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Making better red wine



Numerous techniques have been used by winemakers in an effort to consistently create superior wines. Two of these techniques (and variations of them) have proven most effective at meeting the needs of winemakers: the first is realized through the influence of toasted oak and the second is achieved through the effective use of air and oxygen.

Traditionally, the influence of toasted oak on wine occurred through storage in toasted oak barrels. Furthermore, oxygen or air supplied to the wine was thought to occur only by permeation through the oak barrel. Both traditional approaches can produce wines with well-integrated flavors and aging. However, since all grapes are not the same, conditions must be varied to bring out the best in a particular wine.

American and Australian winemakers have pioneered the use of oak integration systems in the production of superior wines. Four alternatives to barrels (chips, beans, segments and staves) allow the winemaker

to dictate the quality and type of toasted oak that a wine will experience. However, whereas oak integration systems could supply flavor, they could not induce aging or maturation.

It was not until the introduction of a French oxygen micro-metering device that a true alternative to barrels for flavor and aging was made possible. The use of a device to meter in small quantities of oxygen, combined with the proper level of toasted oak, gives the winemaker a degree of control over wine production never before possible. A winemaker can now give the wine what it needs—no more, no less—in terms of flavor and aging.

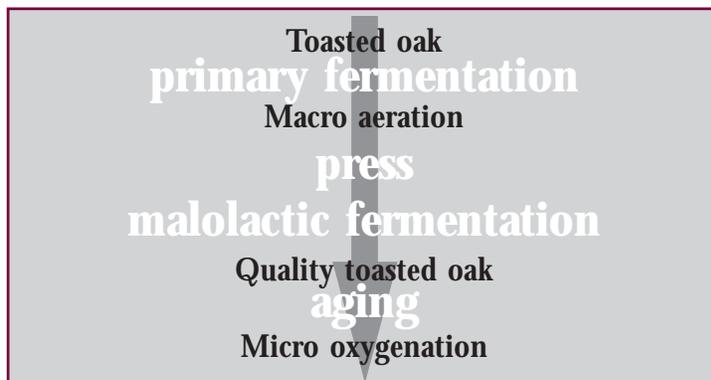
Section II of this booklet, *Fermentation*, recommends how, when and where to apply toasted oak and micro oxygenation to the process of making wine.

Section III, *Micro Oxygenation During Aging*, provides the rationale for adding oak and oxygen, helping the winemaker produce enhanced wines in a consistent manner.

Section IV explains *StaVin Products* for integrating quality toasted oak with your wines.

Section V, *Chemistry*, details the chemical structures when using micro oxygenation and toasted oak in red winemaking.

The purpose of this booklet is to give winemakers more flexibility and control in their constant endeavor to create better wines.



Use of micro oxygenation and oak during red wine production to initiate the stabilization of color and build mid-palate structure

Questions frequently asked by winemakers:

1. How can I retain more color in my red wine?
2. How can I build body in my red wine?
3. How can I improve the fruity character of my wine?
4. How can I reduce harshness?
5. Can you make stave tanks taste like barrel aged wine?
6. Is oak added only for flavor?
7. Can micro oxygenation improve my wine?
8. Can micro oxygenation reduce vegetal descriptors?
9. How can I reduce sulfides during fermentation?
10. How can I mitigate sulfides during aging?
11. Is micro oxygenation oxidizing my wine?



Winemakers often have the issues listed above when they receive grapes or wine which may not have matured ideally during the viticultural period. The products and techniques suggested in this booklet will help you to enhance these wines.

Challenges from the vineyard



Sometimes the shortcomings of a wine originate from certain practices in the vineyard or in vineyard management. In the winery, these problems require time and expense to correct.

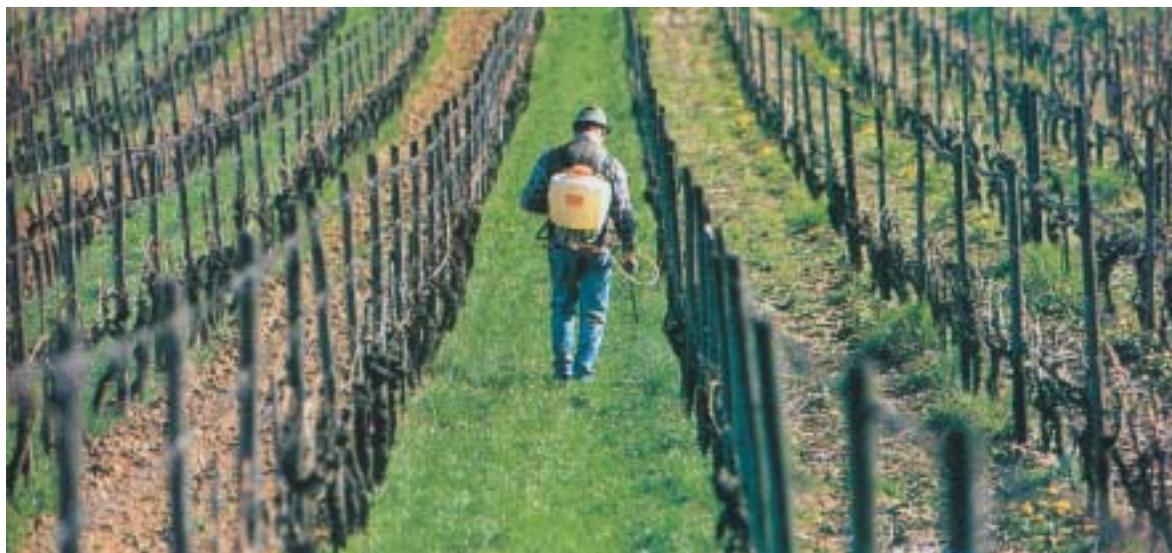
To minimize potential problems before grapes arrive at the winery, it is imperative to have good communication between the winemaking team and vineyard personnel.

Many problems created in the vineyard can be reduced by practicing:

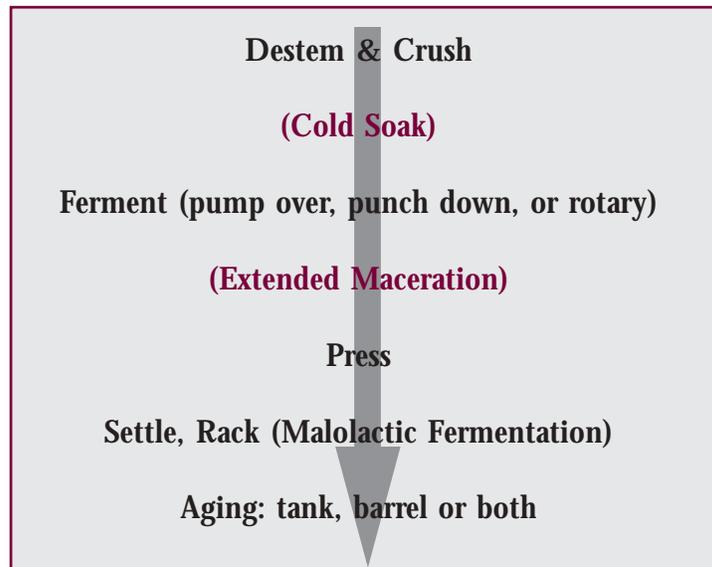
1. Proper vineyard siting: matching the varietal to the micro-climate in order to minimize the amount of immature harvests.
2. Appropriate canopy and vine management (proper trellising, irrigation and fertilization).
3. Adequate, but not excessive, disease control practices.

Canopy management is important in controlling vegetal character. Too much shading can lead to the fruit retaining concentrations which are too high in isobutyl-methoxy pyrazine (bell pepper) and related compounds, as well as other types of green compounds such as cis-3-hexenol.

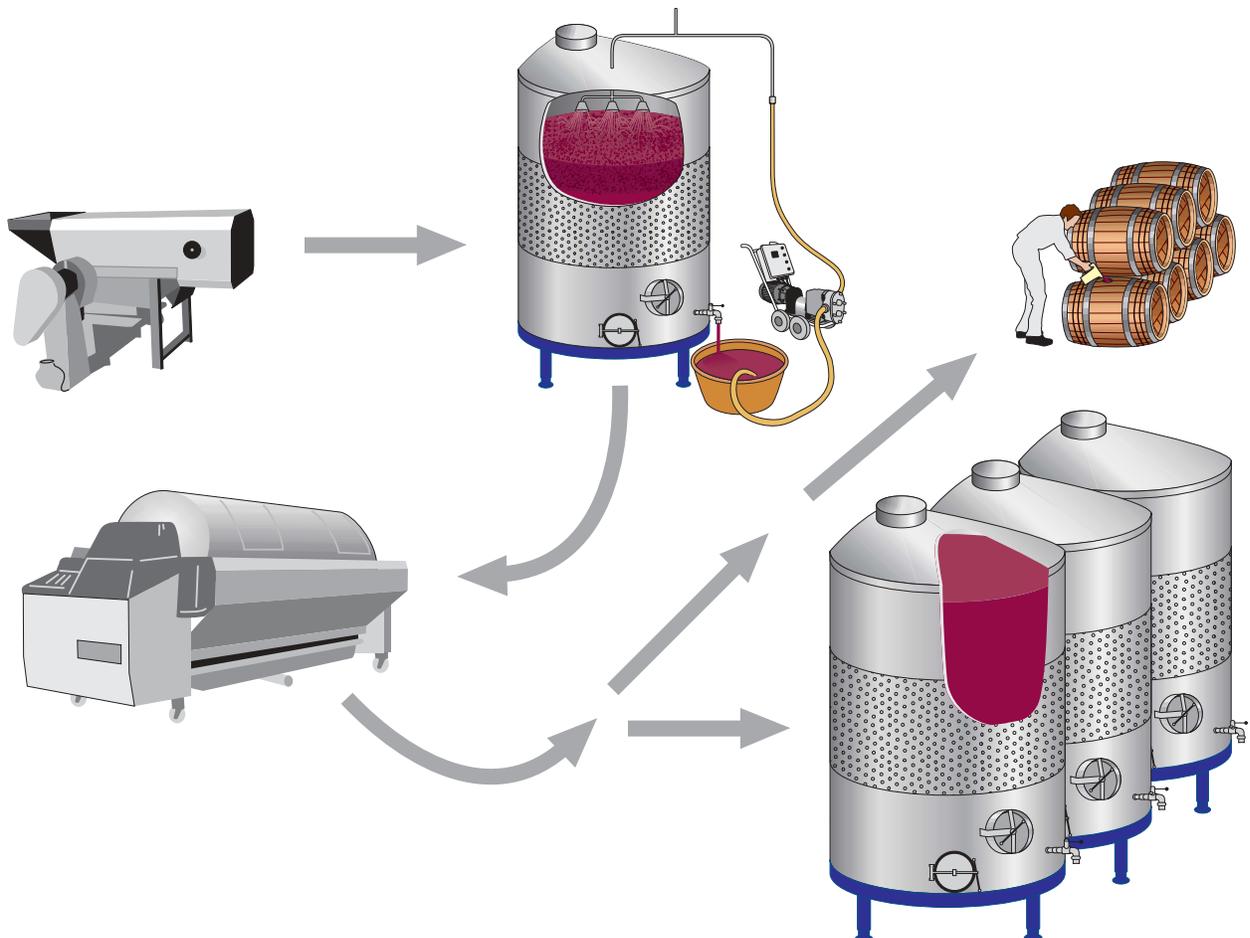
Overdoing disease control or late application of dusting or wettable sulfur can lead to the overproduction of hydrogen sulfide in the fermentation. There is a direct correlation between the μg of elemental sulfur carried on berries into the winery and the production of H_2S . While a winemaker can control H_2S to a degree, overproduction of H_2S can lead to the development of mercaptans, which are often difficult to eliminate.



