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
2014

Programme & Project Leader Information

	Research Organisation Programme leader	Project leader
Title, initials, surname	Prof W.J du Toit	Prof W.J du Toit
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Project Information

Research Organisation Project number	WW-WdT-11/01
Project title	Assessing volatile thiol concentration in South African Sauvignon blanc wines

Fruit kind(s)			
Start date (mm/yyyy)	1/1/2011	**End date** (mm/yyyy)	12/12/2012

Project keywords	

Approved by Research Organisation Programme leader (tick box) ☐

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

Executive Summary

We have developed a novel LCMS method for the quantification of 3MH and 3MHA concentrations in white wines. This is the first time that this method has been used to quantify these compounds in wine, as thiols are normally quantified with GCMS. This method's sensitive is very good, as well as it's repeatability. Levels that we can quantify for both 3MHA and 3MH are below their sensory threshold values in wine. We also quantified the levels of these compounds in 45 South African Sauvignon blanc wines which will help in establishing a database of these compounds.

Problem identification and objectives

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

The role that volatile thiols, responsible for the tropical fruit aroma, play in South African Sauvignon blanc wines is unknown. Volatile thiols responsible for the fruity or tropical organoleptic flavours of Sauvignon blanc wines are 4-mercapto-4-methylpentan-2-one (4MMP) , reminiscent of guava, box tree, passion fruit, broom and black current bud; and 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA), responsible for the passion fruit, grapefruit and citrus aroma. These compounds are thus impact compounds in Sauvignon blanc as well as other white cultivar wines. The analyses of these compounds require expensive and high technology equipment, which led to these analyses being neglected in South Africa in the past. The main objective of this project for would thus be to develop an analytical method to analyse for volatile thiols in South African Sauvignon blanc wines. This would enable other aims, such as correlating tropical fruit aromas in SA Sauvignon blanc wines and compiling a database on thiols levels in SA Sauvignon blanc wines.

Workplan (materials and methods)

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

The initial aim of this project was to set up a GCMS technique for volatile thiol analysis in wine using the method of Tominaga et al. This is technique is technically very challenging and we obtained all the required glassware to concentrate the thiols in wines, as well as the required standards and deuterated standards. We were also assisted by two experts in GCMS with this

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technique. After an extended period of being unsuccessful using GCMS, we decided to try a different approach.

We have currently a very good collaboration with the Department of Food, Environmental and Nutritional Sciences, Milan University. They have developed a novel sample preparation method for the analysis of volatile thiols in wine. This procedure took the researchers in Milan more than 2 years to optimize. However, they did not had sufficient UPLC sensitivity to finally quantify the thiols in wine. In this technique o-phthaldialdehyde was used as an derivatisation reagent and the sample also undergo extensive sample preparation. Samples were then analysed using UPLC coupled with a multi λ Fluorescence Detector 2475). The chromatography separation was performed by a Kinetex Phenyl-hexyl column. The linearity of the detector response was evaluated over the concentration range 25-500 ng/L for 3MHA and 50-2500 ng/L for 3MH, which are concentrations normally occurring in wine.

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.

We initially struggled to obtain proper peaks in real wine for the compounds especially at the lower concentrations using GCMS. 4MMP especially did not deliver good peaks. The limit of detection (LOD) and limit of quantification (LOQ) (ng/L) in real wine were eventually calculated using the signal to noise ratio of 10:1 and 10:3. Results were as follows: 4 MMP: LOD and LOQ: >100; 3MHA: LOD: 59, LOQ 196; 3MH: LOD: 275; LOQ: 919 ng/L. These values are too high and would not suit general thiol analyses in wine, although some wines with very high thiol levels could have levels higher than these LOQ's. Next we tried using a larger sample volume to do the analyses as well as larger injection volumes, but this also did not improve the results. However, due to the fact that a student's Master's degree was linked to this analysis and the student was supposed to graduate end of 2012 we decided to send the student to Slovenia to analyse the thiol levels in the commercial Sauvignon blanc wines she investigated. This yielded interesting results with average values of 10, 159 and 970 ng/L being found for for 4MMP, 3MHA and 3MH respectively (Table 1).

We thus decided to try the LCMS method form Milan University. Using this technique we obtained good results in terms of LOQ and LOD, accuracy and repeatability for both 3MHA and 3MH. The LOD for 3MH and 3MHA were 0.07 and 1.68 ng/L, which is lower than the sensory thresholds of these compounds. However, we did not get good quantification results for 4MMP, probably due to this compound being a tertiary thiol. This is not optimal, but the method is working well for 3MH and 3MHA quantification, which are the two most important volatile thiols. As part of this validation procedure we also analyzed the volatile thiol content of 18 South African Sauvignon blanc wines. Average 3MH and 3MHA concentrations of the wines were 1320 ng/L and 313 ng/L for 3MH and 3MHA respectively (Table 2).

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Table 1: Volatile thiol levels of 2011 Sauvignon blanc wines (ng/L) analysed with GCMS.

