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CURRENT THEORY AND APPLICATIONS
Micro-oxygenation

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Micro-oxygenation, like many procedures in the wine industry, can be an important component of the winemaker's toolbox. The purposeful, measured addition of oxygen to wine is a relatively recent practice that is still not fully developed.

The following is a review of the current theory of micro-oxygenation, its application, and ways in which winemakers can determine if micro-oxygenation is desirable for a particular wine.

Wine stored in barrels generally has more intense color and softer tannins than the same wine stored in stainless steel. The difference between the two is the oxygen exposure that occurs in barrels. Both barrel storage and aeration in tanks cause a decrease in free anthocyanin content, and an increase in color intensity and phenol polymerization (see Table I and Figure 1).

The potential goal of micro-oxygenation is to evolve the phenolic and aromatic characteristics of a wine by controlling oxidative changes. A wine's total capacity for oxygen saturation is approximately 6.3 ml/L at 20°C (68°F). Saturation levels increase with decreasing temperature and increasing pressure.[9]

It has been estimated that ullage from proper barrel storage adds as much as 15 to 20 ml/L per year of oxygen to wines. Therefore, allowing for several rackings, a total of around 30 ml/L could be added to red wine in barrel each year.[8]

The ability of a wine to handle oxygen depends on a number of factors, including the quantitative and qualitative nature of phenols. There is not a simple relationship between a wine's total phenol content and its oxygen capacity.[9]

During a typical racking, it is difficult to control the quantity of oxygen received and the result. The advantage of micro-oxygenation is that it is a controlled process in which a winemaker can quantify oxygen exposure. Figure II illustrates some general relationships among wine type, oxygen exposure, and quality scores.

Phenolic compounds in wine play a major role in both the color and mouthfeel and are important components impacted by oxygen. Phenolic components include a wide diversity of chemical structures and are grouped into two major divisions: non-flavonoids and flavonoids. The phenolic content in juice and wine is made up mostly of non-flavonoids and flavonoid phenols.[5] The non-flavonoids usually include simple phenols with basic structures such as benzoic and hydroxycinnamic acids.

The flavonoids contain a three-ring, 16-carbon structure. This group includes compounds such as anthocyanins, and tannin-building blocks such as polymeric flavan-3-ols, referred to as proanthocyanidins or condensed tannins. Thus, tannins are derived from simple flavonoids mainly from the skins and seeds, and are produced by oxidative or chemical polymerization to form larger structures.

Chemistry of oxygen in wine

Phenolic reactions in wine can generate modified tannins, degrade existing tannins, or generate new ones. As such, polymerization and de-polymerization of tannins, and of tannins and anthocyanins, greatly impact their sensory characteristics. Winemakers have traditionally assumed that the distribution of various-size condensed tannins extracted from the seeds and skins depended entirely on fruit maturity. However, condensed tannins are likely

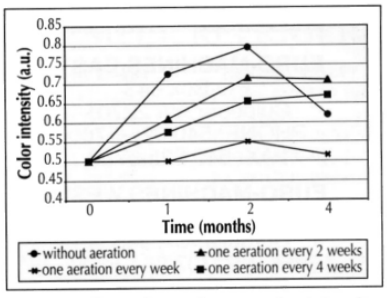

Figure I. Effect of aeration on color intensity of red wine over time; from Ribereau-Gayon and Glories (1983).

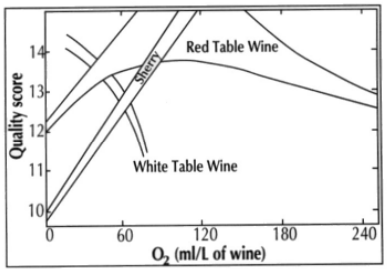

Figure II. Quality responses of two white wines, two red, and a chermat after weekly oxygenation for up to a year; from Singleton (2000).

