

## **Micro-oxygenation in South Africa: part 1. Effect on the colour and phenolic composition of wine.**

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Micro-oxygenation is a process which is receiving more attention today. The following article will deal with some practical aspects regarding micro-oxygenation, as well as the effect it has on the phenolic composition of wine. The second article will focus on the effect it has on the microbial population, sulphur compounds, SO<sub>2</sub> levels, micro-oxygenation in combination with oak wood and its effect on the taste of wine. Some general recommendations will also be given. During micro-oxygenation small, controlled amounts of oxygen (O<sub>2</sub>) is bubbled into wine to bring about positive changes in the wine. This is achieved by filling a known volume with gas at a high pressure. The volume is then transferred via a low-pressure circuit to the diffuser and into the wine. The latter normally consists of a ceramic or stainless steel sparger that produces small bubbles which can dissolve in the wine. The aim of micro-oxygenation is to introduce O<sub>2</sub> into the wine at a rate equal to or slightly less than the wine's ability to consume that O<sub>2</sub>. It has to be managed in such a way that, after addition, all O<sub>2</sub> has been used up, while sufficient SO<sub>2</sub> is still left to protect the wine against oxidation. These changes include the following (some of them claimed by the producers):

Supply yeast with O<sub>2</sub> during fermentation to assist with the production of sterols and other fatty acids

Enhance red colour in red wine

Enhance colour stabilisation

Remove unwanted reductive flavours

Speed up the aging process of red wine

Simulate an oak barrel to a certain degree.

## A few practical recommendations

How is micro-oxygenation brought about? The equipment entails using a gas canister which contains oxygen, linked to a special micro-oxygenation machine which can dose the O<sub>2</sub> accurately. Different machines exist on the market. Some can dose in mL/L while other can dose in mg/L. From the dosing machine a small pipe bubbles the O<sub>2</sub> in a fine bubble form into the wine from the bottom of a tank. The sparger used for this can either be made from stainless steel or ceramic. The latter is reputed to lead to smaller bubbles, which can dissolve better in the wine, but stainless steel is obviously more robust. The tank also needs to be at least 2 m tall to facilitate dissolving of the bubbles in the wine. When micro-oxygenation is applied care should be taken that the bubbles dissolve in the wine and does not simply move to the top of the tank. The pressure from the gas canister to the dosing machine should also be monitored regularly. The sparger should be cleaned after a wine has been treated, by leaving it in a caustic solution for a few hours and then rinsing it thoroughly with clean water. Also make ensure that the pipe leading from the dosage machine to the sparger is free of liquid.

Oxygen can be supplied during different stages of the winemaking process. It can be supplied at 1-5 mg/L/day for a few days just after malolactic fermentation, especially to press wine fractions which are rich in polyphenols. The stage when micro-oxygenation is normally applied is during the ageing period after malolactic fermentation, when between 1-6 mg/L/month is introduced into the wine, although certain researchers recommended adding even up to 10 mg/L/month

The wine's temperature must be around 15°C because temperatures that are too high will lead to poor solubility of O<sub>2</sub> and temperatures that are too low to chemical reactions taking place too slowly.

One of the main aims of micro-oxygenation is to bring about changes in the colour and phenolic composition of wine. It is well known that the addition of O<sub>2</sub> to wine leads to polymerization of certain phenolic compounds, such as anthocyanin and flavanol moieties (catechin and condensed tannins). This polymerization occurs due to the formation of H<sub>2</sub>O<sub>2</sub> from the oxidation of a phenolic molecule. H<sub>2</sub>O<sub>2</sub>, being a strong oxidant, oxidizes ethanol, which yields acetaldehyde. The latter forms a bridge between phenolic molecules, leading to the polymerization. Just after fermentation a large percentage of anthocyanins are still in the colourless form. Slight oxidation, as would happen with a

racking of wine, leads to these colourless anthocyanin molecules binding to tannins and changing into red or brown red compounds. When red wine is matured in oak barrels O<sub>2</sub> also comes in contact with the wine, but this does not happen when it's matured in stainless steel tanks, without O<sub>2</sub> addition such as micro-oxygenation.

