Welcome to VEN124 "Wine Production". This class is the first in the series of advanced courses in enology and viticulture offered by the Department of Viticulture and Enology, and serves to provide an overview of all aspects of wine production. As such, we will touch on many topics that we will not be able to explore in depth. Reading assignments will provide further information on key subjects. This lecture will cover the topics of wine quality and factors influencing the definition of quality, and survey the factors most strongly impacting wine composition and therefore quality. Many of these topics will be considered in more detail later in this course.
Lesson 1: What is Wine Quality?

We will begin our discussion of wine production by first considering the definition of the term "wine quality". Quality is a subjective term, and has many different meanings, depending upon the context in which the term is used. Perceived quality is the reflection of the chemical composition of the wine at the time at which it is being consumed. What one person perceives as quality may not be so thought of by another. This is in part due to physiological differences in the detection of compounds. Some individuals are unable to detect certain bitter compounds, for example. There are also differences in the relative concentration or threshold at which a given compound is detected. The best example of this is trichloroanisole or TCA. There appears to be a thousand-fold range in concentration at which it is detected across the human population.

Perception of characters also depends upon psychological factors and the previous experiences of the taster with that compound. Whether the compound is familiar or not and in what context are important factors. A compound that the taster associates with food or with pleasurable experiences is not as objectionable as one not primarily associated with food that is correlated with a negative experience. For example, some microbes that can be present during wine fermentation produce the ester ethyl acetate. This character is found at high concentration in wine vinegar but it is also a principle aroma compound along with acetone of many nail polish removers. Individuals who associate this character with nail polish remover tend to consider it quite objectionable in wines while those associating it with vinegar do not find it as unpalatable, but consider the wine to be defective. Another example is the rosemary character. This herb character is found in a facial cleansing product. It is also commonly used in cooking. Individuals who associate the character with a soap find it quite offensive in foods and beverages while those who recognize it as the herb rosemary do not object to its presence. Thus the experiences of the individual with certain characters will impact the positive or negative perception of those characters. For this reason quality as applied to wine aroma and flavor is inherently subjective.

However, that said, there are some characters that I term the "gray" taints for "generally recognized as yucky". These are undesirable aromas and flavors on which there is strong consensus, such as the rotten egg character of hydrogen sulfide. Wines with these compounds can be clearly viewed as defective or of low commercial potential or quality. Individuals can decide upon the characters or qualities most desired in a particular style or varietal and quality in this case becomes an estimation of nearness to an ideal target as discussed below.
Good wine cannot be made from bad grapes...
Wine quality is dependent upon viticultural practices and decisions.

You have all heard the statement that wine production begins in the vineyard, and this is certainly very true. There are a lot of factors that influence grape composition, which in turn influences wine composition and, therefore, ultimately perceived quality. You have also doubtless heard this statement that good wine cannot be made from bad grapes, which is also true. The winemaker is limited in the scope and magnitude of changes that can be made to the chemical composition of juice, must and wine. If the composition of the grapes at harvest is not optimal, the finished wine will reflect that lack of optimization. Grape composition is therefore very dependent upon viticultural practices. Winemakers need to be fully engaged in vineyard operations, and control those practices that will impact the ultimate chemical composition of the wine.

But what is the ideal composition of the wine? The answer to this question depends upon the definition of wine quality, and the factors influencing that definition. While perceived quality is clearly subjective, quality as an estimate of nearness to a specific goal can be used more objectively. However it is important to always remember that quality is inherently subjective. There are three broad definitions of wine quality currently in use today.

Wine Quality Defined as:

- Nearness to a specific target or "ideal" wine
- Harmonious complexity
- Inharmonious notes

The first definition characterizes wine quality as nearness to a specific, ideal target. In this case a description of the flavor and aroma composition of the ideal wine of that type exists and the object is to match that ideal. The closer you are to the perfect match in composition, the higher the quality of the wine.

The second definition of wine quality equates quality with complexity. The goal is to produce a wine with a lot of characters, a lot of tastes, a lot of aromas. Harmonious means that all of those characters go together well in a pleasing format. That is, there does not appear to be any character in the wine that is in conflict with the flavor/aroma
mosaic. For example, Grenache is characterized by having a significant amount of what is termed "forward fruit". It has intense red fruit flavors: raspberry, strawberry, cherry, cranberry, plum, as well as a subtle spiciness. These characters all go together well, that is, they form a pleasing mosaic. However Grenache is also characterized by tobacco and smokey notes which when together translates into a burnt cigarette ashes character. On top of fresh fruit, this is usually perceived as a negative. If our definition of quality is harmonious complexity, Grenache is definitely a challenge, which is why I use it as a teaching tool in the pilot winery. However, if we wished to make a Grenache style that is true to the varietal character of this grape, then we may want the ashes character along with the forward fruit. Further, harmonious in one situation might not be harmonious in a different situation. Consider an intense buttery character for example. This might not go as well with intense forward berry fruit (Grenache) as it would with Chardonnay. Whether a given character is good or bad really depends upon the entire composition of the wine. But most importantly, harmonious, like quality in general, is subjective.

The **third definition** of wine quality centers on the concept of the importance of a lack of harmony in wine composition. That is, there is such a thing as being too clean, being too harmonious. An off note or two is perceived as adding character and dimension to the wine. This is frequently associated with the notion that a technically "clean" wine suggests excessive processing and treatment that belies the true character of the fruit. However, many argue that this definition of quality means that virtually anything goes, and thus blurs the division between acceptable and unacceptable.
Lesson 1: Targeted Definitions of Wine Quality

There are several styles of for which there are specific or targeted definitions of quality.

**Targeted Definitions of Wine Quality**

- Regional typicity
- Varietal typicity
- True-to-style

The **first targeted definition** of wine quality that we will consider is *regional typicity*. In this case the wine shows the characters and traits that have been defined as typical for that region. For example, in many areas of France there is a certain expected composition of wines produced from specific regions. The rating system of wine is dependent upon nearness to what is defined as typical for fruit from that region. In most cases the judgment of whether or not a particular wine or vintage is worthy of the regional designation rests with a regional or government body, not the original producer. The beauty of this system is that it safeguards the character of the wine for the consumer, and guarantees a certain nearness to an ideal composition.

The **second targeted definition** of quality concerns *varietal typicity*. The goal here is to produce a wine that best showcases the characters specific to that varietal, in other words, is the best wine that it is possible to make while retaining a strong varietal signature. Obviously varietal styles of wine are restricted to those varietals that are pleasingly complex at the time of harvest, not requiring significant chemical adjustment that can only be achieved by blending.

The **last targeted definition** of quality is *true to style* - style may mean a style that someone else has developed and that is generally regarded as typical for that varietal or region, or it may mean your own style. For example, suppose you desire to produce a Chardonnay that is high in oak and butter because this is quite popular with a certain segment of the market. Oak and butter do not come from the vineyard, so you are not aiming for varietal character, nor are you aiming for regional typicity because these characters are not specifically associated with any one growing region. However, you do have a very specific definition of the ideal wine, which, once in place, can guide viticultural and winemaking operations.
The commonality of all of the cases discussed above is that there is a clear goal for the chemical composition of the wine. Quality is defined as nearness to that goal. It is then up to the consumer to decide if that goal is worth purchasing. Subjective definitions of quality also abound in the wine industry and among consumers. Frequently market factors will dictate the ultimate wine composition. The winemaker must be as aware of market forces as of vineyard operations.
Lesson 1: Harmonious Complexity as an Index of Quality

Harmonious complexity as applied to wine is multi-dimensional. We can think of this type of complexity in two ways, lateral and vertical.

Complexity as Quality

- **Lateral complexity:**
  Wines have multiple intense aromas and flavors that are "forward": immediately apparent upon smelling/tasting the wine

- **Vertical complexity:**
  As wines "breathe" in glass the aroma/flavor profile changes dramatically, positively and continually; also referred to as "layered complexity"

- Both aim for harmony: melding of flavors and aromas

In both cases the wines have a variety of intense and subtler flavors. In the case of lateral complexity we refer to all of the characters as being "forward" or side-by-side. That is, they are immediately apparent in the initial chemical composition of the wine. In this situation, all of the characters present might not be instantly detected. We detect those compounds in highest concentration first. As olfactory sensors become saturated to particular compounds they become desensitized, allowing detection of other less intense aromas in the wine. This physiological property reflects our animal roots - it would be unwise to exclusively focus on the most intense flavor or aroma in a particular environment, instead it is better to detect all the important aromas in that environment in their relative concentrations. The chemistry of the wine is not changing in this case, instead what is changing is our ability to detect specific chemicals. If you set that wine aside and went back to it after sufficient time had passed to clear the olfactory sensors, that is after the desensitization has abated, and you smell that wine again, you will detect the same set of original characters.

In the case of vertical complexity as the wine ages in the glass the chemical
composition of the wine changes so that a different set of aroma characters are present. This is principally due to volatilization of some of the aromatic compounds. Since our olfactory sensors detect volatile compounds, as those compounds are lost from the environment we are no longer able to detect them and will notice characters that remain. Those characters might not have been detected earlier because they were "masked" by other components. The aim is to have harmonious lateral complexity at each step of the volatilization process. As you can imagine, this is the most difficult type of wine to produce. One must also consider that the physiological perception of the characters of a wine will be influenced by the environment in which it is served, particularly by the other flavors and aromas of that environment. This is the basis of the pairing of wines and foods. An appropriate pairing can accentuate positive characters in both the wine and the food while masking those that are negative or inharmonious. In this case the wine would be considered to be of exceptional harmony when served with the proper food but may be thought of as tiring (olfactory sensors saturated with insufficient desensitization time) or defective (excessive negative or objectionable characters) if served alone or with poorly matched foods. To return to our Grenache example, a food that suppressed the ashes character might enhance our perception of the overall harmonious quality of the wine, while one that suppressed the forward fruit tones might leave the wine bland or too strongly accent the ashes note. What is "harmonious" to one consumer (or producer) might not be harmonious for another. There are no absolute definitions. Sensory tests suggest that individuals may be highly irreproducible from one day to the next in their ability to reproducibly detect "quality".
Lesson 1: The Concept of Off-Notes in Wine Quality

Many believe that wines that have negative or "off-notes" are important indicators of the quality of a wine. As mentioned above, this may stem from an association of cleanliness with excessive processing, and a departure from "natural" winemaking practices. However, it is also true that in order to achieve the optimal vertical harmonious complexity the initial wine may contain some negative characters necessitating a period of "breathing" before the wine is consumed. Many negative characters such as hydrogen sulfide (rotten egg) are produced by microbes under the same conditions that lead to the production of desirable microbial characters. If the latter is desired the former must be tolerated. In addition, frequently some of these negative characters are readily suppressed by more strongly flavored foods, such as the pairing of the barnyard medley of characters produced by the yeast *Brettanomyces* with "gamey" meats or abundantly aromatic cheeses.

Off Notes as Index of Quality

- Some believe that a wine free of off-notes is "too clean"
- Off-notes lend character to a wine
- Alternately, off notes accompany microbial activity and therefore track with greater microbially-derived complexity
- Harmonious complexity "boring"
Lesson 1: The Concept of Terroir and Wine Quality

A term frequently used in the description of quality wines is "terroir".

**Terroir**

A term coined by the French, refers to the influence of non-climatic environmental factors¹ (soil, topography) on wine composition and quality


The definition of terroir given above from the "Handbook of Enology" limits the term to the impact of non-climatic environmental factors - soil and topography - on wine composition and quality. Other authors include climate as a key component of terroir and still others include the "human element" that is, the grape growing and winemaking habits and attitudes of a particular region are all considered to be part of the regional terroir. Critics of the terroir concept claim that it is at best merely a statement of the obvious, that the environment will influence grape composition, and that it is frequently defined by characters not originating in the grape. Compounds produced by the yeast *Brettanomyces* and other winery and grape flora often make the list of characters considered typical of a particular "terroir". These characters can be produced in wines made anywhere in the world regardless of the environment of the grape vines by encouraging *Brettanomyces* infestation of the winery. However whether these barnyard, animal, leather characters are considered desirable or not does depend upon the rest of the composition of the fruit which is influenced by the environment. Terroir as a marketing tool, to assure a particular character of the wine, is quite valuable for the consumer. The term has clear usefulness in the marketplace and perhaps should not be interpreted so literally.
Terroir

- Terroir characters are defined by the traits of the wines following elimination of other variables, not from direct demonstration of the influence of environment on those characters.

- Recipes for both vineyard and winery procedures are legislated, minimizing the impact of these decisions on wine composition across vintages.

- Used in marketing to assure consistency of product for the consumer.
Lesson 1: American Viticultural Areas and Wine Quality

The American wine industries have developed a concept similar to terroir also geared toward characterization of wines by growing region. In this case, wines may be marketed as coming from a specific region that ideally leads to the appearance of specific "signature compounds" in the wine as a consequence of the geography of the area. AVA (American Viticultural Area) approval is generally obtained by first demonstrating something unique about the climate, topography or soils of the growing region, and providing information that the region is historically recognized as producing distinctive wines. This falls far short of a detailed descriptive analysis defining the unique characteristics of the wines from the region. As with terroir, the principle value of an AVA designation lies in marketing. AVA status poses no restrictions on vineyard management strategies or wine making procedures.

AVA

- No restrictions on vineyard or winery practices: uniqueness of wine must be apparent regardless of "recipe"
- Allows considerable variation in composition of wine while retaining a regional "signiture"
- Used in marketing to assure consistency of product for the consumer

In the case of terroir, operational decisions are frequently controlled, that is, specified by law and are thus not an influencing factor in wine composition because they do not change. This is not the case in an AVA designation. Both terms therefore attempt to define a "regional signature" for the wines, but do not necessarily document that the characters in question indeed arise because of a specific physiological response of the vine or the fruit to the environment. It is also important to note that one of the most important influences of soil and topography is water-holding capacity. Many of the characteristic changes in composition may be simply due to water limitation or excess.
Lesson 1: Wine Composition

The chemical characters that we find in wine basically come from or are influenced by four sources: the grape, the biological activities of the microorganisms present, processing decisions and wine age. Some wine styles are more dependent upon one source of character than others, but all must be considered as impacting the final chemical composition of the wine at the time of consumption. These sources are also highly interactive. For example, the microorganisms can metabolize only what is present in the must at the time of harvest, so metabolic end products are dependent upon the nature and presence of precursor molecules. The same is true of aging time. How characters change depends upon what characters are initially present either from the grape or that have been produced microbially. What characters appear from the action of yeast and bacteria is dependent upon what organisms are present and how those organisms persist during the processing life of the wine. This is directly influenced by winemaking practices, as we will see in subsequent lectures.

Wine Characters Derive from One of Four Sources:

- Grape
- Activity of microorganisms
- Processing decisions
- Aging

Different wine styles may be more strongly dependent on one sector of the source of wine characters than other styles of the same varietal.
Several factors can impact each source of chemicals of the finished wine. For example, grape composition is influenced by many variables.

**Grape Composition Influenced by:**

- Variety
- Clone
- Rootstock
- Soil
- Canopy management
- Terrain
- Pest Pressure
- Disease Pressure
- Climate
  - Rainfall
  - Humidity
  - Sunshine
  - Wind speed
- Cluster microclimate
- Seasonal Variation
- Vineyard Practices

Obviously of key importance is the **varietal** itself. Different varietals of *Vitis vinifera* display marked differences in the color, flavors and aromas present in the fruit, which is a reflection of the differences in genetic composition and expression of enzymatic
activities in the vine. Vines are propagated clonally, meaning that one vine is used as a source of wood for other vines, so \textbf{clonal differences} may also arise and impact the chemical composition of wine at harvest. Many important wine varietals perform poorly in certain types of soil due to disease or pest sensitivity or poor adaptability to the soil conditions. It is a common practice therefore to graft scion or fruit-bearing stocks to \textbf{roots} derived from a different variety of grape. The hybrid plant now has a stronger root system and produces high value fruit. The root has a dramatic influence on berry composition, as the roots are the principle means of absorption of key micro- and macronutrients. Biochemical pathways are dependent upon having the optimum micronutrient availability, so changes in the pattern of root absorption will impact the enzymatic activities in the fruit and therefore the composition of the berry. \textbf{Soil composition} also impacts nutrient availability, and is in turn a reflection of the geography of the region. As described in more detail in subsequent lectures, pest and disease pressure and vineyard practices likewise greatly influence grape berry composition.

Several classes of microorganisms are contributors to wine composition. The yeast \textit{Saccharomyces} is responsible for the conversion of sugar to alcohol and plays a key role in the transformation of grapes into wine. Members of the lactic acid bacteria are responsible for the "malolactic fermentation" the conversion of grape malic acid to lactic acid, an important deacidification reaction in many wines.

\textbf{Microbial Contributors to Wine Characters:}

- \textit{Saccharomyces}
- Lactic Acid Bacteria
- Grape Flora
- Inocula

Other microbial flora can have a dramatic impact on wine composition either directly through the production of detectable end products and indirectly through the impact on the metabolic activities of other microbes. In this regard, both the grape and winery flora must be taken into account.

Many processing decisions will impact wine composition as well. The following list is a summary of the types of options that will have a profound influence on the finished product. We will consider each of these factors in turn during the rest of the course and will not discuss them in detail here.
Processing Decisions Impacting Wine Characters:

- Harvesting conditions
- Maceration decisions
- Extraction conditions
- Additions to juice/must
- Fermentation conditions
- Lees contact
- Clarification
- Filtration
- Fining
- Blending
- Stabilization Treatments

Likewise, choices made in aging of the wine, such as length of time, cooperage used, exposure to chemically oxidative versus reductive conditions, all impact wine composition.

Aging Decisions Impacting Wine Characters:

- Time
- Temperature
- Cooperage
- pH
- Wine composition
- Evaporation
- Agitation
- Oxygen exposure
- Lees exposure
- Sanitation practices

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Lesson 1: Who Controls Definition of Quality?

In an ideal winemaker's world, the definition of quality would be exclusively in the hands of the producer. However, outside pressures frequently influence the style of wine produced.

**Who Controls Definition of Quality?**

- Government
- Producers
- Consumers
- Intermediaries (Wine Critics / Wine Writers / Distributors / Marketers)

In many regions of the world wine labeling is under strict legal control. This ranges from extreme limitation of both vineyard and winery operations as in France to more limited restrictions of the chemical adjustments allowed to juice, must and wine. The object in all cases is to safeguard the quality of the wine produced which protects producer and consumer alike. In some cases the **producer** of the wine has the ultimate control over the definition of ideal chemical composition. This is clearly the case in the segment of the market that produces "artisan" wines. In this case the **consumer** is purchasing a wine that reflects the artistic talents of the producer. In contrast, in the "fighting varietal" market segment the consumer controls the definition of quality by his/her purchasing decisions. The winemaker often times must rely on an **intermediary** to sell the wine. In this case the wine must appeal to the intermediary, that is, meet their definition of quality. This is frequently the case with wine critics/writers who have a loyal following of consumers. If the wine writer functions as your principle marketing agent, then the wine will need to match the style and composition that will garner a strong recommendation. As long as the definition of the ideal composition of the wine is known, then a clear target can be established at the winery. It is critical that the winemaker understand the market forces that dictate product composition and communicate well with the marketing division of the winery.

In this lecture we have surveyed the definitions of wine quality and the various forces that impact the final chemical composition of the wine. A single winery may have different concepts of quality depending upon the segment of the market for which the wine is being produced. It is as important to understand market forces, as it is to
understand wine composition.

We will end this first lecture with a final thought: while you cannot make good wine from bad grapes, you can make bad wine from good grapes. It has been done many times.

**Bad wine can be made from good grapes...**

Wine quality is also dependent upon enological practices and decisions.
Lesson 2: Introduction

Grape Composition and Ripening: Viticulture from the Plant's Perspective

In this lecture we will consider the factors that influence grape berry composition and the process of ripening. We will discuss berry maturation from the perspective of the plant, that is, what is the purpose of fruit production, and how is berry composition established. We will also cover the topic of the location of various berry components within the berry itself, as this will impact wine making decisions. In the next lecture we will take a different view, berry maturation and ripening from the winemaker's perspective. The principle wine grape species is *Vitis vinifera*, referred to as the European variety. This species originated in the temperate Mediterranean and Black Seas areas. Wine production and the cultivation of grapes begin before recorded history, and the practice is several thousand years old. The most widely grown varieties of *V. vinifera* are Cabernet Sauvignon, Pinot noir, Sauvignon blanc, Chardonnay, Zinfandel and Riesling. Varieties traditionally used in blends are grown on more acreage, but these are the most popular varietal wines. *V. vinifera* is limited to moderate climates and does not survive prolonged cold stress or in high humidity regions. other *Vitis* and non-*Vitis* (*Muscadinia*) species may be grown under these less moderate conditions.
Lesson 2: Grapevine Biology

One of the most critical characteristics of plants that dictates much of their biology, is that they are not mobile, in essence, with the exception of seed dispersal, they are stuck where they are. As animals, if we are in a place that suddenly becomes inhospitable, we can migrate to a different location. Seasonal migration is an excellent strategy in order to avoid environmental extremes of temperature or waterfall, or to escape disease or pest pressure. Plants by their nature are unable to relocate and thus must develop biological strategies such as dormancy to survive environmental stress. The only option available to the plant for relocation is seed dispersal. They must also cope with pests and diseases of their local environment.

Characteristics of Plants

- **Stuck where they are:** use chemical strategies to deal with problems
  - Nutrient limitation
  - Competition
  - Excess/shortage of water
  - Extremes of temperature
  - Disease/Pest pressure
  - Lack of light
- **Prioritize nutrient use for survival**
- **Role of fruit:**
  - Dispersal
  - Fruit is attractive to mobile agents that will disperse seed (animal; insects; bird)
  - Seed itself designed to "taste bad" so it will not be consumed

The main strategies available to plants to handle various biotic and abiotic stresses are chemical in nature. Plants can be viewed as masters of chemical warfare. They produce many compounds inhibitory to other organisms in their environment. They also produce compounds such as proline to allow cellular metabolic activities that are dependent upon water to occur in arid environments. For many compounds, multiple biochemical pathways of synthesis exist, so that the plant can optimally utilize limiting macro- and micronutrients, depending upon availability from the soil. Some biochemical pathways
are sensitive to extremes of temperature and may not be operational under certain growing conditions. Plants will adjust metabolic rates or adjust the pathway in order to optimize whatever biological process they are trying to perform. Many of the phenolic compounds made by plants function in defense against microbial attack, others are produced to address nutrient limitations of the environment. These compounds are important from a winemaking perspective as they will be in the fruit at harvest and therefore in the finished wine.

The next factor to consider is the fruit itself. From the vine's perspective the fruit contains the seed, but provides no nutrition to the seed. The fruit simply serves the purpose of dispersal of the seed. The fruit is designed to be attractive to animal (including human) vectors that will pick the fruit, eat it, but not consume the seed itself. To discourage seed consumption, plants have evolved strategies to make the seeds undesirable. This generally means that the seed contains bitter phenolic compounds that are not pleasant to taste resulting in the vector not damaging the seed. The seed will be rejected by the vector (removed or spit out) or pass unharmed through the gastrointestinal track, depending upon the species of plant and the animal in question. Since animals are motile, the plant has achieved the objective of dispersal of the seed by producing an attractive package for the seed. Seeds are very important in wine production. Their removal reduces phenolic extraction into the wine, resulting in a less bitter end product. Alternately, the seed phenolics may be important contributors to the tannin structure of a wine, depending upon the varietal and the wine style.

Under conditions of severe nutritional stress, the plant may not devote chemical energy to the production of attractive fruit. This is in order to assure that tissues critical for the plant's survival will have a higher priority for nutrients when nutrients are in short supply. It is also likely that the vine under conditions of nutritional excess with the general absence of stress might not produce fruit that is that attractive since the need for seed dispersal is not great. Thus, environmental factors will influence the "investment" the vine makes in the berry and thereby influence the composition of the finished wine. Grape vine physiology is complex and not fully understood due to the difficulty of conducting research on woody perennials. It is believed that moderate stress is required for production of optimal berry composition, as defined by the winemaker. Too little stress leads to inferior fruit, as does too much stress. Achieving the correct amount of stress can be difficult. Different varietals respond to stress differently, and the resulting alteration in chemical composition of the fruit may be varietally or clonally specific. One of the most critical challenges to the winemaker is learning how the physiological responses of the fruit to various environmental factors impact berry composition. It is important to "know the vineyard" in every sense.
Grape vines possess unique characteristics not commonly found among plants in general. Grape vines are successful in a wide variety of soils and climates. Of the cultivated plants, they are commercially grown under the most diverse conditions.

**Characteristics of Grapevines**

- Grown in a wide variety of soils/climates
- Persist in nutrient deficient soils
- Crop set happens in previous season
- Dormant buds developmentally programmed in prior season
- Extensive root structure: can represent up to 90% of the mass of the vine

As perennial plants, factors occurring in a given season can impact vine performance in the following season. In a typical California vintage year, shoots will emerge from the dormant buds on the vines around April 1st, and the vine will flower about six weeks later. About two months after flowering the process of veraison or ripening occurs. Fruit is usually harvested approximately five to six months after bud break. The time of bud break and length of the growing season is strongly influenced by environmental factors. Principle among these is temperature. The dormant buds that will develop into various vine structures, clusters and tendrils for example, are developmentally programmed the season before. This means that environmental stress might not impact fruit in the current vintage but may in the following year.

Varieties differ in the amount of heat needed to achieve maturity of the fruit. The summation of the heat available across a typical season is expressed as degree days. This is calculated by adding the number of degrees by which the average temperature exceeds 50°F for each day. It is difficult to mature grapes with less than 1700 degree days. Fortunately, most growing regions in California range between 2000 and 4000 degree days. California has been divided into five classifications based upon the average degree days of the region. They have been designated Region I (coolest) through Region V (warmest). Some grapes do better in specific regions. Chardonnay and Pinot noir have low heat requirements and are thus better in Region I conditions. Zinfandel and Cabernet Sauvignon do better in Region II and III.

One of the reasons grape vines are successful in a variety of soils and climates is due to their root structure. The root system may be 50 to 90% of the mass of the vine itself. This allows the plant to search far and wide in its immediate vicinity for water and
other nutrients. Grapes can be dry farmed (no irrigation) under conditions that will not support the growth of other crops, depending upon the rootstock. Because rootstocks are genetically diverse and may behave differently, it is important to understand the physiology of the root system used in the vineyard. In particular, it is important to understand where the vine is obtaining nutrients. This will guide vineyard irrigation and fertilization regimens. It is equally important to determine when in the growing season the vines will be active in the translocation of nutrients and time field additions appropriately.
Lesson 2: Grapevine Biology

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  - Nutrient limitation
  - Competition
  - Excess/shortage of water
  - Extremes of temperature
  - Disease/Pest pressure
  - Lack of light
- Role of Fruit:
  - Dispersal
  - Fruit is attractive to mobile agents that will disperse seed (animal; insects; bird)
    Seed itself designed to "taste bad" so it will not be consumed
- Prioritize nutrient use for survival

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are sensitive to extremes of temperature and may not be operational under certain growing conditions. Plants will adjust metabolic rates or adjust the pathway in order to optimize whatever biological process they are trying to perform. Many of the phenolic compounds made by plants function in defense against microbial attack, others are produced to address nutrient limitations of the environment. These compounds are important from a winemaking perspective as they will be in the fruit at harvest and therefore in the finished wine.

The next factor to consider is the fruit itself. From the vine's perspective the fruit contains the seed, but provides no nutrition to the seed. The fruit simply serves the purpose of dispersal of the seed. The fruit is designed to be attractive to animal (including human) vectors that will pick the fruit, eat it, but not consume the seed itself. To discourage seed consumption, plants have evolved strategies to make the seeds undesirable. This generally means that the seed contains bitter phenolic compounds that are not pleasant to taste resulting in the vector not damaging the seed. The seed will be rejected by the vector (removed or spit out) or pass unharmed through the gastrointestinal track, depending upon the species of plant and the animal in question. Since animals are motile, the plant has achieved the objective of dispersal of the seed by producing an attractive package for the seed. Seeds are very important in wine production. Their removal reduces phenolic extraction into the wine, resulting in a less bitter end product. Alternately, the seed phenolics may be important contributors to the tannin structure of a wine, depending upon the varietal and the wine style.

Under conditions of severe nutritional stress, the plant may not devote chemical energy to the production of attractive fruit. This is in order to assure that tissues critical for the plant's survival will have a higher priority for nutrients when nutrients are in short supply. It is also likely that the vine under conditions of nutritional excess with the general absence of stress might not produce fruit that is that attractive since the need for seed dispersal is not great. Thus, environmental factors will influence the "investment" the vine makes in the berry and thereby influence the composition of the finished wine. Grape vine physiology is complex and not fully understood due to the difficulty of conducting research on woody perennials. It is believed that moderate stress is required for production of optimal berry composition, as defined by the winemaker. Too little stress leads to inferior fruit, as does too much stress. Achieving the correct amount of stress can be difficult. Different varietals respond to stress differently, and the resulting alteration in chemical composition of the fruit may be varietally or clonally specific. One of the most critical challenges to the winemaker is learning how the physiological responses of the fruit to various environmental factors impact berry composition. It is important to "know the vineyard" in every sense.
Grape vines possess unique characteristics not commonly found among plants in general. Grape vines are successful in a wide variety of soils and climates. Of the cultivated plants, they are commercially grown under the most diverse conditions.

**Characteristics of Grapevines**

- Grown in a wide variety of soils/climates
- Persist in nutrient deficient soils
- Crop set happens in previous season
- Dormant buds developmentally programmed in prior season
- Extensive root structure: can represent up to 90% of the mass of the vine

As perennial plants, factors occurring in a given season can impact vine performance in the following season. In a typical California vintage year, shoots will emerge from the dormant buds on the vines around April 1st, and the vine will flower about six weeks later. About two months after flowering the process of veraison or ripening occurs. Fruit is usually harvested approximately five to six months after bud break. The time of bud break and length of the growing season is strongly influenced by environmental factors. Principle among these is temperature. The dormant buds that will develop into various vine structures, clusters and tendrils for example, are developmentally programmed the season before. This means that environmental stress might not impact fruit in the current vintage but may in the following year.

Varieties differ in the amount of heat needed to achieve maturity of the fruit. The summation of the heat available across a typical season is expressed as degree days. This is calculated by adding the number of degrees by which the average temperature exceeds 50°F for each day. It is difficult to mature grapes with less than 1700 degree days. Fortunately, most growing regions in California range between 2000 and 4000 degree days. California has been divided into five classifications based upon the average degree days of the region. They have been designated Region I (coolest) through Region V (warmest). Some grapes do better in specific regions. Chardonnay and Pinot noir have low heat requirements and are thus better in Region I conditions. Zinfandel and Cabernet Sauvignon do better in Region II and III.

One of the reasons grape vines are successful in a variety of soils and climates is due to their root structure. The root system may be 50 to 90% of the mass of the vine itself. This allows the plant to search far and wide in its immediate vicinity for water and other
nutrients. Grapes can be dry farmed (no irrigation) under conditions that will not support the growth of other crops, depending upon the rootstock. Because rootstocks are genetically diverse and may behave differently, it is important to understand the physiology of the root system used in the vineyard. In particular, it is important to understand where the vine is obtaining nutrients. This will guide vineyard irrigation and fertilization regimens. It is equally important to determine when in the growing season the vines will be active in the translocation of nutrients and time field additions appropriately.
Lesson 2: Grapevine Performance

There are several factors that influence grape vine performance. Performance, like quality, is a term that has many different definitions depending upon the perspective of the individual and the specific situation. Grapevine performance may be defined in terms of canopy or crop yield or chemically expressed as a function of the composition, usually nitrogen, phosphate and/or potassium, of grapevine tissue. The winemaker, however, will be more concerned with the quality of the fruit than the yield, and desire the highest quality.

Factors Impacting Grapevine Performance

- Soil
- Topography/Location
- Disease/Pest pressure
- Climate
- Microclimate
- The Human Element

Factors Impacting Grapevine Performance: Soil

Soil is one of the most critical factors impacting grapevine performance. Soil has many effects. It not only provides nutrients to the plants, but also dictates the amount of water held in that soil, and the amount of water that is therefore available to the roots. Soil composition will also influence the microorganisms, principally bacteria and fungi, capable of interacting with the root structure of the plant. Some of these organisms are beneficial to the plant, some are neutral and some are harmful. Soil composition affects not only the nature of the organisms present, but their interactions as well. The same is true of insect pests. Soil composition will affect insect availability. Some insects will provide a healthy environment for the vine by limiting other factors that may negatively impact vine performance while other insects may be detrimental both in and of themselves or because they spread diseases. The principle soil feature most impacting vine performance and perceived grape quality is its water holding capacity.

Factors Impacting Grapevine Performance: Topography/Location
The **topography** of the vineyard, (valley floor, hillside), is likewise quite important. Soils tend to be shallower the steeper the slope because of erosion. Soils tend to be deep on the valley floor. The fruit produced under each condition may be very different in composition.

**Factors Impacting Grapevine Performance: Disease and Pest Pressure**

Equally critical is the nature and extent of disease and pest pressure for the vine. Some stress may be desirable and lead to more complex characters in the fruit, but excessive pressure almost always reduces fruit yield and quality and may even be lethal to the vine.

**Factors Impacting Grapevine Performance: Climate**

- Temperature
- Sunshine
- Humidity
- Rainfall
- Evaporation
- Wind
- Water availability

Climate has a tremendous influence on the composition of the fruit at harvest. This is because climate has strong effects on grapevine physiology. Since sugar is produced by photosynthesis, the amount of **sunlight** available to the plant will impact the amount of sugar produced that can be translocated to the berry. As mentioned above, **temperature** impacts biochemical and chemical reactions which will impact the metabolic activities of plant tissues and therefore of berry composition. **Water** is essential for this process and thus water availability will influence berry composition as well. Regional **humidity** is important for several reasons. The higher the humidity the less water is lost from the vine and the soil, but the higher the disease pressure, particularly from the fungi. Fungal infection of clusters can lead to reduced yield and to undesirable alterations in berry chemistry. Fungi consume nutrients needed by the yeast that conduct the alcoholic fermentation, leading to fermentation problems. It is important to note that yeast are fungi, so caution must be exerted when using
fungicides in the vineyard. Excessive use or high residual levels on the fruit at harvest will negatively impact yeast performance and alter the composition of the wine. Recent studies have suggested that encouragement of the yeast flora of the berry will discourage mold growth, which has potential effects on the early flora of the wine fermentation.

**Rainfall** impacts both humidity and soil water content so can have a positive or negative impact, depending upon time of occurrence. Rainfall on mature clusters is generally undesired as this encourages mold proliferation. **Wind** also has multiple effects on the grape vine. Severe wind conditions lead to smaller berries with thicker skins, which may be desirable in some varietals. It also tends to dry clusters, which would limit mold growth, but may also lead to greater losses of vine water through enhanced **evaporation**.

**Factors Impacting Grapevine Performance: Microclimate**

- Climate of individual vines: heating of vineyard floor
- Climate of individual clusters:
  - Shading effects
  - Humidity retention

The local climate of a given vineyard or of sections of the vineyard is also important. Factors such as the composition of the vineyard floor will impact heat retention and may serve to keep the local temperature of the vines warmer than would otherwise occur. Cluster climate is significant as well. Exposed clusters will have a lower humidity but may not be as protected from sunburn as clusters that are not exposed. Many of these effects are varietal specific, meaning that what is beneficial for one varietal might have no or a different effect in another.

One of the, if not the, most important factors impacting grapevine performance and therefore berry composition is the human element.

**Factors Impacting Grapevine Performance: The Human Element**
Decisions made in the vineyard, such as timing and extent of irrigation and fertilization will influence the composition of the fruit at harvest. This can have a direct effect on wine characters, or an indirect effect mediated by microorganisms that are influenced by berry composition and field practices. For example, foliar applications will have a direct influence on berry microflora. Nutrient addition to any ecosystem tends to initially favor the more versatile and rapidly adapting organisms such as the bacteria. The nature of the trellising system and canopy management strategies will impact not only yield per vine but cluster exposure. All of these factors will impact the chemical composition of the fruit, and need to be optimized for each varietal under each growing condition.

**Vine management** practices also impact fruit composition and therefore wine flavor and aroma. The size and shape of a vine can be manipulated by managing the way a vine grows by training, trellising and pruning. Trellising and training impact light exposure which affects sugar production in the leaves via photosynthesis. The more photosynthesis that occurs, the more sugar available for accumulation in the fruit. **Pruning** can also be used to control the size of the vine. It determines the number of dormant buds per vine and the total number of clusters and therefore fruit yield per vine. If a vine has too few buds it can be excessively vigorous. Excessively vigorous vines are believed to produce fruit of reduced quality. Alternately if there are too many buds there will be too much fruit and the vine might not produce enough sugar to fully ripen all of the fruit. This is called "overcropping". One of the most important factors in winegrape production is to balance the amount of foliage with the amount of fruit so that all fruit will ripen. Canopy management or trellising is important in determining the exposure of the foliage to sunlight.
Lesson 2: Berry Structure and Composition

The grape berry is a complex organization of functionally different and therefore compositionally distinct cell types and tissues. The diagram below was taken from an article published in the *American Journal of Enology and Viticulture* by B. G. Coombe (1987; 38(2):120-127).

The berry is composed of a tri-layered skin, a fleshy mesocarp, a septum and an extensive vascular system allowing deposition of materials produced elsewhere in the plant. It serves to house and protect the seed, initially to nurture seed development, then berry maturation.

**Berry Structure**
The skin of the berry is important because it protects both the berry and the developing seed from pests and diseases as well as from undesirable compositional changes. It is important from a winemaking perspective because as we will see it contains chemicals important for the composition of the wine. The color of the fruit can serve to attract vectors for the dispersal of the seed. In this case, color changes follow maturation of the seed. In the white or colorless varieties, the aroma of the fruit rather than visual changes will serve as principle attractant of animal vectors. In this case the development of these characters also follows seed maturation.

**Berry Development: Maturation of fruit follows maturation of seed**

1. **Flowering/Fertilization**
2. **Green Berry Stage**
   - Cell division occurs
   - Acids accumulate
3. **Veraison**
   - Color changes occur
4. **Ripening**
   - Berry swells and softens
   - Sugar (increase), Water (increase), Acids (decrease)

Following flowering and fertilization, seed development and maturation occurs. During this period the berries are green, hard, bitter and astringent. This is to guarantee an
unattractive taste and prevent premature consumption of the fruit by animal vectors. Once the seed has reached a certain level of maturity, a process known as veraison occurs, which is marked most distinctly by color changes. This is the actual ripening process of the fruit. The berry will swell and soften. Sugars will increase. Water content also increases, so the berries become much larger, and the acidity decreases. This is partly due to dilution but also due to catabolism - malate is respired for energy in the berry. The phenolic content softens, making the fruit more palatable. The warmer the climate the faster berries tend to mature, the lower the acidity, the lower the color, the higher the pH. Thus climate has a strong impact on berry development. All things being equal different varietals mature at different rates. Varietals can be described as early or late ripening, depending upon the region.

**Factors Affecting Berry Development and Maturation**

- **Climate:** Warmer: mature faster, less acidity, less color, higher pH, fewer late berry characters
- **Variety:** Mature at different rates
- **Disease/Pest Pressure:** alters composition of fruit, alters timing of development
- **Balance of Vine:** carbohydrate demands of vine versus fruit versus level of photosynthesis

Seasonal and regional differences in disease and pest pressure can impact the timing and duration of berry maturation as well as impact the final composition of the fruit. Fruit that is being produced under conditions of biotic stress will tend to be higher in phenolic content. The carbohydrate demands of other tissues will also impact berry maturation and composition. Carbohydrates produced as a consequence of photosynthesis occurring in the leaves serves as carbon and energy source for non-photosynthetic tissues such as the root. Demands for root growth and metabolism will take precedence over accumulation of sugar in the berry as an attractant.
Lesson 2: Grape Berry Composition

The typical composition of the berry at harvest is shown below, and the most common ingredient, just as with most biological tissues, is water. About eighty percent of berry weight is water.

Berry Composition at Harvest

<table>
<thead>
<tr>
<th>Component</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar:</td>
<td>200</td>
</tr>
<tr>
<td>Organic Acids:</td>
<td>10</td>
</tr>
<tr>
<td>Amino Acids:</td>
<td>5</td>
</tr>
<tr>
<td>Phenolics:</td>
<td>2-5</td>
</tr>
<tr>
<td>Volatiles:</td>
<td>trace</td>
</tr>
<tr>
<td>Water:</td>
<td>800</td>
</tr>
</tbody>
</table>

Sucrose is the circulating product of photosynthesis cleaved to produce glucose and fructose in berry fermentation.

The next most prevalent compound is sugar, which may range from 17 to 26% (w/v) at harvest depending upon the style of wine being produced. Organic acids are found at concentrations of roughly 10 g/L, and will vary with time of harvest. The reasons for this will be discussed below. Amino acids and other nitrogen sources are present at a total concentration of about 5 g/L or less. Phenolic compounds, which are important bitterness and astringency characters and impact the tannin structure of the wine, are present in low amounts. The volatile compounds, which are the most important characters as far as we are concerned, are present in trace amounts.

Sugars (hexoses)

- Glucose
- Fructose
- Sucrose
The principle sugars in the berry are **glucose** and **fructose**. These are hexoses are six carbon sugars. As you know, plants make sucrose following photosynthesis as the circulating sugar. **Sucrose** is a disaccharide of glucose and fructose. When sucrose arrives at the berry and it is cleaved by invertase to glucose and fructose. This strategy allows the berry to accumulate large concentrations of sugar by diffusion. Because actual levels of sucrose remain low, sucrose can continue to enter the fruit without requiring an expenditure of energy.

**Sugars (pentoses)**

- Arabinose
- Xylose

Not metabolized by yeast.

The principle pentoses, or five carbon sugars, are **arabinose** and **xylose**. The pentoses are not used by *Saccharomyces* in the production of alcohol, but can serve as energy sources for other organisms, particularly in the presence of molecular oxygen.

The two principle organic acids are malate and tartrate.

**Organic Acids**

- Malate
- Tartrate

**Malate** as you recall from biochemistry, comes from the TCA cycle. It is a tricarboxylic acid cycle intermediate.
Malate can be consumed via the TCA cycle and electron transport chain to produce energy. It is the principle energy source in the berry rather than sugar. Why? The aim of fruit maturation is to accumulate sugar to serve as an attractant for animal vectors. Consumption of that sugar by the berry tissue itself would defeat the purpose of accumulation. Therefore the berry is metabolically geared to ignore the abundance of sugar and to catabolize instead the available malate. How much malate has been consumed will impact the level of acidity in the fruit at the time of harvest. The longer the fruit is on the vine post-veraison, the lower the content of malate.

The other principle berry acid is tartaric acid. Tartrate, like malate, is a four-carbon dicarboxylic acid.

Tartrate looks similar to malate in structure, but it is really an odd compound. In contrast to malate, tartrate is not metabolized as an energy source and is not very prevalent in the plant, animal or microbial kingdoms. It is only found in grapes and a couple of other plants. Tartrate, in contrast to malate, accumulates in a plant organelle called the vacuole. The vacuole in plants, as well as in yeast, is the site of hydrolysis of a lot of components no longer of use to the cell but that can be recycled. Proteins can be broken down into individual amino acids in the vacuole and stored there for later use in de novo protein synthesis. Components can also be stored in the vacuole for subsequent use in the cytoplasm. Phosphate and minerals will be deposited in the
vacuole then translocated to the cytoplasm when needed. In this case the vacuole is the functional equivalent of a closet. While the exact metabolic role of tartrate is still being debated, one theory postulates that tartaric acid plays a role in cation sequestration and storage in the vacuole. It serves to neutralize positive charges allowing accumulation and retention within the cell of valuable ionic resources. Tartrate is made from ascorbic acid and 5-ketoglutarate.

The amino acid content of the berry is important as these compounds serve as nutrients for the yeast and bacteria that conduct the alcoholic and malolactic fermentations, respectively. These compounds are also important because end products of amino acid catabolism, the fusel oils and esters, impact the aroma of the wine thereby influencing wine composition and quality. Deficiencies of these nutrients can lead to off-character production by the microbes as well as prevent fermentation to dryness. Typically the five most prevalent amino acids are: glutamate, glutamine, alanine, arginine and proline. Together these amino acids generally comprise 90% of the amino acid pool. Ammonia is also important, and levels of this compound will vary dramatically in the fruit at harvest.

**Amino Acids**

- Glutamate
- Glutamine
- Arginine
- Alanine
- Proline

*Typically comprise 90% of All amino acids*

Other amino acids are present in lower concentrations. The relative ratios of available nitrogen compounds impacts yeast metabolic activities and the spectrum of end products appearing in the wine. Another principle nitrogen compound that can be found in grapes is gamma amino butyric acid or GABA. GABA appears to be synthesized in the fruit post harvest and concentrations of this compound reflect the time and temperature exposure of the harvested berries. The longer the time and the higher the temperature post-harvest, the higher the GABA concentration. GABA levels can be quite high in some juices and musts. It is readily utilized as a nitrogen source by yeast and bacteria.
Amino Acids

High Gamma-Amino Butyric Acid (GABA) indicates fruit was held at a high temperature post-harvest

Phenolics

A large variety of phenolic compounds are found in the wine. They are generally present as tartaric acid esters.

The structure shown here is caftaric acid, the most prevalent phenolic. The diversity of phenolic compounds and their roles in wine composition and quality will be covered in subsequent lectures.

The volatile compounds fall into two principle categories, the terpenes and the esters. We'll be talking about these two categories extensively throughout the course.

Volatile Compounds:

- terpenes
- esters

The sulfur-containing compounds of the berry are principally glutathione, a tripeptide containing cysteine. Cysteine and methionine can also be found in the fruit at harvest. Glutathione forms the "grape reaction product" with phenolic compounds, and is therefore not available to the yeast to use as a source of sulfur. These compounds are important because their presence can lead to the formation of sulfur volatiles by yeast, which are almost always objectionable.
Lesson 2: Location of Berry Compounds

As described above, the berry is a complex structure comprised of different tissue types. These tissues have differing chemical compositions. A seminal article on this topic was written by B. G. Coombe and published in 1987 in the *American Journal of Enology and Viticulture*. With their permission, we reproduce some of the key data from that article. The goal of the study was to investigate the composition of different regions of the berry. As shown in this figure, cross-sections of the berry were prepared.

Each fraction of the berry was analyzed for chemical composition. Samples were taken throughout berry development post-veraison.

Let us first consider sugar content.
Early in the season when the specific gravity or Brix value of the grapes is low, sugar content of the fruit is likewise low. As sugar accumulates in the berry the specific gravity of the juice of the fruit increases. As seen in this diagram, sucrose concentration is highest nearest the vascular system of the plant, that is, at the point of entry into the fruit. Glucose (and fructose) levels are high throughout the mesocarp, but are low in the skin. Since sugars provide an excellent source of carbon and energy, their absence in the skin protects this tissue from microbial attack.
Malate is similarly located in the mesocarp, but follows the opposite trend to sugars during berry maturation. As Brix levels increase, malate levels decrease. This is because the malate is being consumed as an energy source. In this example, at 26°B, most of the malate has been consumed. This means that the berry now lacks an energy source and will start to deteriorate. Tartrate tends to be more uniformly distributed across the berry, but levels are slightly higher in the skins. There is a slight decrease over time with berry maturation, most likely due to dilution as water and other components accumulate in the fruit.
In contrast to the sugars and malate, the concentration of phenolic compounds is highest in the skin tissues of the plant. They are also high in the seeds, but the seed data is not shown. If phenolic extraction is desired in the wine, then the skins will need to be exposed to the juice or wine. It is not surprising that the phenolic compounds which are produced principally to counter microbial attack and to maintain berry integrity under conditions of abiotic stress are located in highest concentration in the tissue that serves as the first line of defense, the skin.
Potassium levels are also highest in the skin of the berry. Potassium is required by the yeast for optimal fermentation performance in the presence of high ethanol concentrations. It is important that enough be present to support fermentation to dryness.
Finally, anion content is highest adjacent to the vascular bundles of the berry and in the skins. Anions are translocated to the berry from the rest of the plant so it is not surprising that their content would be highest in and adjacent to the vascular tissue.

This concludes the lecture on berry composition and the influence of environment on the physiological activities of the vine and ultimately, of the composition of the berry. In the next lecture we will consider berry composition and development from the winemaker's perspective and discuss the issues surrounding the harvesting decision.
Lesson 3: Introduction

The Harvesting Decision: Viticulture from the Winemaker's Perspective

One of the most important decisions made by the winemaker is the time of harvesting of the fruit. The chemical composition of the berry at harvest will largely dictate the chemical composition of the finished wine. In addition to varietal qualities, grape composition not only controls the ultimate acid/ethanol balance, but it also determines what characters will be imparted by microbial activity. In this lecture we will consider the factors that are taken into account when scheduling the harvest of a particular vineyard. The ultimate goal is to obtain the ideal berry composition at the time of harvest. Whether that goal can be achieved or not is dependent upon numerous factors.

The Winemakers Perspective

- Grapes must be harvested at the ideal time for the style of wine
- Characters of the finished wine will be largely dictated by the composition of the fruit at harvest
- Goal: to have the ideal composition at the time of harvest
Lesson 3: Changes in Berry Composition During Ripening

Two components typically monitored during berry ripening are sugar and acidity.

Sugar accumulation in the berry commences at veraison and continues throughout the ripening process. At some point, the vine ceases to send further sugar to the berry. Following this point the sugar level may still increase in the fruit but this is due to dehydration or water loss. It is important to determine the time at which net synthesis terminates to avoid excessive raisining of the fruit, unless this is wanted stylistically. If higher sugar concentrations are desired, other techniques such as concentrate addition or blending with a high Brix juice should be considered.
Berry acid levels also change during the ripening process. As mentioned in the previous lecture, malate accumulates in the berry during the green berry stage prior to veraison. At veraison, malate levels will decrease due to catabolism of this acid as an energy source by the cells of the berry. This is an exaggerated graph, with the malate levels dropping sharply. Tartrate levels also rise during early berry development, but then plateau. There may be a slight increase in tartrate as the berry dehydrates, if the fruit is left on the vine that long.

Another compound that is sometimes monitored during ripening is arginine.

Arginine levels rise during the early phase of berry development and remain fairly constant through late developmental stages. If the fruit is held on the vine for a long time, arginine levels will drop. Some winemakers believe that this drop signifies a
deterioration of the fruit, and will harvest immediately.

Probably the most important character to monitor is berry flavor and aroma. Below is a typical profile of the change in flavor characters during ripening that has been described for red fruit. Immediately post veraison the fruit tastes very vegetative, that is, it is reminiscent of vegetation.

Berry Ripening:
Evolution of berry flavors in red grapes
Vegetation ➔ Herbaceous ➔ Unripe Fruit ➔
Red Fruit ➔ Black Fruit ➔ Jam

It then becomes more herbaceous, more like straw and dried vegetation. The fruit then advances to unripe fruit, signifying the beginning of production of fruit characters, but the fruit is still quite bitter and bland. I am most reminded of unripe apples simple because that is the unripe fruit with which I have the most experience. The berry then begins production of the positive fruit components. Initially the red fruit characters emerge, things like cherry, raspberry, strawberry and apple. As the fruit continues to age, more of the black fruit characters, black cherry and plum, are noted. If the fruit remains on the vine, the fruit characters take on the jamminess of processed fruit and some dried fruit characters, such as prune and date, may also become apparent. Spice characters may also appear at this time, enhancing the perception of the wine as processed jelly or jam. The emergence of the ripe characters and the disappearance of unripe components can vary in timing. Some seasons there may be no herbaceousness at the same time as the black fruit traits emerge and in other seasons both may be present at the same time. Some years the fruit may start to deteriorate before the intense jam character appears. None of the flavor and aroma compounds are directly correlated with sugar and acidity either. In some vintages the red fruit characters may appear at a lower sugar value than in other vintages. In order to detect the character of the phenolic compounds, I will frequently remove a bit of grape skin and taste that independently of the sweeter fleshy part of the berry. For me, it is easier to detect the "ripeness" of the phenolic compounds without the interference from the sugar. It may also be important to focus on the absence of undesired or unripe characters rather than just on the appearance of positive characters.

In addition to berry composition as an index of ripeness, the stems must also be considered, even in cases where the grapes are destemmed prior to crushing and
Stems:

Unripe: Green = vegetal, leafy
Ripe: Brown = resinous wood, spices: clove, pepper, cinnamon
Over-ripe: Brittle Brown = dried leaf, tea, herbal

Unripe stems, which are called green stems in the French literature, are very vegetal and leafy tasting. Green in this case refers to much more than the color of the stems themselves. If you are using a crusher that breaks up the stems or the stems are present during fermentation (whole berry fermentation), some of these characters can be noticed. Stem maturation is dependent upon the varietal. In some cases they never mature past the vegetation phase. Thus the practice of whole berry fermentation is not advised for all varietals. Stems can be tasted to determine the impact that they may have on wine composition. Ripe stems are brownish in color. They have resinous characters, clove, pepper, cinnamon, and other components of the spice family. Over-ripe stems are very brittle and brown looking, and they have a lot of the tea and herbal notes, reminiscent of dried leaf characters. These characters are not necessarily bad, depending upon the wine that you are producing.
Lesson 3: The Harvesting Decision

Many factors influence the decision to harvest the fruit. One of the principle factors is sugar content, as this will define the amount of alcohol present in the wine at the end of fermentation.

Decision to Harvest

- Sugar
- pH
- Acids
- Balance of sugar and acidity
- Arginine levels
- Ratio of malate to tartrate
- Taste
- Phenolics/Anthocyanin
- Terpene content
- Environmental factors
- Tank capacity/limitation
- Labor availability
- Cost/Economics
- Availability of fruit
- Style of wine

Sugar

Sugar ranges from 19-26 Brix

- Depends upon style of wine
- Maturity of flavors
- 1.7% sugar = 1% ethanol

Sugar content may be monitored in several ways, either directly through the
assess the specific gravity of the solution. At high sugar levels the specific gravity is largely a function of the sugar content. The Brix scale is set such that 20 g of sucrose in 100 g of total solution at 20°C is 20°Brix. Since specific gravity is a function of temperature, tables have been constructed that give the temperature corrections for the Brix readings. As ethanol is produced, the specific gravity drops below that of water and negative Brix readings will be obtained late in fermentation. Thus Brix is a fairly accurate assessment of sugar in juices and musts, but not so late in fermentation.

Lower Brix levels coincide with reduced varietal character. Fruit would be harvested early when it was desirable to minimize varietal character such as during the production of some sparkling wines. Higher Brix values generally coincide with intense varietal characteristics, and if too high may yield excessive ethanol concentrations that will detract from wine quality.

**pH**

- **pH: 3.0-3.8**
  - Affects solubility of tartrates and proteins
  - Affects microbial populations

The pH of the juice at harvest is also an important variable. Wine pH will impact both tartrate and protein stability and affects the rates of key phenolic reactions. Equally important, juice and wine pH will impact the nature of microorganisms that can persist in the fermentation and subsequently in the wine. Juice pH typically ranges from 3.0 to 3.8 under ideal conditions, but may be much lower (with early harvest in a cool growing region) or higher (with late harvest in a warm growing region). Many bacteria are unable to survive pH values below 3.5. In contrast *Saccharomyces* and the other yeast grape flora are quite acid tolerant, and are not inhibited until the pH drops below 2.5. If the must is at pH 3.5 or above, many, many more microbial species will be present in
the juice, and therefore have the opportunity to impact the chemical composition of the finished wine. This may be either stylistically desirable or undesirable depending upon the situation and the microbes present.

**Acids**

- **Contribute Soursness and Tartness**
- **Titratable Acidity:**
  - Whites (0.7-0.9 g/L)
  - Reds (0.6-0.8 g/L)

The acidity level of the grapes at harvest is very important to the structure of the wine as well as to the composition of the finished wine. Acids contribute sourness, while both acidity and pH influence tartness. There are many ways to evaluate wine acidity, but the most common is to use a simple acid/base titration to estimate the amount of released protons. The amount of base used in titration to a specific end point is then expressed as the equivalent amount of acid. In the United States for example, titratable acidity or TA is expressed as grams tartaric acid equivalents per liter. This is not a direct measurement of the anionic species of tartrate, so it is important to not be confused by this convention. In France the titratable acidity is expressed as grams sulfuric acid. The titratable acidity at the time of harvest is a complex function of the pH, the concentration of anionic species of malate and tartrate, and of the potassium level. TA levels for white wines range from 0.7 to 0.9 grams tartaric acid equivalents/L, and are slightly lower in red wines, 0.6 to 0.8 g/L.

**Balance of Sugar and Acidity**

- **Brix/TA = 30 or less**
  - 22 Brix/0.8 TA = 27.5
- **(Brix)(pH)² = 220-260**
  - (22 Brix)(3.2)² = 225.3

The balance between sugar and acidity in the grape at harvest will reflect the balance between ethanol and acidity post fermentation. There are two conventions for determining the balance of these components. The first is to divide the Brix value by
that for the TA, with a goal of obtaining a number less than 30. This indicates that these two parameters are well matched. The second convention is to multiply the Brix value by the square of the pH, aiming for a number between 220 and 260. These conventions are dynamic and change with the styled wine being produced. Late harvest fruit also does not meet these criteria.

**Arginine**

We have noted above that arginine levels are sometimes monitored as an index of berry deterioration. This practice is not that common across the industry because of the difficulty of the arginine assay, and the variation in arginine levels. That is an important point to bring up - factors that we can readily measure frequently take on a higher value than those that we cannot, but this might not reflect their relative importance as indicators of berry ripeness and quality.

**Ratio of Malate to Tartrate**

In some cases it is desirable to have some malate in the juice at the time of harvest. This is generally to make sure that the malolactic conversion will occur. As mentioned earlier, this conversion is an important deacidification of the wine, so why would one want high acidity if it will simply be removed later in the wine making process? The organisms that conduct the malate to lactate conversion, the malolactic (ML) bacteria, produce other end products desirable in some wine styles. These organisms are responsible for some cream and buttery characteristics, and can produce more complex aromas as well. If these characters are desired, then the malate must be present to stimulate their production. Malate can be legally added to wine to stimulate the ML bacteria, but many winemakers feel that the wine is simply a better product if the malate is present at the onset of fermentation.

**Taste**

One of the most important characteristics of the fruit at the point of harvest is the taste - the flavor and aroma characteristics of the berry are largely responsible for the characteristics of the wine, especially in varietal wine production. The rest of the chemistry might be technically perfect, but if the appropriate flavors are not yet present in the fruit, the wine will not achieve the stylistic goals of the winemaker.

**Phenolics/Anthocyanin**
The **phenolic** composition of the fruit at harvest is important for a variety of reasons. The phenolic compounds are responsible for bitterness and astringency and for the ultimate tannin structure of the wine. In the case of red varieties, anthocyanin content is crucial, since these compounds are responsible for red wine color. The **anthocyanin** pigments are located exclusively in the skins for the vast majority of the varieties commonly used in winemaking. Color changes and is lost upon wine aging so the initial level of extraction, which is dictated by the initial composition of the fruit, is of great consequence.

**Terpene Content**

One of the important classes of volatile aroma compounds is the **terpenes**. Terpenes are responsible for the characteristic Muscat flavors, such as the intense fruity and floral notes. Terpene structure will be described in a later lecture, but for now it is important to note that the terpene content is set at the time of harvest. That is, terpenes are not synthesized in the fruit post harvest. Terpenes exist in one of two forms, bound and unbound. Unbound terpenes are volatile and this is the form that can be detected by our olfactory sensors. Bound terpenes are the same compound with an attached sugar molecule. They are referred to as glycosylated terpenes as a group. Bound terpenes hydrolyze as the wine ages, thus releasing the detectable volatile form. Thus, what is most critical for the harvesting decision is the total amount of terpenes present in the wine and the ratio of bound to unbound. This can be determined by assay, and used to identify the optimal time of harvest.

Other compounds important in wine aroma and flavor are also present as non-volatile glucosyl-glucose (GG) precursors. Enzymatic treatments can be used to cleave the GG moieties from all flavorant precursors. The amount of GG released can be assayed and used as an indicator of the total precursor population. GG values can then be used to define the optimal maturity of the fruit and define the harvest date.

**Environmental Factors**

- Rain
- Humidity
- Temperature
- Disease Pressure
All of the other factors discussed to this point relate to the chemical composition of the fruit and the determination of the optimal composition. In an ideal world, this is all that would matter. However, since this is not an ideal world, other factors must also be taken into account when deciding that it is time to harvest. Principle among these are the environmental factors. While the ideal time of harvest may be based upon the chemical composition of the fruit, Mother Nature may change your plans.

**Rain** is problematic because it generally results in higher humidity, which fosters the growth of molds on the fruit. In contrast to yeasts and bacteria, molds produce mycelia and can invade grape berry tissue. This not only consumes grape nutrients, it can lead to loss of grape varietal characters and can result in a general microbial bloom. This leads to high microbial loads in the initial juice and increases the risk of off-character production. It has been our experience that mold infestation post-harvest is not nearly as damaging to wine quality as infestation of clusters on the vine. Thus, if the weather conditions are such that mold infestation of the fruit will occur, it is better to harvest the berries early than to wait for a balanced chemistry that will never appear in infected fruit.

**Temperature** is also very important. For example, if temperatures drop to near freezing this can damage the berries unless you are interested in making ice wine. In this case you may need to harvest and process the fruit before the change in the weather can cause damage.

**Disease or pest pressure** can also influence the harvesting decision depending upon the region in which the fruit is produced and what type of crops might be grown in the vicinity of the fruit. In this case, the grower may need to harvest the crop before an insect pest displaced by harvest of another commodity in the region invades the vineyard.

In addition to compositional and environmental factors, issues relating to internal operations within the winery may also impact the harvesting decision.

**Tank Capacity/Limitation**

**Tank capacity and tank limitation** are important factors to consider when deciding upon the harvesting date. That is, the fruit may be ready to harvest but the winery is swamped and is unable to process the fruit. For example, if tank capacity is limiting, it will dictate what can be handled by the winery. Tank limitation is difficult to predict from one vintage to the next as different varietals ripen at different times. One could assume
worst-case scenario, but there is a balance between overbuilding your winery and having an excessive amount of unused tank capacity and being short maybe one or two seasons out of every ten. In my experience, overbuilt wineries have a tendency to increase production and thus do not maintain an excess fermentative capacity.

**Labor Availability**

Another important issue is labor availability. Grape harvesting is obviously a seasonal job and in many regions seasonal workers are in short supply. Mechanical harvesting can accommodate this, which will be discussed below, but if the wine style is dependent upon hand-harvested fruit, the availability of labor will definitely impact the timing of harvest.

**Cost/Economics**

The decision to harvest may be influenced as well by economic factors. For example, harvesting costs may vary seasonally. In some regions of the United States, High School and College students provide the seasonal labor for harvesting the grapes. If the grapes are not harvested before classes resume, other laborers may need to be hired at a higher pay rate. Also, if purchasing fruit on the open market, it might not be economically feasible for a grower to continue to farm the fruit, that is, the grower's return from the sale of the fruit will not cover additional expenditures in the vineyard. In this case, the grapes will be harvested and sold to whoever is willing to receive them.

**Availability of Fruit**

Thus fruit availability may be an important factor in the harvest decision if delay in acceptance of fruit means that the winery will not obtain the needed amount. Many wine producers address this issue by having long-term contracts with growers, but this is problematic if the fruit from the vineyard does not meet the winery's chemical specifications.

**Style of Wine**

Stylistic considerations will also influence the decision to harvest. Different wine styles may require that the chemistry of the berries at harvest be different. For example, in the case of Cabernet Sauvignon, one winery might want some of the classic bell pepper and herbaceous notes as this is part of their style while another might desire a
more intense jamminess and no bell pepper. The ideal time of harvest for each winery of the same vineyard would differ because their definitions of quality and style differ.

The last topic to consider under factors influencing the harvesting decision is the **human element**. In our case we harvest the fruit from the Davis vineyards upon the return of the students in the last week in September. It does not matter what the chemistry of the fruit is, we must wait until the students are present. While uncommon in new world growing regions, a similar situation does exist in other winemaking regions of the world. In some cases harvest commences on a specific date, perhaps associated with a particular Saint's feast day or the lunar cycle. In these cases the chemistry of the fruit is secondary to the cultural habits of the region.
Lesson 3: Vineyard Sampling

Many of the above characteristics used in determining the optimal time of harvesting are dependent upon berry chemistry. It is therefore important to assess the composition of a representative cross section of the fruit in the vineyard. This is not as easy as it seems.

Sampling of Vineyard

- Unbiased representation of entire crop
- Statistically significant evaluation

It is important to develop vineyard sampling protocols that result in an unbiased sampling of the vineyard and that lead to statistically significant values for the composition of the fruit.

Types of Sampling

- Berry: 100-200 berries randomly picked
- Cluster: 20-50 clusters also randomly chosen
- Vine: select typical vine and sample all clusters

Individual berries, clusters or vines can be sampled. The most statistically robust method is berry sampling. It is important to take a representative sample of the entire vineyard. This first necessitates walking through the vineyard and determining the relative homogeneity of the soil and topography of the area that is planted. If soil and/or topography differ, then it will be necessary to establish the relative locations of differing growth conditions and the relative percentage of the vines in those conditions. In the final sampling strategy, fruit should be represented in the final mixture at the same percentage that the particular topographical condition under which it was grown is present in the vineyard.

In berry sampling, a typical sample size is 200 berries. If the vineyard is large and 200 berries does not cover 10% of the planted area, multiple samples totaling 200 berries can be taken, processed and analyzed separately and the values obtained averaged.
The berries must be chosen randomly - not always from the same location in the cluster and not always from exposed clusters. Berries should also be harvested without damaging them as this may change the chemical composition before analysis. Computer programs exist that will generate a random sampling protocol from entered vineyard data. These can be quite useful if it becomes apparent that sampling bias is an issue. This becomes quite obvious when the composition of the fruit post harvest does not match that predicted from the sampling.

**Cluster** sampling has the advantages or avoiding berry damage and of bias in the selection of berries from the cluster. However, to be statistically robust, more fruit is harvested in the sampling process. Whole **vine** sampling is only advised if the soil and topography of the vineyard are fairly uniform. If this is the case, then sampling of an entire vine that has been randomly chosen eliminates both berry and cluster bias. The ideal situation seems to be to have multiple individuals perform berry sampling and to average the values obtained from separate analysis of the sampling lots. Of course, this is only feasible in larger vineyard and winery operations.

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**Berry sampling is most accurate but harvester must be careful not to damage fruit prior to processing analysis**

The most statistically robust method of vineyard sampling is a combination of berry and cluster sampling. Clusters are sampled from the vineyard and brought intact to the winery. All berries are carefully removed from each cluster and the berries are mixed together in a tray. Numerical strategies are then used to obtain a 200 berry sample from all of the berries in the tray. Multiple 200 berry samples can be taken from the same set of clusters.