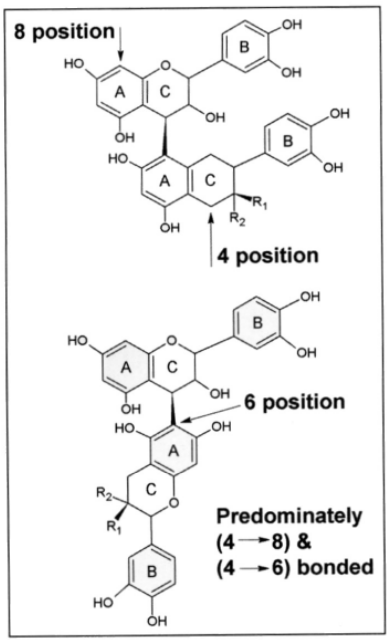

Figure III. Flavonoid phenol polymerization binding sites.

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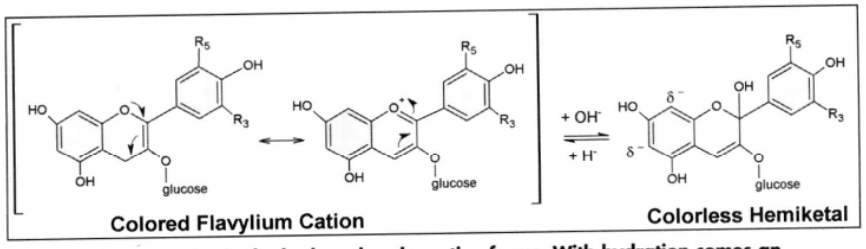

Figure IV. Anthocyanins in the hydrated and reactive forms. With hydration comes an increase in electronegativity at the 6 and 8 positions.

not stable. Hydrolysis at the C4-C8 position or the C4-C6 position likely occurs (Figure III).

With oxygen exposure, several different structural linkages can occur creating tannin polymerization. Polymerization reactions that occur between anthocyanins and tannins may generate stable compounds, which provide more color intensity and are more resistant to degradation.[1]

Anthocyanin molecules have a positive charge that enables them to absorb light and thus have color. This positive charge is usually assigned to the oxygen atom in the aromatic C-ring structure; however, the charge is widely spread throughout the molecule (Figure IV). The positive charge increases the reactivity of the ring structure, which can lead to the destruction of the positive charge. This is countered by binding with tannin molecules, such as can occur with micro-oxygenation. The degree to which tannins and anthocyanins bind together is, in part, a function of the concentration of these molecules in solution.

An anthocyanin also has a carbohydrate (sugar), usually glucose, esterified or bound at the carbon-3 position. Naturally occurring pigments from grapes always have a sugar bonded at the carbon-3 position (though other compounds can be involved, such as

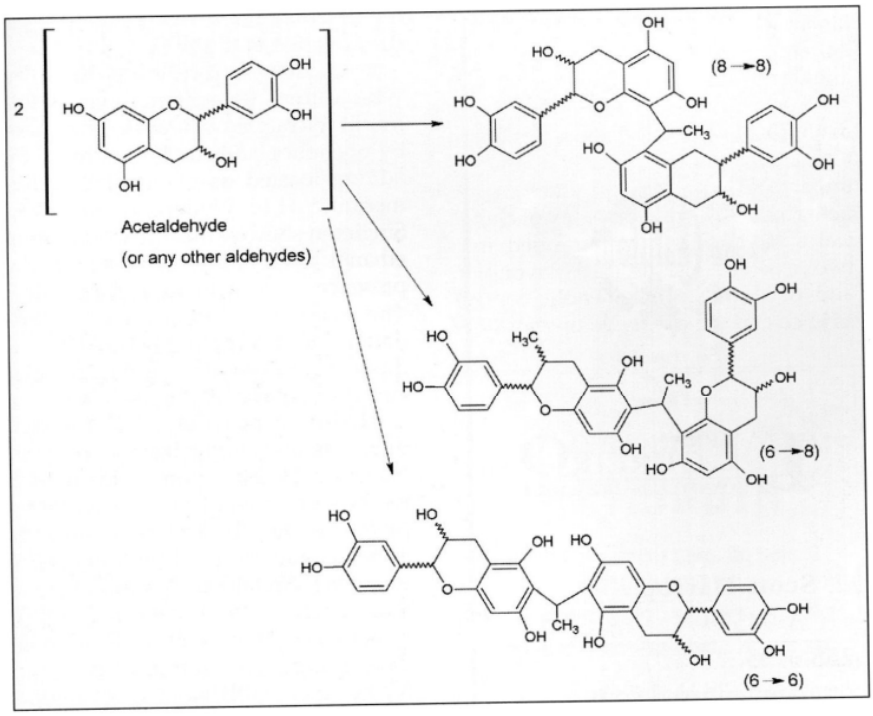

Figure V. Acetaldehyde bridging.

