Wynboer A Technical Guide for Wine Producers

Effect of different oxygen levels on glutathione levels in South African white must and wines

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Glutathione is an important compound in wine and also human health, due to its detoxification actions. Glutathione, a sulphur containing compound naturally occurring in grape must, has been shown to form the Grape Reaction Product (GRP). This is due to glutathione reducing quinones molecules to GRP when enzymatic oxidation of grape must takes place. This reaction is thus very sensitive to the presence of O^2 and SO^2, with the latter inhibiting oxidation enzymes. So called reductive winemaking of especially Sauvignon blanc is used extensively throughout the so-called New World. During this process O^2 and oxidation is shut out by the addition of dry ice, SO^2, ascorbic acid etc to the must and wine. This process is supposed to better preserve the flavour and aroma of especially Sauvignon blanc. It is, however, not well known just how much O^2 is displaced in a commercial cellar set up, both in the crusher and the press by these actions. White juice can easily pick up a few mg/L of O^2 during processing of the grapes. Due to glutathione's sensitivity to oxidation it has been proposed to be used as a possible marker of oxidation. New commercial presses now exist, which enables the winemaker to perform whole bunch pressing at O^2 levels lower than 0.5%. Very few studies, however, investigated the effect of different concentrations of O^2 measured in the press and must on the glutathione concentration. We thus decided to investigate the effect of adding measurable concentrations of dissolved O^2 to different SA white musts and to monitor the glutathione concentration in the must and after alcoholic fermentation.

Materials and Methods

Three different white grapes, two Sauvignon blancs (juice and wine A and C) and a Colombard (juice and wine B) were used for this study. The Sauvignon blanc grapes originated from two different vineyards in Elgin and the Colombard from Robertson. The grapes harvested when deemed optimally ripe by the winemaker and transported to Stellenbosch University. The grapes were not crushed, but whole bunch pressing was applied to avoid O^2 pick up during crushing. Whole bunches were pressed in a specially designed small scale press, where the O^2 level inside the press was maintained at <1% and this yielded juice with a dissolved O^2 concentration of less than 0.3 mg/L. The juice was collected in 4.5L glass bottles (in which the air was previously displaced with N2 gas). By adding different amounts of O^2 to these juices three O^2 treatments were yielded: a reductive treatment where no O^2 was added and the dissolved O^2

concentration in the juice was <0.3mg/L, a control where between 1-1.5mg/L O^2 was added and an oxidative treatment where 3.5-4mg/L O^2 was added. To the reductive and control treatments 60mg/L SO^2 and 50mg/L ascorbic acid was also added. These juices was then allowed to settle over night at 15°C and racked the next day under N2 pressure into other 4.5L glass canisters (in which the air was also dispelled with N2). All juices were inoculated with Vin13 and DAP added at 0.5mg/L two days after the initiation of fermentation. All fermentations were performed at 15°C.

Samples for glutathione analyses, performed with LCMSMS, were drawn from the settled juices, as well as the wine after the completion of alcoholic fermentation. To these samples a 1000mg/L SO^2 and 500mg/L ascorbic acid were added to completely inhibit any further oxidation and frozen at -20°C. Samples were also kept at 4°C and certain samples thawed after two weeks and refrozen before being analysed after four weeks. This was done to investigate the stability of glutathione over time during storage.

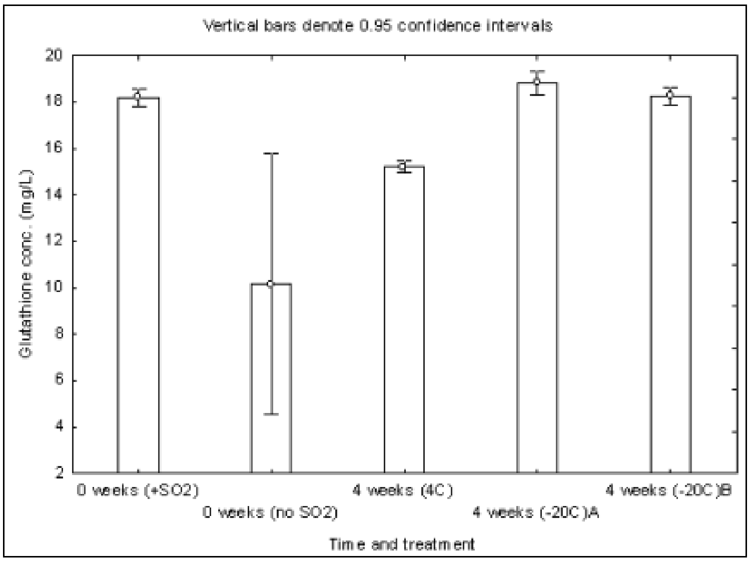

Figure 1: Reduced glutathione levels in grape juice stored under different conditions. 0 weeks (+SO^2): samples analyzed immediately after pressing, with 1000 mg/L SO^2 and 500 mg/L ascorbic acid added;
0 weeks (no SO^2): samples analyzed immediately after pressing, with no SO^2 or ascorbic acid added;
4 weeks (4C): Stored at 4 °C for 4 weeks;
4 weeks (-20C) A: Stored at -20 °C for 4 weeks. Sample thawed only once,
4 weeks (-20C) B: Stored at -20 °C for 4 weeks. Sampled thawed twice. All the samples analyzed at week 4 had 1000 mg/L SO^2 and 500 mg/L ascorbic acid added initially. Different letters indicate significant differences ($p \leq 0.05$). (Du Toit et al, 2007).