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Lesson 3: Harvesting Options

Once the decision has been made that it is time to harvest, the winemaker/vineyard manager must then decide how the fruit is to be harvested.

Several options are available for harvesting that will impact the character of the harvested fruit. Thus the winemaker must also be involved in the decision of how harvesting will proceed.

**Harvesting Options**

- **Temperature**
- **Machine**
- **Hand**

The three main factors that have to be considered when harvesting are **temperature**, and whether the fruit will be harvested by **machine** or by **hand**. These factors will impact the extraction of compounds from the berry and the amount of damage and pre-fermentation microbial activity that can occur. The higher the temperatures at harvest, the higher the temperature of the fruit at the time of crushing. Higher temperatures lead to greater extraction of skin components. Thus, as described in a subsequent lecture, if skin extraction is desired such as in red wine production warmer temperatures may be preferred. In contrast, if extraction is not desired such as in white wine production because of excessive bitterness or astringency, then the winemaker might want to harvest at cooler temperatures to minimize extraction even with damage of the fruit. In some regions fruit can be harvested at night, when the temperature is at its lowest. Mechanical harvesters must be matched to the trellising system. Some are more selective, only heavy, riper clusters are dislodged while others are not.

**Temperature of Harvest**
- Lower temperature
  - Less flavor loss
  - Less extraction from skins
  - Less microbial activity
- Elevated temperature
  - More extraction
  - Initiate fermentation earlier

It is also important to remember that higher temperatures stimulate both the growth and metabolic activities of microorganisms. Harvesting conditions that damage fruit significantly and will result in stimulation of microbial activity. This will be reduced somewhat at lower ambient temperatures, but if the damage to the fruit is extensive, it may be desirable to inoculate the fruit at the point of harvest rather than waiting until it arrives at the winery.

The next decision that must be made is how the fruit will be harvested, that is, will it be harvested mechanically or by hand.

**Machine Harvesting**

- Faster
- Cheaper
- Can be done day or night
- Less gentle
- Mixture of "good" and "bad" clusters
- More "MOG" (material other than grapes)
- Berries can be crushed
  - Juice loss
  - Oxidation
  - Microbial Growth

Machine harvesting has the advantages of being much faster than hand harvesting and much more efficiently done at night. It is cheaper than hand harvesting, as labor costs are minimal. However, it is not selective, bad or unripe clusters may be harvested along with the ripe clusters, and there will be material other than grapes.
(affectionately known as MOG) present in with the fruit. Mechanical harvesters do not treat the fruit gently so there will be considerable berry damage. Depending upon conditions, this may lead to juice oxidation and loss of yield in addition to the encouragement of microbial growth.

**Hand Harvesting**

- Slower
- More labor intensive
- More expensive
- More selective of clusters
- Less MOG
- Lower yield

In contrast to mechanical harvesting, hand harvesting is more expensive, more labor intensive, and slower. However there is much less berry damage and better control over MOG. Hand harvesting allows selection for good clusters eliminating those that display mold infestation or are unripe (second crop). The yield will be lower simply because it is a more selective process. Which method, hand or machine harvesting, is most appropriate depends again upon many issues. Principle among these are desired yield, cost and labor availability. In some areas, hand harvesting is not an option because of the lack of availability of labor. In other areas the terrain is such that machine harvesting is not an option. If mechanical harvesting is to be used, then it is important that the vineyard sampling protocol reflect this. Meaning, that if ripe and unripe clusters will both be picked, the sampler must determine the ratio of ripe to unripe and maintain the same percentage of first and second crop in the sample.
Section 2 - Grape and Must Processing
Lesson 4: Introduction

Grape Processing

The next series of lectures will cover wine production from the point of delivery of grapes to the winery to the initiation of fermentation.

In this first lecture of section 2 we will cover the topics of crushing of the fruit and pressing of the must, and will discuss the issues to consider when purchasing a crusher. Unfortunately we will not be able to devote the same amount of time to all major equipment items needed for wine production, but this discussion will serve as an example of the myriad of factors that need to be considered before a capital investment is made.

Before we begin, I would like to make the distinction between two terms that we have been using, must and juice. Must refers to the juice plus the skins and seeds, while juice simply means the non-solid material resulting from the crushing of the fruit. Must will always be used in the context of meaning juice plus skins and seeds.
Lesson 4: The Crushing Operation

The first step in grape processing is to decide how the fruit will be processed upon arrival at the winery.

To Crush or Not to Crush?
A Matter of Style

In most cases the initial processing step is to crush the fruit. However, there are times when crushing is not desirable for stylistic reasons as discussed below.

Crushing/Destemming

- Purpose
- Stylistic Options
- Equipment Choices
Lesson 4: The Crushing Operation - Purpose

Crushing of the fruit allows extraction of the juice as well as extraction of the components of the skins of the berry.

Purpose of Crushing

- Better extraction of juice
- Better maceration of skins
- Opportunity to remove stems

Crushing also allows maceration or tearing of the skins, which will further enhance extraction of skin components if the skins are present during the alcoholic fermentation. Crushing also provides the opportunity to separate the stems from the must. This may be desirable if the stems will impart unwanted characters to the must or wine.
Crushing allows better extraction of the juice, but it also allows better extraction of the components of skins and occasionally seeds. If extraction of varietal characters is not wanted, the fruit can be taken directly to the press.

**Crushing: Stylistic Options**

- No crushing
- Crushing

**No Crushing**

- Direct to Press
- Whole Berry Fermentation
- Carbonic Maceration

**Direct to Press**

- Minimizes extraction from skins
- Used for white and blush wines
- Used to reduce/manipulate varietal character

This decreases yield considerably, but results in a juice low in bitterness and astringency. This may be desired if the varietal is subject to intense bitterness and the processing regime does not allow sufficient time for polymerization and softening of bitterness and astringency. It is also an important factor when making a blush wine from a red grape if normal crushing practices lead to the release of too much color into the must. The crushing/pressing processes can be used to effectively manipulate varietal character. The more damage done with both or either process to skin and internal berry cell walls the greater the release of berry components. Whether the fruit is crushed or not is a stylistic decision.
There are two additional occasions in red wine production in which immediate crushing of the fruit is not desired: **whole berry fermentation** and **carbonic maceration**.

As the name implies, in **whole berry fermentation** there are a certain percentage of whole clusters present during the initial stages of fermentation. The clusters are generally mixed with must and the must allowed to ferment. After a period of fermentation deemed optimal by the winemaker, the whole berry ferment is pressed which crushes the whole berries releasing additional berry components. The juice is then left to complete fermentation. This allows a slower rate of fermentation and better retention of berry characters in the wine. The volatile aroma characters of wines can be lost during vigorous fermentation due to entrainment in the carbon dioxide produced by the yeast. In addition, volatilization of these characters occurs more rapidly at higher temperatures, and the fermentation process releases heat as one end product of metabolism. In whole berry fermentation, the presence of the intact berries retains some of the volatile compounds within those berries. If the berries are crushed later in the fermentation, once the yeast is past the vigorous stage, the aroma characters will remain in the wine.
The process of **carbonic maceration** also uses intact clusters. In this case the fruit is held in an atmosphere of carbon dioxide but is not bathed by juice from the must. To create the anaerobic conditions, a bit of juice is placed on the bottom of the fermenter and inoculated (or not, depending upon the convention) with *Saccharomyces*. The berries, which require molecular oxygen for continued metabolism, asphyxiate.

**Carbonic Maceration**

- Berries asphyxiate
- Lose some characters due to continued berry enzymatic activity
- No ethanol extraction but ethanol is produced by fruit
- Develop characteristic flavor of silage and strawberry
- Wines do not age well

Enzymatic activities still occur in the berries during this process. Some varietal characters are degraded and thereby lost from the fruit. Since the berries are unable to respire due to the lack of molecular oxygen, they adopt a fermentative mode of energy generation and some ethanol is produced.

**Whole Berry Fermentation vs. Carbonic Maceration**

In carbonic maceration, the slow process of asphyxiation of the fruit leads to loss of varietal characters due to both loss of masking as well as *de novo* synthesis.

In whole berry fermentation the ethanol produced by yeast penetrates berry inhibiting "decay" reactions seen with carbonic maceration.

Presence of whole berries during the most active phase of fermentation traps volatile aroma characters and prevents loss due to CO$_2$ entrainment, increasing the varietal character of the finished wine.
Specific characters that develop during carbonic maceration are strawberry and silage (or fermented hay). Following a period of carbonic maceration, the fruit is then pressed (and may also be crushed prior to pressing). The juice is then allowed to ferment to dryness. The wines are generally light in color due to the deterioration of anthocyanin pigments during the maceration process. Because so much varietal character has been lost, these wines do not age as well as other styles.

Carbonic maceration can be quite tricky. One of the main problems with this technique is the need to make sure that there has been minimal berry damage. If the berries are not sufficiently intact, a microbial bloom can occur and characters more reminiscent of decayed fruit can be present. It is also important to keep the fermentation anaerobic, as the presence of even slight amounts of oxygen will greatly encourage microbial growth and the production of undesirable compounds such as acetic acid and ethyl acetate.

Whole berry fermentation and carbonic maceration produce distinctly different types of wines. In whole berry fermentation, the ethanol produced by the yeast diffuses into the berry effectively inhibiting the enzymatic reactions responsible for the evolution of the traditional carbonic maceration style.

**Crushing**

- **Temperature**
- **Percentage of Intact Berries**

If the decision is made to crush the fruit, other parameters must be considered as well. The *temperature* of crushing will impact the amount of extraction that occurs from the skin. Temperature is one of the most critical factors stimulating extraction. Controlling the temperature of the fruit at harvest controls the temperature of crushing.

As we will see below, many of the newer crusher designs allow the winemaker to manipulate the *percentage of undamaged berries* exiting the crusher. This allows modification of the amount of intact berries present during the initial fermentation for reds or entering the press in the case of white wine production.
In the case of mechanically harvested fruit, oftentimes the fruit passes from the harvester to a crusher in the field. In this case, crushing occurs immediately upon harvest. The must can then be inoculated with *Saccharomyces* and the fermentation commences in the tanker on the way to the winery.

**Location of Crushing Operation**

- In the field - as fruit is mechanically harvested
- At winery - raises issues of delivery of undamaged fruit

The alternative strategy is for the fruit to be crushed at the winery.
Lesson 4: The Crushing Operation - Choice of Equipment

There are principally two general types of crushers: those that destem or remove the berries from the stems of the clusters prior to crushing of the berries by rollers and those that crush stems and fruit together.

Types of Crushers

- **Crusher / Stemmer**
  - Separates stems from crushed fruit
  - Desirable when:
    - Stems impart a negative or undesired character to wine
    - Early removal facilitates downstream processing

- **Crusher / Stem Disintegrator**
  - Breaks up stems along with fruit
  - Desirable when:
    - Stems impart positive character
    - Stems increase yield upon pressing

**Removal of the stems** prior to crushing of the fruit is desirable when the stems will impart an undesired character to the wine or in cases when early stem removal facilitates some other downstream processing operation of the juice or must. In other cases, it is more desirable to leave the stems in the must so that they will be present during fermentation to allow extraction of stem components or when the presence of stems will facilitate downstream processing. The presence of stems during pressing provided better mixing and extraction of juice from the must. The stems provide a firm surface against which pressure can be applied and they also are stiff enough to provide channels through the must that allow rapid passage of the juice. The amount of extraction obtained from the stems is dependent upon the amount of damage to the stems that occurs during the crushing operation. The amount of damage is a function of the nature of the stems themselves and how supple or brittle they are at the time of harvest and upon the design of the crusher being used.
Lesson 4: The Crushing Operation - Equipment Purchase Considerations

Crushing: Choice of Equipment

- We will now consider the factors that should be taken into account when choosing a crusher / destemmer to purchase.

There are numerous factors to take into account when deciding upon any equipment purchase. It is a good idea to make sure that the requirements for the equipment have been well thought out beforehand. In this section of the lecture we will consider the issues that are important in the choice of a crusher. Many of the items that we will discuss as important are self-evident, but it is still useful to develop a list of essential criteria for any major capital investment made by a winery.

Crushing: Choice of Crusher

- Ease of Inspection / Cleaning
- Quality of Must
- Rate of Feed vs. Winery Capacity
- Dependability
- Overbuilt
- Compatibility with Other Equipment
- Is Adjustment Possible While Running?
- Service
- Portability

Inspection / Cleaning
One of the most important features of any piece of winery equipment is ease of cleaning and inspection. Good sanitation practices are a necessary component of the sustained production of quality wines. Any opportunity for the formation of a pool or reservoir of juice is an opportunity for microbial spoilage. If a component of the crusher is difficult to clean and a microbial population develops, that population will serve as a source of inoculation of all materials that pass through the crusher. Winemakers are fortunate in that the organism most likely to colonize winery equipment is *Saccharomyces*, the same yeast that conducts the primary fermentation. If one is not going to adopt strict sanitation procedures, then it is best to encourage domination of the winery flora by *Saccharomyces*. The ease with which a crusher can be taken apart to be cleaned or repaired is also an important feature. The more difficult and time consuming the process for cleaning a piece of equipment is, the less likely it will be done correctly or on a regular basis during crush. You also do not want to be in the situation of a thousand trucks lined up outside of the winery waiting to deliver fruit which you are having difficulty processing because you had to take the crusher apart and it is not a trivial process.

**Quality of Must**

- Hole size: amount of berry damage
- Hole surface (smooth or rough): amount of berry damage
- Type of paddle: plastic might not hold up like stainless steel
- Clearance between basket surface and paddles: amount of shearing
- Rollers: Adjustable? Can they be bypassed?
- What % breakage of berries do you want and can crusher deliver that reproducibly?
- How much clearance of stems do you want and can crusher deliver that reproducibly?
The second most important feature of a crusher is that it be able to provide the desired qualities of must. Specifically, the crusher should result in the "correct" amount of berry and stem damage for the winemaking style. If some whole berries are desired, then it is important that the crusher be designed to allow intact fruit to pass through the system.

The quality of must is most influenced by the amount of berry damage. The size of the holes in the drum of the crusher will in large part dictate the amount of damage that occurs. Obviously larger holes will allow more berries to pass through undamaged after being removed from the stems by the paddles.

The nature of the surface of the holes is also important - the rougher the surface the more likely there will be tearing of the berry skin and damage to the fruit. The type of paddle also has an influence. Stainless steel will hold up longer than plastic but also exerts more shear force on the fruit. Shearing is also a function of the distance between the paddles and the drum of the crusher. The shorter the distance the greater the shear forces. The greater the shear forces the greater the damage to the fruit. There are cases where greater damage to the fruit and skins is desired, such as in the production of red wines, as this may lead to increased color extraction. However there are also times when shear is not desired, such as in the production of many white varietal wines. The winemaker must then make a compromise on an equipment purchase, obtaining a unit that will work for both types of wine, though may not be ideal for any individual type. The other option is to purchase equipment, which allows modification of the components (comes with different types of drums or paddles) and that can be adjusted to maximize or minimize shear forces.

If whole berries are desired in the must, then the option of removable rollers should be considered. In this case, the fruit is destemmed but not crushed by passing through rollers.

**Rate of Feed vs. Winery Capacity**
Is crushing rate-limiting?
  - Slow tank fill allows:
    - Oxygen exposure
    - Growth of aerobes
    - Loss of volatiles
    - Slows processing of harvest

Is flow rate from crusher too fast?
  - Heat from friction
  - Over shoot tank

Another important consideration when purchasing any piece of equipment is to make sure it matches the rest of the **capacity of the winery**. This seems an obvious point, but one that can be overlooked. For example, if the optimal **rate of feed** for the crusher exceeds the capacity of must pumps to deliver the must to the tanks or to the press, then the crusher will have to be continually shut down. This is clearly not an advantageous situation. You also do not want to be in the opposite situation, where the **capacity of the crusher** is below that of the rest of the equipment as this will also lead to delays in processing.

If the rate of tank fill is **too slow**, this may lead to excessive aeration of the juice. This allows oxidative browning of phenolic compounds to occur, loss of volatile components, and encourages the growth of aerobes and facultative anaerobes capable of producing acetic acid. If the rate is **too fast**, the must might heat up too much and there is the risk of overshooting the tank.

**Dependability of Crusher**

- Breakdown frequency
- Ease of mechanics / repair
- Type of materials / construction

It is also important to determine the dependability of the piece of equipment you are purchasing for your production conditions. Some units work well under limited use conditions, but have increased **frequencies of breakdown** if used continuously. In this case, the equipment is under built for your conditions.
Overbuilt

- Will be running at sub-optimal conditions
- May not meet specifications / expectations
- Increases chances of equipment failure
- If remove berries in first third of crusher then the rest of the time the stems are simply being whacked about which will result in the appearance of stem fragments in the must

An aligned issue is to make sure the equipment is not overbuilt for your needs. That is, optimal running conditions exceed normal operating procedures of the winery. As analogy, if it were important to travel at a constant but low rate of speed, say two miles per hour, this would be nearly impossible to attain in a conventional automobile as they are designed to perform optimally at much faster speeds. In this case we might want to consider purchasing a golf cart instead. However, if our major use of the item required running at a speed of 50 miles per hour, then a golf cart would be seriously under-built for our purposes. The same consideration should be given to winery equipment - what are the conditions under which the crusher will be operated and is this in the optimal range for the unit under consideration?

Another important issue is to make sure that the piece of equipment purchased is compatible with other winery equipment and procedures. For example, if fruit is delivered to the crusher using half-ton bins and a forklift, the crusher has to be designed to accommodate this amount of fruit. Either that, or the winery must have the capacity to fabricate corrective solutions to any problem of incompatibility.

Compatibility with Other Equipment

- Using auger, conveyer belt, forklift or hand delivery of fruit to crusher?
- How key is uniformity of feed for optimal performance of crusher?
- Capacity of must pump
It is also important to determine how important the uniformity of feed is to the operation of the crusher. This may be addressed by having an auger system deliver fruit at a more constant rate to the crusher than using a bin system.

Adjustability during operation is another key feature of crushing equipment attractive to winemakers. If too much (or too little) skin, berry and stem damage is occurring, the ideal situation is to be able to adjust the shear forces of the crusher while it is in operation.

**Is Adjustment Possible While Running?**

- Allows for adjustment of must quality without stopping and restarting operation
  - Berry loss with stems
  - Seeds being crushed
  - Stems cracked
- Allows for non-uniform juice composition which may increase desirability of final product

The need to stop the crushing operation so that the equipment can be adjusted is time consuming.

Each time the crusher is stopped for adjustment, there is the potential for the loss of juice or for undesired aeration of the must or juice. The ability to adjust the shear forces of the crusher also allows for the production of a non-uniform juice/must that may increase the complexity of the finished wine.

**Service**
A practical consideration in the purchase of any type of equipment is the availability and quality of service. This is again an obvious point, but one that has frequently caused difficulty for winemakers. Many of the equipment manufacturers for the wine industry are located in foreign countries and problems in the delay of shipment of parts can arise.

Equally problematic is the availability of trained technicians that can repair the equipment. Again, with international products, the availability of knowledgeable technicians may be limited. It is also helpful if the operating manual is clearly written.

**Portability**

- Can Crusher / Destemmer be moved?
- How well can it be leveled
- How sturdy is it?

Another issue to consider when purchasing a crusher is the **portability** of the unit. The ability to be able to relocate a crusher can be invaluable and allow optimized utilization of winery space resources.

The above discussion has covered numerous considerations in the purchase of a single piece of winery equipment - a crusher. This list is by no means complete as there are other winery-specific issues that must be considered. It is frequently helpful to be able to test a piece of equipment prior to purchase, and many suppliers offer this service. The two most common complaints that I hear from members of the California wine industry with respect to equipment purchases concern the item being under-built...
for winery needs and problems obtaining parts and service, especially within the time frame needed during crush.
Lesson 4: De-Juicing

We will now return to the pre-fermentation winery operations. Many winemakers and consumers alike prefer the characters of wine produced from what is termed the "free run". This is especially true in white wine production. The free run is the initial juice fraction obtained from crushed fruit prior to the application of any mechanical pressure. This juice tends to have a reduced phenolic content and is thus lower in astringency and bitterness. The free run can be obtained by taking the initial juice fraction that drains from the press during filling of the press, or it can be separated from the skins and seeds by use of a de-juicing tank.

In some cases, it may be desirable to separate the juice from the skins and seeds using a de-juicing tank. This process can occur prior to pressing in white and blush wine production to yield juices with minimal extraction.

There are two basic types of de-juicing tanks as diagramed here.

The positioning of the screen relative to the must differs in these tanks. More importantly the force of gravity will differ depending upon where the collection screen is relative to the bulk of the must.
Once dejuicing is complete, the tank is then moved over the press and the must dumped into the press.

Continuous de-juicers based on must traveling over a screen are also available.
Lesson 4: The Pressing Operation

The pressing operation serves to remove juice from the skins and seeds, in the case of white and blush wine production, or wine from the grape lees in the case of red wine production.

Pressing

- Purpose
- Stylistic Considerations
- Equipment Options
Lesson 4: The Pressing Operation - Purpose

Pressing not only increases juice yield, but it also increases extraction from the skins. The purpose of crushing therefore is two-fold, the manipulation of juice/wine composition as well as the optimization of juice/wine yield.

**Purpose of Pressing**

- To recover juice / wine associated with pulp, skins and seeds that would not be released by simple draining
- Can separate press fractions to manipulate juice / wine composition

Different press fractions, can be separated. The different fractions will have a different juice composition, which in turn impacts the final wine composition of each fraction.

**To press or Not Press?**

A Matter of Timing.

The timing of the pressing operation will have an impact on juice and wine composition. The more maceration of the skins that has occurred, the more skin components will be released during the pressing operation. If minimal extraction is desired, then the winemaker should limit maceration time. This can be accomplished by pressing early or at a high Brix level in the case of red wines. Alternately, pressing late in the fermentation after ethanol has accumulated will lead to greater deterioration and extraction of the skins.
Timing of the Pressing Operation

- Maceration / Skin Contact first:
  - To allow greater extraction of materials from skins prior to removal by pressing
- Fermentation
  - To enhance extraction by ethanol, CO₂, and heat of fermentation
- Stylistic reasons for late pressing
  - Whole Berry Fermentation
  - Carbonic Maceration

There are stylistic reasons that also drive the timing of the pressing operation as well as the compositional concerns described above.

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Lesson 4: The Pressing Operation: Choice of Equipment

Several different types of presses are available to the winemaker. Which type of press is used depends largely upon the style of wine being produced.

Pressing: Equipment Options

- Batch
- Continuous

Pressing may either be done in batches or continuously. There are two broad categories of batch presses: basket and cylindrical.

Batch Pressing

- Basket Press
- Cylindrical Press

The basket press is most useful for small volumes and causes the least damage to skins and seeds. As a consequence, the yield of wine and juice is the lowest with this type of press. Application and maintenance of uniform pressure is challenging if not impossible with a basket press. They are also quite messy to operate and depending upon the design, may be messy to clean up after.

Basket Press

- Ideal for small volumes
- Low yields / High volume loss
- Low damage to seeds and skins (minimize extraction)
- Uniform pressure difficult
- Messy to clean
The basket press operates by placement of the must into a metal basket with slats or wholes in the basket. A piston is used to apply pressure to the must at the top of the basket. The juice/wine that is removed from the skins and seeds is then collected.

Cylindrical presses are an alternative to the basket press. One style of these presses still uses a piston to apply pressure, but this is in an enclosed system that allows mixing and application of more uniform pressure.

**Cylindrical Presses**

- Piston
- Air Bladder
- Easy to control pressure uniformly
- Elaborate pressing cycles and separation of juice lots possible
- Moderate yield
- Somewhat messy to clean

An air bladder is an alternative to the piston allowing for the application of more uniform pressure to the must because the surface area is greater. As in the case with the piston press, the application of pressure is cyclical, alternating with a period of mixing of the must in the press. The amount of pressure can be regulated and increased with each pressing cycle that follows each mixing cycle. Cylindrical presses
offer a higher yield of juice than basket presses but may still be messy to clean.

Pressing aids, which increase the surface area against which pressure may be applied and facilitate both drainage and mixing may be used in cylindrical presses to increase yield at low pressures.

**Pressing Aids**

- Inert material that can be used as a hard surface against which to press must
- Increases yield of juice

Stems removed at the crusher may be added back to the press to serve as pressing aid. Other inert materials may also be used.

**Continuous Pressing**

- Screw Press
- Belt Press
Continuous presses offer the obvious advantage of higher rates of processing of the must since it is unnecessary to stop after each batch. There are two main types of continuous presses in use in the industry today, screw presses and belt presses.

**Screw Press**

- High yield
- High tissue (seed and skin) damage
- High solids content of juice
- Higher astringency of wines

The screw press offers the highest yield, and the driest pomace, but causes the most damage to skins and seeds. It results in a high solids content for the juice and wine, and generates a high phenolic content in the wines. Use of the screw press is therefore limited to varietals that can tolerate this kind of damage to the skins and the seeds and the resulting increase in bitterness and astringency.

The belt press is a more recent design. The pressure is applied between the rollers and a moving belt.
Belt Presses

- Juice can be drained at different levels of pressure
- Low to moderate to high yield: fully adjustable
- May break seeds due to shear forces
- Microbial population build up can be a problem

The shear forces that can be attained in a belt press are high enough to damage seeds, so care must be taken to avoid this. Microbial build up is also a problem in the continuous presses, but particularly in the belt press because of the large surface area that can support a microbial biofilm.

One advantage of the belt press is that different pressures can be applied at different points along the belt allowing simultaneous obtention of different press fractions.
Lesson 4: The Pressing Operation - Choice of Press

Several factors are taken into account when deciding upon the appropriate press for the desired style of wine.

Choice of Press

- Yield vs. Quality
- Affects juice composition: phenolics, tannins, pH
- Stylistic considerations
- Cost

Clearly an important consideration is the yield of versus the qualities of the juice/wine. The type of pressing operation as well as the amount of pressure applied impacts the amount of damage to the skins and seeds, which directly impacts wine composition. In addition, the type of press used and factors such as the amount of solids or nutrients released indirectly affects wine composition due to effects on the metabolic activities of the microbes present. In addition to these stylistic concerns, the cost/benefit ratio is also important, as this impacts winery profitability.
Lesson 5: Introduction

Juice and Must Treatments and Additions

In this lecture we will cover juice and must treatments and additions. Before starting this discussion, it is important to point out that permitted additions are legislated in most countries and vary. It is imperative that the winemaker know what the legal additions are for the intended marketplace and abide within those limits. In virtually all cases, the additions that are allowed are consistent with sound winemaking practices, but inconsistent with correction of mistakes in judgment made by the winemaker or grower. There is an effort organized via the OIV, the International Organization of Grapes and Wine located in Paris, to normalize wine production practices internationally.
Lesson 5: Juice Treatments

There are three common treatments of juice used in wine production, depending upon the style of wine desired. As noted in earlier lectures, many important phenolic characters are located in the skins of the berry. These compounds can be extracted if the skins are present during the fermentation. Other techniques can also be used to increase the concentration of skin components in the wine.

Juice Treatments

- Skin Contact
- Extended Maceration / Cold Soak
- Clarification

Two common juice or must treatments are aimed at increasing the extraction of chemical components of grape skin cells. Skin contact simply refers to the length of time that a juice is in contact with the skins. The term is used principally in white wine production, and refers to the time between crushing and pressing of the must to separate the juice from the solids. Skin contact is typically conducted at low temperatures since excessive extraction of phenolic compounds is generally not desired. If an extended time of skin contact is desired, the must is held in a tank at low temperature typically for no more than 48 hours, and usually for a much shorter period of time.

Skin Contact

- Allowing juice to remain in contact with skins and seeds
- Increases extraction of material from skins
- Increases astringency
- May be done at varying temperatures
- Term used in white wine production

Extended skin contact has been shown to increase the astringency and bitterness of the wine, but does not provide a high enough phenolic concentration so that
bitterness and astringency can be softened by polymerization during aging.

In contrast, red wines are produced with fermentation on the skins. There are two options used to **maximize extraction** from the skins. The first is to leave the wine on the skins post fermentation. This is referred to as **extended maceration**. Extended maceration times vary, and range from one to several weeks. **Cold soak** refers to incubation of the must at a low temperature, 4°C or lower, for an extended period of time prior to the initiation of fermentation. Again the time of incubation at low temperature may range from a few days to a couple of weeks. At higher temperatures microbial growth will occur. In some cases this may be desired and increase the complexity of the wine.

**Extended Maceration / Cold Soak**

- Post fermentation "skin contact" for red wines = extended maceration
- Holding red must at low temperatures for an extended period of time prior to fermentation to increase extraction of components = cold soak
- Impacts microbial flora

Several studies have shown that both extended maceration and cold soak do not really impact the level of skin components in the wine, as the principle factor enhancing extraction is high temperature. Generally, the majority of the compounds are extracted during the fermentation as a consequence of the ethanol and heat produced by the yeast. Likewise, in controlled studies cold maceration did not lead to a significant change in the levels of phenolic compounds post-fermentation. However, both of these treatments do allow for other types of changes to occur in the wine. Cold soak in particular greatly affects the numbers and persistence of the grape microbial flora which leads to changes in the microbial contribution to wine composition. Extended maceration allows contact of solid components with the wine post-fermentation. This **provides further nutrients** for microbial activity and is stimulatory for the lactic acid bacteria. It also provides substrates for the enzymes released by microbes, which can then alter the composition of the wine. The surface of the skin cells may additionally provide a nucleating center for the precipitation or polymerization of compounds, decreasing their concentration in the wine. Thus, these techniques do not simply affect extraction, and their largest impact on wine composition may be unrelated to increases
in phenolic content.

The other operational decision made prior to fermentation in white wine production concerns whether or not the juice will be clarified, and to what extent solids will be removed. **Clarification** and early removal of suspended solid material may facilitate downstream processing by reducing the release of polysaccharides, which have a tendency to coat and clog filtration apparatus. In some cases it appears that it is easier to clarify the juice rather than the finished wine, this may be especially important in wine intended to be bottled without fining or filtration post-fermentation and aging.

**Clarification**

- Removal of suspended particles in juice
- Can facilitate downstream processing
- May be removing yeast nutrients (over-clarifying)

If done to excess clarification may drop the solids content below 2%. If this occurs the yeast may be deficient in nutrients and be unable to complete the fermentation. Grape solids provide fatty acids and sterols used by the yeast late in the fermentation to maintain ethanol tolerance. Clarification is usually accomplished by a technique called **cold settling**. The recently pressed juice is allowed to settle overnight in a refrigerated tank at a temperature below 10°C. The next day the juice is racked off of the settled lees. This can result in a significant loss of volume. Wineries may typically filter the lees to recover the juice and produce a "lees wine". This wine can be used for blending.

**Juice Clarification**

- Natural (Gravity) Setting:
  - Rack Juice from lees: results in loss of volume
- Batch and Continuous Drainers
  - To separate juice from solids
  - Control size of particles removed
- Centrifugation
  - Can lead to aeration of wine
  - May strip too many solids
- Filtration
• Usually a "rough" filtration
• Juice may be difficult to filter

● Flotation
  • Use of fine suspension of gas (nitrogen) bubbles
  • Suspended pulp becomes attached to bubbles and floats to surface allowing removal

Solids can also be removed from the juice using de-juicing tanks, depending upon the exclusion properties of the screen and the relative size of the solid material. **Centrifugation** is another option. Centrifugal force can be used to concentrate and remove the heavier solid material. The types of centrifuges available for use in the wine industry will be discussed in one of the lectures on post-fermentation cellar operations. The problem with centrifugation is that frequently it can lead to aeration of the wine and browning. Centrifugation under a modified atmosphere, that is, with nitrogen, argon or CO₂ flushing, is possible, but a lot more involved.

A **rough filtration** can be used to clarify the juice, however, juice is notoriously difficult to filter because the materials to be removed tend to gum up filter matrixes. Juice filtration also tends to strip the wine of nutrients so is not advisable unless there is no other option. Filtration also allows oxygen exposure, which may not be desirable. There is another newer technique called **flotation** for solids removal. In this case a fine suspension of nitrogen bubbles generated in the bottom of the tank leads to the flotation of the suspended particulate matter that can then be removed from the top of the tank. The gas bubbles adhere to or are entrapped by the solids material thus making this material buoyant. This process does not lead to aeration and does not strip the juice of nutrients.
Lesson 5: Juice Additions

The next topic that will be covered is juice additions. There are several additions that are routinely made to juice and must, and others that are made under more limited conditions.

Juice Additions

- Nutrient additions
- Microorganisms
- Acidity adjustment
- Defoaming agents
- Enzyme additions
- Inert solids
- Compounds affecting color
- Sulfur dioxide (SO$_2$)
- Dimethyl Dicarbonate (DMDC)
- Ascorbic Acid
- Oxygen
- Sugar
- Water

Nutrient Additions

- Nitrogen as ammonium phosphate (8 lb/1000 gal or 0.96 g/L in USA)
- Yeast extract/nutrient mix (3 lb/1000 gal or 0.36 g/L in USA)
- Yeast "ghosts" (3 lb/1000 gal or 0.36 g/L in USA)
- Thiamine (0.005 lb/1000 gal or 0.6 mg/L in USA)
- Malic acid to stimulate malolactic bacteria
- Specific amino acids and vitamins
Nutrients are one of the most common additions to must and juice. This is done to make sure that sufficient nutrients are available for the yeast so that the fermentation will be conducted to dryness, that is, little to no residual sugar will be left. Nutrient limitation is a common cause of slow and arrested fermentations. Nutrient deficiencies can also lead to off-character production by the microorganisms present in the must and juice. Addition of nutrients pre-fermentation assures that the yeast will not suffer a deficiency.

**Nitrogen** can be added in the form of ammonium phosphate, or diammonium phosphate (DAP), at a maximum level of eight pounds per thousand gallons, 0.96 grams per liter, in the United States. The level of addition permitted varies by country of production. DAP also provides phosphate, which may be limiting in some growing regions. Micronutrients, vitamins and minerals, can also be added to the fermentation. **Yeast extract** and similar complex nutrient mixtures may be added at a level of 0.36 g/L. Specific nutrients such as the vitamin thiamin and some specific amino acids can also be added legally. In many cases yeast "ghosts", also called yeast hulls, can be added. Yeast ghosts are the outer wall and membrane of lysed yeast cells. They contain fatty acids and sterols that can provide these nutrients and enhance yeast ethanol tolerance. They also can serve as a "sink" for the absorption of toxic fatty acids in the medium. An appendix of the textbook lists the additions permitted in the United States by the lead regulatory agency, The Bureau of Alcohol, Tobacco and Firearms (BATF).

In addition to stimulation of the yeast and the alcoholic fermentation, nutrients may also be added to stimulate the growth and metabolism of the malolactic bacteria. **Malate** may be added to the juice for the purpose of stimulating the ML fermentation and the accompanying flavor changes.

One of the problems with nutrient addition is that it is largely done indiscriminately. Nutrients are frequently added as a matter of course, and not because a deficiency was detected or anticipated. There are problems with excessive nutrient additions. Principle among these is the stimulation of the non-*Saccharomyces* flora and the accompanying production of inhibitory end products. If excessive nutrients remain post-fermentation this will of course be stimulatory to the malolactic bacteria but also to spoilage organisms that may be present. Amino acid additions can be particularly problematic in this regard. Amino acids serve not only as nitrogen source but also as a carbon and energy source for many microorganisms. However, the end products that are made from the degradation of amino acids are not neutral. Some may be positive and desired while others are not.
Microorganisms

- **Yeast**: *Saccharomyces cerevisiae* or *Saccharomyces bayanus*: No limit on addition, typically no more than $10^6$ cells/mL.
- **Bacteria**: Malolactic bacteria, generally *Oenococcus oeni*: Also no limit on addition, can be as high as $10^8$ cells/mL.

Addition of microorganisms is also permitted in the production of wine. Both yeast and bacterial cultures can be added. In the case of **yeast**, either *Saccharomyces cerevisiae* or *Saccharomyces bayanus* is used as an inoculum. There is no limit on the size of the inoculum, but it is typically in the range of yielding a final concentration of $10^5$ to $10^6$ cells/mL. Inoculum levels above this value can lead to excessive yeastiness of the wine. There are various types of preparations of yeast, but the most common is as an **active dry** form. The dried yeast is rehydrated (following the instructions on the packet) and used as inoculum. One of the most common mistakes in using active dry yeast is failure to follow the instructions on the packet. If the yeast is not rehydrated properly, the cells will rapidly lose viability.

Cultures of **malolactic bacteria** can also be added. There are two types of ML inocula available, pure and mixed cultures. *Oenococcus oeni* is the lactic acid bacterium most frequently conducting the ML conversion due to its high tolerance of low pH and high ethanol. Other species of lactic acid bacteria may also be present and are commonly found in the mixed culture inocula. Dried forms of the ML bacteria are available as are liquid cultures; again, manufacturer's instructions should be followed in the use of these products. The ML bacteria are far more fastidious than the yeast, and many wineries have their own techniques for preparation of the ML inoculum. These organisms and their roles in wine production will be discussed in detail in a subsequent lecture.

**Acidity Adjustment**
● Increase Acidity
  ◦ Tartaric and malic acid can be added to correct a natural deficiency
  ◦ Ion exchange to a pH no less than 2.8
  ◦ Lactic, citric and fumaric acid can be added to wine only

● Decrease Acidity
  ◦ Calcium carbonate (not below 6 g/L)
  ◦ Ion Exchange to a pH no greater than 4.5

It is frequently necessary to adjust either the acidity or the pH of the juice or must prior to fermentation. The pH of the must/juice will impact the survivability of the bacterial flora and affect protein stability and phenolic reaction rates, in addition to playing a direct role in wine flavor.

Acidity can be increased by addition of tartaric acid to the must or juice. It is also possible to use the technique of ion exchange to increase or decrease the hydrogen ion content of the juice or must, thus adjusting pH. It is not permitted in the US to adjust the pH lower than 2.8 or higher than 4.5, but these extremes are largely ridiculous from a winemaking perspective. Addition of calcium carbonate and the generation of calcium-acid anionic species salts that will precipitate can decrease acidity. In this context it is important to know if the intended goal is simple adjustment of pH or of the anionic species content of the wine. The anionic species are important, as they are perceived as sour, while the pH contributes tartness as well as impacting microbial activity and rates of spontaneous chemical reactions.

At this point we should digress to cover the different ways that acidity is referred to in wine production.

**Definitions of Acidity**

- Titratable acidity (TA)
- pH
- Volatile acidity (VA)
- Fixed acidity
- Total acidity
There are five terms that are used to describe the acid content of wine: titratable acidity, pH, volatile acidity, fixed acidity and total acidity. Each term has a different and specific meaning.

**Titratable Acidity (TA)**

Defines the proton concentration of wine as measured by titration with a strong base to a specific end point, pH 8.2
Expressed as g/L tartaric acid equivalents in USA

Titratable acidity has been discussed earlier in the context of parameters that are used to determine berry ripeness. It refers to the amount of base that is needed to titrate juice, must or wine to a specific pH end point. The convention in the United States is to express the amount of base needed as g/L tartaric acid equivalents.

**pH**

Defined as the "free" proton concentration
Not to be confused with the "titratable" proton concentration (dependent upon dissociation and concentration of organic acids)

The term pH has the same meaning as in chemistry, and is the log value of the concentration of hydrogen ions. This is not the same as the titratable hydrogen ion concentration that is measured by TA since the process of titration will result in the release of protons that are bound to anionic species.

**Volatile Acidity (VA)**

The portion of the acid species that are distillable away from the rest of the organic acids

Volatile acidity refers to the amount of volatile acids present in the wine, juice or must.
In the case of wine, the most common volatile acid is **acetic acid**. Acetic acid is distillable because of its volatility, and that serves as the basis for measurement of this compound. Acetic acid is quite pungent, and is a common component of vinegar. High levels are considered to be a defect in wine and most wine producing regions have upper limits on the amount of acetic acid that can be in the wine at the time of marketing. Therefore it is very important to monitor volatile acidity.

**Fixed Acidity**

The portion of the organic acids that are not distillable

Fixed acidity simply refers to the amount of acidity that is not volatile under normal conditions.

**Total Acidity**

The summation of the organic acid species in juice/wine

Total Acidity = Volatile Acidity + Fixed Acidity

Total acidity has the misfortune of having the same initials as titratable acidity, but the two terms should not be confused. The total acidity of a wine refers to the sum of the **anionic species** that are present and can be equated with sourness of the wine. Hydrogen ion concentration is related to tartness. Total acidity is unrelated to pH, as some of the organic acids will be bound to potassium instead of hydrogen ions. Thus, the amount of hydrogen ions released during titration is dependent upon the concentration of potassium present and the concentration of the anionic species as well as the pH.

**Defoaming Agents**

- To prevent foaming and loss of wine volume
- Silicon dioxide, sorbitan monosterate
- Not to exceed 0.15 lb/1000 gal or 0.0018 g/L in USA
Defoaming agents are added to prevent foaming or frothing that can happen during the rapid yeast fermentation phase. The carbon dioxide produced by the yeast can adhere to or be entrapped by materials in the juice, causing them to rise with the gas. This is similar to the phenomenon behind the flotation of solid materials, and can lead to loss of wine volume as the solid material is extruded from the fermentation vessel. Defoaming agents block this process and can be used to prevent loss of wine volume. The foam contains nutrients usable by other microorganisms, so it is not a sound idea to allow it to coat the outside surface of a tank or the winery floor. Silicon dioxide, sorbitan and glycerol dioleate, are common defoaming agents. Only low amounts of these agents are needed, on the order of 0.0018 grams per liter.

Various enzymes can also be added to wine. In this case the goal is to take advantage of the catalytic activity of the proteins to adjust must composition.

**Enzyme Additions**

- **Amylases**: breakdown complex polysaccharides
- **Cellulases**: breakdown complex polysaccharides
- **Pectinase**: breakdown pectins
- **Protease**: breakdown of proteins
- **Glycosidase**: release of terpenes

There are several enzyme preparations that can be added to juices, both red and white. It is important to know all of the catalytic functions of a given enzyme preparation as some side activities might not be desirable, if they lead to color loss for example.

**Amylases** and **cellulases** will breakdown plant polysaccharides while **pectinases** degrade pectins, which are similar to some of these complex polysaccharides. All three of these enzymes are used principally to improve the settling and filterability of the juice or the wine. **Protease** addition is also legal and will result in protein degradation. This is important in situations where the presence of protein leads to a problem in the wine, most notably formation of a visible haze. Most commercial protease preparations are not that effective in juice, must or wine unless it is heated to 65 degrees, which is not generally considered to be sound wine making practice. While proteases are a legal addition, most people do not use them. **Glycosidases** are legal additions in some
areas. These enzymes catalyzed the hydrolysis of covalently bonded sugar:alcohol complexes. When we discussed terpenes, I mentioned that they occurred in free and bound forms. Glycosidases can remove the sugar moiety from the bound terpene, converting it into a free or sensorially detectable form. This will happen naturally as the wine ages, but an enzymatic treatment can accelerate the process. The use of enzymes to release flavor is a hotly contested issue. To many consumers it suggests a natural deficiency of the fruit or the incompetance of the winemaker. In either case consumers may conclude that only low quality fruit/wine requires such adjustment.

### Purpose of Enzyme Additions

- Increase yield
- Facilitate settling
- Release flavors
- Prevent wine haze from forming later in processing

Enzyme additions serve multiple purposes in winemaking. If filterability and settling of solids are improved, then wine and juice yields are elevated. Enzyme treatment can also reduce the chance of macromolecular components of the wine becoming insoluble over time leading to the formation of an undesirable cloudiness or haze. Enzymes can also release flavors through the direct action on flavor and aroma compounds and indirectly by promoting lysis and extraction of plant cell components. However, commercially available enzyme preparations are frequently not highly purified and side activities may be problematic. **Pectin methylesterases**, important in the degradation of pectins, produce methanol as an end product of degradation, and high concentrations of this compound are both organoleptically undesired as well as legislated against. Enzyme additions should be applied with care and according to manufacturer's instructions and at recommended doses.

### Inert Solids

- Settling aids to increase clarifications of juice
- Increase solids content to facilitate yeast fermentation

Inert solids are also occasionally added to juice. This can be done in the cases of over-
clarification of the juice (a mistake which should be infrequently made) or if there is some component of the juice that the winemaker wishes to remove.

Solid material can facilitate settling by entrapment of suspended solids material in the juice and by forming "tight" lees, that is, a sediment that is relatively difficult to disrupt by the racking procedure. This increases yield. In some cases, a fining agent like bentonite might be added to the juice to remove protein in lieu of bentonite treatment of the wine. This may be important in winemaking styles where post-fermentation fining is not desired. However, as discussed in the lecture on haze formation, soluble protein may be stabilized against denaturation by interaction with polysaccharide materials or yeast manoproteins. Similarly, polysaccharides may be stabilized against agglutination and haze formation by interaction with proteins. Removal or adjustment of either the protein or polysaccharide content of the juice may impact the haze stability of the wine.

**Compounds Affecting Color**

- **Polyvinylpolypyrrolidone (PVPP):** to remove color and "off-color-forming" potential (mostly whites); not to exceed 60 lb/1000 gal or 7.9 g/L
- **Hydrogen peroxide:** to bleach oxidizing color pigment, not to exceed 500 mg/L (ppm)

Additions impacting color are also allowed, but the regulations vary by wine producing region.

*Polyvinylpolypyrrolidone* (PVPP) interacts with phenolic compounds and can be used as a juice treatment to reduce phenolic content. It is especially effective at removal of compounds that turn pink upon oxidation, pinking is obviously undesired in white wine production. Hydrogen peroxide, a strong oxidizing agent, can be used to bleach color, if this is desired such as in the case of production of a blush wine. Another important addition that will affect color is sulfur dioxide, SO$_2$.

**Sulfur Dioxide (SO$_2$)**
Sulfur dioxide serves many functions in wine production. It is typically added in the range of 20 to 50 ppm. Higher levels are sometimes used in some areas to preserve juice for extended periods of time. SO₂ is volatile, and will be lost from the wine over time due to volatilization. It is also reactive and will bind to phenolic and other components of juice, must and wine reducing the effective sulfite concentration. For this reason total and free (unbound) SO₂ levels are monitored during wine production.

Sulfur dioxide is used for its antioxidant as well as for its antimicrobial activity. It impacts the microbial flora of the fermentation, but will also bleach color by reacting with anthocyanins. Since this binding is freely reversible, over time it will not permanently affect color.

**SO₂ as Antioxidant**

- Blocks chemical oxidation reactions by reacting with oxygen radicals (not with O₂)
- Inhibits polyphenol oxidase (PPO) activity
- May bind to oxidized compounds altering the perception of the level of oxidation

Sulfur dioxide can block chemical oxidation of phenolic compounds by binding to oxygen radicals. Its reaction rate with molecular oxygen is so slow under wine production conditions that it will not directly block generation of oxygen radicals in the first place. It also has a perhaps more important role in inhibiting enzymatic oxidation reactions. Grapes possess an enzyme known as polyphenol oxidase, PPO. PPO reacts with molecular oxygen, water and phenolic compounds to produce an oxidized phenolic compound and hydrogen peroxide. The hydrogen peroxide can then react with other components in the juice or wine. PPO functions in defense against microbial attack and is one of the most important enzymes of the vine chemical warfare program. Hydrogen peroxide is the goal of the catalysis as this compound has
strong antimicrobial effects. It is not clear if the oxidized phenolic compounds are also inhibitory. PPO is released to the must or juice upon crushing of the berries, so is present and active in fermentation. Many oxidized phenolic compounds are undesirable as is the production of hydrogen peroxide. Yeast possess an enzyme called catalase which degrades hydrogen peroxide so are not inhibited by the levels of this compound produced in the must or juice. However its effects on the chemical composition of the wine, and inducement of aldehyde and off-color (brown, orange, pink) formation are undesirable. Molecular oxygen serves as a micronutrient for many organisms, required for the biosynthesis or degradation of many compounds. PPO successfully competes with the microbes present in wine for O$_2$. Oxygen is required by the yeast for optimal ethanol tolerance, if PPO activity is unchecked, the yeast may be in a nutrient deficient situation. This is an additional reason to add SO$_2$. It is not clear at the molecular level exactly how SO$_2$ is able to inhibit PPO activity. Further, the binding or interaction of SO$_2$ with an oxidized phenolic compound may prevent detection of the off-color, that is, it bleaches the off color just as SO$_2$ can bleach anthocyanins. In this case SO$_2$ is not functioning as an antioxidant but instead masks the oxidation that has occurred.

SO$_2$ as Antimicrobial

- Inhibits both bacteria and yeast, less of an effect on yeast at low concentration
- Effectiveness dependent upon pH
- "Detoxified" by Saccharomyces metabolic activity
- Will form addition compounds with acetaldehyde, sugars, phenols reducing effective concentration of SO$_2$

Sulfur dioxide also has antimicrobial properties. Yeast, bacteria and mold are all sensitive to inhibition by SO$_2$. The effectiveness of SO$_2$ addition is dependent upon the pH of the solution, as this will impact the molecular form of SO$_2$ present.
The inhibitory form of SO$_2$ is thought to be the aqueous form of free SO$_2$. As seen from this plot taken from the text, the aqueous form is rapidly converted into ionized forms at wine pH. Depending upon the wine pH, SO$_2$ may be completely ineffective at low doses as an antimicrobial agent. SO$_2$ is detoxified by *Saccharomyces*. *Saccharomyces* is not inherently resistant to the inhibitory effects of SO$_2$. It instead has developed mechanisms to eliminate this compound as a problem. This is accomplished by binding of the SO$_2$ to yeast *acetaldehyde* produced during the fermentative catabolism of sugar. The acetaldehyde forms a complex with SO$_2$ essentially inactivating its inhibitory potential. It is important to stress that this is a *detoxification*, because *Saccharomyces* is basically removing this compound from the environment, which is great news for other microbes present because it is no longer inhibitory to them as well.

Multiple molecular forms of SO$_2$ function as antioxidants and in the inhibition of PPO activity, so the pH effect on this function of sulfur dioxide is not as severe. In the case of SO$_2$ addition as an antimicrobial, this will not be effective if *Saccharomyces* is or is about to rapidly catabolize the sugar. SO$_2$ may play an important role as an antimicrobial post-fermentation, after the opportunity for detoxification has passed.
SO₂ Stimulation of Yeast

- Inhibition of microbial competitors
- Elimination of competition with PPO for molecular oxygen

Sulfur dioxide stimulates *Saccharomyces* in two ways. First, by reducing competing organisms if the SO₂ application occurred with sufficient time prior to the onset of fermentation and detoxification. Bacteria are generally more SO₂ sensitive than the wild, non-*Saccharomyces* yeasts, so the inhibition of this class of organisms will be greater. Sulfur dioxide inhibition of PPO activity will leave dissolved oxygen for the use of the yeast and other microbes and is therefore stimulatory to yeast growth and fermentation.

SO₂ Effects on Color

- Aids in extraction by killing grape skin cells
- Chemically bleaches color (reversible reaction)

Sulfur dioxide has complex effects on juice and wine color. SO₂ aids extraction through toxic effects on grape skin cells leading to cell lysis and the release of cellular components. However, as we will see later in the lecture on red wine production, SO₂ can form a complex with anthocyanins resulting in loss of anthocyanin color. This reaction is reversible, and is not a permanent loss of color unless excessive amounts of SO₂ (greater than 200 ppm) are used in the winemaking procedure. It is also important to note that many of the binding interactions of SO₂ are reversible, and in equilibrium. As free SO₂ is lost from the juice or wine due to volatization, SO₂ adjuncts may dissociate.

Another digression is warranted with respect to sulfur dioxide. In the United States, wine must now carry a warning label indicating that the product contains sulfur dioxide. What is the basis of the health concerns that lead to this warning?
SO₂ Health Concerns

- Wines must be labeled as "containing sulfites"
- Chronic asthmatics may be hypersensitive to SO₂
- Lack sulfite oxidase
  - Normal: 0.75-3 units of activity
  - SO₂ sensitive: 0.2 or less units
- Lung tissue: lowest in sulfite oxidase
- Humans synthesize g/day of sulfite: natural antioxidant

Chronic asthmatics are hypersensitive to SO₂, principally because they lack an enzyme called sulfite oxidase. SO₂ is a natural antioxidant produced in our bodies from the degradation of sulfur-containing amino acids. If an excessive amount of this natural antioxidant is produced, the enzyme sulfite oxidase will eliminate it via reaction with molecular oxygen. This is an important safety valve to assure optimal levels of SO₂ are present without generation of too high of a level. Individuals who are deficient in sulfite oxidase have difficulty handling biologically produced as well as ingested SO₂. Failure to detoxify sulfur dioxide leads to symptoms similar to anaphylactic shock, commonly associated with an allergy. Thus low sulfite oxidase has been equated with an allergy to SO₂. However it is not an allergic reaction in the classic sense, and is frequently fatal. For this reason, winemakers willingly agreed to label their product as containing sulfites. Sulfite is also produced by Saccharomyces from the degradation of sulfur containing amino acids and during the course of reduction of sulfate for biosynthesis. For this reason, all wines are typically labeled as "contains sulfites" or the cleverer "contains no added sulfites" which means only biologically produced sulfite is present. In any case individuals with low sulfite oxidase activity should avoid wine and many other products.

Dimethyl Dicarbonate (DMDC)

- Toxic to yeast, including Saccharomyces
- Rapidly hydrolyzed and inactivated
- Less toxic to bacteria especially in absence of sulfur dioxide
Dimethyl Dicarbonate (DMDC) is an **antimicrobial** agent that will hydrolyze to methanol and carbon dioxide. It therefore forms harmless compounds over time. DMDC can be used to eliminate the yeast flora of a must, juice or wine. It is less effective against the bacteria, but appears to act synergistically with SO\(_2\). In this case lower amounts of sulfur dioxide are needed. DMDC is marketed as Velcorin. However, its use as an antimicrobial agent is prohibited in some countries largely due to dangers of the compound if not handled properly.

The next compound that can be added to juice, must or wine is ascorbic acid. Ascorbic acid functions as a chemical antioxidant.

**Ascorbic Acid: Antioxidant**

Ascorbic acid is better at preventing some types of oxidative reactions than is sulfur dioxide. The two may be used in concert.

There are several conditions under which deliberate oxidation of the juice or must may be desirable.

**Oxygen**

- Stimulates microorganisms
- Required by yeast for optimal ethanol tolerance
- Stimulates oxidation reactions so oxidation products can be removed early (does not always work)

Oxygen catalyzes some phenolic reactions and is involved in the ultimate stabilization of red wine color. As mentioned above, it is also an important yeast and bacterial nutrient.

One of the uses of **hyperoxidation** is to attempt to generate phenolic oxidation off-colors at an early stage of the fermentation. These oxidized compounds sometimes then polymerize or are lost from the juice during yeast fermentation, perhaps by attachment to the yeast cell surface. In this case, the wine is protected against development of these compounds later on during aging and it is not so critical to protect them against oxidative damage. However this does not always work and is
dependent upon juice composition. If phenolic compound levels are insufficient to support formation of large precipitable polymers, the off-color may not be removed during fermentation and may remain in the wine following settling of the yeast lees. Winemakers have had mixed results with juice hyperoxidation, so this technique should be attempted initially on a small scale and used with caution.

We will now cover a couple of additions that are legal in some regions of the United States but not in others. One peculiarity of wine production in the US is that it can be regulated at both the federal and state levels. Some treatments or additions that are permissible at the federal level may be prohibited in certain states, so local state as well as federal regulations must be followed. One of these types of additions is sugar.

**Sugar**

- Sugar addition not legal in California, is in other wine-growing regions in the USA
- To correct a deficiency at time of harvest

As a rule of thumb, if normal viticultural practices yield fruit with ample sugar, sugar addition to the must or wine is prohibited in table wines (with the exception of the special case of sparkling wine production). If the fruit at the time of harvest is deficient in sugar, such as occurs in very cool or short season growing regions, then sugar addition is permitted in order to obtain the proper balance the ethanol and acidity in the finished wine.

The addition is permitted to correct a deficiency at the time of harvest, not for a subtle adjustment of sugar levels to correct an error in the timing of harvesting.

**Water**
- Water can be legally added to correct a "high Brix" must or juice to make wine from a juice concentrate
- Water can be added with other additions if water solutions have been made
- Water cannot be added simply to increase volume of production

Another restricted addition is water. Water may be added in a situation of excess sugar at the time of ripening; it may not be added to dilute a juice to obtain a higher yield of wine volume.

Water can also be added back to **juice concentrate** (typically 80% sugar) to allow fermentation to occur. Water obviously can also be added at the time of nutrient or yeast additions, since these components are first solubilized or rehydrated in water.

It is critical that the winemaker understand the process by which additions become permissible and the agencies responsible for enforcement of the regulations. In the United States, no addition is allowed or banned without a period of comment from the public. The practices of the state alcohol boards vary dramatically, but few have the aim of hindering the wine industry but rather safeguarding the quality of the wine produced. Consumers tend to associate wines with the region in which they were produced, and are likely to make purchase decisions based upon area of production. It is imperative therefore that a given region protect its reputation as a producer of premium wines. Encouragement of additions to correct errors in judgment in the winery or vineyard detracts from wine quality and can harm the reputation of an entire region.
Lesson 5: Who Decides Which Additions Are Allowable?

- Bureau of Tobacco, Alcohol and Firearms (BATF) in USA
- State Alcohol Boards in USA
- Office International de la Vigne et du Vin (OIV)
- European Community

We will end this lecture by emphasizing the need to be aware of the permissible additions and juice treatments of the region of production as well as legislation covering the region of intended sales. Many winemakers have lost shipments of wine because they are not allowed entry into a foreign market, and are not stored under appropriate conditions awaiting shipment back to the United States. It is also important that accredited analyses be performed for those compounds, such as acetic acid, that have maximal allowable limits.

Additions which are fully permitted in one country might not be permitted in another. It is important to know the regulations of the country in which the wine is produced as well as of the country in which it will be sold.
Lesson 6: Introduction

Overview of Red Wine Processing

In this lecture we will cover an overview of the steps of red wine production, emphasizing pre-fermentation practices. Several topics of importance red wine production, such as color, will be covered in more detail. Post-fermentation operations will be considered in a later section of the course.
Lesson 6: Overview of Red Wine Production

Red wine production differs from white wine production principally in the amount of desired extraction of components from the skin.

The Basic Steps of Red Wine Production

1. Crushing, Destemming
2. Must to Tank
3. Fermentation / Maceration
4. Pressing and Draining
5. Finish of Fermentation

Following **crushing** and **destemming** (if desired) the must is transferred to a tank or wooden cask and allowed to ferment. At some point during or after the fermentation process, the partly fermented juice (or wine) will be removed from the skins by use of a press. If the wine is not yet dry, it may be returned to a tank to complete fermentation or to allow the malolactic fermentation to occur. The timing of pressing is a stylistic issue, partially dictated by the varietal. Extended skin contact is detrimental to some varietal wines, but highly desired in others. Depending upon the style of wine, the grapes may be pressed early to eliminate or minimize skin contact or the clusters may not be crushed at all.

Red grapes may be used to produce a blush wine, which is light in color and low in tannin content. In this case the grapes would be pressed immediately following
crushing as in the case of white wines. If the must is to be fermented, then a decision needs to be made as to whether or not the stems will be present along with the seeds and skins during fermentation. If whole clusters are preferred, then the winemaker must determine if the goal is to generate characteristics associated with carbonic maceration and berry asphyxiation or if part of the fermentation will contain whole berries. As discussed in a previous lecture, whole berry fermentation will produce wines higher in fruit character.

**Principle Red Wine Varietals in California (% production)**

- **Zinfandel** 23%
- **Cabernet Sauvignon** 20%
- **Merlot** 18.3%
- **Barbera** 0.6%
- **Grenache** 0.6%
- **Rubired** 0.5%
- **Pinot noir** 0.5%
- **Carignane** 0.4%

The principle red variety currently grown in California is Zinfandel. However, the largest red wine production is of Cabernet Sauvignon. This is because a significant amount of Zinfandel is being used to make a blush wine marketed as White Zinfandel. Several varieties other than those on this list are also grown in California, but the production is low. Some of the wines are produced principally for blending and not intended to be marketed as a varietal wine.
Lesson 6: Red Wine Production: Cap Management Strategies

One of the most critical aspects of the production of red wines is the management of extraction of skin and seed components. Several factors will enhance extraction, but there is a problem with excessive extraction and the accompanying bitterness and astringency in some varietals. Red wines are typically aged longer than whites in new world wine production, so excessive extraction can be compensated for somewhat by extended aging of the wine. However, this is largely dependent upon the holding capacity of the winery since most consumers intend to consume wine soon after purchase, depending upon the segment of the market for which the wine is intended.

Red Wine Fermentation on the Skins

- To extract pigments and other phenolic compounds in grape skin cells
- Use of ethanol of fermentation to increase extraction
- Use of cap management strategies to increase extraction

Extraction is facilitated principally by two factors: temperature and ethanol. However, the carbon dioxide produced by the yeast during fermentation tends to make the skins buoyant and the skin and seeds will float to the top of the tank away from the ethanol and active site of fermentation, the major source of heat. This is addressed by mixing the skins back into the fermenting juice. There are several techniques used to accomplish this.

Methods of Cap Management

- Rotary tank
- Punch down
- Pump over

The primary fermentation may be conducted in **rotary tanks**. These tanks can be rotated along their axis, leading to mixing of the skins and seeds with the fermenting juice.
This serves to assure regular contact of the skin "cap" and the ethanol produced by the yeast and to create a more uniform temperature throughout the tank. Alternative mixing techniques can be used for upright or vertical tanks.

A "punch down" may be conducted. In this process a piston or piston-like device is used to physically push the cap down into the center of the tank. The cap will reform on the surface of the wine, but the contact with the high temperature mid-tank and the ethanol enhances extraction. Punch downs may be performed once to several times per day. However, the technique is only effective if the must is actively fermenting, that is, ethanol and heat are being produced. If fermentation has not yet been initiated, there is the possibility of spoilage organisms growing on the cap, such as the acetic acid-producing Acetobacter. This organism is readily killed in anaerobic conditions established by the yeast during fermentation. However, if the fermentation has not
initiated, punching down a contaminated cap may serve no purpose other than simultaneous aeration and dispersal of spoilage organisms.

**Submersion**

- A variation of the punch down technique is total submersion of the cap.

Submersion, pushing down of the entire cap, is a variation of the punch down technique. It is typically gentler (or it can be, depending upon winery operations and personnel), and it can be automated. Excessive breakage and shearing of skins is undesirable as it may lead to increased viscosity of the wine making pressing quite a challenging task.

Another alternative to punch downs is pumping over. In this case juice from the bottom of the tank is pumped over the top of the cap, bathing the cap in juice. This is generally not done with such force that the cap is submerged in the tank, as that will lead to excessive maceration of the solid material hindering the ability of the must to be pressed.

**Pumping Over**

- The process of using fermenting juice from the bottom of the tank to bathe the cap of skins and seeds that forms on the tank exposing the cap to the ethanol of fermentation.

The pump over technique can be quite gentle depending upon how it is done. It may also lead to aeration of the fermenting must, again depending upon how it is done.
If the fermenting juice is passed through a sprinkler device, this is the gentlest bathing of the cap but also leads to the most aeration of the juice. Aeration is not necessarily detrimental to red wines, especially at this stage. The yeast will use the oxygen for biosynthesis and compete with the enzyme PPO. The formation of oxidative "off-colors" (brown, orange, pink) is not a problem in red wine due to the initial color. However, excessive oxidation may encourage production of acetic acid or acetaldehyde, both of which might be undesirable.

Not all compounds of skins are extracted at the same rates, and it is important to determine when the maximum possible has been extracted of the desired components. Continued encouragement of extraction may be deleterious to the quality of the wine.
In the case presented above, compound A is more readily extracted than compound B. Overtime the ratio of the two compounds to each other will change, which may have an impact on the organoleptic characters present in the wine.
Lesson 6: Red Wine Production: Factors Affecting Extraction

Several factors have a dramatic impact on extraction and therefore influence cap management practices.

Extraction Affected By:

- pH
- Temperature
- Length of time
- Maceration
- Ethanol concentration
- Total amount in skin cells
- Enzyme treatments
- SO₂

The pH of the must will impact extraction. Low pH values tend to be toxic to living cells as the cytoplasm acidifies. Non-viable cells are more susceptible to lysis and leaching of internal components. Therefore, the relative pH of a must may impact extraction. In reality, differences in must pH play only a minor role in extraction. A far more major role is played by temperature. Simply put, the higher the temperature the greater the rate and extent of extraction. This is largely because rates of diffusion increase with increasing temperature. High temperatures of fermentation (30°C or higher) result in maximal extraction of skin components in roughly seven days or less depending upon the varietal and other factors. The length of time the skins are in contact with the fermenting must is also an important factor. The longer the time of exposure, the more complete the extraction. Also, the longer the time of exposure, the higher the ethanol concentration of the exposure. Ethanol is also toxic to most living cells, speeding cell death and release of cellular components. Enzyme treatments, specifically enzymes with catalytic activity destabilizing plant cell walls (pectinases), will also increase extraction by increasing deterioration of the cells. Sulfur dioxide, as mentioned in a previous lecture, if used at high concentration can also accelerate extraction by impacting plant cell viability. However, the concentrations needed (200 ppm or greater) are higher than what is normally used in wine production (20 to 50 ppm). Finally, extraction is obviously dependent upon the total amount of a given compound available to be extracted.
Lesson 6: Red Wine Production: Manipulation of Must Composition

Several techniques exist in addition to cap management that serve to manipulate the composition of red must.

Techniques for manipulation of red must composition

- Maceration
- Temperature of Fermentation
- Pressing Conditions
- Thermovinification

Maceration

- Pre-fermentation
  - Temperature
  - Length of time
- Number of pump overs/day
- Type of pump over
  - Spray
  - Punch down
  - Rotary tank
- Ethanol at time of pressing

Maceration, or damage of the cell walls causing leakage, may also be encouraged pre-fermentation by incubation at a low temperature; a temperature low enough to prevent the initiation of the alcoholic fermentation and that will inhibit the growth of spoilage aerobes on the surface of the wine. Temperatures below 10°C are sufficient, but the lower the better as there are some microbes that will grow in this range. The number and nature of the punch down/pump over regime will also impact maceration of the plant tissue, as discussed above, as will ethanol.
One commonly used technique is maceration. Maceration may occur post-fermentation, termed extended maceration, and consists of simply leaving the wine in contact with the skins and seeds for an extended period of time.