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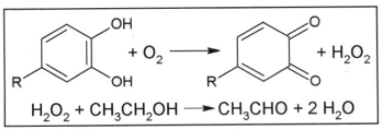

Figure VI. Oxygen leading to the production of acetaldehyde through a vicinal diphenol on a flavonoid phenol.

acetic acid and hydroxycinnamic acid). The presence of this sugar helps the anthocyanin maintain solubility. If the sugar is hydrolyzed or removed, the solubility decreases and the molecule will be destabilized and lost (a problem, for example, noted in wines that have experienced *Brettanomyces* growth).[3]

Anthocyanins and tannins bind together in two ways, depending upon the oxygen concentration. Under reductive conditions (low redox potential), hydrolysis may break down a tannin molecule, producing two products, one charged molecule and one neutral molecule. Depending on the concentrations of tannins and monomeric anthocyanins, the charged molecule formed will react with one or the other. If it is another tannin, a longer oligomer or polymer will be formed.

However, the process differs if an anthocyanin is involved. An anthocyanin, in the hydrated or colorless form (Figure IV), provides an electron-rich molecule which more readily reacts with the charged tannin. The reaction occurs between the two molecules at the carbon-4 and carbon-8 positions, and a covalent bond is formed. Once formed, the larger tannin moiety acts as an electron sink and a stabilized color or anthocyanin-tannin adduct is produced. The terminal molecule, the anthocyanin, no longer has available electrons in excess to further react, meaning that the anthocyanin acts as a terminus for any further reaction at this end of the polymer.

The other tannin-anthocyanin reaction method involves oxidative polymerization. As such, acetaldehyde can play an important role in the formation of phenolic polymers in a wine and, thus, in micro-oxygenation.

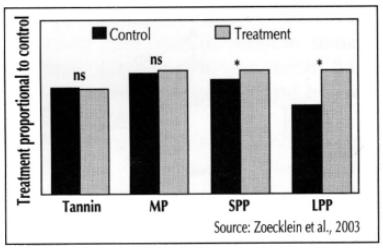

Figure VII. Effect of micro-oxygenation of Merlot wine on the relative concentration of total tannin, monomeric pigments (MP), small polymeric pigments (SPP) and large polymeric pigments (LPP).

Acetaldehyde-bridged molecules form to bind phenolic compounds together. These compounds are relatively stable and are somewhat resistant to bleaching by bisulfite ion (which is why sulfite bleaching is used in some assays). Acetaldehyde bridging can also facilitate the formation of tannin-tannin complexes. Acetaldehyde linkages usually lead to C8-C8 bonding instead of C4-C8.[1] This can lead to different sensorial properties. Cross-linking of procyanidins with aldehydes is illustrated in Figure V.

Acetaldehyde can be produced by yeast during fermentation, can result from the coupled oxidation of ethanol by phenolics, and can be produced by adding toasted oak wood into a fermentor.[10] H.L. Wildenradt and V.L. Singleton showed that the oxidation of ethanol to acetaldehyde occurs in the presence of O_2 at an appreciable rate.[12] This coupled reaction involves the oxidation of a simple phenol (vicinal diphenol) to produce a colored molecule (orthoquinone) (Figure VI).

Hydrogen peroxide (H_2O_2) is produced as an intermediary of coupled oxidation. H_2O_2, a strong oxidant, then reacts with ethanol to form acetaldehyde (Figure VI). The newly-formed acetaldehyde can react with phenolics in a wine. Acetaldehyde forms a polymerization product between anthocyanins and tannins through an aldehyde bridge, for example. These can further react with other procyanidins

Table I: Effect of conservation conditions on the evolution of the color of red wine over a 16-month time period. From *J. Ribereau-Gayon and Y. Glories (1983)*

Conditions of conservation	Antho-cyanins (mg/L)	Color intensity (a.u.)	Poly-merized pigments index
Non-aerated tank	340	0.63	56
Aerated tank	240	0.72	66
Wooden barrel	240	0.75	64

Note: Initial anthocyanins content 500mg/L; initial color intensity 0.6

or anthocyanins, to form more complex trimers.