## **Experimental observations**

### **Materials and methods**

Four different SA red wines were monitored in terms of their phenolic and colour development during micro-oxygenation (Table 1). Note that in wines A and B the treatment started just after malolactic fermentation and wines C and D seven months after malolactic fermentation. Different spectrophotometric analyses were conducted during the course of the experiments. These included wine colour density, modified wine colour density (the modified version of the analysis negates the effect of pH and SO<sub>2</sub> on the analysis), wine colour hue, modified wine colour hue, total red pigments, total phenolics, estimate of SO<sub>2</sub> resistant pigments. The fractions of co-pigmented anthocyanin, free anthocyanins and polymeric colour pigment content in the red wines were also determined. The estimate of SO<sub>2</sub> resistant pigments and modified colour density were used to analyse these fractions. Total anthocyanin concentrations, total tannin concentrations, HCl index value (index of polymerisation of procyanidins) and gelatin index values (index of reactivity of phenolic molecules in wine towards gelatin) were also conducted. Additional phenolic analyses were performed with HPLC.

### **Results**

It is clear from Fig 1 that the colour density of wine A increased with increasing concentrations of O<sub>2</sub> added. In wine A, micro-oxygenation led to a decrease in total phenolic concentrations (which was high in this wine) after seven to nine weeks and were lower in the treated wines after 15 weeks (results not shown). The colour pigment in this wine also increased with O<sub>2</sub> addition. The free anthocyanin fraction was also smaller in the treated wines (results not shown), as well as the difference between the colour density and modified colour densities. The latter shows that the colour became

more resistant towards the bleaching effect of SO<sub>2</sub>. This indicated that more free anthocyanins were incorporated into polymeric pigment form, leading to the increase in colour.

The same trend happened in wine B (Fig 2) where the micro-oxygenation led to a drastic increase in the colour density, but not the modified colour densities (where the bleaching effect of SO<sub>2</sub> is negated). The fraction of colour in the polymeric pigments were much higher than that of the control wine (Fig 3).

However, the addition of O<sub>2</sub> with micro-oxygenation does not always lead to drastic increases in colour density. This was true for wines C and D (Fig. 4).

Micro-oxygenation, if used to increase the colour of red wine, would rather be used just after malolactic fermentation, where a large part of the anthocyanins are still in the colourless form. New research also showed that the addition of O<sub>2</sub> between alcoholic fermentation and malolactic fermentation also lead to less colour being lost during malolactic fermentation. Total red pigments decreased as when red wine is aged in a barrel in all the treatments, but were slightly higher in the treated wines after 15-18 weeks than in the control (Fig. 5).

The HPLC results for wine C can be seen in Table 4.3. Vanillic acid was much higher in the barrel treated wine than in the other treatments, probably due to the higher oak contact. Catechin and procyanidin B1 concentrations decreased with the increasing O<sub>2</sub> addition over time, with the malvidin-3-glucoside concentration being lower in the control wine. The procyanidin B1 concentration can also increase over time due to catechin associations, explaining the higher concentrations in the control wine after 24 weeks. The O<sub>2</sub> treated wines were also higher in polymeric pigment and polymeric phenols, due to acetaldehyde formation and polymerization. The polymerisation of procyanidins in wine A was also reflected in an increase in the HCl index of the treated wines (Results not shown).

A small decrease in the total tannin concentration and a small increase in colour hue in the treated wines were observed (results not shown). The gelatin index of wine C also varied over time, but it must be kept in mind that this index also gives an indication of astringency and is not always directly correlated with it. During micro-oxygenation the wine is supposed to first go through a structuring phase with an increase in astringency. This is followed by a phase where softening of the wine occurred. If too much micro-oxygenation is applied for too long a period of time the wine can become hard or "dried"

out. These phases has however not been scientifically proven, but it has been found that if micro-oxygenation is applied for too long a period of time the mean degree of polymerization of catechins increases to a large extend and the wine can become too hard. Research has also showed that the decrease in anthocyanin level was also higher where larger percentages of oak were added to the wine. This is probably due to oak tannins being oxidized easier than grape tannins, leading to enhanced polymerization. It thus seems that micro-oxygenation is most effective in terms of colour development when applied before or after malolactic fermentation. It is doubtful whether a wine which is older than 6 months will still benefit from micro-oxygenation for its colour. We have also seen that the colour of wines with a high colour density (<15) after malolactic fermentation does not increase to such a degree if micro-oxygenation is applied that it can be observed with the naked eye. Micro-oxygenation can however be a useful tool if applied to young red wines to increase the colour.