**Temperature of Fermentation**

Temperature of fermentation has multiple effects during red wine production. As mentioned previously, the higher the temperature the greater the extraction of cellular components. But other effects also occur. High temperatures are stimulatory to some microbes and inhibitory to others, so the temperature of the fermentation will impact the microbial ecology and therefore the composition of the must. Temperature also affects the rates of chemical reactions, which occur during wine production. There is also an effect on the loss of volatile compounds; the higher the temperature the faster the rate of loss of volatile components.

**Pressing Conditions**

Pressing conditions also affect the amount of damage to skin cells and to seeds. This topic has been covered in a previous lecture so will only be briefly considered here. Pressing conditions can be severe enough to generate shearing of plant cell walls and greater extraction. The shear forces may also be great enough to damage seeds resulting in the release of seed phenolic compounds. Some of the seed phenolics appear to be important in tannin development, so some damage of these components may be desired. This is varietal- and style-specific, however.

**Post-fermentation Maceration**

- As CO₂ bubbles, which provide buoyancy to the cap dissipate, cap sinks
- Maceration can continue for one to four weeks after cap settling
- Also aging on yeast lees

After fermentation is completed and the carbon dioxide dissipates from the fermentation, the cap is no longer buoyant and will sink to the bottom of the tank. There the material is constantly bathed in ethanol and is exposed to enzymatic attack.
In some varietals, those with particularly hearty cell walls, this extended contact allows further extraction of cellular components. In other cases, it serves to allow greater extraction of nutrients important for stimulation of the malolactic fermentation.

Recently, a new technique called thermoflash maceration has become available for use in wine production.

**Thermoflash Maceration**

- New technique for rapid heating/cooling of must
- Use vacuum for rapid cooling
- Combination destroys plant cell walls and permeability barrier
- Greatly increases extraction

The thermoflash unit is designed to **rapidly heat and cool** the wine. Cooling is accomplished by use of a **vacuum**. This procedure causes **massive damage to the plant** cell walls in a relatively short period of time. The unfermented must has the depth of color associated with **maximal extraction** and the skins have the appearance of skin cells following extended maceration. This technique greatly increases and accelerates extraction. While still new and untested, this method will allow cooler fermentations to be run and avoids enzymatic attack of plant tissues and the encouragement of undesirable microbial activity. Further research is needed to fully evaluate the impact of this technique.

**Thermovinification**

- Hold must at high temperature (60-80°C) for a short (seconds) period of time
- Grapes: Steam treatment so surface reaches 75°C, pulp no more than 30°C.

Thermovinification is a technique that greatly impacts must composition.

Thermovinification refers to the holding of the must or intact berries at a **high temperature for a short period of time**, followed by rapid cooling. The process is not
as rapid as thermoflash maceration, nor does it lead to the same amount of cell damage.

Whole berries may be treated rather than must. In this case the surface of the berry reaches a high temperature, but the pulp does not. This provides the heat treatment exclusively to the skin cells rather than to the entire must.

Consequences of Thermovinification

- Heat denaturation of enzymes (PPO)
- Increases color extraction
- Increases stability of "purple dimer"
- Alters microbial flora
- Easier to press
- Characteristic flavor changes

Thermovinification leads to the destruction of polyphenol oxidase. While desirable in white wine production, this means that oxidative reactions common in red wine production will not occur. Oxidized or aged color does not develop in these wines. Thermovinified wines are typically intense in purple color, almost iridescently so, because of stabilization of anthocyanin dimers found in the intact fruit as described below. Heating of the must has a dramatic impact on the microbial flora of the fruit, so these wines are less complex in microbial characters. The must is easier to press and juice and wine yields are high.

Flavor Changes of Thermovinification

- Fruit characters intense but less complex
- Increase vegginess (may be too intense once fruit disappears)
- Hydrolyze terpene glycosides to free terpenes
- Aging/Chemical reactions occur

Characteristic flavor changes occur in the wines as well. The berry fruit character is more intense but less complex following thermovinification. There is also an increase in the vegetal characters that is initially masked by the intensity of the fruit, but upon
aging may become too intense. Thus these wines do not age well. **Terpene glycoside hydrolysis** is encouraged by the heat treatment, imparting Muscat characters to the wine. Some temperature dependent **chemical reactions normally associated with aging occur**, resulting in wines that are a mix of age and fresh fruit characters. Thermovinification is not suitable for all varietals or tastes.
Lesson 6: Red Wine Production: Red Wine Color

One of the most important features of red wines is their color. Juice is initially low in color until pigments are extracted from the skins. Color is affected by many variables.

Red Wine Color

- Due to anthocyanins
- Anthocyanins can be glycosylated
- Anthocyanins can be acylated or have another type of molecule attached to first sugar
- Acylated pigments less sensitive to bleaching and oxidative loss
- Extracted at different rates

Color is due to the anthocyanins present in grapes. Anthocyanins have the following general structure. Anthocyanins can be glycosylated and/or acylated. Acylated pigments are less susceptible to loss during aging.

Six types of anthocyanins are found in wine that differ in the substitutions of the third ring. These compounds are commonly found throughout the plant kingdom, and are responsible for flower as well as fruit color.
Anthocyanins

- Delphinidin: R=OH; R'=OH
- Cyanidin: R=OH; R'=H
- Peonidin: R=OCH₃; R'=H
- Malvidin: R=OCH₃; R'=OCH₃
- Petunidin: R=OCH₃; R'=OH
- Pelargonidin: R=H; R'=H

The anthocyanin pigments are extracted at different rates and occur in varying ratios in different varietals.

Numerous factors affect red wine color.

Factors Affecting Must Color

- Variety
- SO₂: flavene sulfonate is colorless
- pH
  - Protonated form = red
  - Quinoidal base = blue
- H-bonding between protonated form and quinoidal base = purple
- Co-pigmentation

As mentioned earlier, sulfur dioxide binds to anthocyanins. The resulting flavene sulfonate is colorless.
Wine pH also impacts color. It affects the molecular form of the anthocyanin molecule, which in turn affects the color of the solution.

At low pH the anthocyanin molecule is protonated forming the flavylium cation, which is red. At higher pH values, the quinoidal base forms, which is blue in color. The chalcone form is yellowish and the carbonyl pseudobase is colorless. These reactions
are freely reversible as pH changes.

![Effect of pH on Anthocyanin Structure](image)

At wine pH values the colorless carbonyl pseudobase is the dominant form. The grape berry appears purple in color because of hydrogen bonding between the flavylium cation and the quinoidal base. This combines a red form with a blue form, leading to purple. This hydrogen bonding is stabilized during thermovinification, giving those wines their characteristic intensely purple color. In must, because of the low pH, when the anthocyanin dimmer dissociates, the quinoidal base is converted to either a carbonyl pseudobase or a flavylium cation and the blue color is lost leaving a more intense red color.
Anthocyanin Structures

Hydrogen bonding occurs between the Quinoidal base and Flavylum cation stabilizing the purple color.
Lesson 6: Red Wine Production: The Co-pigmentation Phenomenon

An interesting discovery about wine color has been known for sometime. Red wine color does not behave in a linear fashion with dilution. If color were simply due to the presence and amount of anthocyanin monomers, one would expect a linear decrease in absorbence as monomer content decreases. However, this is not the case. Instead, a much greater decrease in absorbence is initially observed.

Co-Pigmentation

- Refers to the observation that color does not appear to be a linear factor upon dilution - absorbence decreases faster than can be accounted for by simple dilution.

The decrease in absorbence that is observed is not linear, implying a dissociation of a complex. Consistent with this belief, co-pigmentation is due to the interaction of anthocyanin molecules with other phenolic compounds present in the must. This interaction changes the intensity of the color largely by shifting the wavelength of maximal absorption. Wine color, therefore, is dependent upon the total phenolic composition of the wine, not just the anthocyanin concentration. Much more remains to be discovered regarding the chemistry of the co-pigments, their stability and influence.
on pigment polymerization.

The remaining aspects of red wine production will be considered in later sections of the course.
Overview of White Wine Processing

In this lecture we will discuss white wine production, with a focus on pre-fermentation operations. There are some concerns that are similar for red and white wine production, but others that are specific for white and blush wines.
Lesson 7: Overview of White Wine Production

The Basic Steps of White Wine Production

1. Crushing, Destemming
2. Pressing
3. Cold Settling / Racking
4. Fermentation
5. Racking
6. Finish of Fermentation

In contrast to red wines, white wines are pressed immediately upon crushing or following a brief period of skin contact. Excessive skin contact is not recommended in white wines as this leads to undue bitterness and astringency. White grapes are frequently destemmed during crushing again to avoid extraction of the phenolic compounds. If minimizing skin contact is desired, the crushing step may be omitted and the fruit taken directly to the press. White must may be subjected to de-juicing to obtain the free-run, which generally yields the highest quality wine. Press fractions may also be fermented separately. The juice is transferred to a tank for cold settling overnight, and then racked off of the grape lees prior to initiation of fermentation. The juice is usually warmed to the temperature of fermentation, typically lower than that for red wines (12-18°C versus 25-30°C). Sulfur dioxide is typically added early, at the crusher or at the point of cold settling.

Principle White Wine Grape Varietals in California

- Thompson Seedless        60%
- Chardonnay                    18.4%
- French Colombard          10%
- Chenin blanc                   4.6%
- Sauvignon blanc             2.2%

The white grape variety with the largest production in California is Sultanina or Thompson Seedless. This is a very high-producing neutral variety used principally for blending. Roughly 18% of the white wine grape production is Chardonnay, which has
the largest varietal wine production. French Columbard, Chenin blanc and Sauvignon blanc round out the list of the top five varietals. Several other varietals of white grapes are also grown in California, but the production levels are relatively minute.
Lesson 7: White Wine Production: Stylistic Options

Options for White Wine Production

- Style
- Residual Sugar
- Carbonation

Style

- Varietal or Blend
- Premium or Picnic
- Price/Volume

As with red wines, the first decision to be made is the type of wine to be produced, as this will dictate quality parameters and cost effectiveness. For example, is the wine to be marketed as a varietal wine or as a blend? If it is a blend, is it a well-known blend style or is it going to be a proprietary blend? Is the goal to produce a premium, ultra-premium or artisan style or will it be a picnic wine? To be economically feasible, the price obtainable per bottle will obviously need to cover the costs of production. The profit margin is not high for wine, just as for other commodities, so there is little room for error or poor judgment.
Other styles of white wine include dessert wines, which are usually high in sugar as well as ethanol, and specialized styles such as Botrytized wines.

Grapes harvested at a very high Brix value (28 Brix or higher) will naturally arrest fermentation. This high sugar levels lead to high ethanol levels, which may be high enough to be inhibitory to the yeast present, depending upon other juice and strain factors. Many yeast strains can tolerate ethanol concentrations as high as 17%, but if the nutritional and physical parameters of the fermentation are not optimum, yeast ethanol tolerance is reduced. In this case, fermentations with 15% or higher ethanol may arrest. As a rough rule of thumb, the final ethanol concentration can be estimated as 0.55 of the initial Brix value (this is a rough estimate, as ethanol yield is affected by factors such as temperature of fermentation and the type of fermentation vessel). A juice with a Brix value of 30 or more will likely arrest naturally. This is the basis of one type of dessert wine production - harvest of the fruit at very high sugar levels. Other ways are to arrest fermentation by artificial means, the addition of distillate for example, or a temperature shock.

Botrytized wines are made from fruit that is infected with the mold Botrytis. Botrytis is able to penetrate the berry surface and impact the composition of the fruit. The yeast
have difficulty completing the fermentation of the juice produced from the mold-infected fruit and characteristic flavors are present in the wines.

**Residual Sugar**

- Dry (<0.2% RS)
- Semisweet (0.5-2% RS)
- Sweet (5-10% RS)

Another decision that needs to be made is the level of desired sugar at the end of fermentation. In **dry** table wines, the goal is a sugar content of less than 0.2%. As we will see in the lectures on fermentation, the residual sugar is generally fructose rather than glucose.

**Semisweet** wines range from 0.5 to 2% residual sugar and wines containing greater than 5% sugar are classified as **sweet**.

**How to achieve desired residual sugar**

- Arrest fermentation
  - Temperature
  - Ethanol addition: fortification
- Add juice concentrate
- Late harvest: natural arrest of fermentation
- Add sweet reserve

Other than the various methods of arrest of fermentation, there are several techniques for adjusting the level of sugar post-fermentation. Juice or juice concentrate may be added or a sweet reserve (sugar plus ethanol); however if ethanol is added the wine will need to be labeled as fortified. If the ethanol content is below that which is inhibitory to continued yeast fermentation, the wines may undergo a spontaneous secondary fermentation. If this is not desired, the wine can be steriley filtered and bottled immediately upon sugar addition.
Carbonation

- None - still wine
- Lightly carbonated
- Heavily carbonated (Sparkling wines)

The level of carbonation may also vary in the finished wines. The amount of CO₂ varies with the style of wine produced. Carbon dioxide enhances flavor perception; so many winemaking regions produce a lightly carbonated style. This style is more popular outside of the United States.

Sparkling wines are heavily carbonated. We will not have time in this course to cover the complex process of Champagne and Sparkling wine production, but there are several methods that are used to generate carbon dioxide in the bottle. The carbon dioxide can be produced naturally by having a secondary fermentation occur in the bottle or can be added artificially at the time of bottling.
Lesson 7: White Wine Production: The Goals

The goals for white wines differ from red wine production in several respects. Generally little to no skin contact is desired. This is because the principle flavor and aroma compounds are located in the pulp of the grape with the skin providing little other than bitterness and astringency. Many white wine styles are designed to be consumed relatively young (less than five years of age), which is insufficient time to allow polymerization and softening of the phenolic content. In addition to bitterness, phenolic compounds lead to off-color production under oxidizing conditions. This color change is generally undesirable in white wines. Given the more delicate flavors of white wines, other spoilage characters are not well masked, such as aldehydes, higher alcohols and acetic acid. It is therefore more important to protect white wines from oxidative damage than red wines.

**White Wine Processing: Goals**

- Limit skin extraction
- Limit oxidation
- Limit volatile flavor/aroma loss

In addition to limiting both skin contact and oxidation, white wines are usually protected against excessive loss of volatile components by fermentation in enclosed vessels generally with refrigeration. If fermented in barrels, care is taken to minimize loss of volume due to evaporation, especially post-active phase of fermentation when a protective carbon dioxide blanket is no longer present.
Lesson 7: White Wine Processing: Processing Options

There are several production techniques that can be varied in white wine production that will affect the composition of the final wine.

White Wine Processing: Variables

- **Solids Content**
- **Skin Contact**
- **Pressing Conditions**
- **Temperature**
- **Oxygen Exposure**
- **Lees Contact**
- **Oak**

**Solids Content**

- **High solids:**
  - Better yeast and ML fermentation
  - Decrease fruity content due to esterases of solids
  - Increase phenolics/astringency
- **Control of solid content**
  - Take free run
  - Settle/Rack at low temperature
  - Centrifugation

The solids content of the must can be varied, using techniques described in an earlier lecture. The higher the solids the less fruity the wine, because the solid material contains esterases which break down aromatic compounds. If varietal character is to be minimized this is an advantage, if not, then it should be discouraged. The higher the solids, the higher the phenolics and the astringency, and the increase in the astringency might not be desirable. Settling and racking, and using a settling aid if necessary can control solids level. Centrifugation and filtration will also reduce solids.
but may lead to oxygen exposure unless performed under a modified atmosphere. High solids are stimulatory to the growth of both yeast and bacteria; over-clarified juices tend to ferment poorly.

Another option we have discussed previously is skin contact. The more contact with the skin, the higher the phenolic content.

**Skin Contact**

- Increase phenolics/astringency
- Impact yeast fermentation products
- Impacts microbial flora

Since the microbial flora of the grapes is located on the skins, skin contact also increases the contact of these organisms with the juice. If the skins are separated from the juice quickly, the microbes are also separated, minimizing their numbers in the primary fermentation. If the presence of the wild flora is desired, then some skin contact is beneficial. Since the skins are a source of phenolic compounds and, as we will see in later lectures, microbes can convert phenolic compounds to aroma compounds, the amount of skin contact will affect the aroma composition of the wine if those microbes are present.

Pressing conditions can also be used to modify the solids content. The more pressure applied, the higher the solids content and the more damage to the skins and seeds due to shear forces. One of your assigned readings is a seminal paper that covers the changes to must and juice composition with varying pressing regimes.

**Pressing Conditions**

- Higher pressure, higher solids content, harder to settle
- Warmer pressing, greater extraction

The warmer the temperature at pressing, the greater the extraction. Temperature of both skin contact and fermentation is important.
Whites are generally produced between 12 and 16°C. Lower temperatures of fermentation are desired, but the yeast are inhibited below 12°C, and fermentation is exceedingly slow increasing the chances of oxidative damage because a protective carbon dioxide blanket does not form - the rate of fermentation versus the rate of equilibration with the atmosphere is too low.

**Temperature**

- 12-16°C
- Lower temperature, greater retention

The lower the temperature the greater the retention of the volatile aroma character. One of the major aspirations of commercial yeast companies is the generation of yeast strains that will ferment well at lower temperatures.

The level of oxygen exposure will also have a profound affect on the composition and therefore quality of white wines.

**Oxygen Exposure**

- Can stimulate yeast
- Can lead to off-color (pink, brown) formation
- Can lead to development of oxidized characters

Oxygen exposure, as we have mentioned before, stimulates yeast. It also leads to off color production, and it can lead to the development of oxidized characters. So how much oxygen exposure is desired depends upon what the main problems are. If the wine tends toward arrest of fermentation, then oxygenation so that the yeast will be stimulated may be desired. However, oxygenation would not be desired if the wine were at high risk for a pink or a brown character developing, or if other oxidized characters, such as aldehydes, appear in the wine.

How can you prevent oxidation of white wine? Chemical antioxidants, which we have already discussed, can be used.
Preventing Oxidation of White Juices / Wines

- Use of chemical antioxidants: to block/mask oxidation reactions
- High Temperature / Short Time (HTST): to inactivate oxidases
- Use of low temperature: to inhibit oxidases
- Carbon dioxide / Nitrogen blanketing: to eliminate oxygen
- Clarification: to remove oxidases

**High Temperature/Short Time (HTST) treatments**, which expose the juice to a high temperature for a short time, can be employed to inactivate polyphenol oxidase activity. PPO can also be inhibited by sulfur dioxide. Molds, particularly *Botrytis*, also produce an enzyme capable of oxidizing phenolic compounds called laccase. Laccase is far more resistant to SO$_2$, and is not inactivated at the levels commonly used in wine production. If laccase is a problem, then HTST is the solution, as this enzyme will be denatured at high temperatures. However, HTST treatments will impact other components of the wine, and can lead to protein denaturation, aggregate formation and cloudiness in the finished wine.

The rate of enzymatic reactions is temperature dependent and will be slower the lower the temperature. PPO activity is reduced at **low temperatures**, so if the temperature is low enough and the yeast hearty enough to compete well for dissolved oxygen, browning can be minimized. Enzymes can also be physically removed, by adsorption to a **fining agent** binding proteins such as bentonite. However, such fining agents are not specific and other components may be stripped as well. If protection against oxygen is desired in wines before the yeast fermentation becomes active, the juice can be provided with an **artificial gas blanket**, by addition of carbon dioxide, nitrogen or argon. This can be done to the top of the tank or from the bottom racking valve, allowing the gas to rise to the surface. This latter technique also serves to mix the juice or must. In this case, dissolved oxygen in the juice will also be driven out of the tank. Again, what options are the most appropriate depends upon the nature of the problems with production of wine from a given vineyard.

**Lees Contact**
One post-fermentation variable used in white wine production is the length of time the wine remains in contact with the yeast sediment or lees. Yeast lees contact influences the aroma profile of the wine.

At the end of fermentation, yeast cells lose viability because an energy source is no longer available. Non-viable cells go through a process known as autolysis. During autolysis, the vacuole of the yeast cell deteriorates releasing the hydrolytic enzymes located in this organelle. These enzymes then degrade components of the yeast cell itself and lead to the lysis of the cell and release of cellular contents. This process affects the mouthfeel of the wine and results in the production of distinctive characters.

Oak

- **Fermentation in barrel**
  - New
  - Used
- **Kind of oak**
- **Kind of toasting**
- **Use of alternatives**
  - Chips
  - Staves

A final important processing variable for white (and red) wines is oak contact. Oak imparts distinctive characters to the wine.

Oak contact may be limited to barrel aging post-fermentation or it may occur during fermentation. Chardonnay for example is frequently fermented in oak barrels. The kind of Oak used and the processing of the Oak critically influence the oak characters that will be present in the wine. The effects of oak will be discussed in more detail in the lecture on aging. Alternatives to barrels, such as the use of oak chips or staves, can also be used. These techniques have the advantage of being cost effective, but many
expert tasters can tell the difference between barrel-fermented wines and those that
have been fermented in a stainless steel tank with wood addition. There are lots of
reasons why this might be the case that are not dependent upon the characters
extracted from the wood exposure. In contrast, many consumers cannot tell the
difference, particularly in the fighting varietal price category (7-10$).
Lesson 7: Blush and Rosé Wine Production

Blush (white wine made from red grapes) and rose (pink) wines are made using similar production practices for white wines.

Rosé Wine Production

There are two ways to produce a rosé wine:

1. As a blush wine of a red varietal
2. As a blend of a white wine with a red wine

The pink coloration of these wines may come from limited skin contact with a red variety (blush) or by blending of a white wine with a red wine (rosé).
In this section of the course we will cover the primary fermentation, the conversion of sugar to ethanol, which is the foundation of the transformation of grapes into wine. The first lecture will cover the basic biology of the yeast *Saccharomyces*. Subsequent lectures will cover all aspects of fermentation management, and the problems that can arise. Principle among these problems is off-character production and slow or incomplete fermentations.

The alcoholic fermentation is conducted by yeast of the genus *Saccharomyces*. The two common species involved are *S. cerevisiae* and *S. bayanus*. These two species are closely related, and the subject of a continuing debate among taxonomists as to whether they constitute separate species or races of the same species. *Saccharomyces* converts the glucose, fructose and sucrose found in grape must and juice into ethanol via the process of fermentation. In fermentation, an organic compound, in this case acetaldehyde, serves as terminal electron acceptor. This leads to the production of ethanol.
Lesson 8: Yeast Biology

Characteristics of *Saccharomyces*

- Eukaryote: possesses a membrane bound nucleus
- Reproduces by budding
- Grows vegetatively as haploid (1N) or diploid (2N)
- Capable of conjugation (1N to 2N) and sporulation (2N to 1N)
- Non-motile

*Saccharomyces* is a Eukaryote

*Saccharomyces* is a member of the kingdom of fungi. Fungi possess plant-like cell walls, but have other features more in common with animals. A significant amount of information is known about *Saccharomyces* due to the utility of this organism as an experimental system. Many of the fundamentals of genetic inheritance in eukaryotic cells were initially identified and studied in this yeast. The fungi are eukaryotic organisms meaning that they possess a membrane bound nucleus.

The nucleus has a double membrane structure. The outer membrane is contiguous with an organelle known as the endoplasmic reticulum. The endoplasmic reticulum
(ER) is involved in secretion of extracellular proteins and in \textit{de novo} biosynthesis of the plasma membrane.

\textbf{Saccharomyces Reproduces by Budding}

\textit{Saccharomyces} reproduces by a process called budding. A mother cell initiates a new replication cycle by formation of an immature bud. This process is called bud emergence.

The emerging bud is referred to as the daughter cell, and it appears at one end of the mother cell. This yeast displays multilateral budding, meaning that the site of selection of a new bud is toward one of the poles of the cell, where the curvature is greatest, but is not restricted to the pole. Each time a new bud is produced, a circular scar, called a bud scar, is left at the site of bud emergence. Counting of the number of bud scars is an indication of the number of cell divisions a particular mother cell has undergone. Yeast cells are mortal, meaning a limited life span. On average, a cell can only undergo roughly 40 cell divisions. After this point, the cell is no longer able to divide.

\textbf{Saccharomyces Grows Vegetatively as Haploid (1N) or Diploid (2N)}

\textit{Saccharomyces} can grow vegetatively as either a haploid or a diploid. Haploid cells have one set of chromosomes (1N) and diploid cells have two sets (2N). Many other organisms can only grow vegetatively as a haploid or a diploid, with the other state serving only for reproductive purposes.
Budding is asymmetric, meaning that the daughter cell is typically smaller than the mother cell, depending upon growth conditions. Daughter cells must grow to a critical mass before initiating a new cell cycle, that is, before becoming a mother cell. This serves to make sure that sufficient nutrients are available for the next cycle to go to completion. Yeast cells divide only under conditions of nutrient sufficiency. Their resting state is as an unbudded cell. After separation from the mother cell, the bud assesses the nutrient composition of the medium before making the decision to enter a non-growing or stationary phase or to divide.

*Saccharomyces* is Capable of Conjugation (1N to 2N) and Sporulation (2N to 1N)

Haploid cells of *Saccharomyces* can mate and produce a diploid cell. There are two yeast sexes or mating types. These have been termed "a" and "α".
Each of the mating types produces a peptide mating factor or pheromone that serves to signal their presence to cells of the opposite mating type. When two cells of the opposite mating type are near each other, they respond to the presence of mating type factor by growing in the direction of each other. This process is called shmoo formation. When the surfaces of the two haploid cells contact, fusion of the cell walls and membranes occurs. This is followed by fusion of the two nuclei and formation of a zygote. The zygote gives rise to 2N or diploid buds.

Diploid cells can generate haploid cells via the process known as sporulation. Under appropriate environmental conditions the diploid cell decides that rather than undergo vegetative cell division, a reductive or meiotic cell cycle will occur. In this process, the replicated DNA is divided into four nuclei, two each of the a and \( \alpha \) mating types. The nuclei are then surrounded by cytoplasm and a plasma membrane and cell wall. The four spores that result are still housed inside the mother cell, which has the appearance of a sac. This is why the yeast *Saccharomyces* is classified as an ascomycete. Ascomycete means that sexual spores are formed within a sac or ascus.
Because four spores are formed, the ascus is called a tetrad. The purpose of alternating haploid and diploid life cycles is genetic reassortment. That is why these spores are called sexual spores. They should not be confused with the asexual spores produced by bacteria and other fungi that function as highly resistant cellular forms. The sole purpose of the haploid spores after germination is mating. *Saccharomyces* strains may be either heterothallic, not self fertile, or homothallic, self-fertile. Heterothallic yeast strains produce spores that need to find a spore of the opposite mating type in order to form a zygote. They can mate with a sister spore (spore from the same ascus) or a non-sister spore. In contrast, homothallic yeast strains can mate with their own vegetative progeny. That is, a mother cell gives rise to a bud of the same mating type as the mother, then the mother cell switches to the opposite mating type, and can then mate with the daughter.

**Saccharomyces is Non-motile**

*Saccharomyces* is not motile, meaning that the cells do not display chemotaxis and the ability to move toward or away from specific environmental conditions. *Saccharomyces* displays the sub-cellular organization of the typical eukaryote. The outer most surface of the cell is comprised of glucan and phosphomannan, forming a tough cell wall. The cell wall is therefore composed of carbohydrate and protein.

**Characteristics of Saccharomyces: Sub-cellular Organization**

- Plant-like cell wall: comprised of carbohydrate (glucan, mannan) and glycosylated protein (phosphomannan protein)
- Mitochondria: site of oxidative reactions
- Vacuoles: site of storage and hydrolysis
- Secretory pathway
- Nucleus

*Saccharomyces* cells possess mitochondria, the site of oxidative phosphorylation and respiration. *Saccharomyces* can generate energy via respiration, with oxygen as the terminal electron acceptor producing water, as well as via fermentation. Other key biological activities are also localized in the mitochondria. Oxidative biosynthetic (fatty acid biosynthesis) and degradative reactions (proline degradation) are confined to the mitochondrion as well. This serves to limit the potential damage to other cellular components of any errant oxygen radicals that might be produced as a consequence
of enzymatic reactions involving molecular oxygen.

When viewed under a microscope, yeast cells contain a darkly visible circular structure. This is frequently confused with the nucleus, but it is instead another organelle, the vacuole. The vacuole houses hydrolytic enzymes and is the site of degradation of cellular components that are no longer needed. The advantages of locating these damaging activities in an organelle are numerous. The vacuole is also the site of cell storage. Excess amino acids, phosphate and other compounds are stored in the vacuole. In this case, the vacuole serves the same purposes in both yeast and plant cells.

The chromosomal DNA is housed in the nucleus. *Saccharomyces* possess 16 chromosomes. The sequence of the entire *Saccharomyces* genome has been determined. All of the candidate genes are now known. Systematic studies are underway to determine the function of each gene.

Yeast also possess a typical eukaryotic secretory pathway. The secretory pathway is comprised of the endoplasmic reticulum, Golgi bodies and secretory vesicles. New cell wall and plasma membrane growth occurs at the junction between the mother cell and growing bud. Cytoskeletal elements target the fusion of secretory vesicles to the region of rapid growth. For proteins destined for the cell surface, protein synthesis is initiated in the cytoplasm. Ribosomes synthesizing surface proteins associate with specific receptor proteins on the surface of the endoplasmic reticulum. The nascent or growing
peptide chain is then inserted across the ER membrane into the lumen of the organelle. The protein is then processed through a series of organelles, the Golgi bodies and secretory vesicles, and arrives at the cell surface fully modified and adjacent to the proper proteins with which it interacts.
Lesson 8: Glycolytic Pathway

The universal biochemical pathway by which sugars are degraded in an energy-yielding process to the three carbon compound pyruvate is called glycolysis. This pathway is found throughout the plant, animal, fungal, bacterial and archae kingdoms. Energy is generated in the form of ATP via a process called substrate level phosphorylation.

Glycolysis

- The set of biochemical reactions converting hexose (6 carbon) sugars to two 3 carbon pyruvate molecules, during which energy is released and recaptured in the form of ATP.

We can think of glycolysis as a process rearranging the energy in the bonds of a sugar molecule, so that a high-energy bond is formed that can then transfer that energy in a conservative manner to ADP generating ATP, the universal energy source. The energy in the ATP bond can be used to drive energetically unfavorable reactions.

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Glycolysis

Glucose + 2ATP + 2NAD⁺ + 2ADP + 2Pi

2 Pyruvate + 4ATP + 2NADH + heat
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This process requires the cofactor NAD⁺ that is converted to the reduced form NADH. Heat is also given off as an end product of glycolysis. One sugar molecule plus two ATP and 2ADP molecules are converted into 2 pyruvate and 4 ATP molecules. Early steps in the glycolytic pathway consume ATP. The first reaction is a phosphorylation of glucose (or fructose) at the six position.
With glucose as substrate, the second reaction is the isomerization of glucose-6-phosphate to fructose-6-phosphate. A second phosphorylation then occurs forming fructose 1,6-fructose diphosphate. Phosphorylation occurs to facilitate downstream rearrangement of bond energies. Fructose 1-6 diphosphate is cleaved into two three-carbon molecules, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Triosephosphate isomerase interconverts dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. Glyceraldehyde-3 phosphate is the entry molecule for rearranging bond energy. One ATP molecule is generated in the conversion of 1,3-diphosphoglycerate to 3-phosphoglycerate. The second ATP molecule is formed from phosphoenol pyruvate with pyruvate as the ultimate end product. At this point four
molecules of ATP have been produced, two replacing the initial ATP molecules used in phosphorylation of the sugars, and two net ATP molecules. Upper glycolysis refers to the ATP consuming steps and lower glycolysis refers to the steps that generate net ATP. It is important that upper and lower glycolysis be coordinated.

"Upper Glycolysis": consumes two molecules of ATP
"Lower Glycolysis": produces four molecules of ATP
NET PRODUCTION: TWO MOLECULES OF ATP

Sophisticated regulatory mechanisms exist to make sure that energy is not consumed in upper glycolysis unless lower glycolysis is fully functional.

Regulation of Glycolysis

- Transport: site of global rate control
- Allosteric enzymatic steps: localized rate control
  - Hexokinase
  - Phosphofructokinase
  - Pyruvate kinase
- Effectors of regulation: ATP, ADP, AMP, fructose2, 6 bisphosphate, citrate, glucose

The most important site of control of the rate of carbon flux through glycolysis is transport of the sugar into the cell. If problems arise limiting the glycolytic pathway, the rate of uptake is adjusted to match the maximal rate of flux through the entire pathway. This is a global response to limiting metabolic conditions. Internal pathway controls also exist to adjust the rates of flux between upper and lower glycolysis. Three key enzymes, hexokinase, phosphofructokinase and pyruvate kinase are regulated by small molecule effectors. These enzymes catalyze important reactions in the pathway. Hexokinase is responsible for the phosphorylation of glucose and fructose and phosphofructokinase converts fructose-6-phosphate to fructose 1,6-diphosphate. These two enzymatic reactions are ATP consuming. Pyruvate kinase generates pyruvate from phosphoenol pyruvate, generating ATP. It is not surprising that these key ATP consuming and producing reactions are tightly coordinated with other metabolic activities of the cell.
The production of pyruvate serves the purpose of generation of chemical energy in the form of ATP. But this process converts two molecules of the cofactor NAD+ to two molecules of NADH. Continued operation of the pathway necessitates converting the NADH back to NAD+.

Where does ethanol come from?

- The end products of glycolysis are pyruvate and 2 molecules of the reduced co-factor NADH.
- Yeast cells regenerate NAD+ by transferring the hydrogen molecule (electron) to an organic molecule: acetaldehyde

In the alcoholic fermentation, pyruvate is decarboxylated to acetaldehyde, which is then reduced to ethanol with concomitant formation of NADH.

Carbon dioxide is also an end product of glycolytic metabolism. Thus, the 6-carbon sugars are converted into 2 one-carbon molecules of carbon dioxide and 2 of the two-carbon molecule ethanol. Other organisms use other means to regenerate NAD+. Some reduce pyruvate directly to lactic acid.

Other organisms use different strategies to generate NAD+.

Their presence in wine leads to a diversity of end products of sugar catabolism.
The majority of the sugar is used to generate ATP. Respiration generates a greater yield of ATP (36-38) per glucose molecule and glucose is converted to six molecules of carbon dioxide. This is more efficient in terms of optimization of energy production when sugar is limiting. However, if substrate is plentiful, respiration is not necessary as energy needs can be met solely from fermentation. Roughly 95% of the hexose consumed appears as carbon dioxide and ethanol.

**Carbon Distribution at End of Fermentation**

- 95% = Carbon Dioxide + Ethanol
- 1% = New Cells
- 4% = Other End Products
  - Pyruvate
  - Acetate
  - Acetaldehyde
  - Glycerol
  - Lactate

Approximately 1% of the substrate is used as building blocks for new cells. About 4% of the initial sugar carbon will appear as other compounds, such as pyruvate, acetate, acetaldehyde, glycerol and lactate. The percentage varies, depending upon environmental conditions. Glycerol is produced from dihydroxyacetone phosphate. NADH is consumed in the process of making glycerol. Therefore glycerol production is increased under conditions limiting NAD\(^+\) generation from pyruvate. For this reason one tends to find production of glycerol accompanied by pyruvate release.

The theoretical maximum yield of ethanol is roughly 0.6 times the initial Brix reading. In practice, the actual yield is around 0.55 times the initial Brix.
One of the interesting properties of *Saccharomyces* is that given a high enough concentration of sugar, they will ferment even in the presence of oxygen.

Yeast will ferment even in the presence of oxygen. Why?

On the surface, this would seem kind of foolish since energy generation is more complete with respiration. If you can obtain between 36 to 38 ATP's per molecule of glucose, why would a cell settle for 2? It is actually more rapid to get ATP simply from glycolysis and fermentation than from respiration. Further, the phenolic compounds present in grapes may interfere with respiration. This is one of the principle classes of anti-fungal compounds produced by the grape. Ethanol is also inhibitory to respiration.

Fermentation vs. Respiration

- Fermentation: 2 ATP/glucose (fructose)
- Respiration: 36-38 ATP/glucose (fructose)

Efficiency of ATP yield is only an issue if sugar is limiting

Thus, *Saccharomyces* has developed biological mechanisms to assure that fermentation will occur in preference to respiration. Glucose itself regulates the switch between fermentation and respiration. High sugar concentrations repress the synthesis
of mRNA from genes that are involved in respiration and oxidative metabolism.
Lesson 8: Choice of Yeast Strain

Individual yeast strains possess different physiological traits. There are several traits that are highly desired in wine strains of *Saccharomyces*.

Yeast Choice: Desirable Traits

- Fermentation to dryness
- Reasonable rate of fermentation
- Predictable fermentation characteristics
- Good ethanol tolerance
- Good temperature tolerance
- Sulfur dioxide tolerance
- Little to no off-character production
  - Hydrogen sulfide
  - Acetic acid
  - Ethyl carbamate
- Little to no inhibition of other desirable microbes
- Killer factor resistant
- Production of desired aroma characters

The most important characteristic is that the strain be able to complete the fermentation, **leaving little to no residual sugar**. It is also critical that the strain display a **reasonable rate of fermentation**. It is problematic if the rate is too fast as well as too slow. A slow rate of fermentation becomes difficult to distinguish from a problem fermentation. If the rate is too fast, the fermentation may reach too high of a temperature due to the rate of heat release from metabolism. Rapid fermentations may also lead to increased loss of volatile components. It is also valuable if the strain is **predictable in terms of its fermentation characteristics**. This also aids in identification of problematic fermentations and in diagnosis of the cause of the decrease in fermentation rate. It is also important to have a predictable fermentation as this allows for optimal use of fermenter space and planning of the harvest. The strain also needs to display a **reasonable tolerance to ethanol**. Wine strains of *Saccharomyces* are typically resistant to 16-17% ethanol. Many wild strains are not as tolerant, and may be resistant only to 12-14% ethanol. If the grapes are harvested at a high Brix level, these strains might not be able to complete the fermentation. Another parameter that can inhibit yeast metabolic activities is temperature. Since wine is
frequently fermented at extremes of temperature to which *Saccharomyces* is tolerant, it is critical that the strain used not be unduly inhibited by heat or by very cool conditions. White wines are generally fermented at as low of a temperature that the yeast can tolerate (12-14°C) while many red wines are fermented at temperatures as high as the yeast can tolerate (35-42°C) in order to facilitate extraction.

Since sulfur dioxide is used as an antioxidant, and has antimicrobial properties; therefore it is important that the yeast strain used be tolerant of sulfur dioxide. As mentioned in an earlier lecture, *Saccharomyces* detoxifies SO$_2$ via the formation of acetaldehyde adjuncts. It is important that the strain used be tolerant of the SO$_2$ levels used in the winemaking procedure. Sulfur dioxide tolerant strains are viable in fairly high concentrations of SO$_2$ (200-500 ppm).

It is desirable that the yeast strains present during fermentation not have any "bad habits", that is, produce little to no off-characters. This will be discussed in detail in a later lecture, but the most important class of off-characters is hydrogen sulfide "rotten egg" and other sulfur-containing volatile compounds. *Saccharomyces* also makes acetic acid, but generally not in a high enough concentration to be above the threshold of detection. Nevertheless, many commercial strains are available with little to no acetic acid production. It is also important that the yeast strain used be compatible with any other microbes that are desired in the fermentation. There are "good" and "bad" match ups of specific yeast strains and the lactic acid bacteria. While the exact reasons for the inhibition of one organism by the other are not known, it is likely due to competition for micronutrients or the production of inhibitory end products. Killer factor refers to a peptide produced by one strain of *Saccharomyces* that kills other strains of *Saccharomyces*. A virus like particle that yeast seem to have acquired at some point in their evolutionary life encodes killer factor. There are strains that both produce and are resistant to killer factor, strains that do not produce the factor but are resistant to it, and strains that are sensitive. Many wild strains of *Saccharomyces* either produce or are resistant to killer factor. The majority of the commercial strains are resistant, but may not produce killer factor. It is important to make sure that any novel strain selected by the winemaker is killer-factor resistant.

One final important property of yeast strains is their ability to confer the desired organoleptic characters to the wine. This is particularly important in sparkling wine production. However, even in table wines certain yeast strains may be preferred. The characters produced by yeast will be discussed in a later lecture.
Lesson 8: Yeast Nutrition

One of the most critical components of management of the yeast fermentations is to make sure that the yeast have all of the requisite nutrients to maintain fermentation rates and optimal ethanol and temperature tolerance. A typical yeast fermentation profile is shown below. Glucose is consumed at a faster rate than fructose, likely due to the different kinetic properties of glucose and fructose transport and metabolism. However, late in fermentation, the relative concentration of fructose will be higher than that of glucose.

The maximal rate of fermentation of both sugars coincides with the maximal viable cell biomass. Cells lose viability late in fermentation, once the usable sugar has been consumed. The maximal cell population for *Saccharomyces* is 1-2 x 10^8 cells/mL. Fermentations may attain this level, but are typically on the order of 2-5 x 10^7 cells/mL. The final cell concentration achieved is dependent upon the nutrients present in the must or juice. There are two principle classes of nutrients: macronutrients and micronutrients.
Yeast Nutrition

- Macronutrients: Building blocks needed for new cell material
- Micronutrients: Catalysts needed to facilitate biochemical reactions

Macronutrients are the compounds that supply the needs for cell division and energy generation. They are needed in high or stoichiometric amounts. In contrast, the micronutrient vitamin and minerals are required in much lower amounts and are catalysts involved in many enzymatic reactions.

Macronutrients

- Carbon/Energy Sources: glucose, fructose, sucrose
- Nitrogen Sources: amino acids, ammonia, nucleotide bases, peptides
- Phosphate Sources: inorganic phosphate, organic phosphate compounds
- Sulfur Sources: inorganic sulfate, organic sulfur compounds

The macronutrients are sources of carbon, nitrogen, phosphate and sulfate. These four elements are required for production of a new cell as well as for maintenance of a cell in stationary phase. In grape juice the carbon sources available for *Saccharomyces* are plentiful: glucose, fructose and sucrose. Grape juice contains other sugars and carbon compounds used by other organisms as carbon and energy sources, but the *Saccharomyces* repertoire is more limited. *Saccharomyces* is able to use the following compounds as energy sources. Only the sugars are fermented, the other compounds can only be used under conditions conducive to respiration.
Macronutrient Energy Sources

- Monosaccharides: glucose, fructose, galactose, mannose
- Disaccharides: sucrose, maltose, melibiose
- Trisaccharides: raffinose
- Pentoses: None
- Oxidative Substrates: pyruvate, acetate, lactate, glycerol, ethanol

The major nitrogen sources are ammonia, amino acids, nucleotide bases and small peptides. There are other nitrogen sources that Saccharomyces is not able to use, but that will support the nutritional needs of other microbes. Not all amino acids can be completely degraded by Saccharomyces, especially under anaerobic conditions. Amino acids can be classified into different categories based upon their ability to be utilized as sole nitrogen source.

Categories of Yeast Nitrogen Sources

- Compound may be used as that compound for biosynthesis
- Compound may be converted to related compounds for biosynthesis
- Compound may be degraded with release of nitrogen

All 20 amino acids can be transported and used directly as that amino acid in protein biosynthesis. Many amino acids may be converted to related amino acids, such as the interconversion between cysteine and methionine. A smaller subset of amino acids can be completely degraded releasing ammonia, which can then be used in biosynthesis. That is, can serve as sole nitrogen source. Sole nitrogen source means that the yeast can grow if that amino acid is the only nitrogen-containing compound in the medium. Amino acids, such as glycine, histidine and lysine, do not serve as sole nitrogen sources, but they can be important nutritional components of juice. Their presence means that they will not have to be synthesized and the available nitrogen sources can be mobilized to synthesize other needed compounds.
Yeast Nitrogen Sources

- Degradation may depend upon availability of other components: vitamins and oxygen
- Utilization impacted by other environmental factors such as pH

Whether a specific nitrogen compound can be used as a nitrogen source or not depends upon other factors. If specific micronutrients are required for the degradation and those nutrients are not available in the fermenting must or juice, the yeast will not be able to use that compound.

*Saccharomyces* can use inorganic or organic sources of phosphate, but is not permeable to compounds other than inorganic phosphate. The yeastsecreted phosphatases that degrade the organic forms of phosphate external to the cell, allowing the inorganic phosphate that is released to then be taken up by the cell. Yeast sulfur sources are sulfate or the sulfur-containing amino acids.

The micronutrient composition of juice is just as important as that of the macronutrients. Micronutrient deficiency might prevent synthesis of a single crucial compound the lack of which will lead to arrest of cell growth and perhaps of fermentation.

**Micronutrients**

- Mineral and Trace Elements: Mg, Ca, Mn, K, Zn, Fe, Cu
- Vitamins: biotin is the only required vitamin, but others are stimulatory

The essential minerals and trace elements are the cations required universally by eukaryotic organisms. Biotin is the only vitamin that yeast cannot synthesize *de novo*, and at least a precursor of this vitamin must be present in the grape juice. While other vitamins can be synthesized, yeast growth and fermentation is accelerated in the presence of these compounds.
Yeast require nutrients during active growth, but also require compounds during the non-proliferative phase of fermentation and to prevent loss of viability as ethanol accumulates in the medium.

The nutrients required for these phases differ somewhat.

**Nutritional Requirements of Different Phases of Fermentation**

- **Growth Phase**: Building blocks and catalysts
- **Stationary Phase**: Survival factors

Growth phase requires that the compounds needed for net synthesis of new cell material be present in sufficient quantities to encourage division. These are the building blocks and essential micronutrient catalysts. The compounds required for maintenance of fermentation rates and viability during stationary phase are called survival factors.
Most of the fermentation is conducted by stationary phase cells

- **Stationary phase:**
  1. rate of growth = rate of death
  2. quiescent, no growth, no death
- **Stationary Phase: Survival factors**

As seen from the typical yeast fermentation profile presented above, stationary phase cells conduct the bulk of the alcoholic fermentation. Stationary phase has two different definitions. It is characterized by the failure to detect an increase in cell number. This may arise because the rate of cell death equals the rate of cell division. In this case, the total number of cells present will remain the same. A second type of stationary phase is truly non-proliferative. That is, there is no cell division nor is there cell death. Cells are simply not dividing. There is evidence for both types of stationary phase in yeast strains during vinification. Survival factors are important for the maintenance of cell viability.

**Role of Survival Factors**

- Maintain viability of cells
- Increase ethanol tolerance
- Maintain energy generation

Survival factors maintain cell viability by providing the nutrients needed to repair cellular damage and support the limited synthesis of needed proteins and other cellular components. Survival factors also increase ethanol tolerance and help maintain fermentation rates and energy generation.
Survival Factors

- Oxygen
- Fatty Acids
- Sterols
- Nutritional Factors

The yeast survival factors are fatty acids and sterols. These compounds are needed for ethanol tolerance. This topic will be discussed in more detail in a subsequent lecture. Molecular oxygen is required for the synthesis of these cellular components, so if oxygen is present, there is no need for fatty acid and sterol supplementation. Nutritional factors such as nitrogen are also required. This is because protein synthesis is required to maintain the transport capacity of the yeast cells and therefore of fermentation.

How Does Ethanol Inhibit Yeast?

- Displaces water of hydration changing the properties of protein-lipid interactions
- Denatures proteins
- Disrupts protein active sites
- Allows increased passage of protons from the medium into cell leading to acidification of the cytoplasm
- Removal of protons requires expenditure of energy

Ethanol has several inhibitory effects on yeast cells. Ethanol disrupts protein-lipid interactions in the plasma membrane. It may also disrupt internal bond interactions in proteins leading to their denaturation and/or inactivation. Further, ethanol allows hydrogen ions to penetrate the yeast cell membrane at a higher rate than in the absence of ethanol. This can lead to acidification of the yeast cytoplasm and cell death if it exceeds the capacity of the cells to correct. Removal of protons that have leaked into the cell is an energy requiring process. This is why the cells quickly lose viability in the absence of an energy source late in fermentation. The required fatty acids and sterols are needed in order to synthesize an ethanol tolerant plasma membrane. The nitrogen is needed because different proteins, not as susceptible to damage by ethanol, must be synthesized.
Survival Factors

- Needed to alter composition of the plasma membrane (sterols, fatty acids and proteins) so that it can withstand the perturbing effects of ethanol
- Both phospholipids and protein content must be adjusted

The winemaker must make sure that the nutritional needs of the yeast are met during active growth as well as for maintenance of ethanol tolerance. Nutrients can be added to stimulate biomass production and prevent fermentation arrest.
Fermentation Management

This lecture will describe the enological parameters impacting yeast fermentation performance and strategies for management of the fermentation. We will survey the tools and options available to the winemaker for manipulation of the metabolic activities of yeast. As with other operations, there are many decisions that must be made by the winemaker that will impact the microbial contribution to the composition of the finished wine. The first decision is whether or not the fermentation will be conducted spontaneously or by use of a commercial culture.
Lesson 9: Native Flora versus Inoculated Fermentations

*Saccharomyces* is isolatable from vineyards in very low numbers, and can be found as part of the winery flora in very high numbers, especially once crush is underway. Spontaneous fermentations are conducted by vineyard and winery flora.

**Sources of *Saccharomyces***

- Vineyard flora
- Winery flora
- Inoculum

The alternative is to use a commercial preparation as an inoculum. In this case, the characteristics of the primary strain conducting the fermentation are known, and potential fermentation problems can be minimized. Native flora or uninoculated fermentations are not necessarily problematic, but it is incumbent upon the winemaker to monitor them a bit more closely and to take appropriate action should a problem arise in the ferment. If a commercial strain has been used in a winery, it requires about 3 years of use of a different strain to displace the original strain in the winery.

**Inoculated Fermentations**

- With active dry yeast (ADY)
- With a starter culture in juice
- With an already fermenting must/juice

There are several ways in which a fermentation may be inoculated. One can use an active dry yeast culture, rehydrated according to instructions on the packet. Some yeast manufacturers recommend rehydration in the presence of a nutrient mix. If the winery has the capability, liquid starter cultures can be prepared in either juice or a defined medium, and used to inoculate juice or must. Not all strains are amenable to commercial production in a dehydrated form. If such a strain is desired, it will need to be prepared as a culture. Some yeast suppliers offer this service to wineries. An alternative method is to inoculate a juice with a juice that is already in the active phase of fermentation. The typical inoculum in all cases is between $10^5$ and $10^6$ cells/mL or 1
to 0.1% on a volume-to-volume basis. Higher strength inocula are also occasionally used. More yeast esters are produced with very high inocula, which may or may not be desirable depending upon the style of wine. Yeast cultures in stationary phase make a characteristic rose oil perfume ester called phenethyl acetate in addition to characters commonly associated with bread. These two classes of characters are not necessarily harmonious, depending upon the rest of the composition of the wine and the goals of the winemaker.

**Level of Inoculum**

- Typically $10^5 - 10^6$ cells/ml or 1 to 0.1% on a volume/volume basis

Inoculated fermentations are more predictable in terms of onset, duration and maximal rate of fermentation than are native flora fermentations. Because of the predictability problems arising in the fermentation, discussed in the next lecture, can be readily recognized and appropriate treatments performed. Many commercial strains are available that are quite neutral in the aroma characters produced. These are desired when the aim is to minimize the contribution of the yeast, such as in cases where the style emphasizes varietal character.

**Inoculated Fermentations: The Benefits**

- Predictability
- Control of spoilage characteristics
- Neutrality: enhanced varietal characteristics

With a clean start to the fermentation, the yeast is able to dominate quickly minimizing the organoleptic contribution of the indigenous microbial flora. Again, depending upon the style produced, this may be desirable or undesirable.
Inoculated Fermentations: The Negatives

- Reduce overall complexity
- Fermentation rates too rapid
- Wine too "yeasty"

The undesirable effects of inoculation are the overall reduced complexity of the wine due to the absence of microbial characters. In a sense, the wines are as predictable as the yeast strain used. In some segments of the marketplace, this is considered quite negative in and of itself. In other segments it is desired by the consumers. Inoculated fermentations, especially if overfed, may occur too rapidly which as we have discussed before, leads to heating of the ferment and the loss of volatile flavor and aroma characters from the juice or must. A final problem is that inoculated fermentations may have too strong of a yeast signature, that is, be too yeasty and bread-like.

Spontaneous or native flora fermentations in contrast are generally not predictable unless they are being taken over by a commercial strain that dominates the winery flora. This is frequently the case in many wineries. The level of yeast inoculum from winery equipment is on the order of $10^3$ to $10^4$ cells/mL, depending upon sanitation practices, the type of equipment used, the commercial strains used and their ability to form a biofilm. Lower initial biomass delays the onset of the fermentation and the domination of the must or juice by *Saccharomyces*. This allows the non-*Saccharomyces* flora to produce metabolites that will contribute to the aroma profile of the wine, but still assures that a strain capable of finishing the fermentation is present.

Native Flora Fermentations: The Benefits

- Increased microbial complexity
- Slower fermentation rates

Therefore, the primary benefit of a native flora fermentation is the increase complexity of microbial characters of the resulting wine. Some of these notes may be quite subtle, and enhance perception of varietal character. In other cases, the microbial contribution may be dominant over that of the varietal. There are many wines that are intensely microbial in the origin of the major aroma characters. A secondary benefit of native flora fermentations is their slow rate of fermentation. This minimizes heating of the
ferment and thus of loss of volatile varietal characters. This again results in an enhancement of varietal character of the wines.

Native Flora Fermentations: The Negatives

- Off-character formation
- Lack of predictability
- Seasonal variation in microbial populations on fruit

However, the native flora can produce undesirable characters in the wine that may detract from wine quality. They are the main producers of acetic acid and ethyl acetate, which are reminiscent of vinegar in low concentrations, but in high concentrations have the aroma of nail polish remover. These characters are volatile and may be lost later in the fermentation or, in the case of acetic acid, reconsumed by other organisms. This is not always guaranteed, of course, especially if a cool fermentation is being conducted. Another problem with native flora fermentations is lack of predictability. Since the size of the inoculum is not known nor are the fermentation characteristics of the dominating yeast strain, it is not possible to predict how quickly a fermentation should commence or if the fermentation rate is maximal or reflects a nutritional problem. Since the non-*Saccharomyces* flora make a spectrum of undesirable characters, especially from the degradation of amino acids, nutrient supplementation may not be a good option. Their unpredictability is also an advantage in a segment of the marketplace, as the composition of the wines will vary from vintage to vintage. When it works well, the wines can command a high price, but vintages of much lower quality are expected and therefore still marketable. This variability in characters formed is due in part to the variability in the chemical composition of the juice or must, but it is also due to seasonal variability in the numbers and kinds of flora present in the must or juice. What organisms are present is influenced by vineyard operations and practices as well as to changes in environmental conditions (humidity, by presence of insect vectors, disease pressure and berry infection, for example).

Many wineries that conduct native flora fermentations take out an "insurance policy". Roughly 10% of the juice or must is inoculated with a neutral commercial yeast. This can be used as an inoculum for the native flora fermentations if a problem (off-character, reduced fermentation rate) arises.
Lesson 9: The Role of Non-Saccharomyces Flora in Wine Production

Second Decision

Encourage or discourage grape berry microflora?

Grape Berry Microflora

- Bacteria
- Molds
- Yeast

Whether fermentations are inoculated with *Saccharomyces* or not, the winemaker must decide if the non-*Saccharomyces* flora is to be encouraged or discouraged. The berry flora is comprised of bacteria, molds and yeasts.

**Bacteria**

- *Bacillus*
- *Pseudomonas*
- *Micrococcus*
- Lactic Acid Bacteria
- Acetic Acid Bacteria

The bacterial species most prevalent on the surface of grapes are members of the genera, *Bacillus*, *Pseudomonas* and *Micrococcus*. The acetic acid bacteria, principally *Acetobacter* are also present. Members of these genera are strict aerobes, so once the juice becomes anaerobic further growth and metabolism is inhibited. Many of them have mechanisms for surviving periods of anaerobicity, so one should not assume that they are completely eliminated in the ferment. In fact, they will be viable at the air interface at the top of the tank until that headspace is displaced by the carbon dioxide of active fermentation. In the case of red wine production, the process of
pumping over bathes the cap in ethanol, which is toxic to many of the aerobic organisms, but not to all.

The final class of organisms that are predominant on grape surfaces are the lactic acid bacteria. **The lactic acid bacteria** are facultative anaerobes, so they can persist under anaerobic conditions, depending upon the pH of the juice or must.

Minor species may also be found, some of which are not berry residents but are indigenous to the vineyard soil or other parts of the plant. Vineyard and harvesting conditions may lead to their inclusion in the must or juice. These organisms do not generally persist under juice or must fermentation conditions but can contaminate other winery surfaces. **Streptomyces** for example is a cellulose degrader and can infect winery filtration apparatus if appropriate sanitary practices are not employed. This organism is responsible for the characteristic odor of dry dirt, which is quite noticeable in the wine upon filtration. The diversity of **Bacillus** species present also depends upon the amount of soil contamination of the fruit. Members of this genus are particularly troublesome if they infect a winery because of the production of spores that are highly resistant to both heat and chemicals. They are therefore difficult to displace from the winery flora. **Bacillus** infection is not common in California, but is present in other wine growing regions of the world.

**Molds**

- **Aspergillus**
- **Penicillium**
- **Rhizopus**
- **Mucor**
- **Botrytis**

Several genera of molds are also found. The molds and the yeast are classified together as fungi. To be called a "yeast" the organism must exist vegetatively primarily as single free-living cells. Molds are found primarily in the mycellial or multicellular form. The four most commonly isolated molds are members of the genera **Aspergillus, Penicillium, Rhizopus** and **Mucor**. These molds are found just about everywhere in the environment. They function in the degradation of complex organic material. These fungi produce both sexual and asexual spores. The asexual spores largely function in dispersal of the colony. They can be carried great distances by wind
or deposited locally. Fungi are communal organisms due to their principle biological activity of degradation of biological matter. The fungi are prolific secretors of hydrolytic enzymes allowing digestion of material outside of the cell. This process is greatly accelerated by a community of cells working in concert. In this case a critical mass of catalytically active enzymes can be produced quickly and serve to feed the entire fungal population.

Some fungi are able to initiate the degradation process on living material. They possess characteristics that allow penetration of the cell walls of plants for access to plant nutrients. At some point these organisms cross the line between free-living and pathogenic. *Botrytis* is able to infect berries on the vine leading to deterioration of the fruit. As noted in an earlier lecture, wines made from Botrytized fruit contain many unique and distinctive characters as a consequence of the infection. Some of these notes derive from the plant and are produced in response to infection, others arise due to the impact of mold metabolites on the flora conducting the fermentation, and some may derive from *Botrytis* itself. Other genera of molds are opportunistic, that is, will participate in an infection once initiated by another organism, but are not capable of causing disease on their own.

The molds are all obligate aerobes and are not present during the active phase of fermentation. They also do not persist in the fermentation in contrast to the bacteria. Initial must platings are dominated by mold, but platings we have done from juice or must just hours after crushing display little to no mold. The aerobic bacteria are frequently still isolatable at this time.

### Yeast

- *Kloeckera/Hanseniaspora*
- *Metschnikowia pulcherrima*
- *Hansenula* species
- *Candida* species
- *Saccharomyces*

Four yeast genera are typically found on the surface of the grape berry: *Hanseniaspora, Metschnikowia, Hansenula* and *Candida*. Taxonomists have historically divided the yeast into two categories, the ascomycetes and the basidiomycetes, based upon whether the sexual spores are formed inside of a
structure or sac (ascomycetes) or are borne on a stalk (basidiomycetes). This classification depends upon the ability to observe sexual spores in an isolate. There are other factors such as the cell wall structure and type of budding of vegetative cells that can also be used to classify yeast isolates as ascomycetes or basidiomycetes. However, the practice that has developed is to separately categorize isolates for which no sexual stage has been observed. These organisms are called the deuteromycetes. Many of the deuteromycetes are identical in all other properties to isolates classified as ascomycetes or basidiomycetes. When this is the case, they are referred to as the imperfect form of the organism for which a sexual cycle has been observed. The strain possessing the sexual stage is called the perfect form. Grape and wine isolates are members of the ascomycetes. Members of the genus *Hanseniaspora* are perfect forms, that is, possess documented sexual cycles. The imperfect forms of these yeasts are classified as *Kloeckera*. These yeasts are identical in all ways except the observation of a sexual cycle. One of the most commonly isolated strains from grape surfaces is *Kloeckera apiculata*, the imperfect form of *Hanseniaspora uvarum*. Another commonly isolated yeast is *Metschnikowia pulcherrima*, the perfect form of *Candida pulcherrima*. In this case the species names are the same making it easier for the novice to recognize that these designations represent the perfect and imperfect forms of the same organism. These names are used interchangeably in the wine literature for the most part. Comparative analysis of DNA sequences from each of these pairs of organisms clearly supports their identity to each other. Be that as it may, the convention is still to use the two different designations, with the "perfect" designation reserved for those isolates for which a sexual stage has been observed. The ability to compare DNA sequences is revolutionizing the field of taxonomy, and in the near future the deuteromycetes may disappear.

*Hansenula* and other members of the *Candida* genus are also frequently isolated from grape surfaces. More rarely isolated are *Pichia* and *Issachenkia* species, yeasts that are commonly found on fruit surfaces. *Saccharomyces* can also be isolated from grape surfaces but not in great numbers. Many yeast ecologists believe that *Saccharomyces* is not a normal resident of grape surfaces as it is not tolerant of UV light and is out-competed by other yeasts when a direct inoculation of the fruit is attempted. *Saccharomyces* is referred to as a domestic yeast, sort of the Cocker Spaniel of the fungal world. *Saccharomyces* is readily isolatable from humans and may be transferred to the vineyard by human contact. If the yeast lees are used in the vineyard as fertilizer, then *Saccharomyces* might also be transferred from the winery to the vineyard and back again. This cycling is thought to have selected for regionally specific strains of yeast.
Grape Berry Microflora

- 95-98% of total organisms are molds and bacteria
- 2-5% are yeast, principally *Hanseniaspora* and *Metschnikowia*
- Non-*Saccharomyces* yeasts present at levels of $10^5$- $10^6$ organisms/mL, *Saccharomyces* present at $10^{-2}$ - $10^{-3}$ cells/mL

The majority of the microbial biomass of the grape surface is comprised of the molds and bacteria. The yeast represents around 2 to 5% of the isolatable organisms. Non-*Saccharomyces* yeasts are present at the levels of $10^5$ to $10^6$ in intact fruit, but can rise to $10^7$ to $10^8$ in later harvest fruit or in fruit with some berry damage. *Saccharomyces* is found at levels of $10^{-2}$ to $10^{-3}$ cells per mL, meaning that there is 1 cell in 100mL to 1 cell in 1 Liter. Numerous factors impact the types and numbers of microbes on the grape surface.

Factors Affecting Grape Berry Microflora

- Rainfall / Humidity
- Insect vectors
- Altitude
- Temperature
- Vineyard fertilization practices
- Varietal factors: tightness of cluster
- Vineyard practices: inoculation of fruit with soil microbes

**Rainfall** or **high humidity** favors growth of the molds, and accompanying damage to the berries then encourages the growth of bacteria and some of the yeasts as well. **Insect vectors** are also important. The molds produce air-borne spores that are able to disperse the culture. The bacteria but principally the yeast rely on insect vectors to be relocated. In fact, there are numerous examples of symbiotic relationships between yeasts and insects. The yeast produces esters that attract the insects to a fruit food source. The insects then feed and pick up the microbial flora on their bodies. As they visit other clusters, the yeast can then be deposited. This phenomenon can readily be observed in the winery. Fermentations producing high amounts of acetic acid and ethyl acetate are strongly attracting to the fruit fly *Drosophila*. *Drosophila* infestation of the
The winery can quickly spread *Acetobacter* and the yeasts throughout the facility.

The **altitude** of the vineyard also influences the flora of the fruit. It is not clear if this is a direct effect of altitude or an indirect effect, that is, different altitudes support different insect populations and feeding activity. Interestingly, in one of the few comprehensive studies that was undertaken, *Hanseniaspora* was isolated from high altitudes while *Kloeckera* was found at lower altitudes. This suggests some selective advantage to maintaining the ability for sexual reproduction in some environments.

The mean **temperature** of the vineyard also impacts the flora present. At very high temperatures under dry conditions, such as those found in Davis and the Central Valley of California, *Saccharomyces* does not persist in the vineyards. This yeast does not display the range of temperature tolerance of the other organisms present, another indicator that the native environment of this yeast is likely not the berry surface. It is not clear if this is due to the heat alone or to dehydration or to inhibition by other microbes more tolerant of heat.

**Vineyard fertilization** practices are also important. What is being applied and how it is being applied can both have an impact. Foliar applications of nutrients favor the growth of those organisms that can utilize the compound being applied as a nutrient. Foliar application of urea is used in fruit crops to encourage the growth of the beneficial native flora. In the case of apples, it was shown to increase the numbers of bacteria. The increase in bacteria resulted in a decrease in mold, and reduces the incidence of mold infestation and spoilage. Urea also used to be used in vineyards, but this practice was terminated when it was discovered that urea in the presence of ethanol will react spontaneously to produce the carcinogen ethyl carbamate.

**Varietal specific factors** also impact the numbers and kinds of flora present. Varieties prone to grape damage, such as those with tight clusters, release more nutrients to the surface of the fruit, making them available to support microbial growth. The tendency of the fruit to produce antimicrobial substances will also impact the flora if any susceptible microbes are present.

Finally, **vineyard practices** also can affect the microbial flora of the fruit. If soil is stirred up so that dust can land on the fruit, soil microbes might be transiently associated with the grape surface. Time of harvesting is also important. We have seen greater numbers of both molds and bacteria on late harvest fruit that otherwise appears to be healthy and free of infection. Vineyard practices also impact the insect vectors that may be available and therefore the spread of microbes through the vineyard. Environmental factors such as the practice of leaf pulling and presence of
wind are also critical, as these will impact local cluster humidity. In addition to factors affecting the microflora of the fruit, must or juice composition and processing factors will impact the persistence of the flora in the fermentation.

Factors Affecting Persistence of Grape Berry Microflora in Must/Juice

- pH
- Temperature
- Oxygen
- Nutrient Levels
- Presence of Inhibitors
- Microbial Interactions
- Inoculation Practices
- Winery Practices

pH

- Low pH (<3.5) inhibits many bacteria
- Yeast not pH sensitive at normal juice pH values (2.8-4.2)

One of the most important factors is pH. The **low pH** of wine is inhibitory to many bacterial species. If the value is below pH 3.5, many of the lactic acid bacteria are unable to grow.

Temperature

- Low temperatures inhibit bacteria
- Low temperature enriches for non-*Saccharomyces* yeasts
Temperature is another important factor, just as it was in the vineyard. Holding the must at low temperatures is inhibitory to the bacteria. The yeast *Saccharomyces* is also inhibited at low temperature and will not initiate fermentation below about 12°C. *Hanseniaspora/Kloeckera* tolerates low temperatures well and will be dominant in musts following a cold soak. Thus, the processing decision to do a cold soak will impact the flora present. The longer the soak, the stronger the effect on the flora. If a procedure encouraging native flora has been performed, addition of nutrients may feed this population and not the intended *Saccharomyces*.