Typical red fermentation process

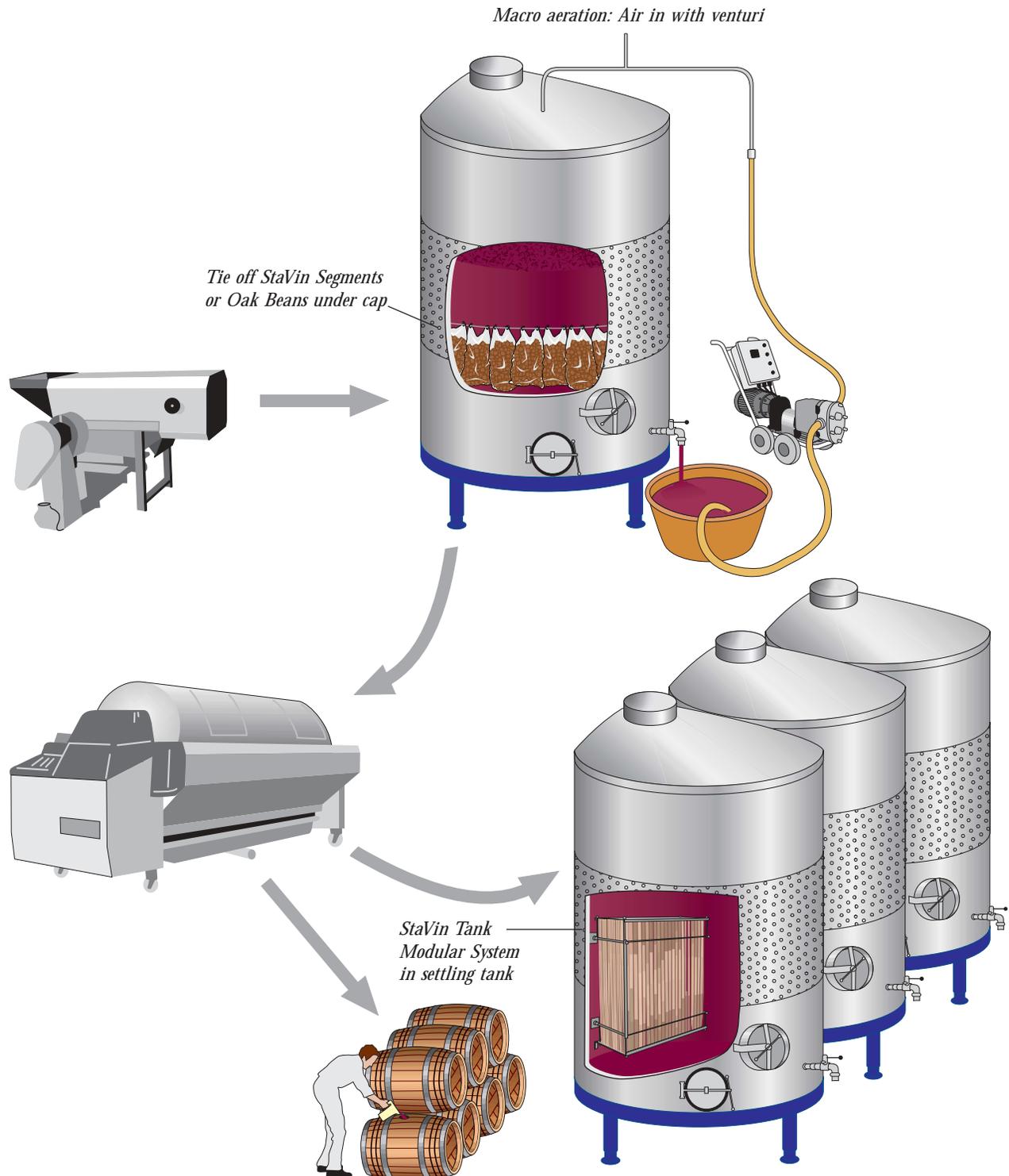


Every winery has variations on the typical process of red fermentation. However, most of these variations are illustrated above.



Enhanced red fermentation process continued

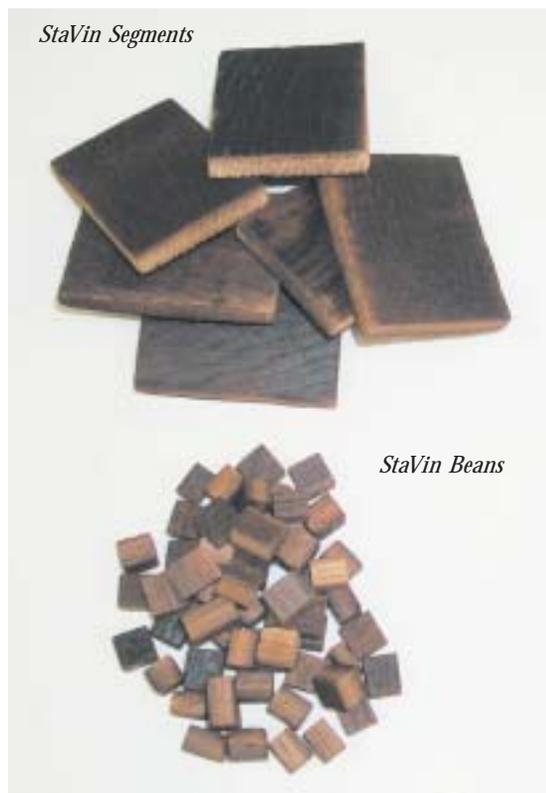
The combination of toasted oak implemented in the fermentor, macro aeration during fermentation, and pressing onto toasted oak, provides much of the same effect as would the structurization process. This technique integrates harmoniously with the way winemakers currently produce their wine, rather than imposing an expensive change on their accustomed methods.



Using oak during enhanced fermentation



1. Chips can only be used during primary fermentation (they are not being used for flavor). Add 4–8 pounds of chips per ton of grapes.
2. Or, StaVin Granular can also be used during primary fermentation. Add $\frac{1}{2}$ to 1 pound of Granular per ton of grapes.
3. Also, you may add tannin supplements (sequential additions provide more consistent results).
4. A combination of toasted Granular oak and tannin supplements may maximize results.
5. For higher quality oak, add bags of StaVin Beans, Segments or Staves to the fermentation.



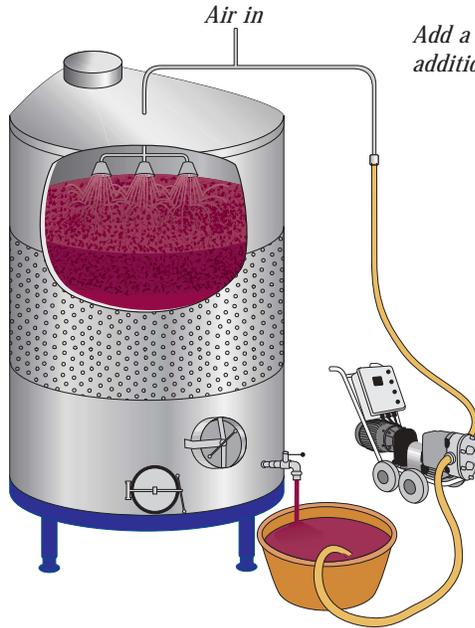
Adding toasted oak into the fermentor provides compounds to aid in the stabilization of tannins and color. Oak during this stage is not necessarily added for flavor. At this point, be careful not to overuse oak chips, since shaved oak pieces can add harsh and bitter characteristics.

Sequential additions of tannin supplements (TS) can also provide color and tannin stabilization. Rather than adding 3 to 5 lbs. of TS per ton of grapes at once, begin by adding 1 pound and adding one pound per day thereafter, or as recommended by the product's provider. This latter dosage is optimal because the material is added as a soluble solution. The reactive (stabilizing) compounds which are present will react with available anthocyanins and tannins. As more tannins and color are extracted from skins and seeds, more reactants will be needed to stabilize them. Therefore, sequential addition of these tannin supplements becomes more effective than a single dose in stabilizing color and tannin.

Sequential addition of TS's also explains why the addition of toasted oak seems more consistent in its ability to stabilize color and build middle body. The slower extraction of compounds from toasted oak enables reactants to be always present as anthocyanins and tannins are being extracted from seeds and skins.

Macro aeration during primary fermentation

Schematic pumpover with topside aerator



Add a venturi (passive oxygen addition) at every pumpover.

A 1/2 inch to 1 inch nipple welded in the top of the pumpover pipe will usually be sufficient. Add 3 to 6 feet of PVC or Tygon tubing to contain burping must.

1. Winemakers generally underestimate, to a large extent, the amount of oxygen a red wine needs during fermentation.
2. Macro aeration helps maintain yeast viability and reduces the production of sulfides.
3. Macro aeration may play a role in the initiation of color stabilization.

Most red fermentations can use more, rather than less, oxygen. This additional oxygen helps minimize production of H_2S , which can lead to potential mercaptan problems later in the wine's life.

When implementing the venturi shown on the next page, make a hole in the top of the pumpover system and attach a small nipple. Connect the nipple to a 3 to 6 foot air intake tube. This arrangement is the simplest passive method for putting air in intimate contact with fermenting must. Rack and returns, while effective at removing stems and green seeds, are not effective at aerating a fermentor. The out gassing of CO_2 during the racking effectively blankets the wine and minimizes contact with air.

Other methods for putting air in contact with the must: opening a valve on the suction side of the pump, or using an active valve that opens when the pump is switched on, injecting compressed air or oxygen.

Plastic version of an inline venturi (stainless steel versions are available) for introducing oxygen.



Check valve, to prevent backflow of must.

This type of venturi works under most conditions. When combined with the check valve, it can be used with most pumps and in a variety of positions. This system also provides excellent aeration when racking a tank off lees.