In order for this to proceed, adequate levels of acetaldehyde must be available. SO_2 will readily bind to any free acetaldehyde, thus removing it as a reactant. (For this reason, sulfite addition can be used to evaluate the sensory impact of acetaldehyde in an "aroma screen.")[13]

In order to achieve the desired results from acetaldehyde-induced coupling, binding must occur before the wine is sulfited, or the free SO_2 level should be low (15 ppm depending on pH). This usually means SO_2 additions are postponed until after micro-oxygenation is complete.

Acetaldehyde can also bind with another procyanidin molecule, instead of the anthocyanin as above, with the reaction proceeding in the same way. The reaction may continue to produce more highly polymerized molecules. It is thought that the reaction stops when anthocyanin molecules "cap" either end of the structure.[3]

A goal of micro-oxygenation is to modify phenolic compounds, especially polymerized tannins, to create a more rounded, softer mouthfeel in the wine. Preventing extensive tannin-tannin polymerization from occurring may help in accomplishing this goal.

Micro-oxygenation/wine structure

The sensory perception of astringency is due to the interaction between polyphenols and salivary proteins in the mouth. These result from the hydrophobic and hydrogen-bonding effects of the phenolic compounds.

A wine's astringency has been found to be largely a tactile sensation. This has lead to the idea that the interaction between polyphenols and salivary proteins results in insoluble aggregates that precipitate and obstruct the palate lubrication, thus causing the unpleasant sensation of roughness, dryness, and constriction.[4] It has been found that the extent to which condensed tannins elicit astringency increases with the degree of polymerization,[7] although this is an oversimplification.

It is believed that micro-oxygenation may affect astringency by changing the way tannins polymerize and create large globular structures[14] (Figure VII). It may help to do this by increasing the reaction of procyanidins with other molecules, thereby limiting aggregation.

Another way oxygen could affect procyanidin interactions is by increasing the proportion of C4-C6 linkages and thus reducing the number of C4-C8 linkages. Procyanidin dimers linked through a C4-C8 inter-flavonoid bond have consistently greater tannin specific activity (TSA) for proline-rich proteins (such as saliva) than their counterparts with a C4-C6 linkage. Thus, C4-C8-linked phenols are perceived as being bigger and more aggressive.

The addition of acetaldehyde linkages promotes tannin polymerization through C4-C6, and C8-C8 bonds. These linkages may also have smaller TSAs than their C4-C8 counterparts, resulting in a more supple perception.

Tannin polymerization stops when anthocyanins occupy the terminal ends of the structure.[3] By increasing the potential for forming these bonds, it may be possible to decrease the size of the condensed tannin polymer. However, this is highly dependent on the stage that the tannin molecules are in, prior to oxidation. If the procyani-

din molecules are only slightly polymerized, their condensation with the anthocyanins leads to formation of stable end-products with greater spectral color.

However, this same condensation, along with tannins that are already highly polymerized, forms unstable pigments that will precipitate. For this reason, aeration has been found to be most effective during the period immediately following completion of yeast fermentation, prior to extensive polymerization.

At that time, tannin molecules are only slightly polymerized, and their condensation with anthocyanins in the presence of air leads to stable, colored pigments. If aeration occurs later, the tannins are then polymerized, and further condensation with the anthocyanins causes their precipitation.[2]

However, many other factors also affect the intensity of astringency besides the size, or degree of polymerization, of tannins in a wine. Ethanol, acidity, viscosity or sweetness, and the concentration of phenolics all affect astringency in wine.

Table II: Correlation coefficients between wine sensory attributes, each correlated independently against others.

		R value (correlation coefficient)						
		Fruit	Vegetative	Oxidation	Off aroma	Green tannin	Tannin grit	Plushness
P value	Fruit		–0.938	–0.448	–0.958	–0.836	–0.590	0.784
	Vegetative	0.0006***		0.354	0.862	0.910	0.796	–0.782
	Oxidation	0.2661	0.3903		0.600	0.127	0.233	–0.389
	Off aroma	0.0002***	0.0059*	0.1184		0.711	0.471	–0.770
	Green Tannin	0.0097**	0.0017**	0.7642	0.0482*		0.841	–0.824
	Tannin-Grit	0.1237	0.018*	0.5783	0.2394	0.0088*		–0.721
	Plushness	0.0212*	0.0218*	0.3410	0.0253*	0.0119*	0.0436*	

*indicates significance at p≤0.01
**indicates significance at p≤0.05
Source: Sullivan, P., 2001, MS thesis.