**Oxygen**

- Lack of O$_2$ inhibits all molds
- Lack of O$_2$ inhibits aerobic bacteria
- Oxygen stimulatory to yeast: not clear how different species are affected

The presence or absence of oxygen is likewise an important factor. As mentioned previously, many of the members of the berry flora are aerobes. If the juice receives an oxygen treatment (is aerated) before the development of *Saccharomyces*, spoilage may occur. It is also important to mention that oxygen is stimulatory to the facultative anaerobes as well. As we will see in the lecture on the lactic acid bacteria, these organisms can produce acetic acid as an end product of metabolism in an energy-generating pathway if molecular oxygen is available. *Saccharomyces* is an excellent competitor for oxygen, so if aeration is needed, it should occur after the yeast has become established.

**Nutrient Levels**

- Must/Juice composition
- Supplementation
- Timing of addition

Nutrient levels are clearly going to impact the organisms present and whether or not they are competing for nutrients or there are ample to go around. Nutrients arise in the
grape itself, but are obviously influenced by nutrient addition practices. The timing of addition is important as discussed above. The dominant species at the time of feeding will be the group that benefits the most from the addition. Again, if *Saccharomyces* is the intended beneficiary, nutrients should be added once this organism has become established or following inoculation with a commercial culture.

**Presence of Inhibitors**

- Fungicide/pesticide residues
- Sulfur dioxide

The use of pesticides and fungicides in the vineyard will impact the relative ratios of organisms on the fruit, but if applied too close to harvest, may also influence the microbial flora of the fermentation. It is important to remember in this regard that *Saccharomyces* is a fungus and may be inhibited by some antifungal agents. Many commercially available compounds have been tested for effects on the flora of the fermentation, but the vineyard manager should make sure these studies have been done before applying agents to the fruit.

Sulfur dioxide has been mentioned before with respect to its antimicrobial activity, and depending upon juice conditions may be quite inhibitory to the flora present.

**Types of Microbial Interactions**

- Production of Inhibitors
  - Acetic acid
  - Ethanol
  - Fatty acids
  - Killer factors
- Competition for nutrients
- Stimulation
  - Removal of inhibitor
  - Release of micronutrients
Interestingly, but not surprisingly, the persistence of microbes in the ferment is affected by the types of other microbes present. Microbes compete for nutrients and if nutrients are limiting, those that are more able to consume them will reduce the numbers of those that are not. In addition to competition, some organisms produce end products that are inhibitory to other organisms. For example, ethanol is inhibitory to most bacteria and molds and many yeasts. Those normally associated with ferments are more tolerant, but the non-Saccharomyces flora can be inhibited at concentrations as low as 7% ethanol. Cell division in Saccharomyces is reduced at this level and above as well. Acetic and other organic acids are also inhibitory. Saccharomyces is less tolerant of acetic acid than are other organisms, but many of the acetic acid producers will produce sufficient compounds to inhibit their own growth as well. Other kinds of inhibitory compounds can also be formed. We discussed killer factor, inhibitory peptides produced by some strains of Saccharomyces that affect other strains of the same yeast. Certain non-Saccharomyces yeasts produce broader spectrum killer factors. Their presence in a fermentation can be inhibitory to other yeasts. Bacteria make analogous compounds, called bacteriocins that are inhibitory to other bacteria. molds, while not present in the fermentation, may have produced mycotoxins on the surface of the berry that can be inhibitory to members of the must or juice flora.

Alternately, the presence of one microbe may stimulate the persistence of others. A classic example of this is the detoxification of sulfur dioxide by Saccharomyces. Specific organisms may remove other inhibitory substances as well. This may be due to active degradation or to depletion of the inhibitor by the initial biomass. In this case, the initial population may decline due to the impact of the inhibitory compound, allowing other populations to then bloom.

**Inoculation Practices**

- Early inoculation minimizes impact of flora
- Higher levels of inoculation limit impact of microbial flora

Another obvious factor impacting population dynamics is the practice of inoculation, either with a commercial preparation or with a tank that is already undergoing active fermentation. The introduction of a new population of organisms will definitely impact the existing population and vice versa.
The earlier and the heavier the inoculation, the stronger the inhibitory affects against the indigenous flora.

**Winery Practices Impacting Microbial Flora**

- Sanitation
- SO₂
- Cap management
- Nutrient additions/Juice adjustments
- Maceration strategy
- Temperature of fermentation

Winery practices in addition to inoculation will also impact the numbers, kinds and persistence of individual members of the flora. Several of these factors have already been mentioned, SO₂ use, cold soak, temperature of fermentation, aeration practices, addition of nutrients. The sanitation procedures used in the winery are also important: how frequently the equipment is cleaned, the type of microbial reservoirs that can accumulate in the equipment, nature of the sanitation process. Are chemicals used? Hot water? Steam? Are hoses stored in a position allowing good drainage? All of these factors impact the numbers of organisms that can inoculate the juice or must. Also, are conditions established that encourage insect infestation of the winery? If so, organisms can then be spread from tank to tank. Just as it was important to walk through the vineyard, it is also important to take a thorough look at winery practices from the perspective of sanitation.

In addition to these factors, skin contact, cap management and maceration strategies also affect the flora. The berry flora usually occurs as a biofilm attached to the surface of the fruit. This biofilm is difficult to dislodge, but in nutrient sufficient liquid conditions such as a ferment, new cells produced from cell division will be released into the culture. Therefore the length of time the skins are in contact with the juice will affect the numbers of progeny cells released. Cap management practices, number and kinds of pumpovers performed, will affect the organisms present on the cap. The ethanol from the fermentation will inhibit many microbes on the surface as will the establishment of anaerobic conditions due to the formation of the carbon dioxide blanket. **Maceration strategies** directly influence the release of phenolic compounds (which may be inhibitory to microbes) and nutrients (which may be stimulatory). The effect of these practices can be difficult to predict. One winery in California began extensive
pumpovers of late harvest fruit before the yeast had become established in the tank. This practice strongly encouraged a bloom of lactic acid bacteria and production of volatile acidity. The levels produced were not as high as those obtained from Acetobacter, but were high enough to be inhibitory to the yeast during fermentation.

Several things can be done if the winemaker desires to encourage the berry flora. No or late inoculation with Saccharomyces will allow the other microbes more time to build up populations and to produce their spectrum of end products. Early addition of nutrients will also favor the non-Saccharomyces flora.

To Encourage Grape Berry Microflora

- No or late inoculation with Saccharomyces
- Add nutrients early (pre-inoculation)
- Hold must/juice at low temperature
- No to low SO₂
- Adjust pH

Sulfur dioxide addition should be avoided, or added late. The pH should be adjusted upwards (above 3.5) if the goal is to encourage bacterial flora. Finally, the must or juice can be held under conditions stimulatory to microbes, but that limit the ability of Saccharomyces to dominate the fermentation. The opposite can be done to discourage the wild flora.

To Discourage Grape Berry Microflora

- Early addition of SO₂, other antimicrobials
- Early inoculation with Saccharomyces
- Use a high level of inoculum
- Add nutrient after Saccharomyces is established
- Avoid incubation at low temperature
Lesson 9: Monitoring the Alcoholic Fermentation

The next decision to be made by the winemaker is to determine how will the fermentation be monitored and what exactly will be evaluated. Another important question: is what will be done with the information?

Third Decision:

How will fermentation be monitored?

Fermentation monitoring may be as simple as measuring Brix or sugar level, or may involve analysis of many other parameters.

Fermentation Monitoring

- What will be monitored?
- How will it be measured?
- How frequently will measurement be taken?

It is important to have a good understanding of how what is being evaluated relates to the information desired. It is equally important to know the reproducibility, precision and accuracy of the method and what types of factors will interfere in the measurement.

Fermentation Factors to Be Monitored

- Sugar consumption
- Nitrogen availability/consumption
- Microbial flora
- Microbial activity
- Acidity changes
Monitoring Sugar Consumption

- Hydrometry (specific gravity/density)
- CO₂ evolution (weight/pressure change)
- Loss of glucose/fructose (HPLC, CE, enzyme assay)
- Ethanol evolution (GC, eubillometry)
- Temperature evolution

Sugar levels can be monitored in one of several different ways. The most common is to use the Brix scale or a similar means to assess the specific gravity or density of the ferment. The amount of carbon dioxide liberated can also be used to determine the amount of sugar consumed. The advantage of measuring CO₂ loss is that this can be evaluated continuously by the change in weight of the tank. This can be done automatically and downloaded to a computer spreadsheet, being instantly available to the winemaker. The levels of the two sugars, glucose and fructose, can also be evaluated, either using an enzymatic assay, which can now be automated, or by HPLC (High Performance Liquid Chromatography) or CE (Capillary Electrophoresis). These latter methods are more accurate and precise, but require sophisticated analytical equipment and someone competent to keep the equipment in proper working order.

Ethanol evolution can also be measured as a means to determine the amount of sugar consumed. Eubillometry is the most common method, but Gas Chromatography, CE and HPLC methods are also available. Fermentation activity can also be evaluated directly by measuring the heat released from fermentation and the change in temperature of the tank. Software programs exist to allow this to be done even in tanks that are refrigerated. If ethanol or carbon dioxide are used as parameters to monitor the fermentation, it is important to know the starting sugar concentration so that the winemaker will know when the fermentation is finished.

Monitoring Nitrogen Availability/Consumption
The nitrogen content of the must or juice can also be measured to provide the winemaker with information on the amount of supplementation required. An amino acid analysis can be performed using HPLC. This provides quantitative information on a variety of nitrogen containing compounds that can be used by yeast and bacteria, including ammonia. However, as with most HPLC analyses, it is a tad time consuming and may not provide information rapidly enough to make an informed decision regarding supplementation. Other chemical assays have been developed that serve to measure the compounds possessing a certain type of nitrogen moiety. The three most common are the free amino nitrogen or FAN analysis, the NOPA analysis developed at UC Davis and the Yeast Utilizable Nitrogen analysis developed by Geisenheim.

### Monitoring Microbial Flora

- Microscopic observation
  - Total counts
  - Qualitative assessment
- Plate counts
  - Total viable counts
  - Differential media

The microbial flora of the wine can also be evaluated. Yeast and bacteria can be distinguished from each other under the microscope so the relative numbers of these two classes of organisms can be easily determined using a counting chamber. However, the yeasts are so similar to each other in appearance that it is not possible with any degree of accuracy to distinguish among the yeast genera microscopically. *Kloeckera* is an apiculate yeast, meaning that it has a point on the ends and is lemon shaped versus the ovoid *Saccharomyces*, but "newborn" *Kloeckera* cells develop the distinct pointed shape only after several rounds of replication. The same is true of the bacteria, it is not possible to distinguish genera solely using microscopic observation.
Qualitative estimates of relative numbers of microbial populations can also be made, but are less reliable. Microscopic observation can be modified by the use of vital dyes to distinguish between viable and non-viable organisms. However, we have found that the ability to take up some of these dyes is influenced by ethanol content and the number of non-viable organisms can be overestimated. Cultivatable or viable organisms can be monitored directly by plating a sample of the must or juice. General media can be used supporting the growth of a broad spectrum of organisms or more selective media can be employed that support the growth of a subset of the microbes. In this regard we have found the WL or Wallerstein Laboratories medium to be most useful. This medium supports the growth of a wide spectrum of organisms but slows the growth of all so that no one subset is able to dominate the plate. The colonies formed display quite distinctive morphologies allowing quantitation of subpopulations. For example, on this medium, colonies of Hanseniaspora/Kloeckera are an intense green, Metschnikowia forms colonies that appear red when viewed from the bottom of the plate, Saccharomyces forms colonies that are off-white to a pale green and Brettanomyces forms small colonies that are a distinctive olive green. The other yeasts commonly present also have quite distinct morphologies. It is possible to plate at a high density and see the unique morphologies over a lawn of the more common ones. Other media exist that contain inhibitors of yeast growth to enrich for the more fastidious bacteria. This type of analysis will allow the winemaker to profile the flora of the juice or must and determine the point at which Saccharomyces becomes dominant. These analyses require the availability of a laboratory equipped for microbiological analysis, which may be beyond the capability of many wineries. In California, there are several commercial services that can provide a full microbiological analysis of the ferment. These services are most commonly used to determine the source of off characters appearing in the wine, so that steps can be taken to eliminate the contaminant. It is important to define a contaminant or spoilage organism. A spoilage organism is simply one that is unwanted. One winemaker's spoilage problem may be another's critical contributor to style.

At this point it is important to again underscore the importance of a statistically valid sampling of the tank for microbial profiling. Tanks are not uniform in the flora present. For example, there may be localized high concentrations of some organisms near the surface or at the bottom of the tank or in relationship to the temperature distribution across the tank. Analysis of a lone sample taken from the racking valve might not provide an accurate picture of the distribution of the flora throughout the tank. For organisms present in low number, it may be necessary to collect the microbial flora from a large sample by sterile filtration and to then plate at more concentrated levels.

Monitoring Microbial Activity
Rather than monitoring the organisms themselves, end products of metabolism can be evaluated. The amount of a specific end product present is an indication of the metabolic activity of the microbes not just their presence or absence. **Volatile acidity** or VA analysis can be used to measure acetic acid content. This is an indication of the presence of *Acetobacter* or of the lactic acid bacteria. One can then look for other compounds produced by one or the other class of organisms to determine which bacterium is responsible for the volatile acidity. The level of **vinyl phenols** is an index of the presence and metabolic activity of *Brettanomyces*. **Hydrogen sulfide** can be produced by many microbes, but in wine conditions, is most frequently associated with *Saccharomyces*. The factors affecting H$_2$S production will be discussed in a subsequent lecture. Finally one of the most important analytical tools available to the winemaker is their own **sense of smell**. Off-characters can readily be detected by nose and it is important that ferments be sniffed on a regular basis in order to detect problematic compounds and metabolic activities.

Another factor that can be evaluated during the fermentation is changes in acidity. This can be done by monitoring titratable acidity as discussed in one of the earlier lectures and by measurement of pH. Levels of malate and lactate are typically directly measured as this is correlated with the presence and level of activity of the lactic acid bacteria.

**Monitoring Acidity Changes**
These acids can be measured by enzymatic assay, by HPLC or by paper chromatography. The former two methods are quantitative, while the latter gives a qualitative estimate of the levels of these acid species. The latter method is easy to perform and is the most common method used in wineries today because of its ease and simplicity, and because frequently qualitative information is all that is required.

**Monitoring Strategy**

- Ease vs. Frequency
- Cost
- Skill level required/Difficulty of analysis
- Is information necessary?

The monitoring strategy of the winery needs to take into consideration several factors. The first factor is **ease of the measurement versus the number of times** the measurement must be made. If it is a cumbersome and time consuming method, it may not be able to be performed in a timely fashion on a large scale, that is, on hundreds of barrels or tanks fermenting simultaneously. Or, it may be performed hastily and therefore inaccurately.

As always, **cost** versus benefit of the information is a critical factor. Many wineries forgo sophisticated nitrogen analysis because of the cost of commercial services, and instead adopt the practice of treating every fermentation as if it will stick. In this case, every must and juice receives nutrient additions. The **skill level required** of the laboratory technician is an equally important consideration. Methods improperly performed yield inaccurate information. The inaccuracy might not be immediately apparent and erroneous decisions may be made as a consequence. The final question
that needs to be answered is "Is the information necessary?" In many cases wineries are performing analyses out of habit more than for any other reason. The information is recorded, but not referred to again or used to guide winemaking practices.
Lesson 9: Temperature of Fermentation

The next critical decision for management of the fermentation is the temperature of the fermentation. Will the temperature be controlled? If so, what is the ideal temperature? And, most importantly, why is that the ideal temperature?

Fourth Decision:

Temperature of Fermentation

We have discussed the numerous effects temperature can have on the extraction of skin and seed materials and on the growth and proliferation of microorganisms.

High Fermentation Temperatures

- Speed fermentation rate
- Discourage diverse flora
- Enhance extraction (reds)
- Greater loss of volatile aroma characters
- May increase risk of stuck fermentation

High temperatures of fermentation encourage rapid rates of metabolism speeding the production of ethanol, and lead to greater loss of volatile characters. They also discourage a diverse flora due to the stimulatory effect toward *Saccharomyces*. However, if the fermentation becomes too hot, further growth and metabolism of *Saccharomyces* will be inhibited. We have found experimentally that very hot ferments tend to arrest late in fermentation, once the juice has cooled. This appears to be an arrest at a specific ethanol concentration, as discussed in the next lecture. Thus, the impact of a poor choice of temperature during the most active phase of fermentation, may impact the finish of the fermentation and the ability of the yeast to consume all of the available sugar.
Low Fermentation Temperatures

- Favor non-\textit{Saccharomyces} flora
- Better retention of volatile aroma compounds
- Slow fermentation rates

Lower temperatures result in slow rates of metabolism, allowing other non-\textit{Saccharomyces} organisms to persist in the ferment. There is obviously better retention of volatile characters, the lower the temperature.
Lesson 9: Type of Fermentation Tank

A final important consideration for fermentation management is the nature of the vessel in which the fermentation will occur.

Fifth Decision:

Fermentation vessel

The nature of the fermentation vessel is oftentimes dictated by stylistic considerations, and the need to control temperature.

Fermentation Vessel

- Wooden cask
  - Size
  - Source of wood: Oak? Redwood?
- Stainless steel tank
  - Refrigeration
  - Size
- Barrel
  - Age
  - Type of oak
- Cement

Wooden fermentation vessels may impart characters to the wine, depending upon the number of times the cask or barrel has been used. Wood supports higher microbial loads or biofilms than stainless steel and is harder to sanitize. It is easier to control the temperature of stainless steel than of wood. If wood is used, the nature of the wood must also be determined, redwood or oak are the options in California. Cement tanks are still used in many parts of the world and in some wineries in California, but are more difficult to maintain.
Lesson 10: Introduction

Problem Fermentations

In this lecture we will discuss the two main types of problems associated with *Saccharomyces* fermentations: fermentation arrest and off-character production. Occasionally a fermentation will slow down dramatically or even stop before all of the grape sugar is converted to ethanol. The former is called a "sluggish" and the latter a "stuck" fermentation. These types of fermentations are problematic for several reasons. First, high residual sugar may not be desired in the wine. Second, high residual sugar can be viewed as an open invitation to many classes of spoilage organisms that would otherwise not be able to infect the wine. Third, the winemaker must protect the wine that no longer enjoys the benefit of a carbon dioxide blanket from oxidation but not take any action that would further inhibit yeast metabolism or ethanol tolerance (such as restriction of oxygen). Fourth, the inability to predict if the fermentation is simply slow but will go dry or is actually stuck is difficult. Needed tank space may be occupied indefinitely by the arrested ferment while the winemaker waits to see what will happen. This limits the flexibility of tank usage.

Objectionable compounds or "off-characters" are likewise problematic. Conditions leading to slow or incomplete fermentations frequently also result in the production of undesirable yeast metabolites such as sulfur volatiles. These characters must be removed or wine quality will be diminished. In both cases, it is better to prevent the problem in the first place than to treat it after the fact.

**Problem Fermentations**

- Slow
- Stuck
- Off-character production
  - Hydrogen sulfide
  - Sulfur volatiles
  - Acetic acid
  - Undesired Esters

Treatments for particular off-characters may be available, but can have undesirable impacts on wine quality. Any time an attempt is made to remove chemical components
of a wine, the risk of unavoidably removing positive characters as well exists.

We will begin with a detailed examination of slow (sluggish) and incomplete (stuck or arrested) fermentations.
A stuck fermentation is defined as a fermentation containing a high or undesired level of residual sugar. In a typical fermentation, residual sugar concentration is less than 0.2g/L. In some styles that tolerate a higher sugar level, 4 g/L may be considered dry.

**Stuck and Sluggish Fermentations**

- Characterized by failure of yeast to consume sugar
- Multiple causes
- Difficult to treat
- Leads to reduced wine quality

Stuck fermentations arise when the yeast fails to consume the available sugar. As we will see, there are multiple causes of cessation of yeast metabolism. Sluggish fermentations are defined as those that are progressing very slowly, requiring a period of several weeks to complete. Fermentations are generally complete in two to three weeks under typical California vinification conditions. This of course depends upon the temperature of fermentation, the nutritional content of the juice or must and the yeast strain used. Both stuck and sluggish fermentations are challenging to treat since the cause is often obscure. Identification of aberrant fermentation kinetics requires first understanding what a normal profile looks like. "Normal" may vary depending upon vinification conditions and profiles may differ for different strains.
A typical fermentation is presented in the graph above. There is an initial lag in sugar consumption. The lag phase comprises the time during which rapid proliferation of the yeast is occurring. The maximal fermentation rate coincides with the time of maximal biomass production. As ethanol increases, the fermentation rate slows and may change abruptly or more gradually, depending upon the conditions of the fermentation. The point at which there is a dramatic change in rate is called the transition point. This point may be very late in the fermentation or may not be observed at all, depending upon the strain and the nutritional conditions. If it occurs early, at a Brix value greater than 5, the fermentation frequently becomes sluggish and at risk of arrest. The Brix value at which the transition occurs therefore has diagnostic value. The difference between the maximal fermentation rate and the post transition fermentation rate likewise has predictive value for the occurrence of sluggish fermentations. The smaller the difference, the healthier the culture. Also, if there is a transition from one rate to another rate, and the second rate is steadily decreasing this may simply reflect a change in the dominating yeast strain present. If the rate post-transition is not steady, but instead continues to decline, the fermentation may be at risk of arrest. Finally, it is important to note the overall time required to achieve dryness. We define this as the time from the initiation of fermentation (exit from lag) to dryness, less than 0.2% sugar.
Fermentation Profile

- Lag time
  - Duration?
- Maximum fermentation rate
  - Rate value?
  - Duration?
- Transition point
  - At what Brix level?
  - How sharp?
- Post-transition fermentation rate
  - Value relative to max fermentation rate?
  - Length of time?
  - Brix/ethanol/nitrogen level at which it occurs?
- Overall time to dryness

What is normal for one strain may not be typical for another so it is important to know the characteristic traits of the strain used.

Fermentation Capacity Is a Function of:

- Yeast Biomass Concentration
- Fermentative Ability of Individual Cells

The maximal fermentation rate is related to both the total number of cells and the fermentative capacity of the cells. Not all cells in a culture are uniform. There may be distinct subpopulations differing in fermentative capacity. What is typically measured is the fermentation rate of a population, which is then averaged over the number of cells or biomass present. This is because it is experimentally difficult, if not impossible, to measure individual rates of fermentation or rates of sub-populations. However, it is not known if all of the cells at a given phase of fermentation are metabolically identical; indeed data suggests that they might not be and distinct subpopulations may be present. As noted in the lecture on yeast cell biology, yeast are mortal and new cell surface growth is restricted to the bud. Thus the bud may have a different protein complement than the mother cell. Since changes in the composition of both the cell wall and plasma membrane affect yeast cell viability and fermentative activity,
depending upon the time of "birth" of a cell it may have a completely different tolerance to ethanol, and a different capacity for fermentation.
Lesson 10: Causes of Slow and Incomplete Fermentations

Several factors have been identified that impact fermentation rate or progression and can lead to slow or incomplete fermentations.

Causes of Stuck/Sluggish Fermentations

- Nutrient limitation
- Nutrient imbalance
- Substrate inhibition
- Ethanol toxicity
- Presence of toxic substances
- Poor adaptation of strain
- Low pH
- Temperature shock

Nutrient Limitation as a Cause of Sluggish Fermentation

Nutrient limitation is frequently a cause of fermentation arrest. If insufficient nutrients are available to support the production of maximal yeast biomass, the maximal rate of fermentation will obviously be diminished. Nutrients are also needed to maintain the fermentation capacity of individual cells. That is, the fermentation may achieve maximal cell density of \(10^8\) cells/mL, but if the cells have diminished capacity for sugar catabolism, the rate may still not attain the maximum possible value. Typically there are between \(10^7\) and \(10^8\) cells/mL during fermentation. The cell number fluctuates, depending upon the strain used. This is partly due to settling of yeast to the bottom of the fermentation vessel. It seems that the culture must drop below a certain threshold before re-growth to \(10^8\) cells/mL. It is important to monitor the suspended cell count as well as the total cell count (obtained upon thorough mixing of the fermentation vessel - which may be difficult under commercial production conditions).
Nutrient Limitation: Nitrogen

- Nitrogen: most often limiting
- Amino Acids
  - Can be degraded as N source via transamination
  - Can be interconverted with related amino acids
  - Can be used as that amino acid
- Ammonia
  - Mobilized by direct amination

The most frequently limiting nutrient is nitrogen. There are several compounds that can serve as yeast nitrogen sources. Some of these compounds can serve as sole nitrogen sources, meaning that if the compound is the only nitrogen-containing compound in the medium yeast will still be able to grow and metabolize substrate. Lysine and cysteine were found to be incapable of supporting growth of any one of five yeast strains that were evaluated when present as sole nitrogen source in a defined medium. The ability to serve as sole nitrogen source means that all other nitrogen containing compounds necessary for cell growth and viability can be made from that compound. Two or more strains were not able to use glycine or histidine as sole nitrogen source. For the rest of the strains, growth was quite poor on these compounds. Similar and rapid growth rates were obtained with ammonia, glutamine, asparagine, arginine, glutamine, serine, alanine, aspartate, allantoin, urea, and ornithine. Other amino acids supported growth at intermediate doubling times. There is significant strain variation in the efficiency of use of amino acids.

Amino acids that cannot serve as sole nitrogen source may still be important to the cell. All amino acids can be used as that amino acid to support protein synthesis. Some amino acids can be inter-converted with other amino acids, meaning that they can be used to synthesize other nitrogen containing compounds without being completely degraded. In other words, the amino acid may feed multiple cellular pools. The fastest growth rates occur on mixtures of amino acids. In this case the yeast is saved the need of having to expend energy to synthesize amino acids and only needs to invest in their uptake into the cell.

Ammonia is the most versatile nitrogen source; it can be directly incorporated into carbon skeletons via direct amination. Both glutamate and glutamine can be produced enzymatically by amination.
The compounds produced, glutamate and glutamine, can in turn be used in transamination reactions. In these reactions the amine group is transferred from one carbon skeleton to another.

**Amination**

\[
\begin{align*}
NH_4^+ + \text{alpha-ketoglutarate} & \rightarrow \text{glutamate} \\
NH_4^+ + \text{glutamate} & \rightarrow \text{glutamine}
\end{align*}
\]

**Transamination**

\[
\begin{align*}
\text{Glutamate} + X & \rightarrow \text{alpha-ketoglutarate} + N - X \\
\text{Glutamine} + X' & \rightarrow \text{glutamate} + N - X' \\
\text{Alanine} + X'' & \rightarrow \text{pyruvate} + N - X''
\end{align*}
\]

Where "X" is an intermediate in amino acid/nucleotide biosynthesis, and "N - X" is an amino acid or nucleotide base.

Alanine can also be a nitrogen donor in transamination reactions. Nitrogen moieties are transferred to carbon skeletons either via direct amination or transamination. Other nitrogen compounds can be degraded to generate free ammonia, alanine, glutamine or glutamate thereby becoming mobilized to synthesize other amino acids.

When presented with a mixture of amino acids, yeast first fills cytoplasmic amino acid pools. Once pools become depleted the yeast must start synthesizing amino acids from the remaining nitrogen sources. Yeast show a distinct preference for some amino acids over others. Preferred nitrogen sources are defined as those that are consumed from the medium first when the culture is presented with a mixture.
Preference for Nitrogen Sources

- How readily can it be converted to NH\(_4\), glutamate or glutamine?
- Expense of utilization (ATP, cofactor, oxygen requirement)
- Toxicity of C-skeleton
- What else is available?

Nitrogen source preference is dependent upon several factors. Principal among these is how readily the compound can be used to **generate ammonia, glutamate or glutamine**, as these are the principle compounds used in biosynthesis. Amino acids that are "inexpensive" to degrade will be used in preference to those that require energy or micronutrients. Proline and some of the aromatic amino acids cannot be degraded under anaerobic conditions. This is because molecular oxygen is required for degradation. Another important factor concerns the possible toxicity of the **carbon skeleton** generated following deamination or removal of the amino group. For those amino acids that require micronutrient vitamins for degradation, the availability of those cofactors will impact whether or not the compound can be used. Therefore nitrogen source preference is influenced by the composition of the medium. Strain differences also exist. Some strains utilize certain amino acids more efficiently than others. The genetic basis of these differences are largely unknown in commercial strains, but, if supplementing a fermentation, it is important to understand the nutritional needs of the specific strain in question. All strains evaluated are able to use ammonia, therefore ammonia supplementation is the most widely effective.

Factors Affecting Nitrogen Compound Utilization and Preference

- **pH**
  - Transport is coupled to H+ ion movements
- **Ethanol**
  - Inhibits amino acid transporter function (80% decreases) at 5% ethanol for the general amino acid permease
  - Increases passive proton flux
- **Other N compounds**
  - Competition for uptake
  - Nitrogen repression
  - Induction
Yeast strain differences

Other factors also affect nitrogen compound utilization. Amino acids need to be transported against their concentration gradient, meaning that the amino acid concentration inside of the cell is higher than that of the medium. Transport against a gradient is energetically unfavorable. *Saccharomyces* has solved this problem by coupling movement of amino acids to a component that displays a steep gradient across the cell membrane: hydrogen ions. The internal pH of a yeast cell is near neutrality (pH 6.5-7.0), while the pH of the medium is 3.0 to 4.0. This creates a large gradient of protons, and entry of hydrogen ions into the cell is energetically favorable. Thus, movement of amino acids is coupled to that of protons. Amino acid transporters transfer both an amino acid molecule and a proton into the cell. The proton is then exported out of the cell via a proton pump. The pump extrudes protons in an energy dependent manner since excretion occurs against the proton gradient. Hydrolysis of ATP provides the energy for operation of the pump. Continued amino acid uptake requires efficient excretion of the protons co-transported with the amino acid; thus amino acid uptake is an energy requiring process. Since amino acid uptake is coupled to protons it is not surprising that medium pH affects amino acid uptake. If the pH is too low, below 2.7, protons tend to enter the cell due to passive proton flux. The proton pump must then be directed towards removal of these protons. The cell has a limited capacity for the removal of protons, thus at very low pH the cell is not able to sustain amino acid uptake due to the lack of capacity of the proton pump.

Ethanol also increases the tendency of protons to enter the cell. At high ethanol concentrations, protons tend to enter the cell again due to passive proton flux. Amino acid uptake is inhibited at high ethanol concentrations. Studies have shown that
addition of ethanol results in a decrease or inhibition of amino acid permeases. Some permeases or transporters may be inhibited up to 80% by the presence of ethanol. This regulatory mechanism assures that internalized protons will not swamp the cell and proton influx will not exceed the capacity to pump protons out of the cell. What is the consequence of failure to remove protons? The cytoplasm of the cell becomes acidified which denatures proteins leading to cell death.

Many amino acids share common transport mechanisms. There are general amino acid transporters and specific amino acid transport mechanisms. Amino acids that share common protein uptake systems compete with each other for uptake. The kinetics of uptake of an amino acid will therefore be affected by the presence of other amino acids in the medium. In addition to competition for uptake, nitrogen compound degradation is also regulated transcriptionally. Preferred nitrogen sources block the degradation of the less preferred compounds. Ammonia, for example, represses the synthesis of many of the enzymes required for degradation of amino acids. In effect this saves those amino acids for protein synthesis. Another regulatory process that occurs is called induction. This means that degradative enzymes will not be synthesized in the absence of their respective substrate. For example, it would not be prudent to synthesize asparaginase unless asparagine was actually present in the medium.

Different yeast strains vary in response to these factors. Some are supersensitive to proton influx and arrest amino acid uptake under conditions that are still permissive for other strains. Whether this is due to differences in proton influx or to the capacity of the proton ATPase pump is not known.

Sources of Nutrients

- Grape
- Nutrient additions (winemaker)
  - Diammonium phosphate
  - Yeast extracts
  - Yeast "ghosts"
  - Proprietary yeast nutrient mix
- Yeast autolysis

Nitrogen compounds come from the grape itself. The winemaker may also augment the nitrogen content of the fermentation by addition of diammonium phosphate or other
yeast nutrient preparations. At the end of fermentation, yeast autolysis may also result in the release of internal components including amino acids.

**Ionic Imbalance as a Cause of Sluggish Fermentation**

- Ratio of K⁺:H⁺
- Must be at least 25:1
- Needs to be adjusted early in fermentation
- Probably important in building an ethanol tolerant membrane

Imbalances in the relative concentrations of ions can also lead to arrest of fermentation. Yeast ferment better if there is an excess of potassium over protons. The function of the potassium is not known. Some researchers think that it blocks the disruptive effects of free protons on protein structure and function. Others believe that potassium is important in regulating proton efflux from the cell. In this model, protons can be exchanged for potassium in a process that does not require ATP. A third model suggests that potassium is incorporated into the cell wall and plasma membrane which makes the membrane better able to resist the disruptive effects of ethanol.

The ratio of potassium to hydrogen ions needs to be on the order of 25:1, on a molar basis (not gram to gram). Adjustment of the ratio late in fermentation does not restore fermentation rate. This suggests that the last model, that potassium is required for synthesis of ethanol tolerant cellular components is more likely to be correct than the other models.

**Substrate Inhibition as a Cause of Sluggish Fermentation**

Another cause of a decrease in fermentation rate is substrate inhibition. This phenomenon appears to have two different causes. It has been well established that high substrate concentration can inhibit sugar uptake if the cells have adapted to a low substrate concentration. This would occur if one were using one fermentation to inoculate another. *Saccharomyces* possesses a large family of sugar transporters named the "HXT genes" for HeXose Transport. There are 18 HXT genes in laboratory strains.
Transporters that are designed to work at low substrate concentrations are said to have a high affinity for substrate. They have an open conformation allowing them to recognize the substrate regardless of the way in which the substrate approaches the transporter. In this case, the transporter has more than one recognition or binding site for the substrate. Binding triggers molecular movement of the transporter and the translocation of substrate to the inside of the cell. If substrate concentration is high then more than one substrate molecule binds to the transporter at the same time. Two sugars occupying the binding sites prevents the molecular movement of the protein from occurring and the transporter is jammed. This is only a problem when the cells shift from a low substrate concentration to a high concentration. This mechanism assures that the cell will have the opportunity to readjust metabolism to the new substrate level. This is important since as noted in the lecture on glycolysis, excessive sugar uptake leads to an imbalance in ATP consumption, which will arrest cell growth. This phenomenon is called substrate inhibition.

A different form of substrate inhibition occurs late in fermentation. Cells metabolize glucose with faster kinetics than fructose. This results in a change in the glucose to fructose ratio from the 1:1 of juice. Under certain conditions the yeast appear to arrest or slow utilization of fructose but not of glucose. In this case the medium accumulates fructose. This imbalance of fructose over glucose appears to inhibit re-initiation of the fermentation. The mechanism by which this occurs is not known.

**Ethanol Toxicity as a Cause of Sluggish Fermentation**
Ethanol:

- Perturbs membrane structure at protein:lipid interface
- Leads to increased "passive proton flux" and acidification of cytoplasm
- Inhibits protein activity
- Affects membrane "fluidity"

Another factor impacting fermentation rate is ethanol itself. Ethanol inhibits many cellular activities including transport of amino acids and other nutrients. Interestingly it does not impact sugar uptake unless at very high concentrations.

**Ethanol Toxicity**

Plasma membrane is the most ethanol-sensitive cell structure:

<table>
<thead>
<tr>
<th>Composition</th>
<th>Protein</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lipid</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>10%</td>
</tr>
</tbody>
</table>

Functions:
- Permeability barrier
- Regulation of uptake
- Mediates response to environment
- Maintains electrochemical gradients
- Mediates cell - cell interactions

The main target of ethanol inhibition is the **plasma membrane**. The plasma membrane is comprised largely of protein and lipid. This cellular structure plays a vital role in regulation of uptake and excretion of cellular components. It also mediates the response of the cells to environmental stress and maintains cellular electrochemical gradients. In addition, the plasma membrane plays a key role in cell-cell interactions such as mating and flocculation. Ethanol impacts function of the membrane in several ways.

It **perturbs the interaction between lipids and proteins** in the membrane. This can lead to leakage or **increased passive flux of protons**, which in turn leads the acidification to the cytoplasm. Ethanol can disrupt the interaction between proteins and water thereby affecting protein activity. One of the principle responses of the cells is to
adjust the fluidity of the membrane, which has lead to the hypothesis that ethanol perturbs membrane fluidity.

To return to the model of glucose transport, interaction of a glucose molecule with the transporter causes a change from a conformation facing the outside of the cell to one facing the cytoplasm. The sugar molecule then dissociates from the transporter and is transferred to the cytoplasm. The inward facing transporter must then make a second conformational change to reface the outside of the cell to pick up another sugar molecule. The conformational change requires that the protein be able to move within the lipid matrix. The membrane must allow movement but at the same time restrict it to prevent the protein from denaturing. This elastic property of the membrane is called membrane fluidity. If the membrane is too stiff, the movement will not occur and there will be no net transport. If the membrane is too fluid, the protein will move too much, and it will be energetically difficult to realign the protein. Anything that alters membrane fluidity will affect transporter protein activity.
Ethanol Toxicity

Adaptation of membrane requires:
- Increasing content of sterols
- Increasing relative content of proteins
- Increasing level of desaturation (number of double bonds) in fatty acid side chains
- Modification of phospholipid head groups?

Plasma membrane composition can be altered in order to minimize the impact of ethanol produced during fermentation. Several studies have shown that membranes isolated from high ethanol growth conditions are higher in sterol content, have a higher ratio of protein to lipid. The yeast sterol is ergosterol, which has the following composition.

Plants also produce sterols that can be used by yeast as substitutes for ergosterol. The actual requirement for ergosterol is quite low, other sterols, including animal cholesterol, can provide what has been termed the "bulk" or structural function.

In addition the nature of the fatty acid chains of the lipid molecules appears to change. The number of double bonds (unsaturated lipids) increases.
There are conflicting reports in the direction of changes of the phospholipid head groups. One study suggests that phosphatidyl inositol levels increase in ethanol tolerant membranes while in another study phosphatidyl choline levels increased relative to other phospholipid head groups. The differences in these conclusions likely reflect differences in the way the studies were conducted and what substrate molecules needed to continue to be translocated into the cell. It is important when reading the yeast cell literature to pay attention to the conditions under which the studies were conducted.
Ethanol Toxicity

- Sterol and fatty acid desaturation are Oxygen-requiring processes
- New protein synthesis requires nitrogen be available
- Phospholipid head group synthesis requires cofactors (S-adenosylmethionine) be available

Both sterol biosynthesis and fatty acid desaturation require the formation of double bonds in carbon chains. Enzymatically this occurs by transfer of hydrogen ions to molecular oxygen. This means that these reactions require that oxygen be present in the medium. They will not occur under strict anaerobic conditions such as occur in grape juice fermentation. The alteration of the protein content of the membrane requires that new proteins be synthesized. This of course will not occur in the absence of a nitrogen source. Phospholipid head group synthesis requires that key cofactors be available. If the yeast are deficient in any of these compounds, the change cannot occur. There is disagreement in the literature as to which factor, lipid or protein modification, is the most important. Part of this disagreement is likely due to the way in which these studies have been conducted. Some have been an "ethanol challenge" where ethanol is added to a culture with low to no ethanol. This leaves no opportunity for adaptation. Other studies use naturally accumulated ethanol, that is, initiate the experiment with a high concentration of sugar. In this case the yeast is able to make adaptive changes during the course of fermentation. It is not surprising that different conclusions would be reached under these conditions. Further, acetaldehyde may accompany natural ethanol accumulation and will compound the interpretation of the inhibitory effect of the ethanol. Acetaldehyde is itself quite toxic.

Presence of Toxic Substances

Substances that inhibit growth or lead to cell death will also impact fermentation rate and can lead to sluggish or stuck fermentations. Toxins can arise from several sources during wine production.
Presence of Toxic Substances

- Toxins may arise from the metabolic activity of other microbes
- Toxins may arise from metabolic activity of *Saccharomyces*
- Toxins may have arisen in vineyard, but are not inhibitory until ethanol has accumulated

Toxins are most commonly thought of as **deriving from other microbes** that impact the biological activities of *Saccharomyces*. Inhibitory factors may also be **produced by strains of Saccharomyces**. These compounds are called **killer factors**. They are small peptides that inhibit the growth of sensitive strains of *Saccharomyces*. With respect to killer factor there are three types of strains. Those that both produce it and are resistant to it, those that are resistant to it but do not produce it, and those that are sensitive to the killer factor. The killer factors produced by *Saccharomyces* only inhibit other strains of *Saccharomyces*. Other yeasts produce similar inhibitory peptides that have a broader spectrum, inhibiting many genera of yeast. Toxins may also arise from the vineyard. As noted before, *Saccharomyces* is a member of the kingdom of fungi. Fungicides used in the vineyard to inhibit mold infestation of the fruit may also inhibit *Saccharomyces* if present in high enough concentration at the time of harvest.

The most common toxins occurring in grape juice/must fermentation are listed below.

**The Most Common Toxins**

- Acetic acid
- Higher organic acids C₂-C₄
- Medium chain fatty acids/fatty acid esters
- Acetaldehyde
- Fungicide/Pesticide residues
- Higher alcohols
- Higher aldehydes
- Killer factors
- Sulfur dioxide

Bacteria commonly produce **acetic acid, higher organic acids** and **medium chain**
fatty acids and fatty acid esters. Some yeast strains, Kloeckera/Hanseniaspora for example, produce acetic acid as an end product of fermentation. Acetaldehyde and higher aldehydes can be produced by Saccharomyces during the course of fermentation and amino acid catabolism. It is thought that many of the inhibitory affects associated with ethanol are actually due to acetaldehyde, which is far more toxic at lower concentrations. Aldehydes are very reactive molecules that can interact with amino acid side chains inhibiting protein activity. Higher alcohols (greater than two carbons) are also inhibitory, and are more inhibitory than ethanol. They can be produced by Saccharomyces, but it is thought that if they are present in inhibitory concentrations they were produced by bacteria or other non-Saccharomyces yeasts. Sulfur dioxide can also be inhibitory. The levels produced by Saccharomyces are never in the inhibitory range; however, it is all too common for multiple SO₂ additions to accidentally be made in a winery that result in an inhibitory concentration being present.

Poor Adaptation of Strain

- Strain may not display ethanol tolerance
- Strain may have high nitrogen/vitamin requirements
- Strain may be a poor fermentor, but capable of dominating the fermentation
- Temperature effects

Another factor impacting strain fermentation performance is a poor adaptation of the strain to the fermentation conditions.

If the cell has insufficient oxygen available, it will not be able to make sterols and unsaturated fatty acids required for optimal ethanol tolerance. Saccharomyces can use fatty acids and sterols that are stored in internal membranes. That is, it can parasitize its own internal membranous organelles. However if it has been grown under poor or sub-optimal growth conditions it will not have anything to recruit fatty acid or sterol components from and is therefore poorly adapted to the growth conditions. Similarly, vitamins accumulate within yeast cells. They frequently have sufficient vitamin stores to undergo up to 40 cell divisions in the absence of vitamin supplementation. In an inoculated fermentation cells typically undergo five to seven generations, nowhere near 40. However, if the population has been undernourished, it will become vitamin deficient during fermentation. Some strains have higher vitamin or nitrogen
requirements than others and may be poorly adapted to fermentation conditions. Some strains are more sensitive to temperature extremes than others. If one of these strains is used in a "hot" or "cold" fermentation, it will arrest once ethanol accumulates in the medium. Thus the ability of strains to ferment is a function of intrinsic genetic characters as well as of medium composition.

**Low pH**

- pH is reduced by metabolism of *Saccharomyces*
- Low pH musts may drop to an inhibitory level
- Dependent upon K+ concentration

*Saccharomyces* produces protons during fermentation. The resulting release of protons can decrease the pH of the fermenting juice by 0.1 to 0.3 pH units. If the pH of the starting wine is too low (3.0 or below) this decrease can lower the pH to an inhibitory level (less than 2.75). At these low pH values further fermentation will not occur.

Inhibition by hydrogen ion concentrations is a function of the potassium ion concentration. The lower the pH the higher the concentration of potassium needed to sustain fermentation rates.

**Temperature Shock**

- Super-cooling/heating of tank due to equipment failure
- High temperature fermentations becoming too warm due to yeast metabolism

Temperature swings during fermentation can also inhibit sugar catabolism. Temperature impacts membrane fluidity. The higher the temperature the more fluid the membrane. High temperatures may alter membrane fluidity impacting ethanol tolerance. At higher ethanol concentrations, the temperature maxima supporting growth is lowered while the minima is increased. In other words, the temperature range supporting growth and metabolism narrows. For example if the temperature range
supporting growth is from 12 to 35°C in the absence of ethanol, in its presence the range will be narrowed to 20 to 30°C. In general, it is more difficult to produce a membrane simultaneously tolerant to low temperature and to high ethanol as these parameters require opposing adaptations. It is easier to maintain growth at higher temperatures in the presence of ethanol, but this too has its limits. Temperature shock refers to a dramatic (greater than 5°C) change in the mean temperature of the tank. This may arise due to supercooling, which can occur as the fermentation slows and the heat release from yeast metabolism decreases.

High temperatures can occur when yeast metabolism generates much more heat than can be dissipated during the fermentation.

The factors leading to arrest of fermentation are interacting. Limitations for nutrients enhances the toxicity of ethanol as does the presence of other toxic substances.

In general if multiple inhibitory conditions are present, the effect on yeast metabolism may be synergistic rather than additive. Many inhibitory compounds are more deleterious at higher ethanol concentrations.
Lesson 10: Off-Character Production

The next section of the lecture will cover the second class of problem fermentations, those that result in formation of off-characters.

The *Saccharomyces* Off Characters

- Volatile Sulfur Compounds
- Acetic Acid
- Higher Alcohols
- Acetaldehyde/Higher Aldehydes
- Unwanted Esters
- Vinyl Phenols

The principle class of off-characters is the volatile sulfur compounds. As a group, these compounds have objectionable odors and have low thresholds of detection by humans.

Volatile sulfur compounds derive from the degradation of sulfur containing amino acids. Hydrogen sulfide can arise from the reduction of sulfate. The following table lists the most common sulfur containing volatiles found in wine.

**Volatile Sulfur Compounds**
- Hydrogen Sulfide: $\text{H}_2\text{S}$
- Methanethiol: $\text{CH}_3\text{-SH}$
- Ethanethiol: $\text{C}_2\text{H}_5\text{-SH}$
- Dimethyl sulfide: $\text{CH}_3\text{-S-CH}_3$
- Dimethyl disulfide: $\text{CH}_3\text{-S-S-CH}_3$
- Diethyl sulfide: $\text{C}_2\text{H}_5\text{-S-C}_2\text{H}_5$
- Diethyl disulfide: $\text{C}_2\text{H}_5\text{-S-S-C}_2\text{H}_5$

Hydrogen sulfide may also come from the degradation of sulfur containing amino acids or from the reduction of organic sulfur used in the vineyard as a fungicide. If other sulfur containing fungicides or pesticides were used in the vineyard, they may also be degraded to a volatile sulfur compound. Some of these compounds may then undergo other spontaneous chemical reactions producing other types of molecules. Hydrogen sulfide formation from inorganic sulfur is thought to be a spontaneous chemical reaction requiring reducing conditions established by yeast metabolism but is not catalyzed directly by *Saccharomyces*. The term "higher sulfides" refers to any compound other than $\text{H}_2\text{S}$.

**Sources of Sulfur Compounds**

- Sulfate reduction pathway
- Degradation of sulfur containing amino acids
- Inorganic sulfur
  - Non-enzymatic
  - Requires reducing conditions established by yeast
- Degradation of S-containing pesticides/fungicides

Other factors also impact formation of volatile sulfur compounds. In most strains, limitation for nitrogen leads to the production of higher levels of hydrogen sulfide.
Higher Sulfides

- Come from degradation of sulfur containing amino acids
- From reaction of reduced sulfur intermediates with other cellular metabolites?
- Formed chemically due to reduced conditions

There are many sources of hydrogen sulfide, and just as many factors impact its formation during fermentation.

Hydrogen Sulfide Formation

- Due to nitrogen limitation
- Sulfate reduction regulated by nitrogen availability
- Lack of nitrogenous reduced sulfur acceptors leads to excessive production of reduced sulfate and release as H$_2$S
- Strain variation

Sulfate is reduced in order to synthesize the sulfur containing amino acids. Therefore it is not surprising that there is a connection between nitrogen availability and release of reduced sulfur in excess of the nitrogen-carbon acceptor molecules. There is also significant strain variation. Some commercial strains tend to produce high levels of H$_2$S while others produce relatively low levels.

Hydrogen sulfide formation is a chronic problem in the wine industry worldwide. Copper fining can remove sulfide via the formation of a CuS precipitate. Copper must then be removed by other treatments. While H$_2$S can be removed, most winemakers would prefer that it not be made in the first place. A significant amount of research has been conducted over the years to develop fermentation strategies to limit or eliminate production of H$_2$S.
Current Understanding of H$_2$S Formation

- Nitrogen levels not well-correlated with H$_2$S
- Under complex genetic control
- Tremendous strain variation in H$_2$S production

While nitrogen limitation clearly leads to higher levels of hydrogen sulfide in most strains, there is not a consistent relationship between nitrogen level in the medium and sulfide release. This is due in large part to the high degree of variability in H$_2$S release across strains. The process of sulfate reduction generates several toxic intermediates and the end product cysteine has also been shown to be toxic if it accumulates in yeast cells. Of these compounds, H$_2$S is the least toxic. Thus, if there is a problem in sulfate reduction, the cells would prefer to make H$_2$S. Other work suggests that hydrogen sulfide is inhibitory to respiration so it may only be non-toxic during anaerobic conditions.

Factors Impacting H$_2$S Formation

- Level of total nitrogen
- Level of methionine relative to total nitrogen
- Fermentation rate
- Use of SO$_2$
- Vitamin deficiency
- Presence of metal ions
- Inorganic sulfur in vineyard
- Use of pesticides/fungicides
- Strain genetic background

In addition to medium nitrogen concentration, other factors impact the appearance of H$_2$S. Obviously genetic background is a key factor although one poorly understood at this time. Inorganic sulfur and pesticide/fungicide residues impact sulfide as mentioned above. In addition, the sulfate reduction pathway is regulated by the concentrations of cysteine and methionine in the cell. Methionine represses expression of the genes encoding the enzymes of sulfate reduction, which reduces the amount of H$_2$S formed.
by the pathway. Cysteine blocks formation of an inducer of expression of the sulfate reduction pathway. When both methionine and cysteine are present in high concentrations, expression of the pathway is at its lowest. However, as stated several times above, if methionine and cysteine are present in excess they can be degraded for their nitrogen content leading to release of hydrogen sulfide or production of higher sulfides.

Some strains show an impact of fermentation rate on sulfide formation, but this is not observed in all conditions, and may be an artifact of how the $H_2S$ is measured as it is driven off of the fermentation by $CO_2$.

Synthesis of methionine requires the vitamin pantothenate. If pantothenate is limiting, sulfide levels increase because methionine and cysteine do not accumulate. Sulfide is also produced by yeast to detoxify metal ions in the environment. This is currently not a problem in the wine industry as juices and musts do not contain high enough concentrations of metal ions. Finally, sulfur dioxide can be correlated with $H_2S$ formation in some strains. In the sulfate reduction sequence, sulfide is produced from the reduction of sulfur dioxide. Hydrogen sulfide can be produced in some strains from the reduction of external sulfite.

The pattern of formation of hydrogen sulfide during fermentation can vary. There are two points in the fermentation where high levels of sulfide might appear in the medium.

![Timing of Formation of H$_2$S](image)

The early peak occurs usually around the time of maximal cell density. This is associated with the active phase of metabolism. The later peak occurs as the cells are finishing fermentation. It is thought to be due to degradation of sulfur containing amino acids. Some strains produce lower levels of sulfide throughout fermentation.
Timing of Formation of $\text{H}_2\text{S}$ Early (first 2-4 days): due to N imbalance

Late (end of fermentation): due to autolysis, degradation of S-containing compounds

$\text{H}_2\text{S}$ produced early can be driven off by carbon dioxide during active phase of fermentation

Hydrogen sulfide produced early in fermentation can be driven off by the carbon dioxide produced during fermentation, so it is not considered to be as problematic as $\text{H}_2\text{S}$ produced after the fermentation rate slows.

The sulfate reduction pathway is presented in the following figure.

Sulfate Reduction Pathway

Sulfide is incorporated into homoserine yielding homocysteine. Homocysteine can be
Methionine is used to synthesize Met-tRNA used in protein synthesis and S-Adenosylmethionine (SAM), which is required for one-carbon transfers used in many biosynthetic reactions. Transfer of the methyl group from SAM produces S-adenosylhomocysteine that can then be reconverted to homocysteine and methionine.

Cysteine is used to produce Cys-tRNA and to produce the tripeptide glutathione. Glutathione regulates the redox status of the cells and is a very important compound. Thus both methionine and cysteine are required to synthesize compounds other than proteins that are vital to the continued growth and metabolism of the cells.

**Acetic Acid**

Another off character arising during fermentation is acetic acid. *Saccharomyces* can produce acetic acid but this is generally at levels below the threshold of detection.

Other organisms are more prolific producers of acetic acid. Still, the amount produced by *Saccharomyces* can contribute to the overall level if other organisms are present.
and making acetate. There are strain differences in the amount of acetic acid produced by *Saccharomyces*.

**Acetic Acid Production by *Saccharomyces***

- Levels made by *Saccharomyces* are low
- Strain differences in amount formed
- Derived from:
  - Fatty acid biosynthesis/degradation
  - Amino acid degradation

Under anaerobic conditions, acetic acid is produced from fatty acid biosynthesis or degradation and from amino acid degradation. It can be produced from acetaldehyde under aerobic conditions.

**Higher Alcohols**

- Fusel oils
- Phenethyl alcohol

Higher alcohols, those with more than two carbons, may also be considered as off-characters depending upon the amount produced and the style of wine desired.
Higher alcohols are produced from amino acid degradation and are collectively called "fusel oils". The amino acid is first deaminated followed by a decarboxylation of the resulting carbon skeleton. This yields an aldehyde that can be reduced to an alcohol. This reduction is catalyzed by the same enzyme family that converts acetaldehyde to ethanol. Under some conditions, the aldehyde is oxidized to an acid rather than being reduced to an alcohol. Aldehydes are more toxic than alcohols or acids so the yeast would prefer to make one or the other of these compounds from the amino acid skeleton.

Alcohols can also be made from aromatic amino acids. One of the most common is phenethyl alcohol. This compound has been described as having a floral aroma, which, if present in high concentration may be too intense for some wines.

**Acetaldehyde/Higher Aldehydes**

Acetaldehyde and the higher aldehydes can be considered as off-characters if present in high concentration. These compounds are desired in some styles, such as sherry production, and are associated with wine age. Many students describe the aldehyde character in wine as "sherry". The formation of these compounds during aging will be discussed in a subsequent lecture.
Aldehyde Production

- Acetaldehyde from glycolysis
  - Released when conversion to ethanol is blocked
  - Released as SO₂ adjunct
- Higher aldehydes from amino acid degradation
  - Released when formation of higher alcohols is blocked

Acetaldehyde is formed during glycolysis. It is released under two conditions, when ethanol formation is blocked due to absence of alcohol dehydrogenase or when NADH is being used for some other purpose and does not need to be recycled during end product production. Acetaldehyde is also released as the detoxification mechanism for sulfites.

Unwanted Esters

- Fatty acid metabolism
- Amino acid metabolism
  - Phenethyl Acetate

Saccharomyces produces many esters during fermentation. Some wine styles are dependent upon the presence and spectrum of yeast esters produced. While in other wines they are undesired. Esters hydrolyze readily under acidic conditions so if the wine is to be aged they may not be present at the time of bottling.

Unwanted Esters

Esters form from the reaction of an alcohol and an acyl-CoA molecule

\[
R_1 - OH + R_2 - C \sim SCoA \rightarrow R_1 - O - C - R_2
\]

Esters are formed from the reaction between an alcohol and an acid species. The acid
species is first activated by attachment to coenzyme A. The most common ester is ethyl acetate formed from the reaction of acetyl-CoA and ethanol. These are the most common acyl-CoA and alcohol compounds, respectively.

**Source of Esters**

- Most common ester is ethyl acetate made from the reaction of ethanol with acetyl-CoA
- Esters can derive from amino acid degradation and reaction of acids with ethanol or of alcohols with acetyl-CoA
- Esters can derive from fatty acid metabolism

Esters are also produced during fatty acid degradation. If an acid species is produced it may react with ethanol. Higher alcohols may in turn react with acetyl-CoA. Fatty acid biosynthesis and degradation involves attachment of the growing (or shrinking) fatty acid chain to CoA. The acid species can react with ethanol, producing a long chain ester. Long chain esters are soapy and are more stable than short chain esters.

**Phenethyl Acetate**

- Degradation product of phenyalanine
- Characteristic "rose oil" odor
- May be too pungent

Phenethyl alcohol can react with acetyl-CoA producing phenethyl acetate, which has the character associated with rose oil. It has a perfumy rose character that again may be too intense for some wines. It tends to be produced by *Saccharomyces* late in fermentation.

Many factors impact ester formation. In the wild, yeast make esters to attract insects to sites of yeast growth. The yeast can then be picked up by the insect and transferred to a new site. Yeasts tend to produce esters late in fermentation or under conditions of some types of stress. Under these circumstances the culture is running out of nutrients.
and would like to be relocated. Esters are volatile and less toxic than either the alcohol or the acid moiety. Under conditions of high cell density, production of a volatile compound as a means to detoxify the environment may be advantageous.

**Vinyl Phenols**

- Responsible for sweaty, horsy, stable off aromas
- Usually formed by *Brettanomyces*
- *Saccharomyces* possesses the enzymes needed to make vinyl phenols and there are reports that it will make them under certain conditions

The final class of off-characters we will consider here are the vinyl phenols. These compounds have very distinctive medicinal or pharmaceutical aromas and are responsible for the "animal" or barnyard characters found in wines.

Decarboxylated phenols are reduced to vinyl phenols by yeast enzymatic activity. The principle yeast producing vinyl phenols is *Brettanomyces*. However the enzymes catalyzing vinyl phenol production have been found in *Saccharomyces* so it is believed that this yeast could produce them as well. Other yeasts are also thought to be capable of producing these compounds, but *Brettanomyces* appears to do so most frequently.
Vinyl phenol formation is dependent upon the phenolic composition of the fruit, and what other compounds are available to be reduced. The reason *Brettanomyces* produced high levels of these compounds relates to its mode of metabolism of sugar substrates. *Brettanomyces* produces acetic acid as a primary end product of metabolism. This results in formation of extra NADH, as acetaldehyde is oxidized to acetate. NAD$^+$ can be regenerated via the production of vinyl phenols. Oxygen is the preferred acceptor of electrons, and oxygen will limit vinyl phenol formation but has the negative side effect of strongly encouraging acetic acid formation.

**Moral:**
Yeast needs are simple, but it can be challenging to keep them happy.
Lesson 11: Introduction

Stuck Fermentation: Diagnosis and Rectification

In this lecture we will cover identification of the cause of a stuck fermentation and strategies that can be used to restart the fermentation. Successful re-initiation of an arrested ferment depends upon knowing why it arrested and what must be done to alleviate the biological or environmental stress to the yeast that is limiting sugar utilization.
Lesson 11: The "Normal" Yeast Fermentation Profile

The ability to recognize a problem fermentation requires that the normal or typical course of fermentation be understood. Winemakers frequently monitor soluble solids or specific gravity of the fermentation rather than directly measuring grape sugars. Glucose and fructose are not consumed at the same rate by yeast cells. Glucose disappears with more rapid kinetics than does fructose. The hexose transporters that translocate each sugar into the cell display a higher affinity for glucose than fructose, thus, some of the difference in rate is due to the biochemical properties of the transporters themselves.

**Normal Fermentation Profile**

- **Glucose is consumed faster than fructose**
- **Arrested fermentations will be high in fructose relative to glucose**

As a consequence, fructose concentrations will typically be much higher than glucose late in fermentation. If an arrest occurs at this time, then the yeast used as a new inoculum to get the fermentation started will need to be able to ferment fructose efficiently. The following graph shows the profiles of glucose, fructose and total sugar accumulation in a typical Chardonnay fermentation.
Analysis of the ratio of glucose to fructose and how the ratio is changing over time can be used to determine the health of the fermentation. The pattern of total sugar consumption is also diagnostic. There are several types of deviations from the normal curve. The type of deviation from normal provides important information on the type of stress being experienced by the yeast.
This graph depicts the normal fermentation of a Chenin blanc juice highlighted in red. Fermentations may be slower than expected because of an unusually long lag before the onset of fermentation. They may start normally, but slow down during the courses of fermentation, or they may be sluggish throughout, never attaining a high rate of fermentation. Finally, a normal fermentation may display a rather abrupt arrest. These types of profiles are associated with different types of stress for the yeast.
A typical inoculated fermentation initiates, that is, shows a dramatic drop in sugar, starting between 24 and 72 hours. As shown here, initiation may not occur for 5 to 7 days or longer. Such fermentations display a lag in initiation of rapid sugar consumption. This is not necessarily undesirable, especially if the reason for the sluggish start is known. There are several causes of a long lag. As shown here, a long lag does not necessarily mean the fermentation will be sluggish or that it will arrest prior to dryness.
Causes of Long Lag

- Poor health of starter culture
- Presence of inhibitors
- Grape quality

Poor health of Starter Culture as a Cause of Long Lag

The most frequent cause of a long lag is **poor health** of the starter culture in inoculated fermentations. The final yeast cell density is roughly $10^8$ cells/ml. An inoculum of $10^6$ cells/ml must undergo seven generations to reach $10^8$. The yeast doubling time is on the order of 3 to 5 hours in a nutritionally rich grape juice fermenting at 25°C. Seven generations under these conditions would require 24 to 35 hours. In most cases growth does not initiate immediately and there is a period of adaptation of the yeast to the juice or must. This may take 12 to 24 hours depending upon the condition of the inoculum (active dry yeast, fermenting juice or must at high ethanol) or may be much shorter if a mid-fermentation must or juice (less than 7% ethanol) is used as inoculum. This also assumes that the cells of the inoculum are fully viable. This may not be the case.

Improperly rehydrated yeast (too high or too low of a hydration temperature or rehydrated in wine rather than water) will rapidly lose viability. In this case the viable count may not be $10^6$cells/ml post-inoculation. We have seen it as low as $10^2$cells/ml. The yeast then simply requires more doubling times to attain maximal biomass and therefore the maximal fermentation rate. If the practice is to inoculate one juice with a previously fermenting juice rather than with active dry yeast viability can also be an issue. Late in the fermentation cells can lose viability due to the loss of sugar as an energy source. We generally see the post-fermentation population drop to $10^3$ cells/ml. If this is used as a 20% inoculum, again the fermentation will be inoculated with roughly $10^2$cells/ml final concentration. It requires on the order of 60 hours for $10^2$ cells/ml to reach $10^6$. 
Native flora fermentations frequently display long lags due to the low numbers of *Saccharomyces* present on the grapes at the time of harvest. Early during the vintage, the levels may be well below $10^2$ cells/ml. However later in harvest the yeast populations build up on winery equipment and the level present in an uninoculated fermentation is more on the order of $10^4$ cells/ml. Native flora fermentations may be difficult to predict for several reasons. The dominant yeast strains present at the start of fermentation may not be the ones best suited to conduct a fermentation to dryness. Proliferation of *Saccharomyces* may be inhibited by the presence of other organisms, and thus the generation time is significantly longer than 3-5 hours. If a low inoculum is being used then the winemaker knows to expect a sluggish start, so this is not problematic. The most frequent cause of unexpected sluggish starts is failure to follow manufacturer's instructions for the rehydration of the yeast. Yeast should never be exposed to extremes of temperature during rehydration. Likewise, rehydration in wine is not recommended, as the yeast does not maintain viability during the rehydration process in the presence of high ethanol concentrations.

In addition to a low inoculum level, long lags might also arise if there is some problem with juice composition particularly the presence of a toxin or toxic condition that the yeast must overcome before fermentation can initiate. We have already discussed how yeast adapts to sulfur dioxide by detoxifying it. The detoxification process has to be complete before growth will initiate.

**Presence of Inhibitors as a Cause of Long Lag**
● Sulfur dioxide concentration too high
● Sulfur dioxide added improperly
● Microbial activity resulting in inhibition
● Pesticide/fungicide residues on grapes at harvest
● Temperature of must/juice too high/low

The yeast will have a longer lag the higher the \( \text{SO}_2 \) concentration of the must or juice. Sometimes in a winery situation during crush communication may not be optimal and more than one person may sulfite the same tank. This leads to a very high level that must be detoxified. Also one must be careful in how the \( \text{SO}_2 \) is added. It should never be mixed in with the yeast inoculum, as the level present will be toxic. Nor should it be layered on to of a yeast inoculum, a practice that will again result in a localized high concentration for the yeast. The \( \text{SO}_2 \) should be well mixed into the fermentation before the yeast is added. The yeast inoculum should also be well mixed post addition.

It is also important that the inoculum be well dispersed, that is, not rehydrated into clumps, which will simply sink to the bottom of the tank. This requires gentle mixing, too vigorous mixing may break the cells leading to a drop in viability.

The non-\emph{Saccharomyces} flora may produce toxins limiting yeast growth. The presence of toxins may be manifest as an inhibition of cell growth and therefore of the start of the fermentation. \emph{Saccharomyces} is inhibited by high concentrations of acetic acid, which can be produced by wild yeasts and bacteria in the must or juice, especially if the juice is aerated significantly. As noted in the previous lecture, \emph{Saccharomyces} is a fungus so incorrectly applied fungicides in the vineyard may wind up in the must or juice and prevent initiation of fermentation. Finally, if the yeast inoculum is exposed to extreme \textbf{temperature} cell viability will decrease. This would occur if the juice or must were too warm (greater than \( 40^\circ \text{C} \)) as might happen following a heat treatment to eliminate polyphenol oxidase activity. Similarly, if the juice or must has been subjected to cold settling or cold maceration, it is best to warm the juice to ambient temperature before inoculation with the yeast.

\textbf{Grape Quality}
The condition of the grapes also impacts the rate of initiation of fermentation. If the grapes have heavy damage, especially while on the vine, the growth of non-*Saccharomyces* organisms will occur which may lead to the consumption of macro and micronutrients. If the deficiency is severe enough, maximal cell biomass will not be attained. Even if nutrients have not been depleted the simple presence of a high bioload of organisms may limit fermentation until ethanol accumulates to a high enough level to eliminate the competition.
Lesson 11: Fermentations Displaying a Sluggish Rate Throughout

Types of Sluggish Fermentations

- Long Lag
- Slow Rate Over Entire Course of Fermentation

The following graph displays a fermentation that would be described as sluggish throughout highlighted in red.

