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FINAL REPORT 2019

Winetech Number : P04000019

1. PROGRAMME & PROJECT LEADER INFORMATION

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2. PROJECT INFORMATION

Project title	Pinking of wine – methods for the detection of causative agents and pre-/ post- pinking treatments
Short title	Pinking of wine

Fruit kind(s)	Grapes (wine)		
Start date (mm/yyyy)	01/04/2015	**End date** (mm/yyyy)	31/03/2019

Key words	Pinking, white wine

Applications submitted to	Winetech

3. TOTAL PROJECT COST

	CFPA	RAISIN SA	SAAPPA SASPA	SATI	Winetech	ARC	THRIP	OTHER
TOTALS **All years**	0	0	0	0	880077	916000	0	0
Total cost of entire project						**R1796077**		

Consider all findings as preliminary

Version updated September 2018

4. EXECUTIVE SUMMARY

Objectives and Rationale
The aim was to investigate winemaking processes that affect the pinking susceptibility of white wine, and to provide practical guidelines for the prevention of pinking to enable cellars to establish pinking risk practices. Sensory evaluations aimed at confirming the notion that pinking does not affect wine flavour, to confirm the optimal absorbance for visually pinked wines, and if tasters can differentiate between pinking and other wine faults. In addition, the identification of the compounds responsible for pinking was attempted.

Methods
Winemaking processes applied throughout the course of this project to investigate their impact on pinking include harvesting at different stages of grape maturity, applying different temperature treatments, subjecting grapes/pomace to different crushing and pressing methods and skin contact times, reductive and oxidative winemaking, and fermentation using different yeast strains.

Fining agents and antioxidants as curative agents for pinking were investigated.

Samples were subjected to different chemical analyses during the course of the project, including standard wine parameters (WineScan), volatile (GC) and less volatile (HPLC) compounds, with further characterisation of compounds using LC-MS and NMR.

Three sensory evaluation experiments were conducted to confirm the notion that pinking does not affect wine aroma and taste, to confirm the optimal absorbance for visual pinking assays, and test whether or not tasters can differentiate between pinking and other wine faults.

The impact of Botrytis cinerea infection on pinking potential of wines was determined in fermentation trials using must from infected and non-infected B. cinearae grapes, inoculated with five yeast strains.

Key Results
Treatments, i.e. grape maturity, temperature and time of skin contact, and their interactions, affected oenological data and colour parameters in both juice and wine, with groupings observed on PCA.

Guidelines provided in this study gives some insight into the best fining agents and stages of application thereof to use for the prevention of pinking.

Practical guidelines for the prevention of pinking to enable cellars to establish pinking risk practices have already been communicated in popular style in Wineland.

Pinking influenced the aroma of the wine, but not the taste. Most tasters visually identified pinking at an AU of 0.03 (67%). Panellists also attributed other wine “faults” to pinked wines.

Yeast strains in winemaking can contribute significantly to the protection of wine from pinking in both non-infected and infected Botrytis cinerea samples.

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Sunlight (UV) does decrease the pink colour considerably as determined spectrophotometrically, but not visually, questioning the effectiveness as a post-pinking treatment method.

Naturally pinked wines are chemically distinct from control or non-pinked wines as shown by winescan and LC-MS analysis and clear grouping on PCA and DA. Although chemical differences are noted, the compounds causing pinking is still evasive, but further investigated.

Key Conclusion of Discussion
Winemaking processes do affect the pinking susceptibility of white wine. Natural pinking under winemaking conditions influence the wine sensorially.

Recommendation to Industry / Key take-home message
Several winemaking processes affect pinking and this information needs to be communicated to industry/winemakers to enable them to take necessary precautions of which some will be a more economically viable option than the use of fining agents/antioxidants.

5. PROBLEM IDENTIFICATION AND MOTIVATION

Problem Identification

The term pinking describes the infrequent but troublesome, subtle discoloration or undesirable red blush that sometimes develops during production or storage, where white wine turns pink due to oxidation. The pinking phenomenon can, however, occur in young white wines carefully protected from oxidation. Pinking can be slight or intense; in both cases, the wine will be unfavourably affected, or commercially unacceptable. Pinking is often expensive to correct because the wine may have to be removed from the bottle, treated, and rebottled. While the problem is not new, it is not easy to find reports describing curative treatments. Although the cause of pinking is still speculative, the physico-chemical properties of the pink material indicate that these materials and their precursors are unstable flavonoid phenols. Since the compounds that are "pink" are unknown, it is difficult to understand and solve the problem. The aim will be to investigate winemaking processes that affect the pinking susceptibility of white wine, and to provide practical guidelines for the prevention of pinking to enable cellars to establish pinking risk practices. Although Sauvignon blanc is not the only cultivar that pink, it is the most widely reported on in this regard, and will be the cultivar focussed on in this study. Additionally, wines identified as candidates for pinking can be given “adaptive” treatment to prevent pinking, or remedial treatment and applicable dosages thereof, after the appearance of pinking. Means for reducing the likelihood of pink colour development will include treatments to reduce the quantities of components capable of producing pink colour. According to anecdotal evidence, white wines that turned pink in colour have no difference in taste and aroma. Sensory evaluations aimed at confirming the notion that pinking does not affect wine flavour, to confirm the optimal absorbance for visually pinked wines, and if tasters can differentiate between pinking and other wine faults.

Motivation

The pinking phenomenon appear in some white wines. The cause of pinking is still speculative. Various factors affect the pinking susceptibility of wines. Different pre- or post-pinking treatments exist. PVPP as a pre-pinking treatment in wine is the method of choice, however, it is costly. This study already shows that

certain winemaking processes affect pinking susceptibility, and aim to report on winemaking processes that affect pinking susceptibility, and provide more practical guidelines for the prevention of pinking to enable cellars to establish pinking risk practices.

6. ACCUMULATED OBJECTIVES TABLE

Performance chart

Objectives	Milestones	Original Target Date	Date achieved
2015/16:			
1. Site visits and discussions with winemakers experiencing little or no, and higher occurrence of pinking	1.1. Collect information on winemaking techniques, viticultural practices, and other factors winemakers feel could be the cause of pinking experienced and should be addressed in Sauvignon blanc and other white wines.	October 2014 up to harvest 2015	October 2014 up to harvest 2015
2. Assay for pinking to identify wines susceptible or more inclined to pinking	2.1. Assay for pinking and optimisation of assay conditions.	Pre-harvest to harvest 2015	April 2015
3. Collect samples from winemakers at wineries that experience little or no, and higher occurrence of pinking, and obtain information that could assist in identifying practices that cause a wine to pink	3.1. Collect grape/ juice/ wine samples from winemakers that indicated little or no pinking of Sauvignon blanc.	Harvest 2015	Harvests 2015/16
	3.2. Collect grape/ juice/ wine samples from winemakers that experience pinking of Sauvignon blanc.	Harvest 2015	Harvests 2015/16
	3.3. Complete voluntary questionnaire on viticultural and oenological practices in an effort to identify practices that could explain the occurrence of pinking in some white wines.	Pre-harvest 2015	October 2016

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4. Investigate different agents used in winemaking on the prevention of, and for the removal of pinking	4.1. Determine the use of fining agents and related products on the pinking susceptibility, and effectiveness as curative agents for pinked wine.	April – June 2015	Harvest 2017
	4.2. Determine the use of antioxidants such as ascorbic acid and SO2 on the pinking susceptibility, and effectiveness as curative agents for pinked wine.	April – August 2015	Harvest 2015
	4.3. Take representative wine samples from milestones 4.1 to 4.2 and analyse chemically for standard wine parameters, must turbidity (where applicable), thiols, esters and phenols. Characterise pinking compounds by LC-MS, and if required preparative isolation and NMR.	April 2015 – March 2016	2016
5. Reporting	5.1. Progress report 1 April to July 2015	July 2015	July 2015
	Some of the experiments stipulated under objective 6 below might already be performed in the first year.		
2016/17:			
6. Determine which winemaking processes can be linked to the pinking potential in white wine	6.1. Investigate the influence of oxidative vs. reductive must treatments on the pinking potential of white wine.	Harvest 2016	Harvest 2016
	6.2. Investigate the influence of temperature on the pinking potential of white wine.	Harvest 2016	Harvest 2017
	6.3. Investigate the influence of different methods of	Harvest 2016	Harvest 2017

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	crushing and pressing on the wine's ability to pink.		
	6.4. Investigate the influence of time of, and temperature of skin contact on the wine's ability to pink.	Harvest 2016	Harvests 2016 & 2017
	6.5. Investigate the influence of yeast strain on the final wine's ability to pink.	Harvest 2016	Harvests 2016 - 2018
	6.6. Investigate the influence of grape maturity on the pinking potential of wine.	Harvest 2016	Harvest 2017
	6.7. Take representative wine samples from each of milestones 6.1 to 6.6 and analyse chemically for standard wine parameters, must turbidity, volatile and less volatile compounds, including thiols, esters and phenols, and characterise pinking compounds by LC-MS, and if required preparative isolation and NMR.	April 2016 – March 2017	April 2016 – 2017
	Investigate additional factors that induce or affect pinking if such factors carry merit and are warranted.		
7. Reporting	7.1. Progress report 1 April 2015 to March 2016	July 2016	August 2016
8. Provide practical guidelines for preventing pinking	8.1. Provide practical guidelines for preventing pinking - establish pinking risk practices.	December 2016	February 2017
2017/18:			
9. Commercial-scale application/testing of technologies for the	9.1. Commercially apply and test technologies proven to be effective at small-scale for	Harvest 2017	Performed laboratory and cellar scale, not commercial scale.

prevention of pinking	the prevention of pinking.		
10. Reporting	10.1. Progress report 1 April 2016 to March 2017	July 2017	August 2017
11. Development of a routine LC-UV method for the identification of pinking compounds	11.1. Develop a routine LC-UV method for identifying pinking compounds.	April 2017 – March 2018	N/A - Causative compounds causing pinking in white wines still evasive
12. Reporting	12.1. Final report 1 April 2015 to March 2018	July 2018	August 2018 - Progress Report (Extension request approved for 1 April 2018 to 31 March 2019)
13. Provide practical guidelines for preventing pinking	13.1. Provide updated practical guidelines for preventing pinking - establish pinking risk practices.	March 2018	March 2018
14. Determine which winemaking processes can be linked to the pinking potential in white wine	14.1. Investigate the interactive influence of grape maturity, grape temperature, different methods of crushing and pressing, and time of skin contact on the pinking potential of white wine.	Harvest 2018	Harvest 2018
15. Extension request	15.1. Sensorial properties of wines with pinking potential 15.2. Pinking potential of wines made from Botrytis infected grapes 15.3. Influence of amino acids, proteins, thiols and phenols on wine pinking potential 15.4. Oxygen concentration and wine pinking potential 15.5. Generation of pinking potential in model/synthetic and/or young wines without pinking potential	May 2018 March 2019 March 2019 March 2019 March 2019	June 2018 December 2018 December 2018 - proteins and phenols
16. Additional experimentation	16.1. Determining the effect of UV on pinking	2016	2017/18

Consider all findings as preliminary

	16.2. Further characterisation of pink wines		

7. WORKPLAN (MATERIALS AND METHODS)

W1.1 - Collect information on winemaking techniques, viticultural practices, and other factors winemakers feel could be the cause of pinking experienced and should be addressed in Sauvignon blanc and other white wines.

