Industry allocated project number

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Indicate (X) client(s) to whom this final report is submitted. Replace any of these with other relevant clients if required.

FINAL REPORT
2013

Programme & Project Leader Information

	Research Organisation Programme leader	Project leader
Title, initials, surname	Prof A. Deloire	Dr W.J du Toit
Present position	Professor	Senior Lecturer
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Project Information

Research Organisation Project number	WW-WdT-10/03
Project title	Methods to improve Sauvignon blanc wine quality and diversity in South Africa

Fruit kind(s)			
Start date (mm/yyyy)	1-1-2010	**End date** (mm/yyyy)	31-12-2012

Project keywords	Sauvignon blanc, glutathione, oxidation

Approved by Research Organisation Programme leader (tick box) ☐

THIS REPORT MUST INCLUDE INFORMATION FROM THE **ENTIRE** PROJECT

Executive Summary

Give an executive summary of the total project.

The main aim of this project was to investigate winemaking practices which may affect Sauvignon blanc wine composition. We found that oxygen (O2) additions to Sauvignon blanc must in the absence of sulphur dioxide (SO2) led to lower volatile thiol and glutathione (GSH) levels in the resulting wines. However, SO2 additions even in the presence of O2, negated this loss. Methoxypyrazine levels were not influenced by these treatments, while SO2 additions also influenced ester and higher alcohol concentrations. The addition of inactive yeast strains also led to higher volatile thiol levels in the wines.

Levels of GSH also varied between different commercial yeast strain during fermentation, while we also developed novel analytical method to assess anti-oxidant levels in must and wine. Different levels of GSH and SO2 in commercial wines influenced O2 consumption during ageing of the wine.

We also assessed the sensory interaction between 3-mercapto-hexanol and 2-isobutyl-3-methoxypyrazine, two aroma impact compounds in Sauvignon blanc wine. These two compounds sensorially supressed each other at high levels.

This project has been very productive, with 2 peer reviewed review articles, 5 peer reviewed experimental articles, a number of conference proceedings and popular articles being published and a number of conference presentations originating from this work. One Italian PhD and three South African Master's students in Oenology have also graduated while working on this project.

Problem identification and objectives

State the problem being addressed and the ultimate aim of the project.

Wine producers will often treat Sauvignon blanc juice and wine very reductively due to the threat of oxidation. However, how SO2, O2 and inactive yeast additions to the juice affects the composition of Sauvignon blanc wine has not been scientifically elucidated at the start of this project. The rate of O2 consumption and factors affecting this rate in commercial Sauvignon blanc wines were also not well-known. Certain anti-oxidant such as GSH and caffeic acid may influence this rate and it was therefore also important to assess the effect of O2 and yeast strain on GSH levels in Sauvignon blanc wines. This led to the need for a fast, reliable and accurate chromatographic method to be developed to analyse for these anti-oxidants in must and wine.

The sensory interaction between important thiols and methoxypyrazines in Sauvignon blanc has also not been investigated in detail before. The ultimate aims of this project were thus to investigate reductive and oxidative juice treatments on Sauvignon blanc wine composition as well as the reaction rate of O2 in commercial Sauvignon blanc wines and how anti-oxidants influence this.

Workplan (materials and methods)

List trial sites, treatments, experimental layout and statistical detail, sampling detail, cold storage and examination stages and parameters.

This work has been divided into four different milestones.

Milestone 1 investigated the effect of different oxygen, inactive yeast and SO2 additions to Sauvignon blanc must on the wine's composition. We added no or about 4 mg/L O2 in combination with no or 60 mg/L SO2 to Sauvignon blanc musts from two different farms in 2009. We repeated this experiment, but also included Optiwhite, an inactive yeast addition in 2010 as well. Analyses performed on the resulting wines included GSH, volatile thiols, methoxypyrazines, esters, higher alcohols, terpenes and phenolic compounds.

Milestone 2 investigated the effect of different yeast strains and inactive yeast additions on GSH levels in Sauvignon blanc wine during fermentation. A wide array of yeast strains were tested over two years in Sauvignon blanc juices containing different levels of initial GSH. We also investigated the GSH levels in a Sauvignon blanc juice containing different levels of inactive yeast added at different stages of fermentation. Part of this milestone also entailed developing new analytical techniques for GSH, caffeic acid and cathechin in must and wine with UPLC.