In this particular juice there was a long lag followed by failure to attain maximal fermentation rate. In the previous long lag example, once maximal cell biomass had been achieved the fermentation progressed more or less normally.
Causes of Slow Rate Over Entire Time Course

- Failure to reach maximum cell density
- Nutrient limitation
- Strain a poor choice for conditions
- Inhibitory fermentation conditions: temperature, pH, ionic imbalances

Sluggish fermentations generally mean that the culture has not been able to attain or maintain maximal biomass production at $10^8$ cells/ml. This may be due to nutrient limitation, that is, insufficient growth factors, or it may mean that the strain is not well adapted to growth in a grape juice or must environment. Slow fermentations may be associated with inhibitory conditions such as a low temperature of fermentation, low pH or some type of ionic imbalance. Slow fermentations may eventually arrest or they may complete, it depends upon the characteristics of the yeast strain. Strains can vary quite a bit in terms of maximal fermentation rate and responses to stress conditions. If the strain is "naturally" slow then a slow fermentation does not mean the fermentation will arrest. On the other hand, if the strain is typically a robust fermentor a drop in fermentation rate may indicate a problem in the fermentation. Yeast strains display differences in ethanol tolerance ranging from 10% to well over 19%. If the initial sugar content is high and a strain with poor ethanol tolerance is selected or present without having been selected, the fermentation may be sluggish and then arrest. This underscores the need to understand what the normal behavior of a given strain is during fermentation. Sluggish fermentations need not arrest, they may go to dryness if the strain is simply a slow fermentor, but is not being inhibited by any stress factors.
Lesson 11: Normal Initiation of Fermentation Becoming Slow

One of the most common types of fermentation problems in California is the seemingly normal fermentation that slows dramatically at a high sugar concentration. These types of fermentations often arrest with a high residual sugar content.

Types of Sluggish Fermentations

- Long Lag
- Slow Rate Over Entire Course of Fermentation
- Rapid Rate Becoming Slow

There are many known and suspected causes of this type of fermentation arrest. It is generally thought to reflect a problem with ethanol tolerance of the culture.

Types of Problem Fermentations

In this case, the stain either displays a poor native tolerance to ethanol or other
conditions in the fermentation impact and limit innate ethanol tolerance.

**Causes of a Decrease in Rate**

- Poor ethanol tolerance
- Loss of viability
- Loss of fermentative capacity
- Nutrient limitation
- Poor strain

**Ethanol tolerance** will be reduced if the cells do not have sufficient resources such as sterols and unsaturated fatty acids, to generate an ethanol resistant membrane. Tolerance will also be reduced if the cells have insufficient nitrogen to synthesize the new proteins needed that are more resistant to the inhibitory effects of cellular ethanol.

The ability to tolerate ethanol is a function of external pH, temperature and the presence of other inhibitors. The following compounds seem to synergistically impact ethanol tolerance: acetic and other organic acids, seed tannins, and inhibitory fatty acids. If these compounds are present, the maximal tolerable ethanol concentration decreases.

If there is some condition that reduces cell viability such that cell numbers drop below $10^8$ cells/ml, the fermentation rate will be likewise reduced since it is a function of total biomass. It is also a function of the rate of fermentation of the individual cells. It is possible to have a normal maximal biomass but to still not observe the maximal fermentation rate because the cells have a "diminished capacity" to ferment. An example of this would be a culture that does not have enough nitrogen to make the hexose transporters with higher substrate affinity needed to optimize fermentation rate as the external concentration drops.

To review some biochemistry, sugar is transported in yeast by a process known as **facilitated diffusion**. In this case, substrate simply moves along its concentration gradient. This process does not require energy and is therefore the most beneficial process to use for uptake of an energy source. Facilitated diffusion systems have a limitation, however, not found with active energy-requiring transport systems. The only work well within a concentration range 10 fold higher and 10 fold lower than their Km (the concentration at which the transport process is "half-saturated" - that is, that yields one half of the maximal transport rate). Grape sugar spans the concentrations from
molar to milimolar, or a thousand-fold range. This is well beyond the capacity of a single transport protein. For this reason *Saccharomyces* has a family of hexose transporters spanning the Km range from 1 M to 1mM. As sugar concentration in the medium drops the yeast cells make different transporters so that the Km of the transporter is well matched to the sugar concentration of the fermenting juice. Since the transporters are proteins, this process requires nitrogen be available. If it is not, yeast will only be able to use the current transporters that will not work as efficiently. Therefore the rate per cell is less than it would be if they were able to make the transporters they needed. There seems to be strain differences in the ability to express the appropriate transporters under conditions of moderate nutritional stress. The biological reason this occurs is unknown.
Lesson 11: Normal Fermentation Arresting Abruptly

In the example above, a normal fermentation becomes sluggish and then may or may not arrest. Occasionally, a fermentation arrests abruptly - there is no "sluggish becoming stuck" phase.

Types of Sluggish Fermentations

- Long Lag
- Slow Rate Over Entire Course of Fermentation
- Rapid Rate Becoming Slow
- Abrupt Stop

This is usually indicative of an environmental shock to the yeast rather than a nutritional problem.

Types of Problem Fermentations

These types of fermentations are among the most difficult to re-initiate. They may not be associated with loss of viability, but rather with the yeast entering a resting
stationary phase. Alternately, if some manipulation of the fermentation occurred leading to cell death, loss of viability may be a cause. An example of this would be a late addition of a high SO₂ level. This might occur if the winemaker became concerned that a spoilage organism might be present. Fermentations will also arrest abruptly if a temperature shock occurs at a high, but not quite finished, ethanol concentration, or if a change in pH has occurred. This would be the case if the pH of the juice were adjusted late in fermentation, as a means to control microbial flora for example.

Causes of Abrupt Stop

- Temperature shock
- Build up of inhibitors: acetic/organic acids
- pH decreases too much
- Strain very ethanol sensitive

An abrupt arrest may also occur if there is significant metabolic activity by other organisms producing an inhibitory end product. Some yeast strains are sensitive to co-inoculation with the malolactic bacteria. In this case, inoculation with a vigorous ML bacteria culture late in fermentation may abruptly arrest fermentation. If this is the practice at the winery, a yeast strain tolerant of lactic acid bacteria should be used. The fermentation will also abruptly stop once the yeast has reached its maximal ethanol tolerance level.
Lesson 11: The Most Common Causes of Fermentation Arrest

Numerous possible causes of fermentation arrest have been described in the literature, but it is important to know what the probable causes are under specific winery conditions. The following slide lists the five most common causes of fermentation arrest under California production conditions.

**Most Common Causes of Stuck/Sluggish Fermentation**

- Nutrient deficiency
- Temperature extreme
- Presence of a toxic substance
- Microbial incompatibility
- Deficient yeast strain

The most common juice or must problem that can lead to arrest of fermentation is **nutrient limitation**. It is current California practice to treat every fermentation as if it is or will be deficient and to add nitrogen and phosphate in the form of diammonium phosphate at the start of fermentation. It could be argued that this practice eliminates nutrient deficiency as a cause of slow or incomplete fermentations. However, the winemaker must time nutrient additions so that the yeast conducting the bulk of the fermentation will benefit from them. Early additions may feed the wrong population. Yeast appears to arrest uptake of nitrogen compounds at high ethanol concentrations, so a late addition is not necessarily beneficial either.

A second common cause is **temperature shock** or exposing the fermentation to a temperature extreme. This is more common in red wine production because many reds tend to be fermented "hot" (30°C or warmer) and the maximal tolerable temperature for yeast is between 35 and 42°C. The heat given off during fermentation may raise the temperature to an inhibitory level. We have found that cooling the tank may lead to restart, but the culture is fundamentally changed and tends to arrest at high ethanol levels, around 11%. The culture arrests at this point and if sugar is still present, it will not be fermented. We have not noted the same types of problems with low temperature shock as would occur if supercooling of the tank (forgetting to turn off a refrigeration system as fermentation rate slows) took place. However, winemakers have reported this problem and it may be strain specific.
There are various types of toxic substances that may be present during fermentation. Fungi make compounds inhibitory to plants as a mechanism to enhance infection of the plant. Some of these mycotoxins are known to be inhibitory to yeast depending upon the composition of the must or juice.

Another common complaint is that fermentation seems to arrest following a "harmless" lactic acid bacterial bloom. It is not clear if this is due to competition for limiting nutrients or production of a toxic substance by the bacteria such as acetic or another organic acid. Nutrient supplementation does not appear to solve this problem, so if it is competition for a nutrient, the nutrient is something other than what is typically found in yeast nutritional supplements.

Winemakers frequently note that certain vineyards or sections of a vineyard tend to cause stuck fermentations more than fruit of the same varietal harvested from a geographically similar area. This may reflect consistent differences in the flora of one section of the vineyard versus another, but a more likely explanation is the presence of inhibitory phenolic compounds. We have found that seed phenolic preparations are inhibitory to fermentation. Plants take up phenolic compounds from the soil and concentrate them in the seed. From the lecture on viticulture we know that the purpose of the seed is to generate a new plant and it is important to make sure the seed contains antimicrobial compounds. Depending upon the "phenolic" history of one region of the vineyard versus another, seed composition may vary which would lead to differences in the presence of inhibitors in the seeds.

Finally, some popular commercial strains are more sensitive to certain types of stresses than others. It is important to match the strain to the fermentation conditions in order to avoid fermentation problem.

It is frequently challenging to restart an arrested fermentation. For this reason, winemakers would prefer to have the fermentation complete in a timely fashion.
Why are stuck fermentations difficult to treat?

- Cells adapt to adverse conditions by reducing fermentation capacity
- Biological adaptation difficult to reverse
- Diagnosis of cause of fermentation problem difficult
- Conditions that cause stuck fermentations are also conducive to cell death
- New inocula respond to cell death by arresting activities

Yeast cells **decrease fermentation rate under conditions of environmental or biological stress.** These conditions frequently lead to formation of a resting state, sometimes called a "deep" stationary phase. Resting stage cells have drastically reduced metabolic activity, but do not readily re-enter a metabolically active state unless environmental conditions drastically change for the better. **Biological adaptations are difficult to reverse** as they represent a survival mechanism. Further, if the exact cause of the arrest of fermentation is not known, it is **challenging to correct the cause of stress for the yeast.** Conditions of stress leading to entry to a resting state frequently are lethal or near-lethal situations. If uncorrected, a significant percentage of the **population may die.** Many microbes are social organisms, and respond to signals generated by other members of the same species in the environment. Massive cell death appears to lead to the **release of compounds that signal** conditions leading to loss of viability. New cells in the environment will respond to these signals, even if conditions are otherwise permissive for growth. The presence of these types of compounds during grape juice fermentation is hypothesized, but has not been directly demonstrated yet. However, it is likely that such compounds are produced and would lead to arrest of cells in the new inoculum.
Lesson 11: Factors Most Impacting Fermentations Rate Under Enological Conditions

Several winery practices have a dramatic impact on yeast fermentation. Some of these practices may be employed with the intent of stimulating the yeast, while others are used for a different purpose with the stimulation of yeast being a secondary benefit.

Fermentation Variables Impacting Progression and Rate

- Oxygenation
- Mixing: Natural or Assisted
- Type of Fermentation Vessel
- Inoculation Practices
- Temperature of Fermentation
- Supplementation/Juice Treatments
- Lees Contact
- Presence of Solids

Oxygenation:

- Oxygen is a micronutrient electron acceptor
- Oxygen is a survival factor
- Oxygen can lead to color changes (brown, pink, orange)

Principle among these fermentation variables is oxygenation. Oxygen is very stimulatory to yeast growth and fermentation, largely because oxygen allows cells to form ethanol tolerant membranes.

While oxygen plays an important role in provision of survival factors needed for ethanol tolerance, it can also lead to oxidative changes in the wine. Sometimes these changes are desired and other times they are not as we have discussed at length in earlier lectures. If the yeast are actively metabolizing they are quite efficient at sequestration
of medium oxygen that would limit formation of oxidative off-characters.

**Mixing (natural or assisted):**

- Separation of yeast from end products
- Brings yeast in contact with new nutrients
- Can facilitate skin extraction in reds and solids extraction in whites and reds

Oxygenation may be deliberate, for the sole purpose of stimulating yeast or it may be a consequence of other activities such as mixing of the tank. Mixing leads to oxygen exposure unless done under a modified atmosphere, which is rare. Mixing will also occur with a vigorous fermentation due to the rapid formation of carbon dioxide. Mixing even in the absence of aeration can be stimulatory to yeast, perhaps because it distributes toxic end products more evenly preventing localized accumulation.

Mixing can also bring yeast in contact with nutrients again by distributing the yeast more uniformly in the tank. It can also increase extraction of components from the skins and solids during fermentation. This provision of nutrients may be stimulatory if nutrients are limiting.

**Type of Fermentation Vessel**

- **Stainless Steel**
  - Can be cooled
  - Easier to sanitize
  - Inert
- **Wood:**
  - Can impart positive flavors and aromas
  - Difficult to clean; impossible to sterilize
  - Develops stable biofilm of microflora

The type and design of the fermentation vessel may also impact fermentation performance.
These effects concern ease of temperature control of the vessel, and presence or absence of a biofilm of flora. A biofilm may be comprised of beneficial organisms, of spoilage organism or of both types. Sanitation is easier for stainless steel than for wood or cement fermenters.

**Inoculation Practices**

- Spontaneous Fermentation
- Inoculated Fermentation

One of the most important winemaking decisions that impact fermentation initiation and progression is inoculation practices.

The two options discussed at length in an earlier lecture are inoculated and spontaneous fermentations. Inoculation allows the winemaker to control the biomass level of *Saccharomyces* and to minimize the impact of non-*Saccharomyces* microbes. It is more difficult to predict the course of a spontaneous fermentation as the bioload of *Saccharomyces* and the fermentation behavior of the dominant strains are unknown.

**Temperature of Fermentation**

- Affects presence and persistence of wild flora
- Affects fermentation and growth rates
- Extremes are inhibitory
- Impacts spontaneous chemical reactions

Fermentation temperature is one of the most important variables impacting rate. Temperature affects growth as well as metabolic rates. Within permissive limits, the higher the temperature the higher the fermentation rate. However, winemakers need to be cautious in manipulation of temperature as yeast adapt to temperature using similar mechanisms as adaptation to ethanol. Great swings in temperature will result in arrest if the strain is intolerant.

In addition, temperature will affect both the types and numbers of non-*Saccharomyces*
flora. The effect of this will depend upon what microbes are present and whether they are inhibitory, stimulatory or neutral towards yeast metabolism. Temperature affects the rate of spontaneous chemical reactions and processes such as volatilization. We have discussed at length the impact of temperature on extraction. Thus, frequently the temperature selected is more influenced by winemaking style issues than by considerations for the comfort of the yeast.

**Supplementation/Juice Treatments**

- Prevents nutritional deficiency
- May impact spectrum of yeast end products
- Residual nutrients encourage growth of spoilage organisms
- Unwanted byproducts may be made

An obvious variable impacting yeast fermentation is nutrient addition strategies and juice/must manipulation practices (cold soak, cold settling, pH adjustment, SO₂ addition, etc). These factors have been discussed previously.

If fermentations are overfed, there may be high levels of residual nutrients at the end of yeast fermentation. These nutrients can then encourage the growth of other organisms, and may increase spoilage problems post-fermentation.

**Juice/Must Supplements**

- Diammonium phosphate (0.96 g/L; 8 lbs/1000 gal)
- Yeast nutritional supplements (varies by producer)
- Yeast autolysates (3lb/1000 gal)
- Thiamin hydrochloride (0.005 lb/1000 gal)

In addition to nutrient supplements summarized above, other juice treatments may have a strong impact on yeast fermentation.
Other Juice Treatments

- Fining
- Centrifugation
- Aeration
- Clarification: settling/filtration

Processes such as fining and centrifugation may impact fermentation performance as these techniques may strip the wine of nutrients or other growth stimulating conditions. Clarification, if carried to an extreme, may have a similar negative impact by stripping the wine of solids.

**Lees Contact**

- Extraction of nutrients
- Extraction of grape characters
- Function as solids

Lees contact, both grape and yeast, also affects yeast performance. Lees may contain nutrients that will be more slowly released during the course of fermentation. The slower release tends to make these compounds available late in fermentation when it is most critical to feed the dominant species present.

Grape lees can function as solids in stimulation of yeast growth and have been proposed as being able to sequester toxins, although this has not been definitively shown. Similarly, it has been proposed that yeast lees can remove toxins from the fermentation thereby allowing the remaining yeast to complete the fermentation. In addition, lees contact will affect the composition of the must/juice that may be desired independently of impacts on yeast biology.

**Presence of Solids**
Natural (grape material) or added (bentonite, yeast hulls)
- Stimulate fermentation
- Stimulate growth
- Source of nutrients
- Removal of inhibitory components

We have noted above several times that solids are important stimulators of yeast fermentation. The exact effect of the solids is not known, but several hypotheses have been put forward.

Solids may be "natural" deriving from the grape or added such as the fining agent bentonite or yeast hulls. They are known to stimulate fermentation and growth. Cultures receiving solids treatment tend to have higher maximal cell numbers, which may explain the faster fermentation rate. Solids are proposed as providing nutrients or removing inhibitors. It has also been suggested that solids may help hold molecular oxygen in the juice or lead to faster nucleation and loss of carbon dioxide, which aids in mixing. Finally, it has been suggested that solids affect the non-\textit{Saccharomyces} flora having an indirect effect on yeast metabolic activities.
Lesson 11: Re-initiation of Stuck Fermentations

Our final topic for this lecture concerns the restart of arrested fermentations. There are some key guidelines for a successful restart. First, it is helpful if the cause of arrest is known, a determination which may not be as easy as it seems.

Re-initiation of Stuck Fermentations

- Correct diagnosis of nature of the problem important
- If re-innuculating, make sure inoculum is adapted to conditions of stuck wine
- Serial re-inoculation
- May need to remove existing biomass

It is also important to make sure that the new inoculum is adapted to the fermentative conditions (temperature, pH and ethanol content) of the arrested ferment. This will prevent shock of the inoculum upon addition to the ferment. The best way to achieve this is via the procedure called serial re-inoculation. In this case, the arrested wine is mixed with an equal volume of juice or actively fermenting must. This 50:50 mix is allowed to ferment to conditions near the arrested culture and a second 50:50 mix occurs of the total volume with an equal volume of arrested fermentation. This procedure is repeated until the arrested fermentation is restarted in total.

This procedure can be tricky to perform. It is important that the fermentation never be
allowed to go dry. If this happens fermentation will not occur in the downstream 50:50 mix.

**Serial Re-Innoculation**

Transfer initial 50:50 blend to second tank when it is 2-4 Brix above the arrested Brix level of the stuck fermentation

Do not let any of the intermediate steps in the series go dry, transfer them at the equivalent or slightly higher Brix than the arrested wine

This procedure or variations thereof work much of the time but not all of the time. It may be necessary to remove the existing yeast biomass through settling and racking or filtration.

This ends the series of lectures on the alcoholic fermentation and problems fermentations. In the next section we will consider the other principle microbial conversion that occurs during wine production, the malolactic fermentation.
Section 4 - The Malolactic Fermentation
Lesson 12: Introduction

Malolactic Fermentation

This section of the course will cover the Malolactic Fermentation, the conversion of malate to lactate conducted by members of the lactic acid bacteria family. In the first lecture we will discuss the biology of this important class of organisms and in the second cover the topic of management of this fermentation.

The lactic acid bacteria are gram positive organisms. They are found broadly in nature and are important in the production of many fermented foods and beverages. The malolactic fermentation refers to the conversion of malate to lactate, which can result in the generation of energy in the form of ATP.

The "malolactic fermentation" refers to the conversion of the grape acid malate to lactate conducted by members of the lactic acid bacteria.

Malate is a dicarboxylic acid, meaning that it contains two carboxyl groups. Lactate has a single carboxyl group and is monocarboxylic. Therefore the conversion of malate to lactate is a decarboxylation producing one molecule of CO$_2$ for every molecule of lactate.
Dicarboxylic acids have two acidic groups that can release protons while lactate contains only one proton that can be released. Note that the other terminal carbon in lactate has three hydrogen ions. One of the "free protons" in the system has been fixed in the conversion of malate to lactate. Thus the acidity is decreased and the pH of the wine is increased.
Lesson 12: The Lactic Acid Bacteria

Lactic Acid Bacteria: Characteristics

- Prokaryotes: no membrane around nucleus
- Gram positive
  - Peptidoglycan
  - Teicholic acid
- Divide by binary fission

The lactic acid bacteria are true prokaryotes: they do not contain a membrane-bound nucleus or other organelles found in eukaryotes like yeast. Their cell walls contain peptidoglycan a characteristic of gram positive organisms. Teichoic acid is another characteristic component of the gram positive cell wall. However some of the lactic acid bacteria do not contain this compound. The lactic acid bacteria may be rods or cocci. These bacteria divide by binary fission.

Cell division is initiated by the synthesis of a new copy of the single circular DNA molecule. The DNA molecules are attached to the surface of the cell. New growth occurs between the two DNA molecules separating them from each other. Once sufficient growth has occurred, the cell synthesizes a membrane and cell wall between the two DNA molecules, forming two new cells. In contrast to the yeast cell division, there are no mother or daughter cells just two daughters. Each daughter contains new cell material and old cell material.

The lactic acid bacteria are chemotrophic meaning that they obtain energy from the
oxidation of chemical compounds just as was the case with yeast. When one compound is oxidized it loses electrons. To balance metabolism a recipient compound that receives the electrons and is thereby more reduced must be formed. In the malolactic conversion, malate is the electron donor and the electron (proton) appears on lactate. These bacteria can also use pyruvate as an electron acceptor, which also yields lactate.

**Lactic Acid Bacteria: Divisions**

- **Group I:** Strict homofermenters
- **Group II:** Facultative heterofermenters
- **Group III:** Strict heterofermenters

The lactic acid bacteria have been divided into different groups based upon the spectrum of end products produced. There are strict homofermenters, which only produce lactic acid and are unable to grow on pentoses. The strict heterofermenters generate several other compounds in addition to lactic acid. The final class is the facultative heterofermentative (or facultative homofermentative) lactic acid bacteria. These organisms are the most versatile and can switch between hetero- and homofermentative modes of metabolism, depending upon the carbon source.

In homofermentative metabolism the majority of the sugar present in the medium is converted to lactic acid. Lactic acid is produced from the reduction of pyruvate. Pyruvate is formed via glycolysis from hexoses using the same enzymatic steps.
previously described for *Saccharomyces*. Two lactic acid molecules are formed from each hexose molecule catabolized.

**Heterofermentative Metabolism**

Organism smetabolize glucose via the pentose phosphate pathway.

End products can vary depending upon level of aeration and presence of other proton and electron acceptors.

Acetyl-phosphate can be converted to acetate and ATP or reduced to ethanol without ATP production.

Heterofermentative organisms also produce significant amounts of lactate, but they use the pentose phosphate pathway for sugar catabolism rather than glycolysis. Five carbon sugars or pentoses can also be metabolized via this pathway. In this case the carbons appear as lactate and acetate.

**Pentose Phosphate Pathway**

Lactic Acid Bacteria can also metabolize pentose such as ribose, arabinose and xylose, via the pentose phosphate pathway.

Acetyl-phosphate leads to the generation of acetate and ATP exclusively in pentose metabolism.

The rest of end products produced depends upon other environmental and nutritional factors. The formation of acetate results in the generation of an ATP molecule so this will be favored, conditions permitting. The strict heterofermentors lack the fructose 1,6-diphosphate aldolase, a key reaction in glycolysis, and therefore only use the pentose phosphate pathway giving lactate, acetate, ethanol and CO$_2$. The strict homofermenters use the glycolytic pathway for hexose catabolism, but not the pentose phosphate pathway and produce two molecules of lactate from hexoses. These
organisms do not ferment pentoses. The facultative heterofermenters can use both pathways. Glycolysis is the preferred mode of metabolism of hexoses but pentoses are fermented via the pentose phosphate pathway. However, if conditions (aeration) exist favoring generation of energy from the production of acetate, these organisms may shunt sugar carbon towards this pathway.

Acetate is also formed from six carbon hexoses in the heterofermentative bacteria. Six carbon compounds are decarboxylated to a five-carbon compound then continue through the pathway. If conditions are not permissive for acetate formation, ethanol may be produced instead. Ethanol production will occur if the cells need to transfer electrons to recipient compounds, that is, when oxidative catabolism is generating reduced cofactors that cannot be reoxidized any other way. If oxygen is present, the bacteria can transfer electrons to molecular oxygen forming water and in some cases hydrogen peroxide. If oxygen is present, this mode of metabolism will be favored since it yields more ATP.
Lactic Acid Bacteria: Genera

- *Oenococcus*
- *Pedicoccus*
- *Lactobacillus*
- *Leuconostoc*

There are four genera of lactic acid bacteria that are important in wine production. I have listed both *Oenococcus* and *Leuconostoc* since both terms appear in the literature. There is only one species of *Leuconostoc* found in wine, *Leuconostoc oenos*. This organism is quite different from other members of this genus so in 1995 taxonomists proposed a new genus and species name for this organism, *Oenococcus oeni*. The proposal was approved and *Oenococcus oeni* has now become the official designation. Both names can be found in the wine literature so it is important to remember that they are the same organism.

*Oenococcus*

- *O. oeni*

*O. oeni* is a facultative heterofermentative lactic. It produces only lactic acid from hexoses and is therefore a homolactic hexose fermenter. There is only one species of *Oenococcus*. Several species of the *Pediococcus* and *Lactobacillus* genera are present in wine. Some are homolactic (producing only lactate from hexoses) and others are heterolactic (producing lactate, acetate, ethanol and CO₂).

*Pediococcus*

- *P. damnosus*
- *P. parvulus*
- *P. pentosaceus*
- *P. acidilactici*
P. damnosus is heterolactic while P. pentosaceus is homolactic.

In contrast to the typical gram positive bacteria, members of Pediococcus do not have teichoic acid in their cell walls. These species can be differentiated based upon the maximal temperature that will support growth.

**Lactobacillus**

<table>
<thead>
<tr>
<th>Homolactic on hexoses</th>
<th>Heterolactic on hexoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>● L. bavaricus</td>
<td>● L. fermentum</td>
</tr>
<tr>
<td>● L. casei</td>
<td>● L. brevis</td>
</tr>
<tr>
<td>● L. homohoiachii</td>
<td>● L. buchneri</td>
</tr>
<tr>
<td>● L. curvatus</td>
<td>● L. fructivorans</td>
</tr>
<tr>
<td>● L. saki</td>
<td>● L. hilgardii</td>
</tr>
<tr>
<td>● L. plantarum</td>
<td></td>
</tr>
</tbody>
</table>

Many more species of Lactobacillus can be found in wine. They are split between homo- and heterolactic fermenters, based on their behavior in the degradation of hexoses. The homolactic species listed above produce only lactic acid, but they are actually facultative heterofermenters. No strict homofermentative organisms have been isolated from wine to date. The heterolactic species listed above are strict heterofermenters, meaning that compounds in addition to lactate are made from hexose catabolism. Many of the Lactobacillus species will not persist in wine at low pH (3.5 or lower). The end products of the biological activities of the lactic acid bacteria will be dependent upon which species are present and which mode of metabolism, homolactic or heterolactic occurs.

**Lactic Acid Bacteria: Prevalence in Wine**

- Only O. oeni is found at low (<3.5)pH
- Pediococcus and Lactobacillus grow at pH values above 3.5.
Lesson 12: The Malolactic Fermentation

The malolactic fermentation has many effects on the flavor and aroma of wines. As noted above, it is a deacidification reducing the net concentration of carboxyl groups and therefore acidity. This is important in regions where the fruit is high in acid at harvest.

**Effects of Malolactic Fermentation**

- Deacidification

Deacidification may not be desired in wines already low in acidity, but this conversion may occur whether desired on not if conditions support the growth of the organisms.

**Deacidification**

- Decrease titratable acidity by 0.01 to 0.03 g/L because of H+ fixation
- Increase pH by 0.1 to 0.3 units
- Important for high acid wines
- May be undesirable in low acid situations

The fixation of hydrogen ions on lactate can reduce the titratable acidity by 0.01 to 0.03 g tartaric acid equivalents/ L. The pH is also increased by as much as 0.3 units. This is very important because if a wine is low in pH (below 3.5) the metabolic activity of the lactic acid bacteria can raise the pH to a level supporting the growth of many more species.

**Effects of Malolactic Fermentation**

- Deacidification
- Bacterial stability
Lesson 12: Bacterial Stability

The malolactic fermentation is also important because it confers bacterial stability to the wine, meaning that the growth of other organisms is inhibited. This is due to the consumption of nutrients so that conditions are not permissive for other microbes, but it may also be a consequence of the production of bacteriocins, compounds that are toxic to members of other species.

Bacterial Stability

- Consume nutrients that would otherwise be available for other organisms
- Produce toxins (bacteriocins) that may inhibit growth of other bacteria
- Prevent malolactic fermentation from occurring in bottle

Another important consideration is the timing of the malolactic fermentation. If it occurs prior to bottling it prevents microbial growth in the bottle. There are several reasons why growth in the bottle is undesirable.

Malolactic Fermentation in Bottle:

- Increases turbidity due to cell growth
- Produces noticeable gas as CO₂
- May produce polysaccharides material
  - Haze
  - Ropiness
- May raise pH allowing growth of spoilage organisms
- Does not allow for control of flavor/aroma profile of wine

Cloudiness or turbidity is objectionable in wine. Many consumers do not understand the source of the cloudiness so equate it with spoilage. The decarboxylation of malate yields carbon dioxide, which will produce noticeable bubbles in the wine. This is again undesired because many consumers do not understand the source of the CO₂, so equate it with an inferior or spoiled product. The bacteria may produce other unwanted
products that are noticeable in the bottle. If these characters appear in the wine prior to bottling, they can be removed. But if they appear in the bottle, this may require recalling the production lot and uncorking, treating and re-bottling, an expensive process. Also if the reaction occurs in the bottle, the winemaker has no control over the process. As we will see in the next lecture, the malolactic fermentation can be managed and directed towards desired versus undesired compounds. If a problem arises, the winemaker can increase the amount of SO\textsubscript{2} or decrease the pH if it is high enough to be stimulatory to the "bad" lactics. None of this is possible if the wine is already in the bottle.

**Effects of Malolactic Fermentation**

- Deacidification
- Bacterial stability
- Flavor changes

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Lesson 12: Flavor Changes of the Malolactic Fermentation

There are several important flavor changes that occur in wine that has undergone a malolactic fermentation in addition to deacidification. If heterofermentative organisms are present, acetic acid can be produced.

Flavor Changes Associated with Malolactic Fermentation

- Acetic acid
- Diacetyl
- Acetoin
- 2,3 Butantediol
- Ethyl lactate
- Diethyl succinate
- Acrolein
- Other compounds

Acetic Acid

- From sugar metabolism
- Amount formed versus ethanol depends upon aeration and presence of other electron acceptors
- Level produced can be significant
- Can also be produced from citrate metabolism
- Low levels can be made by Saccharomyces

Acetic acid itself is pungent but not offensively smelling. However its production can lead to the formation of other compounds that are objectionable.
Acetic acid is formed from sugar catabolism. As discussed above, the organism has the option of producing acetate or ethanol. Ethanol will be produced if other electron acceptors are not present. If molecular oxygen is available, acetic acid will be produced. This is one reason that extensive aeration of the wine during the ML fermentation is not recommended. However, oxygen stimulates growth of the malolactic bacteria much as it does in yeast. Limited oxygenation can actually stimulate the production of lactate, but it must be applied with caution. With limited aeration, *O. oeni* tends to produce lactate and ethanol and requires more full aeration for the production of acetate. However, other lactics may produce acetate under conditions where *O. oeni* will not.

The amount of acetic acid produced can be significant and above the sensory threshold of detection. As discussed below acetate can also be formed from citrate metabolism by the lactics.

**Diacetyl**

- **Made from pyruvate**
- **Multiple pathways to pyruvate**
- **1-4 mg/L adds complexity "buttery"**
- **Over 4 mg/L dominates "movie popcorn butter/rancid"**
- **Low amounts can be produced by yeast**

Another very important compound produced by the lactic acid bacteria is diacetyl. This compound has a characteristic buttery note, which can become as strong as popcorn butter in high concentration, and may take on a rancid taint.

Diacetyl is formed from metabolites of pyruvate. There are multiple ways in which pyruvate can be formed.
Pyruvate can come from acid catabolism as well as from sugar catabolism. Its formation will therefore be dependent upon the amount of these precursors present. Diacetyl is formed from the reaction of two two carbon compounds, active acetaldehyde and acetyl CoA, or from the reaction of pyruvate and active acetaldehyde producing the 5 carbon acetalactate which is the converted to one four carbon diacetyl molecule plus one molecule of carbon dioxide.

Acetaldehyde* refers to "active acetaldehyde" which indicates the enzymatically bound form of acetaldehyde with the coenzyme thiamine pyrophosphate.

Diacetyl and other dicarbonyl compounds have recently been shown to interact with the sulfur-containing amino acids producing a spectrum of characters such as hazelnut, chocolate, cheese, potatoe, cabbage, popcorn and roasted nut. Whether these compounds are formed or not depends upon the amino acid composition of the juice or wine.

Acetoin
Another compound that can be produced by lactic acid bacteria is acetoin. It can be produced from pyruvate as well and it can also come from diacetyl.

**Acetoin is usually present below the threshold of detection in most wines.**

![Pathways to Acetoin](image.png)

### 2,3 Butantediol

- Derived from acetoin
- Generally present below threshold of detection
- Mild alcohol flavor that borders on bitterness
- Can be produced by yeast
Lactic acid bacteria also produce 2-3 butanediol. This compound comes from acetoin. It is generally below the threshold of detection, but it has a flavor that has been described as a mild alcohol that borders on a bitter finish.

![2, 3 Butanediol](image)

This compound can also be produced by yeast, but not in very high quantities. Note that the formation of these compounds is reductive, that is, electrons are fixed in the formation of both acetoin and 2,3 butanediol. They allow the organism to continue to catabolize substrates oxidatively and to produce acetic acid if oxygen is present by serving to recycle reduced cofactors.

The lactic acid bacteria can also produce some ester characters that contribute positively to the aroma profile of the wine.

**Ethyl Lactate**

Ethyl lactate is described as "generic fruit". It is an ester formed from the reaction of the acid lactate and the alcohol ethanol. Since both of these compounds are present during active lactic bacterial metabolism, the formation of this ester is not surprising.
Generic fruit notes tend to increase the overall perception of the fruit character of the wine. That is, ethyl lactate may not be identifiable as such in a wine, the wine just appears to have more intense fruity and perhaps varietal character. However as we will see in the last section of the course, esters tend to hydrolyze under wine conditions so are not stable over time.

**Diethyl Succinate**

Diethyl succinate can also be produced by the lactic acid bacteria. This compound also imparts a generic fruit character.

![Diethyl Succinate](image)

This compound is formed from the reaction of the dicarboxylic acid succinate with two ethanol molecules.

**Acrolein**

- Made from glycerol
- Creates an intensely bitter taste when combined with phenolic compounds

Acrolein can also be produced by lactic acid bacteria, but is fortunately for winemakers only rarely found. This compound is made from glycerol and is intensely bitter.
Not all strains can produce this compound, and glycerol levels in wine are generally low enough to prevent its formation. However the use of yeast strains releasing large quantities of glycerol to improve mouth feel should be used with caution if the wine is also subjected to a malolactic fermentation, especially under conditions leading to the presence of *Pediococcus* and *Lactobacillus*.

The lactic acid bacteria can produce many other characters, depending upon what energy sources are available in the medium. Some of these compounds are the degradation products of amino acids.

**Other Compounds**

The Lactic Acid Bacteria are capable of producing numerous other aroma compounds, especially from the degradation of amino acids. It is likely that some of these compounds are also being produced during growth in wine.

Some of the notes found in cheeses may be present in wine, including the characters that border on fecal. These characters are classically described as "pig feces".

In the laboratory section of the course we have had the dubious pleasure of seeing the fecal character that can be produced by these organisms. This has more commonly occurred in nitrogen rich juices inoculated early in the fermentation or pre-fermentation by the yeast consistent with derivitazation from the amino acids. Another nasty character that can be produced from amino acids is the mousy taint that comes from the degradation of lysine. Again, production of this compound requires the presence of
lysine. Lysine is an important nutrient for *Saccharomyces* as we discussed in the yeast lectures, and supplementation with lysine can stimulate fermentation rate is some strains, but if over-supplementation occurs, the incidence of mousiness will increase. For those of you fortunate enough to be unfamiliar with this taint, it smells and tastes like a "used" mouse cage - a mixture of rodent and rodent urine. The character that we perceive is an oxidation product of the compound produced by the bacterium. It can be noticed by placing a drop of wine on one palm and rubbing palms together. When the palms are then smelled, it has the odor of a mouse cage. It is quite unpleasant by mouth as it is not initially detectable, but once oxidized imparts a nasty character that seems to originate in the back of the throat and is quite persistent.

**Tartrate**

Some strains of *L. plantarum* and *L. brevis* are capable of metabolizing tartrate to acetic acid, referred to as "tourne disease" by Pasteur. This is always undesirable.

Some lactic strains can metabolize tartrate to acetate. This is also always undesirable in wines.

The spectrum of compounds produced by the lactic acid bacteria are dependent upon the species present and the composition of the wine, juice or must at the time of the malolactic conversion.

**Flavor Changes Associated with the Malolactic Fermentation**

Amounts produced are strain dependent and dependent upon the composition of the juice and level of aeration.

In the next lecture we will discuss management of the malolactic fermentation and the options available to the winemaker.
Lesson 13: Introduction

Managing the Malolactic Fermentation

The factors impacting the rate and progression of the malolactic fermentation and strategies for stimulation and inhibition of this biological process will be covered in this lecture. The timing of this fermentation with respect to the alcoholic fermentation is also important, and will be addressed. Some yeast strains are sensitive to the presence of the bacteria and will arrest fermentation upon inoculation of the must or fermenting medium with lactics. Others are resistant and still others are inhibitory. We will also survey the current state of knowledge on the possible microbial interactions occurring in wine as related to the malolactic conversion relevant to these phenomena of stimulation and inhibition.
Lesson 13: The Biology of the Malolactic Fermentation

The conversion of malate to lactate occurs after the lactic acid bacteria have reached stationary phase. This reaction requires the cofactor NAD+ and manganese.

The Malolactic Fermentation

- Requires NAD+, Mn++
- Occurs after exponential growth phase
- Used to generate energy

Malate is directly converted into lactic acid (L-lactic acid) by the malolactic enzyme. This is a direct decarboxylation that does not pass through pyruvate. Production of the malolactic enzyme is induced by malate and stimulated by sugars. This conversion is used by the cell to generate energy.

The fixing of a proton on lactate creates what is called a "proton motive force" across the membrane. This force means that protons are in higher concentration on the outside of the cell and their entry into the cell is energetically favorable. The lactic acid bacteria are able to capture this energy and form ATP. However, it is not
stoichiometric, meaning that one ATP is not produced for every lactate molecule generated. A plasma membrane ATPase is able to convert the energy from hydrogen ion movements into the biochemically active form of energy, ATP. However, not all organisms that conduct the malolactic conversion have been shown to obtain energy by this process. There may be other metabolic factors driving this conversion.
Lesson 13: Factors Influencing the Malolactic Fermentation

As noted above, the malolactic conversion involves generation of a proton motive force, which requires maintaining a strong proton gradient across the membrane.

Factors Affecting the Malolactic Fermentation

- pH
- SO₂
- Nutrient composition
- Oxygen
- CO₂
- Alcohol
- Temperature
- Organic acids
- Phenolic compounds
- Presence of other lactic acid bacteria
- Bacteriophage

pH

- Affects which strains/species will grow
- Affects rate of growth
- Affects survival of organism
- Affects metabolic behavior of strains that are growing

One of the most important factors affecting the progression of the malolactic fermentation is pH. The pH has many effects on the progression of the malolactic fermentation in addition to contributing to the proton gradient. The pH of the medium determines which lactic acid bacteria will be present. It also impacts growth rate and is inhibitory if too low (less than 2.9). The pH also affects viability in addition to the
inhibition of growth. Finally, the pH will influence the metabolism of the organisms. Malate can be catabolized at pH values (3.2) below what is permissive for sugar catabolism (3.5) in many lactic acid bacteria. The malolactic conversion is actually faster at pH 3.8 than at lower pH values, however. The conversion will occur approximately 10 times slower at pH 3.2 as compared to 3.8. There is also considerable species and strain variation with respect to pH tolerance.

SO₂

- Sulfur dioxide is inhibitory
- All genera/species/strains appear to be equally sensitive
- Even if SO₂ is not added, it may be produced by yeast at an inhibitory concentration

Another factor that is very important in managing the malolactic fermentation is the use of sulfur dioxide. These organisms are very sensitive to sulfur dioxide, much more so than Saccharomyces. All strains appear to be equally sensitive and there are no SO₂ tolerant strains of Oenococcus. The molecular or free form of SO₂ is the inhibitory species. As discussed in an earlier lecture the amount of free SO₂ present is dependent upon the pH. The amount of free SO₂ is also a function of the presence of compounds that will bind sulfite. Bound SO₂ can also be inhibitory to the lactic acid bacteria, but is less so.

The SO₂ produced by Saccharomyces may be inhibitory to the lactic acid bacteria. Yeast produce on the order of 20 mg/L sulfite, which, if the pH conditions are appropriate, may be high enough to inhibit some malolactic strains.

The Nutrient Composition
Lactic acid bacteria are fastidious: numerous growth requirements
Aging on yeast lees increases micronutrient content via autolysis
Extended skin contact enhances malolactic fermentation
Higher solids/less clarification enhances malolactic fermentation

Nutrient availability is as important to the encouragement of the lactic acid bacteria and the malolactic conversion as it was for the yeast and the alcoholic fermentation. The lactic acid bacteria are more fastidious than the yeast, requiring many more micronutrients and growth factors.

This is one reason that the malolactic conversion is stimulated by autolysis of the yeast. Extended skin contact and higher solids levels are also stimulatory to the lactic acid bacteria. In contrast to the yeast, the bacteria require the presence of several amino acids. That means that they cannot synthesize all 20 amino acids from ammonia. Strains vary in which amino acids are required. Yeast release amino acids at the end of fermentation, so amino acid limitation is not usually a problem unless the bacteria are inoculated and the conversion expected before this release occurs. We have found that the malolactic conversion occurs more readily pre-yeast fermentation or after the release of the amino acids (post-fermentation), but is less likely to occur if inoculated during the active phase of the alcoholic fermentation. Several proprietary mixes of lactic acid nutrients are available, but if added early in the fermentation may also stimulate yeast growth and metabolism. Again it is important to time nutrient additions so that the desired population is the one that is fed.

**Oxygen**

- Stimulatory to growth
- Affects spectrum of end products
- Can produce more energy (and acetic acid) in presence of oxygen

Molecular oxygen is stimulatory to the malolactic fermentation, depending upon the
organisms present. It stimulates the growth of some lactic acid bacteria, behaving as a growth factor just as in the case of yeast. This is not observed with all species however, and some appear to be inhibited by oxygen. Limited oxygenation generally appears to be stimulatory to the malolactic fermentation in wine production. However, if too much oxygen is applied, and if strict heterofermentative organisms present, acetic acid may be produced as discussed in the previous lecture.

Aeration regimes during the malolactic fermentation should be limited to avoid unwanted end products. Winemakers frequently report acetic acid accumulation when the malolactic conversion occurs prior to the onset of the alcoholic fermentation. This usually coincides with an aeration of the juice or must.

**CO₂**

- Stimulatory to malolactic fermentation
- Mechanism unknown

Carbon dioxide is also stimulatory to lactic acid bacteria. This may be because CO₂ is associated with much better mixing, rather than it providing some other sort of nutritional benefit.

Carbon dioxide might also impact the buffering capacity of the wine and therefore stimulate growth and metabolism, but the actual mechanism of stimulation is not known.

**Alcohol**

- High alcohol slows malolactic fermentation
- Affects bacterial viability
- Affects which species/strains are present

Ethanol also affects the malolactic bacteria. As in the case of the yeast, these organisms have an upper limit of ethanol that can be tolerated. The malolactic bacteria are generally inhibited above 14% ethanol, but some species display a much stronger
sensitivity. If the malolactic conversion is desired in a late harvest or high Brix juice, it may be necessary to conduct this fermentation prior to the onset of the alcoholic fermentation.

In general, the higher the alcohol content the slower the malolactic fermentation.

**Temperature**

- Growth of malolactic bacteria better at higher temperatures
- Malolactic fermentation faster at higher temperatures

Temperature is an extremely important variable in the stimulation or inhibition of the malolactic fermentation. The optimum temperature for growth of the malolactic bacteria ranges from 20 to 37°C. Growth of the bacteria is inhibited below 15°C. Within the permissive range, the higher the temperature the faster the growth rate and the metabolic conversion of malate to lactate.

It is frequently necessary therefore to warm a fermentation before the malolactic fermentation will occur.

**Organic Acids**

- Fumarate inhibitory at low concentrations
- Can be produced by yeast
- Fatty acids can also be inhibitory
- Malate stimulates growth prior to malolactic fermentation

The presence of specific organic acids other than malate can influence the malolactic fermentation as well as other metabolic activities of the organisms. Malate will stimulate the growth of most of the bacteria prior to the onset of the conversion of malate to lactate. Interestingly, those that are not stimulated by malate are the same strains that do not appear to be able to generate ATP from the malolactic conversion.

Fumarate, which is produced in the tricarboxylic acid cycle by yeast, is inhibitory to the
malolactic bacteria. It is not clear if one reason specific yeast strains tend to inhibit the lactic acid bacteria is due to the release of fumaric acid during the alcoholic fermentation. Citric acid can be metabolized by the lactic acid bacteria as described in the previous lecture. Certain fatty acids may be inhibitory to the lactic acid bacteria.

**Phenolic Compounds**

The bacteria are also affected by the phenolic compounds of the wine. They can be inhibited by seed phenolics just as was the case for *Saccharomyces*. Stimulation by anthocyanins and gallic acid has been reported in the literature.

**Presence of Other Lactic Acid Bacteria**

- **Mixed cultures may yield "better" complexity**
- **Can be stimulatory**
  - Increase in pH
- **Can be inhibitory**
  - Bacteriocin production
  - Competition for nutrients

The presence of other bacteria will influence the malolactic conversion. This can be stimulatory, such as would occur if *O. oeni* raised the pH to a level permitting growth of other lactics or could be inhibitory, competition for nutrients or production of bacteriocins. There is a belief in the California wine industry that mixed culture malolactic fermentations provide greater complexity than single culture conversions, but it is not clear how many species in these preparations actually contribute to the overall character of the wine versus impacting the metabolic activities of the dominant organisms.

**Bacteriophages**
Bacterial "viruses" that can be spread from one bacterium to another and that cause cell death

Not known if this is a problem in wine production or not; it is a problem in other lactic acid bacteria fermentations

Another factor that is mentioned as possibly problematic in lactic acid bacteria fermentations is the presence of bacteriophage. Bacterial phages are virus-like particles that infect and lyse target bacteria.

They spread from one bacterium to another due to release of mature virus particles and lysis of the host cell and can eliminate an entire population. This is certainly a problem in other types of lactic fermentations, but it is not clear if this is problematic in wine. Bacteriophages infecting wine lactic acid bacteria have been discovered, but they have not been associated with prevention of the ML fermentation.
Lesson 13: Management of the Malolactic Fermentation

There are several considerations that must be taken into account by the winemaker with respect to the malolactic fermentation. The first question is whether or not this conversion is desired.

First Decision:

Do you want the MLF?

In some cases the malolactic fermentation is desired for the resulting increase in wine complexity; in other cases it will occur whether the winemaker wishes it to or not due to the composition of the wine. It is my experience that this fermentation demonstrates Mother Nature's sense of humor: it seems to go most readily under conditions where it is not desired and is more difficult to encourage when most wanted. Many winemakers contend that the best way to stimulate the ML fermentation is to bottle the wine!

Reasons MLF is Desirable

- Acidity reduction
- Addition of flavors
- Bacterial stability of product

There are three main reasons for desiring the malolactic fermentation: reduction in acidity, flavor addition and bacterial stability.

Reasons MLF is Undesirable

- Acidity reduction
- Addition of flavors

With the exception of bacterial stability, these are also the reasons the malolactic fermentation might not be desired!
If the malolactic fermentation is desired, there are several things that can be done to encourage it. Obviously little to no use of SO₂ should occur and the winemaker should use strains of *Saccharomyces* that do not produce high levels of sulfite.

**MLF Stimulated By:**

- Low to no use of SO₂
- Warm temperatures
- Addition of nutrients
- Use of inocula
- Low ethanol (avoid late harvest wines)
- Delay racking off yeast lees
- Acid/pH adjustment

The wine should be held at a permissive temperature for the bacterial conversion (above 15°C). Nutrients can be added, if needed, that is, if the wine has been racked off of the yeast lees prematurely or the wine was not fermented with a high solids content or with significant skin contact. As with *Saccharomyces*, an ML inoculum can be used rather than relying on a spontaneous initiation of the fermentation. The ethanol content of the wine should not be at an inhibitory value if the malolactic fermentation is desired. We have found that there are lactic bacteria that seem to grow in wines of high ethanol content, but they do not appear to conduct the malate to lactate conversion to completion, and they can produce objectionable characters in these wines. Finally, the pH should be adjusted upward if it is too low (below 3.2 for *O. oeni*). Doing the opposite of the above will be inhibitory to the malolactic conversion.

**MLF Inhibited By:**

- Use of SO₂
- Early racking
- Downward pH adjustment
- Low temperature
- Filtration
- Addition of fumaric acid
If inhibition is desired, the winemaker may want to filter the wine off of the yeast lees. But it is important to remember that the yeast tend to release amino acids prior to reaching true dryness, so this practice may be ineffective. One could use fumaric acid inhibition, but this acid will precipitate under wine conditions dropping its concentration in the wine, thus it is not a permanent guard against the ML bacteria. The same is true for SO₂, sulfite is volatile and reactive and its free concentration will decrease over time in the wine. If it drops below the inhibitory concentration, the ML bacteria may bloom.

The second major decision to be made by the winemaker is whether to inoculate with ML bacteria or to allow a native fermentation to occur.

**Second Decision:**

**Inoculated *versus* Spontaneous Malolactic Fermentation**

There are benefits and disadvantages to both, just as there was for the alcoholic fermentation.

**Inoculated MLF**

- Better control over both timing and organisms present
- Difficult to maintain inocula
- Starter culture must be "pure"
- Percent inoculation: 1-50% depending upon vigor of culture

Inoculation allows better control over the timing and the organisms (homolactic on hexoses (facultative heterofermenters)) versus the obligate or strict heterofermenters. That said, it is far more challenging to use a bacterial starter than a yeast starter culture. The bacteria are more fastidious so must be grown in a nutrient rich, part juice medium. This medium is impossible to fully sterilize, meaning that there is a certain loss of control over the purity of the species present. If a "bad" lactic becomes dominant, it will then be used to infect the entire production lot. It is imperative that all ML inocula be sensorially evaluated before being used as an inoculum. The best percent inoculum to use depends upon the vigor of the culture. Very high inocula tend
to initiate the fermentation faster and it is thought that they are less problematic than low inocula. There are now some active dry ML bacteria products in the marketplace with which some winemakers have had excellent experience.

The basic steps of ML inoculum preparation are as follows:

**Inoculum Preparation**

1. Start culture from slant in medium supporting good growth of organism
2. Inoculated "diluted" juice (with water) from starter with addition of nutrients
3. Use #2 to inoculate full strength wine or juice with addition of nutrients

Again I cannot emphasize enough that the inoculum should be smelled at each step. Your nose is the best indicator of the presence of a problem microbe.

**Spontaneous MLF**

- Uncontrolled timing of process
- Risk of unwanted species/strains
- Off-characters can be produced if MLF occurs when undesired

Allowing a spontaneous ML fermentation to occur runs many of the same risks as a spontaneous yeast fermentation. There is no control over the timing of the process and whether it will occur, has just occurred, or will not occur. Given the fastidious nature of the bacteria, the timing is far less certain than for the alcoholic fermentation. Also, conditions that stimulate the desired ML bacteria (the facultative heterofermenters that basically function as homolactic hexose fermenters in wine) also stimulate the bad lactics (the strict heterofermenters). This can lead to the formation of objectionable characters that would not be present if the "good" lactics were preferentially encouraged and consumed nutrients and produced factors inhibiting growth of the "bad" lactics. Good and bad are far more distinct with respect to the lactic acid bacteria than was the case for the yeast.
The next decision for the winemaker is the timing of the malolactic fermentation.

**Third Decision**

**Timing of Malolactic Fermentation**

An inappropriate choice in timing may result in the inhibition of the alcoholic fermentation or alternately may greatly reduce the chance that the ML will occur at all. The options are to encourage the ML prior to the initiation of the alcoholic fermentation, fully post fermentation, to encourage simultaneous yeast and ML fermentations or to inoculate with the ML bacteria at some specific point in the ML fermentation. Every year in the laboratory section of VEN124 we conduct a trial on the timing of inoculation of the ML fermentation versus success in completion of the fermentation. Our experience is that if it is going to go at all it will most likely occur pre- or post yeast fermentation. In most years the simultaneous inoculation does not lead to a successful ML fermentation nor does a mid fermentation inoculation. There is a trend in the industry to inoculate the ferment with about 5°Brix of sugar remaining. We have not had much success with inoculation at this time. Sometimes the post-fermentation ML will not occur and other times the pre- yeast fermentation sample will not undergo an ML fermentation.

**Timing of MLF: Options**

- Prior to yeast fermentation
- Simultaneous with yeast fermentation
- Mid-way throughout yeast fermentation
- After yeast fermentation

The timing of the ML fermentation will obviously depend upon the conditions of the juice and whether or not the temperature, pH and nutrients are permissive for all organisms.
- Decreases yeast nutrients
  - Stuck/sluggish fermentation
  - Production of off-characters
- May lead to production of inhibitory compounds (acetic acid) due to presence of oxygen

Pre-fermentation inoculation with the ML bacteria can decrease the nutrients available for the yeast. This can lead to off-character production or arrest of fermentation. It has been well established that lactic acid bacteria produce compounds that are inhibitory to yeast growth and fermentation. This is an area currently under active investigation.

Other types of problems may be associated with a simultaneous inoculation.

**Timing of MLF: Simultaneous with Yeast Inoculation**

- See increases in acetic acid
- See a decrease in viability of both yeast and bacteria
- Yeast "rebound" better than bacteria

Winemakers frequently report an increase in acetic acid production with simultaneous inoculation. A decrease in the viable populations of both organisms can be noted as well. It appears that the yeast are able to rebound more readily than the bacteria under these conditions, but the culture may still be prone to arrest. There are important strain factors that impact the fate of the yeast and bacteria. We have found that the commercial yeast, Premier Cuvee, seems to be relatively unaffected by the activity of the ML bacteria, but may be quite inhibitory towards many strains of the bacteria. Other commercial strains are much more sensitive to fermentation arrest upon inoculation with the bacteria.

**Timing of MLF: Mid-Fermentation**
Nutrients left for bacteria  
Ethanol low and not inhibitory  
Yeast-produced SO$_2$ may be inhibitory  
May lead to arrest of yeast fermentation

Inoculation mid-way or late in the alcoholic fermentation but before it is completed can be quite risky.

At this point there are hardly any nutrients left for the bacteria since the yeast have not yet released any. Ethanol may be low and not inhibitory at the point of inoculation, so it may seem like a great idea especially for high Brix musts and juices, but the yeast will continue to metabolize and raise the content of alcohol. Yeast-produced sulfur dioxide may also be highest at this time, especially if the rate of carbon dioxide production and therefore loss of SO$_2$ due to CO$_2$ evolution has slowed. While we have not seen this in our experiments, many winemakers report an arrest of the yeast fermentation upon inoculation with the ML bacteria mid or late into the alcoholic fermentation. Since high inoculum strengths are typically used, it may be that some inhibitor produced by the bacteria in the inoculum preparation is carried over into the yeast ferment. Alternately, at this point the yeast is most dependent upon available oxygen in the must and on fatty acids needed for ethanol tolerance the introduction of biomass at this point may rapidly deplete the fermentation of needed survival factors.

**Timing of MLF: Post Fermentation**

- Nutrients have been depleted
  - Add nutrients
  - Encourages yeast autoysis
- Ethanol concentration high
- Concentration of other yeast inhibitory compounds also high
- Better temperature control

Post-fermentation inoculation is another strategy. This prevents any inhibitory effect of the malolactic bacteria against the yeast, but will be problematic if the ethanol content is too high.
At this point other nutrients have been depleted and yeast autolysis requires on the order of six months to occur. Other inhibitory factors may have been produced by the yeast that may impact the ML bacteria. Post-fermentation inoculation allows better temperature control in that it permits the primary alcoholic fermentation to be conducted at a temperature leading to greater retention of grape volatile characters but too low for growth of the ML bacteria. Warming of the wine post alcoholic fermentation to make conditions permissive for the malate to lactate conversion does not lead to the same loss of characters that occurs if the wine is warm during the maximal rates of yeast catabolism and CO$_2$ evolution.

Another important choice for the winemaker is the selection of the ML strain to be used during the ML fermentation. It can be argued that this is more of an issue than it was with *Saccharomyces* because there is a greater diversity in the amounts and nature of sensorially detectable characters produced by different strains of lactics.

**Fourth Decision:**

**Choice of Strain**

This is complicated by the fact that the bacteria are so fastidious and the correct strain or pattern of metabolic activity may not actually be what is being encouraged to grow.

**MLF: Choice of Strain**

- Compatible with yeast
- Production of desirable characters
- Ability to complete ML fermentation
- Vigor
- Availability as freeze-dried inoculum

It is important that the ML strain selected be compatible with the yeast strain utilized. It is also important that the strain not have a tendency to produce off-characters and display reasonable vigor in growth as well as in conducting the ML conversion. Since the bacteria are fastidious in their requirements and numerous grape compositional factors impact the ML fermentation, it is not easy to be sure the strain will perform as
expected. If the strain is to be prepared as a commercial inoculum it is important that the strain be able to survive the production process and display good viability upon rehydration. One other note, it has frequently been reported that "cultured" ML strains lose the vigor and aroma profile of the "native" organisms.

As with the yeast fermentation, the winery needs to develop a strategy for monitoring the ML fermentation.

Fifth Decision:

Method of monitoring MLF

Monitoring the MLF

- By conversion of malate to lactate
  - Loss of malate not appearance of lactate*
  - HPLC, Enzymatic, Paper chromatography
- By flavor changes
  - Tells you bacteria are active
  - Does not tell you when they are done

* Lactate can be produced from other sources

The fermentation can be monitored by following the conversion of malate to lactate. This should be done by evaluating loss of malate, not the appearance of lactate. Recall that the yeast can make lactate and the bacteria can make this compound from hexoses so its appearance is not indicative of a malolactic fermentation. The loss of malate can be followed by simple paper chromatography or more quantitatively using an enzymatic assay method or HPLC or CE analysis. It is also important to note the appearance of other characteristic or "signature" ML compounds. This can frequently tell the winemaker that the bacteria are indeed present and metabolizing. However this will not tell you if the ML fermentation has been initiated or completed.

A final decision that can be made concerns the primary reason for conducting the malolactic fermentation.
Sixth Decision:

Alternative Method of Acid Reduction

If it is being conducted simply to reduce acidity, then alternative methods of acid reduction might be considered.

Alternative Methods of Acid Reduction

- Immobilized enzyme
- Immobilized cells
- Yeast mediated conversion of malate to ethanol
  - Conducted by *S. pombe*
  - *S. cerevisiae* has been genetically engineered to perform this conversion
- Expression of ML enzyme in *Saccharomyces*
- Chemical precipitation

The options noted above are mainly in the design stage or are undergoing testing and are not in commercial use as yet. It is possible to reduce acidity enzymatically by use of an immobilized enzyme system. The enzymes being evaluated convert malate to lactate, so the primary end product will not change. Immobilized cells can also be used. In theory it is easier to control and limit the biological activities of immobilized cells. The goal is a more controlled and guaranteed ML conversion. Dr. H. van Vuuren and colleagues have genetically engineered a yeast strain that will convert malate to ethanol. While this might not be desired in a high Brix juice or must, it may be an option for fruit low in sugar but high in acid at the time of harvest. The enzymes used were derived from the yeast *Schizosaccharomyces pombe*. Earlier Dr. Kunkee and colleagues were able to engineer a strain of *Saccharomyces* expressing the bacterial malolactic enzyme. These genetically engineered strains are not commercially available and are not being used in the production of wines except on a laboratory scale.

This concludes our section on the Malolactic bacteria. The most important goal of the winemaker is to make sure that this conversion occurs prior to bottling. If it has not, the only way to guarantee that it will not occur in the bottle is to steriley filter and steriley
bottle the wine.
Section 5 - Post-Fermentation Processing
Lesson 14: Introduction

Post-Fermentation Processing

In this section of the course we will cover the main operations in wine production that occur post-fermentation. This includes clarification, filtration, fining, stabilization, aging and blending of wines.

The first lecture will present an overview of all of the post-fermentation operations and focus on the complex issue of wine stability. Wines must be stable against microbial activity as well as undesirable chemical and physical chemical reactions from occurring in the bottle.
Lesson 14: The Goals of Post-Fermentation Treatments

We will begin with a discussion of the goals or rationale behind post-fermentation winery operations.

The 5 Goals of Post-Fermentation Operations:

1. Clarity
2. Stability
3. Compositional adjustment
4. Style
5. Packaging

There are five goals of "finishing" a wine: clarity, stability, compositional adjustment, style development and packaging. It is important, especially in white wines, that the wine at the point of consumption not be cloudy or contain any haze or precipitate. In the United States, haze is a visual defect associated with spoilage in the eyes of the consumer. It is also important to prevent unwanted microbial growth from occurring in the wine after the primary fermentation is complete as this will impact the flavor and aroma profile in unpredicted ways. *Saccharomyces* autolysis will replenish nutrients in the wine making them available for other organisms. And, as noted in the yeast lectures, *Saccharomyces* does not consume all possible bacterial energy sources. Many spoilage organisms are obligate aerobes so the wine must be protected against exposure to air once the carbon dioxide blanket generated during fermentation has dissipated.
Lesson 14: Wine Clarity

The 5 Goals of Post-Fermentation Operations:

1. Clarity

The goal of the clarification practices is simply to remove existing cloudiness.

Clarification

GOAL: to eliminate existing cloudiness

There are several methods by which this may be achieved. Existing haze may be removed by filtration or centrifugation. These topics will be discussed in the next lecture. It is also desirable to remove components from the wine that will lead to the development of cloudiness over time. Cloudiness can arise from microbial growth or from the polymerization and agglutination of macromolecular components of the wine. The macromolecular components or "haze forming potential" can be removed by fining agents. This will be discussed in detail in the lecture on fining. The potential for microbial turbidity can be eliminated by stabilizing the wine against bacterial and yeast growth. This brings us to the next topic of stability.
Lesson 14: Wine Stability

The 5 Goals of Post-Fermentation Operations:

1. Stability

It is important that the wine be stabilized against unwanted changes prior to bottling.

Stability

GOAL: to stabilize the clarity and desirable sensory characteristics

The objective is to stabilize both the clarity of the wine as well as desirable sensory characteristics. There are three types of problems that can impact the clarity of a wine post-fermentation.

Stability: Types of Problems

- Microbial Stability
- Chemical stability
- Macromolecular stability

Loss of clarity can come from three sources: microbial growth and production of polysaccharides; precipitation of chemical compounds and denaturation and complex formation between macromolecules (proteins, polysaccharides and polyphenolics).
Lesson 14: Microbial Instability of Wine: Bacteria

MICROBIAL STABILITY

GOAL:

to prevent microbial growth and/or metabolism especially in the bottle to prevent both turbidity and off-character production

Prevention of microbial growth not only eliminates turbidity but also avoids the production of microbial off-characters.

Spoilage Organisms

- Bacteria
- Yeast
- Molds

Microbial spoilage may be caused by bacteria, yeasts or molds. There are several bacterial species that can be problematic.

Bacteria

- Lactic Acid Bacteria
- Acetic Acid bacteria
- Bacillus
- Streptomyces

The lactic acid bacteria can be considered as spoilage agents if the ML fermentation occurs in the bottle or if undesired characters are produced during the fermentation.

Lactic Acid Bacteria
• **Off-character production**
  - Mousiness
  - Acetic acid
• **Turbidity**
• **Effervescence (CO₂)**
• **Polysaccharides**
  - Haze
  - Ropiness

In addition to acetic acid, the lactics may produce other off-characters such as the mousy character from the degradation of lysine. Lactic acid bacteria may also produce histamine from histidine along with other amines. These amines have no sensory impact but have been associated with headaches in some individuals. Thus, winemakers would prefer that these compounds not be produced. This is a "defect" not associated with an off-odor or off-taste, but in many respects just as important. As noted in the previous section, the ML characters may be desired or not, depending upon the style of the wine. If a lactic bloom occurs in the bottle, visible turbidity will occur due to the growth of the organisms. Carbon dioxide will also be produced, which can be considered a defect. In addition the bacteria may produce polysaccharides, which can lead to a haze or the phenomenon known as ropiness. Ropiness is rare in wine production and limited to only a few lactic acid bacteria. In severe cases the wine can become semi-solid, with the consistency of poorly coagulated Jello.

**Mousiness**

Several compounds (oxidation products of lysine) have been implicated in this off-character:

- 2,4,6-trimethyl-1,3,5-triazine
- 2-ethyl-3,4,5,6-tetrahydropyridine
- 2-acetyl-3,4,5,6-tetrahydropyridine
**Prevention:**

- Use of SO₂
- pH adjustment
- Control of ML

The growth of lactic acid bacteria in the bottle can be prevented by employing procedures to prevent growth of the organisms like use of sulfite. The pH can also be lowered to prevent growth of the organisms as long as this is compatible with the style of wine being produced. Finally, as mentioned in the last lecture, the best way to prevent this fermentation from occurring in the bottle is to encourage it prior to bottling. If this has not been done, then the wine needs to be sterilely filtered and steriley bottled.

**Acetic Acid Bacteria**

- *Acetobacter aceti*
- Require O₂
- Acetic acid accompanied by ethyl acetate

It is also important to prevent the growth of the acetic acid bacteria post fermentation. These organisms are obligate aerobes and will not grow in bottled wine unless the seal is compromised.

