Industry allocated project number	PHI allocated project number

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FINAL REPORT
(2017)

1. PROGRAMME AND PROJECT LEADER INFORMATION

Research Organisation Programme leader	Project leader
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2. PROJECT INFORMATION

Research Organisation Project number	WW-ASB-1601
Project title	Determining levels of volatile compounds associated with smoke taint in South African commercial and experimental wine by chemical analysis and sensory evaluation
Short title	Chemical analysis and sensory evaluation of smoke tainted wine

Fruit kind(s)	Wine grapes		
Start date (mm/yyyy)	01/2015	**End date** (mm/yyyy)	03/2017

Key words	Smoke taint, volatile phenols, GC-MS, UPLC-MS, sensory evaluation

Approved by Research Organisation Programme leader (tick box) ☐

Consider all findings as preliminary

3. ABSTRACT

The main wine production areas of South Africa have a Mediterranean climate. Long hot summers are associated with strong south-easterly winds, and the propensity for the local flora (*fynbos*) to burn regularly, mean that veldfires are a frequent event when grapes are ripening on the vines during the first few months of each year. Wines produced from grapes that are exposed to smoke can exhibit undesirable smoky, burnt aromas and an ashy character on the palate.
In order to measure the impact of veldfires on wines and/or the success of amelioration strategies by winemakers, it is necessary to have chemical/analytical and sensory methods in place to analyse for compounds, precursors and attributes associated with the taint. This project enables the development of this methodology in order to provide the South African wine industry with tools for monitoring taint levels in grapes and wine.

In this study, Shiraz grapes from Welgevallen vineyard in Stellenbosch were smoked in 50 L plastic containers using a commercial beekeeping smoker. Wine were made from the smoked grapes and analysed for levels of volatile phenols using an existing GC-MS method. Grapes were also analysed for smoke volatiles. Wines produced from unsmoked and smoked grapes differed in colour, non-volatile and volatile phenol compounds. Grapes and wine from seven commercial vineyards that had been affected by veldfires were also collected and analysed.
This project forms part of a larger collaboration which includes investigations into the effects of various winemaking microorganisms on the volatile compounds associated with smoke taint being carried out at the ARC.

4. PROBLEM IDENTIFICATION

Wines produced from grapes harvested from vineyards exposed to smoke can exhibit undesirable smoky, burnt aromas and an ash character on taste. This taint is commonly described as “smoke taint” and can result in significant financial losses, with many winemakers deciding to abandon affected grapes rather than harvest and risk making tainted wine.

The aims of this project, in order to help South African winemakers during problematic vintages, were:

- to develop methods for detection of volatile phenols (VPs) by GC-MS and show evidence of VPs in smoke affected wines, as well as monitor levels in industry samples
- to train a panel in detection of smoke-related attributes in affected wines by DA and rapid methods
- to set up a method for detection of VP glycosidic precursor levels by UPLC-MS

This project involves collaboration between ARC Infruitec-Nietvoorbij, Stellenbosch University (Institute for Wine Biotechnology (IWBT) & Department of Viticulture and Oenology (DVO)) and Institute for Grape and Wine Science (IGWS).

5. PROGRESS

Performance chart (for the duration of the project)

The project was divided into the 'Pilot' aspects which happened in 2015 (laying out conditions for the actual experiment), and the experimental work in 2016. A number of changes were made to the original scope of the experimental project due to unforeseen circumstances. No postgraduate students were available due to very limited numbers of students graduating in 2015. The project objectives therefore had to be met with technical assistance from the IGWS (Ms Alex Schulze) and staff and students in the DVO, who had full-time jobs and commitments to meet. Plans to set up smoke structures similar to those constructed by De Vries et al (published 2016) had to be abandoned in favour of small scale smoking in clear plastic containers. This fulfilled experimental design requirements, and microbiological work was able to continue.

Table 1: Performance Chart for project

Objectives	Milestones	Original Target Date	Date achieved
PILOT PROJECT			
Hydrolysis trials (acid/enzyme) and analysis	Pilot project*: establishing 'bound' volatile phenols levels	May-June 2015	July 2016
Method development for detection of glycosides (LC-MS)	Pilot project*: establishing 'bound' volatile phenols levels	Sept-Oct 2015	Discontinued due to lack of resources (please see 7.1.3)
Locating a vineyard suitable for smoke trials	Pilot project*	Oct /Nov 2015	March 2015
EXPERIMENTAL WORK			
Direct GC-MS analysis of affected wine samples from 2015	Augmentation of existing GC-MS	May- June 2015	Nov 2015
Migrate LC-MS to MS/MS	Increase the reliability of the results	2016	Discontinued (see 7.1.3)
Obtain smoked grapes from commercial cellars	Smoked grapes/juice samples from commercial vineyards	Feb 2016	Feb- April. 2016
Smoking of grapes for 2016-harvest	Smoke tainted grapes of 2016-harvest	Feb2016	Feb. 2016
Making wines from treatment and control plots (2016)	Samples for Sensory and Chemical analysis	March-May 2016	May 2016
Training of sensory panel for screening purposes	Sensory screening of initial wine samples	Dec 2015	Sept 2016
GC-MS analysis of experimental and commercial wines	Completed wine data set	2016-17	Data set collected for 2015 and 2016 (**App. 1 A & B and 2).**
Sensory evaluation of experimental and commercial wines 2016	Evaluation of experimental and commercial wines	Oct 2016	November 2016

6. WORK PLAN MATERIALS AND METHODS

***Pilot Project: 2015**

Samples from industry for 2015 were analysed by existing direct GC-MS analysis (Appendix 1). A call for smoke-tainted grape and wine samples went out in February 2016, and samples were received from seven farms

Samples supplied for assessment / VP analysis	No. farms	No. batches
Smoke-affected grapes (not yet fully ripe)	1	1
Smoke-affected grapes (ripe)	4	7
Smoke-affected juice	2	2
Smoke-affected wine	5	11

Hydrolysis trials (acid/enzyme) and analysis of hydrolysed samples was initiated. Enzymes were tested, as well as initial acid hydrolysis conditions. Development of a method for detection of glycoconjugated precursors of volatile phenols by liquid chromatography- mass spectroscopy (LC-MS) was initiated. Please see Section 7.1.3 for further information.
Optimisation and application of a GC-MS method for commercial samples (juice and wine), and development of a sample preparation method for GC-MS analysis of grapes took place.
Suitable grapes for smoke trials were sourced from Welgevallen (Shiraz) and grape-smoking in large plastic crates was trialled.

Experimental project (2016)

6.1 Samples affected by smoke from commercial cellars/vineyards-2016

Commercial cellars/vineyards that had problems with veld/vineyard fires were contacted, and grapes and/or juice samples were collected. Grapes with smoke exposure or juice were submitted to CAF for Gas chromatography-mass spectrometry (GC-MS) analyses to determine the levels of volatile phenols. Mr L. Mokwena and Ms A. Schulze were responsible for this part of the project. Smoke-exposed grapes from seven commercial vineyards and 10 wine samples were collected and their volatile phenol levels analysed (see Appendix 1). The identity of the cellars/vineyards are confidential, but please contact Ms M. Mckay if additional information is needed.

6.2 Smoking of grapes (Experimental treatments): 2016

Initial plans were to smoke the grapes in the vineyard, but due to lack of technical assistance (No post-graduate available and MSc student from Tunisia cancelled due to family commitments), logistical problems and weather concerns, it was decided to smoke the grapes in plastic containers (Figure 1). The plastic containers gave us more control over the level of smoke exposure and smoking of grapes was not affected by adverse weather conditions (heat waves and gale force winds in the vineyards).

Grapes from the Shiraz block at Welgevallen farm (US) were used for smoking experiment. Grapes were picked from the same side of the row and then divided into 50 L plastic containers. Each container was weighed. Pine needles and fynbos were placed into a standard commercial beekeeper's smoker (used to smoke bee-hives) and set on fire. The smoke stream was directed into the container by lifting the lid corner slightly of each container. Smoke was blown into the treatment containers until the atmosphere inside the container was completely saturated inside and grapes were invisible. The lids were taped closed, and the smoke allowed to diffuse through and into the grape mass overnight

Table 2: The following treatments were carried out (2016).