Discussions were held with winemakers at different wineries in different regions in 2015/16, including De Grendel Wines, Diemersdal Estate, Durbanville Hills, Spier and Buitenverwachting to collect information on winemaking techniques, viticultural practices, and other factors winemakers feel could be the cause of pinking experienced and should be addressed in Sauvignon blanc and other white wines.

W2.1 - Assay for pinking and optimisation of assay conditions.

The assay as described in Methods of Analysis For Wine Laboratories by the South African Wine Lab Association in association with SASEV. For this assay the control sample contained 15 ml wine, and the test sample 14 ml wine. To the test sample, 600 ul of hydrogen peroxide was added. Both samples were incubated at room temperature, overnight, in a dark cupboard. This assay provides a means of showing which wines are liable to develop pink colour during storage. White commercial wines collected from various sources, and experimental wines, were subjected to pinking tests and their inclination to pink.
W3.1 - Collect grape/ juice/ wine samples from winemakers that indicated little or no pinking of Sauvignon blanc.

See 6. Accumulated objectives table above

W3.2 - Collect grape/ juice/ wine samples from winemakers that experience pinking of Sauvignon blanc.

See 6. Accumulated objectives table above

W3.3 - Complete voluntary questionnaire on viticultural and oenological practices in an effort to identify practices that could explain the occurrence of pinking in some white wines.
See 6. Accumulated objectives table above

W4.1 - Determine the use of fining agents and related products on the pinking susceptibility, and effectiveness as curative agents for pinked wine.
In this experiment 29 different chemicals were tested at three different winemaking stages, which is settling, fermentation and fining. The juice was mixed thoroughly before 264 x 5 L PET bottles (261 treatments plus 3 for control) were filled up to the 4 L mark. Twenty nine different chemicals were weighed for 4 L volumes and added to 90 of these 5 L PET bottles. The 90 bottles and the rest of the 183 bottles were left overnight at 14ºC. The next morning the juice was racked off the lees to clean 5 L PET bottles and active dried yeast from Anchor (VIN13) was added at a dose of 30 g/hL. DAP (1 ml/L) was also added and 1 ml/L of a 5% bentonite solution was added 3 days later. The 29 fining agents were added to a further 90 bottles during fermentation. After the completion of fermentation, wines were tested for RS to ensure fermentation to dryness, and racked to clean 5 L PET bottles. The 29 chemicals were added to a further 90 bottles, and all wines cold stabilised. After the acquired period the wines were filtered into 750 ml glass bottles with Roté capsules and stored until further analysis.

W4.2 - Determine the use of antioxidants such as ascorbic acid and SO2 on the pinking susceptibility, and effectiveness as curative agents for pinked wine.

Grapes for the 2015 harvest season were sourced from two farms in the Durbanville area that reported pinking and no pinking potential of their wines, respectively. Grapes were processed into wines as indicated for each treatment. Every treatment was done in triplicate. Treatment 1 or control wines were prepared according to the standard Nietvoorbij procedures for small-scale, white wine production. Treatment 2: Juice was hyper-oxidised at settling by bubbling air through the juice for 10 minutes until the juice had a brown colour, followed by vinification according to the Nietvoorbij standard white winemaking protocol. Treatments 3-5 entailed the addition of ascorbic acid to SO2 ratios of 0.5:1, 1:1 and 2:1, respectively, at the destemming and crushing stage. Before destemming and crushing, the crates of grapes were divided into different batches, and SO2 and ascorbic acid added in ratios. The calculation was done with the assumption that there is no SO2 or ascorbic acid in the grapes or juice. During this addition the SO2 concentration was kept the same and the ascorbic acid concentration varied. In the first group (1:0.5) 40 mg/L SO2 and 20 mg/L ascorbic acid were added to the grapes. To the second (1:1), 40 mg/L SO2 and 40 mg/L ascorbic acid, and to the third (1:2) 40 mg/L SO2 and 80 mg/L ascorbic acid were added. Vinification followed according to the Nietvoorbij standard white winemaking protocol, however, under conditions as reductive as possible by the use of CO2. The reductive juices in treatments 3-5 were made under blankets of CO2 during destemming and crushing, with all pump-overs, and racking after settling, fermentation and cold stabilisation (0ºC store for 14 days), as well as bottling, performed under CO2 pressure. A CO2 blanket forms during fermentation. The bottled wines where stored at 14ºC.

W4.3 - Take representative wine samples from milestones 4.1 to 4.2 and analyse chemically for standard wine parameters, must turbidity (where applicable), thiols, esters and phenols. Characterise pinking compounds by LC-MS, and if required preparative isolation and NMR.

See 6. Accumulated objectives table above, and W16.2

W5.1 - Progress report 1 April to July 2015

See 6. Accumulated objectives table above

W6.1 - Investigate the influence of oxidative vs reductive must treatments on the pinking potential of white wine.

See 6. Accumulated objectives table above. Also incorporated under W4.2 and W6.4.

W6.2 - Investigate the influence of grape temperature on the pinking potential of white wine.

The grapes were sourced from a farm in the Robertson district which was known to have a pinking potential. Half the grapes (one batch, 105 kg) were cooled to 4ºC, and the other half/batch (107 kg) exposed to ambient temperature (20ºC) before processing. For both batches the standard Nietvoorbij procedures for small-scale, white wine production were followed.

W6.3 - Investigate the influence of different methods of crushing and pressing on the wine's ability to pink.

The grapes were sourced from a farm in the Robertson district which was known to have a pinking potential. Harvested Sauvignon blanc grapes were divided into 2 groups. One group/batch (109 kg) was processed and vinified according to the standard Nietvoorbij procedures for small-scale, white wine production. Another batch (103.5 kg) of Sauvignon blanc grapes was prepared the same as above, except

that the cooled grapes were whole bunch pressed (without the destemming and crushing stage) the next day.

W6.4 - Investigate the influence of time and temperature of skin contact under standard and/or reductive winemaking conditions on the wine's ability to pink

In one experiment, grapes were obtained from two different producers. One producer claims to have little or no pinking potential in his wine, while the second producer claims to have pinking potential every year. Each producer's grapes where divided in two batches, one batch for standard winemaking and one for reductive winemaking. Each of these batches were then further divided in treatments, including control (no skin contact) and 3, 6 and 12 hours of skin contact and kept in a cold store room of 10ºC. During skin contact or cold maceration, the grape pomace (skins, pips and juice) were kept for the different contact times at a temperature of at least 10ºC. During maceration the aqueous medium extract more phenols from the skins and pips. The standard Nietvoorbij procedures for small-scale, white wine production were followed, except for the different skin contact times, and reductive winemaking. With the aid of a specially made manifold the batch and treatments destined for reductive winemaking were kept under reductive conditions during skin contact times. After each skin contact time, the pomace was pressed and the normal white winemaking protocol was used. For reductive winemaking CO2 cylinders were used to blow gas at the destemming and crushing while a second cylinder was used to blow gas in the receiving bucket. These buckets were closed immediately with a lid to prevent O2 entering. A N2 cylinder was connected to the bag press so that the pressing could also be done reductively. In the reductive winemaking process, CO2 was blown into all 4.5 L glass bottles before and during filling with pressed juice. A specially constructed gas manifold, connected to a gas bottle, kept a reductive environment inside multiple 4.5 L bottles during experimentation. All wine transfers were performed by gas. During sampling, a special invented laboratory filter was used to filter wine for sampling purposes as well as a special unit to transfer wine from one bottle to another under CO2 gas pressure. The wines were cold stabilised and bottled and analyses for pinking potential, and semi-quantitatively for phenols.

In a second experiment, the effect of skin contact time at different temperatures, were investigated. Sauvignon blanc grapes (523 kg) were used for this experiment. For the ambient temperature (20ºC), 312 kg of grapes were used of which 101 kg was for the control wine, (destemmed and immediately pressed, 0 hours skin contact), 102.5 kg was destemmed and left on the skins for 3 hours, and 108.5 kg grapes was destemmed and left for 6 hours on the skins and juice. The rest of the grapes were cooled to 10ºC. After 10ºC was reached, 109 kg of grapes were destemmed and left for 3 hours on the skins, and 102 kg were destemmed and left for 6 hours on the skins in a 10ºC cool storage room. Grapes were further processed and vinified according to the standard Nietvoorbij procedure for small-scale, white wine production.

W6.5 - Investigate the influence of yeast strain on the final wine's ability to pink.

Fermentations were carried out in synthetic grape juice and in real grape juice (Sauvignon Blanc) until the weight loss was constant. Saccharomyces cerevisiae BM45 was used and wine-related strains to ferment the juice. Yeasts used in the different fermentation trials were: C. parapsilosis Y824, C. prunicola Y1162, H. opuntiae Y1045, S. cerevisiae BM45, M. fructicola Y1005, I. hanoieses Y1105, H. uvarum Y1159, M. pulcherrima Y1094, C. oriensis Y827, C. zamplina Y1082, M. pulcherrima Y1103, M. pulcherrima Y1063, R. mucilaginosa Y1027, H. vinea Y1021, Td Bodiva, MP Flavia, C azyama Y1014, EC1118, C. flavescences Y1004, H. dermontiae Y1069, H. uvarum Y1048, C. azyama Y1132, H. vinea Y1087, Candinda Albicans Y1023, P. fermentans Y1154, Zygoscus meyerae Y1084, K. thermotolerance Y107, H. uvarum Y1062, H. uvarum Y1041, C. pyralidae Y1064, M. pulcherrima Y1102. The Saccharomyces cerevisiae BM45 strain was used as a control strain to compare the potential of the wine-related strains to reduce the pinking potential of the wines. During the course of fermentations samples were taken for

chitin and for crude grape GFP-chitinases quantification using the Flow cytometry and also for microscopy. At the end of the fermentations, the wines were subjected to the pinking potential assay.

W6.6 - Investigate the influence of grape maturity on the pinking potential of wine.

Sauvignon blanc grapes were harvested at three different ripening stages from the same block on a farm in the Robertson district known to have a pinking potential. After crushing and pressing, the juices were vinified using the standard Nietvoorbij white winemaking protocol. The first batch of grapes was harvested on (8/2/2017) at 18°B. The second batch of grapes was harvested on (7/3/2017) at 22°B, while the last batch was harvested on (20/3/2017) at 25°B. Grapes were processed and vinified according to the standard Nietvoorbij procedure for small-scale, white wine production.

W6.7 - Take representative wine samples from each of milestones 6.1 to 6.6 and analyse chemically for standard wine parameters, must turbidity, volatile and less volatile compounds, including thiols, esters and phenols, and characterise pinking compounds by LC-MS, and if required preparative isolation and NMR.

See 6. Accumulated objectives table above, and W16.2.

W7.1 - Progress report 1 April 2015 to March 2016

See 6. Accumulated objectives table above

W8.1 - Provide practical guidelines for preventing pinking - establish pinking risk practices.

See 6. Accumulated objectives table above

W9.1 - Commercially apply and test technologies proven to be effective at small-scale for the prevention of pinking.

See 6. Accumulated objectives table above

W10.1 - Progress report 1 April 2016 to March 2017

See 6. Accumulated objectives table above

W11.1 - Develop a routine LC-UV method for identifying pinking compounds.

