THEORY AND APPLICATIONS OF MICRO-OXYGENATION

Section 2.

Micro-Oxygenation and 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 leads 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 (Gawel, 1998). It has been found that the extent to which condensed tannins elicit astringency increases with the degree of polymerization (Lea and Arnold, 1978).

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

Another way oxygen could effect 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 TSA (tannin specific activity) for proline-rich proteins 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 perception of more suppleness.

Tannin polymerization stops when anthocyanins occupy the terminal ends of the structure (Fulcrand et al., 1996). By increasing the chance of forming these bonds, it may be possible to decrease the size of the condensed tannin polymer. However, this is highly dependent on the stage of the tannin molecules before oxidation. If the procyanidin molecules are only slightly polymerized, their condensation with the anthocyanins leads to the 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, the tannin molecules are only slightly polymerized, and their condensation with the 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 (Dournel, 1985).

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

Micro-Oxygenation and Wine Aromas

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:

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

The above reaction is governed by the wine's redox potential and is reversible. Oxygen may also affect the sensory properties of micro-oxygenated wines 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 grape maturation conditions (Lacey et al., 1991). The authors have reported reduction of herbaceous character as a result of micro-oxygenation (Zoecklein, 2002).

Initially, we presumed that this reduction was the result of transformations or changes in the pyrazines. That appears not to be the case. Rather, the change in the aromatic profile of some wines with micro-oxygenation is 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.

Micro-oxygenation can change the redox potential and can 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.

$$2\ CH_3SH + ½\ O_2 \Rightarrow CH_3SSCH_3 + H_2O$$

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.

It is well-known that wine exposed to air will spoil quickly. *Acetobacter* will aerobically convert ethanol to acetic acid in wine, leading to increased volatile acidity in the wine (Zoecklein et al., 1995). High levels of volatile acids can lead to wine spoilage. Yeasts can also oxidize ethanol to create acetaldehyde, as is carried out in the production of sherries.

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

Table 2. Correlation Coefficients Between Wine Sensory Attributes, Each Correlated Independently Against Others (Sullivan, 2000)

Attribute	Fruit	Vegetative	Oxidation	Off Aroma	Green Tannin	Tannin Grit	Plush-ness
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 statistically significant ($p \leq 0.05$) ** indicates highly statistically significant ($p \leq 0.01$)
***indicates very-highly statistically significant ($p \leq 0.001$)

Micro-Oxygenation Equipment

Several types of micro-oxygenation equipment are available. The winemaker would need to take into account the temperature, pressure, and resistance to flow (such as wine head pressure) in order to calculate the number of milligrams per liter of oxygen delivered. Equipment consisting of a gas chromatograph flow-controller is the least expensive, but puts the burden on the winemaker to be sure that the wine is receiving the proper dose.

OenoDev developed its micro-oxygenation equipment with electro-mechanical devices that would allow automation of some control parameters, but not of others. Their equipment has a very precisely-calibrated volume chamber that is filled to a defined pressure; then, under a controlled sequence, a valve is opened and the pressure is released through the transfer tube to the tank.

With some units, design criteria for the calibrations were based on a 2.5m liquid head height at 20°C. Accordingly, the amount of oxygen delivered depends on the temperature and head height. If either parameter changes, then additional calculations must be made to ensure that the desired amount of oxygen is delivered.

Parsec developed a microprocessor-based control system that uses a defined volume chamber in which the gas is pressurized to a defined pressure. When the gas is slowly released, pressure transducers provide a continuous data output of pressure and temperature changes. Parsec's process algorithm uses the data output from the transducers to calculate the actual milligrams of oxygen that have passed that release point in real time.

Because the Parsec unit has two additional control settings, the winemaker can select a desired number of milligrams of oxygen per liter of wine, and a tank size in hectoliters. Thus, the winemaker has a precise delivery mechanism to assure that the oxygen is accurately delivered and that the dosage can be repeated when desired. Accurate delivery from batch to batch, and vintage to vintage, translates into valuable data that can be applied to future vintages to reflect the winemaker's choices for a wine.

Applications of Oxygenation in Wine

From the earlier discussion under chemistry, it is apparent that oxygen does affect the tactile sensation of wines, and that transfers into various complex interactions between the flavor and feel of tannins, the wine's color, and the perception of aged quality in the wine. All of these interactions happen at an accelerated rate when the wine is being treated with oxygen.

