = 3.41$, $pK_h = 2.93$, $K_I = 0.61$ (Glories, 1984)
Titratable acidity (TA)

- Tartaric and malic acid are the main organic acids present in grapes
  - Citric acid is 3rd most prevalent
    - Rest formed during fermentation by yeast or bacteria
- Why is knowing the TA important?
  - Guide to acid taste of wine
    - Desired amounts depends on wine style
    - Wine balance
  - Adjust pH of juice/wine
  - How much to add to adjust pH?
    - Difficult to predict
Titratable acidity (TA)

- **Adjust potential wine pH**
  - Change in pH not directly related to acid addition
    - Depends on wine’s buffer capacity
    - Rule of thumb: 1 g/L of tartaric acid, decrease pH by 0.1
    - Efficiency of acidification can improve with CaSO₄ addition
  - **Add L(+) - tartaric acid**
    - DL-tartaric acid addition increase calcium tartrate instability
  - **De - acidify when pH < 2.9 + TA > 10 g/L**
    - Add 1.3 g/L of KHCO₃ – lower TA 1 g/L H₂T
- **Ion-exchange**
Titratable acidity

- **Measure malic acid (and lactic acid) conc**
  - High malic conc will impact pH if planning on MLF
    - ↓ 1 g/L malic acid, ↓ TA 0.56 g/L and ↑ pH
  - Also if high conc of malic and very low pH
    - MLF at pH<3 difficult
Nitrogen (NH₃, NH₄⁺ and amino acids)

- N₂ deficiency (< 100 mg/L)
  - Stuck fermentation
  - Utilization of sulfur containing amino acids – formation of H₂S

- Too much N₂
  - Modify aroma character
    - ↑Fusel alcohols, esters
  - Formation of ethyl carbamate

- N₂ needed
  - Depending on yeast needs and starting Brix
    - 21-27° Brix, need 200-350 YAN (mg N/L)
Yeast Assimilable Nitrogen (YAN) levels in juice

- Need to measure YAN of each fermentation
- Can vary greatly across fermentation lots even if from same vineyard
- YAN vary by season, varietal, rootstock and region
Adjusting N levels

- DAP (di-ammonium phosphate) most popular
  - Easy and cheap
  - Approximately 5 mg/L DAP for 1 N mg/L added
  - Adjustment needed before inoculation for cell growth
  - Any additional addition must be made before the half way point (< 8% alc)
Laccase activity

• Why measure?
  • If Botrytis in the vineyard – knowing the number and potential risk
  • Better adaptation during the winemaking process
So what is needed for a healthy fermentation?

- Yeast goal is survival
- Enable max cell growth
  - Meet nutritional needs
  - Minimize inhibitors
  - Minimize shocks like temp
- Sustain permissive conditions ~ minimize stress
  - Enhance ability to resist premature metabolic arrest
Yeast nutrition

- Growth is dependent upon available nutrients
- Insufficient nutrients causes:
  - Limit cell numbers (biomass)
  - Impact fermentation speed
  - Result in premature arrest of fermentation
  - Decrease ethanol tolerance due to increase in stress sensitivity
- Macronutrients are building blocks for new cell material
- Micronutrients catalyzes biochemical reactions
Yeast Nutrition

• Macronutrients are building blocks for new cell material
  • Carbon/energy sources - glucose, fructose, sucrose (never limiting)
  • Nitrogen Sources - amino acids, ammonia, nucleotide bases, peptides (often limiting)
  • Phosphate Sources - inorganic phosphate, organic phosphate compounds (occasionally limiting)
  • Sulfur Sources - inorganic sulfate, organic sulfur compounds (rarely limiting)
Yeast nutrition

- Micronutrients catalyzes biochemical reactions
  - Minerals and trace elements - Mg, Ca, Mn, K, Zn, Fe, Cu
  - Vitamins - biotin is the only required vitamin, but others are stimulatory
  - Sufficiency is site and varietal/rootstock dependent
Yeast nutritional phases

- Lag
- Log
- Stationary
- Death
- Dormant

Cell # vs. Time

Brix
Yeast Nitrogen Requirements

- Strain used
- Level of starting sugar/final ethanol
  - At ≤ 21 Brix need starting YAN ~ 200
  - At > 25 Brix need starting YAN ~ 300
- Accompanying deficiencies
  - Presence of other microorganisms
- N needs increase with ↑ alcohol
  - Decreased amino acid transport
  - Decreased ammonia uptake
- Stress can increase amino acid demand
Inoculation practices

- Active Dry Yeast
  - Rehydration is important
    - Can lose cytoplasmic components during rehydration
    - Will decrease viability if held too long prior to use
    - Water versus juice depends upon preparation: follow manufacturer instructions
  - Temperature equilibration is critical
  - Osmotic equilibration is critical for high Brix juices
  - Success of implantation depends upon conditions
Inoculation practices

• Starter culture – fermenting juice
  • May exacerbate nutritional deficiencies if juice is deficient
  • If too far along (too high in ethanol) return to high osmotic concentration poses a biological shock
  • Need to assess viability (stressors present)
  • Need to assess nature of organisms growing

• Starter success
  • Length of lag time before biomass production and fermentation initiation (↓2 Brix)
Use of sulfur dioxide