Micro-oxygenation/wine aroma

The introduction of oxygen may help to modify wine aromas. It has long been known that aeration can impact some sulfur-like odor compounds, either by volatilization, oxidation, and/or slight changes in the oxidation-reduction potential. For example, hydrogen sulfide can react with oxygen to form water and elemental sulfur, although the extent of that reaction is likely limited:

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$$2H_2S + O_2 \leftrightarrow 2H_2O + 2S$$

The above reaction is governed by the wine's redox potential, and can go back the other way.

Micro-oxygenation can change the redox potential and convert some wine thiols to disulfides. For example, the transformation of methanethiol (methyl mercaptan) to dimethyl disulfide results in a change in the sensory character and threshold due to a small change in molecular structure. The perception threshold goes from about 2 ppb to 12 ppb.

$$2\ CH_3SH + \tfrac{1}{2}\ O_2 \leftrightarrow CH_3SSCH_3 + H_2O$$

Methyl mercaptan **Dimethyl disulfide**

This helps to explain how micro-oxygenation can "clean-up" and help aromatically "balance" some wines. It should be noted, however, that the above reaction is reversible. As such, wines bottled and stored under reducing conditions can have the disulfide reduced back to the thiol.

Another way in which oxygen may affect the sensory properties of micro-oxygenated wines is through the transformation of certain vegetal aromas, or their perception. Compounds such as 2-methoxy-3- (2-methylpropyl) pyrazine can be detected by humans in water in concentrations as low as 2 ng/L. This chemical contributes to the characteristic aroma of vegetables such as bell peppers. The highest concentrations are found in the coldest fruit maturation conditions.[6] The authors have reported reduction of herbaceous character as a result of micro-oxygenation.[13]

Initially, we presumed this reduction was the result of transformations or changes in the pyrazines. That appears not to be the case. Rather, the lowering of the perception of herbaceousness of some wines with micro-oxygenation may be the result of changes in thiols (sulfur-like off-odors). This demonstrates the sensory relationships between thiols and herbaceousness. Thiol odor in wines complements the herbal perceptions from pyrazines and, indeed, some thiols may contribute to "green"-type odors.

Brettanomyces can decarboxylate 4-vinylphenol to 4-ethylphenol. One important concern with regard to micro-oxygenation is that it can stimulate *Brettanomyces* growth. Careful monitoring of 4-ethylphenol production and "Brett" plating should occur for wines treated by micro-oxygenation.

Table II lists some wine sensory descriptor correlations. These relationships illustrate some changes in wine sensory attributes that may be achieved with micro-oxgenation.

Is micro-oxygenation for you?

Micro-oxygenation can be an important addition to a winemaker's tool box, but it must be used with discretion, and it will take a learning curve to determine how it will work in

a given winery. There are several ways in which you can determine if micro-oxygenation is in your future.

The easiest way is to arrange for a trial through a company that sells micro-oxygenation equipment. The basic outline of the experiment follows, but the details would need to be developed in concert with your chosen supplier.

Pick a wine that will most likely give you the biggest income or quality boost, take one barrel of that wine and follow it through a cycle that ideally would begin post-fermentation and pre-malolactic, and then treat it with small to medium amounts of oxygen for each time frame. Take dual samples every week, and top off the barrel with wine to replace those samples.

Run the experiment through to the bitter end where the wine seems as if it is totally over the hill (it is important to work out the actual steps with your supplier). Take those samples, put them aside for several months, and then open them all at once and evaluate them against your control. You should see the influence of the oxygen on the wine and you should be able to make a judgment from that experiment. ■

To receive reviews on micro-oxygenation and other topics, see the Enology-Grape Chemistry Group, Virginia Tech website: www.vtwines.info.

This text was revised from original publication in Wine East, *September/October 2002.*

Patrick Sullivan was a Master's student under Bruce Zoecklein and others at California State University Fresno, and is now an enologist at Paul Hobbs Winery in Sebastopol, CA. Some content was adapted from his MS thesis.[11]

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