# Overview of Yeast Selection and Malolactic Fermentation on Aroma, Flavor and Phenols

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# Introduction: Unlocking The Potential

Wine is largely defined by its appearance, aroma, flavor and palate/mouth-feel properties. These arise from three major sources; the grapes, yeast and bacteria and, when used, wood.

(1) Grape-derived compounds provide varietal distinction in addition to basic structure arising from the contribution of phenolics.

- Present as free volatiles, which may contribute directly to aroma, or as higher molecular weight sugar- or cysteine-bound conjugates.
- Thus, floral monoterpenes largely define Muscat-related wines whereas volatile thiols may play a significant role Sauvignon blanc and related cultivars.

(2) The role of yeast in modifying the chemical, mouth-feel and flavor of wine has been more recently established. The action of yeast on wine occurs at several levels:

- Extraction of compounds from solids present in grape must/juice with formation of the characteristic metabolites of fermentation; alcohols, esters, fatty acids, carbonyls, etc.
- Modification of grape-derived compounds: many yeast, including *Saccharomyces* sp., possess limited capabilities in terms of

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enzymatic hydrolysis of higher MW-precursors and formation of volatile (sensorially detectable) products.

- Studies have suggested that the ability of commercial strains to hydrolyze glycosides varied but was near 7%.

Volatile thiols are important aroma components of varieties such as Sauvignon blanc where they contribute to varietal characteristics.

- Since initial identification 30 years ago, volatile thiols (and their higher MW-conjugate precursors) have been isolated from wines made from a variety of cultivars, including Sauvignon blanc, Gewürztraminer, Riesling, French colombard, Semillon, Pinot gris, Cabernet sauvignon, and Merlot.
- Potentially volatile thiols are present in grapes/juice as cysteine-containing precursors.
- Their subsequent release during fermentation suggests that metabolic activity of yeasts is necessary to cleave the precursor.
- Hydrolysis of volatile thiols during fermentation appears to be strain dependent and a particular yeast's ability to release one thiol does not appear to be linked formation of a second, different thiol.
  - Research suggests that by using different strains, variation in the release of these enzymes can be achieved.

# Yeast and Bacterial Starters

- These observations suggest the opportunity for developing wine yeast starter strains with optimized volatile-thiol release capabilities.
  - A more predictable tool in the winemaker's quest for regularly definable flavor specifications and styles.
- Since development and release of the first commercial wine active yeast starters (WADY or ADY) in 1965 by Red Star Yeast (Universal Foods Corporation), over 100 available ADY cultures have been commercialized.
- Reported properties:
  - Alcohol resistance (up to 17% v/v)
  - Compatibility with MLF and indigenous (native) yeast
  - Low fermentation temperatures
  - Ester production
  - Low $H_2S$ production
  - Enhanced palate structure (mouth-feel).
  - Color and structure compatible (including enhanced release of polyphenol-reactive polysaccharides).

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- Enhanced fructophilic properties
- Intensified varietal character (S. blanc, Pinot noir, Muscats and their related cultivars).

Suppliers are now recommending pairing selected strains with specific cultivars. Despite the apparent advantages of commercial *Sacc* cultures, the winemaking community still remains divided with regard to the philosophy and practice of using starters.

- At one extreme are those that rely solely on yeasts (including *Sacc* sp.) and bacteria native to the winery and vineyard.
  - Success, here, relies on belief that, without intervention, such indigenous or "native" fermentations occur as an uninterrupted succession of yeast populations beginning with relatively weak, although numerically superior species present on the fruit and, eventually, giving way to indigenous populations of *S. cerevisiae* that, due to their alcohol tolerances, finish the fermentation.

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- Others prefer to encourage the growth of some non-*Sacc* yeasts early in alcoholic fermentation and, eventually, inoculate with a *Sacc* starter as desired flavor/aromatics and structure develop.
- Still others use *Sacc* starters but at lower than recommended inoculum levels.

Due to the demanding nature of modern winemaking practices and a increasingly sophisticated consumer base, there is a growing need for wine yeast strains possessing a wide range of optimized, improved or novel enological properties.