Because the effect of oxygen is as an accelerant of natural reactions in wines, this technique is not for the faint of heart. The act of infusing oxygen into a wine sets reactions into motion. It is as if the winemaker has started an ocean liner moving into the harbor. To be sure the liner stops at the dock and does not run aground, the winemaker must stop the treatment sufficiently before achieving the desired effect. If not, the momentum that the wine gains from oxygen infusion will

result in overshooting the desired balance, and will likely ruin the wine.

There are three stages in the winemaking cycle where oxygenation can be used: first, during fermentation; second, post-fermentation, but before malolactic fermentation (MLF); and third, post-fermentation and post-malolactic. Each has its own advocates and reasons for its use, and a suggested protocol.

The problem with this technique of adding oxygen is that it is so new – and we know only a fraction of the chemistry necessary to make definitive judgments about what is, or is not, good practice – that a lot of continued personal experimentation or reliance on others for their input will be necessary for some time to come.

Micro-Oxygenation During Fermentation

Please note that this section deals with large scale injection of oxygen into primary fermenting juice. It has been included to note that oxygen is important in the making of good wine. Adding oxygen by splash pump-over or by splash-racking has been practiced by winemakers for a very long time.

However, the concerted use of oxygen in wine through either the OenoDev clicquer device, or the macro-oxygenation setting on the Parsec equipment, will change the rate of oxygen delivery from mg/L/month to mg/L/day. The maximum oxygen addition is about 6 mg/L/day, with an average of 2 to 3 mg/L/day. The purpose of the oxygen addition is to help the yeast cells build healthy cell walls. The sterols necessary for this purpose are aided by the availability of more oxygen.

The target window for oxygen infusion is during the first third of primary fermentation. After that point, the yeast have built the necessary cell wall

structure to complete fermentation. Experiments on oxygen availability during fermentation have shown that fewer stuck fermentations occur when oxygen is used, and that the amount and extent of reductive aromas is significantly reduced.

It is important for the winemaker to remember that the addition of oxygen during primary fermentation should be undertaken with caution. Placing a diffuser into a fermenting wine, and turning it on to the levels mentioned, will send grapes and juice out of the fermentor like a Roman candle. Oxygen must be introduced *slowly* after fermentation begins, and then ratcheted up.

The purpose of this type of treatment has more to do with obtaining a clean dry finish with low propensity for reductive aromas. It does not materially affect color intensity or tannin modification. This treatment is the most forgiving if over-treatment happens.

Micro-Oxygenation Post-Fermentation, Pre-MLF

Micro-oxygenation during post-fermentation will, in general, have the greatest impact on a wine's style and structure, because this is when many of the formative reactions are occurring. The most aggressive proponents of micro-oxygenation advise that up to 60 mg/L/month be given to the wine for a relatively-short period of time. Other, less aggressive, proponents advocate 25 mg/L/month.

The reactions happening at this time are fixing the anthocyanins to the procyanidins, and also polymerizing the tannin molecules with each other. The aggressive addition of oxygen is used here to polymerize the tannins and, we hope, cap the slightly-polymerized tannin structures with anthocyanins so they will be protected from bleaching by sulfite during the aging process. However, as

has been discussed earlier, acetaldehyde is part of this series of reactions, and one needs to be mindful of the danger of over-oxygenating a wine and ruining the product.

As nerve-wracking as this time is, use of micro-oxygenation during this phase of the winemaking cycle will definitely have the biggest effect on the wine.

Micro-Oxygenation Post-Fermentation, Post-MLF

The longest-term use of micro-oxygenation occurs in this time frame. Much of the color-fixing has occurred by this time in a wine's cycle. If micro-oxygenation has not previously been used in a wine, then its use now will scavenge remaining anthocyanins and polymerize some tannins. It will intensify the color, and also help to form the tannin plates, where the molecules begin stacking into large structures that aid in the rounding of the mouthfeel of the wine.

At a rate of a few tenths of a milligram per liter per month, to the low single digits per liter per month, the amounts being added do not act as quickly on the wine now, and this part of the cycle allows more leeway in determining when the treatment should end. The amount of time required for this treatment varies from several weeks to more than a month.