See 6. Accumulated objectives table above

W12.1 - Final report 1 April 2015 to March 2018

See 6. Accumulated objectives table above

W13.1 - Provide updated practical guidelines for preventing pinking - establish pinking risk practices.

See 6. Accumulated objectives table above

W14.1 - Investigate the interactive influence of grape maturity, grape temperature, different methods of crushing and pressing, and time of skin contact on the pinking potential of white wine.

Grapes (harvest 2018) from a farm in the Robertson district known to have a pinking potential, were harvested at three different stages of maturity, i.e. 18B, 20B and 25B. At each stage of maturity, half the grapes were cooled to 4ºC, and the other half/batch exposed to ambient temperature (20ºC) before processing. For each temperature treatment, one group/batch was processed and vinified according to the standard Nietvoorbij procedure for small-scale white wine production (control wine, destemmed and immediately pressed, 0 hours skin contact), a second batch destemmed and left for 18 hours on the skins, and a third whole bunch pressed (without the destemming and crushing stage). Grapes were further processed and vinified according to the standard Nietvoorbij procedure for small-scale, white wine production.

W15.1 - Sensorial properties of wines with pinking potential
Sensory evaluation 1: Sensory evaluations were conducted in the Department of Viticulture & Enology at Stellenbosch University. A trained panel of 28 tasters evaluated 50 mL wine samples served in individual boots at 23°C wines in black ISO (International Organization for Standardization, 1977) wine tasting glasses for differences in aroma and taste. The tasting glasses were covered with a petri dish for 1 h before testing. Half the tasters had two control wines and one pink wine, and the other fifty percent of the tasters one control and two pink wines.

Sensory evaluation 2: In a separate experiment, two oxidised wines were included with a second control wine. The 28 tasters were asked to identify all wines for visual wine faults, to see when the evaluators perceive the colour of the wine as a fault, and then comment on what the wine fault may be. The tests were done with random numbering and then was re-randomised so that no two tasters had the same sequence of wines.

Sensory evaluation 3: According to the SASEV Laboratory manual, pinking potential of white wines are tested at 500 nm on a spectrophotometer (SASEV, 2002). When the AU is 0.05 and above, the wine showed a pinking potential and the winemaker must use PVPP to eliminate the compounds that could turn the wine pink. The question is if 0.05 is the optimal AU at which pinking is visible. Never before was a sensory study undertaken to evaluate the point where pinking became noticeable. A sensory analysis was designed to see at which level consumers and winemakers perceive the wine as pink. A Sauvignon blanc was artificially coloured to correlate with 0.01, 0.02, 0.03, 0.04 and 0.05 AU, measured on a spectrophotometer at 500 nm (SASEV manual). A control wine with no pink colour was used, and a Rosé as an example of a very pink wine. The wines was randomly shifted and the consumers/winemakers where asked to rank the wine from the control wine to the Rosé wine and then to indicate at what point do they see the wine as pink.

W15.2 - Pinking potential of wines made from Botrytis infected grapes
Grapes were infected with 104 spores/ml of Botrytis cinerea over a period of 5 days, incubated at 25℃ and a humidity of ~95%. Half the grapes was not infected (controls, non-infected). Grapes were crushed, pressed and racked, and the resultant must (200 ml) flash pasteurization (1 minute at 100 ℃) and inoculated (6 log cfu/ml to obtain and optical density of 1) in triplicate with different yeasts, i.e. Saccharomyces cerevisiae EC1118, Saccharomyces cerevisiae ALS, Candida zemplinina Y1072, Cryptococcus randawii Y823, Candida zamplina Y1106, Metchnikowia pulcherrima Y1102 and Saccharomycodes ludwigii Y154. After fermentation wines were centrifuged at 5900rpm for 8 minutes and filtered with 0.45 um filters.

Must and wine samples were analysed with an Oenofoss for routine parameters, i.e. total acids (g/l), pH, malic acid (g/l), ethanol (%v/v), glucose/fructose (g/l) and volatile acidity (g/l). Wines were also analysed for pinking potential. Wine samples were also analysed for phenols with HPLC. For the pinking assay, two different protocols were used: SA Wine Lab Association and AWRI. For the SA Wine Lab Association

assay 600 ul of hydrogen peroxide is added to 14 ml test sample. The control sample (15 ml) gets no hydrogen peroxide. Both test and control samples are incubated overnight at room temperature in a dark cupboard. The AWRI pinking assay is similar to the SAWLA method, except that 240 ul of hydrogen peroxide is added to 6 ml wine. After incubation, samples were measured at 500nm and 520nm to determine the pinking potential. Control samples were used as blank against which the test sample were read.

W15.3 - Influence of amino acids, proteins, thiols and phenols on wine pinking potential

See 6. Accumulated objectives table above

W15.4 - Oxygen concentration and wine pinking potential

Oxygen concentrations were measured at different stages of winemaking in the 2018 cellar experiment (see W14.1). The winemaking stages where oxygen was measured were at crush, pumpover after crushing, 1 h after pumpover, after settling, racking and alcoholic fermentation.

W15.5 - Generation of pinking potential in model/synthetic and/or young wines without pinking potential

See 6. Accumulated objectives table above

W16.1 - Determining the effect of UV on pinking
Wine with a high pinking potential (0.123 AU measured at 500 nm) was used. The wine pinked naturally. The pinked wine was decanted in 100 ml Schott bottles and exposed to direct sunlight for 0, 15, 30, 60 and 120 minutes. After the set time in direct sunlight, the wine was spectrophotometrically analysed at absorbance 420, 500 and 520 nm against the original wine (control).

W16.2 - Further characterisation of pink wines
Attempted identification of the causative compound(s) that causes pinking in white wines were approached as described below.

Extraction of phenolic compounds with Solid Phase Extraction tubes
Solid phase extraction (SPE) was carried out with a 24-position SPE vacuum manifold from Supelco. The SPE procedure used was similar for each kind of cartridge. The cartridges were activated with 5 mL methanol, by following the product instruction, and conditioned with 5 ml 5% methanol solution. Two hundred and fifty millilitres of samples were loaded into the cartridges and, before elution, sorbents were washed with 5 mL ultrapure water and eluted with 15 ml methanol. The eluted samples were concentrated with N2 gas up to 0.5 ml. Concentrated samples were kept in a 1 ml Eppindorf tube until analysis by LC-MS (Prelle et al., 2013).

Liquid Chromatography Mass Spectrometry (LC-MS) analysis
Anthocyanin method
A Waters Synapt G2 quadrupole time-of-flight mass spectrometer (Waters Corporation, Milford, MA, USA), fitted with a Waters Acquity UPLC and photo-diode array detector (PDA), was used for LC-MS analyses. Separation was achieved on a Waters BEH Amide UPLC column (2.1 × 100 mm, 1.7 μm) at 35°C. Solvent A consisted of 10 mM ammonium acetate in water; solvent B consisted of 10 mM ammonium acetate in 95% acetonitrile. The gradient consisted of a flow rate 0.25 ml min–1, starting with 95% B to 40% B over 9 min, applying gradient curve 7, followed by re-equilibration to initial conditions over 5 min. Electrospray ionisation was applied in the negative mode, using a capillary voltage of 2.5 kV, a cone voltage of 15 V, desolvation temperature of 250°C and desolvation gas (N2) flow of 650 l h–1. The

rest of the MS settings were optimised for best sensitivity. Data were acquired in MSE mode, consisting of a scan using a low collision energy and a scan using a collision energy ramp from 25 to 60 V, which has the added advantage of acquiring low energy molecular ion data as well as fragmentation data for all analytes all the time. Data were scanned using a scan rate of 0.2 s over the range m/z 100–1000. Leucine enkephalin was used as lock mass for accurate mass determination on the fly using a lock mass flow rate of 0.002 ml min–1, acquiring lock mass data every 20 s. Sodium formate was used to calibrate the instrument. The PDA detector was set to scan over the range 220–450 nm.

Phenolic method
A Waters Synapt G2 quadrupole time-of-flight mass spectrometer was used for LC-MS analysis. It was fitted with a Waters Ultra pressure liquid chromatograph and photo diode array detection. Separation was achieved on a Waters HSS T3 column, 2.1x100 mm with 1.7 um particles. A gradient was applied using 0.1% formic acid (solvent A) and acetonitrile (solvent B). The gradient started at 100% solvent A for 1 minute and changed to 50% B over 22 minutes in a linear way. It then went to 100% B after 23 minutes where it was held until 24.5 minutes, followed by re-equilibration to initial conditions for 4 minutes. The flow rate was 0.25 ml/min and the column was kept at 60ºC. The injection volume was 3 µL. Data was acquired in MSE mode which consisted of a low collision energy scan (6 V) from m/z 150 to 1500 and a high collision energy scan from m/z 40 to 1500. The high collision energy scan was done using a collision energy ramp of 30-60 V. The photo diode array detector was set to scan from 220-600 nm. The mass spectrometer was optimized for best sensitivity, a cone voltage of 15 V, desolvation gas was nitrogen at 650 L/hr and desolvation temperature 275ºC. The instrument was operated with an electrospray ionization probe in the negative mode. Sodium formate was used for calibration and leucine encephalin was infused in the background as lock mass for accurate mass determinations.

Winescan analysis
WineScan™ has a FTIR (Fourier Transform Infrared Spectroscopy) interferometer that scans the full infrared spectrum from 200 to 4000 cm-1. Collection of data from the entire spectrum allows the analysis of many parameters in a short period of time. Therefore, for routine wine analysis, only about 30 data points are used to quantify the twelve odd parameters. There is thus a whole range of data points that are not used and which could be used to identify unknown compounds.

Wine sample preparation
Wine samples were selected from production cellars that had a high pinking potential. Control samples were kept with two treatments. The treatments were as follows. Two hundred and fifty ml of wine was left with an airspace in the open for three days until the sample naturally pinked, showing a visible pink colour (PN). Ten ml of hydrogen peroxide (H2O2) was added to 250 ml of wine sample and left overnight until the wine turned pink (pink induced - PI). These samples were put through the solid phase extraction tubes as described above. The samples were concentrated with N2 gas until a concentration of 0.5 ml was achieved. These samples were analysed with LC-MS.

8. RESULTS AND DISCUSSION

Site visits and discussions with winemakers experiencing little or no, and high(er) occurrence of pinking/ Collect information on winemaking techniques, viticultural practices, and other factors winemakers feel could be the cause of pinking experienced and should be addressed in Sauvignon blanc and other white wines

Factors listed by winemakers that prevent pinking in their wines are: Using enough SO2, addition of

ascorbic acid at bottling, keeping wines on yeast lees for longer, the use of the fining agent PVPP, low pH, low levels of the metals iron and copper and clearer musts (less turbid musts). Causative factors listed by winemakers experiencing pinking problems include region, oxidation, quality control during bottling, skin contact, pH, wines prepared under reducing conditions and machine harvesting of grapes. Some cellars when buying in wine, request that the wine should contain no ascorbic acid due to reasons stipulated, including shortened shelf-life. “Breaking” of foliage of the vine is said to affect pinking. Nieu-Zealand winemakers do not allow skin contact; possibly the reason that Nieu-Zealand Sauvignon blanc’s do not pink. Although, reportedly, incidences of pinking for wines entered in competitions decreased from about 15-16% over the number of years up to 2015, to about 1% in 2015, some local winemakers and international purchasers (Bolaget) reported high and regular incidences of pinking in 2014/15; with pinking taking place months after bottling and approval by SAWIS. Some producers reported pinking, even after the use of ascorbic acid, SO2 and PVPP. Whether or not higher incidences of pinking can also be related to cold stabilisation of white wine cultivars, i.e. Sauvignon blanc, Chenin blanc and Chardonnay in 2014/15, to higher incidences of rot in 2014/15, or to the degree of must turbidity, were questions raised by some winemakers. Exposure to UV light has been reported to cause pinking, after which pinking disappears, never to reappear again. For various stated reasons, not all winemakers are keen on the use of PVPP and ascorbic acid.