The principle organism involved is *Acetobacter aceti*. This microbe is responsible for the conversion of wine into wine vinegar. Acetic acid production is accompanied by formation of ethyl acetate. Acetic acid itself simply gives the burning sensation of pungency by nose; ethyl acetate is quite aromatic and objectionable. It has a strong solvent note. The French describe it as "glue" but in American culture it is more reminiscent of nail polish remover. Either way, it is not a desirable addition to the flavor and aroma profile of most wines!
**Prevention:**

Use of SO$_2$
Topping off to prevent O$_2$ exposure
Market it as wine vinegar!

*Acetobacter* is a problem in barrel aging of wines if a headspace develops. This allows oxygen to enter the barrel, which stimulates the growth of the organisms on the surface of the wine. This can be prevented by limiting headspace development by topping off of the barrels (adding wine to keep the barrels full). If this all fails, the wine can be marketed as vinegar.

**Bacillus**

- Turbidity
- No off-character production
- Produces resistant spores
- Relatively rare

Another bacterium that can cause problems post fermentation is *Bacillus*. This type of spoilage is not common, but is quite problematic if it occurs in a winery. This is because the organism can produce heat and chemical resistant spores that are able to survive the commonly employed sanitation regimes.

This organism does not produce any off-characters but can produce turbidity.

**Prevention:**

Use of SO$_2$
Limit O$_2$ exposure

The only preventive measure is use of sulfite and restriction of oxygen as members of this genus are also aerobes.
Another bacterium that can cause problems in wine production is *Streptomyces*. *Streptomyces* is a soil bacterium that produces compounds reminiscent of the "earthy" aroma of dirt. *Like Acetobacter*, this type of spoilage problem is completely preventable.

*Streptomyces* is not found in wine or in barrels but will infect cellulose-based filtration matrices. It is capable of degrading cellulose in the wild as a carbon and energy source and makes no distinction between native sources of this substrate and processed cellulose filtration sheets. It is a problem only if appropriate sanitation procedures are not being used in the winery.

**Prevention:**

Clean equipment *after* each use!

Cleaning and sanitizing equipment immediately after each use, not waiting until it is to be used again, can prevent this spoilage. This strategy prevents the build up of microbial populations. It is wiser to prevent the bloom of unwanted organisms rather than to attempt to reduce their numbers.
Lesson 14: Microbial Instability of Wine: Yeasts

Prevention of microbial growth not only eliminates turbidity but also avoids the production of microbial off-characters.

Yeasts may also be the causative agents of wine spoilage.

**Spoilage Yeast**

- *Zygosaccharomyces*
- *Pichia*
- *Candida*
- *Brettanomyces/Dekkera*
- *Saccharomyces*

**Spoilage Yeast: Zygosaccharomyces**

- Turbidity
- Little to no off-characters
- Resistant to potassium sorbate
- Most common in semi-dry wines
- Predominant in juice concentrate
- More resistant to SO$_2$

One of the principle spoilage yeasts of wine is *Zygosaccharomyces*. This yeast is tolerant to sulfite and sorbate at levels that are inhibitory to other yeasts and to bacteria. It is frequently mistaken for *Saccharomyces* because it has a similar aroma profile.

The problems associated with *Zygosaccharomyces* are turbidity and carbon dioxide production. It does not produce off-characters. It is very tolerant of high sugar conditions and will be found as a common contaminant in juice concentrate. It is important to analyze juice concentrate for the presence of this yeast prior to using it to
adjust the residual sugar content of a finished wine.

**Spoilage Yeasts: Pichia**

- Can produce turbidity
- Can produce off-characters
- Sensitive to SO₂
- Sensitive to dimethyldicarbonate (DMDC, "Velcorin")

Another yeast that can cause wine spoilage is *Pichia*. There are several species that can be found in wine. Some are only problematic because of turbidity while others can produce off-characters and films.

These yeasts are sensitive to SO₂ and to other antifungals used in wine production.

**Spoilage Yeasts: Candida**

- Some strains can produce off-characters
- Can form a film *"C. mycoderma"*
  - Oxidizes acid reducing acidity
  - Forms ethanal (apple)
- More common in barrel fermentations/aging
- Sensitive to SO₂ and DMDC

Members of the genus *Candida* can also be found in wine post-fermentation. These yeasts are commonly found on the surfaces of grapes but they can also be part of the winery flora.

Some of these yeasts can also produce off-characters. A skin-like film may form on the surface of wine that results in acetaldehyde or "ethanal" (term used in the French literature) production. The French describes this as rotten apple and in low concentrations it does have this note. In higher concentrations it has the nutty character associated with sherry production. *Candida* infection is commonly associated with barrel aging. These organisms are sensitive to sulfite and to DMDC. Some of the
*Candida* species have been reported to be able to produce vinyl phenols usually associated with our next spoilage organism, *Brettanomyces*.

**Spoilage Yeasts: Brettanomyces/Dekkera**

- Multiple off-characters
  - Vinyl phenols
  - Amino acids degradation products
  - Oxidation of wood aldehydes
- More common in barrel aging
- More common in red wines

*Brettanomyces* is the imperfect name and *Dekkera* the perfect form of one of the most "celebrated" of the wine spoilage yeasts. Brett, as it is affectionately called, is the major contributor to the aroma profile of some high-end French wines. Many novice consumers find the traits quite objectionable, however.

This organism produces a wide array of interesting characters in wine. Off-characters can be derived from metabolism of phenolic compounds, from degradation of amino acids and from oxidation of wood aldehydes. It is commonly associated with barrel aging and fermentation in wooden casks. It is more common in red wines than in whites, probably because of the higher phenolic content. However, we have isolates in our culture collection that are quite content to grow in white juices. *Brettanomyces* produces acetate as the principle end product of sugar catabolism. Recall from the discussion of glycolysis, production of acetic acid does not allow the organism to regenerate NAD+ from NADH. Many of the Brett characters produced are reduction products that serve to regenerate NAD+ for this yeast. What compounds are produced will depend upon the composition of the wine.
The Brett Off-Characters

- Horsy, Horse Blanket
- Barnyard, Fecal
- Wet Dog
- Tar
- Tobacco
- Creosote
- Leathery
- Pharmaceutical
- Mousy

Brett can also produce the same spectrum of lysine metabolites as the lactic acid bacteria and can be a cause of mousiness. Which organism is responsible can generally be determined by noticing the other characters that are also present. Some of the characters listed above are highly valued in some wine styles, but not in others.

Control of *Brettanomyces*

- Use sanitized cooperage
- Avoid topping off with contaminated wine
- Filtration of contaminated wine
- Use of SO₂

*Brettanomyces* infection of the winery can be difficult to eliminate. It is important that sanitation procedures be developed that prevent the establishment of the organism. One common way that it is spread is by using Brett infected wines to top off other wines in the barrel room. Brett is a facultative organism and is not inhibited by the absence of oxygen. However, the presence of oxygen greatly stimulates acetic acid production because it can be used as a terminal electron acceptor. Contaminated wine will need to be steriley filtered prior to blending with other wine in the winery. *Brettanomyces* is also difficult to eliminate because these organisms produce cellulases and are therefore able to degrade the complex polysaccharides in the barrel wood and use the resulting sugars as an energy source. This means they are able to persist, if not flourish, under conditions inhibitory to other spoilage microbes.
Spoilage Yeasts: Saccharomyces

- Turbidity
- Effervescence (CO₂)
- More of a problem in wines with high residual sugar
- Can be prevented by use of SO₂ and sterile bottling

Even *Saccharomyces* can be problematic if growth occurs in the bottle. Turbidity and CO₂ are the defects produced; off-character formation (hydrogen sulfide) generally does not occur. Wines with high residual sugar (glucose, fructose) are at risk for a secondary yeast fermentation occurring in the bottle.

*Saccharomyces* spoilage can be prevented by use of SO₂ post-fermentation, as they will not be able to detoxify this compound in the absence of sugar. However, if the goal is to produce a wine high in residual sugar, then the wine should be sterilely filtered and sterilely bottled. I emphasize the sterility of both processes: filtration and bottling - the wine must be protected from picking up organisms post-filtration but pre-bottling. A steriley filtered wine that is transferred and held in a non-sterile tank, moved through non-sterile hoses and pumps, is no longer sterile. A check of the bottling line involves performing plate counts on samples of the wine before and after bottling. If the net number of organisms increases, then the wine is being infected during bottling. This means that there is a reservoir of organisms somewhere in the process and that line sanitation procedures are not effective.

Combinations of DMDC and SO₂ can be used which are more effective than either compound alone at the time of bottling. DMDC requires the use of a special dosing machine, so this might not be practical in all winery situations.
Lesson 14: Microbial Instability of Wine: Molds

Molds can also be a cause of wine spoilage. These organisms are obligate aerobes and sensitive to ethanol and sulfite, so are not a problem in wine or inside of barrels during aging. However they can grow on ethanol vapor so can be found on the outside of barrels and coating barrel room walls.

Molds

- Not a problem if wines is protected against O₂ exposure
- Impart "moldy" taints
- Can produce "corkiness": 2,4,6-trichloranisole

The molds can impart a moldy taint if the wine comes in contact with the mold growth, which would happen if the mold growth occurs in a bottling line for example. The principle mold spoilage characters are associated with corks. The corks may not appear moldy, they may have been exposed to mold at some point in their production or storage. It is important for wineries to develop a mechanism to evaluate the quality of corks being brought into the winery. This can be simply done by soaking a statistically significant fraction of the corks in a neutral wine over night. If the character is present it will be quite noticeable the following day. Some individuals are not as sensitive as others to the corky character, so the quality control personnel associated with evaluation of the corks should include individuals with a low threshold (parts per trillion) of detection for this compound.

2,4,6 - Trichloroanisole

The corkiness character is caused by 2,4,6-trichloroanisole. Other negative characters can also be produced by molds growing on cork surfaces. These compounds are
produced as a means of detoxification of chlorine used in the cork bleaching process.

2,4,6-Trichloroanisole

- Intense aroma of "moldy rag"
- Only one of several off-characters that can be associated with bad corks
- Can be formed in absence of cork if have the right conditions: phenolic compounds, mold and chlorine bleach

TCA has the characteristic odor of a damp or moldy rag. Some describe it as an "old book" note. It can be formed anytime the mold is in contact with chlorine. Wineries using chlorine as a sanitization agent need to be aware that if mold populations are present TCA can be produced independent of cork contamination.
Lesson 14: Sources of Spoilage Organisms

The spoilage organisms arise from different sources in the winery. If a spoilage problem occurs it is critical that the source of the microbe be determined so that it can be eliminated.

Source of Spoilage Organisms

- Grapes
- Winery surfaces/equipment
- Airborne contaminants
- Barrels
- Corks/materials entering winery
- Blending wines
- Humans

Any material entering the winery can be a source of microbes. This includes the grapes, barrels (especially used barrels), blending wines, any materials used in wine production. Some spoilage organisms, such as the molds, are airborne. Or can be spread by insects such as fruit flies. It is also important to consider winery employees as a potential source of microbial contaminants.
Lesson 14: Strategies for the Prevention of Spoilage

Prevention of Spoilage

- Do not allow biologically active waste to accumulate
- Clean equipment immediately after use, not just before next use
- Identify source of contamination promptly
- Minimize outside sources of contamination (know your bulk wine!)
- Use SO$_2$ or other anti-microbial
- Monitor O$_2$ exposure of wine

Several practices can be employed in the winery that will reduce the chance of microbial spoilage. Biologically active waste should not be allowed to accumulate in the winery. Equipment should be cleaned immediately after each use. The source of contaminants should be determined promptly and it is important to know the microbial history of any wines brought into the winery from another location. Oxygen exposure of the wines should be carefully monitored. Sulfite or other antimicrobial compounds can be used as well.
Lesson 14: The Chemical Instability of Wines

Stability

- Microbial stability
- Chemical stability
- Macromolecular stability

Wines must also be stabilized against unwanted chemical reactions post fermentation.

Chemical Instabilities

- Metal ions
- Tartrate
- Polymerized Phenols
- Oxidation Products

Chemical Instabilities: Metal ions

- Fe and Cu can form a precipitate "casse"
- Caused by use of iron or copper containing materials in winery or from pesticides
- Elimination: Ferrocyanide precipitation (not legal everywhere)

Metal ions can catalyze the formation of spoilage characters as well as lead to the formation of a haze or "casse".

Current industry practice is to use tank and line materials that do not lead to the presence of metal ions in the wine. Copper can come from the use of copper-containing fungicides. Casse formation is virtually unheard of in California wine production. Copper in the presence of sulfite will form colloidal copper sulfide, which
will react with proteins forming a brownish-red sediment. Metal ions can be removed from wine by fining, using cation exchangers and other methods. Removal of the protein of wine also prevents casse formation. It is important to note if the technique being used to eliminate a problem is a legal treatment. Not all treatments are allowed in all countries.

### Chemical Instabilities: Tartrate

- At low temperature, tartrate will crystallize
- Mistaken for ground glass by consumers
- Unstable in presence of Ca++
- Solubility depends upon pH, K+, tartrate concentrations
- Can get co-crystallization with other organic acids

Another source of chemical instability is the major grape acid tartrate. Tartrate can form crystals during aging of a wine. This is typically seen as the glass-like crystals that form on the surface of the cork. They are harmless, but frequently confused with ground glass by consumers so it is considered a potential problem that must be prevented from happening in the bottle.

Several factors influence the precipitation of tartrate. Crystal formation may be catalyzed by the presence of cations like calcium, and it may be nucleated by addition of tartrate crystals (cream of tartar). Solubility of tartrate is also dependent upon the pH and potassium ion concentrations. It is also a function of the concentration of the anionic species and of other acid species as co-crystallization may occur.

### Tartrate: The Solution

- Super-chill wine to catalyze crystallization
- Nucleate process with tartrate crystals
- Add cations to initiate crystallization

Temperature also affects solubility, so a common method for catalyzing tartrate precipitation is super-chilling of the wine. The process can also be initiated by addition of cations or crystals.
Chemical Instabilities: Polymerized Phenols

- Can precipitate during aging
- Undesired in bottle

A precipitate or sediment may also form from the polymerization of phenolic compounds.

During aging of the wine a sediment will form. The sediment will coat the surface of the glass of the bottle if the wine is bottled prior to achieving phenolic stability. The exact composition of this material is not known, but it is likely mainly a mixture of tannin and protein. It is more common in red wines of moderate phenolic content that have not been aged in oak. Other compounds may also yield sediments.

Chemical Instabilities: Oxidation Products

- Off-color
  - Brown
  - Pink
  - Orange
- Off-characters
  - Aldehydes
- Prevented by using antioxidants

Undesired oxidation products may also form in wine during aging.

Off-colors may form from the oxidation reactions. Browning occurs from the oxidation of phenolic compounds. There are several ways in which brown pigments may be formed, as discussed in the textbook. Pinking is obviously a problem only in white wines as this off-color is undetectable in reds. It has been suggested that the pink character is derived from the oxidation of leucocyanidin to cyanidin, but several studies suggest that this is not the pink compound formed. The orange character is rare, and the source of the off-color is not known. Aldehyde also forms from the oxidation of phenolic compounds and will be discussed in the lecture on aging. Oxidative defects
can be prevented by the use of antioxidants such as SO₂ and ascorbic acid, or fining agents such as PVPP that eliminates the "pinking potential".

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Lesson 14: Macromolecular Instability in Wine

Stability: Types of Problems

- Microbial stability
- Chemical stability
- MACROMOLECULAR STABILITY

The macromolecule proteins and polysaccharides can form complexes in wine leading to the appearance of a visible haze.

Macromolecular Stability

- Protein
- Polysaccharides

Macromolecular Stability: Protein Instability

- Proteins involved are from grapes
- Denature over time causing visible haze
  - Hydrophobic regions interact
  - Aggluination complexes formed
  - Complex becomes visible
- Accelerated by treatment of wine at high temperature (HTST)
- Can be prevented by fining

Over time proteins will denature in wine exposing hydrophobic groups. The hydrophobic groups will interact with other hydrophobic material in the wine leading to aggregation and production of cloudiness. There is not a good correlation between total protein content and haze formation, as a subset of the proteins of wine appear to catalyze this process. Protein denaturation is complex and a function of the
temperature to which the wine has been exposed, the pH, and the composition of the wine.

The proteins causing haze are derived from the grape, not from microbial activity. Protein denaturation is accelerated at higher temperatures. The fining agent bentonite can be used to remove protein from wine, eliminating the "haze forming potential". HTST treatments remove significant amounts of protein but lead to the formation of a bentonite-resistant haze. This may be due to the fact that protective colloidal materials have also been removed.

**HTST**

- "High Temperature Short Time"
- Used on juices with high oxidase levels
  - Polyphenol oxidase from plant
  - Laccase from *Botrytis*
- Used on wines
  - Pasteurization (Kosher wines)
  - Inactivation of added enzymatic activity

HTST treatments are used to eliminate laccase activity in white wines. Heat treatments are also used in the pasteurization of wines, which results in similar haze problems. Protein hazes are largely comprised of protein but can also contain phenolic compounds and polysaccharides. Polysaccharide hazes may also form. These hazes are largely comprised of polysaccharide but can contain some protein and polyphenolic material. A quick test that we use to distinguish between the two is to evaluate the solubility of the particulate matter in hot water. Denatured proteins are not soluble under these conditions, but polysaccharide will go back into solution due to the increase in temperature and reduction in ethanol content of the medium. Chemical assays can also be used to distinguish between the two.

**Macromolecular Stability: Polysaccharide Instability**
Polysaccharides may derive from the plant or from microbial activity. Plant polysaccharide content is high under conditions leading to maceration of the skins. The microbial polysaccharides are produced by bacteria. Polysaccharides are insoluble in ethanol, which is increased at low pH. In contrast to protein hazes, fining agents effectively removing "polysaccharide haze forming potential" do not exist.
Lesson 14: Compositional Adjustments of Wine

It is frequently desirable to modify the chemical composition of wine post-fermentation.

The 5 Goals of Post-Fermentation Operations:

3. Compositional Adjustment

Several types of adjustments may be made.

Compositional Adjustment

- Acidity
- Sugar level
- Ethanol level
- Tannin removal
- Sulfide/mercaptan removal

Compositional Adjustment: Acidity

- To increase acid add:
  - Malate
  - Tartrate
  - Citrate
- To decrease acid add:
  - Calcium carbonate
- To remove volatile acidity
  - Reverse osmosis

The acidity of the wine may be adjusted. This is desired for several reasons, to encourage the malolactic fermentation, to achieve balance to the wine, to prevent an
instability from occurring. Acidity can be adjusted in several ways.

Malate, tartrate and citrate are all legal additions in the United States. Acidity can be reduced by treatment with calcium carbonate as was allowed for juice. Volatile acidity, or acetic acid, can be removed by the process of reverse osmosis.

**Compositional Adjustment: Sugar level**

- Add juice concentrate
- Arrest fermentation
  - Fortified wine
  - Fortified juice
- Temperature shock

Generally, final sugar levels are not altered in dry table wines. However, some styles call for a higher sweetness than usually remains a robust fermentation. Sugar can be adjusted in different ways, depending upon what is allowed for the region.

The fermentation may be arrested by addition of alcohol (if it is marketed as a fortified product) or by high temperature shock. Alternately, juice concentrate may be added. The wine may be blended with a wine that naturally arrested during fermentation. It is important to protect wines with a high residual sugar from undergoing a secondary alcoholic fermentation in the bottle. Concentrate users need to be concerned about *Zygosaccharomyces* infection.

**Compositional Adjustment: Ethanol level**

- Evaporative removal with return/replacement of co-stripped volatiles
- Reverse osmosis followed by adjustment of flavors/aromas

The ethanol level of the wine may also be adjusted, which is obviously especially important in the production of low ethanol wines or ethanol-free products. These methods can also be used to reduce the ethanol content if it has risen to an inhibitory
level and has caused arrest of the yeast fermentation.

There are several procedures for the production of alcohol-free wines that depend upon some form of evaporative removal with the return of lost aroma compounds. Reverse osmosis can also be used. With table wines, ethanol content can be adjusted by blending.

**Compositional Adjustment: Tannin removal**

- **Time of aging**: to allow polymerization to occur
- **Ultrafiltration**: 500-2000 mw cut-off

Tannins are very important compounds in wine. Procyanidins are polymers of flavan-3-ols that range from 2 to 8 units in size. During aging, procyanidin molecules will polymerize and undergo condensation reactions leading to the formation of tannins. Tannins are responsible for wine astringency. But bitter compounds may be removed via the formation of tannins. Tannins will react with proteins and precipitate during aging reducing both bitterness and astringency.

Tannins can be removed simply by appropriate aging of the wine. Alternately they can be removed by ultrafiltration.

**Compositional Adjustment: Sulfide/Mercaptan removal**

- **Copper sulfate**
  - H$_2$S
  - Some thiols
- **Copper sulfate + SO$_2$ + ascorbate**
  - Disulfide removal
  - VERY SLOW

If off-characters have formed in the wine it is of course necessary to remove them prior to bottling. The most common off-characters are hydrogen sulfide and higher sulfides produced by the yeast during fermentation.
These compounds can be removed by copper sulfate treatment, depending upon the nature of the compound. Charcoal fining can be used to remove persistent off-characters, but such a treatment may strip the wine of positive notes as well.
Lesson 14: Post Fermentation Operations for the Expression of Style

Several post fermentation operations are conducted to impart nuances to the wine or simply as a part of the style of the wine to be produced.

The 5 Goals of Post-Fermentation Operations:

4. Style

Stylistic factors include aging regime, blending and fining treatments. Some fining agents are not neutral and will add nuances to the wine. Others are designed to remove specific wine components.

Style:

- Aging
- Blending
- Fining

Each of these topics will be the subject of a following lecture.
Lesson 14: Packaging

The final and ultimate post-fermentation process is packaging of the wine.

The 5 Goals of Post-Fermentation Operations:

5. Packaging

With respect to marketing, the packaging may be as important as the contents of the bottle. Many consumers are strongly influenced by the way in which the wine is presented. Wines served from screw-capped jugs are generally ranked as lower in quality than those from corked bottles, even when the identical wine is presented in each type of bottle.

Packaging:

- Bottling
  - Sterile
  - Non-sterile
- Closure
  - Cork
  - Synthetic cork
  - Screw cap
  - "Bag-in-box"

It is important to consider the type of closure that will be used. Corks are susceptible to contamination with mold taints, but are generally associated in the eyes of the consumer with "quality". The synthetic corks eliminate these problems and allow the winemaker or marketing folks to become quite creative in the use of colors and designs. Unless the wine is to be aged for a very long time such that cork integrity issues become a factor, consumers and experienced tasters alike cannot tell the difference wines bottle-aged with a cork or screw cap. The consumer perception of the level of quality associated with each type of closure cannot be ignored, however.
Lesson 15: Introduction

Clarification and Filtration

In this lecture we will cover the two principle means of removing particles from wine: centrifugation and filtration. Natural settling may be adequate to eliminate the particulate matter of wine. This depends upon the difference in density between the particle and the wine, the particle diameter and the viscosity of the wine. Frequently, however, natural settling is insufficient to clarify a wine. In this case centrifugal force can be used to remove particulate matter. Alternately, particles may be removed by filtration by the sieving action of the filter matrix or via adsorption.

Clarification Options

- Natural Options
- Centrifugation
- Filtration

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Lesson 15: Natural Settling

Natural settling is the gentlest and most simple form of clarification. Particles are removed due to the action of gravity. This will be successful only if the density of the solids is sufficiently different from that of the wine.

**Natural Settling/Racking**

- Decanting wine off of solids
- May add a settling aid to "tighten" lees
- Volume loss high
- Gentle process

The wine is then decanted or racked off of the solids or "lees". Racking is typically performed immediately after the alcoholic and malolactic fermentations unless extended sur lies aging is desired, after several months of aging in a barrel or wooden cask to remove sediment, following treatment with a fining agent, to disperse sulfite added to the wine post-fermentation, and prior to bottling or filtration. More frequent racking may occur depending upon the style of the wine being produced and the need to expose the wine to aeration, or simply because of tank management issues.

Excessive racking and movement of the wine should be avoided. Each time the wine is racked it is exposed to oxygen and there is a potential loss of volatile compounds due to the more exposed surface area of the wine as it is being transferred, depending upon how the racking is performed. To minimize oxygen exposure the wine can be racked from the bottom of one tank or barrel to the bottom valve of the new tank or barrel, allowing the liquid to fill from the bottom gently. Alternately the wine can be transferred to the new tank or barrel through the top or bung hole allowing the stream of wine to "splash" into the new container. If still more aeration is desired, a screen can be used on the top of the new tank or barrel making a fine spray of the wine exposing more wine surface area as droplets are formed.

A settling aid such as silica sols can be used to promote gravitational settling. The wine volume loss may be high. However, this process is the most gentle to the wine and does not expose the wine to as much oxygen or generate as large of a surface area as other clarification operations.
The rate of settling is a function of the terminal velocity of the particles in suspension. Settling rate can be increased using centrifugal force. This can dramatically increase the settling force several hundred fold.
Lesson 15: Centrifugation of Wines and Juices

Centrifugation: Types

- Desludging
- Decanting

There are two types of centrifuges: desludging and decanting. Desludging centrifuges accumulate the solids material inside of the centrifuge bowl and are therefore batch operations. The solids are then removed by the process of desludging. Desludging may be done continuously, but this is not common practice. In contrast, decanting centrifuges are operated continuously discharging both a paste of solid material and the clarified wine. If juice is to be centrifuged, decanting is preferred to desludging.

Centrifugation: Function

- Removal of particles using centrifugal force
- Can be adjusted to remove larger or smaller particles

The rate of speed of the centrifuge can be adjusted which will determine the amount of centrifugal force applied. The higher the force, the smaller or less dense the particles that can be removed. This allows the winemaker to determine the amount of solids remaining in the juice or wine.

Centrifugation: Problems

- Aeration
- Cost
  - Modified atmosphere
  - Low temperature

Centrifugation may expose the wine to aeration. Centrifuges represent a capital investment that may not be justified at moderate to small wineries. If it is necessary to
conduct the operation under a modified atmosphere or under refrigeration, this will add to the cost of the production of the wine. On the positive side, centrifugation does not lead to the accumulation of a large volume of waste with a high biological and chemical demand for oxygen, and minimizes the loss of wine.
Lesson 15: Filtration of Wine

Suspended particles may also be removed by the process of filtration. Filtration works by trapping suspended particles, allowing the liquid to pass through the matrix. We will first discuss the types of filtration processes and then the types of units available.

Filtration

- Types of filtration processes
- Kinds of filters units

There are two types of forces at play in filtration: sieving and adsorption. Sieving refers to the exclusion of particles simply based on size. The matrix has a specific pore size and larger particles will not pass the matrix. In adsorption the particles adhere or attach to the matrix.

Filtration Processes

- Sieve
- Adsorption

The pore sizes of filter matrices can range from 0.45 to 200 mm. The lower pore sizes will exclude yeast and bacteria and comprise a sterile filtration if done properly. Large pore size filters are called rough filtration. Intermediate pore sizes might be called polished or tight filtration in the literature.
Adsorption can also be involved in the removal of particulate material of wine. This is typically based upon a charge interaction between the particles and the matrix or between the particles and other material excluded from the filter. The charge arises on the surface of the particles due to the flow of the fluid around the solid material.

Most filtration matrices involve both adsorption and sieving.
There are two types of problems associated with filtration: fouling and clogging. In clogging accumulation of the solid material at the pore surface blocks the openings preventing the liquid from passing into the matrix. In fouling, the applied pressure forces denaturation of the particles on the surface, which more completely plugs the pores.
Lesson 15: The Types of Filters

There are four basic types of filtration processes used in wine production: Diatomaceous Earth (or depth bed), pad, membrane and cross-flow filtration.

Kind of Filter Units

- Depth-bed

In depth bed filtration Diatomaceous Earth (DE) forms the matrix or porous cake through which the wine is filtered. It is a depth filtration because the DE is added continuously with the wine and the matrix thus grows in size during the filtration process. This is a rough filtration.

Depth-Bed Filtration

- Filter matrix mixed with wine
- Filter matrix builds as wine is filtered through coated screen
- Constantly laying down new matrix with wine

This type of filtration is the least susceptible to clogging and fouling. DE is derived from fossil algae shells of diatoms. Cellulose particles can also be used as a filter matrix in a depth-bed filtration. Perlite, the term for silicate particles made from the processing of volcanic rock, can also been used.
Perlite is more porous than DE but has a lower adsorbent capacity. Depth filters contain a screen upon which the cake builds. It is first necessary to precoat the screen, which is usually done using a material of a finer grade than the matrix used for the filtration. The screens or pressure leaf filters may be vertical or horizontal.

**Depth-Bed Filtration**

- Diatomaceous earth; cellulose; perlite
- Cost effective
- Minimal clogging
- "Rough" filtration: sieving action is minimal
- Principle of "torturous path" for particles to travel

These matrices can trap particles smaller than the exclusion limit of the pores. This is because of the length of time it takes a small particle to travel through the matrix that is established as the cake builds. This can be thought of as creating a "torturous path". In this case a certain percentage of the particles do not make it all the way through the matrix before the wine is completely filtered, because they are moving at a much slower rate. However, while this process may reduce the number of small particles present it will not remove them all. It is not a sterile filtration and should not be thought of as such.
The next type of filter process is pad filtration. As the name implies, in this case the wine is passed through a pad. The pad may be comprised of DE or cellulose and this is similar to depth-bed filtration, except new matrix is not being constantly laid down with the wine as it is filtered.

**Kinds of Filter Units**

- Depth-bed
- PAD

Many of the same types of phenomena occur in pad filtration.

**Pad Filtration**

- Filter matrix is a preformed sheet or pad
- Sieving as well as adsorption
- Pads come in a variety of porosities, but pore size is heterogeneous
- Flow of wine perpendicular to pad
- "Dead end" filtration

As with depth-bed filtration the flow of wine is perpendicular to the pad. Pads come in a variety of porosities, but pore size is heterogeneous. This is also a dead-end filtration.
This is a simpler method, as it does not require pre-coating. However, pad filtration is more costly than depth-bed filtration. Filter pads are designed to collect particles in their interior rather than to develop a cake at the surface. In the diagram above the wine enters the bottom of the pads and flows through them to be collected. In plate and frame filters the name derives from the use of support plates to retain the filter medium and to collect the filtrate interspaced with frames, which distribute the wine across the filter medium. Pad filters operate much like the depth-bed filters in that particles are trapped using the same forces and principle of traveling a torturous path.

Kinds of Filter Units

- Depth-bed
- Pad
- MEMBRANE

The next type of filtration uses a membrane filter. Membrane or cartridge filters contain a synthetic polymer matrix. They are produced with more uniform pore sizes and are typically used for a finishing or sterile filtration. Particles collect on the surface rather than getting caught in the path. Therefore these filters clog and foul quite easily. Some adsorption to the matrix can occur, but the principle method of particle removal is exclusion or sieving.
Membranes are rated based on the largest pore size and therefore the size of the particles that can pass through the membrane. Pore size may vary, but what is most important is the largest pore size as this dictates the exclusion limits of the membrane.

**Membrane Filtration**

- Like pad filtration, but uses a membrane
- Fixed pore size
- Sieving as well as adsorption
- Clog easily
- "Finishing" filtration

The bubble test can be used with membrane filters to determine the intactness of the filter as well as the pore size. In this test, compressed gas (nitrogen) is passed into the filter unit loaded with wine. The pressure is increased until bubbles appear. The circumference of the pores of the membrane dictates the pressure at which bubbles are formed. The "bubble point" is the pressure at which bubbles are released. Other types of tests can be performed that are more amenable to automation and analysis, as shown on the CD. Other tests of cartridge integrity, such as pressure hold and diffusion, can also be used. These tests can be automated and linked to a computer program that will automatically determine if a unit passes or fails the tests based upon specifications for the cartridge.
The final type of filtration unit is cross-flow. In contrast to the other types of filtration units, cross-flow is not a dead end filtration meaning that the wine does not flow perpendicular to the membrane but flows across the filter matrix, thus the name "cross flow".

The cross-flow minimizes clogging of the membrane, as there is a constant flow of particles across the surface. Fouling is also prevented, as there is no direct pressure against the particles causing them to denature and block the pores. The filtered wine is called the "permeate" and the fraction which does not pass through the filter is called the "retentate". There are several types of cross-flow units, and the filter medium can vary.
Cross-Flow Filtration

- Same porosities as membrane filtration
- Wine flows across matrix, not through it
- Wine retentate can be re-circulated
- Back flux can be used to clear membrane
- Does not clog that easily

Reversing the flow of the wine can be used to clear the membrane. Cross-flow filtration can also be used to achieve an ultrafiltration of the wine. Ultrafiltration is defined as the removal of large molecular weight solutes like proteins rather than large particles. Ultrafiltration can be used to remove proteins and thereby confer stability against protein haze formation. However, this can also remove phenolic polymers as well, which might not be desired. Lower molecular weight cut off filters can be used for tannin and color removal.

Another type of filtration is reverse osmosis. Osmosis refers to the movement of water across a membrane from a solution of low solute content to one of higher salt content. Reverse osmosis is the opposite, the removal of water from solutions of higher salt content. This process can be used to make juice concentrate. These membranes have molecular weight cutoffs of 10 to 100 atomic weight units. Water has a molecular weight of 18 and will pass across the membrane. Sugar monomers have a molecular weight of 180 and will be retained. Very small molecules such as acetic acid or ethanol can also pass through the membrane in reverse osmosis. This process can be used to reduce the content of these molecules as well.

Filtration processes should be ordered so that the largest particles or particles that may foul a later type of filter unit are removed first. Thus, rough filtrations should be performed before finishing or tight molecular weight cut off filtrations.
Order of Filtration: Rough Before Finishing

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Lesson 15: Impact of Filtration on Wine Quality

One frequently hears the statement that filtration should be avoided as this detracts from wine quality.

The Question:

Does filtration impact wine flavor and aroma?

The belief is that passage through the filtration matrix removes aroma and flavor components from the wine. Immediately after filtration the wine may appear to have lost aroma components. As equilibria are re-established the aroma compounds return with the same intensity.

The Belief:

Filtration removes flavor and aroma compounds and is therefore undesirable
"Unfiltered" wines are more complex than filtered

Unfiltered wines are thought to retain complexity, however, this conclusion has not been supported by controlled studies with blind sensory evaluations.

The Facts:

Several studies have shown that expert tasters are not able to recognize filtered versus unfiltered control wine
Unfiltered wine allows continued microbial activity, which may explain differences perceived in unfiltered wines in general.

Unfiltered wine may allow continued microbial activity, which may change the character of the wine if it is aged significantly post-fermentation.
Lesson 16: Introduction

Fining

Particulate matter in solution deflects light making the solution appear hazy. The goal of the clarification operations discussed in the last lecture is the removal of existing cloudiness and sediment from a wine. This is obviously possible by the techniques of centrifugation and filtration if the particulate matter is already present. Frequently, however, the material that will form colloids and eventually agglutinate into particles is in the wine in a soluble form. In this case it is necessary to design operations to remove the "potential" for formation of a haze or sediment. This may be accomplished by removing one or more of the participants in colloid formation or the use of techniques to stabilize the colloids against agglutination. The latter approach is more risky than the former.

Fining refers to the addition of an adsorptive agent to wine or juice followed by settling or precipitation of the agent. Undesired wine components bind to and then settle with the fining agent and are thereby removed from the wine. As discussed below there are several different types of fining agents. In some wines it may be necessary to use more than one agent. Other wines might not benefit from fining at all. Many fining agents are not highly specific, meaning that if not appropriately used, positive and well as undesired characters may be removed from the wine. Fining can also be used to accelerate the conversion of colloidal substances into agglutinated complexes so that clarification processes can be used to remove the particulate matter. It is important that the winemaker understand the uses of fining agents to avoid unwarranted treatment of the wine.
Lesson 16: The Fining Process

Goal of Fining

Removal of soluble components that are undesired stylistically or that will lead to an instability.

Fining can therefore be used to remove components that will result in instability of the wine or that are simply undesired from a stylistic perspective. This includes removal of proteins that would otherwise result in formation of a haze, tannins or phenolic compounds reducing astringency and bitterness, off-colors or off-color forming potential, metal ions, or off-flavors and aromas. Fining can also be used to add nuances, depending upon the agent used.

Components to Be Removed

- Protein: "haze-forming potential"
- Phenolic compounds (tannin): soften wine by reducing bitterness and astringency
- Metal ions
- Off-character or off-character-forming potential

The winemaker should carefully consider what agents will be used and in what order. It is important to conduct fining operations before clarification processes since some residual fining agents may impact wine clarity. Some clarification or fining treatments may lay lead to a destabilization of another component of the wine, so this must be taken into account. For example, it is thought that wine polysaccharides can coat the surface of colloidal protein-tannin complexes preventing those complexes from further agglutination and haze formation. Disruption of the coating effect of the polysaccharides will lead to the appearance of a visible cloudiness of the wine. Also, some proteins are stabilized by interactions ions on the hydrophilic or exposed surface of the protein. Removal of those ions can lead to unfolding and denaturation of the protein, which leads to colloid formation.
Fining agents operate by taking advantage of hydrophobic or hydrophilic interactions between the agent and the species to be removed. The fining agents used are generally not soluble in wine or are of limited solubility. The agents initially dissolve in the wine; interact with wine components then come out of solution bringing wine components with them.

**Mechanism of Fining**

- Take advantage of either hydrophobic or hydrophilic interactions to remove offending component
- Wine will initially be cloudy, but particles will eventually become large and sink
- Clarify by racking or filtration
- Add a charged component that will interact with oppositely charged components followed by precipitation of the neutral complex
- Add a denaturing component that will expose hydrophobic surfaces that will then interact allowing a hydrophobic complex to form

The wine generally has to undergo a clarification treatment following addition of the fining agent.

Components of one charge may be used to remove components of the opposite charge. Due to the low pH wine proteins are generally positively charged and can be
removed by negatively charged fining agents.

In this case, both the fining agent and the component to be removed carry multiple charges. This allows a large insoluble complex to form. Similarly, denaturation of proteins reveals non-polar regions or areas of hydrophobicity that strongly interact with other hydrophobic regions due to Van der Waals forces. Multiple domains are exposed capable of interaction with multiple components again leading to the formation of a large lattice or complex.

In some cases, more than one agent is added. The first agent may be responsible for denaturation of the undesired component allowing interaction with the stripping agent.
Lesson 16: Choice of Fining Conditions

Choice of Fining Conditions

- Difficult to predict outcome due to complexity of process and number of unknowns
- Temperature influences process
- Amount and type of mixing critical
- Relative molecular weight and charge density of particles important for complex/lattice formation

Fining is one of the most challenging of winemaking operations because the outcome is difficult to predict. This is due to the fact that many variables impact component solubility in wine and these variables are not easily measured. Temperature has a striking effect as it impacts rates of denaturation and the stability of complexes formed. At higher temperature denaturation or unfolding is favored while at low temperature many components are less soluble so will more likely precipitate. Since fining requires that the fining agent make direct contact with the components to be removed, how the fining agent is prepared and added to the wine is critical. It is also important to know that the undesired component will not just interact with the fining agent but will be capable of forming a lattice structure that will settle from the wine.

The efficiency of agglutination is also affected by the nature of the components present in wine and their relative ratios. There is not a linear relationship between total tannin content and protein content and colloid formation and agglutination. Tannin protein interaction occurs more readily at lower pH values, so the pH of the wine impacts stability of the complexes. Divalent as well as monovalent cations can catalyze flocculation and precipitation of tannins. The nature and content of polysaccharides is also important since these components can both participate in and dampen colloidal interactions.

The purpose of this discussion is to underscore the importance of conducting fining trials for each wine to be treated in the winery. This is generally done using small volume lots from the wine to be treated. It is important to remember that small scale or laboratory fining conditions may not mimic the actual behavior of agents on a commercial scale due to difficulties in the speed or extent of mixing. However it is possible to determine the relationship between small scale fining trial and commercial
scale fining. This need not be done every time a wine is fined. We have found reasonable reproducibility in scaling up from a fining trial once the "scaling" factors are understood. It is also possible to adapt the small scale fining trial (adjust rates of agent addition and mixing) to **match that of the commercial operation**. Under typical winery conditions this has to be done empirically, by measuring loss of haze forming potential with step wise addition of an agent and repeating the step wise addition under a commercial scale and comparing the amount of undesired component (tannin, protein) remaining in the treated wine. This procedure can also be used to test for the potential of over-fining. **Over-fining** is a term that has different meanings in different regions. Many California winemakers consider over-fining to mean that there was a noticeable and negative effect on wine quality. This is usually due to the removal of desired flavor and aroma components. I prefer to call this particular problem **"stripping"**. The French use over-fining to mean that the wine has become destabilized in some way, that is, some of the added protein fining agent has not been removed and will lead to subsequent colloid formation upon interaction with tannins. This is especially problematic if the wine is exposed to tannins post fining, such as by blending or barrel aging. Over-fining is particularly problematic in white wines fined with gelatin. For the purposes of this class we will use the French definition of over-finining. Over-fining should not be confused with failure of the fining process or agent to remove existing components of the wine that will likewise lead to haze formation later on in the aging of the wine. We will call this phenomenon **incomplete fining**. It is important to note at this point that rarely does any fining operation reduce the concentration of the undesired component to the analytically undetectable range. The goal is to reduce the concentration to a value below which it will not be noticeable as a problem.

**Problems Associated With Fining**

- Lack of specificity
- Over-fining
- Oxygen exposure
- Loss of wine volume to fining lees
- Expense and need for clarification
- Additions of flavors/aromas if process is not neutral
- Potential addition of microbes

Two other consequences of fining also need to be mentioned. Fining agents are not sterile. Many are actually quite good carbon, nitrogen and energy sources for
microbes. If the winery is experiencing problems with spoilage, the fining agents should not be ignored as a potential source of contaminants. This is usually not a problem unless the fining agent has not been stored properly (allowed to become wet, stored near other contaminated or possibly contaminated materials, splashed with wine during the fining operation then put back in storage, etc.). Some agents that are used in fining discussed below such as egg whites or spoiled milk can add flavor or aroma "nuances" to the wine. In this respect, many consider the wine to be "over-fined" if the fining agent can be detected sensorially in the wine. This is in the same spirit of the French meaning of over-fining, just a different type of problem caused by residual fining agent material remaining in the wine.
Lesson 16: The Fining Agents

There are several different classes of fining agents used in wine production. The most common agents are the proteins.
Lesson 16: The Fining Agents: Proteins

There are four protein fining agents that are used.

The Protein Fining Agents

- Casein
- Gelatin
- Albumin
- Isinglass

The Protein Fining Agents: Casein

- Mixture of milk proteins
- Proteins have hydrophilic regions and areas of high negative charge due to extensive phosphorylation
- Insoluble in wine
- Can remove phenolics via hydrophobic interactions
- Can remove proteins via charge and hydrophobic interactions

Casein is derived from milk and actually represents different protein species. These proteins are of low molecular weight (less than 30 Kd) and are not soluble at low pH.

The casein proteins have regions of net negative charge due to the fact that they are phosphorylated. These regions can undergo charge interactions with positively charged species in the wine. The proteins also have hydrophobic or nonpolar regions that are exposed when the caseins denature at wine pH. These regions can interact with phenolic compounds and other components. Finally, most proteins will have a net positive charge at wine pH due to the pKa values of the amino acid side chains. Thus casein has areas of both positive and negative charge density on the protein surface as well as nonpolar regions. Casein is generally used to remove phenolic compounds and off-colors or bitterness. Casein use is quite problematic however. Since the protein rapidly denatures at wine pH it will flocculate rapidly and with itself (due to the
The possession of areas of net positive and net negative charge). If this occurs then it can lead to incomplete fining. It is not soluble in water so must be used in a slightly alkaline solution (with ammonium hydroxide) so it is important to do this in a manner not impacting the pH of the wine.

### Casein: The Problems

- Tends to clump requiring good mixing
- Tendency to strip wine
- May impart characters to wine

Casein does not lead to over-finishing in the classic French definition, but can strip wine of aroma and flavor. It can also be detected depending upon how the casein was prepared and how pure the preparation is. Casein is generally produced from coagulated skim milk typically made from commercially unacceptable (that is, spoiled) milk.

### The Protein Fining Agents: Gelatin

- Animal by-product
- Net positive charge at wine pH
- Somewhat soluble in wine
- More neutral than other proteins
- Not as effective as other proteins

Gelatin is derived from animal collagen (skin or bones). Gelatins are classified as heat soluble, cold soluble and liquid, based upon molecular weight of the principle species present and charge. The gelatins are produced in various ways (chemical hydrolysis or enzymatic degradation) and have many uses in food industries. Gelatins have a high content of glutamic acid and will therefore be slightly positively charged or neutral at wine pH. The pKa of the gamma-carboxyl group of glutamic acid is 4.25. It also contains a high percentage of nonpolar amino acids, glycine, proline and hydroxyproline.

The more highly charged the gelatin the more active it is in removal of tannins from
wine. Gelatin can be dissolved in hot water and is frequently used in conjunction with silica sols. The purpose of the silica gel is to prevent over-finishing with gelatin (high residual levels of gelatin).

**Gelatin: The Problem**

- Overfining: requires use of an additional fining agent to get rid of it

The next protein fining agent is albumin.

**The Protein Fining Agents: Albumin**

- From egg whites
- Net positive charge at wine pH
- Removes bitter phenolics
- Softens astringency

Albumin is produced from egg whites. In powder form it is obtained from the drying of egg whites. It is comprised largely of two protein species, ovalbumin and conalbumin. Fresh egg whites can also be used, but these will have a different composition than the dried product. One to as many as eight egg whites may be used per barrel. Most experienced tasters can detect egg whites at two to three per barrel, depending upon the wine, however. Egg white protein can be dissolved in water, but excessive mixing should be avoided as this will lead to significant foaming. Better solubility is obtained if a little potassium or sodium chloride is added to the water.

The albumin proteins also have a net positive charge at wine pH and can remove phenolic compounds. Egg white fining is often mentioned as the method of choice for the production of high end red wines. The only problem with egg white fining is as noted above, if overdone, expert tasters will be able to detect it, and this may be considered a fault or defect of the wine. The egg white character is similar to the aroma of meringue.
Albumin: The Problems

- Not neutral, especially if egg whites rather than pure albumin is used
- Experienced tasters can tell if a wine has been fined with egg whites

The final protein agent is isinglass.

The Protein Fining Agents: Isinglass

- From fish air bladders
- Net positive charge at wine pH
- Large surface area
- Forms stable, tight lees
- Least tendency to over-fine
- Neutral, does not add nuances

Isinglass is a protein produced from the air bladder of fish. Like the other proteins, it has a net positive charge at wine pH. It also tends to denature into "sheets" or strands and thus has a large surface area for adsorption.

It has the least tendency to over-fine, and forms more stable (tight) lees than the other agents. It is also neutral, not adding any nuances to the wine. There are two main problems with isinglass, expense and availability. It is commonly produced from sturgeons so its availability depends upon the availability of the fish. Some other types of isinglass (or fish protein products) are available, but these tend to have lighter lees and offer no advantages over other protein fining agents.

Isinglass: The Problems

- Expense
- Availability
Which protein fining agent is used should be decided after a fining trial. With respect to removal of phenolic compounds, they are equivalent so the decision should be based on other considerations. Some winemakers feel particular proteins are easier to use or better in terms of diminished stripping or over-fining problems, but this will differ on a case by case (wine by wine) basis.

There is intense research interest aimed at identifying plant proteins that may substitute for animal proteins as wine fining agent. This is being fueled by concern over contamination of animal based products with the agent causing mad cow disease in the European community.

The removal of soluble components such as protein by the protein fining agents is facilitated by the presence of tannin. Tannins can be added to wine in conjunction (the day before) protein fining agents. This practice is commonly used in France to increase the efficacy of the protein agents and to guard against over-fining. Tannin fining has other effects as well. Tannins can function as oxidation targets reducing oxidative loss of wine components. Tannins may form complexes with negative characters, and enhance removal of oxidative enzymes. Because tannin dramatically impacts wine structure, additions should be made several weeks prior to bottling.

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Lesson 16: The Earths

The next class of fining agents are the earths.

The Fining Agents

- Proteins
- EARTHS

The most common fining agent in this category is bentonite. Bentonite is a natural montmorillonite clay.

The Earths

- Bentonite

Bentonite is an aluminum silicate that also contains magnesium, calcium and sodium. Bentonite composition differs depending upon the source of the clay, and may contain higher calcium or higher sodium.

Bentonite

- Silicate (SiO$_2$)
- Large surface area: occurs in sheets
- Net negative charge at wine pH: ideal for interaction with wine proteins
- Different forms occur differing in salts associated with silicate: Na+, K+, Ca++

Bentonite dissociates into large sheets and has a net negative charge at wine pH. It will exchange the calcium, magnesium and sodium ions for positively charged proteins in wine. The cation exchange mechanism means that the displaced cation will be in the wine.
Bentonite is not specific, and will bind many wine components.

**Bentonite Levels**

- Typically 1-4 lbs/1000gal (0.12-0.48 g/L) is ample to remove wine protein
- If >10 lbs/1000 gal (>1.0 g/L) is needed, haze problem might not be due to protein!

Because of its affinity for protein, it is not surprising that bentonite is most commonly used to achieve protein stability in wines. It is typically used in the range of 1 to 4 lbs/1000 gal or 12 to 48 g/hL. If significantly higher concentrations are needed, then the haze is likely not proteinaceous in origin or the proteins involved are not highly charged at wine pH. This is rare, but we have seen it happen in white wines made from juices that have undergone a high temperature short time treatment to eliminate laccase. Bentonite is less effective in the removal of proteins protected by polysaccharide components. The heat treatments may lead to the appearance of protective polysaccharides that prevents full agglutination of the proteins present. It is our experience that these types of colloids are very difficult to remove from the wine, but will eventually agglutinate during aging so will be a problem if the wine is bottled.
Bentonite: The Problems

- Must swell properly in water or water/wine mixture before use
- High lees volume
- Addition of ions that may encourage tartrate instability

Bentonite can be challenging to swell properly. It will form cement especially with insufficient mixing. It is typically prepared as a 5 to 15% slurry. Swelling is faster at higher temperatures. Some winemakers prefer to swell the bentonite in wine, but it will coagulate much faster and loses some adsorptive capacity. Other winemakers feel that the bentonite is more effective at removal of protein if juice rather than wine is treated, but this appears to be juice-specific and not a general finding. It may be necessary following bentonite fining to add a protein such as casein to fully strip the bentonite from the wine. Bentonite fined wine can typically be clarified by racking, but in some cases filtration might be necessary. Since bentonite adds cations to the wine, it is wise to tartrate stabilize post bentonite treatment. Bentonite has a tendency toward a high lees volume, perhaps as high as 20% of the volume of the wine treated depending upon the conditions used. Some winemakers recommend bentonite treatment be done in shallow versus deep tanks. This provides an initial larger surface area of bentonite to wine which can improve adsorption.
Lesson 16: The Colloids

Colloidal materials can also be used as fining agents.

The Fining Agents

- Proteins
- Earths
- COLLOIDS

Polysaccharide material can be used in a fining treatment. The polysaccharides generally derive from agar or gum Arabic.

Colloidal Fining Agents

- Natural polysaccharides
- Agar
- Gum Arabic
- Sparkolloid: alginate based
- Ferrocyanide colloidal preparations
- Naturally dispersed or "protective" colloids can hold proteins, tartaric acid crystals, other colloidal materials in suspension
- Colloidal fining agents neutralize surface charges on naturally dispersed colloids thereby allowing them to dissolve or coagulate

In the United States, one proprietary colloidal preparation is known as Sparkalloid. Colloidal fining agents can neutralize surface charges on other naturally dispersed colloids causing agglutination or dissolution of the existing particles. They aid in the removal of more finely suspended particles. It is important to know the mechanism of action or stability of a colloidal preparation in wine, as some effects may be temporary.

We also include ferrocyanide preparations in the section on colloids. These preparations are used to remove transition metal cations such as residual copper from copper fining. In some countries non-colloidal forms of ferrocyanide are permitted, but
only colloidal forms are allowed in the United States. Wines treated with ferrocyanide must be assayed for residue levels. Use of colloidal ferrocyanide preparations is prohibited in many countries. It is generally thought that it is best to adjust winemaking operations so that ferrocyanide treatment is unnecessary. It is rarely used in the United States. Use is so rare that commercial preparations are no longer routinely available.
Wines may also be treated with synthetic polymers.

**Fining Agents**

- Proteins
- Earths
- Colloids
- SYNTHETIC POLYMERS

These agents are used to remove specific phenolic components.

The principle synthetic polymers used are polyglycine, polyamide and polyvinyl-polypyrrolidone (PVPP). The carbonyl oxygen atoms on the surface of these polymers act as adsorption sites for phenolic compounds.

**Synthetic Polymers**

- Polyglycine
- Polyamide
- Polyvinylpolypyrrolidone (PVPP)

All have carbonyl oxygen atoms on surface that act as adsorption sites

These agents remove subsets of phenolic compounds and are particularly effective at removal of monomeric phenolics that will oxidize to off-colors.
Lesson 16: Silica Suspension

Silica suspensions (sols or gels) were mentioned previously as being a useful adjuvant to gelatin fining.

The Fining Agents

- Proteins
- Earths
- Colloids
- Synthetic polymers
- SILICA SUSPENSIONS

The silica sols are principally used to accelerate fining processes as well as to remove excess fining agent. This can improve filterability of the wine.

Silica Suspensions

- The "sols"
- Used primarily with gelatin
Lesson 16: Activated Carbon

Activated carbon can also be used to remove unwanted components from wine. Carbon is very effective at stripping wine so should be used only as a last resort.

The Fining Agents

- Proteins
- Earths
- Colloids
- Synthetic polymers
- Silica suspension
- ACTIVATED CARBON

Activated carbon is not very selective and will remove a wide range of compounds. It is the method of choice for highly tainted wines.

Activated Carbon

- High and broad affinity
- Removes color, wide range of phenolics
- Strips wine: used only as a last resort to salvage a wine for blending

If activated carbon is used, then the wine is frequently not of the quality needed for production of a varietal wine. It can be used in blends.

In contrast to filtration, fining can impact the flavor and aroma profile of a wine. Production levels used are typically low enough to have little to no impact, but it is possible to strip a wine if the winemaker is not judicious in the use of these agents.

In contrast to filtration, finning can have an impact on the flavor and aroma of wine.
Lesson 17: Introduction

Aging

In this lecture we will cover the very important topic of aging of wines. The age-ability of a wine needs to be considered even if the wine is destined to be consumed young. Many authors define aging as the time between the end of fermentation and bottling. However, the wine continues to change in the bottle so we define aging as the period post alcoholic and malolactic fermentation but pre-consumption and include a discussion of events that can happen in the bottle.

Chemical changes occurring during aging strongly impact the flavor and aroma profile of the wine. Aging can be divided into two stages, bulk and bottle. Bulk aging is usually conducted in wooden cooperage allowing limited contact with air while bottle aging occurs in the absence of oxygen. Wine can be aged in stainless steel tanks, which would be similar to bottle aging with respect to exposure to oxygen. Many important aging reactions are oxidation-reductions, some of which are dependent upon the presence of molecular oxygen. Other important chemical reactions are independent of oxygen. These reactions may still occur in the presence of oxygen versus those that require a chemically reduced state.

There are basically three goals of aging of a wine: to assure stability, to correct a defect or problem in the wine and to evolve the wine style or complexity. The first two clearly must be considered prior to bottling. Complexity will continue to increase with age post-bottling up to a point beyond which the wine begins to lose complexity. Bottle bouquet refers to the components that appear during aging in the bottle. The most characteristic notes of bottle age are described as cedar and sun-dried sheets. Some of the classic French wine styles do not owe their celebrated woody character to oak but to bottle aging.
Lesson 17: Aging as a Means of Achieving Stability

As mentioned in the lecture on fining agents, components that form hazes and precipitates or sediments denature over time forming colloids and then large agglutinated complexes. If sufficient time is allowed for these polymerization and agglutination reactions to occur during the aging of the wine and, depending upon the conditions of aging, the particulate matter that forms can be removed using clarification techniques. This may reduce the need for fining agents, and may allow racking to be the only clarification method needed. This process will remove the unstable components and the wine will therefore be stabilized.

Aging can therefore be thought of as a means of achieving wine stability.

Aging: To Achieve Stability

- To allow reactions that are going to happen to occur before bottling
  - Polymerization of tannin
  - Polymerization of pigment
  - Stabilization of color
  - Loss of volatile esters

Aging in the presence of a limited amount of oxygen encourages polymerization of phenolic compounds, which leads to stabilization of color and softening of the tannins (from bitter to astringent, then loss of astringency due to degree of polymerization). Volatile compounds can also be lost during this process.
Lesson 17: Aging as a Means to Correct a Wine Problem

Aging can also be used to correct a problem in the wine. For example, loss of volatile esters may be desirable for full evolution of wine characters. Ester loss may be desired for multiple reasons: because these compounds are not stable, they are too dominating of wine character masking other components, or they simply are not desired sensorially. These characters are largely microbial in origin, not deriving from the grape and therefore not considered to be components of varietal character. If the goal is to produce a true to type varietal wine, esters may detract from the perceived quality of the wine.

In addition to esters, aging may be used to allow other negative characters to disappear from the wine through one or more of the chemical reactions described below.

**Aging: To Correct a Problem**

- Allow "negatives" to disappear
  - Volatilization
  - Hydrolysis
  - Oxidation
  - Precipitation
  - Other chemical reactions

In addition to volatilization compounds may be lost due to hydrolysis, oxidation, precipitation or other chemical reaction. Bitter and astringent phenolic compounds may form larger complexes thus sedimenting from the wine.
Lesson 17: Aging to Achieve Style and Complexity

Aging may be done to allow evolution of wine style and complexity. It may be done under conditions (oak) that are not neutral and add nuances to the wine. Allowing time to lose fermentation or microbial characters so that varietal character is more dominant is also an example of aging to achieve style. Sometimes it is desirable from a stylistic perspective to age wine to lose some of the "forward fruit" traits that originate in the grape, again because these notes tend to mask other characters in the wine, and therefore the complexity.

New characters can also be derived from yeast lees if they are present during aging.

Aging: As Stylistic

- Allow formation of new characters
- Addition of new characters from cooperage
- Addition of new characters from yeast lees/autolysis
- Increase/Decrease complexity depends upon varietal/composition

How the wine changes depends upon the composition of the wine. As new characters develop, complexity increases. Thus aging can be used to subtract or add characters and to increase complexity. The increase in complexity can be considered as a multiplication effect. This is because a single compound can interact with several others, producing a spectrum of reactants. For example, if one compound can react with six others, the wine may contain the original seven compounds plus six reactants for a total of thirteen compounds from the original set, assuming the reactants are not completely consumed in the process. If some of the reactants can undergo further reactions, complexity is greatly amplified. This of course requires that the compounds be subtle rather than dominating for the complexity to be enhanced.
Oxidation-reduction reactions are important components of wine aging. Oxidation is defined as an increase in the oxidation number and an apparent loss of electrons by an atom, molecule, compound or ion. Reduction is the opposite: the apparent gain of an electron. We have discussed in the lectures on microbial metabolism the role of the cofactor NAD+ in biochemical reactions. NADH, which has gained an electron, is called the reduced form of the cofactor and NAD+ 'which has "lost" the electron and now has a positive charge, is the oxidized form. We learned that microbes must maintain a balance and for every biological oxidation a reduction must occur. The same is true in chemical oxidation-reduction reactions. For one compound to be oxidized another must be reduced.

The chemical that gains the electron is called the oxidizing agent and that which loses the electron is the reducing agent. Atoms that react with oxygen donating an electron to the oxygen atom are said to be oxidized. Therefore molecular oxygen gains electrons and is an oxidizing agent. Other more reactive species of oxygen are important oxidizing agents in wine. Antioxidants are reducing agents that react with oxidizing agents more readily than other compounds. Sulfur dioxide is an important antioxidant because it will react with, that is donate electrons to, oxidizing species thereby becoming oxidized. Ascorbic acid is also an important antioxidant because it can react with oxidized molecules converting them back to their reduced form. Ascorbic acid will reduce quinones back to hydroquinones and become oxidized itself in the process. Ascorbic acid is consumed, that is, it remains oxidized. Reaction with an oxidizing agent may lead to the formation of a covalent bond between the molecules that may be difficult to reverse or it may be freely reversible. In many cases reaction with a reactive oxygen species "fixes" the oxygen on the atom on the compound. The reversibility of oxidation-reduction reactions depends upon how energetically feasible the reactions are. Oxidation-reduction reactions can be balanced using the principle of conservation of charge. Extensive oxidation is an important component of some wine styles such as Sherries and Ports. It is considered a defect in many other styles, particularly in white wines. For most wine styles there is an optimum level of oxidation and further oxidation detracts from wine quality. This may be due to simple loss of complexity or to the appearance of undesired compounds. It is frequently difficult to predict the optimum oxygen exposure for a specific wine lot. How a wine responds to oxygen is dependent upon wine composition. Several other factors also impact the aging reactions occurring in wine.
Lesson 17: Variables Affecting Wine Aging

Several factors impact the processes occurring during aging.

Aging Variables

- **Time**
- **Temperature**
- **Oxygen**
- **Cooperage**
- **Yeast lees**
- **pH**
- **Catalysts**
- **Chemical composition of wine**

Aging Variables: Time

One of the most important factors affecting the composition of a wine is the length of time of the aging process, that is, the time between the end of microbial activity and human consumption.

Rates of reactions differ, so the composition of the wine may change dramatically over time.
Depending upon the concentration of initial reactants and the rate of the reaction, the wine may require a long time to achieve a steady state concentration of a particular component. In the graph above, the two components that are appearing in the wine do so with different kinetics. If both are detected sensorially, the aroma and flavor profile of the wine will differ depending upon the time at which it is consumed.

**Aging Variables: Temperature**

As is true of all chemical reactions, the aging reactions occurring in wine are influenced by temperature.

In general, the rate of a reaction will increase with an increase in temperature unless the reaction becomes energetically unfavorable at high temperature. These kinds of reactions are rare in wine, but increasing temperature may appear to be inhibitory to product formation for other reasons discussed below. The magnitude of the increase in rate with increasing temperature may be difficult to predict. A general rule of thumb is that the typical reaction rate will double for a 10°C increase in temperature, but this is highly variable. Some reactions may show a much stronger dependence on temperature and others may appear to be fairly temperature independent.

Some of the variation in response to temperature may be because reactants are affected. For example, if one of the reactants were volatile, loss of the reactant from the wine would be greater at higher temperature thus reducing the concentration of the compound in the solution. In this case, the reaction would appear to be inhibited by temperature. Instead the reaction may be stimulated by temperature but product levels fall because of loss of one of the reactants. Similarly, temperature may more strongly favor one type of reaction that a specific compound can undergo. As an example, if
hydrolysis of a compound is more strongly temperature dependent (meaning it is greatly stimulated at higher temperature) than the reaction between two molecules, higher temperatures lead to preferential reaction of one of the components with water and therefore decrease the level of production of the other product. Aging is generally conducted at moderate temperatures (7 to 24°C) to achieve a balance between formation of desired products and loss of reactants.

**Aging Variables: Oxygen**

One of the most important variables of wine aging is oxygen exposure. Molecular oxygen (O$_2$) is able to catalyze many key oxidation-reduction reactions directly or through the generation of a reactive species such as hydrogen peroxide (H$_2$O$_2$).

Saturation of a typical table wine with air results in a dissolved oxygen content of 6 ml/L (8 mg/L). In practice this level of saturation is not achieved during normal transfer from tanks and barrels, but a wine can be more fully oxidized if a splashing technique is employed or the process of micro-oxidation is used. In micro-oxidation the wine is deliberately oxidized using a device that releases very fine bubbles into the wine. The dissolved oxygen is consumed by reaction with the oxidizable components of wine such as the phenolics. Since red wines have a higher phenolic content, they can "consume" more dissolved oxygen.

**Air Saturations**

- One "saturation" = 6mL O$_2$/L
- Capacity for O$_2$ is dependent upon the phenolic composition
- A single saturation occurs with each air exposure
  - Racking
  - Fining
  - Filtration
  - Centrifugation
  - Movement to tank/barrel

Red wines can also tolerate a higher amount of oxidation because the off-colors produced (brown, pink, orange) do not detract from wine quality and are indeed desired. The amount of molecular oxygen in a typical saturated wine falls to
undetectable in approximately one week at room temperature. Oxidation reactions may continue once the molecular oxygen is gone, as not all oxidation-reduction reactions require molecular oxygen as one of the reactants.

**Oxygen Exposure Leads To:**

- **Polymerization of phenolics**
  - Good: softens astringency
  - Bad: too much loss of color
- **Browning/Pinking**
  - May be desirable or neutral (reds)
  - May be undesirable (whites)
- **Acetaldehyde**
- **Stabilized color**
- **Oxidized flavors**

Oxygen can have many effects in wine. We have discussed browning and pinking, but oxygen also stimulates polymerization of phenolic compounds. Oxygen can also lead to the formation of aldehydes, principally acetaldehyde.

This occurs in a two-step process. A polyphenolic compound reacts with molecular oxygen donating two electrons (via hydrogen) to the oxygen species. This forms hydrogen peroxide, which is still very reactive as an oxidizing agent in spite of having picked up the electrons. Hydrogen peroxide can then interact with ethanol, gaining an additional electron (via hydrogen) for each oxygen atom. This forms acetaldehyde and water. Acetaldehyde is the principle aldehyde formed because ethanol is the principle alcohol, but other aldehydes can also be generated via the reaction of alcohols with
hydrogen peroxide. The appearance of acetaldehyde indicates oxidation of the wine. It is an odor all winemakers should know. Hydrogen peroxide can react with many other wine chemicals. It is more reactive than molecular oxygen. The quinone also produced from the oxidation of the phenolic compound is also electrophilic and reactive. Thus this oxidation-reduction reaction produces two reactive species that then further impact the composition of the wine.

Stabilization of Color Reaction of oxygen with anthocyanins leads to polymerization and stabilization of red color.

Anthocyanin compounds also react with oxygen and oxidizing agents. Oxidation of anthocyanin monomers converts them to a colorless form. Polymerization of anthocyanin monomers can produce colored species with enhanced color. The polymerized pigments are stable against bleaching by SO$_2$ and against other reactions leading to loss of color. Alternately, excessive polymerization may lead to loss of color as sediment.

Oxygen may dissolve into the wine through a wine:air interface. If there is an air space or "head space" above the wine post-fermentation the wine will be exposed to and "pick up" oxygen. Oxidation reactions can then happen at the surface of the wine. If this is undesired, then the winemaker must eliminate the air interface by topping off tanks or barrels. Space will form due to the loss of volatile components, principally water and ethanol, during aging. The amount of volume lost depends upon relative humidity as well as temperature.