Controls:	4 containers x ~10 kg unsmoked Shiraz grapes stored in laboratory at 20C for 48h
Treatment 1:	3 containers x ~10kg exposed to fynbos and pine-needle smoke, and left for 48h at 20C
Treatment 2:	containers x ~10kg exposed to fynbos and pine-needle smoke, and left for 24h at 20C then exposed again, and left for a further 24h at 20C.

Figure 1: Crates containing smoked grapes in the laboratory.

The crates for Treatment 2 were smoked again after 24 hours. Thereafter the grapes were kept at 15 degrees until crushed into separate fermentation containers. The four crates containing reference/control treatment was also stored in the laboratory in plastic crates but did not receive any smoke exposure. Samples of berries were taken from the smoked grapes and controls and analysed at CAF for volatile phenols by GC-MS using a method developed for this project.

6.3 Winemaking (2016)

The grapes from Welgevallen were used for wine production trials at SU. Winemaking was carried out using standard SU experimental cellar winemaking protocols. Control and smoked grapes were received at the JH Neethling Experimental cellar, crushed and destemmed and dosed with 30mg/l sulphur dioxide. Yeast strain QA23 was used for all fermentations, with an addition of 0.3g/l Goferm nutrient. Industry grapes were processed as they came in to the cellar. Standard juice and wine analyses (sugar, pH, total acidity) were performed. Juice and wine samples were also collected, stored and analysed for volatile phenols and glycoconjugated precursors. Samples were prepared and sent to Central Analytical Facility (CAF, US).

6.4 *Chemical and sensory evaluation of 2016 wines:*

6.4.1 *Chemical procedures*

- Grapes samples were collected after smoking, and then frozen before being macerated and prepared for GC-MS analysis.
- Juice, must and wine samples were collected throughout the winemaking process for GC-MS analysis.
- Standard juice and wine analyses (sugar, pH, total acidity, volatile acidity, were carried out during winemaking.

- Grapes,juice, must and wine samples were analysed for volatile phenols by GC-MS at CAF, during the vintage, and samples were stored in order to monitor in-bottle release after 12 months of bottle ageing
- Hydrolysis of glycoconjugated precursors by acid and enzymic means were trialled, and samples stored for follow-up volatile phenolic analysis.

An overview of juice and wine analyses follows in Results Section 7 of this report.

6.4.2 Sensory:

Sensory aspects (screening of samples, training panels, post-treatment evaluation) were carried out at US and Ms V. Panzeri was the panel co-ordinator. Initial screening of a panel of judges for evaluation of experimental wines started on September 30, 2016. Formal training of sensory panel for wine-taint screening purposes and evaluation of the aromas of treated and untreated experimental wine samples was conducted over the next three months. Each panel session involved at least ten judges, and lasted two to three hours. There were twelve initial training sessions, followed by three testing sessions.

We also included DA sessions for the industry wines, amounting to around 20 sessions in total over 2016/17. A large proportion of the funds paid to the US was used for this purpose, as each panel session costs between R2,500 and R3,000.

The sensory evaluations were performed on commercial wines from fire-affected areas around the Cape as well as experimental wines. This included focus group sessions in an informal tasting, during which the wines were evaluated blind and discussed immediately after tasting. It also included Descriptive analysis (DA) of wines by a panel of trained judges.

Development of rapid assessment methods (which also involved training) were also carried out over the next eight months. Data from both analytical and sensory assessments were subjected to statistical analysis.

7. RESULTS AND DISCUSSION

7.1 Chemical analysis

7.1.1 Odour Detection Thresholds

There was a clear difference in colour between the unsmoked and smoked juice and resulting wines, with wines produced from smoked grapes having a better colour than wines produced from unsmoked grapes. The levels of volatile phenols were much higher in the wines produced from smoked grapes than wines produced from grapes that were unsmoked in the microbiological trials conducted at the ARC (Table 5 and 6, ARC Report 101648 submitted by Mr H. du Plessis) in grapes from Nietvoorbij vineyards.

Table 3 Shows the ODTs and characteristics of some of the volatile phenols associated with smoke, and smoked products. Some of them have fairly low detection thresholds, for example, the cresols, but some (like phenol) are very high. Not all the thresholds have been established in red wine and researchers are often working in a very different matrix to what the threshold has been detected in.

The lower the detection threshold, theoretically, the more easy it should be to detect in wine, but unfortunately this is not always the case. In Appendix 1B, results of around thirty commercial wines brought in by industry and/or suspected of having smoky or ‘bretty’ characteristics are presented. It can be clearly seen that the vast majority of phenols present come in below their detection thresholds (Table 3). There are some interaction effects at

subthreshold levels which may contribute to smokiness when VPs are present together. It is not easy to predict from the matrix what will happen to the wine's aroma, and a lot more research is needed in this area to understand the agonistic and antagonistic effects.

Table 3: Odour detection thresholds in parts per billion (ppb) and aromatic attributes for volatile phenols found in smoked products (aq= in water; eth = model wine matrix; red = in red wine)

Compound	Guaiacol	2,6 Dimethyl phenol (2,6-xylenol)	4 Methyl Guaiacol	o-cresol	phenol	4 Ethyl Guaiacol	m-cresol	p-cresol	2,3 Dimethyl phenol (xylenol)	Eugenol	4 Ethyl phenol	3,4-dimethyl-phenol (xylenol)
Descriptors	smoke, sweet, burnt	sweet, tarry odour	sweet-spicy, phenolic-leathery	rubber, medicinal, ink	sweet, cloying, chemical	clove,medicinal, woody sweet	leather, rubber, ink-like	horse-stable, fecal	ink, sweet, leather	clove, phenolic, sweet	Band-aid, medicinal, bacon, smoke,	horse, stable, fecal, ink
ODT	**9 (aq) -23 (red)**	**400 (aq)**	**21 (aq)**	**31 (aq)-62 (red)**	**5900(aq)**	**50 (aq)**	**15 (aq)-68 (eth)**	**4(aq)-10 (eth)**	**500 (aq)**	**6 (aq) - 700 (red)**	**450(aq)-605(red)**	**1200 (aq)**

7.1.2 Results of volatile-phenol analysis by GC-MS:

a) Berries

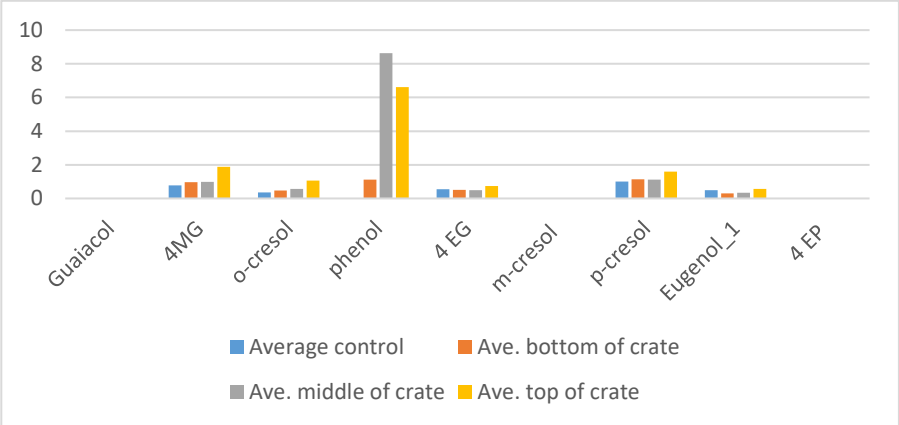


Figure 2: Differences between bottom, middle and top of smoking crates in VP levels in berries

A sample preparation method was developed specifically for this project by Mr L. Mokwena of CAF for measuring volatile phenols in grape berries, with technical assistance from Ms Alex Schulze. Although the levels of volatile phenols in the berries generally were low, it is clear from Appendix 2A and Figure 2 above, that berries at the top of the crates had increased concentrations, especially of phenol. This is because of greater exposure to the smoke which was introduced into the crates from the top of the crate. Berries in the middle and the bottom of the crate did not absorb as much of the smoke volatiles.
As a result of this information, it was imperative that during winemaking that all crates were mixed very well and the the berries were crushed, destemmed and fermented in a tank per treatment. During fermentation, punching down and pumping over also were used to ensure good mixing of berries and juice, negating any differences in the VP levels within treatments.

b) Experimental juice and wines:

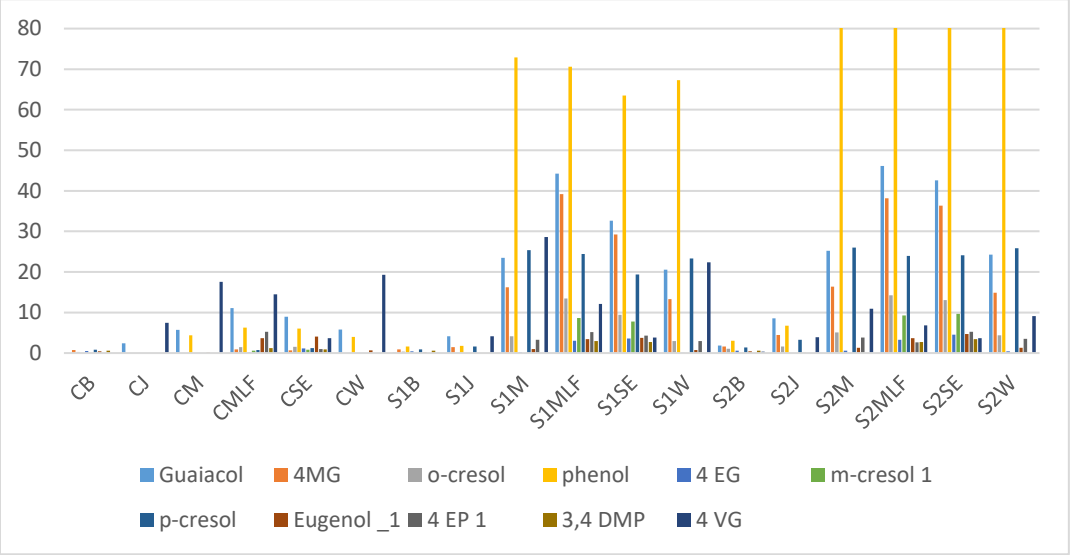


Figure 3: Averages for all volatile phenols in berries (B), juice (J), must (M), after fermentation (W) after MLF, before SE.

Volatile phenols were measured at six stages of the winemaking process (Appendix 2(A-F): In berries (B), grape juice (J), before pressing(M), after pressing (W), after malolactic fermentation (MLF) and after bottling, before sensory evaluation (SE). Averaged results (3 repeats) are shown in Figure 3- 7. We have not shown results before and after pressing, as trends are similar to those in the wines before ML and after bottling. The GC-MS method for measuring phenols in grape berries is newly developed

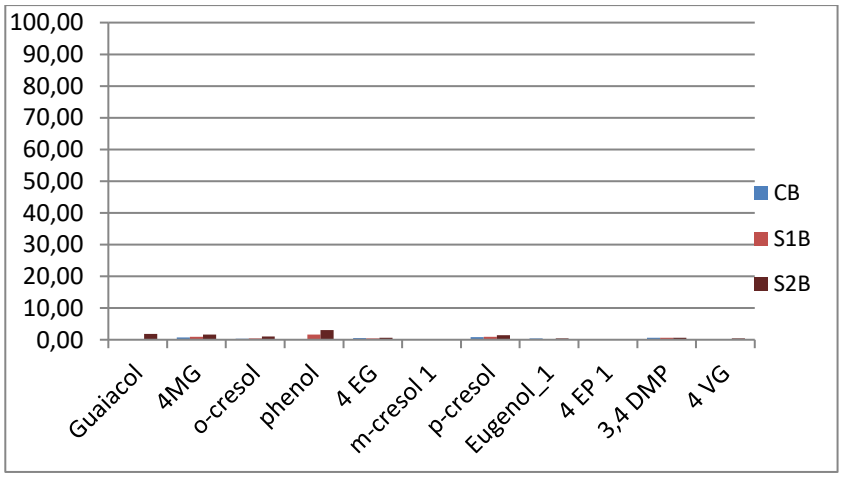


Figure 4: Volatile phenol levels in berries (ppb) where C= control (no smoke exposure); S1= Treatment 1-one exposure to smoke; S2= Treatment 2-two exposures to smoke.

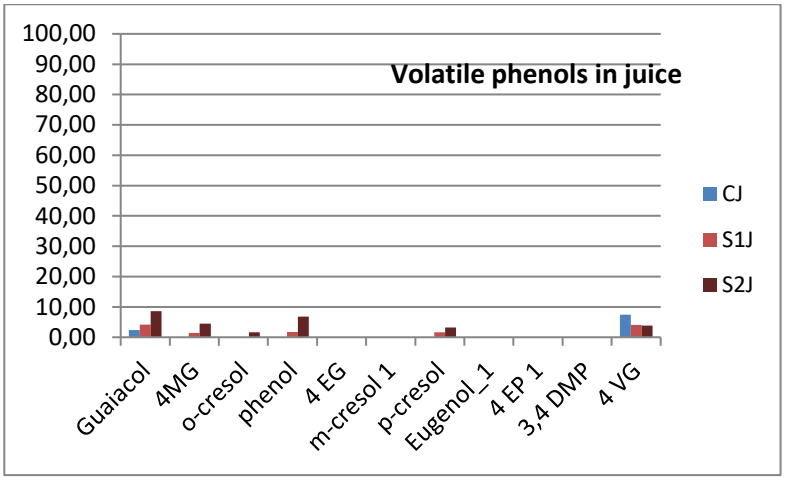


Figure 5: Volatile phenol levels in grape juice (ppb) where C= control (no smoke exposure); S1= Treatment 1-one exposure to smoke; S2= Treatment 2-two exposures to smoke.

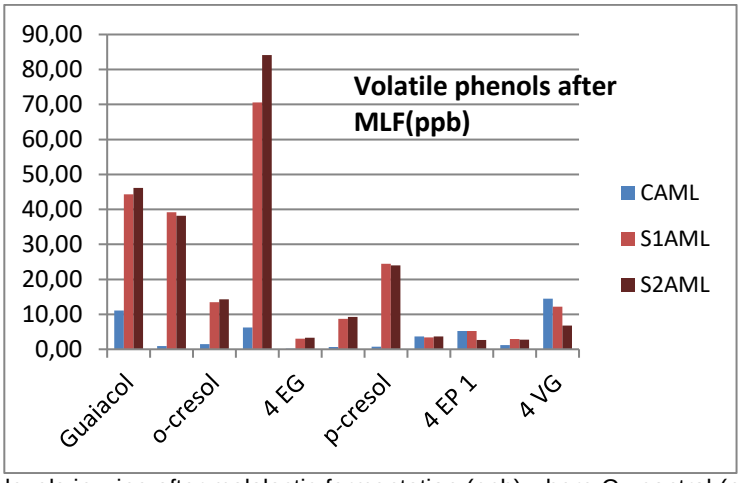


Figure 6: Volatile phenol levels in wine after malolactic fermentation (ppb) where C= control (no smoke exposure); S1= Treatment 1-one exposure to smoke; S2= Treatment 2-two exposures to smoke.

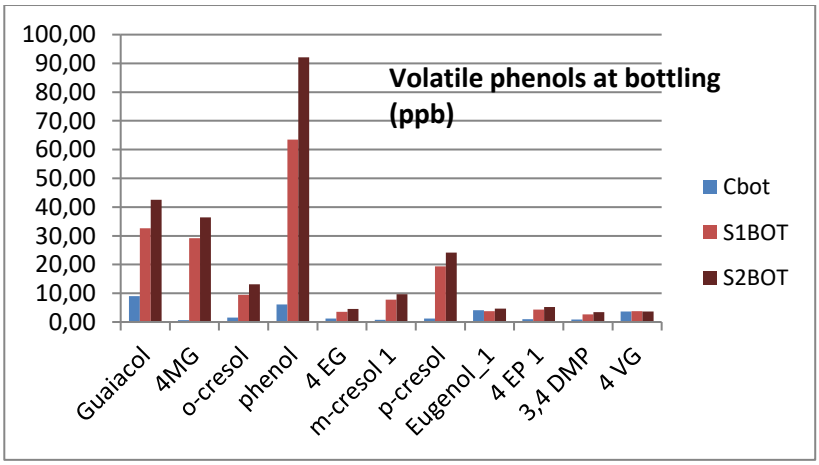


Figure 7: Volatile phenol levels in wine on bottling (ppb) where C= control (no smoke exposure); S1= Treatment 1-one exposure to smoke; S2= Treatment 2-two exposures to smoke.

From the figures above, it is evident that volatile phenols increase during the winemaking process, if grapes have been exposed to smoke. Levels in the figures indicate that our experimental smoking is adequately exposing the grapes to smoke, and in all but one case (16/564), our smoke treatments are within the same ppb ranges as wines made from grapes exposed to real fires.