It is clear that the sensory monitoring of wine during micro-oxygenation is still very important and will be discussed in more detail in the next article.

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TABLE 1: Different commercial wines with their origins and treatments.

NoCultivar and year	Origin of wine	Treatment
A Cabernet Sauvignon 2002	Paarl	0, 1.5 and 3 mg O <sub>2</sub> /L/month with oak staves starting just after the completion of malolactic fermentation.
B Red blend 2003	Stellenbosch	0 and 4 mg O <sub>2</sub> /L/month with oak staves starting just after the completion of malolactic fermentation.
C Pinotage 2004	Paarl (	0, 1.5 and 3 mg O <sub>2</sub> /L/month with oak staves starting seven months after the completion of malolactic fermentation. The same wine was also matured in an oak barrel of the same wood as the staves used (USA MT+).
D Shiraz 2003	Worcester	0 and 3 mg O <sub>2</sub> /L/month with oak staves starting seven months after the completion of malolactic fermentation.

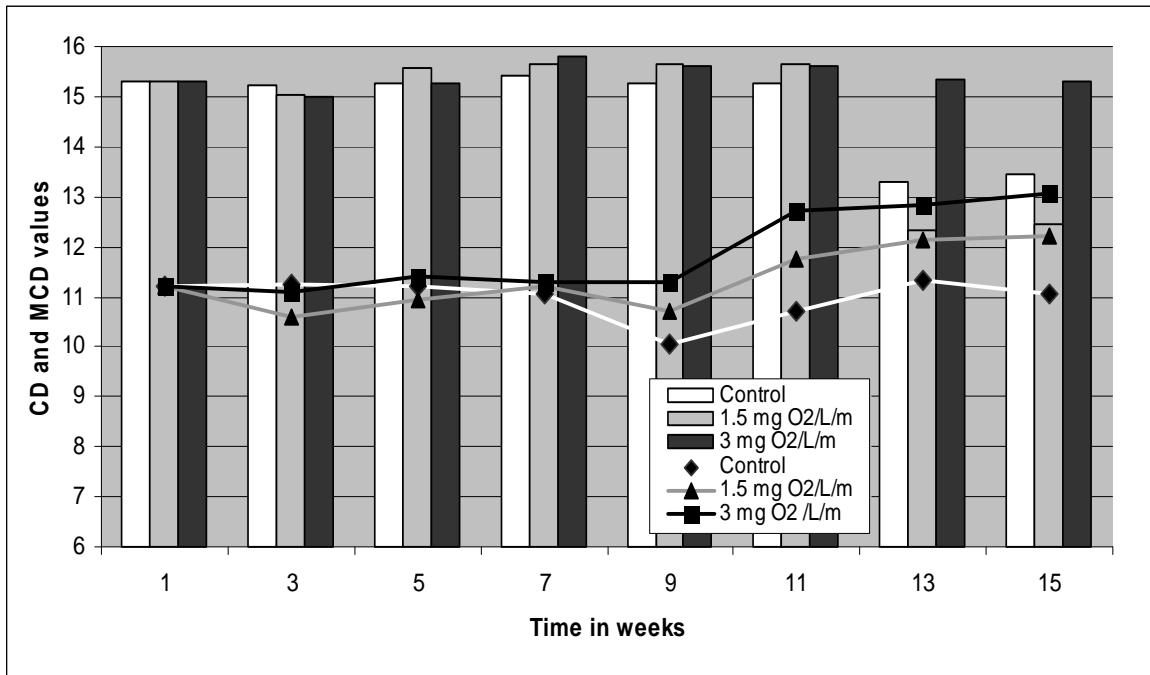


FIGURE 1

Colour (CD) (lines) and modified colour (bars) density (MCD) of wine A during micro-oxygenation treatment.