Mazzei injector model 2081-A. Venturi courtesy of Bob Bertheau, Belvedere Winery.

A venturi without a backflow preventor

This venturi system uses a nipple welded onto to the 3 inch stainless pipe. Pascal Chatonnet at the University of Bordeaux first suggested its use to minimize sulfide production during fermentations. This system works well with a diaphragm air pump when it has a correctly matched irrigator.



Pump and pressure need to match. Photos courtesy of Marcello Monticelli, Gallo Sonoma Winery.



Irrigator with venturi in place. A venturi can also be used to induce more efficient aeration during racking.

Press, settle and rack wine



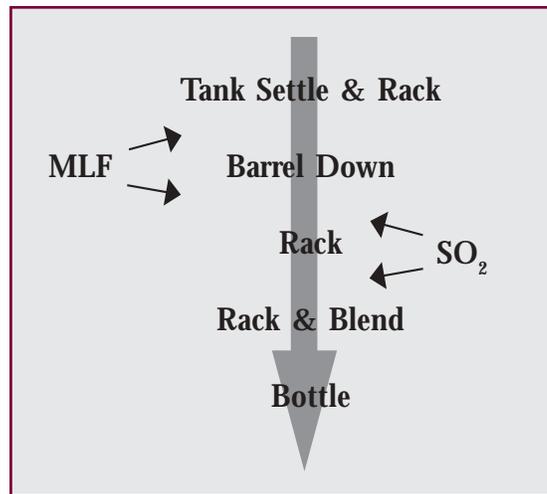
1. Move pomace to press as gently as possible. Bitter/harsh character is extracted from macerated skins (not necessarily skin tannins).
2. Drain and press directly to the tank with quality oak integration system. Or settle, then rack into the tank with quality oak integration system.
3. Rack or micro oxygenate just enough to control sulfides until malolactic fermentation has completed. (Do *not* add copper after any aerative procedure.)

Gently moving drained pomace usually produces a higher percentage of press wine that may be used later, without it adding overtly harsh phenolic tastes. It is commonly believed that grinding skins enhances the extraction of skin tannins, thereby producing excessive harshness.

However, recent research by Veronique Cheynier at the Montpellier Research Institute (INRA), and Elizabeth Waters at the Australian Wine Research Institute (AWRI), have shown this common belief is not accurate. They isolated fractions of skin and seed tannin of average lengths: degree of polymerization (dp) 3, 7 and 15. Sensory evaluation of these fractions by Leigh Francis at AWRI has shown that none of these tannin fractions are bitter. As predicted, they do increase in astringency as the polymer size increases, proving that skin tannins are not responsible for the bitterness and harshness in the press fraction of wines. However, there are many other phenolic based compounds which could be responsible (research continuing).

Copper additions to wine should only be made with forethought! For copper to act as we expect it to, it binds with free thiols or mercaptans and must be added to wine at low redox potentials (reduced conditions). If copper is added too soon after an aerative process, *eg* racking, it will act as a catalyst to form disulfides rather than bind the free thiols or mercaptans. If disulfides are formed, free thiols may reappear later in the life of that wine.

Typical red wine aging process

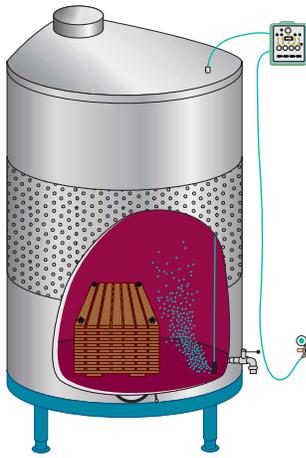


The above diagram may be a typical process after pressing for most wineries.

The timing of malolactic fermentation is usually the winemaker's prerogative. In addition, it is the winemaker's decision whether MLF occurs in the tank or barrel.

Smaller wineries usually are able to move wine to barrels shortly after pressing and settling. However, depending on the winery's size and a particular wine's price point, the wine may stay in tanks for as long as four months before barreling. Or it may never see a barrel at all.

Enhanced red wine aging process



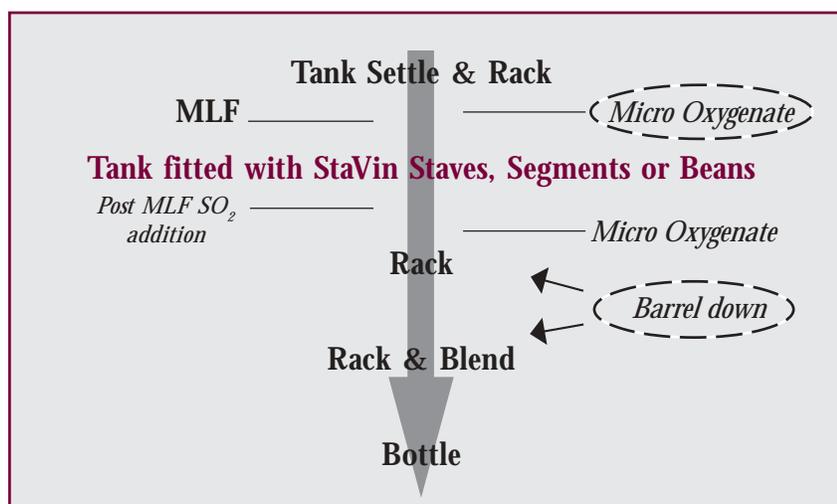
Below is a process flow chart depicting the enhancement of red wine color, body and aging characteristics.

As stated before in the fermentation section, structurization can take place pre-malolactic fermentation, as the French recommend. However, most winemakers prefer to finish malolactic fermentation at an early stage since slowing or stopping malolactic fermentation is not desirable logistically. We still advise that winemakers test the potential benefit of this French approach of structurization.

Preferably, a winemaker will inoculate for malolactic fermentation toward the end of primary fermentation (before pressing), utilizing the warmer wine temperatures to help the bacteria get started.

If a winemaker knows there will be time (1–4 months) before barreling, pressing the wine onto a quality oak integration system is recommended. As described earlier, pressing onto oak supplies compounds which will, during fermentation, aid in the stabilization of color and modify tannins. We suggest that the winemaker use high quality oak integration systems such as StaVin Beans, Segments or Staves. Our products will provide a barrel-like flavor profile, offering the same results achieved when barreling occurs immediately after pressing.

A 24 to 48 hour settling of gross lees before racking into the oak tank is recommended to minimize the potential for sulfides. Once malolactic fermentation has completed, settle and rack back to the tank that contains the oak integration system, adjust the SO_2 and begin micro oxygenation until the wine is ready for blending and bottling, or ready for barreling.



Take control of oxygen input: turn your tanks into barrels

1. Use micro oxygenation levels between 1 and 10 mL O₂/L wine/month.
2. The starting point depends on volatile sulfides, anthocyanin concentration, tannin concentration (how big is the wine?), end use of the wine and time line for the wine

Post-MLF micro oxygenation brings the technique of oxygenating wines to a more reasonable and familiar level for most winemakers. Micro oxygenation is not new. Throughout their careers, winemakers have been using micro oxygenation in some manner. However, the methods were not controllable: racking, barreling, topping, etc.

Now, with the StaVin OxBox, the winemaker will have precise control over how much oxygen a wine will be exposed to. One mL /L/month is approximately what a new barrel can deliver to a wine, including topping and racking. While oxygenation can be applied to wine at a rate more than 10 mL/L/month, a higher rate has a potential for generating compounds which react faster with SO₂ than the phenolic compounds.

The major question is: *Where do I start?*



Segment bags tied to stainless cable inside 20,000 gallon tank.

The answer depends on: First, the presence of sulfides. The rate of oxygenation may be set higher for a brief period to help eliminate these compounds, allowing better fruit expression and better judgment of the optimal rate with which to treat the wine overall.

Second, the rate of oxygenation depends on how much color and tannin are in the wine. The bigger the wine, the higher the rate a winemaker might use. In contrast, the lower in color and tannin, the lower the rate of oxygenation.

Third, what is the final market for the wine and when will it be needed for blending or bottling? Think of wine in a tank being treated with an oak integration system and micro oxygenation as wine in a barrel. Experience has taught winemakers that a particular lot of wine requires eight months for proper flavoring and maturation. Therefore, if the winemaker has the correct amount of oak in the tank and will micro oxygenate this tank at 1 mL/L/month, the wine will develop over eight months approximately the same as the wine would develop in a barrel.

Micro oxygenation cannot cure all problems



Micro oxygenation can help improve wines, especially when used in combination with toasted oak:

1. Stabilizes color.
2. Allows control of O₂ supplied to the wine.
3. Builds middle body.
4. Minimizes vegetal character.
5. Puts a wine into balance.
6. Corrects slight sulfide problems.
7. *Reduces dependence on barrels for flavor and aging.*

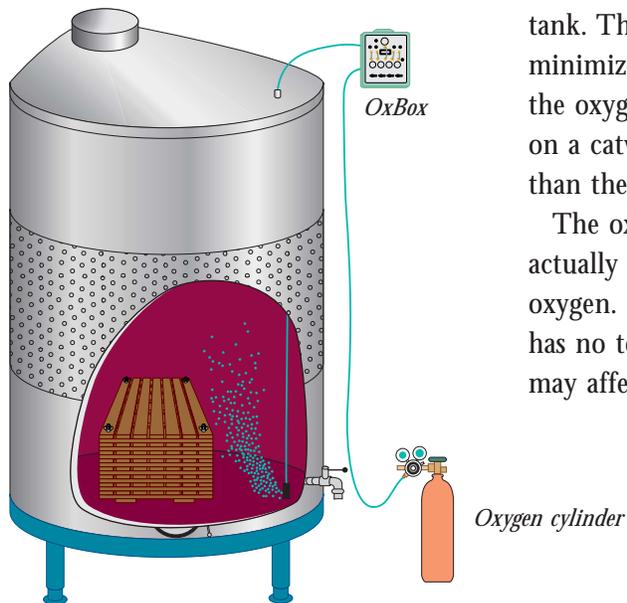
Many winemakers are searching for successful methods to minimize vegetal aromas and flavor characters in their wines. The combined use of toasted oak and micro oxygenation does appear to minimize vegetal characters.

Vegetal characters appear to be due to the combination of three primary components: 1) isobutyl methoxy pyrazine and related compounds (*eg*, bell pepper aromas); 2) cis-3-hexenol and related compounds (*eg*, bright green, leafy aromas); and 3) sulfides, methyl mercaptan and related compounds (*eg*, asparagus aromas). Micro oxygenation appears to affect two of the three components (sulfides and hexenols) through oxidation to minimize their contribution to vegetal characters. It is difficult to foresee a dramatic drop in the pyrazine component due to the components' stability and extremely low aroma threshold (in the low ppT).