Berries may not show strong evidence of volatile phenol contamination after smoke exposure (although this is not a rule, as can be seen in the industry data below). The increases are due to the hydrolysis of glycoconjugated precursors of the volatiles as a result of exposure to acids in the juice and wine and hydrolytic enzymes released by yeast during fermentation. After glycoconjugates are hydrolysed, volatile phenols will be released into the juice, and may exceed their threshold limits and become aromatic. This is substantiated by the enzymatic hydrolysis results carried out in Section 7.1.4 below.
It is also evident that one exposure to smoke is enough to significantly affect levels of most of the compounds for which we were able to analyse, and that the second exposure to smoke does not seem to increase VP levels consistently or dramatically. Interestingly, the trend seems to be that the there is a slight reduction on bottling, possibly as a result of compounds being adsorbed onto yeast lees and being racked or filtered off, or being metabolised during MLF. Work by Mr du Plessis and his colleagues at ARC should help to clarify this situation.

As can be seen in Appendix 1A and 1B (samples of grapes, juice and wine exposed in the vineyard to veldfires during the ripening period that were submitted by industry), trends are much the same, with berries and juice showing generally lower levels of volatiles, but wines exhibiting greatly increased levels. In the cases where vineyards had not been exposed to smoke (as in our controls), VP levels are generally low.

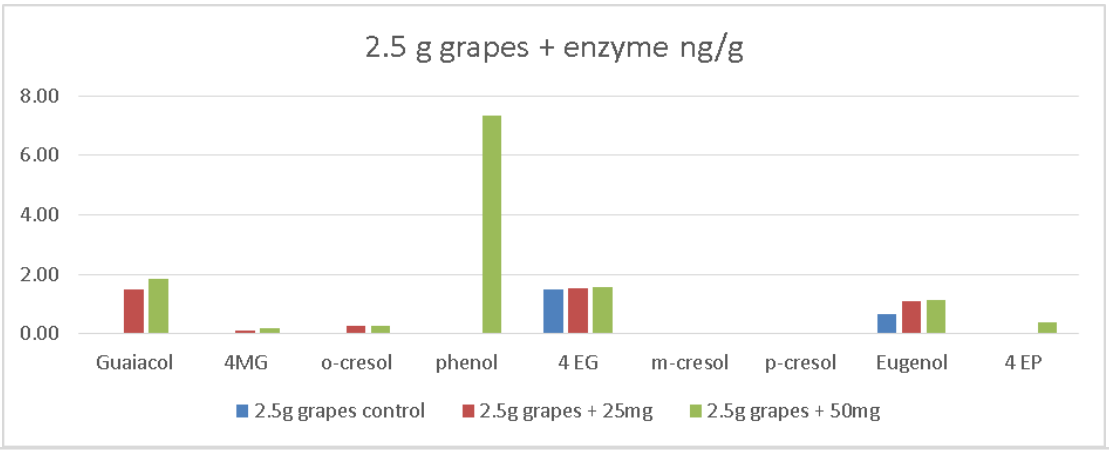
7.1.3. *LC-MS and LC-MS/MS method development:*

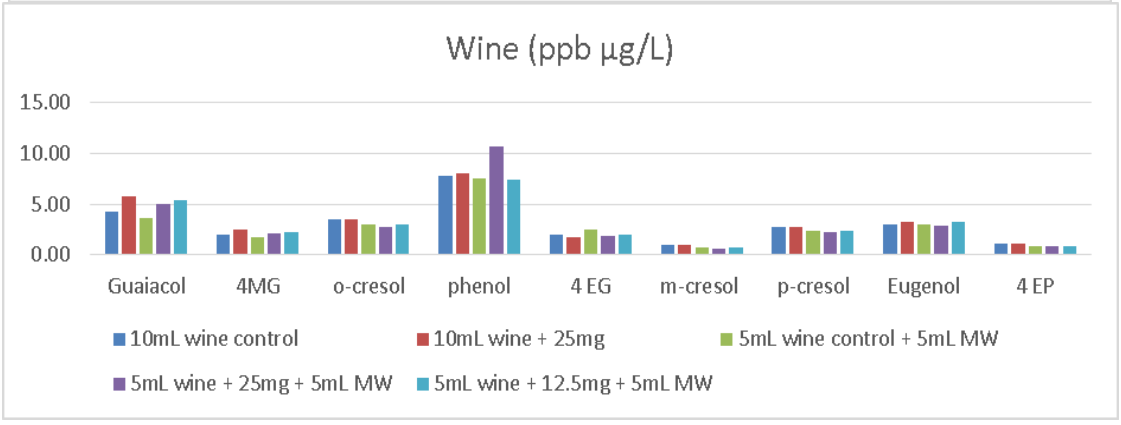
Setting up the LC-MS and LC-MS/MS analysis of the glycoconjugated precursors was unsuccessful since the precursors could not be identified by either technique. The work was done at CAF with the support of their staff scientist Dr M Stander, our expert in the LC-MS field. The running conditions and the equipment was similar to the one used in the publications from AWRI, but the lack of authentic standards made the identification of the compounds impossible. Since these compounds have already been identified and characterised, we expected to be able to use their MS properties for our application, but it was not the case.
Briefly, for the LC-HRMS (high resolution MS), the signal was too complex for interpretation in the absence of authentic standards. The idea was to use each compound's characteristic m/z to identify and then quantify the precursors. The m/z values are available from the literature for a variety of precursors, such as glucosides, glucosylglucosides, rutinosides, and glucose-pentose disaccharides of guaiacol and methyguaiacol but the MS signal obtained after chromatographic separation could not be interpreted in the absence of the standards.
The LC-MS/MS approach is slightly different from LC-HRMS. The MS/MS transitions can be chosen for the glycoside part of the precursor molecule (neutral loss approach) or for the signal resulting from the ionization of the precursors molecules (multiple reaction monitoring, MRM, approach). Both approaches were unsuccessful again due to the lack of authentic standards.
This is a setback since we will not be able to evaluate the presence of specific precursors for future work, but the indirect quantification of the precursors as a group is still possible using the GC-MS technique coupled with hydrolysis.

7.1.4 GC-MS of hydrolysates:

For the GC-MS work, the instrumental method was tested for a variety of matrices, such as grapes (as homogenate), juice, and wine. It was necessary to do the testing to make sure that the instrumental method is adequate, since components relevant to the analysis (for example the sugar level and the presence or absence of alcohol) differ for these matrices. This was done with the help of recovery tests – a spike test in which we compared the results obtained from interfering and non-interfering matrix, such as real wine *vs* model wine. The results were satisfactory and the method is considered appropriate.
Since the direct measurement of precursors was problematic, we focused on the hydrolysis approach. The idea behind this is to analyse twice the same sample, with and without hydrolysing

the precursors. The direct analysis gives us the level of VP as they are present in the sample, while, after hydrolysis, the level of VP will be a combination of initial level plus what was released from the precursors. As mentioned above, this approach will not indicate which precursors are present in the sample, only how much VP these precursors can release. From a practical point of view, the advantage of this analysis is that it is performed on only one instrument (GC-MS as opposed to GC-MS for free VP and LC-MS for bound VP) and that the quantification is easier, since we have the authentic standards for the measurement.
The hydrolysis method was also tested for the amount of sample to be used and for the ratio between sample and glycosidic enzyme to obtain the maximum consistent release of VP from precursors.