Wines	4-mercapto-4-methylpentan-2-one (4MMP)	3-mercapto-hexylacetate (3MHA)	3-mercaptohexan-1-ol (3MH)
Altydgedacht	9.1	226.0	896.6
Neethlingshof	4.9	51.3	604.8
Phizante Kraal	34.6	266.1	1023.1
Diemersdal	9.8	304.4	1143.6
Bergkelder Fleur du Cap	10.2	218.2	1171.8
Spier Lonely Blue Gum	6.1	141.8	668.9
Neil Ellis	8.8	46.4	510.2
Vergelegen	19.0	200.1	1153.6
Hermanuspietersfontein No.3	10.2	75.4	842.3
Dawid Nieuwoudt Ghost Corner	10.4	136.8	543.9
Cederberg Cellar Driehoek	14.9	324.3	1234.0
Groot Constantia	11.3	146.5	1037.3
Springfield Life from Stone	5.3	390.9	3451.5
Klein Constantia	11.5	112.3	826.3
Anura	14.3	38.8	506.2
Graham Beck Pheasant's Run	15.0	720.1	1298.6
Tokara	8.5	121.9	1120.6
Kleine Zalze	10.8	154.5	1045.9
De Grendel	13.5	98.3	740.2
Boschendal	15.4	64.9	915.4
Overhex Soulo	5.4	96.3	1624.2
Du Toitskloof	3.2	84.3	800.5
Robertson	3.3	11.6	569.8
Obikwa	4.7	37.7	453.6
Two Oceans	4.7	61.7	660.5
Flutterby	4.4	88.7	759.6
Savanha	3.9	45.5	582.0

Table 2. Description and thiol levels in the wine samples analysed with UPLCMS.

Name	Vintage	Type of sample	3MHA (ng/L)	3MH (ng/L)
Cellar 1	2012	Bottled	83.01	893.44
Cellar 1	2013	Bottled	571.86	1645.89
Cellar 2	2013	Bottled	552.93	3137.00
Cellar 3	2012	Bottled	231.40	1890.66
Cellar 3	2013	Bottled	300.13	824.71

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Cellar 4	2013	Tank	111.57	364.66
Cellar 4	2013	Bottled	88.93	753.96
Cellar 5	2013	Bottled	322.52	820.10
Cellar 6	2013	Tank	676.32	1288.58
Cellar 6	2013	Tank	457.49	1153.67
Cellar 7	2012	Bottled	236.00	1802.02
Cellar 7	2013	Bottled	1028.66	2262.22
Cellar 8	2012	Bottled	52.35	1459.91
Cellar 8	2013	Bottled	184.45	1468.70
Cellar 9	2013	Tank	218.92	1001.49
Cellar 9	2013	Tank	277.12	1064.45
Cellar 10	2012	Bottled	18.98	717.92
Cellar 11	2013	Bottled	231.08	1216.36

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Complete the following table

Milestone	Target Date	Extension Date	Date Completed	Achievement
1. Set up a GCMS method for thiol calibration.				Not achieved
2. Set up a LCMS method for thiol calibration.				achieved
3. Assess thiol concentrations in well-known bottled SA Sauvignon blanc wines wines.				achieved
4. Assess thiol concentrations in Sauvignon blanc wines from different vintages.				Achieved for 3MH and 3MHA
5. An article has been accepted in the Australian Journal of Grape and Wine Research on the UPLCMS method.				achieved

Accumulated outputs

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

Conclusions

We have developed a novel LCMS method for volatile thiol levels in SA wines. Levels of 3MH and 3MHA are on par with those of some foreign wines.

Technology development, products and patents

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

We developed a novel LCMS method for thiol analyses in wine which could be commercialised in South Africa.

Suggestions for technology transfer

This method could be advocated in South African Wine Laboratory meetings, while the thiol levels can be made possible for winemakers as part of a database on SA wine composition

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.

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Student level (BSc, MSc, PhD, Post doc)	Cost to Project
1.M. Sc. Agric	R100 000
2.	
3.	
4.	
5.	

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

Federico Piano, Daniela Fracassetti, Astrid Buica, Marietjie Stander, Wessel J. du Toit and Antonio Tirelli. (2014). Development of a novel liquid/liquid extraction and UPLC-MS/MS method for the assessment of thiols in South African Sauvignon Blanc wines. Accepted for publication in the Australian Journal of Grape and Wine Research.

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.

Presentations/papers delivered

E. van Wyngaard, J. Brand, D. Jacobson, W.J. du Toit. (2012). Important compounds contributing to South African Sauvignon blanc wine aroma. 34th SASEV conference (14-16th of November 2012).

Total cost summary of the project

TOTAL COST IN REAL TERMS	COST	CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
YEAR 1	2011					100,000.00	65,000		
YEAR 2	2012					120,000.00	60,000		
YEAR 3									
YEAR 4									
YEAR 5									
TOTAL									

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