Toasted oak used both in the fermentor and the tank for flavor and aging appears to provide aromas and flavors to help mask the vegetal characters. Toasted oak also provides compounds which will crosslink tannins, just as micro oxygenation will provide acetaldehyde which will crosslink tannins. The combination of these two sources of cross linkers should push tannins to form different structures. This process is described on page 34. It may be possible that these crosslinked tannins will form a different source of compounds to interact with primary flavor compounds for that wine. Stronger or weaker interaction with these compounds will change perceived aromas and flavors of the wine, potentially explaining why we see less vegetal character in wines treated with toasted oak and controlled oxygenation.

The OxBox

Tank with toasted oak integration system



The toasted oak is shown setup as a StaVin Matrix in this tank. The control box is mounted at the top of the tank to minimize potential siphoning of wine through the box if the oxygen tank is depleted or shut off. Locating the OxBox on a catwalk will lessen the likelihood of anyone, other than the proper user, changing settings.

The oxygen used for the system is industrial grade, actually purer and less expensive than medical grade oxygen. It is purer because industrial or welding grade has no tolerance for water or hydrocarbons, since they may affect welding.

The OxBox configuration

1/8 inch food grade tubing

Regulator (90 psi) going into the box.



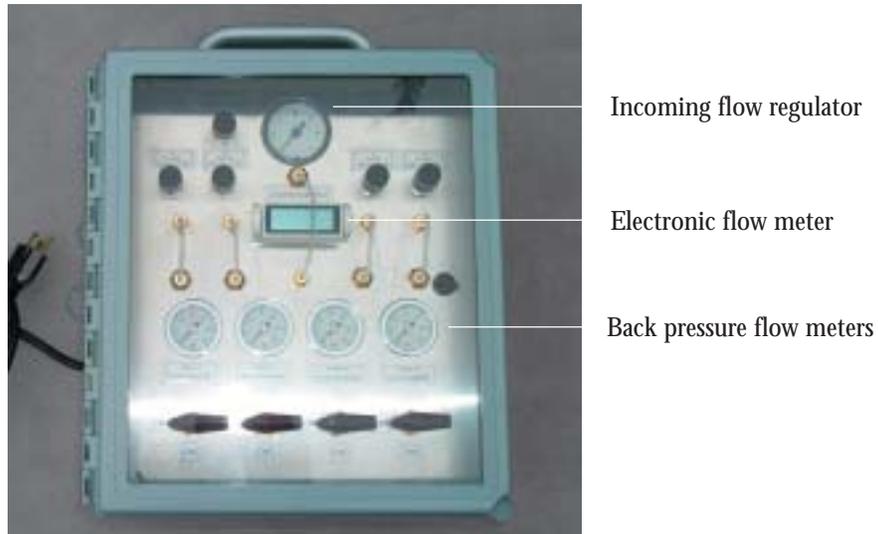
Oxygen tank



Sparge tips (sintered stainless steel)

The StaVin OxBox is housed in an industrial strength plastic waterproof box with a transparent front panel. The sparge tips are HPLC filters made of sintered stainless steel with a 10 μ m pore size. This micron size provides a very fine bubble or mousse as oxygen is metered into the wine.

Micro oxygenation unit (the OxBox)



The OxBox is based on classic gas chromatograph technology. Like the HP 5700 GC, the OxBox uses a high precision flow regulator combined with mechanical flow controllers to set oxygen flow for each tank. The flow meter is electronic and needs to be on only when setting flows to each tank.

The “jumper” tubes either directly connect the flow control to the tank, or are used to orient the oxygen flow through the flow meter when setting the flow to each channel.

The four bottom gauges measure back pressure due to the depth of wine exerting pressure against the stone. Once set, these gauges measure a small amount of back pressure. Simply note this beginning back pressure. If the pressure rises more than 5–6 psi, the sparge stone may be clogged or fouled and should be cleaned.

Calculation of flows for micro oxygenation

The OxBox will not measure flows in mL O₂/L/month (the standard units used with micro oxygenation of wine). The flow meter measures in mL /minute. To set the flow rate, the winemaker must know the size of the tank and desired oxygenation rate. Once determined, one of the two spreadsheets above can be used to calculate the required flow (mL/min.), depending on whether or not the type of sparging (oxygenation) desired is constant or periodic.

Tank size in gallons	<input type="text" value="12000"/>
Desired rate of oxygenation (mL O ₂ /L/month)	<input type="text" value="1"/>
Flow rate in mL/min to set	<input type="text" value="1.05"/>
Calculation of flow rate for periodic micro oxygenation	
Tank size in gallons	<input type="text" value="12000"/>
Desired rate of oxygenation (mL O ₂ /L/month)	<input type="text" value="1"/>
Days per month to micro oxygenate	<input type="text" value="4"/>
Hours per day desired to sparge	<input type="text" value="24"/>
Flow rate in mL/min to set	<input type="text" value="7.89"/>
Instructions To calculate the flow rate needed in mL/minute, fill in the tank size in gallons and desired parameters.	

Constant oxygenation is probably the safest, since flow rates will be lower during the process. Therefore, change will be slower and easier to control. However, periodic oxygenation may be more similar to a barrel program where wine gets occasional aeration during racking and topping. Which method is more advantageous remains to be determined.

The OxBox electronic flow meter cannot accurately measure oxygen flow below 0.25 to 0.5 mL/min. For tank volumes smaller than 3,000 gallons, the meter's limitation makes constant oxygenation difficult to control due to the lower rates required. However, this tank could still be oxygenated using the periodic formula, enabling the winemaker to provide the same amount of oxygen over a one month period.

French, American and Hungarian Oak Beans™



Unlike a chip, StaVin Beans provide a slow and controlled extraction of flavor components. Whereas oak chips deliver a monochromatic, harsh flavor profile, the Beans' gradation of toast delivers a delicate mixture of aromatic compounds. The gradation results from our gradual toasting process. *StaVin's Beans impart to wine complex flavor and aroma profiles similar to that of the best barrels.*

The oak we select is equal in quality to premium wine barrel staves. American oak, harvested from Missouri and Minnesota, French oak and Hungarian oak are seasoned naturally for three years.

One 20 lb. (9 kg.) Bean bag will yield 100% new barrel extraction to 900 gallons (3407 l.) of wine.

During red wine fermentation, Oak Beans are highly effective for initiating the stabilization of color and crosslinking grape tannins to help build mid-palate structure. We recommend tying off the Bean bags beneath the cap. After primary fermentation is complete and the wine has been drained and pressed into another tank, move the bags with the wine. Alternatively, leave the Beans in the tank and *immediately* follow with another red wine fermentation.

The StaVin French Oak Stave Segments



Designed for convenience, the StaVin Segments are easily installed in tanks. Simply place the 15 lb. (6.8 kg.) bags directly into tanks and use during fermentation, or at any time during the winemaking process. In order to achieve new barrel extraction levels, StaVin recommends 2 lb. per 60 gal. (.9 kg. per 227 l.).

Because the Segments are larger than our Oak Beans, they offer a lengthier extraction period which delivers additional complexity to a wine's flavor profile. We recommend a minimum contact time of three months. The Segments have a useful life of eighteen months, at which time they are fully extracted.

The StaVin Tank Matrix



Loaded with our three year seasoned French, American or European Oak Staves, the Matrix integrates subtle, elegant oak flavors into your wines with the same balanced level as new barrels.

To achieve new barrel extraction level, add 1– 44 sq. ft. Oak Stave Packet per 180 gal. (1– 4.1 sq. m. Oak Stave Packet per 681 l.) of wine. For example, in a 9,000 gal. (34,065 l.) tank, fill 2 Matrixes with 50 of our 44 sq. ft. Packets.

Due to the efficient usage of oak surface area, our Tank Matrix is a practical, economical and ecological tool. Used as prescribed to its full potential, our oak costs five to ten times less than the initial cost of barrels. Your winery will also reduce costs in labor, logistics, warehouse space, electricity and water.

Depending on the number of barrels in a typical warehouse, the savings from reduced ullage alone can range from *tens of thousands to hundreds of thousands of dollars per year.*

Why use barrels at all?

StaVin Tank Modular System



Oaking your wines inside a tank with our Modular System can deliver the same oak-to-wine ratio as a barrel. Each Modular System, filled with our seasoned French, Hungarian or American Oak Staves, will impart a “new barrel,” balanced level of oak to 1,260 gal. (4,769 l.) of wine.

The StaVin Tank Modular System is built with 304 stainless steel. Using our mounting tabs, the System can easily be installed on the walls of stainless tanks or oak uprights.

To achieve new barrel extraction level, add 1– 44 sq. ft. Oak Stave Packet per 180 gal. (1– 4.1 sq. m. Oak Stave Packet per 681 l.) of wine.

Calculations for treating wines with StaVin products

StaVin product	Package size	Surface area per package (square feet)	Weight per package (pounds)	Volume treated per package (gallons)
Beans	20 lb. bag	96.2	20	600-1200
Segments	15 lb. bag	34.2	15	204-450
Oak stave packet	44 sq. ft. pack	44	20	180-264
Barrel Insert	16 sq. ft. pack	16	8	60-70
Replica	11 sq. ft. pack	11.1	5	60-120
Barrel	60 gallons	24	na	60-70

Oak integration systems offer: *flexibility, style, time, intensity, efficiency and cost effectiveness*

StaVin product	Volume treated per package in gallons	Addition rate: grams per liter	Addition rate: pounds per 60 gallons	Addition rate: pounds per 1000 gallons	Addition rate: square feet per 1000 gallons	Extraction time in months
Beans	600-1200	2-4	1-2	17-34	80-163	12
Segments	204-450	4-9	2-4.5	32-75	76-168	18
Oak stave packet	180-264	9-13	4.5-6.5	75-108	166-244	24
Barrel Insert	60-70	14-16	5	na	na	24
Replica	60-120	5-10	5	na	na	24
Barrel	na	na	na	na	na	36

How oak and oxygen enhance red wine characters

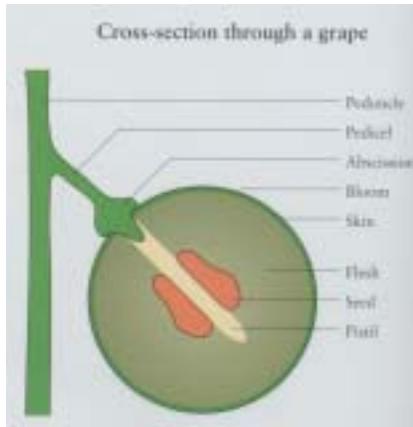


Louis Pasteur conducting experiments on alcoholic fermentation in his laboratory.