- Although winemakers often have strain preferences for particular applications, the issue continues to be one of debate. Anecdotal evidence has existed for some time that yeast strains differ in their capacity to influence wine flavor.
  - Recent studies suggest relatively low genetic diversity amongst some of these strains compared with other species

# Yeast and Bacterial Starters

- Decades old winemaker and research experience, alike, suggest that selected non-*Sacc* ("native") species may contribute, in positive fashion, to quantitative/qualitative diversity of sensorially-active products and by-products.
  - Wines produced from selected co-fermentations have, classically, been described as having improved structural ("mouth feel") properties. This may well be the consequence of higher concentrations of glycerol and other polyols produced by indigenous non-*Sacc* yeasts.
  - Additionally, the extended lag phase before the onset of native fermentation promotes reaction of oxygen with anthocyanins and other phenols which, in the absence of ethanol, may lead to enhanced color stability in red wine as well as accelerating phenol polymerization properties.
  - Co-fermentations are also known to yield enhanced aromatics (particularly ethyl esters and phenylethanol) and overall complexity compared with monocultures of *Sacc* controls.

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- However, there were practical problems associated with early attempts to utilize non-*Sacc* cultures.
  - Non-*Saccharomyces* monocultures have limited fermentation capabilities in terms of both fermentation rate, particularly late in the process, and complete utilization of sugars (Rs <2 g/L).
  - Early attempts at commercialization of non-*Sacc* ADY were limited by viability issues during drying and rehydration.
- Results from our own lab (and others) in the late 80s suggested that while use of non-*Sacc.* monoculture fermentations might not be feasible, their use in co- or mixed cultures appeared promising.
- Strains of *Torulaspora delbrueckii* were among the first to be recognized as playing a positive role in sensory properties of sequential mixed-culture fermentations.
- Inoculation strategy utilized initial addition of *Torulaspora* followed by *Sacc* at 48+ hours.
- Today, Chr. Hansen produces mixed-culture starters that include *Sacc* x *T. delbrueckii, Sacc* x *Kluyveromyces thermotolerans, Sacc* x *K. thermotolerans* x *T. delbrueckii* as well as a monoculture of *Pichia kluyveri.* Laffort also markets a *Torulaspora* culture.

# Yeast and Bacterial Starters

**Fermentation Management Concerns:**

- **Non-*Saccharomyces* strains have a high demand for Yeast Assimilable Nitrogen (YAN). This is a leading cause of stuck/ protracted fermentation where native flora is promoted.**
- **Aside from influencing fermentation kinetics, must nutrient content can impact wine composition and volatiles content.**
- **Diammonium phosphate (DAP) addition has been the cornerstone of fermentation management for decades.**
- **While recognizing the importance of nutritional management, some are now questioning DAP's impact on wine flavor relative to the use of "balanced" nutritional amendments.**
  - **At present, there is no clear resolution to this question.**
- **HOWEVER, regardless of the form of supplement, maintaining adequate levels of yeast nutrition is critical to successful fermentation.**
- **AND, establishing/maintaining a nutritional monitoring and supplementation program is, or should become, a primary goal of the winemaker.**

# Malolactic Starters

**Malolactic Fermentation and LAB Starters: As was the case with yeast, before the availability of lyophilized commercial LAB cultures in the 80s, wineries relied on native microflora to induce MLF.**

- **With the widespread use of wooden storage tanks and barrels, a ready source of "in-house" inoculum was commonly available.**
- **Under these conditions, promotion of MLF was accomplished by maintaining a temperature of 21°C/70°F, not adding sulfites, and maintaining a pH greater than 3.2.**
- **Given that MLF can occur during, immediately following, or up to several months after completion of alcoholic fermentation, there was always a significant risk of spoilage.**
- **Moreover, spontaneous MLF by unidentified lactic acid bacteria led to unpredictable and/or undesirable flavor characteristics in wines.**
- **Because of this, we continue to advise winemakers to regularly monitor the wine microscopically in addition to routine QC tracking of malic and acetic acids.**

# Malolactic Starters

- Although some wineries continue the tradition of using native microflora and in-house isolates, winemakers increasingly inoculate grape must or wine with LAB starter cultures to improve the success of MLF.
  - Numerous strain of *O. oeni* are available in lyophilized, frozen concentrates, and liquid forms.
- Lyophilized starter cultures usually contain high populations of viable bacteria (>$10^8$ CFU/g) and are easy to ship and store.

Keys to Successful MLF:

1. Starter Viable Cell Number: Minimum VCN $10^6$/mL
2. Nutritional concerns: LAB are fastidious. Maximum viability and conversion is improved with the use of LAB-specific rehydration medium (for lyophilized preparation) and, subsequently in wine, LAB-specific nutritional supplement.