Milestone 3 focussed on the oxidation rate in Sauvignon blanc wines containing different levels of SO2 and GSH. We used a model wine, as well as 14 different commercial wines for this study. GSH, oxidised GSH (GSSH) O2, phenolics, colour and SO2 levels were monitored in these wines after they had been saturated with O2.

Milestone 4 investigated the sensory interaction between 3-mercapto-hexanol and 2-isobutyl-3-methoxypyrazine in a de-aromatised Sauvignon blanc wine. For this experiment these compounds were each added at 5 different levels normally found in Sauvignon blanc wines and their sensory impact assessed with a trained sensory descriptive analyses panel.

Results and discussion

State results obtained and list any industry benefits. If applicable, include a short discussion covering ALL accumulated results from the start of the project. Limit it to essential information only.

Milestone 1: Oxygen additions in the absence of SO2 led to large decreases in GSH and phenolic compounds concentrations in both the juice and wines, while volatile thiol concentrations also decreased drastically in the wines. However, this trend was negated by the addition of SO2, proving its effective anti-oxidant function. This trend was seen in both Sauvignon blanc juices investigated. The additions of SO2 and to a lesser extend O2, also influencing esters such as isoamyl acetate and 2-phenylethyl acetate. The degradation of phenolic compounds such as caftaric acid and catechin was also prevented by the addition of SO2. These results show the importance of treating Sauvignon blanc juices reductively, but whether the additions of inert gasses to Sauvignon blanc must/ juice is really necessary when only SO2 could be used could potentially save the industry in production costs. It should however be kept in mind that press juices normally contain less SO2 and should therefore by more protected again oxidation with inert gasses. (Further detail can be found in Coetzee et al., 2013). We also observed slightly higher levels of volatile thiols where inactive yeast additions were made. Part of the analyses of this work was done at Auckland University, which established a good collaboration between them and the Department of Viticulture and Oenology DVO at Stellenbosch University.

Milestone 2: In this work we found that GSH levels can vary during the course of alcoholic fermentation. Levels normally decreased and sometimes increased at the latter stages of alcoholic fermentation, but levels were most of the time lower in wine than in the corresponding juices. Certain yeast strains also led to slightly higher levels, but this depended on the type of juice used. Model grape juice with different YAN levels also did not influence the GSH levels at the end of fermentation as expected. We also found that the additions of inactive GSH enriched yeast should take place in the juice of first quarter of fermentation, which led to higher GSH levels in the wines. Later additions did not affect GSH levels. As part of this study we developed a novel GSH and other anti-oxidant analytical methods, which can be used by the SA wine industry to measure GSH levels in their wines (Further detail can be found in Fracassetti et al., 2011; Kritzinger et al., 2012 and 2013).

Milestone 3: In this work we found that SO2 can increase the O2 consumption rate in both model and real Sauvignon blanc wines. However, O2 consumption rates differed to a large extend between different commercial wines. Positive correlations were also observed between oxygen, SO_2, GSH and Cu concentrations in the beginning of the experiment in the wines, which were again negatively correlated with increases in absorbance at 420 and 440 nm (brown and yellow colour) and oxidised GSSG concentrations at the end of the experiment. This work can give winemakers an idea which factors in Sauvignon blanc wines will affect oxygen consumption and possibly oxidation of their wines. ((Further detail can be found in Fracassetti et

al., 2013). An Italian student conducted this research as part of her PhD at the DVO, which established a good collaboration between Milan University and the DVO.

Milestone 4: We found that 3-mercapto-hexanol (3MH) and elicited tropical, passion fruit, guava, grape fruit when added alone to de-aromatised Sauvignon blanc wine, while descriptors such as green pepper, grass and green were used to describe 2-isobutyl-3-methoxypyrazine (IBMP). These compounds supressed each other when present at higher levels in the wines. These results can give commercial winemakers an idea of which aromas to expect when thiols and methoxypyrazines are present at a certain level in their Sauvignon blanc wines (Further detail can be found in Van Wyngaard et al., 2013).