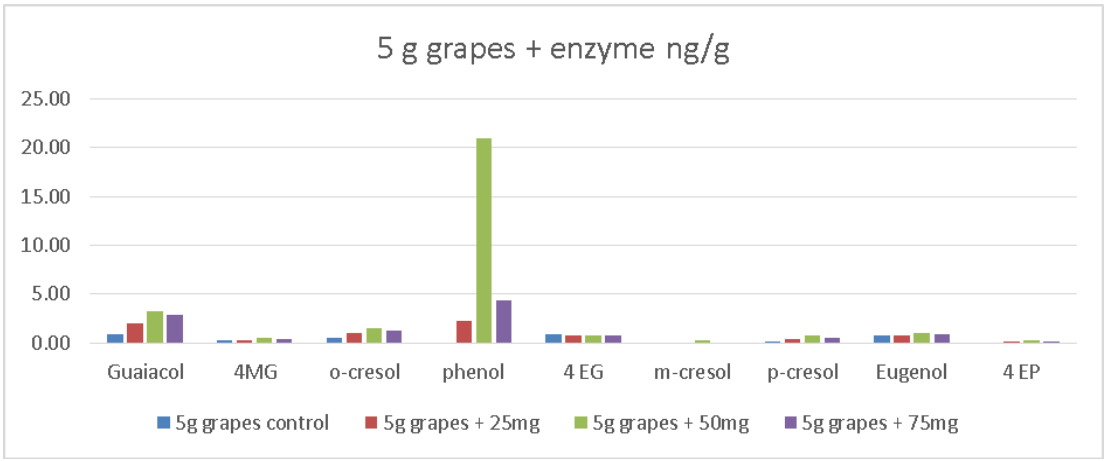


Figure 8: Results for grape and enzyme-level trials

As can be seen from the figures above, for grapes, 5g homogenate and 50mg enzyme yielded the best results, and for wine 5mL and 12.5mg enzyme. Trying to reduce further the quantity of grapes used for the analysis lead to difficulties in the detection (response close or below LOD).

7.2 Sensory Analysis

Panels were presented with both industry samples of suspected smoke-tainted wine, and control and treated wines from the smoking experiments. Panel training ensured panels reached consensus about quality and intensity of attributes. Figure 9 shows an example of a Tucker plot generated during one of the sessions involving industry samples, and although some of the attributes are not agreed upon, it is clear that judges are well-aligned in most cases regarding the presence, identity and intensity of smoke-related attributes.

In some cases for the commercial wines, the trained panel could positively link smoke taint characteristics to the sample set of wines presented. However, variations among wines seemed to be associated mostly to the variety and characteristics of the cultivars used for making the wines, and the presence of woody or oaky characteristics from wood maturation and not to any presence of contaminants in the form of smoke affected grapes. The sensory evaluation definitely seems to correlate and reinforce the conclusions given by the chemical analysis of volatile phenols reported in commercial and experimental samples. In commercial wines presented to us, the levels of volatile phenols are usually relatively low and below detection threshold and not appearing to have a significant influence on the sensory profile of the wines. In experimental wines made from deliberately smoked grapes, no winemaking interventions, and generally higher VP levels, it was easier for the panel to detect smokiness and ashiness.

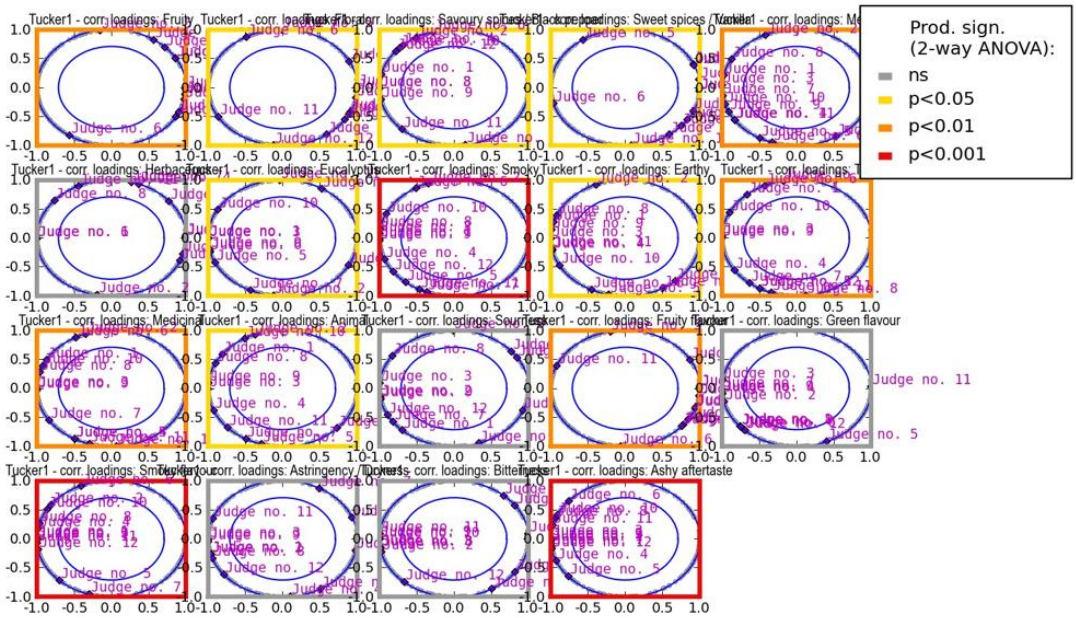


Figure 9: Tucker 1 plots representing the judge's consensus on the different attributes. Plots circled in red, orange and yellow show significant values p≤ 0.05 (Panel Check software).

With the experimental wines,sensory analysis of control and treated wines showed clearly that samples differed from controls in a Principle Component Analysis of their aroma attributes (Figure 9), where control samples were described more often with 'fruity' attributes, and separated from treatments that were more closely associated with 'smoky, ashy', or 'animal' attributes. These are all characteristics that have been associated with smoke taint and volatile phenols by previous workers. The controls also separated clearly from smoked samples in a sensory biplot based on flavour and palate characteristics (Figure 10).

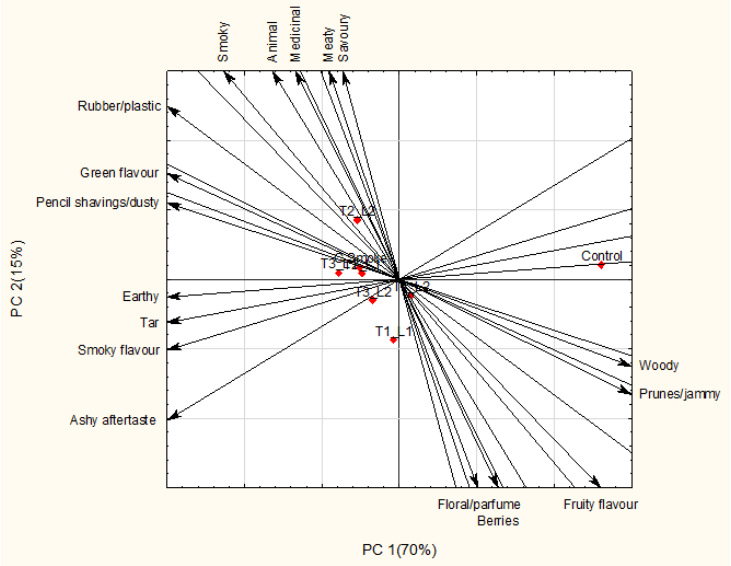


Figure 10: PCA showing separation of controls from treatments according to aroma attributes

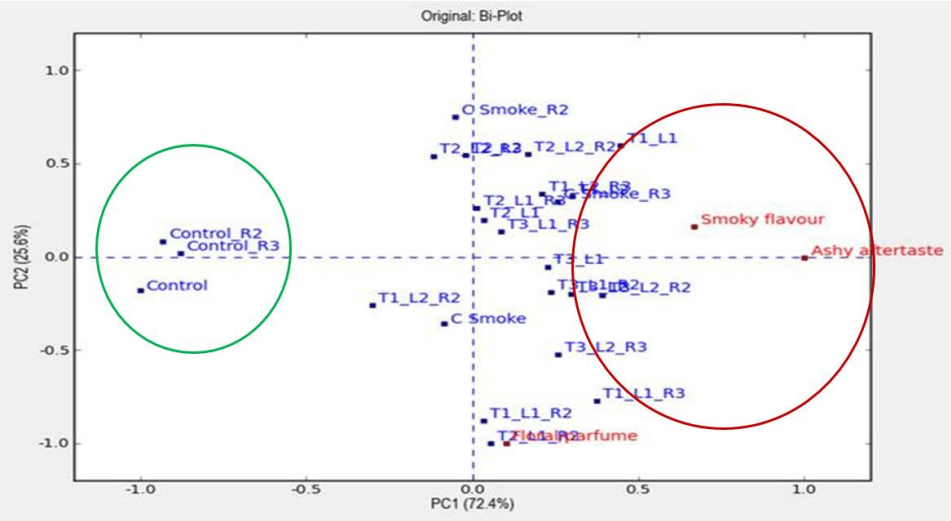


Figure 11: Sensory Biplot showing separation of controls from smoke-treated samples according to flavour and ashy aftertaste

8. CONCLUSIONS

Smoking of grapes in a plastic container can be used to simulate smoke exposure in a vineyard. Smoking has an effect on the volatile phenolic compounds of grapes, juice and wines. Volatile phenols generally increase during the winemaking process, reaching their maxima after malolactic fermentation, in this set of experiments. On bottling, small decreases were shown, probably as a result of compounds being adsorbed onto cell wall surfaces and being removed by racking and/or filtration operations. A trained panel was able to differentiate controls from smoke-treated samples through aroma attributes and flavour characteristics, but this was more challenging with commercial samples where cultivar, vintage and vinicultural interventions made the matrix more complex. These experiments open the way for experiments into amelioration of smoke taint through winemaking interventions.