Assay for pinking and optimisation of assay conditions

The assay as described in Methods of Analysis For Wine Laboratories by the South African Wine Lab Association in association with SASEV for pinking was used. Optimisation experiments were first conducted looking at optimum H2O2 concentration additions, the effect of syringe filters vs. a 3-piece filtration system, the influence of pre-opening of wine bottles on a wine’ pinking potential, time studies to determine the time till maximum absorbance, and absorbance spectra to look at the wavelength of maximal absorbance when pinking occurs. The type of filtration, i.e. syringe filters vs. filtering apparatus, does introduce more or less oxygen, and therefore impact on the reaction, and subsequent absorbance and final result (pinking) values. One millilitre 0.072% H2O2 is adequate for assaying the pinking potential of white wines. Therefore, although the SA Wine Lab Association method, stipulating five different volumes/concentrations, is used currently in this study, only 1 ml additions of 0.072% H2O2 would also be adequate for determining the pinking potential of wines. Samples opened and closed a number of times over time, started to lose their potential to pink, therefore, pinking potential should be determined on freshly opened samples. Time studies showed that samples in the presence of H2O2 reached maximum absorbance at 10-12 hours, however, due to slight differences in absorbance compared to eight hours, decreases in absorbance after ten to twelve hours, and man-hour days, readings were taken after eight hours as specified in the SA Wine Lab Association method. Samples can also stand overnight, and absorbance readings taken the next morning. Centrifugation instead of filtration of tank samples was also tested, and works well.

The effect of different additions of ascorbic acid to juice on the pinking potential of wine

See workplan “W4.2 - Determine the use of antioxidants such as ascorbic acid and SO2 on the pinking susceptibility, and effectiveness as curative agents for pinked wine.”

Ascorbic acid or vitamin C has long been used in the wine industry in white winemaking as a quick-acting and powerful anti-oxidant able to rapidly remove molecular oxygen (O2) from juice or wine, protecting the wine against light and short aerations during racking and bottling, and its colour from oxidative effects. Ascorbic acid is highly recommended for oxygen sensitive cultivars and wines that discolor easily. One of the aims of this study was to determine the effectiveness of preventative technologies, including antioxidants such as ascorbic acid, on the pinking susceptibility of wine. This experiment looked at the

effectiveness of ascorbic acid under reductive conditions, compared to a control prepared under standard small-scale Nietvoorbij white winemaking conditions. Further experimentation below compared ascorbic acid and other fining agents and related products on their pinking susceptibility, and effectiveness as curative agents, during various stages of winemaking (see “The effect of different fining agents”).

In this experiment, wines prepared reductively and with different ascorbic acid to fixed SO2 ratios added at the destemming and crushing stage, were compared to a control prepared according to the standard Nietvoorbij procedures for small-scale, white wine production, and wines from hyper-oxidised juice. Wines were prepared from grapes sourced from two farms in the Durbanville area that reported pinking and no pinking potential of their wines. Although none of the samples for the two producers showed absorbance values as measured against the control larger than 0.05 (A500nm > 0.05), ascorbic acid treatments showed lower pink potential than control and hyper-oxidised wines. This confirms that ascorbic acid is a very good antioxidant, more so when used in conjunction with SO2.

The decreased potential to pink in hyper-oxidised wines can be explained by the oxidation of phenols and other colour forming precursors before the settling stage, giving it a chance to settle out (Ricardo-da-Silva et al., 1993; Schneider, 1998).

Research by another group showed ascorbic acid to first act as an antioxidant, but when its concentrations are too low, enhanced oxidation occurs in an artificial wine medium. Byproducts other than dehydro-ascorbic acid and H2O2 have been identified. Ascorbic acid also seems to react at a ratio of 1:1.7 with SO2 instead of 1:1. This should prompt winemakers to re-assess the use of ascorbic acid, as well as SO2 levels in wine. Ascorbic acid is also believed to shorten the shelf-life of white wine (personal communication: winemakers).

The effect of different fining agents

See workplan “W4.1 - Determine the use of fining agents and related products on the pinking susceptibility, and effectiveness as curative agents for pinked wine.”

Polyvinylpolypyrrolidone (PVPP) is the most well-known fining agent used by winemakers in their fight to prevent pinking in Sauvignon blanc wines. The questions arises, is PVPP the only fining agent that can be used. In this experiment PVPP was compared to other fining agents at different winemaking stages. After contacting chemical companies to get as much info on the effect of fining agents on pinking potential, 29 samples were collected and tested. The fining agents were added at three different winemaking stages, i.e. settling, fermentation and fining. These stages were selected as these are the most popular stages were winemakers do additions to their juice or wine.

Four fining agents had a significant effect, added to any of the winemaking stages, on reducing the pinking potential of the wines. These fining agents were PVPP, Vegecoll, Ascorbic acid and Gelatine grade 75-WF. Everclar work well at the settling and fermentation stages, while Fishcol is most efficient at the settling and fining stages. Oenoprotect, Polylact and Aromatic UVA work best at fermentation and fining stages. Ascorbic acid was very efficient, decreasing pinking potential to a near zero, but studies have shown (Peng et al., 1998; Comuzzo et all., 2015) that wines treated with ascorbic acid age faster and turns brown very quickly after bottling. When ascorbic acid are added to wine, it breaks down to dehydro-ascorbic acid and 2H+ ions. These 2H+ ions bind with O2 to form hydrogen peroxide (H2O2) which in turns are a very strong oxidising agent. Vegecoll is a product derived from potato protein that assist in the fining of white wines. Gelatine is widely used in the wine industry to bind phenols and remove them from wines. Aromatic UVA is a mixture of potassium pyrosulfite, L-ascorbic acid and mix of hydrolysable tannins. Polylact is a PVPP and potassium caseinate on cellulose support that efficiently contributes to the adsorption and removal of oxidised and oxidisable phenolic compounds. The fining

agents, therefore, most efficient in reducing pinking potential should contain gelatine, PVPP or ascorbic acid and/or potato protein as part of the chemical's make-up.

The effect of skin contact time on the pinking potential of Sauvignon blanc wines

See workplan "W6.4 - Investigate the influence of time of and temperature of skin contact on the wine's ability to pink."

During this experiment, two winemaking procedures were followed. The one was standard winemaking practice, which is oxidative, and the other one was reductive, where oxygen was prevented as far as possible. The pinking potential of the wines were determined. Skin contact time had very little ($p \geq 0.05$) influence on pinking potential in wines prepared under standard, more oxidative conditions. Under reductive winemaking conditions, however, for both producers, there was an increase in pinking potential from the control to 12 hours skin contact. Although improved susceptibility to pinking was observed, none of the wines actually showed pinking potential as defined by the assay describe in SASEV laboratory manual (2002). However, sensory evaluation studies showed that evaluators could start to pick up a pink hue in wines that correspond to 0.03 AU, suggesting/recommending that the literature reported values of 0.05 AU can be lowered and the assay improved and refined for pinking potential.

Significant increases (compared to no or 0 h skin contact) in catechin (producer 2), caffeic acid (producer 2), p-coumaric acid (producer 2) and chlorogenic acid (producer 2) was observed during a 12-hour cold maceration time under reductive winemaking conditions. Compared to no skin contact, significant increases in gallic acid (producer 1), catechin (producer 2) and p-coumaric acid (producer 1) was observed during a 12-hour cold maceration period under normal, more oxidative winemaking conditions. Significantly higher concentrations of the following phenols were observed after 6-12 hours skin contact time: Catechin (producer 1, standard winemaking, 6 h) (producer 2, reductive winemaking, 12 h), caffeic acid (producer 1, reductive winemaking, 6 h; producer 2, reductive winemaking, 6+12 h), p-coumaric acid (producer 1, standard winemaking, 6-12 h; producer 1, reductive winemaking, 6h; producer 2, reductive winemaking, 6-12 h), and chlorogenic acid (producer 2, reductive winemaking, 6-12 h). Delphinidin 3-O-glucoside, cyanidin 3-O-glucoside, petunidin 3-O- glucoside, peonidin 3-O- glucoside and malvidin 3-O- glucoside were not detected.

During cold maceration and prolonged skin contact phenols like, protocatechic acid, catechin, epicatechin, caffeic acid, gallic acid, ethyl gallate, p-hydroxibenzoic acid, quercetin-3-rutinoside, quercitin-3-glucocide, quercetin-3-L-rhamnoside, quercetin-3-D-galactoside, cis-coutaric acid, trans-coutaric acid, m-coutaric acid, p-coutaric acid, caftaric acid, ferulic acid, fertaric acid and coumaric acid, are extracted from the skins and seeds (Hernanz et al., 2007; Gómez-Míguez et al., 2007; Maggu et al., 2007; Ružić et al., 2011). The extracted phenols can influence the colour of white wines under different conditions including exposure to polyphenol oxidase enzymes (Simpson, 1982; Lee & Jaworski, 1988; Muñoz-Pina et al., 2018), storage, when exposed to light (Grant-Preece et al., 2018), temperature (Scrimgeour et al., 2015) and oxygen ingress (Serra-Cayuela et al., 2013; Nenadis &·Paraskevopoulou, 2016). The correlation between phenols and browning is well documented (De Villiers, 1961; Petersen & Caputi, 1967; Lee & Jaworski, 1988; Cheynier et al., 1990; Bradshaw et al., 2001; Razmkhab et al., 2002; Lopez-Toledano et al., 2006; Li et al., 2008; Nenadis & Paraskevopoulou, 2016). These extracted phenols can also contribute to the pinking phenomena (Simpson, 1977; Simpson, 1980; Andrea-Silva et al., 2014).

The effect of grape temperature on pinking potential

See workplan "W6.2 - Investigate the influence of grape temperature on the pinking potential of white wine".

Consider all findings as preliminary

This experiment was performed to see if grape temperature at pressing influences pinking potential. After harvest a batch of grapes was cooled down to 0°C in a cool room before crushing and destemming, while another batch of grapes was crushed and destemmed at ambient temperature (20°C). After vinification, wines were cold stabilised and bottled. The routine wine parameters were analysed, and the pinking potential of the wines assayed. Standard wine analyses show little difference ($p \geq 0.05$) in routine parameters between 4ºC and ambient temperature. The pinking potential at 0ºC was about 0.05 AU. The AU for wines from grapes pressed at ambient temperature was 0.02 AU. The solubility of gasses decreases as the temperature goes down, so cold grapes/juice can hold much more oxygen than warm grapes/juice, thus explaining the significantly higher pinking potential of wines prepared from cold grapes (Fig. 1). Measured oxygen did confirm that not only did the oxygen concentration decrease during winemaking up to fermentation, but also that the oxygen concentration was generally higher in samples from grapes at 4℃, compared to 20℃ at the time of processing. The presence of higher levels of the metals aluminium, vanadium and iron as catalysts for oxidation in 4ºC as compared to 20ºC grapes, can also explain the significantly higher pinking potential of the cooler grapes.