- It is an antioxidant
- Protects musts and wines from browning
- Binds oxygen and acetaldehyde
- Antiseptic activity
- Prevent microbiological spoilage in wines
  - Acetic acid and lactic acid bacteria, molds, wild yeast
Use of sulfur dioxide

• **When to add SO2**
  • Crushing – amount depends on condition of grapes, temp and pH (50-80 mg/L)
  • Immediately after alcoholic fermentation
    • Amount based on wine style and variety
    • Usually aim for 30 mg/L free

• **Other antimicrobials**
  • Lysozyme
  • Heat treatments
## Inhibitory levels of sulfur dioxide

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Molecular SO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saccharomyces</td>
<td>0.825</td>
</tr>
<tr>
<td>Acetobacter</td>
<td>0.05-0.6</td>
</tr>
<tr>
<td>Lactic Acid Bacteria</td>
<td>0.01-0.2</td>
</tr>
<tr>
<td>Brettanomyces</td>
<td>0.1-0.6</td>
</tr>
<tr>
<td>Non-Saccharomyces yeast</td>
<td>0.1-0.6</td>
</tr>
</tbody>
</table>
Temperature control

• Important to control fermentation rate
  • Viability of yeast
    • For red wine heat accumulates in the cap
      • Temp gradients of up to 12°C have been observed
      • Yeast growth from 12 to 42 °C (53 to 107 °F)
      • Temp tolerance reduced at high EtOH
      • Yeast strains vary in their ability to adapt to temp shifts
  • Non-Saccharomyces yeast and bacteria
    • LAB grow at temp 18 to 48 °C (64 to 118 °F)
    but varies by strain
Temperature control

- High temp > 30 °C can stress yeast resulting in more off-flavors such as H$_2$S
- Flavor stripping can occur at high temp
  - Cooked fruit character
- Temp control by jacketed tanks or submerged cooling/heating units
Temperature control

- Optimum temp for white and red wine fermentation is different
  - Whites $\leq 15 \, ^\circ C$ (59 °F)
    - Preserve aroma and flavor compounds
  - Reds 20-30°C (68-86°F)
    - For extraction of color, phenolics and tannins
    - In Cab we found opt temp to be 28-30 °C
    - At 35 °C or 95 °F signif decreases in color

Lerno et al., 2015 AJEV 66:4
Cap management

- Mixing of the cap for red wine fermentations
  - Remove accumulated heat from cap
    - Prevent yeast inhibition in the cap
    - Remove extraction saturation close to the cap
  - Studies show the 1 vol twice a day are adequate to remove concentration and temp gradients
  - Complex pump over regimes with different frequencies and volumes have limited impact on extraction

Sacchi et al. 2005 AJEV 56:3; Lerno et al., 2018 AJEV 69:3; Lerno et al. 2017 AJEV 68:4
Oxygen management

- Amount of oxygen depends on fruit condition
- White vs red grape processing
- Crushing and pressing - two processes with potentially significant O₂ exposure
  - As soon as berries are damaged, numerous oxidase enzymes including polyphenoloxidase (PPO) activates starting oxidative chain reactions which form quinones that can lead to browning
Oxygen management

- Protective inert gas blankets and SO$_2$ have shown to preserve thiols and glutathione (GSH), a natural grape antioxidant
  - GSH 51% in juice and 40% in skins
- Hyper-oxygenation – 50 mg/L just before cold settling
  - Protects against future browning
  - Lack of scientific study
  - Sensory of Chardonnay indicated ↑banana and ↓herbaceous and floral
- O$_2$ exposure during red grape processing is less of concern for healthy fruit

Day et al., 2015 AJGWR 21:693
Oxygen management

- **White or red wine fermentation**
  - Oxygen good for yeast viability
    - Lipid production needed for cell growth
    - \( O_2 \) addition at end of exponential growth phase – most effective stimulation of fermentation
      - 5-10 mg/L \( O_2 \) is sufficient (macro-oxygenation)
        - When combined with DAP addition, faster fermentation completion to lower RS
    - In non-Saccharomyces– a range of aeration regimes, applied continuously for various durations starting at inoculation
  - Do not know how laboratory studies translate to large scale
  - Translation to native fermentations?
Oxygen management

- Both yeast and bacteria grow better under aerobic environment
- Anaerobic conditions best to control acetic acid bacteria
Oxygen management

- Excess $O_2$ removed by $CO_2$ during fermentation
- Too little $O_2$ can result in reductiveness
- Too much $O_2$ can result in oxidation
  - Loss of varietal character for aromatic whites
Oxygen management

- Optimal use of $O_2$ can impact wine style greatly
  - Enhance fruit characters, limit reductive characters
  - Develop mouthfeel profiles
  - $O_2$ during fermentation can have the same impact as MOX following fermentation to remove reductive aromas
- The optimal amount of oxygen during red winemaking without leading to excessive addition and concomitant spoilage still has to be determined
Concluding remarks

• Successful fermentations
  • Selecting correct yeast strain for EtOH range
  • Meeting nutritional needs of yeast
  • Limit stress and follow fermentation progress
  • Intervene early if not ‘normal’

• Most off-flavors can be minimized or prevented by
  • Using clean fruit
  • Sufficient nutrient, oxygen and temperature control during fermentation
  • Good winery sanitation and adequate SO₂ use
Thank you