THEORY AND APPLICATIONS OF MICRO-OXYGENATION

Note: Adapted, in part, from an article by B.W. Zoecklein, R. Cary, and P. Sullivan.

Learning Outcomes: *The student will understand the principles and practices of micro-oxygenation as a winemaking tool, and its effects on aroma and structure.*

Chapter Outline

Introduction to Micro-Oxygenation

Chemistry of Oxygen in Wine

Micro-Oxygenation and Wine Structure

Micro-Oxygenation and Wine Aroma

Micro-Oxygenation Equipment

Applications of Oxygenation in Wine

Why Micro-Oxygenation?

Is Micro-Oxygenation for You?

Reductive Strength

Tannins and Wine Quality

Section 1.

Introduction to Micro-Oxygenation

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, as illustrated in Table 1 and Figure 1.

Table 1. Effect of Storage Conditions on 16-Month Color Development in Red Wine (Ribéreau-Gayon et al., 1983)

Conditions of conservation	Anthocyanins (mg/L)	Color intensity (a.u.)	Polymerized pigments index
Non-aerated tank	340	0.63	56
Aerated tank	240	0.72	66
Wooden barrel	240	0.75	64
Initial anthocyanin content 500 m/L; initial color intensity 0.66.			

Figure 1. Effect of Aeration on Color Intensity of Red Wines Over Time (Ribéreau-Gayon et al., 1983)
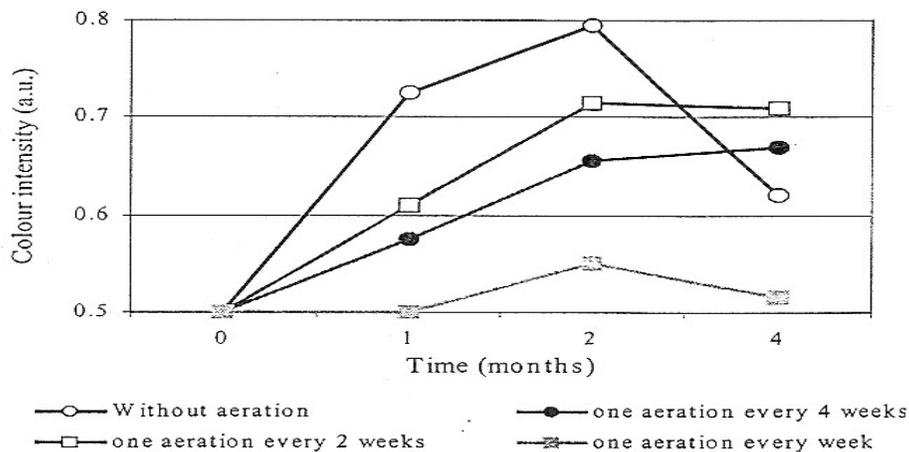
The fact that some aeration can help improve red wines has long been known, and it was the basis for the initial research on micro-oxygenation. Often, wines are splash-racked, or moved from one tank or barrel to another, to facilitate aeration. However, too much racking or aeration can be detrimental to a wine. Knowing how much aeration a red wine needs is important in making high-quality wine.

The potential goal of micro-oxygenation is to evolve the phenolic and aromatic characteristics of the wine by controlling oxidative changes. A wine's total capacity for oxygen saturation is around 6.3 mL/L at 20°C (68°F). Saturation levels increase with decreasing temperature and increasing pressure (Singleton, 2000).

It has also been estimated that ullage (headspace) 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 a barrel each year (Ribéreau-Gayon et al., 1983).

The ability of a wine to handle oxygen depends on a number of factors, including the quantitative and qualitative nature of its phenols. There is not a simple relationship between a wine's total phenolic content and its oxygen capacity (Singleton, 2000). Wine phenols range from unreactive to highly reactive with oxygen, have different reaction rates, and are dependent upon what happens to the initial reaction products.