Complete the following table

Milestone	Target Date	Extension Date	Date Completed	Achievement
1. Different SO2 and O2 additions to S. blanc juice and wines made	2009		2010	All samples analysed and published
2. Testing effect of yeast strain and yeast additives on GSH levels in S blanc and developing analytical methods	2010		2011	All analyses completed and published. New methods developed and available to the wine industry.
3.Assess O2 consumption in commercial SA S. blanc wines	2010		2012	All analyses completed and published.
4. Sensory interaction between 3MH and IBMP	2012		2013	All analyses completed and published
5. Journal publication/s – final milestone				

Accumulated outputs

List ALL the outputs from the start of the project. The year of each output must also be indicated.

Conclusions

This project has yielded a large number of publications and conference presentations. A number of post graduate students have also graduated who worked on this project.

Technology development, products and patents

Indicate the commercial potential of this project, eg. Intellectual property rights or commercial product(s)

New GSH analytical methods have been commercialized by the Central Analytical Facility (CAF) of Stellenbosch University.

Suggestions for technology transfer

List any suggestions you may have for technology transfer

Human resources development/training

Indicate the number and level (eg. MSc, PhD, post doc) of students/support personnel that were trained as well as their cost to industry through this project. Add in more lines if necessary.

Student level (BSc, MSc, PhD, Post doc)	Cost to Project
1. me C. Coetzee (MSc)	R50 000
2. me E Kritzinger (MSc)	R50 000

3. me E. van Wyngaard (MSc)	R50 000
4.me D. Fracassetti (PhD)	none
5.	

Publications (popular, press releases, semi-scientific, scientific)

Peer reviewed scientific articles:

D. Fracassetti, N. Lawrence, A.G.J. Tredoux, A. Tirelli, H.H. Nieuwoudt, W.J. Du Toit . 2011. Quantification of glutathione, catechin and caffeic acid in grape juice and wine by a novel ultra-performance liquid chromatography method. Food Chemistry 128,1136–1142.

Coetzee, C. & W.J. du Toit, 2012. A comprehensive review on Sauvignon blanc aroma with a focus on certain positive volatile thiols. Food Research International 45: 287-298.

Kritzinger, E, Stander, M and Du Toit, WJ. 2012. Assessment of glutathione levels in model solution and grape ferments supplemented with glutathione-enriched inactive dry yeast preparations using a novel UPLC-MS/MS method**.** Food Additives & Contaminants. Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment. 30: 80-92.

Kritzinger E.C; Bauer, FF, du Toit, WJ. 2013. Role of glutathione in wine: a review. Journal of Agricultural and Food Chemistry. 61, 269–277.

Coetzee, C, Lisjak, K., Nicolau, L., Kilmartin, P and du Toit, WJ. (2013). Oxygen and sulphur dioxide additions to Sauvignon blanc must: effect on must and wine composition. Fragrance and Flavour Journal, 28, 155–167.

Kritzinger, E., Bauer, F.F., and Du Toit, WJ. (2013). Influence of yeast strain, extended lees contact and nitrogen supplementation on glutathione concentrations in wine. Aust. J. Grape Wine Res. 19, 161–170

Fracassetti, D, Coetzee, C; Vanzo, A; Ballabio, D, du Toit W.J. 2013. Oxygen consumption in South African Sauvignon blanc wines: role of glutathione, sulphur dioxide and certain phenolics. Accepted for publication in South African Journal of Enology and Viticulture.

E. van Wyngaard, J. Brand, D. Jacobson and W. J. du Toit 2013. Sensory interaction between 3-mercaptohexan-1-ol and 2-isobutyl-3-methoxypyrazine in dearomatized Sauvignon blanc wine. Submitted to Aust. J. Grape Wine Res.

Published conference proceedings:

Kritzinger, E.C., F.F. Bauer & W.J. du Toit. 2011. Influence of yeast strain, extended lees contact and juice composition on glutathione levels in wine. Proceedings of the Wine Active Compounds 2011 (WAC), Beaune, Burgundy, France. pp 83-85, ISBN 2-9054284-30. (24-26 March)

W.J. du Toit, A. Buica, C. Coetzee, E. van Wyngaard, T. Moffat, A. Strever. 2011. Handling grapes from a warm area: how not to burn your fingers. Proceedings of the 16th International Oenology Symposium in Bolzano, p 53-56.