Control of Oxygen Exposure

- Use inert gas flush
- Limit headspace
  - Top-off barrels
- Monitor saturations

Inert gas blanketing, argon, nitrogen, carbon dioxide, can be used to limit oxygen exposure of wine. It is also important to note the number of oxygen exposures a wine has undergone (number and nature of rackings for example). This will allow the winemaker to determine the optimum amount of exposure desired and to plan for it
during the processing of the wine.

Aging Variables: Cooperage

- Glass
- Stainless steel
- Wood

The cooperage used to age the wine is obviously very important. In some cases the wine may gain characters from the cooperage itself, the cooperage may reduce or increase the likelihood of specific reactions occurring in the wine. Finally, some types of cooperage may allow better temperature control thereby indirectly influencing wine composition and aging.

We will consider three primary types of aging containers: glass, stainless steel and wood. Glass and stainless steel are neutral, not adding any nuances to wine. Both are impermeable to air and are more readily sealed therefore trapping volatile components and reducing volume loss. Both equilibrate with external temperatures rapidly.

Wood Variables

- Source of wood
  - French
  - American
  - Other
- Aging of wood
- Toasting level
- Number of times it has been used
- Barrel, Staves, Chips

Wood on the other hand is not necessarily neutral and can impart important flavors to wine. A full discussion of the uses and impact of wood on wine composition and perceived quality is beyond the scope of this course. We will be limited to a brief overview.
Phenolic compounds can leach from the wood into the wine that can affect oxidation-reduction reactions and participate in polymerization of other components. These compounds can have an indirect rather than direct role in wine aroma and flavor. The source of the wood is important but this may be more related to processing than composition. The length of time the wood has been aged prior to being used to construct the barrel, upright or vat, impacts the composition of the wood and therefore the characters that can be contributed to the wine. The process of wood bending has a strong influence on wood composition. There are four basic bending techniques or processes used to heat the wood so it is more flexible: direct flame bending, dry heat oven bending, wet heat (steam) bending and hot water bending. In direct flame bending the length of time the wood is exposed to the flame or toasted is important as this will lead to the development of different kinds of characters in the wood. Direct flame bending is somewhat inexact, meaning that there will be considerable variation within the same "lot" of barrels.

Wood exposure is not limited to aging in barrels. There are many barrel alternatives on the marketplace: oak chips, oak sticks (barrel "renewal" systems), and tank inner staves - planks of wood that can be used inside of a stainless steel tank. These alternatives allow extraction of wood components but not the oxygen exposure that can occur in a barrel that is not topped off regularly. Wine appears to pick up little oxygen directly through the staves of the barrel so most of the oxidation that occurs is due to the headspace. Other woods may also be used such as the redwood tanks in California or Acacia in Europe. A neutral wood is preferred.

- Allows limited oxygen exposure
- Allows some evaporative loss
- Adds nuances
- Surface area versus volume of wine important

Obviously the amount of wood components that are extracted into the wine will depend upon the surface are of the wood exposed to the wine as well as the age of the wood, prior usage and treatment.

**Aging Variables: Yeast Lees**
Yeast autolysis adds flavors
  - Long chain esters
  - Stimulates Malolactic Fermentation
- Activity of yeast enzymes continues post-lysis
- Impacts mouth feel

Another important factor affecting wine composition during aging is the presence of the yeast lees. Yeast autolyze in wine releasing yeast components, particularly long chain esters. These components may have a direct impact on wine flavor, aroma or mouth feel or an indirect effect via chemical reaction with other wine components. The yeast also release enzymes, which have been shown to persist in the aging of sparkling wines. The role of these enzymes in table wine aging is unclear.

Yeast autolysis can stimulate the growth of other microbes, such as the lactic acid bacteria, so some of the characters appearing in the wine may be due to microbial activity rather than aging per se.

**Aging Variables: pH**

- Affects rates of some reactions
- Phenolic oxidation 9 times faster at pH 4.0 versus pH 3.0
- Affects microbial persistence and activity

A frequently overlooked variable during wine aging is pH. Wine pH can affect the oxidation-reduction potential of wine, which influences oxidation-reduction reactions.

The pH can also affect the rate of some chemical reactions as well as the activity of the microbial flora, which may be present during aging. In general pH effects are not important if the wine is to be aged to chemical equilibrium, but are important if it is not.

**Aging Variables: Catalysts**
Metal ions can increase rates of some chemical reactions

The oxidation-reduction reactions of wine can be strongly influenced by the presence of catalysts such as metal ions.

The presence of metal ions such as iron can strongly influence the rate of some reactions and can catalyze reactions that might not otherwise occur. Since wines are currently largely protected against the pick up of metal ions due to the use of inert materials, (stainless steel) their influence is minimal in modern wine production.

Aging Variables: Chemical Composition of Wine

It's what in there that counts!

We will end this discussion of aging by noting that the most important variable impacting the changes in wine during aging is the composition of the wine itself. Products cannot form in the absence of their reactants. For example, terpene glycosides are hydrolyzed during aging then undergo further pH, oxygen and time dependent reactions. If there are no or only very low levels of terpene glycosides, these products will not be produced at levels above the threshold of detection. The relative ratios of reactants will dictate what components are produced. The other variables considered merely impact these reactions.
Lesson 18: Introduction

Blending and Sensory Evaluation of Table Wines

In this lecture we will cover the important topic of blending of wines. Blending can be used to achieve many goals in winemaking. The text makes the statement that blending is "where art replaces science in winemaking". In many respects this is actually quite true. It is in blending that a winemaker can best showcase her/his talents versus those of Mother Nature. We will also discuss the sensory evaluation of wines. It is important to use a statistically valid and robust method to determine if the wine composition is truly dependent upon winemaking operations, especially if those operations are costly.
Lesson 18: The Objectives of Blending

There are many reasons to consider blending of wines. Blending can increase the complexity of wines within a vintage and can correct a deficiency or excess in the wine. Blending across vintages can freshen an old wine or age a young one.

Blending Objectives

- Complexity within vintage
- Correct a deficiency or excess
- Freshen old wine
- Age young wine
- Fortification
- Amelioration
- As part of style

Blending may also be done for a very specific stylistic purpose such as fortification or sweetening of a wine, or simply as a matter of style.
Lesson 18: Labeling Regulations in California

It is important to know the regulations of a region with respect to the labeling of wine prior to blending of the wine. The regulations vary in different wine producing regions of the world.

Varietal Wine Labeling in California

- Vintage: 95% must be from that vintage
- Varietal: 75% must be from that varietal
- Viticulture appellation: 85% must be from that growing region
- "Produced and Bottled By": must control 75% of the fruit
- "Estate Bottled": 100% must be from that appellation controlled by the winery

In California, to be labeled as a vintage wine, 95% of the wine must be from that vintage. To be labeled as a varietal, 75% of the wine must be derived from that varietal. If the wine is a varietal vintage, the 75% does not have to comprise the 95%, meaning that both criteria must be met, but 5% of the varietal could be from a different vintage if desired. To be labeled with an AVA or viticultural appellation, 85% of the wine must be from that region. If the wine is to be labeled "produced and bottled by" the winery must control 75% of the fruit. Control in this case means direct all vineyard operations, but does not mean the vineyard must be owned by the winery. In the case of "estate bottled" 100% of the fruit must be controlled by the winery.

"Controlled by the Winery"

Do or direct all vineyard work-do not have to own all vineyards

Therefore there are many factors that must be considered in making a blend. In addition to the desired labeling, compositional features of the blend have to be well thought-out.
Factors to Consider When Choosing a Blend

- Acidity
- Residual sugar
- Alcohol
- Appellation
- Flavor
- Style
- What are the most critical components?

Residual sugar, alcohol and acidity are very important considerations for the finished wine. These characters must be in balance in the wine. Flavor and aroma traits must also be evaluated, and matched to style of the winery. The winemaker may have to compromise goals in producing the final blend so a critical first question is to decide what the most significant factors are.
Lesson 18: The Blending Process

The Blending Process

- Bench tasting to "guesstimate" best blends
- Make trial blends in a small scale
- Period of "marrying": 3 weeks to 6 months depending upon style
- Re-evaluation of blends
- Determination of final blends

Blends are first made on a bench scale and evaluated. Blends considered desirable are produced in larger quantities and aged for a period of time (several weeks to several months). At the end of the "marrying" period the blends are re-evaluated and the final blend determined. This is an oversimplification of the process in many wineries. The blends may be tasted over a much longer period of time and readjustments made if a problem appears. Others simply blend their entire production based on a target goal such as ethanol concentration. Blending need not be confined to wines. Some winemakers believe that the flavors of the wine are better integrated if the juices or musts are blended rather than the finished wines. It depends upon the size of the winery and the style of wine being produced.

Why Do Blends Need to "Marry"?

To determine if an unexpected problem develops over time

There are several reasons for the marrying period. Some unpredictable changes may occur, which would create an instability or other problem in the blend that was not apparent in either of the original wines.
Types of Unpredictable Changes with Blending

- Instability
  - Protein/polysaccharides haze
  - Microbial: bringing microbes and nutrients together
  - Tartrate: bringing tartrate and ions together

- Flavor changes
  - Masking
  - Unmasking
  - Creation of novel characters

Instabilities may arise because components are brought together. Examples would be bringing proteins and tannins or polysaccharides together resulting in colloid formation and agglutination. It is also possible that both parent wines are microbially stable but the blend is not. This would occur if one wine contains microbes but no nutrients and the other nutrients but no microbes. Once brought together in the blend, growth can occur. A final example would be bringing tartrate and ions together leading to crystallization.

Unexpected flavor changes can also arise in the wine. Flavors or aromas can "disappear" in the blend. This may be due to masking, that is the blocking of the perception of a character by another component or it may be due to simple dilution of the trait. If the concentration falls below the threshold of detection, it will no longer be perceived in the wine.

**Masking**

One flavor is masked by another: seems to disappear in the blend

Due to dilution

Due to competition for detection

Characters may still be present above the threshold of detection but go unnoticed if there is another compound present that competes with that compound for detection by our taste and olfactory receptors. Characters may be masked by a dominating trait in one of the blends.
The converse situation also occurs; blending may dilute a dominant character so that it is no longer chemically dominant. In this case other characters that were already present in one of the original wines but undetected, become unmasked and are now apparent in the flavor and aroma profile of the wine. Existing characters in one of the wines can be lost (masked) or gained (unmasked) in the blend.

**Unmasking**

A character present in one of the wines becomes more noticeable in the blend
Dilution of a competing factor that prevents/limits detection
Character due to a combination of chemicals and the concentration of those components increases in the blend

Factors may be diluted in the blend revealing other traits that are perceived more strongly. In this case the first compound has not dropped below the threshold of detection, but is now lower and therefore not competing with the second compound that now appears "unmasked". Alternately the dominating character may arise due to a combination of components in the blend giving the appearance of a new component. Some flavors and aromas are not due to a single compound but are the consequence of the presence of multiple compounds.

**Novel Characters**

Chemical reactants brought together resulting in new aromatic products
Chemicals brought together that are perceived as something other than the original aromas.

Novel characters may also arise in the blend that were not apparent in the parental wines. This may be due to the fact that two reactants have been brought together that have formed a unique product or that the chemicals in combination are perceived as a different character.
Some traits are not "linear" that is they do not respond to dilution in a linear fashion. In this case a 1:1 dilution might not produce a wine with half of the intensity of the character. This is similar to the phenomenon of co-pigmentation. Dilution may drop the compound below the level of detection, which would obviously eliminate it from the aroma profile of the wine. The character may be due to a mixture of chemicals and dilution of the mix may drop one below detection thus eliminating the entire character. There may also be matrix effects that impact the appearance or volatility of the component. Also, a character may be present in both wines, but below the limit of detection in one of them, the blend may then be above the limit of detection because it contains the average amount of the chemical originally present in the parental wines, as weighted by the ratio of the wines in the blend. In each of these cases the component will not appear to display a linear effect upon dilution. The specific chemicals themselves respond in a linear fashion, our perception of them is not linear.

Even with a trait that shows a linear response it is important to be in the linear range of detection of the component. At some concentration the character will be present above our threshold of detection at lower concentrations we cannot detect the character. At a certain point the character reaches saturation - until it is diluted to the linear range we
will not notice a decrease in the level of the character with dilution. In contrast, ethanol, residual sugar and total acidity are linear factors because they are determined by quantitative chemical means rather than by taste or perception.
Lesson 18: Calculation of Blend Ratios

There are several different methods that can be used to calculate blend ratios for linear factors. A simple method described in the text is called Pearson's Square.

Computation of Blend Ratios

- "Pearson's Square"
- By algebraic equation
- Graphical method for multiple components
- Software program

The Pearson's Square method is based upon a simple algebraic equation of the relationship between the ratio of the two volumes, the initial concentrations of the compound in question in each of the parent wines, and the concentration in the blend.

In this case "a" and "b" represent the lowest and highest concentrations in the two parental wines respectively, and "m" is the desired concentration in the blend. The concentrations are subtracted from each other as indicated. "b-m" gives the ratio of "a" to be added, and "m-a" the ratio of wine "b" for the blend.

Let's consider a specific example. Two wines are available with 11 and 15% ethanol. What ratio of the two wines must be blended if the desired final ethanol content cannot exceed 12%?
The proper blend is a mix of 3 parts of wine "a" to 1 part of wine "b". This is logical, as more of the wine that is closer to the final desired ethanol content is used in the blend.

Instead of a square, a series of algebraic equations can be solved:

\[
\begin{align*}
V_A + V_B &= 1 \\
V_A &= 1 - V_B \\
11V_A + 15V_B &= 12(V_A + V_B) \\
11(1 - V_B) + 15V_B &= 12((1 - V_B) + V_B) \\
11 - 11V_B + 15V_B &= 12 - 12V_B + 12V_B \\
4V_B &= 1 \\
V_B &= 1/4 = 1 \text{ part of } V_B \text{ to } 3 \text{ parts of } V_A
\end{align*}
\]

Multiple equations can be solved if needed. It is a good idea to check all calculations to be sure an error has not been made.
This can be done simply by multiplying the concentration by the ratio (number of "parts" of that wine) of the wines then summing the concentrations and the total number of "parts", and then dividing.

Pearson's Square can also be used for multiple wines as shown:

\[
\begin{array}{c}
A = 11\%; B = 15\%; C = 14\%; D = 13\% \text{ and want 12\% ethanol for final blend} \\
\end{array}
\]

\[
\begin{array}{c}
\text{Dealing with Multiple Wines} \\
\text{3(11):1(15)  2(11):1(14)  1(11):1(13)} \\
\end{array}
\]

A common error in use of the Pearson's Square is to ignore negative numbers. Negative numbers would arise if both wines on the left side of the square exceeded or were less than the desired content of the blend, or if the wine values were placed in the wrong order.
If a group of wines is to be blended it is then important to determine the maximum volume that can be produced. One of your assignments will test your skills in this area. In some cases the ideal blend might not be possible to make. For example the appropriate blend giving the desired ethanol concentration might not give the desired residual sugar concentration or acidity. In this case, the winemaker has to make a judgment call as to which of the factors is the most important for the blend. It is also important to evaluate all possible blends that will generate a wine of the desired composition and to maximize yield. In the example above, the next step would be to evaluate the relative volumes of each of the wines to optimize yield. The 13% alcohol can be used in a 1:1 ratio with the 11% while the 15% ethanol wine requires 3 times the volume of the 11%.

Dealing with Multiple Components

Frequently, blend decisions are made considering multiple wines and multiple components (sugar, ethanol, acidity, etc.). In this case, graphical methods can be used to estimate the best overall blend. However, the ideal value of each component might not be attainable.

Graphical methods for determining the optimum blend of several factors exist (one is described in the text) and there are now several blending software programs that are on the market.

There are no set rules for the blending of table wines unless the winemaker is emulating a particular blend style such as Chianti. Subtle differences in blending ratios can have a strong impact on the aroma profile of the resulting wine. This is why
blending is considered to be more of an art than a science.
Lesson 18: The Sensory Evaluation of Table Wines

Our next topic is the sensory evaluation of table wines. It is important that sensory evaluation be done correctly. There are different methods that can be used, depending upon what the goal of the analysis is.

**Sensory Analysis**

It is important to use scientifically sound procedures for the evaluation of wines.

It is important to not bias the sensory evaluation in any specific direction. This can be quite innocently done for example like asking tasters or judges which wine has the highest concentration of citrus character versus first determining if citrus character is detectable in either wine.

**Wine Attributes for Analysis**

- Appearance
- Odor
- Taste
- Aroma
- Flavor

Wines can be evaluated for their appearance: color, clarity, presence of sediment. They can also be evaluated for odor and taste. For a compound to be detected as an odor it must be volatile. The primary tastes are: sweet, sour, salty and bitter. Astringency can be perceived but is not actually a taste. Taste can be discriminatory, for example, sweet and sour can be detected at the same. This can be more challenging with odors - the combination may be perceived as a different character rather than the sum of the two initial odors. Therefore wine aroma - the synthesis of the individual odors can be evaluated. Similarly, flavor has been defined as the interaction of taste and aroma, and this can be assessed in wines as well.

The temperature of the wine may influence taste, depending upon what components
are present. Temperature also has a dramatic affect on aroma as it affects compound volatility. The time the wine has been in the glass prior to evaluation is also important. If too long of a period of time has elapsed, many of the more highly volatile components may be lost from the aroma profile. Much has been written about the shape of the glass and how the aroma is "presented" to the nose - this is usually not an issue with an experienced taster as experienced tasters know how to evaluate aroma regardless of the shape of the glass, but it may be important with a novice.
Lesson 18: Methods for the Sensory Evaluation of Wine

There are several techniques for the evaluation of wine. Which technique is most appropriate depends upon the aim of the analysis and what kind of information is being sought.

Sensory Evaluation of Wines

- Descriptive analysis
- Difference tests
- Intensity rating
- Hedonic tests

Descriptive Analysis

- Goal: to describe the aroma and flavor profile of a wine
- Using panel discussion decide upon flavor/aroma characters of wine
- Train tasters using standards (wine spiked with characters of wine)
- Blind tasting to determine if characters can be reproducibly recognized in wines

Descriptive analysis can be used to determine which compounds are present in a given wine. This technique profiles the primary aroma and flavor components of a wine. It is critical that real descriptive terms (cherry, wet dog, plastic) be used rather than esoteric expressions (finesse, arrogant, petulant) since the goal is not to win a creative writing award but to evaluate the wine. Since sensory receptors and therefore the ability to perceive components vary across the human population, it is important to perform the descriptive analysis with a group of individuals (10 to 15).

The panel of tasters initially taste and smell the wine and make note of the primary components that they detect. The tasters then compare notes and determine the
consensus description of the wine. Standards are then generated using the descriptors in neutral wines and the wines evaluated again by the tasters after they become thoroughly familiar with the character in the control wines. Discussions by the panel again occur after this process. The ultimate goal is a strong consensus of the foremost aroma and flavor traits of the wine. Blind tastings are then done to evaluate wines based on the characters identified in the descriptive analysis and to confirm their importance.

Sensory analysis, if done correctly, can be time-consuming but is well worth the effort. It is important to be aware of the problem of fatigue and to not attempt to analyze too many wines at once. Tasting is generally more fatiguing than smelling of the wine. This is because taste may continue to be perceived after the wine has been expectorated.

**Difference Tests**

- Use trained judges
- Determine if two wines are reproducibly selected as different
- Requires statistical analysis

Another type of sensory evaluation is difference testing. In this case the goal is to determine if two wines can reproducibly be identified as different from each other. These types of tests are important when trying to determine if a vineyard or winery treatment has had a significant impact on the flavor or aroma of the wine. Significant in the statistically reproducible sense, not in the magnitude of the change in the character. One is frequently evaluating wines for quite subtle changes in aroma or taste.

Difference testing uses trained judges and involves a statistical analysis of the data. Two types of difference tests are commonly employed in the analysis of wine. One is called the triangle and the other the duo-trio.

**Difference Tests for Wine Evaluation**

- TRIANGLE
- Duo-Trio
In the triangle test, the taster is presented with three wines. Two of the wines are identical and one is different. The taster is told this and is asked to identify the wine that is different.

![The Triangle Test](image)

Statistical tables can be used to determine if the percentage of correct answers is significant or not, that is, is unlikely to have occurred by chance.

In setting up these kinds of tests it is important to use a random numbering scheme so that the tasters are not biased by some numerical consistency of the analysis. Students frequently ignore my direction to use randomly generated numbers and come up with a pattern to the code that is convenient for them to use. However, tasters usually detect the patterns either consciously or sub-consciously in the evaluation of the wines. For example, one student group in performing a triangle test made sure that the two wines that were identical had the same middle number: 306, 407, 658; most tasters keyed in to this and were selecting as different the wine that did not have the identical middle number. No patterns in the "random" three digit codes should ever be used.

**The Triangle Test**

A statistical analysis can then be used to determine if the number of times wine 359 was selected as different is significant or not.

The next test is the duo-trio. This test also involves comparison of two wines. But in
this case one is a reference wine and two are samples.

**Difference Tests for Wine Evaluation**

- Triangle
- DUO-TRIO

The taster is told that the reference is identical to one of the sample wines and asked to select the wine that is different from the reference.

**The Duo-Trio Test**

Tasters are provided with a reference and two sample wines. They are asked to determine which sample wine is DIFFERENT from the reference.

```
R   184   352
```

R = 352 = Wine B
184 = Wine A

The taster can also be asked to describe the magnitude of the perceived difference or to note the component that is most discriminating for the wines.

**The Duo-Trio Test**

A statistical analysis can then be used to determine if the number of times wine 184 was selected as different is significant or not.

As with the triangle test, a statistical analysis can be performed to show that the correct wine was selected with a frequency much higher than expected from random chance. Alternately, is it just as important to learn that the wines are not significantly different. These tests can be fatiguing. The triangle is more fatiguing than the duo-trio. The order of presentation may also be important so should be evaluated in the
analysis by having replicate sets with differing orders of presentation (i.e. reference-identical sample-different sample versus reference-different sample-identical sample). It is important to include a test of fatigue - present two identical sets one early and one late in the presentation of wines and determine if the judges have lost the ability to discriminate the second set.

Wines can also be evaluated for the relative intensity of a specific character. This is called intensity rating.

**Intensity Rating**

![Intensity Scale]

Important to train judges to know what a term is and what value they will assign to specific intensities in wines. Can then convert rating into a numerical score for statistical evaluation.

This involves extensive training of the tasters or judges. The judges need to know what the term means, and they need to be shown different concentrations in a base wine to determine the linear range of detection. The linear range may vary by the judge, and it is important to know this prior to the start of the analysis.

The judges then need to translate an impression of the concentration into a numerical value. In the beginning this is done with standard solutions of varying strength to "train" the judge, before the real samples to be evaluated are presented.
The statistical analysis of the data generated from an intensity analysis is more involved than the descriptive analyses and unfortunately beyond the scope of this course. More involved analytical methods, such as principle component analysis, can be used in the evaluation of wine, but this is beyond the scope of this course as well.

The final type of sensory analysis is hedonic or preference testing. This simply asks the tasters which wine they prefer. The test must be done under appropriate conditions so that preference is genuine and not influenced by other factors (such as the packaging) unless that is part of the study. It should not be done in a busy, noisy, public tasting room at the winery.

Hedonic Tests

- Uses untrained consumers
- Evaluates whether a taster likes a particular wine or not
- Can use an overall evaluation scale

Frequently this is the method of choice for determination of consumer preference with respect to style of a wine. It is important that the consumers not be pre-biased by the individual conducting the analysis. For example, "I hope you pick my favorite wine, the one with the citrus character".

An overall evaluation scale can be used such as the one that follows:

Overall Evaluation Scale

Assign wine to one of the following categories:

1. Like intensity
2. Like moderately
3. Like slightly
4. Neither like nor dislike
5. Dislike slightly
6. Dislike moderately
7. Dislike intensely
One common "rookie" mistake with this type of analysis is the attempt to use cute or attractive phrases to describe the wines. This can lead to consumers selecting their favorite phrase rather than evaluating the wine. The following scorecard was used by a student group in the evaluation of their research wines in VEN124:

<table>
<thead>
<tr>
<th>Please check the appropriate box:</th>
<th>1 = excellent</th>
<th>7 = horrifying</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LOVE this stuff!!</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sure, I'd drink this stuff.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Well...if there is no other stuff...</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Hey, it's stuff...</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>This stuff's a little freaky.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>What's going on with this stuff?</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Gross...You call this stuff???</td>
<td></td>
</tr>
</tbody>
</table>

Most tasters were attracted to one of the phrases indicated by numbers 5 through 7. The terms are too close to each other and too vague in exact meaning to be discriminatory. I let them use the scorecard knowing that they would realize they had a problem when they did the analysis of the data.

Selection of Type of Sensory Analysis

- What are you trying to determine?
- Judge/taster fatigue

The tests presented above are obviously geared towards providing different types of information. Difference testing is used to determine if wines produced using different methods are detectably different in character. This does not mean one is bad and the other good, just that they are different and the difference is detectable. Intensity tasting can be used to determine if the composition of a specific character has changed by treatment, such as determining if the aging regime has lead to a detectable difference in astringency. Preference testing may be used to refine style either using expert tasters with a clear stylistic goal in mind. It can be used (with caution!) to allow
consumers to direct the winemaking style toward a more marketable product. You will no doubt notice that I have not included a numerical ranking. I refer you to the article by Ann Noble "Missing the Point" in your readings. These numerical scales are excellent training tools, but are difficult to use for the sensory evaluation of wine without very extensive training of the judges. They are most frequently used as indicators of commercial acceptability rather than as a formal sensory analysis.

Finally, the combination of consumer preference profiling (a hedonic test) with descriptive analysis by trained judges can be quite powerful. In this case these two processes are independent. The consumers processes are independent. The consumers are not involved in the descriptive analysis but simply indicate wine preferences. A statistical analysis combining the preference and descriptive analysis data can be conducted to determine if there is a general consensus of preference for a given style of wine.

This ends our section on post fermentation processing. The final section of the course will focus on the flavor and aroma compounds of wine, their sources and means to manipulate wine composition and character.
Lesson 19: Introduction

Section 6 - The Flavor and Aroma Compounds of Wine

In this final section of the course we will cover the principle flavor and aroma components of wine, consider the sources of each class of compounds and their roles in wine quality.

There are several classes of flavorants in wine that are responsible for the character of the finished product. These compounds arise in the grape, are microbial metabolites, are the products of chemical reactions occurring during aging, and may derive from specific processing steps such as the use of oak.

Only a few odor impact compounds have been identified in wines. Among these are compounds that contribute to floral (terpene) or vegetal characters to white grapes. Some varietals are high in one or the other class, some are high in both, and others are low in both, as diagrammed in the following slide.
Lesson 19: Pyrazines

The first class of compounds we will consider are the pyrazines that are associated with the vegetal character of wine.

The first class of compounds...

Pyrazines have the following chemical structure:

2-methoxy-3-isobutylpyrazine has been shown to be responsible for the bell pepper character of wines. While other pyrazines have been implicated in the potato, chili, carrot, peanut, barley characters of wine, these compounds have not been unequivocally shown to be responsible for these traits in wine.

- Derived from grape
- Not microbial in origin
- Vegetal characters
  - Bell pepper
  - Chili
  - Bean
  - Carrot
  - Potato
  - Peanut
  - Roasted Barley
The pyrazines originate in the grape and are not thought to be synthesized by the microbes present.
Lesson 19: Terpenes

Another important class of grape aroma compounds is the terpenes.

Terpenes

- Fruit/floral aromas from grapes
- Can be produced by some yeasts and molds (but not *Saccharomyces*)
- Derived from isoprene units
- May be unbound or bound (as glycosides)
- Only unbound terpenes can be detected

The terpenes are largely responsible for the fruity (citrus) and floral aromas of wine. They may be bound to sugar groups or free (unbound). While it has been shown that some yeast can produce these compounds, they originate in the grape in wine production. These compounds are derived from isoprene units.

Isoprene units are composed of five carbon compounds. With two carbons attached to one of the terminal carbons. Two isoprene units joined head to tail give a monoterpene. Changes in the location of the double bond, degree of unsaturation and oxidation may also occur, yielding hundreds of possible different monoterpene structures.
Monoterpenes are comprised of two C5 units (C10), while higher terpenes have greater than 2 isoprene units. Sesquiterpenes have 3 C5 units (C15) and diterpenes 4 C5 units (C20).

One can usually spot the order of the isoprene units in the molecule as shown above. Terpenes can be modified in different ways. Branching is generally, but not always, at
Terpenes can occur as hydrocarbons, alcohols, aldehydes, ketones or esters.

**Monoterpene Aldehydes**
- Geranial
- Neral

**Higher Terpenes**
- Grapefruit
The higher terpenes have been described as fruity (citrus) characters but may also be responsible for the diesel or fuel characters.

**Higher Terpenes**

- Includes napthalene derivatives
- From plants
- Fruit characters
- Fuel characters

Trimethyl dihydronapthalene has been described as "kerosene".

While the yeast do not synthesize terpenes they can produce glycosidases that will clean the glucose moiety converting bound terpenes to the free volatile and thereby detectable form. It was recently shown that *Saccharomyces* mutants defective in sterol biosynthesis could convert sterol intermediates into terpene characters.
Lesson 19: Shikimic Acid Derivatives

The next class of aroma compounds are the shikimic acid derivatives.

These compounds are derived from aromatic amino acid metabolism.

- Derived from aromatic amino acid metabolism
- Produced by plants, microbes, oak extraction

Because of the universal nature of the amino acid biosynthetic pathways, these compounds can be made by the plant, by microbes or they can be extracted from wood.

The most important of these characters is vanillin. This character can be quite noticeable in wines aged in new oak barrels.
Lesson 19: Lactones

The lactones are also important characters in wine.

The lactones can be a five- or six-member ring structures with oxygen one of the atoms of the ring. gamma-lactones have five member and delta-lactones, six member rings.

![Lactones](image)

The side chains may have different functional groups. These compounds can be produced by the plant, can derive from microbial metabolism, or may be extracted from oak beta-methyl-gamma-lactone is called oak or whiskey lactone.

Lactones

- Oxygen-containing 5- or 6-member ring compound
- From grapes, microbes, oak extraction
- Typical characters: cotton candy, generic candy, generic fruit, coconut, buttery

Lactones are responsible for the cotton and "generic" candy notes of wine and contribute to butteriness along with diacetyl. At high concentrations they contribute a more coconut-like character.
Lesson 19: Esters

Esters are highly volatile compounds and therefore are important aroma components in wine. Esters can be produced by the plant or by microbes. Given their chemical instability at wine pH, the esters present in wine generally are microbial in origin.

- From reaction of an alcohol and an acyl-CoA molecule
- Formed mainly by microbes, but can come from plant
- Readily hydrolyzed at wine pH

Esters are formed from the reaction of an alcohol and an acyl group attached to a coenzyme A molecule.

![Esters](image)

The alcohol can be ethanol or come from amino acid metabolism or purine or pyrimidine degradation.

- Alcohol: ethanol or alcohol from amino acid, purine, pyrimidine degradation
- Acid: acetic acid or acid from amino acid degradation, fatty acid biosynthesis

The acid can be acetic acid or also come from amino acid degradation. The acyl-CoA
molecule may derive from fatty acid biosynthesis. The esters are responsible for a variety of different types of characters.

**Esters**

- Ethyl acetate: nail polish remover
- Ethyl laurate: soap
- Isoamyl acetate: banana
- Phenethyl acetate: rose oil

In general, short chain esters have a generic fruity note in low concentration. In high concentration they can take on a solvent note like ethyl acetate.

**Esters**

- Short chain: fruity, floral
- Long chain: perfume, soap
- Lower concentration: fruity, floral
- Higher concentrations: perfume

Longer chain esters that derive from fatty acid metabolism have a perfume or soapy note, which can again be overpowering if in high concentration.

Esters are very important components of certain wine styles: sparkling wines made in the Champagne style and those that are to be consumed young. Many factors influence ester formation.
Ester Formation Influenced By:

- **Temperature**
  - Higher temperature: increased loss
    - Volatilization
    - Hydrolysis
- **Oxygen exposure**
  - Stimulate fatty acid biosynthesis
- **Nitrogen source availability**
  - Precursor availability
- **Strain/genetic background**
  - As much as 10-fold difference in level of production

High temperatures lead to greater loss of these compounds due to increased rates of hydrolysis and volatilization. Transient oxygen exposure can lead to the synthesis of fatty acids and thus long chain acyl-CoA molecules are available for the generation of esters. The amino acid content of wine is also an important factor as many of the alcohol and acyl groups drive from the degradation of these compounds. The yeast strain background also has an impact. The production of esters can vary as much as ten-fold.
Lesson 19: Higher Alcohols

The higher (greater than 2 carbons) alcohols can also be components of wine aroma.

**Higher Alcohols**

- Made mostly by microbes, can be made by plants
- From amino acid degradation/biosynthesis
  - Fusel oils
  - Aromatic amino acid derivatives

Higher alcohols can be made by plants but mostly come from the metabolic activities of yeast. They are produced in the course of amino acid metabolism. The higher alcohols are also referred to as fusel oils. There are four principle higher alcohols:

**The Fusel Oils**

- 3-Methylbutanol
- 2-Methylbutanol
- Isobutanol
- Propanol

The production of these compounds was discussed in lecture 10 under the section on off-character production.
Lesson 19: Acids

The acid species present are important in wine flavor.

We have discussed several of the acids already. In addition to malate, tartrate and lactate, other acid species may be important contributors to wine flavor.

Acids

- From plant or microbes
- Sourness
- Other characters:
  - Rancid (butyric acid)
  - Pungent (acetic acid)

Acids originate in the plant or are the products of microbial metabolism. The anionic species contributes to the sourness of the wine, but the volatile acids are also odorants. The acid odor may simply be pungent as in the case of acetate, or may connote a different character.

Lactate is the most and citrate the least sour of the acids commonly found in wine. Most wine producing regions place a legal limit on the amount of acetic acid that may be present in a table wine.
Legal Limits:

<table>
<thead>
<tr>
<th></th>
<th>US</th>
<th>CA</th>
<th>OIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>1.2 g/L</td>
<td>1.1 g/L</td>
<td>0.98 g/L</td>
</tr>
<tr>
<td>Red</td>
<td>1.4 g/L</td>
<td>1.2 g/L</td>
<td>0.98 g/L</td>
</tr>
</tbody>
</table>

Threshold of Detection:
~0.6 to 0.9 g/L

In the United States, the limit for red wine is higher than for white wine. In the European Community there is no distinction between red and white wines. In the US, individual states may set their own limits. The limit for California wines is less than the national level. These limits are above the threshold of detection.
Lesson 19: Phenolic Compounds

Phenolic compounds are also important contributors to wine flavor. They can be classified with the Shikimic acid derivatives, but we will consider them as an additional group for the purposes of this lecture. They can be volatile as described for vanillin above or non-volatile (polyphenols).

Phenolic compounds

- Bitterness
- Astringency
- Produced by plant
- Can be converted into vinyl phenols by microbes (spoilage characters)

We have noted the importance of these compounds in bitterness and astringency and as the precursor to microbial traits that may be considered important in some wines and spoilage in others. While the phenolic compounds may be modified by microbes, they are produced by the plant.
Polymerized polyphenolic compounds or tannins are perceived as astringent. These molecules can interact with proteins in the saliva and this interaction is thought to be responsible for the sensation of astringency. The compounds responsible for bitterness are primarily located in the seed.
Lesson 19: Sulfur-Containing Compounds

We have largely been confining this discussion to positive aroma and flavor characters, but we must also consider families of compounds that are more frequently thought of as negative or detracting from wine quality. Principle among these characters are the sulfur volatiles.

These compounds are responsible for a host of off-characters. They are the products of yeast metabolism of sulfur containing precursor compounds produced by the yeast or found in the grape. They are also responsible for some "varietal" characters, most notably in Sauvignon blanc. These volatile thiols are released from precursors in the grape by the action of yeast metabolism.

**Sulfur-containing compounds**

- Low threshold of detection
- Generally produced by microbes
  - Degradation of sulfur containing amino acids
  - Spontaneous reactions from microbially-derived sulfides

As a class, these compounds have low detection thresholds. Some may undergo other types of reactions in wine producing different but still offensive traits. Lactic acid bacteria can also make these compounds but do not appear to do so under wine conditions.

**Sulfur-containing compounds**

- Sulfides
- Thiols
- Sulfoxides
- Thio alcohols

The sulfur-containing compounds can be divided into three classes: the sulfides, the thiols and the sulfoxides.
**Sulfur-Containing Compounds: Sulfides**

- $\text{H}_2\text{S}$ (hydrogen sulfide): rotten egg
- $\text{CH}_3\text{-S-CH}_3$ (dimethyl sulfide): cabbage, canned corn
- $\text{CH}_3\text{-S-S-CH}_3$ (dimethyl disulfide): clam

Hydrogen sulfide is responsible for the "rotten egg" character. This compound comes from the reduction of sulfate or from the catabolism of sulfur-containing amino acids. Other sulfides come from the degradation of methionine and cysteine as nitrogen sources by the yeast.

**Sulfur-Containing Compounds: Thiols**

- $\text{CH}_3\text{-SH}$ (methanethiol): rubber
- $\text{CH}_3\text{-CH}_2\text{-SH}$ (ethanethiol): onion, rubber, skunk

The thiols are also likely yeast metabolites or derive from the reaction of yeast metabolites.

These characters are also objectionable in wine. Other thiols are responsible for varietal character. The tropical fruit, straw and box wood characters typical of Sauvignon blanc are thiols derived from the degradation of S-cysteine conjugates by the yeast during the alcoholic fermentation. Some already exist at low concentrations in the juice, so the role of the yeast is primarily to amplify these notes in the wine. Dicarbonyl compounds appear to play an important role in the degradation of the sulfur-containing amino acids.

**Sulfur-Containing Compounds: Sulfoxides**

- $\text{CH}_3\text{-SO-CH}_3$ (dimethyl sulfoxide): plastic, rubber hose
Sulfoxides can be responsible for some plastic as well as rubber notes.

The thio alcohols should also be mentioned. These are alcohols of the types of sulfur compounds already mentioned.

**Sulfur-Containing Compounds: Thio alcohols**

- HS-CH₂-CH₂-OH (mercaptoethanol): barnyard
- CH₃-S-(CH₂)₄-OH (thiomethylbutanol): garlic, chive
- CH₃-S-(CH₂)₃-OH (methionol): raw potato, soy

These compounds are also spoilage characters, although they might not be that objectionable in low concentrations. Indeed some of these characters are found in some high-end wines, as are other sulfur-containing compounds.

In addition to the shikimic acid derivatives and fusel oils, other amino acid metabolites may be important in wine composition. One of your reading assignments covers this topic in considerable detail from the perspective of yeast metabolites. The lactic acid bacteria can also convert amino acids into a host of odor compounds.
Lesson 19: Amino Acid Derivatives

In addition to the sulfur-containing amino acid, other amino acids may also generate aromatic compounds. The appearance of these characters is dependent upon the presence of amino acid precursors in the medium, on the type of organisms present and on the availability of other more preferred nitrogen and energy sources (in the case of the bacteria). This is true of microbial metabolites in general. DAP supplementation has been criticized as limiting or eliminating full evolution of the microbial contribution to wine aroma and flavor because it provides an alternative to the degradation of amino acids. The shikimic acid derivatives could be included in this section. I have treated them separately as they can derive from oak, plant or microbial activity. The compounds considered in this section are microbial (yeasts and/or bacteria) in origin.

Amino Acid Derivatives

- Several other types of compounds can be formed from amino acids by yeast and bacteria
- Appearance depends upon which microbes are present and what nitrogen/carbon sources are present

One "famous" off character that we have discussed is the mousy taint that comes from the microbial degradation of lysine. It can be produced by Brettanomyces or by certain species of Lactobacillus.

Amino Acid Derivatives: Mousiness

2-Acetyl-tetrahydro-pyridine

Not all amino acid derivatives are considered spoilage characters. The compounds or their esters may be quite important to the aroma profile of the wine. Phenethyl alcohol and phenethyl acetate are floral. Tyrosine can be degraded to a honey note. Isoamyl
acetate has an over-ripe banana note. Apple and pineapple notes are also esters of derivatives of amino acid catabolism. Active amyl and isoamyl alcohol are responsible for the "marzipan" character of wines.
Lesson 19: Other Compounds

Other kinds of compounds are also important that are not necessarily represented by a family of substances, as are the previous classes. The specific chemicals responsible for many (perhaps most) characters have not been elucidated. These traits may originate in the fruit or derive from microbial metabolism.

Other Compounds

- Other plant compounds associated with aroma
- Not well characterized

One important compound is the Concord grape or "foxy" character. This is present in many non-vinifera species, but is considered a defect if present in a wine made from *V. vinifera*.

![Methyl Anthranilate](image)

2-amino acetophenone also contributes to the foxy note.
Many, many other classes of flavorants await discovery. The compounds described here have been attributed to specific wine components by inference - the compound has that odor in pure form, so is thought to be responsible for it in wine.
Lesson 19: Factors Affecting Wine Aroma and Flavor

We have considered the chemical classes of compounds, but many factors impact the presence as well as the stability of these components. We have discussed in earlier portions of the course the sources of these compounds and, by extension, how their contents can be increased or decreased, depending upon what is desired.

Source of Flavor/Aroma Compounds

- Grape
- Microorganisms
- Aging (Time)
- Addition: Oak, Fining, Aeration
- Removal: Fining, Aeration

It is also important to consider the stability of these characters. Several phenomena impact the persistence of a specific compound in a heterogeneous environment.

Factors Affecting Wine Characters

- Volatilization
- Chemical Reactivity
- Chemical Stability
- Metabolism
- Downstream Processing: fining; oxygen
- Sensitivity to Dilution

Compounds may be lost due to volatilization. All odors are volatile and will eventually be depleted from a solution as the concentration in the headspace is in equilibrium with the dissolved concentration. Frequent turnover of headspace or exposing the wine to a large headspace will lead to the loss of volatile components. The rate at which components are lost depends upon their volatility, which is in turn influenced by solubility in the matrix of the wine. Chemicals can also be lost if they react with other chemicals forming new products. Chemicals may also seem to breakdown, that is, react with water and hydrolyze or otherwise regenerate the precursors from which they
were made. If components are metabolizable and are metabolized they will of course be lost from the wine. Compounds at or near their threshold of detection will disappear with simple blending or dilution of the wine. And, of course fining processes and other processing decisions can lead to the loss of wine characters.

The above overview showcases the limited extent of our knowledge on wine aroma and flavor. Wine is very complex and the chemical basis of many characters is unknown. Some components can be influenced by the presence and concentration of other wine factors, hampering research efforts in this field.

Factors Affecting Wine Characters
Most wine characters are derived from or are metabolites of compounds present originally in the grape.
Grape composition is critical in the production of a quality wine.

It should also be apparent that the evolution of wine aroma and flavor is a complex interaction between the precursors present in the grape at harvest, microbial activity and the conditions of aging and processing. All of these elements are inter-related and interdependent. An unmetabolized precursor will not be detected. Oxygen-requiring reactions will not occur if no oxygen is present. Decisions made by the winemaker also influence the complex interplay among the varietal, microbial and aging influences on wine aroma. Nutrient addition may block development of amino acid based characters.

Factors Affecting Wine Characters
Wine production processes have a strong impact on the metabolites produced and characters remaining in the wine at the time of bottling.
Winemaking practices are critical for production of a quality wine.

Topping off strategies impact oxygen pick up by the wine. Aging in oak and the type of oak used will affect not only the level of phenolic compounds, but can drive oxidation-reduction reactions. Blending is perhaps the most important of all. Thus, while winemaking begins in the vineyard, it definitely continues in the winery!
In our final lecture we will return to the themes of the first: the definition of wine quality and, more importantly, how quality goals can be achieved. One definition of quality is the absence of a noticeable defect or imbalance. Much of the previous lectures have focused on recognition of negative characters and understanding the source of these components and the means to limit their appearance in wine. Indeed, our program has been criticized for taking this approach and thereby advocating the production of "technically correct" fault-free wines. Of course we would not recommend the opposite, but understanding the use and potential abuse of winemaking technologies is an important first step in the production of sound wines.

It can be argued (and has) that all other definitions of wine quality are highly subjective and therefore not germane to a discussion of the science of wine production. Quality, being subjective, is indeed in the nose and mouth of the taster. This is in large part true even when we are considering targeted definitions of quality - how close a particular wine comes to the ideal is dependent upon our own physiology and sensory perception as influenced by our environment and culture. Our goal is to provide winemakers with the best information available to allow them to develop and attain their own vision of quality.

That said, I do indeed have my own vision of quality and have had ample opportunity to work towards that goal while teaching the laboratory component of this course. With your permission in this final lecture I will depart from the realm of science and technology and pay a visit to the artistic side of wine production, and share my own personal opinions and experiences on the creation of "quality" wines (as defined by me). The opinions expressed herein are simply that. I have no interest in becoming a "quality czar" and imposing my definition of wine quality on the rest of the world. There is no shortage of others vying for that role.
Lesson 20: "California Wines Have Personality, French Wines Have Breeding"

I will begin with my favorite quote concerning the difference between California, or new world, and French, or old world, winemaking. I apologize for not knowing who to originally credit with this quote because I have heard it many times, always attributed to a different individual. I like this quote because it nicely expresses the way I feel about the two philosophies of wine making.

**French vs. American**

**Breeding vs. Personality**

**Harmony vs. Complexity**

Personality is the end result of our genetic constitution as influenced by our environment, culture or upbringing. Webster defines personality as "the quality or state of being of a person; the totality of an individual's behavior and emotional tendencies". Someone who has "personality" has "distinction or excellence of personal and social traits". "Distinction and excellence of inborn traits" is my definition of California wines. It is the underlying philosophy of American winemaking.

**Personality**

- Inherent Genetic Factors
- Influenced by Environment

Breeding on the other hand is defined as "ancestry; education; a training in or observance of proprieties". It is less dependent upon excellence of inborn traits, and excels at "training" or optimizing innate qualities to produce the best possible outcome. In my view this is what distinguishes French wine production from that of California: the French excel at the creation of exceptional products from starting material that, to put it kindly, may have been somewhat genetically and/or environmentally shortchanged by Mother Nature. In other words, they can take something that has neither distinction nor excellence of inborn traits and craft greatness.
Breeding:

- Inherent Genetic Factors
- Influenced by Environment

Is only the beginning...

My goal is to combine these two philosophies and to take fruit that is the best that a specific varietal can offer and to develop wines that meet my expectations of quality. What then is my definition of a quality wine? My definition of quality is "well-integrated harmonious complexity". My ideal wine has both breadth and depth of characters and is a symphony for the senses of smell and taste. Aroma is more important to me than taste. My late maternal Grandmother, a great lover of dogs, frequently told me that I was so in touch with my canine side that I must have spent several past lives as a dog. While I can neither confirm nor refute this, I certainly am aroma-oriented. We of course produce a lot of varietal wines in the Wine Production course on campus, but I am not convinced that my ideal will ever be attained if restricted to 75% of a single varietal in the final blend.

The variety I have elected to work with is Garnacha (Grenache). There are very practical reasons for this choice: it can be harvested late (in fact the later the better), it produces fruit of exceptional quality in my region (Region IV), it is best with little to no irrigation under our conditions which conserves water in an area where such conservation is important. These factors mean that I will have quality fruit coincident with the start of my course in late September. However, I would select Garnacha independent of these considerations. This varietal has depth of complexity in a narrow range of characters (forward red fruit and spice). It is frequently criticized for its narrowness and thought incapable of producing a "great" wine. That may be true if one means "great varietal" wine. It is an excellent tool to use to teach the meaning of "depth" because students in our culture are thoroughly familiar with the red fruit family and the characteristic spices. It also has a distinctive off or inharmonious note so it can be used to teach the meaning of harmony. In addition to cherry, raspberry, strawberry, cranberry, plum, currant, violets and cinnamon, all descriptive analyses of Garnacha mention the "tobacco" and "smoky" notes characteristic of the varietal. Individually these two "nuances" are noticeable in the Garnacha but not objectionably so. In combination they impart what I describe as the "used ash tray" character, which greatly detracts from the harmony of the wine. It is relatively easy for students to spot this note and to be sensorially disturbed by it. Although I have had students (avid smokers no doubt), that find the cigarette ashes character to be positive and claim the
inharmonious notes are the fruit and spice tones so favored by me. Either way, there is a definite consensus that something is amiss and the wine is not well integrated. Some students do not like the "forward fruit" character of wines - they complain that such notes remind them of childhood beverages, not those consumed by "adults". Perception is definitely only one aspect of wine quality; preference is the other. An additional benefit to Garnacha is that it pairs so well with some foods but so poorly with others. It is an excellent means to teach students about the influence of food on the perception of wine composition.

My ideal Garnacha would retain the depth and harmony of the existing forward fruit and spice but have more breadth or lateral complexity. We are currently experimenting with this varietal both in the vineyard and in the winery. In order to achieve the intensity of fruit that I desire the fruit has to be harvested late. Garnacha is generally recommended to be harvested at 26 to 28° Brix, and the yeast are able to complete fermentation at this sugar level. In our conditions I have found that I prefer the intensity of fruit picked at 29-30°Brix. This sometimes means that the must will attain a Brix value of 31 or higher. Aside from the difficulty in finding commercial hydrometers that read accurately in this range, this level of sugar is daunting even to the most vigorous of yeast, and the resulting high ethanol levels definitely detract from the "integration" of the wine. We are testing both pre- and post-fermentation blending strategies. The students in the course are helping me in this endeavor. Each year we create and appraise an array of different blends. To facilitate the discussion of the depth and breadth of the complexity, I developed a means of categorizing the principle flavors and aromas of wine. Our vineyard and winery operations managers and crews are all heavily involved in this project as well. I feel it is important to include everyone engaged in the production of the fruit or the wine in the creative process.
Lesson 20: The Flavor and Aroma Families

In order to better communicate what I am trying to achieve I have organized the characters of wine into flavor and aroma families. The goal was to group similar characters together. Characters in the same family are harmonious and provide depth when present together. This categorization is heavily biased by my own physiology and preferences, but I offer it as an example of how one might go about doing what I am attempting to do - attain an ideal that is not currently on the market with an existing reproducible or easily modifiable recipe.

### Wine Flavor/Aroma Families

- **Food: Plant**
- **Food: Non-Plant**
- **Non-Food**

I initially divide wine characters into one of three groups: food: plant and food: non-plant and non-food. Some of the "non-food" characters are clearly biased by culture and what I have been exposed to as a "food" versus in a non-food situation. For example, I only discovered wine vinegars as an adult so my earliest association of ethyl acetate is with nail polish and nail polish remover. Deep in my psyche this note is set as "solvent" rather than food. Since I am a microbiologist I recognize many of the microbial traits as a consequence of the metabolic activities of microbes rather than as a natural integrated flavor or aroma of the food itself. Understanding ones own biases is important in the communication process.

Listed below are the principle plant characters that I note in wines. I have separated them into the "families" that make the most sense to me from a sensory perspective, but my categorizations may not be universal.
I make a distinction between herbal and vegetal: the vegetal characters are the ones clearly associated with dietary vegetables: corn, bell pepper, etc., while herbal contains the traditional herb characters as well as onion and garlic used primarily as flavorings in foods. I also like to make a distinction between fresh and dried herbs, as the characters are quite different. The spices are cinnamon, cloves, ginger, black pepper. I include floral since many flowers are edible, but this is the one category that some students find difficult to consider as "food". I divide the fruit into different categories. Fruit tones within a category provide depth or layering and are well integrated. The traits in these categories most commonly derive from the grape, but this is not always the case. Several of the fruit notes can be microbial in origin, such as the non-berry tree fruit apple, tropical banana and pineapple. Some of the nutty characteristics are also microbial.

This approach allows me to think about what families meld well together. In the case of Garnacha, obviously the red fruit, purple fruit, floral and spice notes integrate well. In general, blends that increase the number and intensity of characters in these families score well with the students and other tasters. In contrast, blends with wines that are vegetal or grassy are more complex, but are not generally thought of as positively so. Some herbal characters meld well in the Garnacha blends, others do not. It is important to show what does not work and to discuss why the wine is objectionable.
While preference and perception are clearly strongly influencing factors, the students can learn to trust their senses and determine the source of the unpleasantness. This is what I call the olfactory equivalent of determining which instrument is out of tune in the orchestra.

**Food: Non-Plant**

- **Meat**
  - Beef
  - Salami
  - Bologna
- **Dairy**
  - Cheese
  - Cream
  - Butter
  - Butterscotch
- **Sea Food**
- **Yeasty**
- **Fungal**
  - Soy
  - Mushroom
- **Other**
  - Cotton Candy
  - Rotten Egg
  - Chocolate
  - Caramel

There are also several food characters not associated with plants per se that can be found in wine. This is not to imply that plants never make these components, on the contrary many do derive from the fruit. The classification is based upon the most common food association of the character (for the American/Californian culture). Clearly the salami and bologna notes are due to the presence of generic meat plus some of the plant characters (onion, garlic). Yeasty to me means bread-like or toasty. This term has different meanings in different wine cultures. The French have far more terms to describe mushroom and there are many subtle differences in flavors. Some of my "meat" notes may easily be considered "mushroom" by someone else. What is most important is that if more than one individual is involved in the assessment of the wines, the terms and their meanings must be clearly understood by all.
The "non-food" characters are especially important. These are the trickiest components to integrate in a harmonious way with food tones. If done incorrectly it gives the impression of spoiled or tainted food. What is considered spoiled or tainted is largely culturally dependent. A classic example of this is cassis. Cassis is a liqueur made from black currents and this character can be present in table wines. Most students in the United States identify the cassis character as cat urine, as that is what it most reminds them of. Needless to say, if the primary association is as a urine character, it is not considered to be positive or a food note. This underscores the importance of cultural experiences and food consumption habits in the definition of wine "quality".

Vegetation refers to the leafy or unripe fruit character of wines. Wood can be oak, cedar, pine or eucalyptus as all can be found in wines. The paper/cardboard note is a mold product - the taint of moldy paper. The animal notes are the wet dog, sweaty horse, pig skin characters, but I also include the animal "product" characters (fecal, urine, barnyard) in this category. The metallic note is the "canned" character. This character is usually associated with one of the food notes - canned asparagus, canned pineapple, canned clam, etc, depending upon what is the dominant "canned" trait.

I use these categories in the class to go beyond a simple discussion of what is present in the wine. The students are asked to determine what characters detract from the wine. There are no absolutes; what one student finds attractive another might not. What is most important is the ability to communicate about the wine composition and to discuss quality in meaningful and understandable terms.
This brings us to the end of the Wine Production course. Other courses in the program cover many of the same topics but in far greater depth. I hope you have enjoyed the class.

This concludes the Wine Production course. Life is too short to make or drink bad wine!
VEN 124 LAB MANUAL

An Introduction to Wine Production

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INTRODUCTION

The purpose of the VEN 124 lab course is to familiarize the student with winemaking processes and to train students to think on their feet under production situations. This lab series will include crushing and pressing of both red and white grapes, observation and analysis of the fermentation, and analysis of wine to determine the concentrations of various compounds important to wine. Since fermentations must be treated and monitored on a daily basis, you will be expected to come in, outside of the regular lab period, at least 2 to 4 hours per week. The TA’s will know how much extra time is required for each experiment; be sure to ask them each week.

The first three laboratory sessions will be conducted in the pilot winery. These lab periods will be devoted to producing the wine that will eventually be used for various analyses and fermentation experiments later in the course.

The fourth, fifth, and sixth lab periods will explore various types of fermentation techniques. Natural and malolactic fermentations and the effect of sulfur dioxide on yeast fermentation will be studied.

The next two labs involve analyzing samples of the red and white wines for glucose and fructose, and malate. Each of these procedures provides an accurate method of determining very small amounts of the substrate in question quantitatively. **In all of these assays, note on your report which sample you assayed.** This is very important for proper interpretation and collation of assay data for each lab section.

Other labs explore various blending techniques for wine, and a comparison of fining technique for badly flawed wines. There will also be a demonstration of depth filtration, bottling and options for oak aging.

As well as these specified experiments, students will design and conduct a small scale project. In consultation with the instructor, groups of two or three students will get together and decide on some aspect of winemaking that they would like to explore. An outline of the proposed experiment, detailing methods to be used and materials required, will be due on the second week of class. Oral presentations of the project results will be given during the last week of classes.
LAB REPORTS

Lab reports will be required and graded for all experiments conducted in the laboratory. The reports should be no longer than three pages (unless otherwise specified), typewritten, single-spaced and exclusive of tables, figures and legends. This will be strictly enforced (i.e., if your report is four pages long, you may be marked down for handing in an incomplete report). Therefore, reports should be brief, yet clearly written and complete. Reports should be written according to the following format:

TITLE

The title of the experiment (as given in the syllabus).

INTRODUCTION

State your hypothesis and the reason for doing the experiment. Cite other research or background information.

MATERIALS AND METHODS

This section should allow the reader to duplicate your experiment, but it should not be overly detailed. Focus on pertinent, important procedures that are not explicitly given in the lab manual. (Such as type of juice used, yeast strain, etc.).

RESULTS

Describe the data. Use tables, charts, graphs or other visual representations when they help to explain or organize the data. It is not sufficient to simply have tables and figures without any text in the results section. You need to state what the data means. In most cases, you should not show all the data, but should summarize it. Do not include judgments, conclusions, or lists of sources of error in this section. This section should include some statistical analysis of the results.

DISCUSSION

Discuss the results in reference to your experiment and your hypothesis. Be careful to keep all of your discussion pertinent to the topic. Cite literature when appropriate. You may also want to include:
• comments on experiments or statements by other researchers
• comments on your experimental design
• comparison of your results with those in literature
• new questions raised by this experiment
• possible sources of error

CONCLUSION

Give the main conclusions of this experiment.

REFERENCES

List all works cited your text.

A laboratory notebook must also be kept that details all experimental procedures, observations and data. This notebook will be collected and should be accurate and complete including all of your own data, pertinent calculations, etc.

SMALL SCALE PROJECT REPORT:

The write up of the small scale project should follow the same format as the lab reports, but will be between 20-25 pages in length (double-spaced). You will need to include a thorough review of the literature as well as explain why you were interested in undertaking the project. As with the lab reports, the RESULTS section must contain a text description of the data - not simply be a collection of charts, graphs and tables. The DISCUSSION must include an analysis of error and sources of error. The CONCLUSION should state the overall findings of the paper. If no firm conclusions are possible, that should be so stated.
DESCRIPTION OF SMALL SCALE PROJECTS

Students should plan on working in pairs, and need no more than 3 to 6 twenty liter (5 gallon) carboys. For most projects, either red or white juice may be chosen. REMEMBER: ONLY ONE VARIABLE AT A TIME, PLEASE, AND DON'T FORGET THE CONTROLS! Choose your project wisely, as some projects may involve significant outside time for juice manipulation or monitoring of fermentation. Projects may be chosen from the following list or developed by students in consultation with instructors.

Suggested Project Topics

1. Effect of skin contact time on fermentation: monitor fermentation of juice with 0, 5, and 15 hours of skin contact time. Changes in sugars, ethanol, and cell density should be followed. This would be most effective for white wines.

2. Effect of % solids on fermentation: use filtered juice at 0.5% and 2% solids and monitor fermentation.

3. Effect of temperature on fermentation: choose three temperatures (12, 15, 20, 25, 30°C) and monitor fermentation.

4. Effect of aeration:
   
   A. Compare fermentation of untreated juice to juice initially aerated (compressed air bubbled through), and to juice initially flushed with nitrogen.
   
   B. Compare fermentation of untreated juice to juices aerated during fermentation (two times daily?).

5. Comparison of different rates of fermentation of different yeast strains with respect to one other variable (temperature, juice type):
   
   A. Compare different industrial strains.
   
   B. Compare uninoculated must (natural fermentation) to inoculated must.
   
   C. Compare affects of addition of non-Saccharomyces or "wild" yeasts.

6. Effects of conditions of inoculum preparation:
   
   A. Compare effects of different inoculum sizes.
B. Compare the effect on fermentation of differently cultured inocula. Inocula can be cultured in various temperatures, pH's, carbon sources, oxygen concentrations, or nitrogen concentrations.

C. Compare canned or rehydrated inocula to inocula from an already fermenting wine.

7. Effect of juice treatment of fermentation:
   
   A. Compare fermentation of juice with no sulfur dioxide to juices with varying amounts of sulfur dioxide (25, 50, 100, 200 mg/L).
   
   B. Explore the effects of additions of yeast ghosts or other solids.

8. Effect of juice pH on fermentation: measure pH of juice, and then adjust with either tartrate, citrate, HCl or KOH to desired pH and compare fermentation rates.

9. Effect of nutrient additions:
   
   A. Compare effects of diammonium phosphate, potassium phosphate, and ammonium chloride on fermentation.
   
   B. Compare growth factor additions, such as vitamins, ergosterol, oleic acid, or Tween-80.
   
   C. Explore the effects of amino acid additions, particularly aromatic amino acids. You may want to measure hydrogen sulfide produced as a consequence of addition of MET, CYS, AND, THR, and sulfate as compared to no addition.

10. Effect of wine processing variables on wine characters:

    A. Comparison of whole berry, carbonic maceration and crushed fruit fermentations.
    
    B. Effect of extended maceration on wine quality.
    
    C. Effect of fining agents on wine components.
    
    D. Effect of filtration regimes on wine aroma and flavor.

This is by no means an exhaustive list of the possibilities available for this project. If none of these ideas suit you, feel free to design your own experiment, but you must check with the
TA’s or the instructor on the feasibility of your proposed project.

Students should get together to decide on a topic sometime during the first two lab periods. A one-page outline (format below) of the project proposal will be due the second lab period. **The proposal should include the hypothesis of the experiment, all the materials required to complete the experiment, and a brief section listing the analytical methods that will be used. It will be particularly important to list all the materials you will need, so that the TA’s can make sure they are available.**

The project will be written up according to the format for other labs with a 20-25 page limit, and will be due during the last week of the quarter. **Your small scale project will also be orally presented to the class**, according to the presentation guide in Appendix 3.

**Format for Proposal Outline:**

**NAMES:**

**PROJECT TITLE:**

**OBJECTIVE/HYPOTHESIS:**

**MATERIALS NEEDED:**
- Juice/Must (type and volume)
- Fermentation Equipment (lot size and number of fermentations):
- Yeast/Bacteria Strains:
- Reagents: (chemicals, fining agents)
- Special Needs: (I. e., controlled temperature bath; filtration, etc.)

**ANALYSES TO BE PERFORMED:**
- Fermentation monitoring: (Brix, temperature)
- Ethanol/Acids/etc.
- Microbiology: cell counts
- Sensory
INTRODUCTION TO THE PILOT WINERY

The first three laboratory sessions will be conducted in the pilot winery.

These lab sessions will involve messy work, as we will be crushing and pressing grapes. Be sure to wear old clothing that can get wet and dirty. It is imperative that caution be taken at all times when working around the winery equipment. When it is used properly, this equipment is perfectly safe, but if it is abused, it can cause serious injury. FOLLOW DIRECTIONS OF WINERY PERSONNEL AT ALL TIMES. These lab sessions are meant to be fun and informative, and there is a lot to be learned about the basics of winemaking here. A flow diagram of the processes involved in red and white (blush) production is given as Figure 1.

Clean-up is also an important part of working in the pilot winery. The winery should be left spotless after each lab session, and it is everyone's responsibility to help.

Each lab section will make, during these first weeks, one lot (500 gal) of both red and white wine. For the first two weeks, one lot of red wine will be made. The red grapes will be crushed in the first lab period, fermented on the skins for one week, and pressed during the second lab period. While the red juice is fermenting on the skins, the skins and the must have to be mixed daily by "pumping over," and samples must be taken during each pump over. These samples will be used in subsequent laboratory periods in both VEN 123 and 124 for the estimation of sugar, acid and ethanol content. After the red wine is pressed, no pump over is necessary, but samples must still be taken twice daily for a week. During the third lab period, one lot of white grapes will be crushed and pressed off the skins, settled overnight, racked, and then inoculated and fermented. Two samples of the fermenting wine will be taken daily, as with the reds. The pumping over of the red wine, and the sampling of both the red and white fermentations will require time during the week other than the scheduled lab period. Be sure to sign up for a pump over time and a sampling time on the sign-up sheet provided by the TA. If you cannot make your assigned time, it is your responsibility to find a student to take your place.

The pilot winery was constructed due to the generous contributions of a number of corporations. For more information on the equipment we will use and the various contributions to the pilot winery, see Acknowledgments.
WINE PRODUCTION

**WHITE WINES**
- Grapes
  - Crushing
  - Pressing
  - Clarification
  - Fermentation
  - Finishing
- Aging
- Blending
- Fining
- Filtration
- Malolactic Fermentation
- Bottling

**RED WINES**
- Grapes
  - Crushing
  - Fermentation
  - Pressing
  - Complete Fermentation
  - Clarification
  - Bottling
I. Crushing and Fermentation of Red Grapes

**Purpose:** Red grapes will be crushed and fermented. Proper use of winery equipment for crushing, storing, and "pumping over" red musts is demonstrated and experienced. Wine from this lab will be used for later experiments.

**Theory:** For red wines, grapes are fed into a crusher/stemmer, and then pumped directly to a fermentation tank, where they are inoculated with the fermenting yeast, *Saccharomyces cerevisiae* (2% inoculum, typically). Since the juice is not pressed off the skins at this point, the skins and seeds remain in the fermentor with the juice, and float to the surface, creating a cap. Because a thick surface cap of skins can increase the fermentation temperature and lead to the formation of undesirable products, it is periodically necessary to cool the must and break up the cap by "pumping over." In pumping over, the must is pumped out the bottom of the fermentor, and sprayed back in through the top with sufficient force to disrupt and flood the cap. This procedure is done generally twice daily, until the juice is pressed off the skins and seeds. The procedure is diagrammed in Figure 2.

In any winery, it is important to monitor the progress of a fermentation. This is accomplished by sampling each tank twice daily, following each pumping over. Brix readings, which estimate sugar concentration in g sucrose per 100 grams of liquid give an indication of how quickly and how smoothly the fermentation is going. pH and other variables are often measured also, but for this class we need only concern ourselves with Brix. Brix readings are taken with a hydrometer. Samples to be filtered and stored will also be taken at this time, and the wine variety, lab section, tank and date should be labeled on the sample with a Sharpie™ permanent marker, along with your initials. Place sample in freezer. Fermentations can also be monitored by assessing the change in weight of the fermenting vessel as CO$_2$ is evolved. The pilot winery tanks are equipped to allow this type of monitoring.