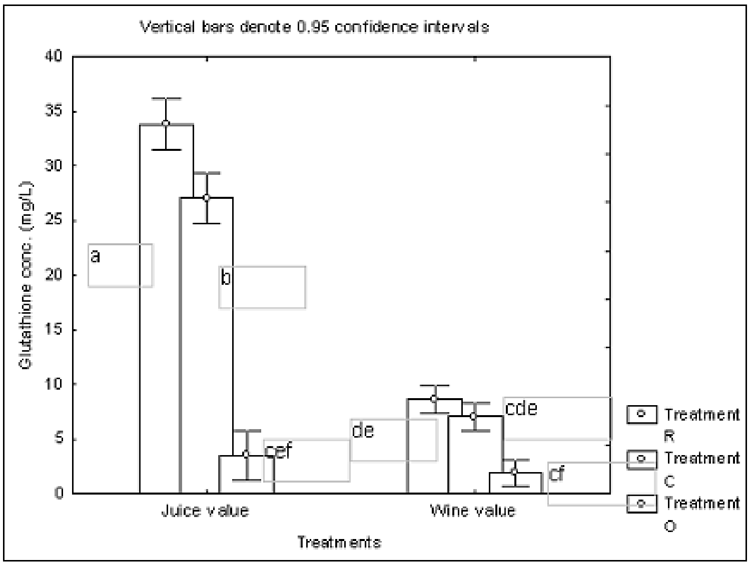

Figure 2 Reduced glutathione concentrations in juice and wine A, which underwent different treatments. Treatment R: Reductive treatment of juice, Treatment C: Control treatment of juice, Treatment O: Oxidized treatment of juice. Different letters indicate significant differences (p? 0.05). (Du Toit et al, 2007).

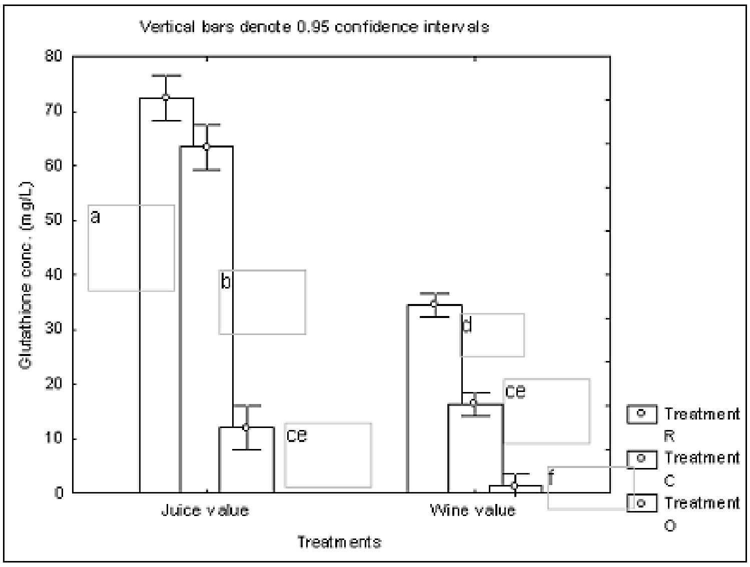

Figure 3. Reduced glutathione concentrations in juice and wine C, which underwent different treatments. Treatment R: Reductive treatment of juice, Treatment C: Control treatment of juice, Treatment O: Oxidized treatment of juice. Different letters indicate significant differences ($p \leq 0.05$). (Du Toit et al, 2007).

Results and discussion

We tested first the effect of storage on glutathione concentrations in grape must before analyses with LCMSMS. In Figure 1 the glutathione concentration in grape must stored under different conditions can be seen. It is clear that storage with no SO^2 addition and at 4°C led to a decrease in glutathione concentrations. Storage at -20°C, even if the must was thawed once and re-frozen, did not lead to significantly lower glutathione concentrations. It is thus advisable to keep must or wine samples destined for glutathione analyses at -20°C if one wants to store samples for glutathione analyses.

In Figures 2 to 3 the glutathione levels in the reduced, control and oxidative treatments of the juice and wine from grapes A and C can also be seen. It is clear that even low amounts of O^2 added to the must (such as in the control) led to a decrease in glutathione, even in the presence of SO^2 (60mg/L added). In the control 1-1.5 mg/L O^2 was added, which is relatively low compared to a commercial cellar set up, where a higher concentration of glutathione can easily be added to the must. Higher additions with O^2, as in the oxidative treatments in the absence of SO^2 and ascorbic acid led to a drastic reduction in glutathione levels in the must. The same results were obtained in the juice and wine made from grapes B. It thus seems that glutathione could be a possible marker of oxidation in the must.

Alcoholic fermentation, in this case with Vin13, also led to a reduction in glutathione concentrations. This does not mean however that Vin13 will always reduce glutathione concentration in the must. Yeast strain, initial glutathione concentration and composition of the must can all influence the evolution of glutathione during alcoholic fermentation.

Why is higher glutathione content desired in white wine? As mentioned earlier, glutathione forms the so-called GRP with oxidised phenolic compounds. This leads to the must having a less brown colour. In wine, glutathione has been proven to protect certain terpenoids, such as linalool and a-terpineol, responsible for the muscat, rose and citrus characters in certain wine.

In bottled Sauvignon blanc wines, glutathione also seems to protect volatile tiols, the latter imparting the typical passion fruit to certain Sauvignon blanc wines. Commercial yeast extract, reputed to contain glutathione and which protects white wine from premature ageing and becoming yellow, can also be purchased. How effective these treatments are on the ageing capacity of South Africa white wines should, however, be investigated further. This could be crucial to the South African wine industry, where premature ageing of certain white wines seems to pose a major problem.

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