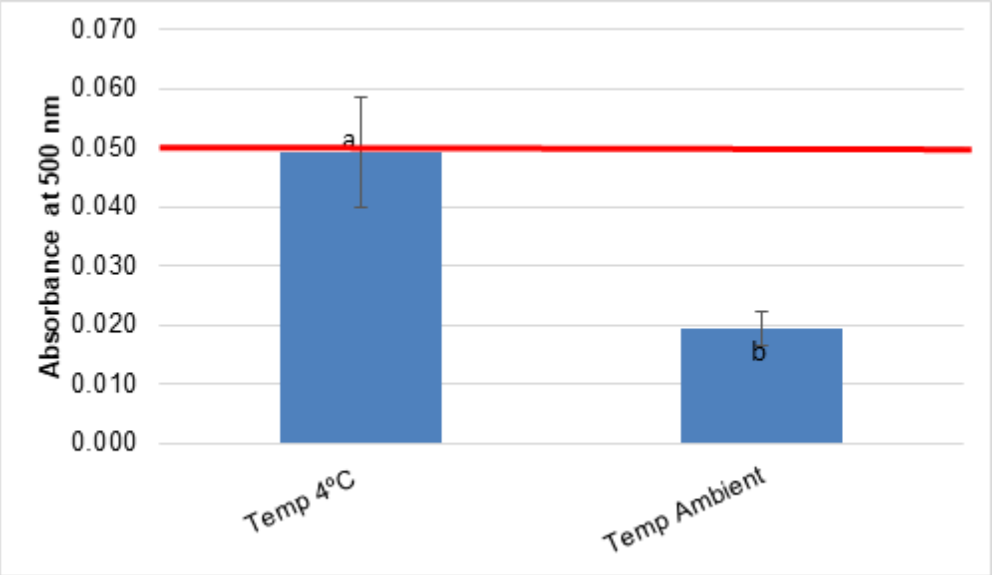

Figure 1: Pinking potential measured at 500 nm for Sauvignon blanc wines made from grapes kept at 4°C and 20°C. The red line indicates the pinking potential limit as defined in the method described in SASEV laboratory manual (2002).

The effect of whole bunch versus crushed and destemmed bag press

See workplan “W6.3 - Investigate the influence of different methods of crushing and pressing on the wine’s ability to pink.”

Juice and wines from whole bunch pressed grapes generally had significantly lower levels of dissolved solids and TA. The influence of pressing on other compounds have also been reported. Authors Smith & Waters (2012) and Kelly et al. (2014) reported the phenolic content to be a little bit higher in crushed and pressed than whole bunch pressed juice.

Wine from whole bunch pressed grapes had significantly higher pinking potential than wines made from bag pressed grapes (Fig. 2). Although less pressure are exuded on the grapes itself during whole bunch pressing, as compared to crushed and destemmed grapes, stems are pressed with the grapes during whole bunch pressing. The stems also have quantities of phenols in them that could be extracted into the juice, possibly explaining the increase in pinking potential of the resulting wines. According to Simpson

(1977, 1980) and Andrea-Silva et al. (2014), some phenols can contribute to the pinking phenomena. Therefore, pinking is also related to phenols qualitatively, not just quantitatively as reported by Singleton et al. (1979), Singleton et al. (1980) and Delteil (2004). The presence of higher levels of the metals aluminium, vanadium and iron as catalysts for oxidation in whole bunch pressed as compared to destemmed and crushed grapes, can explain the significantly higher pinking potential of the whole bunch pressed grapes.

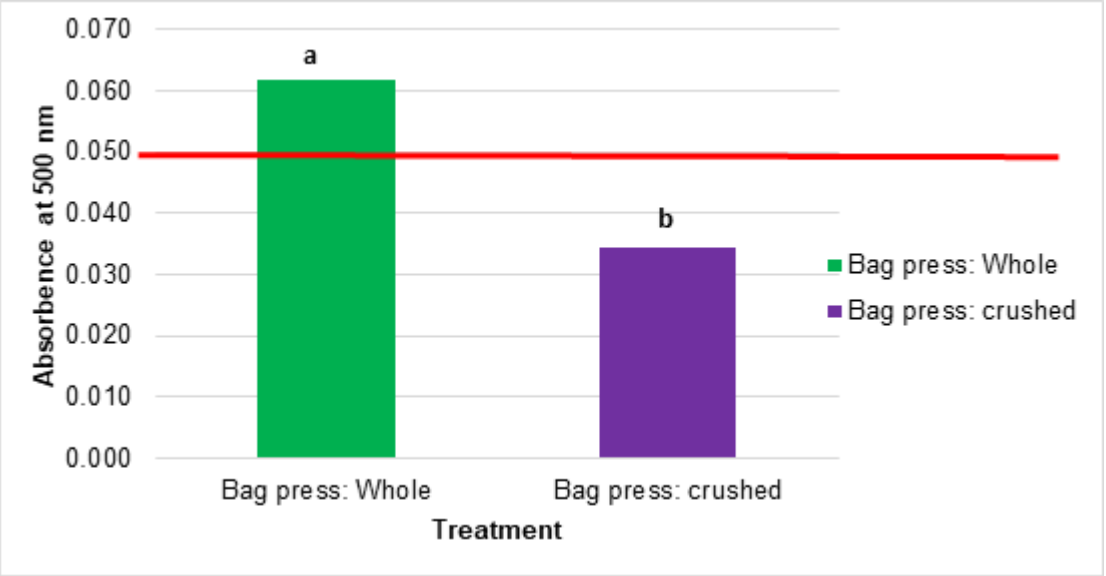

Figure 2: Pinking potential of Sauvignon blanc made from whole bunch pressed and destemmed/crushed and bag pressed grapes. The red line indicates the pinking potential limit as defined in the method described in SASEV laboratory manual (2002)

The effect of different ripening stages

Based on anecdotal evidence that ripening have an effect on the pinking potential of Sauvignon blanc wines, Sauvignon blanc in this experiment were harvested at three different ripening stages (i.e. 18°B, 22°B and 25°B) and analysed for standard wine parameters and pinking potential. Significant differences ($p < 0.05$) exist between the different ripeness levels for the different parameters (except for lactic acid), with pH, VA, glucose, fructose, ethanol and glycerol increasing, and TA and malic acid decreasing with increasing ripeness. Ripening did have a significant effect on the pinking potential of the wine (Fig. 3). An increase in ripeness increases the extraction of phenols into the wine, possibly explaining the wines' increased potential to pink (Singleton et al., 1979; Singleton et al., 1980; Delteil, 2004). Authors Simpson (1977; 1980) and Andrea-Silva et al. (2014) reported the possible contribution of phenols to the pinking phenomena.

During the early stages of grape berry development, the berry accumulates sugars and metabolises the acids. Phenols and thiols are being synthesised at this stage and translocated to the berry. As the berry ripens, the balance between the sugars and acids increases while the berries start to develop the prominent aroma of Sauvignon blanc. Gallic acid, caffeic acid and p-coumaric acid increased significantly during berry ripening from 18°B to 22°B to 25°B. Higher gallic acid, caffeic acid and p-coumaric acid concentrations may be correlated to the increase in pinking at the 25ºB ripening stages. This was confirmed by studies by Hernanz et al. (2007), Gómez-Míguez et al. (2007), Maggu et al. (2007) and Ružić et al. (2011).

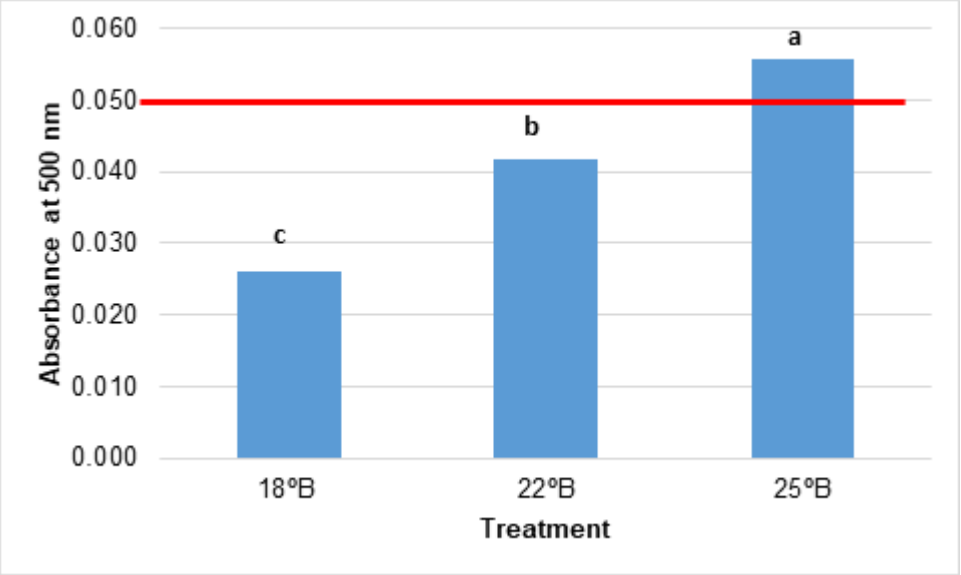

Figure 3: Pinking potential of Sauvignon blanc at different ripening stages. The red line indicates the pinking potential limit as defined in the method described in SASEV laboratory manual (2002)

The effect of skin contact time at different temperatures

Skin contact up to 6h at 10 and 20ºC generally did not impact significantly on standard wine analysis or pinking. The impact of pomace temperature on pinking potential is less significant than the impact of grape temperature before processing (see section “The effect of grape temperature on pinking potential”). The impact of skin contact time up to 6h under standard winemaking conditions on pinking potential of Sauvignon blanc is less significant than longer skin contact times up to 12h under reductive winemaking conditions (section “The effect of grape temperature on pinking potential”). Increased skin contact and lower temperature favours pinking potential of Sauvignon blanc as these grapes absorb more oxygen from the surrounding air as well as extracting more phenols from the skins and seeds.

Investigate the interactive influence of grape maturity, grape temperature, different methods of crushing and pressing, and time of skin contact on the pinking potential of white wine

At 18ºB, lower grape temperature (4ºC as compared to 20ºC) favoured pinking significantly in control wine (destemmed/crushed and immediately pressed, 0 hours skin contact). At 20ºB and 25ºB, a skin contact time of 18 h at 20ºC increased the pinking potential of the final wines significantly. Differences in behaviour, therefore, were noted between grape maturity levels. Longer skin contact times was more of causative factor of pinking in grapes harvested at 20ºB and above. Final wines from cooler grapes harvested at 18ºB was more at risk of pinking. Pinking potential increased with grape maturity, being significantly lower for grapes harvested at 18ºB. No significant differences in pinking potential existed between wines from whole bunch pressed and destemmed/crushed grapes. In this experiment, grape temperature and time of skin contact were more prominent factors affecting the potential to pink than the method of pressing.

Investigate the influence of yeast strain on the final wine’s ability to pink.

The experimental approach is indicated in workplan W6.5 - Investigate the influence of wine kept on lees and of yeast strain on the final wine’s ability to pink”.