9. PLANNED OUTPUTS

a) Articles:

- McKay, M., Mokwena,L. Buica, A. Methods for amelioration and quantification of smoke-taint compounds through enzyme-initiated release and remove strategies
- McKay, M., and Mokwena, L. Sample preparation for GC-MS analysis of grape berries
- McKay, M. Panzeri, V. Improving smoke-taint detection in wine through low-level attribute training.

b) PRESENTATIONS/PAPERS THAT COULD BE DELIVERED

Conference presentations (SAJEV 2018) and proceedings The findings of the proposed research would provide suitable material for oral presentations at national and international conferences for the duration of the project.

c) IMPACT OF PROJECT

- Better understanding of the causes and effects of volatile phenols in solution as a result of this project.
- Availability of GC-MS and LC-MS methods for transfer to another laboratory, or for direct use by industry at CAF/DVO/IWBT.
- Alternative and improved winemaking practices for smoke tainted grapes.
- Sensory training for the SA wine industry in detecting smoke taint
- Availability of trained panels for SA industry
- Availability of rapid screening method for SA wine industry
- Will contribute to the stability of the wine industry by limiting loss of income due to smoke taint.

10. PERSONS PARTICIPATING IN THE PROJECT (Not including ARC)

Initials & Surname	Highest Qualification	Degree/ Diploma registered for	Race (1)	Gender (2)	Institution & Department	Position (3)	*Cost to Project R (SU)
A. Buica	PhD		W	F	DVO, SU	PL	-
M. McKay	MSc	PhD	W	F	DVO, US	PL	-
L. Mokwena	MSc		B	M	CAF, US	Coll	-
A. Schulze	MSc		W	F	IGWS/US	Coll	
V. Panzeri	MSc		W	F	IGWS/US	Coll	-

(1)Race B = African, Coloured or Indian
W = White

(2)Gender F = Female
M = Male

(3)Position Co = Co-worker (other researcher at your institution)
Coll = Collaborator (participating researcher that does not receive funding for this project from industry)
PF = Post-doctoral fellow
PL = Project leader
RA = Research assistant
TA = Technical assistant/ technician

Consider all findings as preliminary

BUDGET (2016) DVO-IWBT

Use of Winetech funds:

- Smoke-taint-related funds have been used to pay for consumables including 30 plastic containers, grapes and a smoker.
- Approximately a third of the funds went on panel training and testing
- A large portion of the funds went on GC-MS costs
- Funds were also used for voluntary contributions towards financial and administrative support, SU levies (17%) and winemaking and technical assistance.

2016	Budget	Spent	Remaining
Research Personnel (panel costs)	45,000	60,000	-R15,000
Research, admin Technical Assistance	35,000	45,000	R-10,000
GC-MS costs	45,000	50,000	R-5,000
Other (levies, overheads, publ costs etc)	40,000	35,000	R5,000
LC-MS	20,000	10,000	R10,000
Consumables	20,000	15,000	R5,000
Totals	R 205,000	R 215,000	-R10,000

a) ANNUAL BUDGET FOR PROJECT RECEIVED FROM INDUSTRY TO DATE (OR THE PREVIOUS 5 YEARS)

US budget

YEAR	CFPA	DFTS	SAAPPA SASPA	SATI	Winetech	THRIP	OTHER	TOTAL
2015					40,000			40,000
2016					135,000			135,000
2017					30,000			30,000
								205,000

EVALUATION BY INDUSTRY/PHI

This section is for office use only

Project number	

Project name	

Name of Sub-Committee*	

Comments on project

Committee's recommendation (Review panel in the case of PHI)

- Accepted. ☐
- Accepted provisionally if the sub-committee's comments are also addressed. Resubmit this progress report by_____ ☐
- Unacceptable. Must resubmit progress report. ☐

Chairperson_____ Date_____

*SUB-COMMITTEES

Winetech
Viticulture: Cultivation; Soil Science; Plant Biotechnology; Plant Protection; Plant Improvement;
Oenology: Vinification Technology; Bottling, Packaging and Distribution; Environmental Impact; Brandy and Distilling; Microbiology

Deciduous Fruit
Technical Advisory Committees: Post-Harvest; Crop Production; Crop Protection; Technology Transfer

Consider all findings as preliminary

APPENDIX 1A: RESULTS OF COMMERCIAL WINES FROM FIRE AFFECTED AREAS 2015

Results for 2015 smoke-affected wine samples submitted by farms (Duplicate numbers in light and darker blue). Certain samples were not resubmitted by the farm after six months and therefore do not have the darker blue duplicate. *Sample preparation and data collection carried out by Alex Schulze, IGWS; GC-MS analysis by Lucky Mokwena, CAF.*

2015	Guaiacol	2,6 dimethyl phenol	4 methyl guaiacol	o-cresol	phenol	4 ethyl guaiacol	m-cresol	p-cresol	2,3 dimethyl phenol	Eugenol	4 ethylphenol	3,4 dimethyl phenol
	concentration (ppb)											
1-05-15	10.30	2.03	3.10	1.85	6.81	0.85	2.29	0.99	nd	1.13	0.95	0.22
1-11-15	5.12	1.56	2.05	1.28	5.93	0.90	1.61	0.56	nd	1.10	0.56	
2/05/15	10.76	1.88	3.62	2.00	6.38	1.09	2.55	1.29	nd	0.99	1.09	0.27
3-05-15	8.10	1.94	2.09	2.45	7.21	0.16	2.69	0.86	nd	2.12	0.69	0.47
4-05-15	11.31	nd	1.90	0.56	5.28	0.03	1.51	0.41	nd	0.78	0.16	0.15
4-11-15	5.14	nd	1.15	0.30	5.58	0.47	1.08	0.18	nd	1.76	0.08	
5-05-15	7.21	nd	1.85	0.40	5.34	0.11	1.49	0.34	nd	6.70	0.31	0.58
5-11-15	5.69	0.32	1.40	0.41	8.27	0.66	1.33	0.46	nd	9.01	0.54	
6-05-15	1.62	0.17	1.27	0.14	3.55	0.34	1.23	nd	nd	20.21	0.87	0.85
6-11-15	1.86	0.23	0.92	0.12	3.44	0.82	0.84	nd	nd	14.61	0.73	
7-05-15	2.26	nd	1.20	0.11	2.29	0.08	1.07	nd	nd	7.72	0.29	0.78
7-11-15	2.34	nd	1.25	0.15	2.96	0.48	0.85	nd	nd	9.79	0.16	
8-05-15	5.98	nd	2.57	0.86	4.68	0.57	2.69	0.50	nd	20.22	1.04	5.72
9-05-15	44.90	0.69	4.28	5.12	11.87	0.88	5.90	3.17	nd	6.98	2.78	3.52
9-11-15	18.21	0.55	3.88	2.96	10.06	0.93	3.48	1.66	nd	11.65	1.58	
10-05-15	8.06	2.27	3.38	2.19	8.06	0.81	2.87	0.86	nd	7.31	2.06	0.19
10-11-15	8.62	2.30	5.46	2.44	12.02	2.06	3.69	1.74	nd	32.44	1.77	
12-05-15	7.85	2.78	3.04	2.29	9.54	0.60	2.59	1.00	nd	9.13	0.81	0.21
12-11-15	8.48	2.69	4.94	2.22	12.34	1.27	3.06	1.28	nd	44.03	1.65	
13-05-15	8.11	1.84	4.41	1.72	6.83	0.67	2.36	0.58	nd	17.95	0.59	0.08
13-11-15	7.54	2.59	3.22	2.58	15.80	1.07	3.18	1.62	nd	24.20	1.49	
14-05-15	3.00	3.18	1.32	1.34	5.94	0.13	1.66	0.06	nd	1.86	0.45	0.01
14-11-15	5.37	2.44	2.03	1.75	11.64	0.82	2.34	0.76	nd	16.49	1.65	
15-05-15	4.06	1.98	1.72	1.30	5.50	0.29	1.41	nd	nd	8.48	0.60	nd
16-05-15	11.85	0.77	4.06	3.12	10.07	1.43	1.94	1.34	nd	9.20	1.05	2.33
16-11-15	8.55	0.69	3.31	3.27	15.89	1.40	2.30	1.98	nd	14.72	1.30	
17-05-15	14.77	nd	4.58	3.55	7.54	2.69	1.79	1.49	nd	20.82	1.56	1.23
17-11-15	17.32	0.63	5.04	4.29	14.12	3.45	2.25	2.83	nd	22.05	3.20	
18-05-15	10.74	0.33	3.58	3.14	8.40	1.24	2.19	1.40	nd	4.41	0.83	1.72
18-11-15	9.25	0.51	4.73	3.65	15.45	1.55	2.56	2.54	nd	14.92	1.31	
19-05-15	22.01	1.09	8.94	6.37	14.53	2.58	3.76	2.68	nd	12.18	2.33	4.37
19-11-15	14.54	0.62	6.48	4.86	14.82	2.60	3.62	2.72	nd	14.82	2.02	
20-05-15	13.36	0.23	3.56	3.07	11.41	1.05	2.23	1.91	nd	5.44	1.12	2.34