**Procedure:** A short section of hose links the exit flange of the Healdsburg crusher/stemmer to the feed pipe on the progressive cavity pump. Two long sections of hose are connected to the exit flange of the pump, and they feed into the top of one of the Mueller fermentation tanks. Once all the hoses are in place, all the valves on the equipment must be opened. This is obviously an important step in any pumping operation, but is easy to forget.

The grapes will arrive at the large service door of the winery in crates on pallets. Two or three people will work together, and groups will rotate through the following tasks:

1) Pouring the grapes from the crates into the crusher, and operating the on/off switch for the crusher.

2) Washing and restacking emptied crates on the pallets.
Figure 2: Schematic Representation of the Crushing of Grapes

Grapes are weighed, then dumped into the crusher by hand or by forklift.

Grape must is transferred via pump to the tank; stems exit crusher.

Tank fill is monitored so as not to overflow the tank.
3) Watching the feed line from the crusher to the pump to make sure it does not run dry, and operating the pump on/off switch.

4) Controlling and shoveling the stems as they exit the stemmer.

5) Handling the hose at the top of the tank to make sure there is no spillage and that must flow is continuous.

6) Weighing full boxes, and determining empty box weight and stem weight to calculate weight of grapes crushed.

7) At the end of the crush, everyone must help clean up. The winery equipment and floors must be thoroughly rinsed and cleaned.

As the tank fills, sulfur dioxide will be added to a final concentration of 50 mg/L. The yeast is added early in the pumping process, so that it is well mixed with the juice.

A sign-up sheet will be handed out for pump over times. Pumping over and sampling of each tank must be done every day until the next lab period. Everyone should sign up for at least one pump over time.
Figure 3: Crusher/Stemmer
Figure 4: Must Pump
Figure 5: Fermentation Tank Design
II. Pressing Red Must

**Purpose:** Partially fermented red must will be pressed. The operation of an automated bladder press will be explained and demonstrated. The pressed wine will be transferred to another tank to finish the fermentation.

**Theory:** The pressing of red musts, or any wine must, should be done carefully, as pressing techniques and the time of pressing will affect wine quality. The Bucher Roto Pressmatic presses the must with air pressure, and thus avoids excessive mechanical bruising of the must, high press temperatures, and excessive extraction of compounds from the skins. It is, however, a batch press, and therefore does not offer the convenience of the less gentle continuous pressing methods.

Time of pressing is important, as well: the longer a must ferments on the skins, the more extraction from the skins occurs. As tannins and phenols from the skins affect flavor, astringency, and color, it is important that pressing be properly timed for the type of wine being made. Pump overs will help speed the extraction process, and various specific chemical compounds from the skins will be extracted at different rates. The desirability of certain compounds in wine, and their threshold levels determine how long this extraction should be, and therefore, when a fermenting must should be pressed.

Wine quality is also affected by how hard a must is pressed. In any pressing operation, the wine that comes out without pressing, called the free run juice, generally produces the highest quality wine from that lot. Moderate and hard pressings produce the second and third press run lots, respectively, and these wines are usually of lower quality, and are often used for jug wines, blending, or distillation. For the purposes of this lab, these various lots will not be separated. The dry material left after pressing is called "pomace," and is composed of the left over skins and seeds.

**Procedure:** The fermentor holding the wine, skins, and seeds is drained through the racking valve. A section of hose, connected to the racking valve, leads to the press, which is filled through large doors on the side (Figure 6,7). The drain valve on the press bin is closed, to catch all of the free run juice.

As the juice is pressed, the pump and hoses are attached to the drain valve of the press bin, so the pressed wine can be pumped into the final fermentor. To press the grapes, the partially fermented must from the first lab period is drained from its fermentor into the press. When the fermentor is almost completely emptied into the press, there will be a lot of skins and seeds left in the bottom of the tank below the racking valve, as well as some juice. The juice must be carefully drained through the large port at the bottom of the tank and transferred to the press, and the skin and seeds must be shoveled into the press.
Figure 6: Schematic Diagram of the Pressing of Red Must

Juice is drained from racking valve of fermentor into the press. When all the juice has been drained, the pomace is shoveled into the press from the cleaning valve.

Juice is collected in the press bin.

When press bin is full, juice is pumped to storage tank or barrel.
Figure 7: Operation of the Bucher Air Bladder Press

Must is pumped from the tank to the press.

Air pressure expands membrane forcing juice into press pan.

Dry pomace is removed from the press.
When the press is full, it will be set for appropriate pressing times and pressures. As the press runs, the wine drains into the bin, and the pomace is left in the press. When the press finishes, the pomace is removed and thrown away, and the wine is pumped from the bin to a clean fermentor to finish fermentation.

Once again, everyone must help with clean-up, so that it will go quickly, and the winery will be ready for the next press run.
III. Crushing and Pressing White Grapes

**Purpose:** White grapes will be crushed, pressed and fermented. Previous experience with winery equipment will be reinforced, and differences in white and red wine production are presented. Wine from this lab will be used for later analyses.

**Theory:** White wine production differs from red wine production: in most cases, white wine should not ferment on the skins at all. Unlike red wines, tannins, astringency, and color are generally undesirable, and the wines must be processed quickly and delicately.

White grapes are dumped into the crusher/stemmer, and then pumped directly to the press. After pressing they are pumped to a fermentor, where they are inoculated with *Saccharomyces cerevisiae* (2% inoculum), and sulfur dioxide is added to a final concentration of 20 mg/L. (Figure 8)

In the subsequent fermentation, it is once again important to follow the fermentation with Brix readings, and to take samples of the wine for later experiments.

The major difference between this procedure and red wine production is the immediate pressing of the must; other differences are more subtle (less sulfur dioxide is added to whites than to reds, as sulfur dioxide binds with many compounds in reds, so that more is needed in reds to get similar concentrations of free sulfur dioxide). Whites are also fermented at lower temperatures than reds, as whites are very sensitive to oxidation and bruising.

White wines are held at low temperatures after pressing to allow solids to settle out, and are racked the following day.

**Procedure:** The set-up and procedure of crushing the white grapes is the same as for red grapes, except that the crushed grapes are pumped directly into the press rather than a fermentor, and inoculation and sulfur dioxide addition follow pressing of the must.

Pressing the grapes also uses the same set-up and procedures as red wines. The pressing of the white grapes needs to be done in two batches. The crusher/stemmer and progressive cavity pump will therefore be shut down while the press is operated and emptied of wine and pomace. Since the procedure for this lab is fairly long, and a lot of equipment is used, all the procedures should be clearly understood.

Once again, this lab requires a lot of clean up, and everyone is expected to help.
Figure 8: Diagram of the Crushing and Pressing of White Grapes

Must is immediately transferred from crusher to press and then to the tank following pressing.
IV. Processing Rosé or Blush Wine

**Purpose:** A batch of rosé or "blush" wine will be produced. Techniques for making rosé wine will be discussed and demonstrated.

**Theory:** Rosé wines can be produced from either varietal or non-varietal red or pink grapes. The most common non-varietal grapes used for rosé wine in California have been Grenache and Gamay Beaujolais. Both of these varieties grow well in cooler regions (I and II). Grenache has been the most popular of these two grapes, but can have a flat flavor and give a bitter aftertaste. Gamay Beaujolais gives good fruitiness to wine, but may not ripen well in cooler years. A rosé wine made from the red grape Zinfandel is the most popular varietal blush currently on the market. Pinot noir and Cabernet Sauvignon have also both been used to make a varietal rosé, but in both cases the varietal characters prized in the red wines made from these grapes are suppressed by early racking of the wine off the skins. Grenache is also now being marketed as a varietal rosé.

Rosé wines should be fruity, light, and slightly tart. Their color should be pink, although slight orange tint is allowable. Excessive orange to amber color indicates excessive oxidation. Color intensity can range from nearly red to a very pale pink blush. Classically, rosé wines have been dry, with less than 0.5% to 1.5% sugar. It has been suggested that the sugar content of rosé wines be required on the label, and that a minimum total acidity of 0.60 be required for labeling a wine "rosé" to assure proper tartness.

The most common method of producing rosé wines is fermenting red grapes, and then racking within 24 to 36 hours to minimize the amount of color and astringency extracted from the skins. Rosé wines can also be made by blending red wine with white or decolorized red wine. Because rosé wines contain limited amounts of extracted compounds, they should be treated as delicately as white wines after racking. High sulfur dioxide content can destroy the color of a rosé. Rosé wines are also highly sensitive to oxidation, and are very light sensitive. Since rosé wines are meant to be light and fruity, they do not age well over long periods of time.

**Method:** One lab period will make one lot of red wine and a "blush" wine from the same variety. For the blush, grapes will be crushed and pressed immediately, and fermented at a low temperature. A second lab section will follow the same procedure, but use a different variety.
INTRODUCTION TO FERMENTATION TECHNIQUES

The fourth through sixth lab periods will explore various types of fermentation techniques. You will observe, in these fermentation experiments:

1) How sulfur dioxide addition can affect *Saccharomyces cerevisiae* inoculum in wine must, and what a natural fermentation is, and how it affects wine quality.

2) How yeast strains other than the commercially available wine strains can affect the quality of a finished wine, particularly the aroma, or the "fermentation bouquet."

3) How to properly run a malolactic fermentation and the various effects such a fermentation can have on a finished wine.

Once again, these experiments will require time during the week other than the scheduled lab period. It will be up to you and your lab partner to decide on a satisfactory weekly agenda for the sampling or analysis of your experiment. The instructor and the TA are not responsible for the completion of your experiment, so be sure that you or your lab partner is following these experiments throughout the week.
V. Sulfur Dioxide Addition and Natural Fermentation

**Purpose:** This experiment illustrates how various sulfur dioxide concentrations in both red and white wines can affect the quality and rate of fermentation.

**Theory:** Sulfur dioxide is widely used in the wine industry to prevent microbial spoilage and browning in wines during and after fermentation. Free sulfur dioxide is the inhibitory form in solution. Because free sulfur dioxide reacts with many different substances in wines and the amount is pH dependent, exact theoretical prediction of preservative effects of sulfur dioxide on any given wine is not simple. Sulfur dioxide, when added to wine, binds with tannins, thiamin, and various oxidative enzymes, and is also depleted by evaporation. All of these effects serve to lower the amount of free sulfur dioxide in the wine. Therefore, it is important to be sure to have enough sulfur dioxide present to adequately inhibit microorganisms even after it undergoes all its various chemical reactions.

Proper additions of sulfur dioxide will not only inhibit microbial spoilage, particularly lactic acid bacteria, but will also bind tannins and certain oxidative enzymes, thereby inhibiting browning. Inhibitory levels can be maintained by addition and chemical determination of free sulfur dioxide.

However, it is also important not to have too much sulfur dioxide in wines. Sulfur dioxide has a very low threshold of detection, causing a very sharp, unpleasant odor in wines. Large additions can also inhibit the fermenting yeast, and thus cause a problematic fermentation.

Some individuals are allergic to sulfur dioxide, and thus wine must be labeled "contains sulfites." *Saccharomyces cerevisiae* will produce SO$_2$ during fermentation and thus it is present in ALL wines.

DO NOT MOUTH PIPET SO$_2$ SOLUTIONS. NOTIFY TA IF YOU SUSPECT THAT YOU MIGHT BE ALLERGIC TO SO$_2$ (ANYONE SUFFERING FROM CHRONIC ASTHMA).

**Procedure:** 10 juice lots of either 5 (white) or 10 (red) gallons each will be available for inoculation. Each lab pair will be assigned two to three fermentations to inoculate and monitor for Brix. Each lab section will be assigned one commercial yeast strain to use as inoculum (5g yeast packet/ 5 gallons). The experiment will be conducted according to the following table:
Brix readings should be taken daily during the course of active fermentation. You should note the aroma of your assigned fermentations every time you take a sample. At the end of the experiment, we will smell all of the wines and taste those that have finished.

For the purposes of this experiment, we will assume that each carboy has approximately 4.5 gallons juice or roughly 18L (1gal = 4 L). Our stock solution of sulfur dioxide is 5% (5g SO2/100mL solution or 50mg SO2/mL solution). To achieve the desired final concentrations of SO2 use the following table:

<table>
<thead>
<tr>
<th>Sulfur dioxide (ug SO2/mL Juice)</th>
<th>Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>none</td>
</tr>
<tr>
<td>0 +</td>
<td></td>
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<tr>
<td>20 none</td>
<td></td>
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<td>20 +</td>
<td></td>
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<tr>
<td>50 none</td>
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<td>50 +</td>
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<td>100 none</td>
<td></td>
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<tr>
<td>100 +</td>
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<tr>
<td>200 none</td>
<td></td>
</tr>
<tr>
<td>200 +</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Final SO2 concentration (mg SO2/L Juice)</th>
<th>Volume of 5% SO2 (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>assuming 18 L of juice</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>7.2</td>
</tr>
<tr>
<td>50</td>
<td>18</td>
</tr>
<tr>
<td>100</td>
<td>36</td>
</tr>
<tr>
<td>200</td>
<td>72</td>
</tr>
</tbody>
</table>

At the end of the experiment, that is, when those fermentations that are going to finish are finished, the wines will be cold stabilized and filtered and aroma evaluated during class. Prior to
the class discussion, you should evaluate each wine and note the main aroma characteristics and any differences that are apparent. The fermentation data for the entire class will be collected and given to each student for the lab write-up. The written report should include your tasting notes and preferences.
VI. Fermentation Bouquet

**Purpose:** Samples of white juice will be fermented in constant, controlled conditions using various yeasts. The effects of these various yeasts on "fermentation bouquet" will be judged by preliminary sensory evaluation.

**Theory:** Determining significant amounts of volatile esters for any wine is most easily accomplished by sensory analysis. [Chemical methods of ester determination do exist, but ethyl acetate is present in such large amounts compared to other esters that separation of the esters becomes necessary before each ester can be quantitatively determined, and such separation can only be accomplished by chromatographic methods (such as HPLC)]. Esters also interact with one another: certain esters can raise or lower the detection thresholds of other esters; therefore the quality of an aroma of a wine is simply not predictable.

Yeasts vary dramatically in the quantity and nature of esters they produce, and certain yeasts do produce very characteristic odors. Most well known are the off-odors associated with wine spoilage, although different yeast strains are also capable of producing widely varying amounts of desirable esters. Whether yeast ester production is useful or desirable is dependent upon the style and type of wine being produced, and the persistence of the compounds during aging.

**Procedure:** Cultures of a number of different yeasts in grape juice media will be provided. Students will smell each culture, and write a brief description of the various aromas. You will be assigned a yeast strain to use as inocula, but you will smell each fermentation during the course of the experiment. An uninoculated control will also be available. The aroma of this sample should be monitored each time the inoculated samples are examined.

Juice samples in one gallon lots will be provided. Each group should record descriptions of the variety and initial aroma of the juice. Pour off 1/4 gallon and inoculate the remaining juice with your assigned yeast culture.

The samples will ferment over the next two weeks. All the samples must be examined for growth and aroma development every weekday by each group. Aroma should be evaluated by pouring a small sample of each wine into a wine glass (aromas cannot be properly evaluated directly out of a fermentor). The TA’s will assign students to pour the wines into the glasses at the start of each day, and to rinse them out at the end of each day. Your observations will be written up as a formal lab report.
VII. Malolactic Fermentation

**Purpose:** The goal of this experiment is to evaluate the effect of the timing of addition of an ML inoculum on the progression of the ML and yeast fermentation. Juice or wine at various stages of fermentation will be inoculated with an assigned culture of a "malolactic" bacteria. Fermentation progression will be monitored weekly.

**Theory:** For many wine types there is a second fermentation that occurs in addition to the alcoholic fermentation conducted by yeast. This is the conversion of malate to lactate by "malolactic" bacteria (mostly genera *Oenococcus*, *Lactobacillus* and some *Pediococcus*). Some winemakers view this fermentation as essential for "complexity," while others consider it a nuisance that must be prevented from occurring in the bottle.

Deacidification of wines is the main aspect of malolactic fermentations. Malate, a dicarboxylic acid, is converted to lactate, a monocarboxylic acid. There are, however, a number of other compounds formed during this fermentation. Most of these compounds will add complexity to a wine, as long as they are not present in large amounts. It is difficult, however, to predict what amount of these various compounds will be formed, and it is this unpredictability of the malolactic fermentation that winemakers generally want to avoid.

Just as in alcoholic fermentations, the malolactic fermentation is started with an inoculation of the bacterium of choice so that the fermentation runs smoothly. The malolactic fermentation can be run before or simultaneously with the yeast fermentation, or it can be conducted afterwards. The malolactic fermentation itself is enhanced in wines that are not filtered or drained off their lees, not refrigerated, and not given acidity adjustments. It is likewise discouraged in wines that are stored cold, filtered, fined, adjusted for pH or sulfur dioxide, or are otherwise protected from microbial spoilage. Proper sterile filtration before bottling generally assures that no malolactic fermentation will occur in the bottle.

There are two common detection methods for malolactic fermentations. One is a chromatographic method, which is qualitative and only capable of indicating the end of a fermentation, and which will be demonstrated in this lab. The other commonly used method is enzymatic and will be demonstrated in another course. The enzymatic method is quantitative.

**Procedure:** Four five-gallon lots of juice will be inoculated with 100 mL of malolactic inoculum, as follows:

1. Malolactic bacteria added to juice, no yeast addition.
2. Bacterial inoculation simultaneous with start of yeast fermentation.
4. Bacteria added after yeast fermentation is complete, at ~0 Brix.
For each of the previous four fermentation samples, an uninoculated (no bacteria) control will be run, for a total of eight samples/lab section. The progression of the fermentation will be monitored by paper chromatography. Samples of all eight fermentations will be taken twice a week by a group of student under supervision of the TA. The students will spot and run the chromatogram according to the Kunkee procedure as instructed in class. After the chromatograms are dry, they will be pinned to the board in the lab. EACH STUDENT SHOULD CHECK THE BOARD EACH WEEK AND RECORD THE PROGRESS OF THE FERMENTATIONS. When the fermentations are completed, the wines will be smelled and tasted in the lab and the observations of the changes discussed.
Paper Chromatography for Monitoring Malolactic Fermentations
(note: Chromatography reagent will stain your hands and clothes. Use gloves and a lab coat to avoid stains. Always use splash goggles when working with chemicals)

1 Preparation of the chromatography solvent:
   - 100 ml n-butanol (reagent grade)
   - 100 ml water
   - 10.7 ml formic acid (reagent grade)
   - 15 ml indicator solution (1 g bromocresol green in 100 ml water)
   - Mix chemicals and place in separatory funnel. Make sure stopper is securely closed. Mixture will separate into two layers; discard lower (aqueous) layer in proper waste container labeled “aqueous phase chromatography waste”
   - Store freshly made reagent in container labeled “new or rejuvenated chromatography reagent”.

2 Setting up chromatography paper (Figure 1):
   - Clean bench top; place paper towels down on surface
   - Take a sheet of chromatography paper and lay on towels
   - Mark a pencil line across the bottom of the page, about 1 inch from the bottom
   - Make tic marks at 2 cm intervals along the pencil line
   - Label tic marks with standard or sample code using pencil
   - Label sheet at top with initials, sample date and other information
   - Only use pencil for labeling.

(note: Sweat contains lactic acid. Handle only the edges of the paper with your fingers. You may want to wear gloves to avoid lactic acid contamination of your test.)

3 Spotting of chromatography paper:
   - Using small (1.1-1.2 mm I.D.; 75 mm length) glass capillary tubes, one for each sample or standard
   - Draw liquid from sample in to the capillary and touch capillary to paper
   - Allow no more than a spot of 1 cm to form on paper
   - Allow spot to dry
   - Repeat spotting 4-5 times

4 Running the Chromatogram
   - After all spots are completely dry, curve paper into a cylinder
   - Staple ends to hold cylinder together – do not overlap ends of paper
   - Transfer 70 ml of new or rejuvenated solvent to jar (large mayonnaise type)
   - Carefully insert cylinder (Figure 2) spot side down
   - Close lid carefully
5 Development of the chromatogram:
- After approximately 4 to 6 hours at room temperature the solvent will have ascended the chromatography paper carrying the spots with it.
- Carefully remove chromatogram careful not to touch the wet portion
- Carefully tear away edges from the staples
- Hang chromatogram in a well-ventilated area (chemical hood) to dry
- Yellow spots on a blue-green background should be visible and indicate the position of the acids
- All chromatograms should have standards (solution of individual acids) spotted to identify position of the acids (Figure 3).

6 Cleaning up:
- Carefully pour chromatography reagent into storage container marked “used chromatography solvent” (Chromatography reagent may be reused, but must be separated in a separatory funnel and occasionally reacidified before each use.)
- Take the empty mayonnaise jar to the sink and rinse it thoroughly with warm water.
- Place the cleaned jar upside down on clean paper towels for the next person to use.

Figure 1: Paper Chromatography Set up

<table>
<thead>
<tr>
<th>T</th>
<th>M</th>
<th>L</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
</table>

T = tartrate; M = malate; L = lactate
Figure 2: Paper Chromatography Jar Set Up
Figure 3: Paper Chromatography: Spot Location

Interpretation: Samples 2, 4, and 5 have completed the malolactic conversion; there is no detectable malate spot and the presence of lactic spots. Sample 1 has not started the malolactic conversion. Sample 3 may be midway through the fermentation or the lactate may have derived from a different source.
INTRODUCTION TO FERMENTATION MONITORING

In addition to off-flavors and aromas, another important type of problem fermentation is a sluggish or stuck fermentation. Sluggish or stuck fermentations are those which proceed very slowly or fail to go to dryness and are at high risk for microbial contamination and spoilage due to the presence of a high concentration of residual sugar. It can be very difficult to re-start a stuck fermentation. There appears to be a direct correlation between the length of time a fermentation has been stuck and the ease at which it can be re-initiated. That is, the longer a fermentation is arrested, the more difficult it is to treat. For this reason it is important to monitor the progress of the yeast or malolactic bacteria in order to quickly identify a problem situation.

The progress of any fermentation can be monitored in any one of several ways. The rate of disappearance of a substrate can be followed. Likewise the rate of appearance of a product can also be evaluated. In the case of the alcoholic fermentation, either loss of sugar or the appearance of carbon dioxide or ethanol can be used to determine the rate and progression of the fermentation. Similarly, in the case of the malolactic fermentation, in theory either the disappearance of malate or appearance of lactic acid could be monitored. However, when choosing which variable to follow, it is very important that that variable be highly correlated with the process being measured. For example, lactic acid can be derived from a variety of cellular processes, not just from the decarboxylation of malate. In fact, lactic acid is an end product of the fermentation of glucose by the lactic acid bacteria. Thus, there are several sources of lactic acid in a fermentation only one of which is malate. Therefore, lactic acid levels are not well-correlated with the conversion of malate to lactate. As a consequence, disappearance of malate is the only reliable means to assess the progress of the malolactic fermentation.

In contrast, both ethanol and carbon dioxide are well-correlated with glucose and fructose metabolism during the alcoholic or yeast fermentation. While other cellular reactions might lead to the production of carbon dioxide, the amount produced is minuscule when compared to the amount produced during fermentation of sugars. Thus, either loss of sugar or appearance of the end-products of fermentation can be used to monitor the progression of the yeast fermentation. When either disappearance of substrate or appearance of product correlate equally well with the process being investigated, the method of choice generally becomes that which is easiest to do. Since grape juice and must is very high in sugar, loss of sugar can be followed by monitoring changes in the specific gravity of the fermenting medium. The Brix scale allows an estimate of changed in specific gravity of a solution. While this does not allow a ready calculation of actual sugar content in the must, it does allow the rate and progression of a fermentation to be easily assessed.

In the first experiment, sugar consumption during fermentation of both red and white must will be monitored using a Brix hydrometer. In the next experiment, the actual level of glucose will
be measured using a glucose analyzer. Finally, in the last experiment the progression of the malolactic fermentation will be monitored by following disappearance of malic acid.
VIII. Fermentation Sampling: The BRIX Scale

**Purpose:** This experiment will familiarize the student with use of the Brix hydrometer as a means to monitor progression and rate of juice and must fermentations.

**Theory:** It is important to any winery to regularly monitor their fermenting wines, so that a fermentation can be properly manipulated and controlled. Hydrometers are almost universally used to measure approximate amounts of fermentable sugars in a juice or wine. pH and titratable acidity are also commonly measured, and can provide valuable information in the production of fine wines. For the purposes of this course, however, hydrometer readings will give sufficient information about the progress of the fermentation.

A hydrometer works on the principle that any solute added to a given solution will cause a proportional change in the solution's density. The hydrometer is carefully balanced so that at 20°C it will give a **fairly good approximation** of fermentable sugar in grams sugar to 100 grams of liquid. However, since the hydrometer measures density, the readings will be affected by temperature, alcohol, and other solutes in wine. In fermenting wines, considerable error can be caused by carbon dioxide bubbles adhering to the hydrometer, if the analyst is not careful. Generally, however, these problems cause only small errors, and the final Brix reading can be obtained using the appropriate correction factors. Hydrometer readings are a useful indicator of remaining sugars and, therefore, of the progression of a fermentation.

Although hydrometer readings are the only quantitative analyses performed on our wines, it is just as important to smell and taste a sample of the wine. Sensory evaluation remains as the quickest way to discover many fermentation problems, especially hydrogen sulfide formation.

**Procedure:** Each student will be responsible for signing up for one or two sampling dates for both the red and white wines. The reds can be sampled during the pump-overs. The sample will be transferred from the winery to the lab room in a large pitcher. You will also need a hydrometer tray, a thermometer, and a hydrometer cylinder. Now you are ready to take a hydrometer reading. The wine should be poured into the hydrometer cylinder (strained if skins, seeds, or berries or present) until the flask very slightly overflows into the rim catch. (DO THIS OPERATION IN THE SINK, PLEASE.) The temperature of the wine should be taken, and if the wine is excessively warm (greater than 30°C), it should be allowed to cool. Once it has cooled sufficiently, the temperature should again be taken and recorded on the fermentation card (Figure 9).

After the temperature is recorded, a hydrometer must be selected according to the expected sugar content of the wine sample. Different hydrometers are capable of measuring different ranges of Brix, usually spanning about 10 Brix over the range from +30 to -5 Brix. The hydrometer tray holds all the hydrometers necessary to measure within this range. An approximate Brix reading can be estimated from the previous reading.
Figure 9: Hydrometer and Hydrometer Cylinder
The selected hydrometer is immersed in the full flask, and allowed to come to rest. The meniscus of the sample in the flask should be curved up to facilitate reading the hydrometer. The hydrometer is spun in the flask to loosen any bubbles which may be buoying it up. The hydrometer reading is taken immediately after the hydrometer comes to rest, and recorded on the fermentation card. Try to take the reading from the bottom of the meniscus, if possible.

If the wine was not exactly 20°C, then a correction of the hydrometer reading for temperature effects will be necessary. This correction can be found on a table which is posted in the lab room.

In addition to hydrometer readings and temperatures, each sample will be smelled and tasted, and the comments recorded on the card (Sample card is attached - Figure 10).

Please note:
- The hydrometers must never be taken into the pilot winery - the wine sample must always be brought to the laboratory.
- All materials that come in contact with the fermenting juice be thoroughly rinsed, including the sink.
- Keeping the lab clean will help alleviate an ongoing fruit fly problem. It is imperative that any spills be cleaned up immediately.
- Each tray contains sets of four hydrometers and one thermometer. Please keep these sets together.
- If you break a hydrometer (or any other lab glassware), notify a TA. Please use the broom and dust pan to place the broken glass in the LABELED BROKEN GLASS CONTAINER. Record on the sheet on the bulletin board exactly what was broken and how it got broken so we can replace it and prevent future breakage.
### Figure 10: Sample of Cellar Records Kept

<table>
<thead>
<tr>
<th>Cellar No.</th>
<th>Wine Type:</th>
<th>Variety:</th>
<th>Produced for:</th>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Vineyard:</th>
<th>Condition:</th>
<th>LBS.</th>
<th>Date Picked:</th>
<th>Date Crushed:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Must Analysis: Brix</th>
<th>TA</th>
<th>pH</th>
<th>Gallons</th>
<th>SO₂ ppm:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Fermentation Record</th>
<th>Yeast Strain:</th>
<th>Date of Inoculation:</th>
<th>Date Pressed:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Chemical Analysis</th>
<th>Date:</th>
<th>TA</th>
<th>pH</th>
<th>EtOH</th>
<th>Color</th>
<th>VA</th>
<th>MLF</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Date:</th>
<th>Comments:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Cellar No.:</th>
<th>Varietal:</th>
<th>Produced for:</th>
</tr>
</thead>
</table>

**CELLAR OPERATIONS**

<table>
<thead>
<tr>
<th>Date:</th>
<th>Initials:</th>
<th>Operation:</th>
<th>Gallons:</th>
<th>Location:</th>
<th>Comments:</th>
</tr>
</thead>
</table>

| Date: | | | | | |
|-------| | | | | |
VIII. Measurement of Glucose using a YSI Industrial Analyzer

The YSI glucose analyzer relies on an immobilized enzyme, glucose oxidase and a platinum electrode measuring hydrogen peroxide amperometrically (electronically).

\[
\text{Glucose} + \text{O}_2 \rightarrow \text{gluconic acid} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 \rightarrow 2\text{H}^+ + \text{O}_2 + 2e^-
\]

Current flow in the platinum anode is proportional to the concentration of \( \text{H}_2\text{O}_2 \), therefore proportional to the glucose concentration. The YSI Analyzer gives a direct readout of glucose concentration in mg/100 mL or mg/L. We will use the mg/L scale. The machine is accurate over the range of 0 to 8000 mg/L (0 to 800 mg/100 mL), or from dry to approximately 0.8 Brix. Keep in mind that the Brix scale is in a wt/wt percentage, not a wt/volume percentage, so the conversion of Brix results to units of wt per volume is not simple, and that the density reading measures all sugars (glucose and fructose). If your wine or juice sample is greater than 0.8 Brix, it will have to be diluted with water so that it is in the linear range of the machine. If it is over the linear range, you will get an error message telling you that the concentration is too high. You will need at least 3 mL of your sample to read on the analyzer.

To calculate your dilution, use this estimation converting Brix wt/wt to wt/vol and assume that the glucose is half of the sugar measured:

\[
[\text{glucose}] = \frac{^\circ\text{B} \times 1000}{2} = \text{mg/100 mL}
\]

For example, you have a sample at 16 Brix, only approximately 50% of the sugar is glucose, the rest being fructose, then:

\[
[\text{glucose}] = \frac{16 \text{ Brix} \times 1000}{2} = 8000 \text{ mg/100 mL} = 8 \text{ g/100 mL} = 80 \text{ g/L}.
\]

You want to be below 8 g/L, so aim for the midpoint of 4 g/L (or 400 mg/100 mL). For a solution of 4 g/L (the midpoint of the range of sensitivity) you need to make a dilution.

\[
\text{Dilution factor} = \frac{(\text{glucose}) \text{ g/L}}{4 \text{ g/L}} = \frac{80 \text{ g/L}}{4 \text{ g/L}} = 20
\]
You would need to dilute your wine 20 fold (1 part of wine to 19 parts of water). It is a good idea to always check your dilution calculation. For a 16 Brix wine, of which roughly 8 Brix is glucose, a 1/20 dilution would give 0.4 glucose Brix ($8/20 = 0.4$). This is in the linear range of Brix for the instrument (0 to 0.8 Brix).

To calculate the glucose concentration of your wine sample:

$$\text{YSI reading} \times \text{dilution factor} = \text{[glucose] wine}$$

REMEMBER the YSI reading is in mg/L. You should convert this reading to approximate Brix (for our purposes, g/100mL, not g/100g) then double the Brix to account for the fructose and see how close you are to the original Brix reading of the sample. Record your results on the table at the front of the room. The table will ask for original reading from the YSI, your dilution factor and final Brix (YSI reading in g/100mL times 2).
IX. Enzymatic Determination of Malate

**Purpose:** Malate levels will be determined for the samples from the malolactic fermentations in Lab 7. Graphs of the resulting malate concentrations will indicate the progress of each of the fermentations.

**Theory:** Malate levels can be determined easily utilizing the malate dehydrogenase reaction to produce oxaloacetate (OAA) and NADH. Since this reaction is reversible, glutamate-OAA transaminase is added to deplete the OAA and drive the malate dehydrogenase reaction in the direction of OAA. The amount of NADH formed is measured spectrophotometrically, and is directly correlated to the amount of malate consumed. The reaction proceeds as follows:

\[
\text{Malate} + \text{malate dehydrogenase} \rightarrow \text{Oxaloacetate} + \text{NADH} \\
\text{NAD}^+ \rightarrow \text{Glutamate-OAA transaminase} \rightarrow \text{Glutamate} \rightarrow \text{Aspartate} + \alpha\text{-Ketoglutarate}
\]

**Procedure:** Samples taken during the malolactic fermentation will be assayed. The buffer for the assay will be prepared by the TA:

**Malate Buffer:** Glycylglycine - NaOH (pH 10.0)  
L-glutamate  
NAD  
L-malate Dehydrogenase (L-MDH)  
Glutamate Oxaloacetate Transaminase (GOT)

Each pair of students will do one blank (no wine sample added), and some samples from the malolactic fermentation. All samples will be diluted 1/100 with water, and the buffer should be added last. Samples should be prepared as shown on Table 2.
Table 2

Dilution Scheme for Test Samples

<table>
<thead>
<tr>
<th>Tube</th>
<th>Buffer</th>
<th>Diluted Sample</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>1250 µL</td>
<td>-</td>
<td>1000 µL</td>
</tr>
<tr>
<td>Sample</td>
<td>1250 µL</td>
<td>100 µL</td>
<td>900 µL</td>
</tr>
</tbody>
</table>

Add buffer, diluted sample and/or water to cuvettes, mix by covering with parafilm and gently inverting 2-3 times, and incubate them at room temperature for 10 minutes. When 10 minutes has passed, absorbance readings are taken. The blank is used to zero the spectrophotometer, and the sample is read immediately following zeroing by the blank. All absorbencies should be taken at 340 nm.

The malate concentrations of the samples can be calculated using the general equation:

\[ C = \frac{V \times MW \times A}{e \times d \times v \times 1000} \]

Where:

- \( V \) = test sample volume = 2.25 mL
- \( v \) = wine sample volume = 0.1 mL
- \( A \) = measured change in absorbance
- \( e \) = absorption coefficient = 6.3 cm\(^3\)/ umole
- \( d \) = pathlength = 1 cm
- \( MW \) = molecular weight of malate = 134.09 g/mol
- \( 1000 \) = not your dilution factor (converts units of \( e \))

Therefore,

\[ C = \frac{2.25 \times 134.09 \times A}{6.3 \times 1 \times v \times 1000} \]

\[ = \frac{A (0.048)}{v} \]

for malate in g/L of diluted sample.

Remember to calculate final concentration using your dilution factor. Record your data and calculations, and be sure to include which sample you analyzed (sample date, Brix, and lot number).
INTRODUCTION TO WINE PROCESSING

The final set of laboratory experiments will explore the effect of various wine processing techniques on the flavor and aroma profile of wine. Some choices in downstream processing are simply stylistic allowing the winemaker to emphasize certain characteristics or minimize others. At times downstream processing steps are dictated by the need to remove an unwanted off-character generally regarded as objectionable by the consumer. Examples of this type of character are hydrogen sulfide (rotten egg), acetic acid (vinegar) and ethyl acetate (nail polish). It may also be necessary to remove characters that, while not objectionable in their own right, are objectionable in a given style of wine. For example, to use a food analogy, sugar is expected and desired in ice cream and cake but would be out of place if poured on a steak.

Several post-fermentation treatments can be employed to favorably impact the flavor and aroma characteristics of the wine. In the next few experiments we will examine the effect of fining of a wine, learn about wine filtration, and assess the impact of oak treatment of a wine. The impact of oxygen exposure during wine aging will be evaluated. Finally, the principles of blending will be demonstrated.
X. Wine Fining

**Purpose:** The purpose of this experiment is to observe the changes in wine brought about by the practice of fining.

**Theory:** It is a common practice to treat wine with various agents to improve the characters of the wine or to enhance consumer appeal and acceptance. The process of "fining" refers to the deliberate addition of an adsorptive agent followed by natural settling of the agent or removal by filtration. Fining is used to remove substances like proteins that can later cause a visual defect of a haze in wine. Fining can also be employed to remove excess tannins or polymeric phenolic compounds as well as monomeric phenolic compounds that might impart a harsh or bitter taste. Fining agents are also used to eliminate off-odors and flavors and to "soften" a wine. Finally, fining agents can operate as a molecular sieve, removing suspended colloidal or particulate matter as the agent settles in the tank.

Fining agents have many important uses in wine making, but, if used indiscriminately can actually result in a less desirable product. In this first experiment, we will compare the effect of indiscriminate use of fining on a wine. In the next experiment we will evaluate different fining agents and their effects on a defective wine.

**Procedure:** Bentonite is a clay used to achieve protein stability of wine. Bentonite has a net negative charge. At the low pH of wine, most proteins have a net positive charge. Therefore, wine proteins will displace positively charged ions on the surface of the bentonite and be removed from the wine. Bentonite expands in wine to yield a very high surface area. Bentonite is not specific for protein, and functions as a general ion exchanger in wine. It is difficult to predict how much bentonite will be needed to achieve protein stability of wine. Too high of a bentonite concentration may have deleterious effects on the wine.

In this experiment you will be given four lots of bentonite-fined wine to taste. One lot will have received no bentonite addition, and the remaining three will have received one, three and five pounds/gallon of bentonite. You will taste each wine and record your observations. You should also check the wine visually for the presence of haze or cloudiness.
XI. Elimination of Off-Flavors and Odors

**Purpose:** A wine will be treated with different fining agents to demonstrate removal of wine components. Adsorption and its use in treating wine defects will be discussed.

**Theory:** Adsorption is used in fining wines to eliminate excesses of any compound that would otherwise cause problematic fermentations, off-odors, or off-flavors. There are many types of adsorbents available that specifically bind the various important constituents in wine. The most commonly used commercial adsorbents are proteins, earths, or synthetic polymers. These and other adsorbents are added to the wine in a powder form, and are then removed by filtration or racking. Activated carbon is not a commonly used adsorbent. It is very non-specific in its binding, and is used to eliminate pigments and a wide range of phenolics. However, if a wine has a very obvious defect that defies other fining treatments, activated carbon can be used to almost always remove the problem.

The first step of any fining technique is determining how much should be used to clean the wine without over fining it. A small adsorption column is set up and packed with the desired fining agent. A known volume of the problem wine is passed through the column in small measured portions. The first few portions to pass through the column will be heavily fined, and will not display the defect of the original wine. Eventually, as the column adsorbs more material, the defect will begin to appear in later portions. The volume of treated wine at which the defect again becomes obvious is the amount of wine which a given amount of fining agent can treat. From this volume, kilograms of fining agent per gallons of wine can be calculated.

To convert this result to a commercially useful figure, the number derived from the above procedure must be increased approximately twenty percent, as the adsorbent cone used for the test procedure is more efficient than a batch operation.

**Procedure:** The adsorbent columns will be prepared by the TA using cut 25 mL glass disposable pipettes. A small wad of glass wool is tamped into the bottom of the column. Four fining agents will be compared: bentonite (a montmorillonite clay), PVPP (polyvinyl polypyrrolidin), celite 545 (acid washed), and a mixture of activated charcoal and celite, 1 g charcoal to 6 g celite. Each pair of students will be provided with one of four compounds prepacked in columns.

The packed column is then set in a stopper in a collection flask, which is connected to an aspirator. The wine to be fined can then be added to the column in 5 mL portions. The aspirator should then be turned on, and the flow rate of the column adjusted so that a portion takes at least 6 minutes to pass through the column. After each portion is fined, it should be poured into a test tube and labeled. When 50 mL of wine have passed through the column, each portion is smelled and tasted for off-odor or flavor. Depending upon the wine, you may also be asked to monitor changes in absorbance at a given wavelength. Be sure to follow the directions
of the TA. The last portion of wine that does not exhibit the defect represents the amount of wine that the column is capable of fining. From this, the grams of compound required to fine one liter of wine can be calculated, and then increased to calculate the fining agent addition for a batch process:

\[
0.20 \times \left( \frac{1 \text{ g Compound}}{X \text{ mL Wine}} \right) \times 1000 = Y \text{ g/L}
\]

Where: 
- \( X \) = volume of wine fined
- \( Y \) = g compound/L of wine for batch treatment

Compare your results to those of other groups using different fining agents. What characteristics were affected by each of the fining agents? Record all observations in your notebook.

References:


Many thanks to Dr. Singleton for help in the design of this experiment.
XII. Wine Blending

**Purpose:** In this experiment different base wines will be blended and the aroma and flavor characteristics of the wines monitored.

**Theory:** The most common use of blending in commercial wineries is to produce a wine with consistent flavor and texture from one year to the next. Blending is, however, also useful for masking deficiencies or excesses in wines, or for freshening older wines with younger, fruitier wines. In general, blending is used to give complexity to wine, and can often add special nuances.

In order to deduce a formula for a blend, a winemaker must experiment with different proportions of his base wines until he finds a blend he or she likes. This often takes extensive tasting, and tires the palate. Therefore, the recipe for this blend is recorded, and reevaluated at a later date to assure the quality of the blend. If this formula is still satisfactory, a small trial lot (10 gal) of the blend is made, to assure that the wine will blend satisfactorily in large scale production. If time allows, this blend will also be given three weeks to six months to "marry": the flavors of the base wines will blend and mellow during this time. Classically, the winemaker has relied on personal taste to guide him in blending his wines. Certain wine parameters such as percent alcohol, titratable acidity or residual sugar that a winemaker may wish to adjust by blending can be handled in a mathematical fashion once the concentrations of these components have been determined in the starting wines (see Appendix 5). Computer programs have been developed to solve simultaneously for blending of more than one constituent at a time. These predictive methods are useful as aids in determining a blend formula, but they cannot completely predict the quality of a blend. The concentrations of many other components of wine, where detection is dependent upon a taster's physiological threshold for that particular compound or compounds do not respond to dilution according to a strict mathematical blend. Characteristics may develop in a blend that were not originally detectable in either of the base wines. Therefore, it is important to do careful sensory evaluations of prospective blends.

**Procedure:** Each pair of students will be provided with samples (approximately 400 mL) of three white and three red wines. Record the aroma characteristics of each of the wines provided. Each pair will mix different ratios of the base wines and make one bottle of their favorite blend. The blends will be collected and allowed to age (marry) for a few weeks. All blends will then be given to the class to evaluate in a blind tasting. Each lab section will choose 1 or 2 most popular blends. The percent composition of the blend will then be revealed to the class. You should record your observations of your own blend. Did any characteristics change during the period of "marrying"? In your report, be sure to include a discussion of why you chose the particular blend that you did.
XIII. Wine Aging

**Purpose:** Proper aging of a wine is a critical component of its production. Determination of the best length of time for barrel or bottle aging for a given wine is an important task for the winemaker. While no "quick-aging" method has been developed that will exactly duplicate conditions found in a standard barrel, many winemakers do attempt to assess the aging properties of a red wine particularly to identify lots of wine that will not hold up to limited oxygen exposure. This experiment could also be titled “the effects of oxygen exposure on wine”. You should be looking for several things: the appearance of any microbial spoilage (both by nose and by formation of a film or turbidity); the appearance of “oxidized characters” like acetaldehyde; the impact of loss of volatile compounds on the wine (as the most volatile and labile characters disappear, is what is left still desirable?). Be sure to smell all of the wines that are available in the room, not just your own.

**Procedure:** A simple technique common in many wineries to obtain a quick estimation of the changes a wine may undergo during aging is to pour a sample of the wine into a wine glass, cover the glass with a watch glass, and leave the glass at room temperature for a period of approximately three to six weeks, evaluating the aroma characteristics of the wine every one to two weeks. Each pair of students will be given 2 to 4 red wines, wine glasses and wine glass covers. At each lab period for five weeks the wines should be smelled and the aroma characteristics recorded. At the end of the five weeks, samples of the original wines will be provided and used for comparison to the exposed samples. An experienced winemaker can use this technique to quite accurately assess his or her wines for the appearance or loss of certain characteristics. This simple method can tell you if the wine is microbially stable as well as allow estimation of the way a wine will age chemically. Be sure to note the appearance of microbial characteristics indicative of spoilage: acetic acid and strong ethyl acetate. Also note any color changes: loss of red /purple pigment, appearance of brown, in addition to flavor changes. Again, record all observations in your notebook.
XIV. Effects of Oak Exposure

**Purpose:** In this experiment, the impact of exposure to oak on the flavor and aroma profile of a wine will be evaluated.

**Theory:** When a wine is aged in young oak barrels, some of the characters of the oak are extracted. These compounds impart both flavors and aromas to the wine. There are three fundamentally different means to achieve oak exposure: traditional aging in an oak barrel or cask, introduction of wooden staves into wine in vessel not made of wood, and introduction of oak chips into a wine.

In each case the amount of extraction is a function of surface to volume ratio, the age of the wood, the processing of the wood, the number of times the wood has been used, and the original source of the wood.

**Procedure:** In this experiment, students will be provided with four samples of wine: wine untreated with oak, wine receiving oak chips, wine stored in oak barrels and wine stored in stainless steel with the addition of oak staves. The wines will be tasted and the aroma and flavor characters noted.
XV. Quality Control: Estimation of "Corkiness" in Cork Lots

**Purpose:** Off-aromas and flavors arising from poor quality corks is a rising problem in the California wine industry. Most wineries have developed tests to assess the suitability of a given lot of corks. Visual inspection is often insufficient for detection of a "bad batch" of corks. In this experiment we will use a simple technique of submerging a number of corks from a “lot” of corks in a white wine base, followed by assessment of any characters (changes in color, aroma or flavor) imported to the wine by the corks.

**Procedure:** The day before the start of the lab period, volunteers from each lab section will place one cork in each jar provided and fill each jar with the same base white wine. The jars will be left at room temperature overnight.

During the lab period, the wine in each jar will be poured into a wine glass and covered with a watch glass. Each pair of students will smell all the samples and a control sample of the base wine treated similarly but without cork addition. Record observations of color and aroma. A "corkiness" standard will also be provided. Determine the fraction of corks yielding an off-characteristic. Describe the nature of the defects. Record all observations in your laboratory notebook.

Some wineries use a variation of this technique where they place more than one cork in the same sample of wine. Why is this a mistake (or is it)?
XVI. Bottling of Wine

**Purpose:** The last step in production of wine is bottling. Bottling of wine is a critical step in the production process. The act of bottling may expose the wine to oxidation, elevated temperature or microbial spoilage. Bottling lines are difficult to clean if allowed to become contaminated with spoilage organisms, thus it is important to pay strict attention to sanitation practices. Wine that is not microbially stable is at particular risk for bottling-associated problems.

**Procedure:** Most small to moderate size wineries have not invested in their own bottling lines, but instead “rent” mobile bottling services. We will have a mobile bottling line set up in the parking lot to bottle the wine made during class. This will be part demonstration and part hands on work for the students. For the written report, you will be required to outline the overall steps in setting up the bottling line, and the potential problem areas for contamination or damage to the wine.
APPENDIX 1

Dilution calculations

During this class and throughout the rest of your career as a winemaker, you will frequently be required to calculate the amount of a stock solution needed to achieve a desired final concentration of that compound in wine or juice. You will also need to understand how to make a dilution and how to read a protocol that requires a dilution to be made.

There are two common conventions used to specify a dilution. For example, a ten-fold dilution can be represented as either: 1:9 or 1/10.

"::" means to, that is: one part of stock solution to nine parts of water or buffer

"/" means in, that is: one part of stock solution in a total of ten parts

Both of these designations ultimately mean the same thing: the stock solution is now ten-fold less concentrated. Use of "::" is the convention in chemistry as it readily tells you how to make the solution: mix one part and nine parts. Use of "/" is the convention in microbiology as it readily gives you the dilution made, in this case ten fold. That is, if you want to know the final concentration of the compound, you divide the original concentration by 10 (NOT BY 9!). You must consider the total volume of the solution in calculating the concentration, not just the volume of diluent that you added. For example, if 1 mL of a 1mg/mL solution is mixed with 9 mL of water, that 1mg is now in a total volume of 10 mL. Thus the concentration of the solution is 1mg/10mL or 0.1mg/mL. You will see both of these conventions used in enology.

Example:
You have a 5% (5g/100mL) solution of sulfur dioxide (SO₂). You want a final concentration of 25 ppm (25μg/mL or 25mg/L) in a 5000 gallon (approximately 20,000L) tank. How much of the 5% solution do you have to add to the tank?

First, always remember to convert everything to the same units. It does not matter which units you choose, g/mL or mg/L, just as long as they are the same for all of the solutions.

If you choose to work in mg/L:
What you HAVE: 5 % SO₂:
5g/100mL = 50g/1000mL (multiply both top and bottom by 10 to determine concentration per L)
= 50x10⁻³mg/L (convert g to mg to work in the chosen units)

WANT: 25mg/L
To calculate the dilution needed, simply divide "have" by "want":
\[50 \times 10^3 / 25 = 2000\]

The stock solution needs to be diluted 2,000 fold or 1/2000.

If you choose to work in g/mL:
What you HAVE: 5% SO2:
\[5g/100mL = 0.05g/mL\]
Want: 25mg/L = 0.025g/1000mL or \(2.5 \times 10^{-5}\) g/mL (converted into g/mL)

Again, dividing "have" by "want":
\[0.05 / 2.5 \times 10^{-5} = 2 \times 10^3\]

Or, the same answer as obtained above.

To determine how much of the stock solution has to be added to the tank, calculate how much stock is needed for a 1/2000 dilution with 20,000L as the final volume. This can be determined by dividing the final volume by the dilution factor of 2000 (or by multiplying by 1/2000):
\[20,000 / 2000 = 10\]

Equals 10 what? 10L since the units of the final volume are in L.

It is always prudent to mathematically check the dilution that you just calculated by determining the amount of sulfur dioxide in 10L and dividing that by the final volume.

\[50g/L \times 10L = 500g\]

\[500g / 20,000L = 0.025g/L = 25mg/L = 25ppm\]

Finally, to have an exact concentration of 25ppm would require accurately measuring the volume of wine in a tank to 19,990L. This is not practical. The amount of error introduced by being slightly off in final volume is insignificant in this case.
APPENDIX 2

Filtration of Samples

Samples taken from the alcoholic and malolactic fermentations will need to be filtered before they are frozen at -20°C. The filter is a fairly simple device, but it is imperative that the filtering be done properly, so that samples will not be turbid (cloudy) when they are assayed.

The filter unit consists of a plastic barrel and a syringe. The tip of the syringe attaches snugly to the barrel. The barrel itself consists of two threaded halves.

Two filter pads are inserted on the wire mesh inside the barrel. The fine filter is smooth and very thin, and is placed on the wire mesh first. The second filter is a prefilter, and it must be placed on top of the fine filter. If you carefully examine the prefilter, you will notice it has a smooth side and a rough side. The rough side, or "water side," should face up, away from the mesh, so that it is the first surface to contact the filtered material (Figure A2-1).

When the filters are in place, screw the barrel back together, making sure that the rubber o-ring in the upper half of the filter barrel has not fallen out. The barrel is now ready to filter a sample.

Pull the plunger out of the syringe, and plug the end of the syringe with your finger. Pour a small scoop of celite filter aid into the syringe, and then add the sample to be filtered. Continue holding the end of the syringe, replace the plunger, and turn upright so that all of the air in the syringe can be evacuated with the plunger, without losing any of the sample.

The syringe is attached to the barrel of the filter by inserting the end of the syringe into the top of the barrel, and gently twisting until the syringe is snug. The sample should be gently filtered into a sterile plastic sample tube. If the filtration requires excessive pressure, there may be too little celite in the syringe. This can best be corrected by filtering another sample.

Use a Sharpie™ permanent marker and clearly label the tubes with:
- date,
- tank code,
- Brix reading, and
- your name or initials.
Figure 11: Sample Filtration Procedure

Attachment Fitting for Syringe

O-Ring Gasket

Glass Fiber Filter

Sterile (0.45 µ) Filter

Filter Screen

Test tube
APPENDIX 3
Guide to Oral Presentations of Small Scale Projects

Each pair/group will be given approximately ten minutes to present the results of their small scale project to their fellow students during the last laboratory period, with three to five minutes additional time allowed for questions or discussion of the presented material. One person can present the entire summary, or the time may be split, such as one partner giving the introduction, and the other the results and conclusions. Although a ten minute oral presentation might seem like an agonizing eternity for some, it is actually not very much time, so you will have to be well organized. You should practice your timing a several times beforehand. Plan on using the overhead projector rather than the blackboard. You should prepare figures, tables, diagrams, or any other visual aids you want to use ahead of time. We will make time to help you print overheads at second-to-the-last lab period, so have your data organized by then.

The presentation should be a summary of your written report, and organized as follows:

TITLE: State the title and the subject of your experiment.

INTRODUCTION: Includes what you were doing and why you did it, and what you hoped to see. Include previous work by other investigators in this area.

RESULTS: Explain how you conducted the experiment and present your data.

DISCUSSION/CONCLUSION: Did you see what you expected? Why or why not? What can you conclude from your own data? How might the experiment be improved \(i.e.,\) did you need more time, more replications, more equipment?\) Mention other parameters that could or should have been monitored. In other words, knowing what you know now, were your results useful, and how would you change your procedures to make them more reliable?

THINGS TO AVOID: 1. If you are asked a question, you will be expected to attempt an answer. Do not expect the TA to answer for you.
2. Do not dwell on minor points or extraneous information.
3. Do not dwell on negative or meaningless results. If your addition or treatment had no effect (an acceptable finding), it should be reported.
4. DO NOT EXPECT THE TAs TO GIVE THE EXPLANATION OF WHY YOU DID WHAT YOU DID OR BAIL YOU OUT IN ANY WAY. If you need our help, seek it before the presentation, not during.

If you think practice in oral presentation is unnecessary in this major, ask any winemaker about "Meet the Winemaker" dinners.
APPENDIX 4

Method for Determination of Titratable Acidity-Simplified procedure
Add approximately 8 drops phenolphthalein to approximately 500 milliliters of distilled water; heat water to boiling with constant stirring. Add 0.01 N NaOH drop by drop until hot water is pale pink. Add exactly 5 mL of degassed* wine or juice sample to a clean 250 mL flask. Dilute to approximately 100 mL with boiling adjusted pink water. Titrate this solution back to the faint pink endpoint with standardized 0.1 N NaOH, and record the titration volume. To calculate titratable acidity of the sample (expressed as g tartaric acid/L):

\[ TA = 15 \times (V) \times (N) \]

Where: \[ V = \text{Volume of NaOH used for titration in milliliters} \]
\[ N = \text{Normality of NaOH} \]

(*Wine can be degassed by connecting a flask with the sample in it to a vacuum hose, and agitating the sample in the vacuum for 3 minutes; be sure to use a vacuum filtration trap.)

Alcohol Determination by Ebulliometer-Simplified procedure
Check with the TA to be sure that the ebulliometer has been standardized. If it has not been, perform the following procedure with distilled water instead of wine sample, and set the resultant boiling temperature to 0% alcohol on the ebulliometer wheel.

Rinse the ebulliometer with tap water, and then with a small portion of the sample to be tested. When rinsing, close the tap at the bottom, cover the thermometer well hole and the condenser hole with fingers of opposite hands, and shake or invert to insure chamber and condenser are rinsed. Drain rinse sample through tap. Close the tap, and add 50 mL of the sample into the boiling chamber through the thermometer hole. Taking care to not spill water into the condenser or the chamber, fill the condensing chamber with ice cold tap water. Insert the thermometer in the fill hole at the top, and set a flame under the drain spout at the bottom of the ebulliometer. The temperature of the sample will rise steadily for a time. When the solution reaches its boiling point, the temperature will suddenly stop rising and hold steady. Record this temperature. The alcohol concentration on the ebulliometer wheel that corresponds to this temperature is approximately the concentration of alcohol in the wine sample. This procedure works best if the sample alcohol concentration is about 7%; this would require most wine samples to be appropriately diluted in a precise manner. Residual sugar can interfere with the ebulliometer reading, however. To correct for this:

\[ \text{ethanol(\%) = } \frac{100 - (\text{RS}) (0.62)}{100} \]

Where: \[ \text{RS} = \text{residual sugar reading from enzymatic analysis} \]
\[ \text{E\%} = \text{observed percent ethanol} \]

APPENDIX 5

Calculating Blends with Pearson’s Square

Draw a square; at the top left corner of the square write the high concentration of the constituent being blended for. At the bottom left corner, write the low concentration. In the middle of the square, write the concentration of constituent in the blend.

For example, if two wines are being blended, one with 10% alcohol and the other with 17% alcohol, and the prospective blend is to have 14% alcohol, the square would look like this so far:

```
17%  
\  
14%  
/  
10%  
```

To calculate the blend ratio, simply take the absolute values of the differences of the middle figure and the corner figures and write them in diagonally from the blended wines:

\[
\begin{align*}
17 - 14 &= 3 \\
14 - 10 &= 4 \\
17 - 10 &= 7
\end{align*}
\]

```
17%  
\  
14%  
/  
10%  
```

The final blend ratio necessary to achieve 14% alcohol is 4:3 17% wine to 10% wine. In other words, 4/7 of the blend must be 17% alcohol wine, and 3/7 must be 10% alcohol wine. (See Appendix I for an explanation of the meaning behind the colon sign (“:”) and the backslash sign (“/”) in dilution expressions).
Calculating a Blend for Three Constituents in Three Wines

To do a calculation of this kind, it is first necessary to identify all of the different variables. The whole purpose behind this calculation is to determine what volume of each of three wines should go into the blend. If the three wines are identified as wine A, wine B and wine C, then:

\[
X_a = \text{Volume of wine A used in the blend} \\
X_b = \text{Volume of wine B used in the blend} \\
X_c = \text{Volume of wine C used in the blend}
\]

Assuming that you are making a unit volume of wine, then:

\[
X_a + X_b + X_c = 1 \quad (\text{eq. 1})
\]

In these calculations, the constituents being blended are alcohol (%), Titratable Acidity (g/L), and residual sugar (g/L). The blend is to be made of the following three wines:

<table>
<thead>
<tr>
<th>Wine</th>
<th>Alcohol % v/v</th>
<th>TA g/L</th>
<th>RS g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>13%</td>
<td>7.0</td>
<td>1.0</td>
</tr>
<tr>
<td>B</td>
<td>9%</td>
<td>12.0</td>
<td>6.0</td>
</tr>
<tr>
<td>C</td>
<td>11%</td>
<td>6.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

The parameters for the blend have been arbitrarily set:

\[
\text{Alcohol} = 10.5\% \\
\text{TA} = 8.0 \text{ g/L} \\
\text{RS} > 4.3 \text{ g/L}
\]

Now, keeping eq. 1 in mind, equations for each constituent can be derived, as follows:

For alcohol: 13X_a + 9X_b + 11X_c = 10.5
For TA: 7.0X_a + 12.0X_b + 6.0X_c = 8.0
For RS: 1.0X_a + 6.0X_b + 5.0X_c = 4.3

Each equation can then be solved for Xa and Xb by solving eq. 1 for Xc and substituting:

\[
X_c = 1 - X_a - X_b
\]
Alcohol:
\[13X_a + 9X_b + 11X_c = 10.5\]  
(Substitute \(X_c = 1 - X_a - X_b\))
\[13X_a + 9X_b + 11(1 - X_a - X_b) = 10.5\]  
(Solve for \(X_a\))
\[2X_a - 2X_b = -0.05\]
\[X_a = X_b - 0.25 \text{ (eq. 2)}\]

TA:
\[7.0X_a + 12.0X_b + 6.0X_c = 8.0\]  
(Substitute \(X_c = 1 - X_a - X_b\))
\[7.0X_a + 12.0X_b + 6.0(1 - X_a - X_b) = 8.0\]  
(Solve for \(X_a\))
\[1.0X_a + 6.0X_b = 2.0\]
\[X_a = -6X_b + 2.0 \text{ (eq. 3)}\]

RS:
\[1.0X_a + 6.0X_b + 5.0X_c = 4.3\]  
(Substitute \(X_c = 1 - X_a - X_b\))
\[1.0X_a + 6.0X_b + 5.0(1 - X_a - X_b) = 4.3\]  
(Solve for \(X_a\))
\[4.0X_a - 1.0X_b = 0.7\]
\[X_a = 1/4(X_b) + 0.175 \text{ (eq. 4)}\]

Graphing these equations gives a visual interpretation of how the desired blend can be obtained (Figure 12). The intercepts of these lines are the points at which the two parameters equal one another. The blend represented by any point on the graph is possible as long as neither \(X_a\) or \(X_b\) is greater than or equal to one, and not less than zero. (Remember that the sum volume of all the wines cannot be greater than 1). The graph is most easily generated by solving for the intercepts of all three equations, and then drawing the lines through the intercepts:

1. Alcohol and RS:
\[X_b - 0.25 = 1/4(X_b) + 0.175\]
\[X_b = 0.57 \text{ or 50\% of blend}\]

Then, solve for \(X_a\)
\[X_a = X_b - 0.25 = 0.57 - 0.25\]
\[X_a = 0.32 \text{ or 32}\]

Solving for \(X_c\)
\[X_c = 1 - X_a - X_b = 1 - 0.32 - 0.57\]
\[X_c = 0.11 \text{ or 11\%}\]
2. Alcohol and TA:

Xb - 0.25 = -6Xb + 2.00
Xb = 0.321 = 32.1% of blend
Xa = 0.071 = 7.1% of blend
Xc = 0.607 = 60.7% of blend

3. TA and RS:

-6Xb + 2.00 = \( \frac{1}{4} \)Xb + 0.1751
Xb = 0.292 = 29.2% of blend
Xa = 0.248 = 24.8% of blend
Xc = 0.460 = 46.0% of blend

Further calculation gives the following results:

Blending 1: Alcohol = 10.5%
TA = 0.97 g/100 mL
RS = 0.43 g/100 mL

Blending 2: Alcohol = 10.5%
TA = 0.80 g/100 mL
RS = 0.50 g/100 mL

Blending 3: Alcohol = 10.9%
TA = 0.80 g/100 mL
RS = 9.43 g/100 mL

Of these blends, blend 2 best satisfies the original criteria for the blend, and would therefore be used to produce the blend.

References:
Figure 12: Calculation of Blending Ratio
APPENDIX 6

Filtration Demonstration

**Purpose:** Filtration with diatomaceous earth will be demonstrated. Applications of diatomaceous earth filters in winemaking will be discussed.

**Theory:** Diatomaceous earth (DE) filters are used in commercial wineries for crude filtration operations. These filters are simple to use, and the diatomaceous earth, though it is discarded after every filtration, is extremely inexpensive. Diatomaceous earth is composed of the skeletons of microscopic sea animals (diatoms), and is mostly silicon or silicates. It is easily mined in large quantities from dry seabeds, and is then purified and graded according to particle size. Different grades of diatomaceous earth will have an average particle size ranging from two to forty microns. The quantity and size of the DE used is directly proportional to the quantity and size of the particles being filtered.

The filter unit is prepared for a filtration operation by trapping the DE on a supporting paper or septum. Although it is possible to filter wine with no further preparation, filtrations are often more effective if some of the filter medium is added to the wine prior to filtration. The filter unit therefore has a tank in which a slurry of wine and DE can be prepared. As the wine is filtered, this slurry is carefully metered into the wine. Suspended solids in the wine become trapped in the DE. This type of filtration is called a depth filtration, as filtered solids are caught within the filter medium, and not on the surface. Filtrations of this type are capable of handling either large volumes of wine, or excessively turbid wines, but are not useful for such delicate operations as finishing or sterile filtering wine. DE filtrations are most commonly performed after racking.

**Method:** The filter is primed with wine, and the precoat of filtering aid is added to the holding tank of the filter. The filter is then run, so that the filtering aid is drawn onto the filter pad. Then the filter is recirculated, and more filter aid is added to the holding tank. This filtering aid will blend slowly with the wine just prior to filtration.

The wine is then filtered. When filtration is complete, the fluid is discharged, the cake is discharged, and water is circulated through the filter to rinse it clean.
APPENDIX 7
Wine Aroma Wheel

More information on the Wine Aroma Wheel can be found at:

http://wineserver.ucdavis.edu/Acnoble/waw.html

or

APPENDIX 8
Forklift Certification

All students will have the opportunity to become forklift certified during this class. Many operations in wineries and other production facilities are being conducted by forklift to prevent worker injury. As with all things, proper training in forklift operation is critical. Students will be expected to watch the two videos provided by UCD Environmental Health and Safety, to read the attached materials and to complete a written (true or false) test and pass a driving test administered by winery staff.
APPENDIX 9
Wine Sensory Evaluation Score Cards

Name________________              DUO TRIO TEST             Set________________

Please swirl the first sample, remove the lid, smell and taste the wine, then evaluate the aroma and taste of the reference and other sample. Circle the code of the sample that is DIFFERENT from the reference. Evaluate the remaining sets in the same manner. Please SNIFF the water between sets to refresh your nose.

<table>
<thead>
<tr>
<th>Set</th>
<th>Wine Codes</th>
<th>Size of Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>SMALL  MEDIUM  LARGE</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>SMALL  MEDIUM  LARGE</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>SMALL  MEDIUM  LARGE</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>SMALL  MEDIUM  LARGE</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>SMALL  MEDIUM  LARGE</td>
</tr>
</tbody>
</table>
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Blue Grass Cooperage Company
Burris Cellars, Ltd.
Danieli Brothers, Inc.
Dempots Cooperate
Dutro Company
FP Packaging, Inc.
Hach Company
Hanley Brewton
Healdsburg Machine Co., Inc.

20-52 Gallon White oak Barrels
Cash Donation
500 Gallon Stainless Steel Tank
10 French Oak Barrels
15 Wine Barrel Racks
Mobile Climate 20 Cooling System
Turbidimeter and Accessories
Sterling Variable Speed Drive
Indefinite Loan of Grape
Crusher/Destemmer
Indefinite Loan of Grape Press (Bucher RPL36)
Catwalk Provided at Cost
2 Centrifugal Pumps, Valves & Connectors
One Fiver Disc Filter
4 - 500 Gallon Fermentation Tanks
Construction of Modified Waukesha Pump
Progressing Cavity Pump
500 Gallon Dejuicing Tank & 560 Gallon Stainless Steel Fermentor
Velo Media Vertical LF Filter
Plate & Frame Filter
French Oak Wine Oval
Electrical Components for Waukesha Pump
2-M-500 Instant Hot Water Makers
2 Gamajet III Tank Washing Machines
Crusher/Destemmer
Ammonia Detector
Waukesha Pump
Two Pump Carts & Welding Work
Air Diaphragm Pump
Must pump and Accessories

KLR Machines, Inc. & Bucher-Guyer AG
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Ladish Company, TriClover Division
Nucleopore
Paul Mueller Company
Process Engineers, Inc.
Robbins & Myers, Inc.
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Wine and the People

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