Non-Saccharomyces wine-related yeast strains showed better protection for pinking potential compared to

S. cerevisiae BM45. Cr. oriensis Y872, M. pulcherrima Y1094 and C. prunicola Y1162 protected the wines from pinking. C. prunicola Y1162 was able to protect the wine from pinking with or without the addition of S. cerevisiae BM45.
Several previous studies reported that during winemaking the yeast strains can absorb the grape polyphenols (Minnar et al., 2018; Vernhet et al., 2018; Baiano et al., 2018; Checchi et al., 2018; Loira et al., 2018; Medina et al., 2018) which are hypothesized to be responsible for pinking.
High levels of chitin do not necessarily result in high protection of white wines from pinking.
The ability to reduce pinking is an attribute of yeast strains that can provide an achievable, natural and cost-effective alternative to chemicals, bentonite and other agents for wine fining, and a desirable approach according to the industry and consumers point of view. No studies to date have reported the impact of yeast strains on wine pinking.

The effect of UV on pinked wine

Based on anecdotal evidence some winemakers leave the pinked wine in direct sunlight for 15 minutes for the pink colour to disappear. This experiment show that, although there was a significant ($p \leq 0.05$) reduction of 43% in absorbency after 15 minutes, longer exposure times to sunlight might be needed to bring the absorbency below the accepted level of 0.05 AU. Although spectrophotometrically a reduction in pinking was seen, with only a very slight change in hue noticeable from a pinkish to a more yellowish colour, visually there was no difference, and the wine did not change back to the natural Sauvignon blanc colour expected for this white wine.

The wines were also analysed for phenolic compounds by HPLC. Compounds analysed include: Gallic acid, epicatechin-3-O-gallate, caftaric acid, epigallocatechin-3-O-gallate, caffeic acid, p-coumaric acid, ssyringic acid, vanillic acid, chlorogenic acid, delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside and malvidin-3-O-glucoside. Although the wine was pink, no anthocyanins were detected by the HPLC. Simpson (1982) suggested that pink colour of a pinked wine will disappear in direct sunlight after 10 minutes. The theory was tested in this study and the results showed that different treatments (sunlight exposure times) had little effect on the concentrations of caftaric acid, epigallocatechin-3-O-gallate, caffeic acid, p-coumaric acid, syringic acid, vanillic acid and chlorogenic acid, indicating that these phenolic compounds has little to do with the colour or reverting back to the colour of the wine. The only two phenolic compounds that showed any significant differences were gallic acid and epicatechin-3-O-gallate, with gallic acid concentration decreasing significantly from zero to two hour exposure to sunlight. Although a study done by Grant-Preece et al. (2018) showed that an increase in these phenols increased the browning potential of a white wine, no studies so far, apart from this investigation, identified the phenols that change significantly in concentration upon exposure to direct sunlight for various time periods.

Causative compound(s) causing pinking in white wines

WineScan analysis

The WineScan scan in the mid-infrared (MIR) spectrum from about 200 – 4000 cm-1. About thirty odd spectra are used to quantify the programmed parameters. This leaves a whole lot of spectra points that could be used to identify an unknown compound. A wine with a high potential for pinking was used in this experiment. About 250 mL of the wine was left outside for about three days to pink naturally. After natural pinking was accomplished, the wine as well as the control (no pinking) wine was sent for WineScan analysis. In figure 4, a graph of the scanned spectra can be seen with two distinct peaks. These two peaks fall between the wave numbers of 1612 – 1682 cm-1 and 3070 – 3427 cm-1.

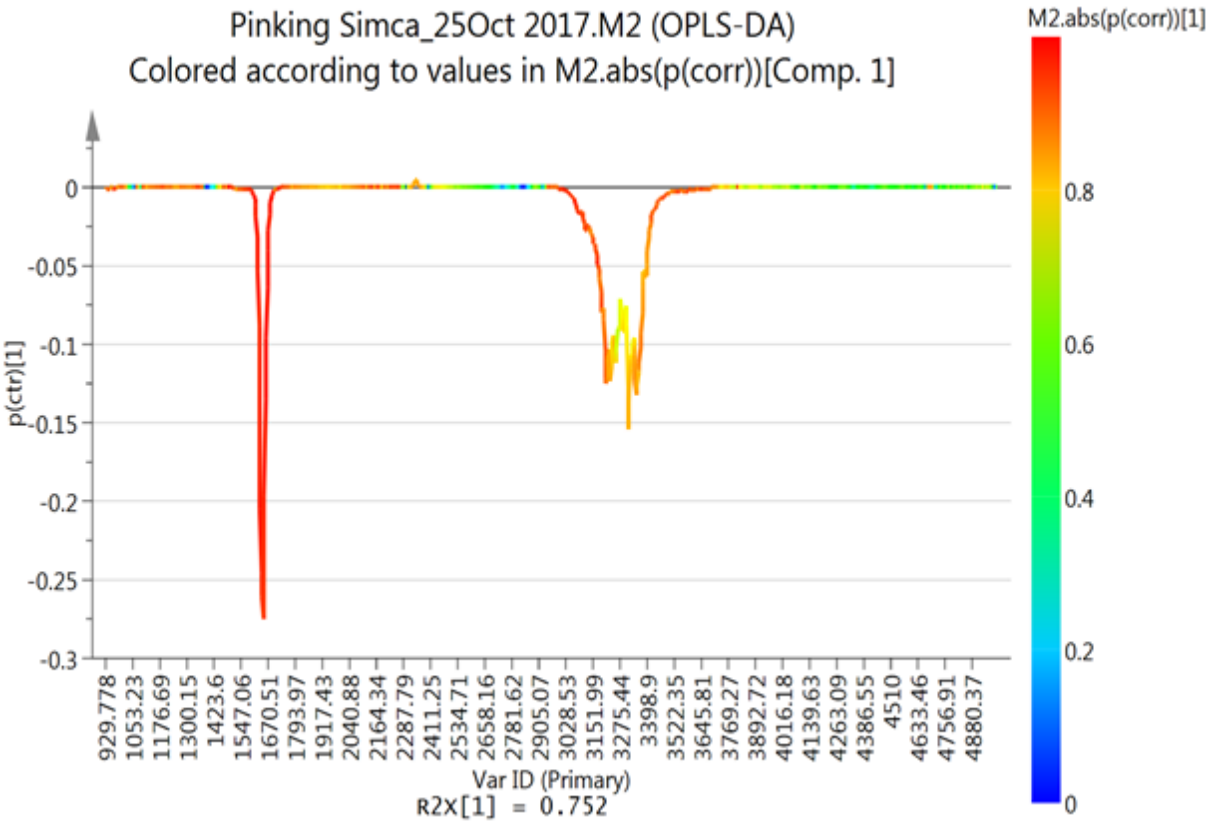

Figure 4: A spectra graph of the scanned control (no pinking) and naturally pinked wines with two distinct peaks.

A PLS-DA was done on all the data points and the descriptive analysis (DA) showed how the control and the pink wines are separated in the two quadrants (figure 5). The PLS-DA graph shows that there is a definitive grouping between the control wine and the pink wine.

Consider all findings as preliminary

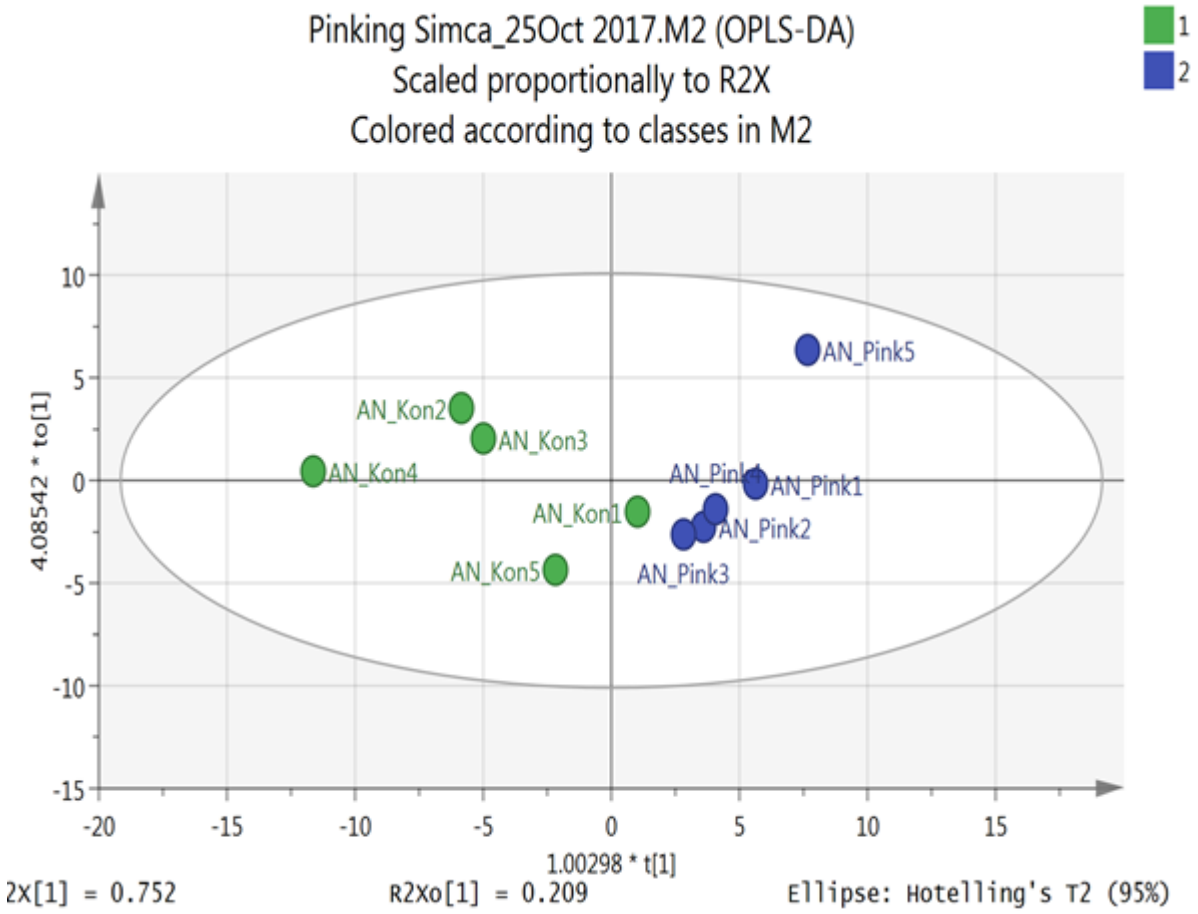

Fig. 5: A PLS-DA plot of control and pinked wines

The two peaks fall outside the fingerprint region (between 400 cm-1 and 1500 cm-1) (Fragoso et al., 2011; Banc et al., 2014; Culbert et al., 2015). The peaks formed between wave numbers 1612 to 1682 cm-1 and 3070 to 3437 cm-1. Every compound has a unique fingerprint and it is measured by the breakdown and absorbency of the functional groups. Each of these functional groups absorbed at different wave numbers and from there the compound can be established. The main theory about pinking is that the colour change is formed from an oxidation of phenols. In Fourier Transform Infra Red (FT-IR), phenyl/aromatic derivatives and phenols absorbed between 750-900 cm-1, while polyphenols and esters absorb between 600-900 cm-1 (Banc et al., 2014). According to Culbert et al. (2015) phenolic compounds absorb between 900-1500 cm-1. Fragoso et al. (2011) reported that the total phenolic compounds absorb between 1133 – 1457 cm-1.