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3. Sulfur Dioxide: Best results are associated with delaying additions until MLF conversion is complete or at a point where further activity is unlikely.
    - Where MLF is required, low sulfite-producing ("ML-friendly") yeast strains should be selected. Consult suppliers for recommendations.
    - Depending upon lactic strain, tolerance of $TSO_2$ from 30-70 mg/L is reported.
4. Wine Alcohol Content: Most commercialized strains can handle alcohol levels approaching 14% v/v.
    - Where levels are higher, there are a couple of strains that can be utilized. Consult suppliers for recommendations.
5. Wine pH: Most strains perform well to pH 3.1- 3.3. Growth is promoted at higher pH but such an environment also tend to support growth of spoilage species/strains.
    - Thus, well prepared, aggressive cultures are required at both extremes.
6. Temperature: Optimal 62-70$^{o}$F. Temperatures below 50$^{o}$F result in slow growth and, potentially, stuck MLF.

# Malolactic Starters

- Diacetyl Production: One of the most apparent sensory changes that occurs during MLF is the development of a 'buttery' or 'butterscotch' character arising from bacterial formation of diacetyl.
- Consumer interest in fruit-forward wines has driven suppliers to identify and propagate strains that either do not produce (or produce low levels of) diacetyl.
  - Such low diacetyl or "diacetyl-free" strains either do not utilize (or only partially utilize) citric acid precursor.
  - Since citric acid utilization is reduced, such strains also produce lower levels of acetic acid.
- Aside from LAB strain selection, winemaking practices can also enhance or diminish levels of diacetyl. These include:
  - Lower inoculation rate ($10^4$ vs $10^6$ cfu/mL) favors diacetyl production.
  - MLF at 65°F versus 75°F may favor formation.
  - Lower pH may enhances production.
  - Contact with active yeast culture either through co-inoculation or with lees contact lowers levels.

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- Shift in Redox potential during MLF. Oxygen favors production of diacetyl via conversion from α-acetolactate.
- Sulfur dioxide addition: Formation of the reversible $SO_2$-diacetyl adduct diminishes the carbonyl's impact. However, this is a reversible reaction and hydrolysis may release diacetyl a later time.
- Partial MLF brings about complexity while retaining acidity.
  - Accomplished by co-inoculation during alcoholic fermentation.
  - Since the wine is biologically sensitive, this approach requires the ability to sterile bottle.

Timing the Inoculation: When MLF starter cultures are used, the winemaker is faced with the decision as to the timing of bacterial inoculation:

- Post-Alcoholic Fermentation ("Old World"): Driven by concerns regarding formation of acetic acid by LAB growing on grape sugars

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and potential antagonistic interactions between yeast and bacteria, many winemakers opt to inoculate upon completion of alcoholic fermentation.

- Co-Inoculation ("New World"): Research, dating back to the 80s, suggests that early inoculation of LAB along with, or shortly after, yeast starters is best for inducing and rapid completion of MLF.
  - This approach relies on availability of nutritional stores needed by LAB that have not yet depleted by yeast growth.
  - Ethanol and $SO_2$, known to be inhibitory to *O. oeni* are present in lower concentrations.
- Preliminary results from 2011 FS trials using both approaches:
  - Lower alcohol (13.69% v/v) in wine produced from LAB x *Sacc*. co-inoculation at the start of alcoholic fermentation compared with LAB addition post-fermentation (14.28% v/v).
  - Co-inoculation lot described as "fruit-forward and relatively simple," post-fermentation inoculation described as "more complex (particularly mid-palate) featuring dark fruit."
  - VA in both was slightly elevated: co-inoculation 0.78 g/L vs 0.82 g/L for post-inoculation lot.
- CAUTION: Must/juice pH should be a consideration with co-inoculation. In cases where pH >3.5, growth of heterofermenters on grape sugars during alcoholic fermentation may increase the potential for VA.

# Concluding Thoughts…


1. Given the array of yeast cultures (*Saccharomyces* monocultures, non-*Sacc* and mixed), lab or pilot-scale trials are recommended before general commercialized use.
2. Nutritional analysis (YAN) should always be part of pre-fermentation monitoring program. Balanced nutritional formulations are recommended and should be added incrementally over the first-half of fermentation.
   Nutritional requirements and supplements for yeast are different than those for LAB. Specific supplements are available for each application. CONSULT YOUR SUPPLIERS!
3. A proactive program of microbiological monitoring should be implemented.