APPENDIX 1B: RESULTS OF COMMERCIAL WINES FROM FIRE AFFECTED AREAS 2016

Table 1A: Volatile phenol concentrations in industry samples (analysed between 30/3 and 19/7 2016)

Code Wine	no	Matrix	Guaiacol	4MG	o-cresol	phenol	4 EG	m-cresol 1	p-cresol	Eugenol _1	4 EP 1
			Concentration (ng/g) (ppb)								
Attributes associated			smoke, sweet, burnt	sweet-spicy, phenolic-leathery	rubber, medicinal, ink	sweet, cloying, chemical	clove, medicinal woody sweet	leather, rubber, ink-like	horse-stable, fecal	clove, phenolic sweet	Band-aid, medicinal bacon, smoke,
ODOUR DETECTION THRESHOLD			**9 (aq) - 23 (red)**	**21 (aq)**	**31 (aq)- 62 (red)**	**5900(aq)**	**50 (aq)**	**15 (aq)- 68 (eth)**	**4(aq)- 10 (eth)**	**6 (aq) - 700 (red)**	**450(aq)- 605(red)**
1	19	Wine	3.24	1.20	0.65	21.48	0.12	2.53	0.46	1.81	5.42
2	1	Berries	2.18	2.12	2.51	3.82	1.07	0.00	2.93	0.85	0.15
3	9	Juice	0.08	0.28	0.17	0.00	0.00	0.00	0.00	0.00	0.00
4	36	Wine	9.91	2.30	2.38	8.30	1.20	0.62	1.80	0.87	0.21
5	37	Wine	10.78	2.29	2.22	6.64	1.23	0.64	1.49	0.69	0.28
6	5	Berries	6.69	0.40	1.91	8.63	nd	nd	5.77	nd	nd
7	15	Juice	0.67	0.40	0.52	nd	nd	nd	nd	nd	nd
8	38	Wine	11.75	2.87	3.72	13.88	1.38	1.35	2.29	0.80	0.49
9	39	Wine	8.94	3.15	3.85	13.88	1.67	1.68	2.62	0.95	0.30
10	16	Juice	nd	0.10	nd	nd	nd	nd	nd	nd	nd
11	20	Wine	1.02	0.27	0.18	2.65	nd	nd	nd	0.10	-0.05
12	17	Juice	0.74	0.26	0.03	nd	0.63	nd	nd	0.10	nd
13	18	Juice	0.69	0.39	0.25	nd	1.04	0.19	0.10	0.23	nd
14	21	Wine	nd	0.35	0.25	3.66	nd	nd	nd	1.99	0.14
15	22	Wine	0.21	0.53	0.46	3.24	nd	nd	nd	0.15	0.06
16	14	Juice	1.43	0.70	0.84	nd	nd	nd	nd	1.18	nd
17	30	Wine	4.50	1.54	2.91	7.75	1.26	1.15	2.01	3.54	0.77
18	3	Berries	nd	0.79	0.59	nd	0.47	nd	1.07	0.68	nd
19	4	Berries	8.26	3.80	4.34	0.70	0.86	nd	3.82	0.60	nd
20	13	Juice	0.83	0.60	0.76	nd	nd	nd	nd	0.69	nd
21	29	Wine	3.94	1.81	3.30	6.98	2.10	0.96	2.53	2.74	1.00
22	2	Berries	nd	1.09	0.91	nd	0.54	nd	1.43	0.88	nd
23	31	Wine	5.50	1.14	3.57	8.35	1.26	0.77	2.25	1.00	0.91
24	6	Berries	0.06	0.46	nd	nd	nd	nd	nd	0.00	nd
25	10	Juice	nd	0.11	nd	nd	nd	nd	nd	nd	nd
26	32	Wine	4.85	1.28	3.35	7.91	1.11	0.69	2.24	0.97	0.75
27	33	Wine	85.19	50.38	29.44	121.89	8.68	8.79	31.47	1.71	8.67
28	7	Berries	28.77	12.48	11.60	35.38	2.13	nd	13.48	0.59	nd
29	8	Berries	nd	0.45	nd	nd	nd	nd	nd	nd	nd
30	12	Juice	0.30	0.26	nd	nd	nd	nd	nd	nd	nd
31	11	Juice	4.82	1.29	1.88	nd	nd	nd	0.78	nd	nd
32	35	Wine	14.04	5.42	7.12	20.07	4.29	2.24	5.52	1.03	1.72
33	34	Wine	91.57	55.28	31.45	130.51	11.47	9.25	35.76	2.70	9.76

Consider all findings as preliminary

Appendix 2: Volatile phenol concentrations in experimental samples during winemaking

A) Berries:

Code	Sample Name	Guaiacol	4MG	o-cresol	phenol	4 EG	m-cresol	p-cresol	Eugenol_1	4 EP
BERRIES (Analysed 15/02/2016)										
		Cnc. (ng/g) (ppb)								
7	Control_1	nd	0.95	0.52	nd	0.71	nd	1.15	0.73	nd
8	Control_2	nd	0.74	0.30	nd	0.52	nd	nd	0.37	nd
9	Control_3	nd	0.70	0.26	nd	0.47	nd	0.86	0.40	nd
10	Control_4	nd	0.69	0.34	nd	0.47	nd	nd	0.47	nd
1	Smoke_1x_Bottom_1	nd	0.82	0.28	nd	0.49	nd	0.91	0.25	nd
1	Smoke_1x_Middle_1	nd	0.73	0.29	7.37	0.46	nd	0.93	nd	nd
1	Smoke_1x_Top_1	0.65	1.52	0.83	nd	0.67	nd	1.40	0.46	nd
2	Smoke_1x_Bottom_2	nd	0.93	0.38	nd	0.49	nd	1.03	0.24	nd
2	Smoke_1x_Middle_2	nd	0.90	0.37	nd	0.49	nd	1.02	nd	nd
2	Smoke_1x_Top_2	0.46	1.11	0.36	4.46	0.52	nd	1.14	nd	nd
3	Smoke_1x_Bottom_3	nd	0.79	0.24	1.14	0.47	nd	nd	0.29	nd
3	Smoke_1x_Middle_3	nd	0.80	0.30	nd	nd	nd	0.90	0.24	nd
3	Smoke_1x_Top_3	nd	0.78	0.30	nd	0.47	nd	0.95	0.28	nd
4	Smoke_2x_Bottom_4	nd	0.77	0.30	1.99	nd	nd	nd	nd	nd
4	Smoke_2x_Middle_4	0.26	0.92	0.59	nd	0.51	nd	1.10	nd	nd
4	Smoke_2x_Top_4	1.86	2.11	1.22	nd	0.85	nd	1.67	0.58	nd
5	Smoke_2x_Bottom_5	1.27	1.13	0.72	0.25	0.56	nd	1.19	0.42	nd
5	Smoke_2x_Middle_5	3.63	1.70	1.47	9.90	0.54	nd	1.87	0.31	nd
5	Smoke_2x_Top_5	4.10	2.88	1.90	12.63	0.93	nd	2.19	0.85	nd
6	Smoke_2x_Bottom_6	1.14	1.35	0.97	nd	0.54	nd	1.46	nd	nd
6	Smoke_2x_Middle_6	nd	0.85	0.37	nd	0.49	nd	0.93	0.45	nd
6	Smoke_2x_Top_6	4.23	2.91	1.79	2.73	1.00	nd	2.17	0.65	nd