The absorbance of the functional groups in the wave numbers of interested are: alkenes (C=C) and alkyne (C-H), amides (C=O), amines (N-H), imines and oximes (C=N). Further research is needed in identifying the compounds with the above mentioned functional groups that may lead to pinking in wines.

Liquid Chromatography Mass Spectrometry (LC-MS) analysis

S-plots were drawn from the HPLC-MS raw data and the mass of the opposite sides where noted (Table 1).

Table 1: Mass with retention times in brackets noted from S-Plots of HPLC-MS data

Mass [M-H] (Rt) - Right	Mass [M-H] (Rt) - Left
429.2139 (14.66)	287.0749 (12.21)
229.1078 (17.95)	401.1458 (8.54)
383.1547 (7.54)	383.1547 (7.54)
487.0681 (8.41)	616.1073 (10.07)

M-H – An H+ is subtracted during the HPLC-MS analysis. Rt – Retention time

Mass 616.1073 correlates with the mass of GRP. Figure 6 depicts the chromatogram showing the difference between the control sample and the pink sample. During the oxidation of the control sample to produce a pink sample, the glutathione (GSH) concentration are low and the GRP concentrations are high (Cheynier et al., 1989). The closest phenolic compounds that fits the masses in table are: Daidzein 4'-O-glucuronide (429.2139 – not found in wine), dihydro-resveratrol (229.1078), 5-(3',5'-dihydroxyphenyl)-?-valerolactone 3-O-glucuronide (383.1547 – not found in wine), 6''-O-acetylglycitin (487.0681 – not found in wine), phlorin (287.0749 – not found in wine) and 5-heneicosenylresorcinol (401.1458 – not found in wine). The above compounds closely resembles the relevant masses, but not exactly, leaving the distinct possibility that masses could represent other unknown, non-phenolic chemical compounds not yet researched in wine. In a series of articles written on pinking in onions the authors showed that certain amino acids reacts with thiosulfunates to form compounds that turn pink in the presence of oxygen (Shannon et al., 1967; Kubec et al., 2004a; Kubec et al., 2004b; Dong et al., 2010; Kubec and Velíšek, 2007 & Kubec et al., 2015). This opens up a new path in the further research for the pinking compound.

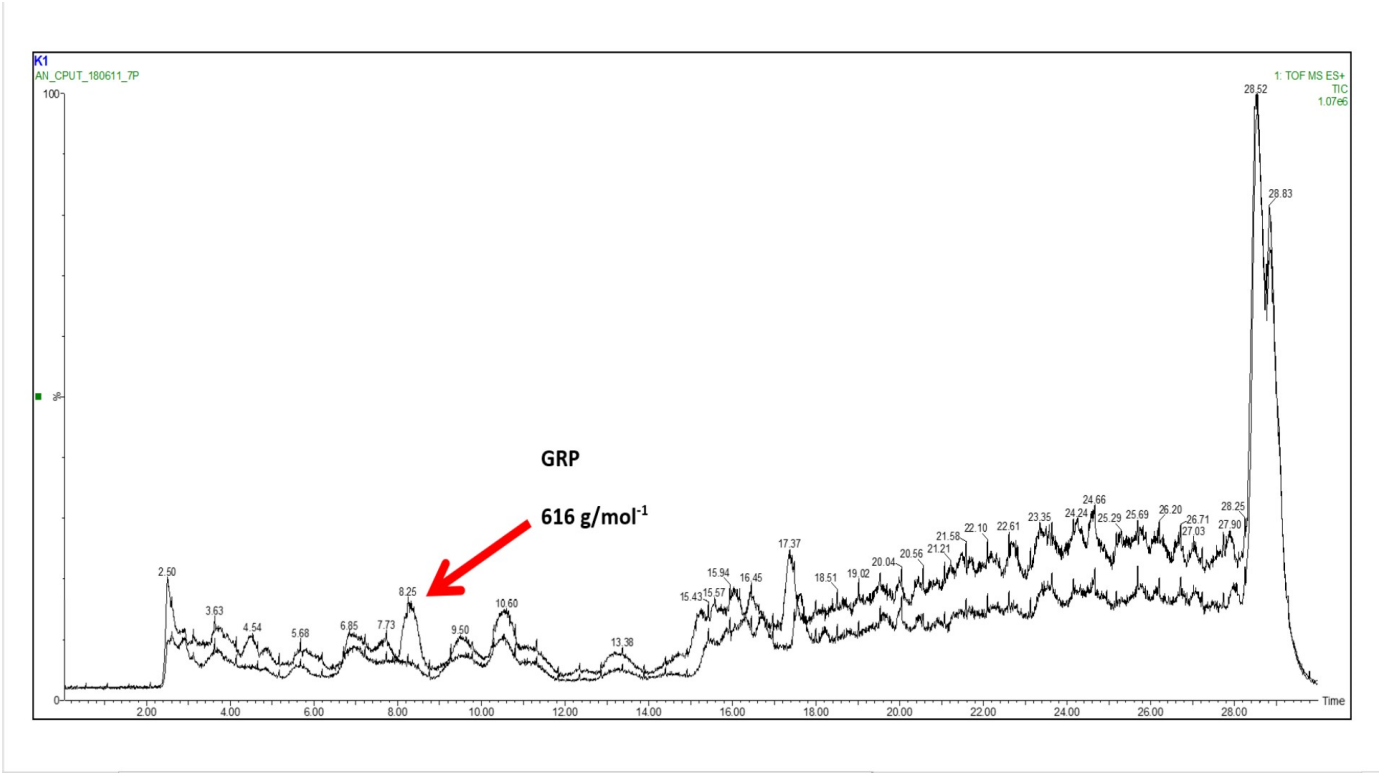

Figure 6: Chromatogram of a control versus pink samples. The top graph is the control and the bottom is the pink sample.

Sensorial properties of wines with pinking potential

The experimental approach is discussed in the workplan “W15.1 - Sensorial properties of wines with pinking potential”

Sensory evaluation 1: According to anecdotal evidence, white wines that turned pink in colour have no difference in taste and aroma. As this was never tested before, a sensory tasting was performed to confirm. The wines were presented to 28 tasters in a duo-trio tasting with black ISO glasses (Tables 2 and 3). According to the Roessler tables for minimum tasters to agree on a two-tailed tasting, 20 tasters must agree for a P=0.05 and 22 tasters for a P=0.01 to be of a significant difference (Roessler et al., 1978). Three different wines that showed a visible pink colouration was presented to 28 professional tasters. In table 2 12, 15 and 16 tasters could correctly identify the odd sample on aroma for wines 1, 2 and 3, respectively. Results showed that a white wine that turned pink will definitely have an aroma influence. The pinking in the wine commence during a brief ingress of oxygen and therefore that oxidation will have a change in aroma (Singleton et al., 1980; Simpson et al., 1982; Simpson et al., 1983; Razmkhab et al., 2002 & du Toit et al., 2006). However, only 16, 13 and 12 tasters could correctly identify the odd sample on taste for wines 1, 2 and 3, respectively. Therefore, pinking had no significant influence on the taste of the wine. In conclusion, one can expect that where oxidation is taking place in a white wine, the effect could be picked up on the aroma of the wine. As pinking is a phenomenon that can turn sensitive white wines pink in the early stages of oxidation, the taste is not that evident yet.

Table 2: Duo-trio tasting results for aroma

Aroma						
	Wine 1 n=28		Wine 2 n=28		Wine 3 n=28	
	Right	Wrong	Right	Wrong	Right	Wrong
2K1P	4	9	6	7	8	5
1K2P	8	7	9	6	8	7
Total	12	16	15	13	16	12

Table 3: Duo-trio tasting results for the taste

Taste						
	Wine 1 n=28		Wine 2 n=28		Wine 3 n=28	
	Right	Wrong	Right	Wrong	Right	Wrong
2K1P	6	7	5	8	5	8
1K2P	10	5	8	7	7	8
	16	12	13	15	12	16

Sensory evaluation 2: Twenty five percent and 11% of the evaluators described the two control wines as oxidised. Six percent and 61% described the Rosé wine as oxidised or pink, respectively. These two wines was the two extreme wines and between 6 – 61% of the evaluators attributed a fault to the wines. With the two oxidised wines 92% of the evaluators and 14%, respectively indicated that the wines were oxidised, while 17% indicated the second oxidised wine to be pink. Of the pinked wine 25 – 67% indicated that the wines show pinking, while 6 – 33% described the wines as oxidised.

Sensory evaluation 3: Sixty one percent of the tasters atoned pinking to an AU of 0.05 (Table 4). The same number or percentage of tasters (61%) thought that the Rosé wine was actually a white wine with a pinking potential. Tasters did not pick up any pink colour in the control wine. Only 25% of the tasters noted pinking at an AU of 0.01, most probably identifying a slightly darker colour compared to the control, rather than pink. Most tasters visually identified pinking at an AU of 0.02 (58%) and 0.03 (67%). In a study done by Simpson (1977), he found that a value of an excess of 0.05 AU shows that the wine has a definitive pinking potential. This study confirmed Simpson's study, but because winemakers are taking more notice of their wines, these values can be lowered to improve or refine the assay to suit the winemaker's preventative measures. Making this values lower will give the winemaker a firmer hand on the quality of his/her wines.

Consider all findings as preliminary

Table 4. Results of the ranking evaluation in percentage

Treatments	Pinking (%)
K1	0
0,01	25
0,02	58
0,03	67
0,04	53
0,05	61
Rosé	61

K1, control wine; 0.01-0.05 are absorbance units

Pinking potential of wines made from Botrytis infected grapes
The aim of the study was to identify possible differences between Botrytis cinerea-infected compared to non-infected white wines, in terms of pinking potential, and hence also to determine which proteins might be implicated in these defects.

Pinking potential in Botrytis-affected white wines were higher than the non-infected white wines. Pinking was also influenced by yeast strain, with Saccharomycodes ludwigii Y154 showing reduced pinking potential in both Botrytis-affected and non-infected white wines. Proteins quantified using 1D- SDS PAGE indicated the presence of proteins, as well as significant degradation of proteins in the Botrytis-affected white wines. Analysis of phenolic compounds in Botrytis-infected and non-infected white wines showed higher levels of phenolic compounds for Botrytis-affected white wines, thus indicating increased risk for oxidation and hence increased probability for pinking potential.

S. ludwigii Y154 might be used as either co-inoculation or sequential inoculation with Saccharomyces cerevisiae to result in reduced pinking in white wines.

References:

Lee, C.Y. and Jaworski, A.W., 1988. Phenolics and Browning Potential of White Grapes Grown in New York. Am. J. Enol. Vitic. 39 (4): 337 – 340.

Muñoz-Pina, S., Ros-Lis, J.V., Argüelles, A., Coll, C., Martínez-Máñez, R., Andrés, A., 2018. Full inhibition of enzymatic browning in the presence of thiol-functionalised silica nanomaterial. Food Chemistry 241: 199 – 205.

OIV. 2012. Compendium of international methods of analysis, Volume 2. 18, Rue D'Aguesseau – 75008.

Paris.