The following points are based on available research world-wide:

1. Extracted oak compounds may help to solubilize procyanidins and maintain stability of monomeric anthocyanins (*act as co-factors*).
2. Aldehyde compounds extracted from toasted oak products can react in much the same manner as acetaldehyde to crosslink the anthocyanins and procyanidin monomers and oligomers (*act as cross-linkers*).
3. Covalent bonds formed with these compounds are more stable than those formed with acetaldehyde.
4. Oligomers formed through crosslinking have been shown to be structurally different, possibly more soluble than natural seed and skin oligomers and polymers.

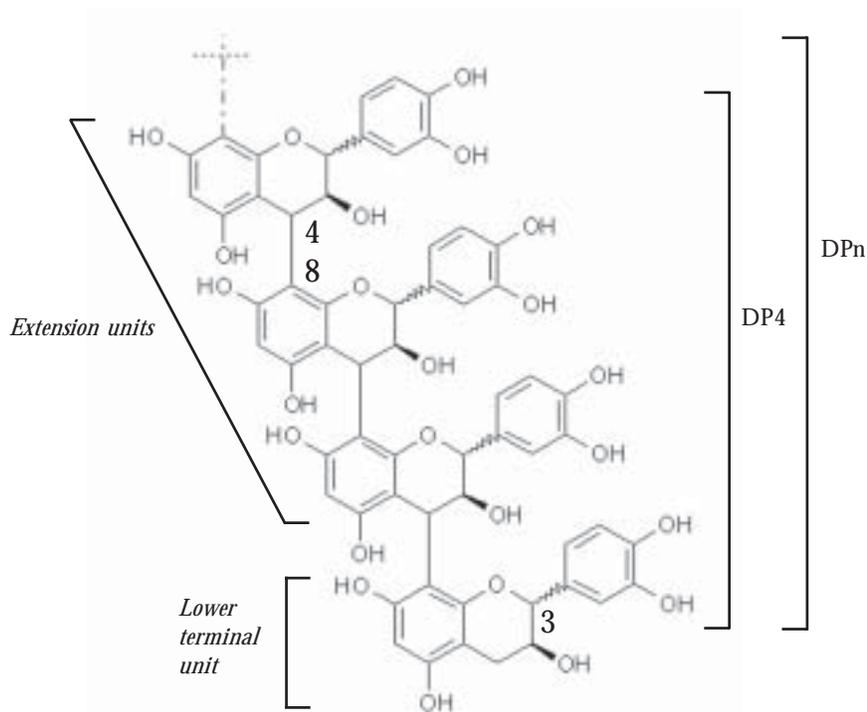
Procyanidins = Seed Tannins Prodelphinidins = Skin Tannins



There are two major classes of condensed tannins (or as they are known more properly, *polyphenolics*) naturally occurring in grapes.

The only difference between the two classes is the presence of one additional hydroxyl group on the B ring of the three ring structure for prodelphinidin.

The names for the two classes are derived from the following: when a polymer of procyanidin is hydrolyzed in strong acid, it will yield the anthocyanidin *cyanidin*; prodelphinidin will yield delphinidin.

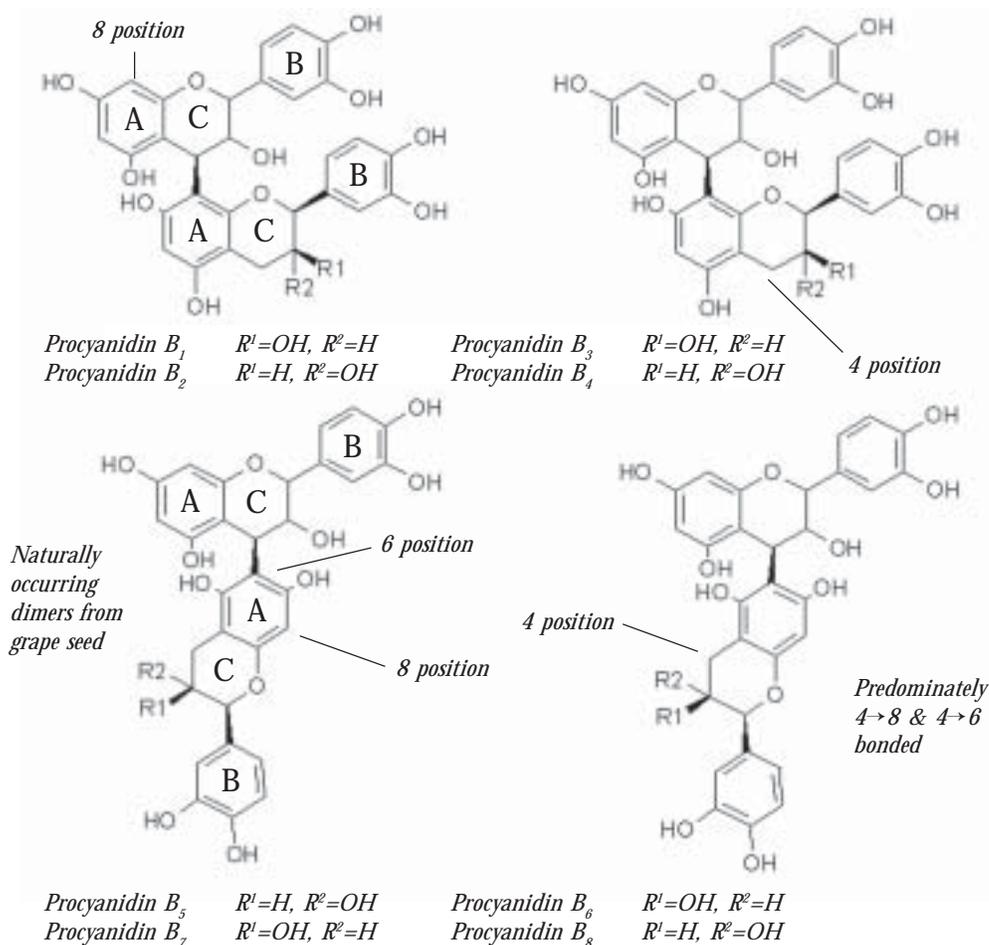


The general structure of a homologous procyanidin is 4→8 bonded, though there can be 4→6 branch points, and a gallic acid esterified at the 3 position.

This structure represents a longer procyanidin structure with the lower terminal unit and subsequent extension units of either catechin or epicatechin.

A gallic acid may be esterified to the 3 position, which would increase the hydrophobicity at the center of the oligomer or polymer.

Structure of the main procyanidin dimers from *V. vinifera*

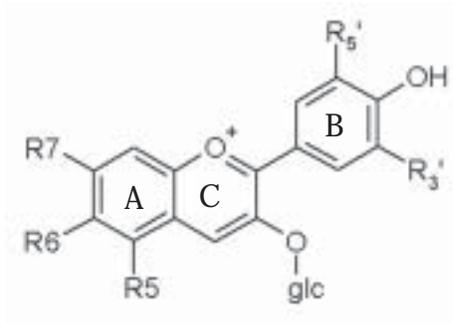


Bombardelli E, Morazzoni P. *Vitis vinifera*. *Fitoterapia* 1995;66:291-317

The upper two structures illustrate examples of dimers of epicatechin and catechin, each bonded between the 4 position of the upper molecule and the 8 position of the lower molecule. The configuration cis or trans of the bond between the C and B rings with the hydroxyl located on the 3 position of the C ring determines whether or not the structure is epicatechin (cis) or catechin (trans).

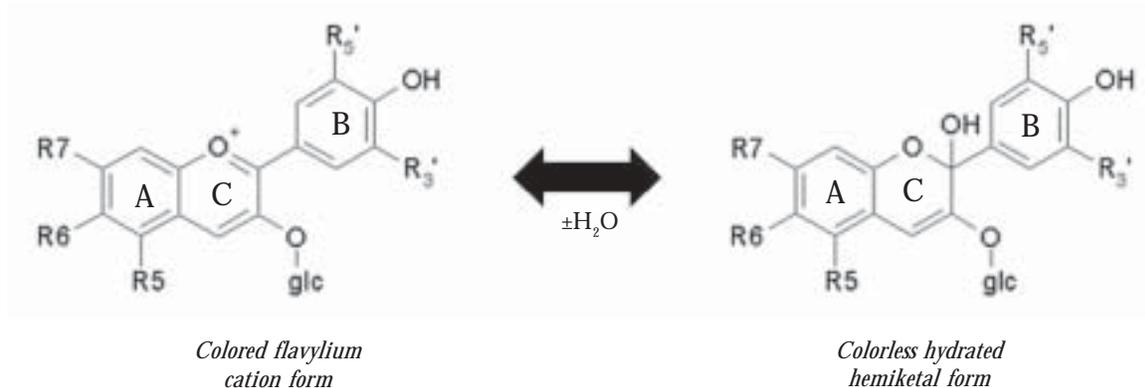
The lower set of structures shows bonding between the 4 and 6 positions which would cause branching of longer oligomers of polymers.

General anthocyanin structure



The second major class of compounds found in red wines are anthocyanins. They have a positive charge on the molecule which enables it to absorb light and thus have color. An anthocyanin has a carbohydrate (sugar, usually glucose) esterified at the 3 position. An anthocyanidin, termed the aglycone, does not have a sugar at the 3 position. Naturally occurring pigments from grapes always have a sugar bonded at the 3 position, though other compounds such as hydroxycinnamates and acetate may be involved. The presence of this sugar helps the anthocyanin maintain solubility in water. If the sugar is hydrolyzed or lost, the solubility decreases and the molecule will be destabilized and lost.

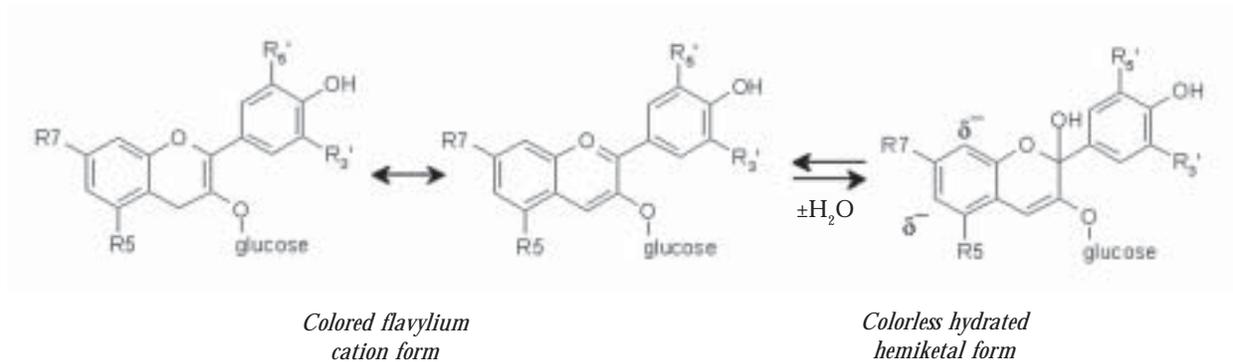
Major anthocyanin forms occurring at wine pH



(Bouillard et. al. *J. Am. Chem. Soc.* 99:8461-8468, 1977)

All naturally occurring anthocyanins are in equilibrium between the colored flavylium cation and the colorless hydrated form. The equilibrium is driven to the left as the pH of the wine is decreased and to the right as pH is increased. At pH's above 4.5 other destabilizing reactions begin, such as ring opening of the C ring.