Therefore, 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 the winemaker can quantify the oxygen exposure. Figure 2 illustrates some general relationships among wine type, oxygen

exposure, and quality scores.

Figure 2. Quality Responses of Two White Wines, Two Red, and a Sherry After Weekly Oxidation with Air at Saturation for up to a Year
(Singleton, 2000)
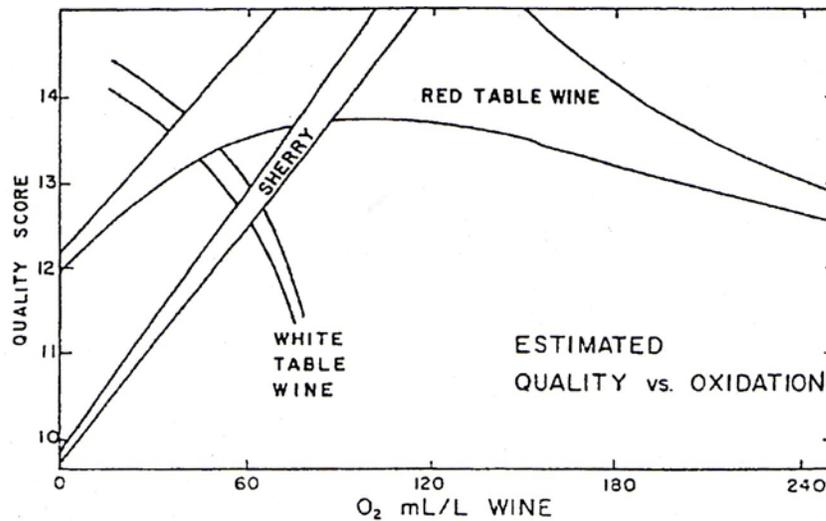
Phenolic compounds in wine play a major role in both the color and mouthfeel, and are important components impacted by oxygen. Phenolic components include a wide diversity of chemical structures and are grouped into two major divisions: non-flavonoids and flavonoids. The non-flavonoids usually include 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, through oxidative or chemical polymerization, 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-sized condensed tannins extracted from the seeds and skins depended entirely on fruit maturity. However, condensed tannins are not likely stable. Hydrolysis at the 4→8 position or the 4→6 position likely occurs (Figure 3).

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 (Cheynier et al., 2000).

Figure 3. Structure of the Procyanidin Dimers from *V. vinifera*

8 position

Procyanidin B₁   R¹=OH, R²=H
Procyanidin B₂   R¹=H, R²=OH   4 position

6 position

Predominately
(4→8) & (4→6)
bonded

Procyanidin B₅   R¹=H, R²=OH
Procyanidin B₇   R¹=OH, R²=H

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 4).

Figure 4. Major Anthocyanin Forms at Wine pH; Hydration Increases the Electronegativity at the Carbon 6 and Carbon 8 Positions
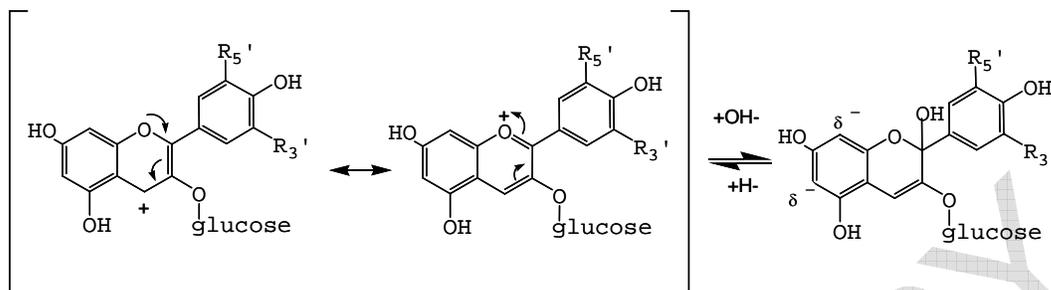
The positive charge increases the reactivity of the ring structure, which can lead to the destruction of the positive charge. This is countered by binding with tannin molecules, such as can occur with micro-oxygenation. The degree to which tannins and anthocyanins bind together is, in part, a function 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 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 *Brett* growth).