Peng, Z., Duncan, B., Pocock, K.F. & Sefton, M.A., 1998. The effect of ascorbic acid on oxidative browning of white wines and model wines. Austr. J. Grape Wine Res. 4: 127–135.

Prelle, A., Spadaro, D., Garibaldi, A. and Gullino, M.L., 2013. A new method for detection of five alternaria toxins in food matrices based on LC–APCI-MS. Food Chemistry 140: 161 – 167.

Ricardo-da-Silva, J.M., Cheynier, V., Samsom, A. and Bourzeix, M., 1993. Effect of Pomace Contact, Carbonic Maceration, and Hyperoxidation on the Procyanidin Composition of Grenache blanc Wines. Am. J. Enol. Vitic. 44(2): 168 – 172.

SASEV. 2002. Methods of Analysis for wine laboratories. Compiled by the South African Wine Laboratory Association

Schneider, V., 1998. Must Hyperoxidation: A Review. Am. J. Enol. Vitic. 49 (1): 65 – 73.

Simpson, R.F., Miller, G.C. and Orr, G.L., 1982. Oxidative pinking of white wines: recent observations. Food Techn. Austr. 34 (1): 44 – 47.

Bradshaw, M.P, Prenzler, P.D. and Scollary, G.R., 2001. Ascorbic Acid-Induced Browning of (+)-Catechin In a Model Wine System. J. Agric. Food Chem. 49 (2): 934 – 939.

Cheynier, V., Rigaud, J., Souquet, J-M., Duprat, F. and Moutounet, M., 1990. Must Browning in Relation to the Behavior of Phenolic Compounds During Oxidation. Am. J. Enol. Vitic. 41 (4): 346 – 349.

De Villiers, J.P., 1961. The control of browning of white table wines. Am. J. Enol. Vitic. 12 (1): 25 – 30.

Lopez-Toledano, A., Mayen, M., Merida, J. and Medina, M., 2006. Yeasts used to delay browning in white wines. Food Chemistry 97: 498 – 504.

Nenadis, N. and Paraskevopoulou, A., 2016. Browning susceptibility of commercial monovarietal white wines under accelerated oxidation conditions: correlation with compositional data and effect on total phenol content and radical scavenging activity. Eur Food Res Technol. 242: 1821 – 1828.

Peterson, R.G. and Caputi jr, A., 1967. The browning problem in wines II. Ion exchange effects. Am. J. Enol. Vitic. 18 (3): 105 – 112.

Razmkhab, S., Lopez-Toledano, A., Ortega, J.M., Mayen, M., Merida, J. and Medina, M., 2002. Adsorption of Phenolic Compounds and Browning Products in White Wines by Yeasts and Their Cell Walls. J. Agric. Food Chem. 50 (25): 7432 – 7437.

Serra-Cayuela, A., Aguilera-Curiel, M.A., Riu-Aumatell, M., Buxaderas, S. and López-Tamames, E., 2013. Browning during biological aging and commercial storage of Cava sparkling wine and the use of 5-HMF as a quality marker. Food Research International 53: 226 – 231.

Muñoz-Pina, S., Ros-Lis, J.V., Argüelles, A., Coll, C., Martínez-Máñez, R., Andrés, A., 2018. Full inhibition of enzymatic browning in the presence of thiol-functionalised silica nanomaterial. Food Chemistry 241: 199 – 205.

Please indicate YES or NO if a PROJECT EXTENSION is required (if YES, contact Winetech)
NO

9. CONCLUSIONS AND RECOMMENDATIONS

According to this Nietvoorbij study it is clear that the practices used by winemakers to make Sauvignon blanc can indeed increase the pinking potential of their wines. Sauvignon blanc is an oxygen sensitive cultivar, which means that the wine may oxidise very rapidly following the slightest uptake of oxygen. The wine then has the ability to become pink first before browning. The degree of ripeness and the temperature of the grapes had a significant impact on the pinking potential of the wine in this study, with the press method and skin contact time also increasing the wine's ability or potential to pink. The potential of the wine to pink increased with riper, cooler and whole bunch pressed grapes, and duration of skin contact. Winemakers should therefore preferably harvest grapes at lower to normal degrees of ripeness and destalk grapes at the cellar at ambient temperature, with as little as possible skin contact time, followed by pressing. Different press methods, as well as periods of skin contact, increase the wine's phenolic composition, which in turn constitute substrates for oxidation. Phenolic extractability of grapes increases as they ripen. Consequently vinification practices impact on the chances of pinking in Sauvignon blanc that has pinking potential.

Making Sauvignon blanc in a reductive manner (i.e. using of inert gasses or dry ice) also increases the pinking potential of the wine, on sudden exposure to air. If wine is made under reductive conditions, preventative measures must be taken to eliminate as much phenols as possible. In conjunction with the use of ascorbic acid (a strong reducing agent used in reductive winemaking), the SO2 content of the wines is also of importance (to bind and neutralise the H2O2, a strong oxidising agent formed from the use of ascorbic acid).

Not all the chemical agents work equally well and at all the winemaking stages where winemakers make juice/must additions, i.e. settling, fermentation and fining. Guidelines provided in this study give some insight into the best fining agents and stages of application thereof to use for the prevention of pinking.

Some yeast strains showed promising results in protecting the wines from pinking. More work, however, needs to be done regarding the protection of wines from pinking using yeast strains, particularly on other yeast cell wall factors, in addition to yeast cell wall chitin and mannoproteins, that might be involved. Yeast strain selection, therefore, in addition to the above-mentioned vinification practices, will reduce/eliminate the need for the use of fining agents for pinking protection.

Sunlight (UV) does decrease the pink colour considerably as determined spectrophotometrically, but not visually, questioning the effectiveness as a post-pinking treatment method.

Botrytis cinerea do influence the pinking potential of the final wine. Considering the phenolic compounds implicated in colour formation, the higher phenolic compound concentrations observed in Botrytis-infected wines may be a possible contributor to high pinking in analysed white wines. Without doubt, the yeast strain S. ludwigii Y154, should be used in winemaking to protect wines from pinking in Botrytis-affected and non-infected white wines.

Contrary to the believe that pinking does not affect the flavour of wine, sensorial evaluations showed that pinking influenced the aroma of the wine, but not the taste. Most tasters visually identified pinking at an AU of 0.03 (67%). Panellists also attributed other wine “faults” to pinked wines, e.g. referring to the wine as oxidised and not pink.

Naturally pinked wines are chemically distinct from control or non-pinked wines as shown by winescan and LC-MS analysis and clear grouping on PCA and DA. Although chemical differences are noted, the compounds causing pinking is still evasive.

10. ACCUMULATED OUTPUTS

a) TECHNOLOGY DEVELOPMENT, PRODUCTS AND PATENTS

Preliminary guidelines on how to minimise the pinking potential in white wines.

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

Through popular articles and scientific publications, and oral and poster presentations at conferences, including SASEV. We make a point of also directly sending participating producers copies of the articles as soon as it is published. Presentations at farmers and open days and at association seminars.

c) HUMAN RESOURCES DEVELOPMENT/ TRAINING (STUDENTS)

Student Name and Surname	Student Nationality	Degree (eg MSc Agric, MComm)	Level of studies in final year of project	Total cost to industry throughout the project
Honours				
Ms DD Mitchell	South African	National Diploma: Biotechnology - Diploma conferred April 2016.	3 (3)	R0
Ms Letitia Sabina Minnaar	South African	National Diploma: Biotechnology - Diploma conferred December 2018.	3 (3)	R0
Mr Abel John	Zimbabwean / South African	National Diploma: Biotechnology	3 (3)	R0
Ms Zovuyo Notshokovu	South African	National Diploma: Biotechnology	3 (3)	R0
Masters				
Mr F.M. October	South African	Hons. Biochemistry. Finalising MSc thesis	3 (3)	R0

Consider all findings as preliminary

		for examination 2019.		
Ms Amanda Kufa	South African	MSc Wine Biotechnology degree conferred April 2019	3 (3)	R40000
Ms Amalie Solberg	Norwegian	MSc	3 (3)	R0
PhD				
Anton Nel	South African	PhD	3 (3)	R0
Postdocs				
Dr B.S. Chidi	South African	PhD / DST-NRF-Post-doctoral Fellowship - Finalised 31 June 2017	3 (3)	R0
Dr T. Ndlovu	Zimbabwean / South African	DST-NRF-Post-doctoral Fellowship - Ended April 2019	3 (3)	R0

d) **PUBLICATIONS (POPULAR, PRESS RELEASES, SCIENTIFIC)**

Nel, A.P. Riglyne om die pienkingpotensiaal van witwyne te verminder, Wineland, February 2017 issue, 67.

Nel, A.P. How to reduce the pinking potential of white wines, Wineland, 1 February 2017. Oenology research, Winetech Technical.

Nel, A.P. & van Jaarsveld, F. Guidelines to reduce pinking potential in white wines. Wineland, 1 Mar, 2018, Oenology research, Winetech Technical.

e) **PRESENTATIONS/PAPERS THAT COULD BE DELIVERED**

VAN JAARSVELD FP, NEL AP, DU TOIT W. 2016. Pinking potential of South African Sauvignon blanc. 38th SASEV/WINETECH CONFERENCE, 23-25 August, 2016. (NEL A.P., CPUT, DU TOIT W., US).

11. PROJECT OUTCOME AND IMPACT

New Knowledge	Benefits Supply Chain	Direct Grower Application	Direct Packhouse/ Winery / Cellar Application	Other
X	X	X	X	

Other is:

The Value of the project to industry
Guidelines on how to minimise the pinking potential in white wines.

12. PERSONS PARTICIPATING IN THE PROJECT

Initials & Surname	Highest Qualif	Race (B, W)	Gender (M, F)	Institution & Department	Position	Cost to Project
Mr F.M. October	Hons. Biochemistry	B	M	PHATs	Co	R0
Mr R. Olivier	BTech Analytical Chemistry	B	M	PHATs	TA	R0
Mr C. Paulsen		B	M	PHATs	Co	R0
Ms M. van der Rijst	MSc Biometry	W	F	Biometry	Co	R0
Mr Marvin Piet	MSc Chemistry	B	M	NRF	RA (Intern)	R0
Ms Simonne Sherriff	BSc Molecular Biology	W	F	NRF	RA (Intern)	R0
Ms Sandiswa Mbewu	Diploma: Biotechnology	B	F	NRF	RA (Intern)	R0
Ms Suanne Honiball	BSc Molecular Biology and Biotechnology	W	F	NRF	RA (Intern)	R0
Mr Al-Girvan Tobias	BSc Medical Biosciences	B	M	NRF	RA (Intern)	R0

** (Only applicable to persons who participate as Consultants or on Contract)

[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/ student
TA = Technical assistant/ technician

Consider all findings as preliminary

13. BUDGET

TOTAL COST SUMMARY OF THE ENTIRE PROJECT

TOTAL ANNUAL COSTS (ALL YEARS)	CFPA	RAISIN SA	SAAPPA-SASPA	SATI	Winetech	ARC	THRIP	OTHER	TOTAL
TOTALS	0	0	0	0	880077	916000	0	0	1796077
2016					287495	299230			586725
2017					319439	332478			651917
2018					273143	284292			557435
2019									0
2020									0
2021									0
2022									0
2023									0
2024									0
2025									0
2026									0
TOTAL									1796077