A: JUICE (INITIAL)

			JUICE (Analysis date 22/02 2016)								
		Matrix	Guaiacol	4MG	o-cresol	phenol	4 EG	m-cresol 1	p-cresol	Eugenol_1	4 EP 1
							(ppb)				
1	smoke 1x juice 1	juice	5.53	2.41	0.14	2.93	nd	nd	2.71	nd	nd
2	smoke 1x juice 2	juice	3.31	1.02	nd	2.04	nd	nd	1.26	nd	nd
3	smoke 1x juice 3	juice	3.60	0.98	nd	0.38	nd	nd	0.86	nd	nd
4	smoke 2x juice 4	juice	5.92	3.07	0.78	4.36	nd	nd	3.52	nd	nd
5	smoke 2x juice 5	juice	11.03	5.43	2.35	8.44	nd	nd	5.62	nd	nd
6	smoke 2x juice 6	juice	8.81	4.91	1.63	7.49	nd	nd	0.62	0.11	nd
7	control juice 7	juice	1.70	nd	nd	nd	nd	nd	nd	nd	nd
8	control juice 8	juice	1.76	nd	nd	nd	nd	nd	nd	nd	nd
9	control juice 9	juice	2.43	nd	nd	nd	nd	nd	nd	nd	nd
10	control juice 10	juice	3.65	nd	nd	nd	nd	nd	nd	nd	nd

Consider all findings as preliminary

B: MUST: DURING ALCOHOLIC FERMENTATION

			BEFORE PRESS (Analysed 22/02/16)								
		Matrix	Guaiacol	4MG	o-cresol	phenol	4 EG	m-cresol 1	p-cresol	Eugenol_1	4 EP 1
			Concentration (ppb)								
1	smoke 1x before press 1	wine	27.25	19.71	5.32	76.69	0.51	nd	28.28	1.31	4.00
2	smoke 1x before press 2	wine	18.44	12.63	2.89	79.01	nd	nd	24.56	nd	2.60
3	smoke 1x before press 3	wine	24.64	16.28	4.18	63.06	nd	nd	23.18	1.73	3.12
4	smoke 2x before press 4	wine	18.53	11.00	3.89	83.08	0.00	nd	21.19	1.01	2.82
5	smoke 2x before press 5	wine	31.08	20.61	6.52	111.14	0.94	nd	31.73	0.93	4.67
6	smoke 2x before press 6	wine	25.99	17.62	4.81	79.66	0.77	nd	25.20	1.93	3.97
7	smoke control before press 7	wine	5.04	nd	nd	4.25	nd	nd	nd	0.01	nd
8	smoke control before press 8	wine	5.37	nd	nd	4.64	nd	nd	nd	0.12	nd
9	smoke control before press 9	wine	5.70	nd	nd	3.99	nd	nd	nd	nd	nd
10	smoke control before press 10	wine	6.88	nd	nd	4.65	nd	nd	nd	0.22	nd

C: AFTER PRESSING/COMPLETION OF AF:

			AFTER PRESS (Analysed 22/02/16)								
		Matrix	Guaiacol	4MG	o-cresol	phenol	4 EG	m-cresol 1	p-cresol	Eugenol_1	4 EP 1
			Concentration (ppb)								
1	smoke 1x after press 1	wine	21.54	14.43	3.28	64.69	0.23	nd	22.96	0.57	2.94
2	smoke 1x after press 2	wine	18.51	12.22	2.58	79.68	nd	nd	25.74	0.03	3.17
3	smoke 1x after press 3	wine	21.63	13.20	2.93	57.44	0.00	nd	21.37	1.52	2.74
4	smoke 2x after press 4	wine	17.84	10.05	3.26	71.89	0.01	nd	19.80	1.39	2.61
5	smoke 2x after press 5	wine	28.64	16.84	4.96	103.09	0.55	nd	32.54	0.96	4.47
6	smoke 2x after press 6	wine	26.35	17.84	4.87	77.77	0.85	nd	25.17	1.65	3.43
7	smoke control after press 7	wine	4.36	nd	nd	3.83	nd	nd	nd	0.73	nd
8	smoke control after press 8	wine	5.40	nd	nd	3.43	nd	nd	nd	0.86	nd
9	smoke control after press 9	wine	6.33	nd	nd	3.98	nd	nd	nd	0.14	nd
10	smoke control after press 10	wine	7.19	nd	nd	4.73	nd	nd	nd	0.82	nd

D: WINE: AFTER MLF

		AFTER MLF									
		Matrix	Guaiacol	4MG	o-cresol	phenol	4 EG	m-cresol 1	p-cresol	Eugenol1	4 EP 1
			Concentration (ppb)								
1	MMK Shiraz Day 15 after MLF 1 smokex1	wine	54.13	49.28	16.07	71.63	3.63	7.21	26.86	3.84	9.61
2	MMK Shiraz Day 15 after MLF 2 smokex1	wine	39.47	35.06	12.81	82.31	2.95	11.75	26.20	2.94	2.79
3	MMK Shiraz Day 15 after MLF 3 smokex1	wine	39.18	33.32	11.45	57.78	2.42	7.03	20.30	3.49	3.21
4	MMK Shiraz Day 15 after MLF 4 smokex2	wine	40.52	33.18	13.91	78.04	2.98	9.40	21.40	4.16	2.66
5	MMK Shiraz Day 15 after MLF 5 smokex2	wine	51.81	41.61	15.12	100.76	3.56	9.59	28.14	3.51	2.90
6	MMK Shiraz Day 15 after MLF 6 smokex2	wine	46.16	39.70	13.82	73.49	3.31	8.86	22.38	3.39	2.47
7	MMK Shiraz Day 15 after MLF 7 control	wine	9.26	0.78	1.00	4.07	nd	0.42	0.57	3.41	2.47
8	MMK Shiraz Day 15 after MLF 8 control	wine	11.23	0.76	1.08	5.76	0.01	0.63	0.93	3.73	3.20
9	MMK Shiraz Day 15 after MLF 9 control	wine	10.27	0.81	1.44	5.34	nd	0.48	0.54	2.90	1.25
10	MMK Shiraz Day 15 after MLF 10 control	wine	13.60	1.28	2.45	9.85	0.31	0.93	1.00	4.52	13.93

E: BEFORE SENSORY EVALUATION (after bottling)

Consider all findings as preliminary

		AFTER BOTTLE (Analysed 19/07/16)									
		Matrix	Guaiacol	4MG	o-cresol	phenol	4 EG	m-cresol 1	p-cresol	Eugenol_1	4 EP 1
						Concentration (ppb)					
1	MMK bottled wine 2016/6 smoke x1 1	wine	34.27	32.88	9.55	55.67	3.68	5.88	18.47	4.05	4.47
2	MMK bottled wine 2016/6 smoke x1 2	wine	29.31	25.18	9.01	75.48	3.60	10.87	21.00	3.13	4.26
3	MMK bottled wine 2016/6 smoke x1 3	wine	34.25	29.57	9.73	59.21	3.42	6.49	18.63	3.98	4.20
4	MMK bottled wine 2016/6 smoke x2 4	wine	33.60	28.54	11.68	85.42	4.00	9.17	20.08	4.79	4.67
5	MMK bottled wine 2016/6 smoke x2 5	wine	52.16	43.69	15.10	113.11	5.13	10.47	29.57	4.70	6.14
6	MMK bottled wine 2016/6 smoke x2 6	wine	41.92	36.91	12.46	77.89	4.39	9.46	22.66	4.64	5.01
7	MMK bottled wine 2016/6 control 7	wine	8.08	0.75	1.45	4.99	1.32	0.75	1.28	4.70	0.62
8	MMK bottled wine 2016/6 control 8	wine	8.28	0.50	1.31	6.21	1.04	0.83	1.24	3.93	0.81
9	MMK bottled wine 2016/6 control 9	wine	9.47	0.70	1.54	5.93	1.13	0.82	1.19	3.65	0.46
10	MMK bottled wine 2016/6 control 10	wine	10.11	0.80	1.90	7.10	1.13	0.86	1.20	3.99	1.96