Major anthocyanin forms at wine pH *continued*



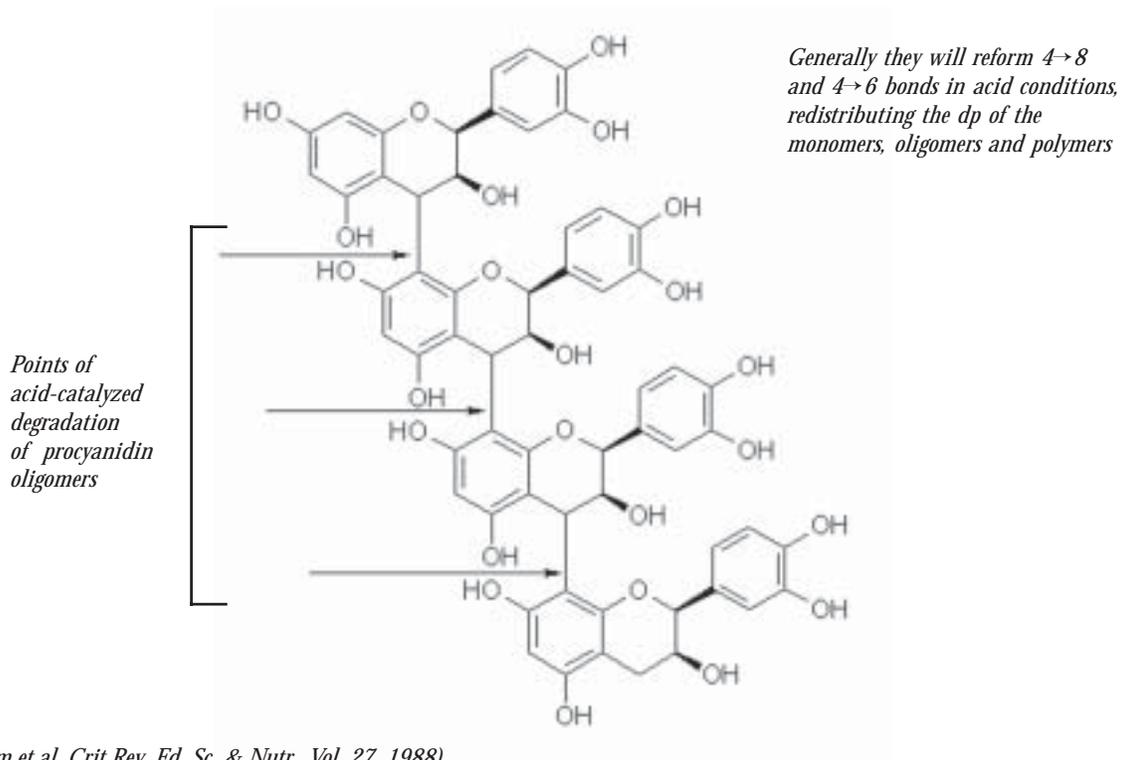
(Cheynier et. al. Proceedings ASEV 50th anniversary annual meeting)

Why is the hydrated or colorless form of the anthocyanin important? Veronique Cheynier has proposed that the hydrated form is actually the reactive form of the anthocyanin. When water hydrates the anthocyanin, it adds 2 electrons to the molecule, which in turn increases the electro-negativity at the 6 and 8 positions on the A ring. Generally, it appears that the 8 position is the favored position of reaction.

Hydrolysis of Procyanidins

Historically, winemakers thought the distribution of various sized, condensed tannins extracted from seeds and skins depended to a high degree on maturity of the grapes. However, Haslam first suggested in 1980 and later wrote in a 1988 review paper, that condensed tannins (polyphenolics) are not stable to hydrolysis. The conditions found in wine (low pH, high acidic conditions) favor hydrolysis at the 4→8 position or the 4→6 position.

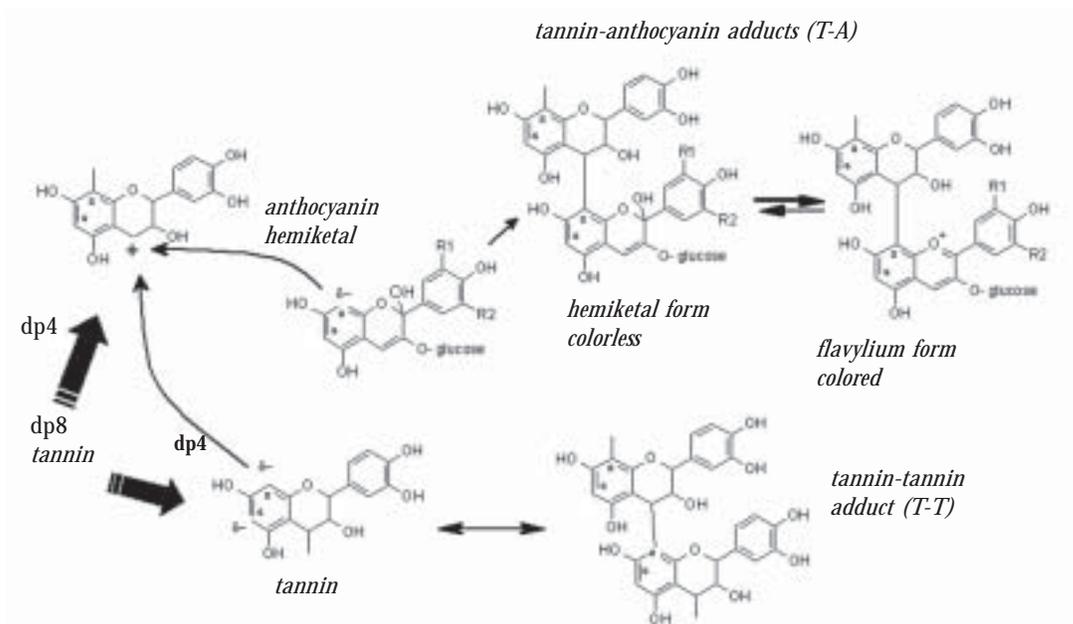
Why is this phenomena important?



Proposed mechanisms leading to T-A and T-T adducts under non-oxidative conditions

Hydrolysis of tannin oligomers or polymers is the central point of a mechanism proposed by Veronique Cheynier under reductive conditions. This mechanism helps to explain compounds isolated from red wines and how these compounds may be formed. As Haslam suggested, a tannin molecule, *eg.* dp 8 (8 catechin or epicatechin units) in length, may be hydrolyzed under acidic conditions in wine. For example, as shown in the above figure, this hydrolysis may produce two shorter oligomers, dp 4 in length. However, the hydrolysis produces one neutral oligomer and one positively charged oligomer (carbocation).

Depending on the concentrations of available tannins or anthocyanins, the carbocation formed will react with one or the other. If the reaction is with another tannin, a longer oligomer or polymer will be formed.



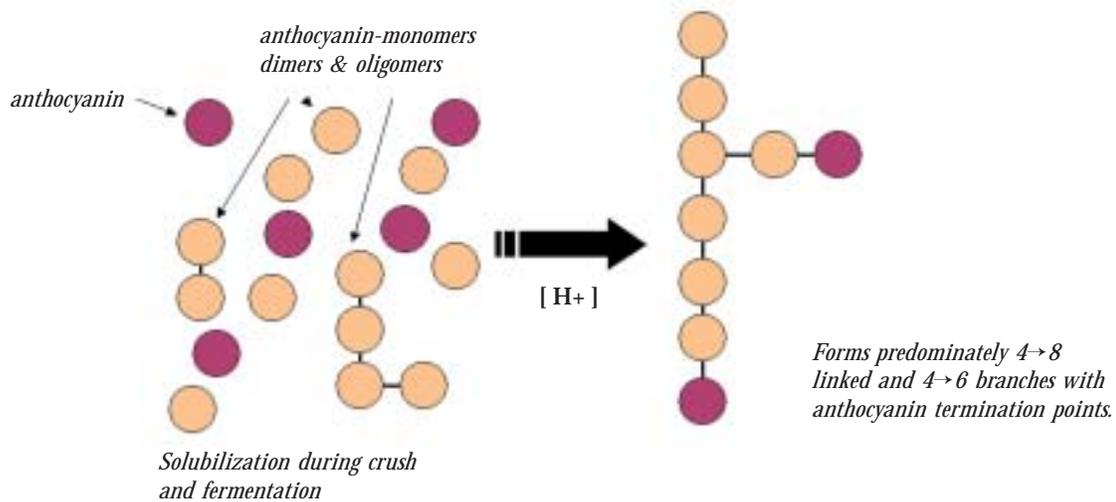
(Cheynier et. al. Proceedings ASEV 50th anniversary annual meeting)

However, the process differs if an anthocyanin is involved. First, note that the anthocyanin must be in the hydrated or colorless form. This form provides an electron-rich molecule which more readily reacts with the formed carbocation.

The reaction occurs between the two molecules at the 4 and 8 positions and a covalent bond is formed. Once formed, the tannin part acts as an electron sink and favors the loss of water (the hydration of the anthocyanin) and a stabilized color or anthocyanin-tannin adduct is formed.

Losing the water of hydration has another unique aspect: the terminal molecule (the anthocyanin) no longer has an excess of available electrons. Thus, the anthocyanin acts as a terminus or terminal quencher for any further reaction at this end of the oligomers or polymers being formed.

Proposed condensation reactions under non-oxidative conditions



Here is a more simplistic representation of the previous page. Reactions under reductive conditions occur more slowly, depending on conditions and availability of reactants.

However, scientists think that tannins formed under these conditions will be longer, with a more uniform structure. The longer and more uniform the tannins, the more likely there will be a strong interaction between the tannin molecules.

This interaction causes an association and aggregation of similar molecules attempting to protect hydrophobic centers (the aromatic rings). Hydroxyls located on the outer portions of the polymers will hydrogen bond, aiding in the stabilization of the molecule, but at the same time excluding water molecules.

Once the aggregate of molecules becomes large enough and excludes enough water it will lose its ability to stay soluble and precipitate from solution.

We see examples of this during extended maceration, especially under highly reductive conditions. At first, it appears that we are extracting more tannin and color. Yet within one to three months, most of this material precipitates from the wine and color is lost.