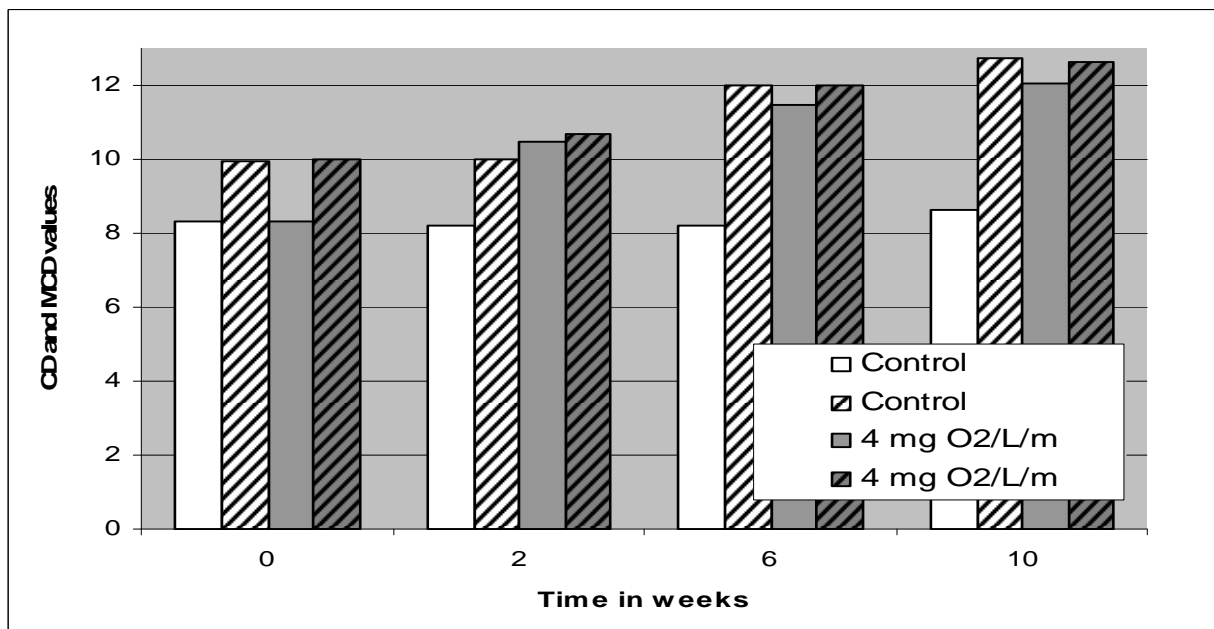


FIGURE 2

Colour (CD) and modified colour density (MCD) of wine B during micro-oxygenation treatment.