Du Toit, W.J., C, Coetzee, K. Lisjak & L. Nicolau. 2011. Different oxygen and sulphur dioxide concentrations in Sauvignon blanc must: effect on the composition of the must and wine. Proceedings of Macrowine 2010: Third International Symposium on Macromolecules and Secondary Metabolites of Grapevine and Wines, Torino, Italy. Quaderni di Scienze Viticole ed Enologiche 2009-2010, Università di Torino, 31, pp 217-220, ISBN 1970-6545. (16-18 June).

Popular articles:

Wessel du Toit en Engela Kritzinger (2013). Glutatioon in wyn: nuwe belangstelling Submitted to Winelands.

We foresee at least two more popular articles submitting from this work to Winelands

Presentations/papers delivered

National conferences

Coetzee, C., K. Lisjak, L. Nicolau & W.J. du Toit. 2010. Different oxygen and sulphur dioxide concentrations in Sauvignon blanc must: effect on the composition of the must and wine. Thirty Second Conference of the South African Society for Enology and Viticulture, Lord Charles Hotel, Somerset West. (**POSTER**) (17-19 November)

Fracassetti, D. & W.J. du Toit. 2010. Oxygen consumption in Sauvignon blanc wines: role played by glutathione, sulphur dioxide and phenolic compounds. Thirty Second Conference of the South African Society for Enology and Viticulture, Lord Charles Hotel, Somerset West. (17-19 November)

Fracassetti, D., N. Lawrence, A.G.J. Tredoux, A. Tirelli, H.H. Nieuwoudt & W.J. du Toit. 2010. Quantification of gluthathione in must and wine by ultra-performance liquid chromatography. Thirty Second Conference of the South African Society for Enology and Viticulture, Lord Charles Hotel, Somerset West. (**POSTER**) (17-19 November)

Kritzinger, E.C. F.F. Bauer & W.J. du Toit. 2010. Influence of yeast strain and extended lees contact on glutathione levels in Sauvignon blanc wine. Thirty Second Conference of the South African Society for Enology and Viticulture, Lord Charles Hotel, Somerset West. (**POSTER**) (17-19 November)

WJ du Toit, C. Coetzee, E. Kritzinger, D. Fracassetti. (2012). Glutathione: recent developments on our knowledge on this important anti-oxidant. 34th Conference of the South African Society for Enology and Viticulture, Paarl, South Africa.

International; conferences

Coetzee, C. and Du Toit, W.J. (2009). Effect of different oxygen and SO_2 levels on Sauvignon blanc must. 4th International conference of the South African Society for Enology and Viticulture- beyond 2010, Cape Town.

Du Toit, W.J., C. Coetzee, K. Lisjak & L. Nicolau. 2010. Different oxygen and sulphur dioxide concentrations in Sauvignon blanc must: effect on the composition of the must and wine. Third International Symposium MacroWine2010 on Macromolecules and Secondary Metabolites of Grapevine and Wines, Torino, Italy. (16-18 June)

E. Kritzinger, F. Bauer& WJ du Toit. 2011. Influence of yeast strain, extended lees contact and juice composition on glutathione levels in wine. Second International Conference on wine active compounds. Beaune, France. 24-26 March 2011.

D. Fracassetti, A. Vanzo, K. Lisjak, A. Tirelli, W. du Toit 2011. Effect of glutathione and sulfur dioxide on the oxidation of white wine. "Enoforum 2011" (3-5 May, Arezzo, Italy).

E. van Wyngaard, J. Brand, W.J. du Toit. (2012) Assessment of interaction between 3-mercaptohexan-1-ol and 2-isobutyl-3-methoxypyrazine in dearomatized Sauvignon blanc wine. Macrowine Conference. 2012 Bordeaux, France, June 18-21.

Total cost summary of the project

TOTAL COST IN REAL TERMS	COST
YEAR 1	
YEAR 2	
YEAR 3	
YEAR 4	
YEAR 5	
TOTAL	

CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
				120000.00	60000.00		180000.00
				115000.00	57500.00		172500.00
				235000.00	117500.00		**352500.00**