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 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 -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 formed. The terminal molecule, the anthocyanin, no longer has available electrons in excess to 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. Acetaldehyde-bridged molecules form to bind phenolic compounds together. These compounds are stable at higher pH values and are more resistant to bleaching by bisulfite ion.

Acetaldehyde bridging can also facilitate the formation of tannin-tannin complexes. Acetaldehyde linkages usually lead to C8-C8 bonding instead of C4-C8 (Cheynier et al., 2000). This can lead to different sensorial properties. Crosslinking of procyanidins with aldehydes is illustrated in Figure 5.

Figure 5. Crosslinking Procyanidins with Aldehydes
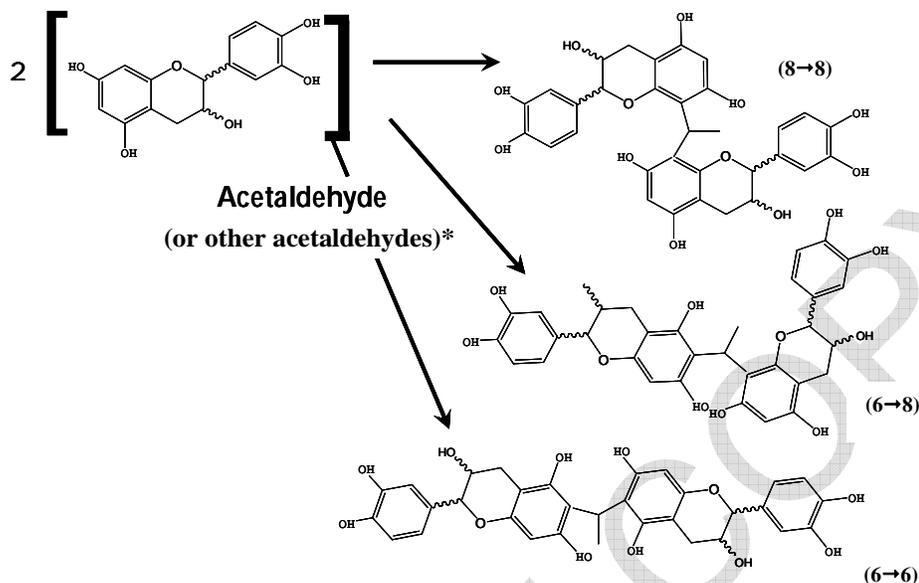
Acetaldehydes can be produced by yeast during fermentation, can result from the coupled oxidation of ethanol by phenolics, and can be produced by adding toasted oak wood into a fermentor.

Figure 6. Coupled Oxidation (Wildenradt and Singleton, 1974)

$$H_2O_2 + CH_3CH_2OH \longrightarrow CH_3CHO + 2 H_2O$$
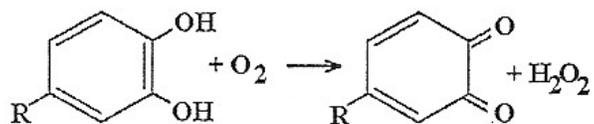
Wildenradt and Singleton (1974) showed that the oxidation of ethanol to acetaldehyde occurs in the presence of O_2 at an appreciable rate. This coupled reaction involves the oxidation of a simple phenol (vicinal diphenol) to produce a colored molecule (orthoquinone) (Figure 6).

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 6). The newly-formed acetaldehyde reacts with phenolics in the wine. Acetaldehyde forms a polymerization product between, for example, anthocyanins and tannins through an aldehyde bridge. These can further react with other procyanidins 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. In order to achieve the desired results from acetaldehyde-induced coupling, binding must take place before the wine is sulfited, or the SO_2 level must be low. 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. In this case, the reaction proceeds 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 (Fulcrand et al., 1996).

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 accomplish this.