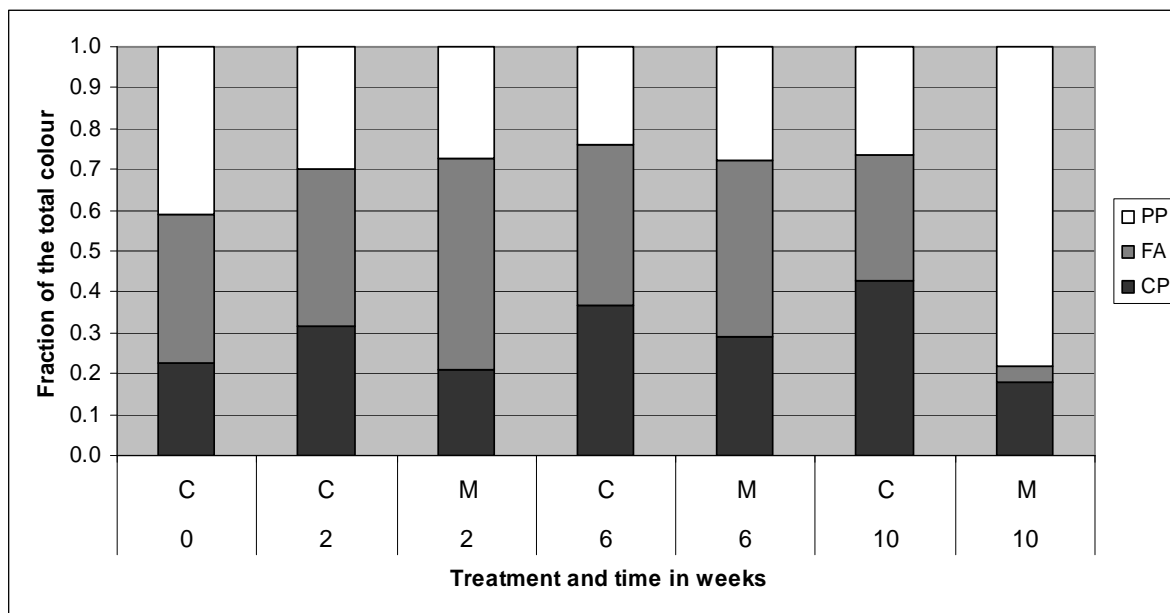


FIGURE 3

Development of the fraction of colour of wine B during micro-oxygenation treatment. C: control tank, M: micro-oxygenation tank receiving 3 mg O<sub>2</sub>/L/month. PP: Fraction of colour due to polymeric fraction, FA: Fraction of colour due to free anthocyanins, CP: Fraction of colour due to co-pigmentation.

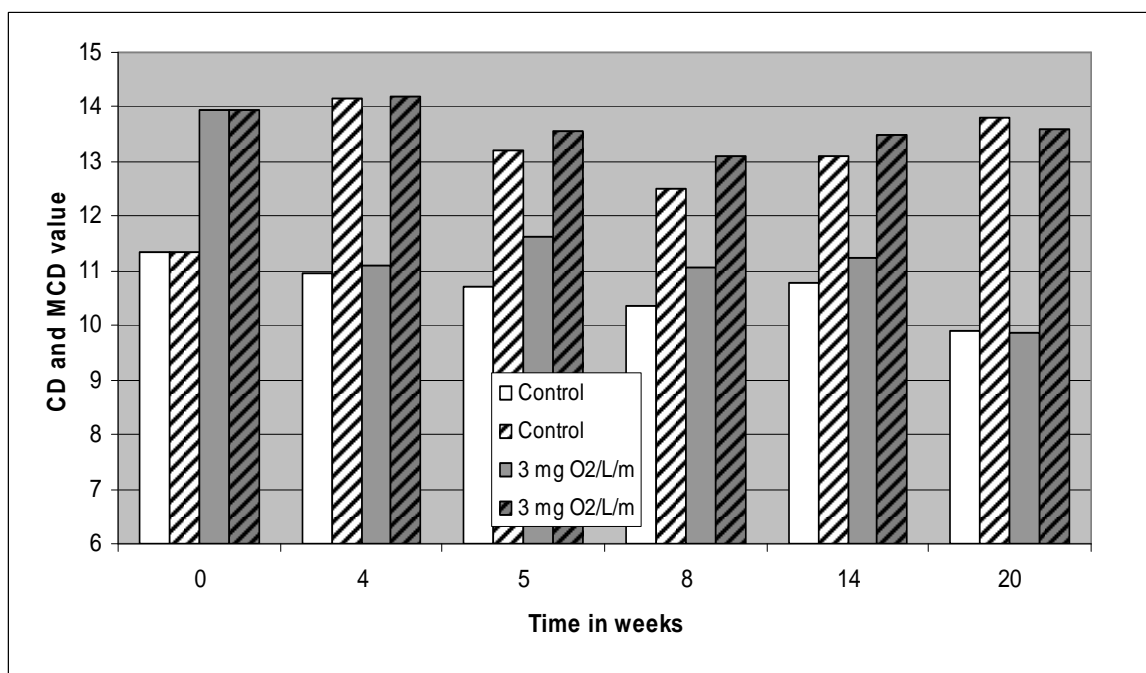


FIGURE 4

Colour (open bars) and modified (striped bars) colour density of wine D during micro-oxygenation treatment.