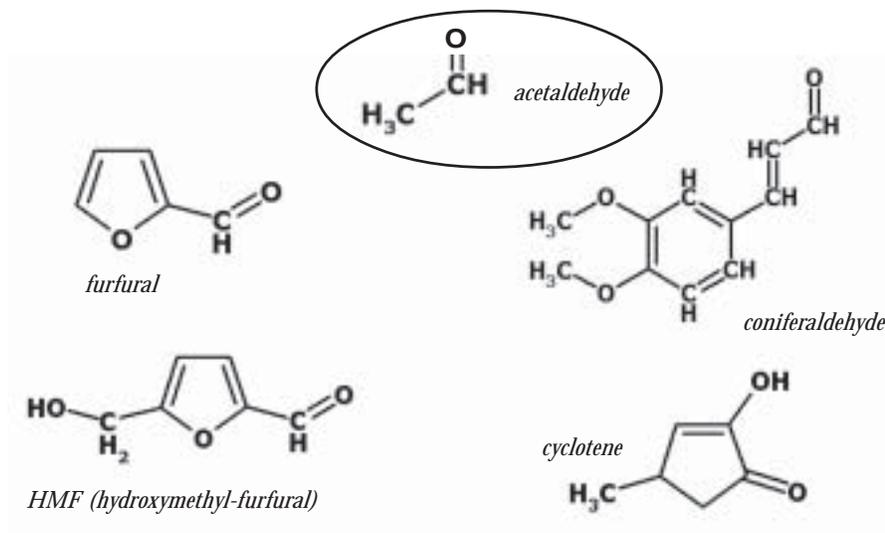
Summary of reactions under non-oxidative conditions

1. Under reductive conditions (no crosslinking by acetaldehyde). A distribution of procyanidin oligomers and polymer sizes will tend to redistribute to a median size. For example, range of sizes of dp 2 to 15, will tend to favor formation of a median size such as dp 7.
2. Anthocyanins will interfere with this process by acting as terminal quenchers. Once an anthocyanin has linked to a terminal end of a polymer or oligomer no further reactions with that molecule will occur, other than losing the sugar moiety.

In model wine solutions, with no interfering compounds, a distribution of procyanidin oligomers should, over time, form an average size molecule.

However, if interfering compounds such as anthocyanins are present, then the distribution of molecule sizes will be much more unpredictable manner.

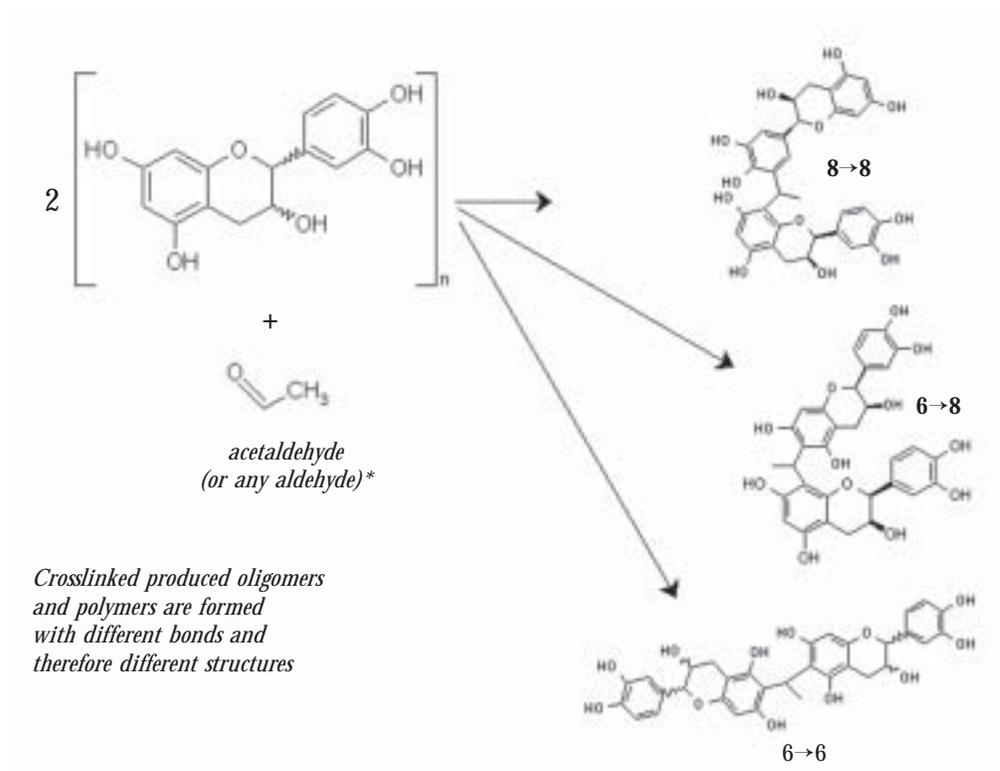
Aldehyde compounds, extracted from oak, capable of crosslinking tannins and anthocyanins



Sugar dehydration products, formed during toasting of oak, *e.g.* furfural and HMF (shown above), are able to crosslink tannins and tannins with anthocyanins. However, Cheynier's group has shown the aromatic aldehydes, *e.g.* vanillin and coniferaldehyde (shown above), have not been found to crosslink tannins. Evidently, there is steric hindrance by the aromatic group. However, the same aromatic group may allow hydrophobic interaction with tannins and anthocyanins, allowing it the aromatic aldehyde to act as a co-factor in co-pigmentation.

Crosslinking procyanidins with aldehydes

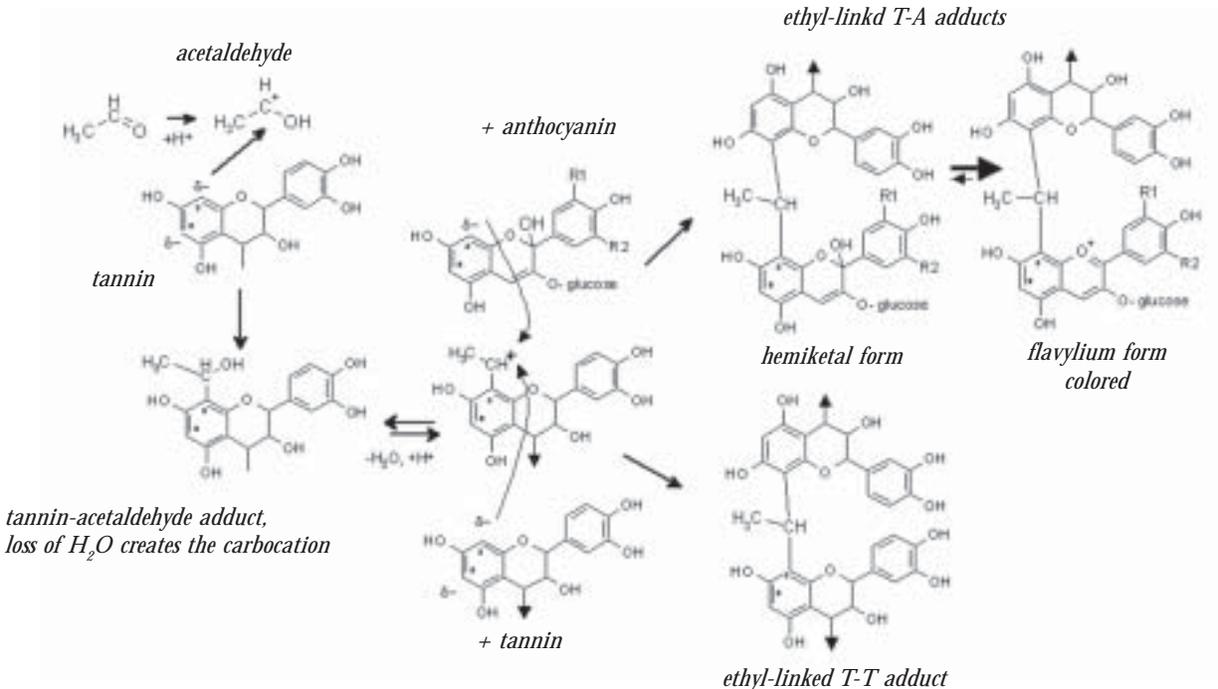
There are clear structural differences between crosslinked procyanidins and natively formed (or reductive linked) procyanidins. The important point here is that different procyanidin or tannin structures can be formed when acetaldehyde or oak extractives are present.



(Montounet et al. *J. Agric. Fd. Chem.*, Vol. 47, pp. 2088–2095, 1999)

*(Montounet et al. *J. Agric. Fd. Chem.*, Vol. 48, pp. 5946–5954, 2000)

Tannin-anthocyanin(T-A) /tannin-tannin (T-T) crosslinking under oxidative conditions

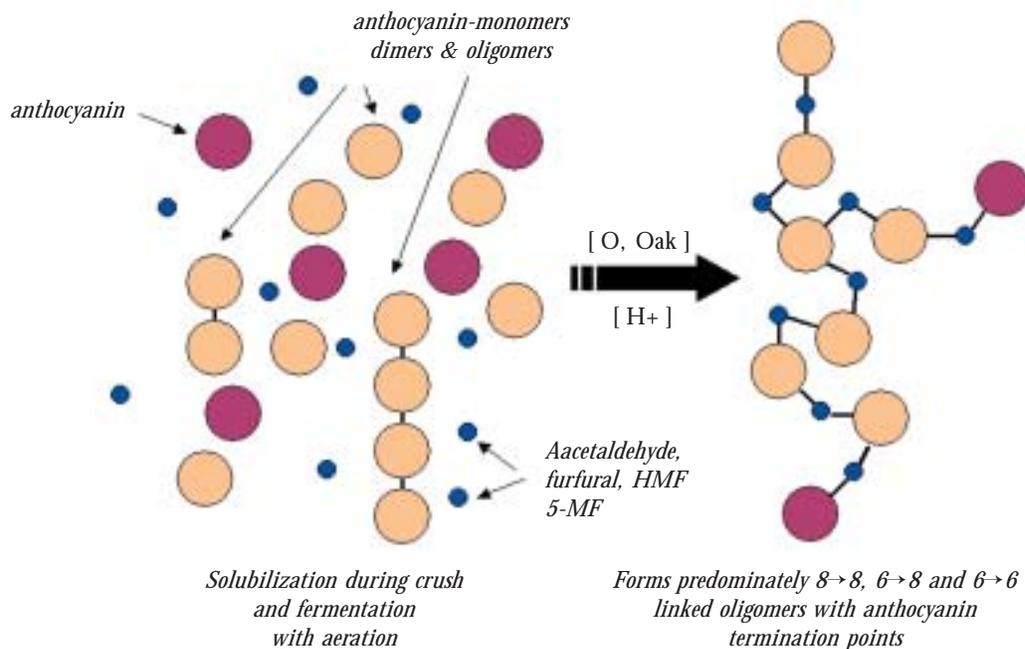


(Cheynier et. al. Proceedings ASEV 50th anniversary annual meeting)

The proposed mechanism of crosslinking between procyanidins and anthocyanins follows:

Acetaldehyde has been shown to react first and preferentially with the procyanidin or tannin, not with anthocyanins. However, as Cheynier's group established, the crosslinker could be furfural, HMF and other oak derived compounds. Secondly, the acetaldehyde tannin adduct will dehydrate to form a carbocation. From this point, the reaction is much the same as described previously for reductively formed adducts. Depending on concentrations and proximity for reaction, the carbocation has the choice of reacting with either tannin or anthocyanin. As before, the anthocyanin reacts in the hydrated or colorless form and the tannin acts as the electro-sink to favor elimination of water, thereby forming the flavylium cation and stabilizing the color.