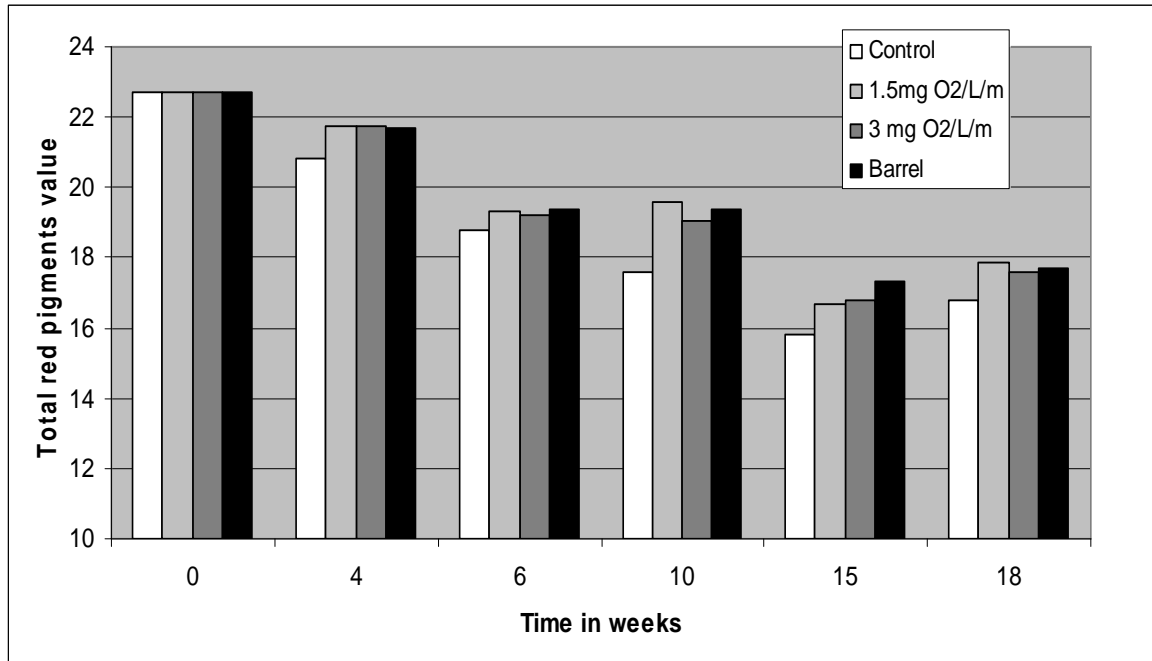


FIGURE 5

Total red pigment development of wine C during micro-oxygenation treatment

TABLE 2

Concentrations of different phenolic compounds in wine C initially and after 24 weeks treatment.

Compound	Concentration (mg/L) for each treatment				
	Initially	Control	1.5 mg O <sub>2</sub> /L/month	3 mg O <sub>2</sub> /L/month	Barrel
Gallic acid	46.4	56.1	50.3	57.2	47.3
Gentisic acid	1.5	2.6	nd	1.5	nd
Caftaric acid	17.3	16.3	17.5	16.8	17.3
Vanillic acid	2.6	4.1	5.1	3.3	43.7
Catechin	790.2	784.0	704.4	698.6	659.5
Caffeic acid	64.2	59.0	56.8	57.0	52.6
Procyanidin B1	60.0	91.0	93.5	55.6	78.5
p-Coumaric acid	3.6	3.8	4.2	6.4	12.2
Procyanidin B2	49.1	40.8	40.4	40.0	39.8
Epicatechin	90.1	73.6	76.2	68.8	80.5
Delphinidin-3-glucoside	11.2	7.7	7.4	9.6	7.2
Petunidin-3-glucoside	13.8	7.6	9.3	8.5	8.7
Peonidin-3-glucoside	5.5	2.9	4.1	3.4	4.1
Malvidin-3-glucoside	117.9	63.4	83.8	72.0	79.6
Ellagic acid	3.7	4.8	4.5	5.4	3.3
Quercetin-3-glucoside	15.8	12.3	13.6	11.1	14.7
Myricetin	4.3	3.2	5.1	5.1	4.3
Quercetin-3-rhamnoside	5.0	4.2	3.4	4.7	4.0
Malvidin-3-Acetate	40.1	18.4	23.8	24.2	22.4
Quercitin	7.8	4.8	6.4	5.8	5.2
Malvidin-3-p-coumaric acid	19.6	6.8	11.1	9.6	10.0
Polymeric pigment	28.8	29.0	33.9	34.8	35.3
Polymeric phenols	791.6	782.9	947.1	1021.5	1132.0

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