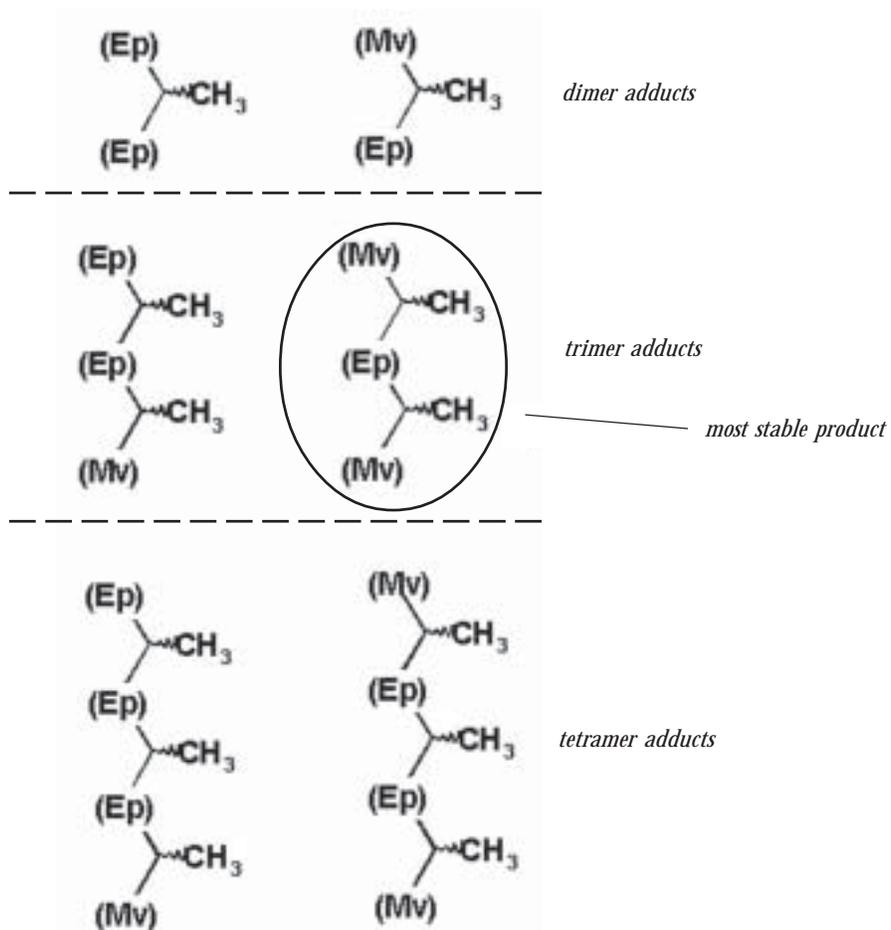
Proposed condensation reactions under oxidative conditions



Above is a simplified pictorial of the crosslinking reaction. The main point of interest is the difference in structure comparing crosslinked vs. reductively formed or native tannin oligomers. The many kinks added into the oligomer or polymer prevent tight association and hence aggregation minimizing precipitation.

The crosslink formed between procyanidins is no more stable than those existing in the native procyanidin. Therefore, they are labile and prone to hydrolysis, just as the native oligomers and polymers are. However, Cheynier's group has found that crosslinks formed with furfural, HMF and 5-methyl furfural are more stable (less prone to hydrolysis) than those made with acetaldehyde. Remember that linking an anthocyanin to a tannin prevents further polymerization at that end. We should see a higher percentage of smaller oligomers formed when acetaldehyde or oak derived crosslinkers are present. This should be especially prevalent during fermentation, when anthocyanins are in high concentration and available for reaction.

Acetaldehyde-induced condensation of epicatechin and malvidin-3-glucoside



(Montounet et al. *J. Agric. Fd. Chem.*, Vol. 47, pp. 2096-2102, 1999)

In model solutions, we should see more smaller polymers formed when enough reactants (procyanidins and anthocyanins) are available. The trimer adduct should be the most stable that is formed.

Building bridges: A summary of important points about crosslinked procyanidins or procyanidin/anthocyanins

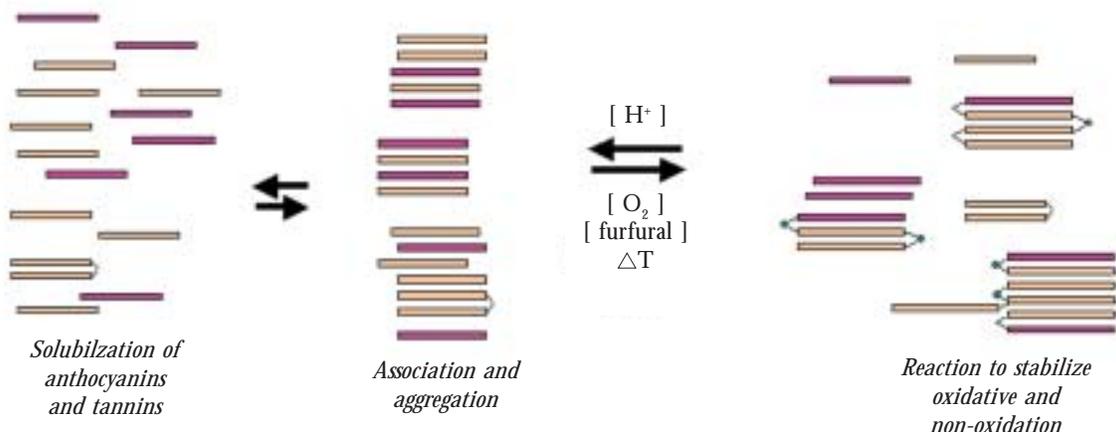


1. Crosslinked oligomers or polymers form different structures than originally extracted from the grape.
2. Like the naturally occurring (4→8) links between monomers, acetaldehyde crosslinks between monomers are hydrolysable under acidic conditions.
3. Flavan-3-ol's, oligomers and polymers linked to anthocyanins act as electron sinks, stabilizing the cation form (flavylium or colored form), thereby minimizing the formation of the hydrated or colorless form of an anthocyanin.
4. Anthocyanins act as terminal quenchers (stopping further polymerization).

Proposed role of co-pigmentation

1. Anthocyanins contain aromatic rings (hydrophobic) which are highly hydroxylated (hydrophillic).
2. The sugar esterified to the 3 position of the C ring helps to maintain solubility.
3. Must conditions favor water soluble compounds; wine conditions are more favorable for hydrophobic compounds.
4. Other compounds present with similar properties will tend to aggregate and stack to protect the hydrophobic centers.
5. Conditions during later fermentation (increasing alcohol and temperature) favor disruption of these aggregated or stacked complexes.
6. Presence of crosslinkers and close proximity of anthocyanins and phenolics polymers (co-pigmentation) favors the crosslinking reaction.
7. Toasted oak provides compounds able to both co-pigment (stack) and crosslink, favoring the formation of stabilized pigments.

Co-pigmentation



A pictorial of tannins and anthocyanins solubilizing is on the left; stabilizing themselves through stacking and aggregation is in the middle. On the right (depending on conditions), tannins and anthocyanins will either bond directly or cross link if acetaldehyde, furfural, HMF or 5-methyl furfural are present.

It is the relative proximity and orientation of the molecules that may be the most important role of co-pigmentation. This closeness and arrangement of the molecules may aid in the crosslinking reaction, much in the manner that enzymes work when catalyzing reactions between molecules.

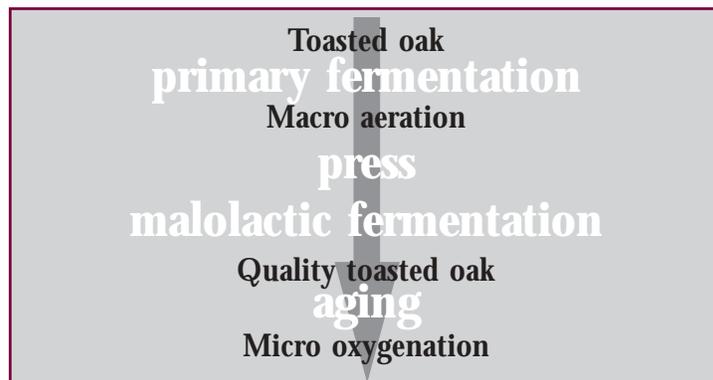
Creativity and innovation leads to discovery



1. Presence of toasted oak extractives helps to induce the production of more stable pigments and procyanidin/prodelphinidin oligomers and polymers.
2. The combined use of toasted oak and micro oxygenation will produce high quality wines with: enhanced body and color, substantially reduced labor costs, increased control of sanitation and *minimized dependence on barrels.*

Evidence now exists to support what many winemakers have experienced when 1) adding toasted oak and macro aeration during fermentation, and 2) adding high quality toasted oak combined with micro oxygenation for aging. *Red wines will be improved with the proper use or introduction of these techniques during fermentation and aging.*

However, we still do not have a clear view of where to push a wine. What is the optimal ratio of crosslinked to direct linked oligomers or polymers? What is the desired ratio, or percentage of crosslinks to natively formed links? Or, what is the preferred ratio of skin tannins to seed tannins? Finally, is there even a proper ratio of these tannins to seek – or does it change with variety and vintage?



Opportunities for your winery to explore

Suggested trials to determine if oak, aeration, and micro oxygenation will work for you:

1. Experiment with lots of grapes and/or wine where you can conduct side-by-side trials.
2. Oak vs. no oak in the fermentor.
3. Air vs. no air during fermentation.
4. Press onto oak vs. no oak.
5. Compare barrel lots to stainless tanks.
6. Compare barrel lots to stainless tanks with oak and oxygen.

We at StaVin hope this information provides a greater understanding of using toasted oak and oxygenation to produce better red wines.

