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# FINAL REPORT 2020

**Winetech Number : WW WdT 17/01**

## 1. PROGRAMME & PROJECT LEADER INFORMATION

| | Research Organisation leader | Project leader |
|---|---|---|
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## 2. PROJECT INFORMATION

| | |
|---|---|
| **Project title** | Modelling colour stability and ageing potential in SA red wines |
| **Short title** | Phenolics and ageing |

| | | | |
|---|---|---|---|
| **Fruit kind(s)** | | | |
| **Start date** (mm/yyyy) | 2017-01 | **End date** (mm/yyyy) | 2020-08 |

| | |
|---|---|
| **Key words** | phenolics, ageing |

| | CFPA | DFTS | HORTGRO | SATI | WINETECH | ARC | OTHER |
|---|---|---|---|---|---|---|---|
| **TOTALS All years** | R 0 | R 0 | R 0 | R 0 | R 1406000 | R 0 | R 0 |

| | |
|---|---|
| **Total cost of entire project** | R 1406000 |

## 3. EXECUTIVE SUMMARY


**Objectives and Rationale**

The main objective of this project was to follow the phenolic evolution of a large number of red wines and to predict and corroborate their ageing capacity .

**Methods**

A wide range of phenolic analyses (spectrophotometric and HPLC) were employed as well as sensory assessments by winemakers on a range of red wines. Advanced statistical analyses were employed to analyse the large datasets generated. We also assessed winemakers' prediction of certain red wines' ageing capacity and confirmed how well these wines aged after a period of time.

**Key Results**

Evolutions of phenolic compounds were seen in both commercial and experimental wines. Winemakers rated wines with a relative low or high ageing potential correctly, which was confirmed after three years of ageing, but in a large number of wines they did not agree on this. The phenolic analyses performed could not explain the sensorial results obtained, but we were able to develop a phenolic concentration and evolution ageing index.

**Key Conclusion of Discussion**

Wines with a very low or high ageing capacity can be sensorially predicted by winemakers to age accordingly. However, factors other than only phenolics probably also play a role in the ageing capacity of wines, which might also explain the variation seen between winemakers' ability to predict the ageing ability of most other red wines tested. However, the phenolic evolution of most commercial red wines seems to follow a certain pattern. An aging index based on phenolic concentration and evolution (stability) only was obtained. This aging index correlates well with some of the most relevant phenolic measurements. Wines with high phenolic based aging index values are generally wines with initial high levels of tannins, anthocyanins, flavonols and therefore phenols and polymeric pigments. The index provides values in the range of 0 to 7.5. Values of the index were obtained for low aging potential wines (0-2,5), medium (2.5- 4,5) and high (4,5- 7.5). However, this phenolic aging index values could not be correlated with the sensory scores provided by the winemakers. Factors other than only phenolics probably also played a role in the winemakers' ability to predict the ageing ability of most of the red wines tested.

**Take Home message for Industry**

Wines with increased mouthfeel and persistence seem to age better, but not only phenolic analyses should be kept in mind when assessing a wine's ageing capacity. However, high or low phenolic wines should still have a similar phenolic profile after ageing. By using the proposed phenolic ageing index, wine producers might be able to better understand how their wines' phenolic profiles might look like after an ageing period of 12 to 36 months. General ageing capacity of the wine should, however, be complimented with sound sensorial analyses as well.

## 4. PROBLEM IDENTIFICATION AND MOTIVATION

### Problem Identification

The evolution of phenolics and loss of colour after red wine fermentation and during aging is a relevant issue for wine producers. Although good colour and phenolic extraction is often achieved during the fermentation process the stability of the colour material is not always secured. Simultaneously the aging potential of a red wine in terms of phenolic composition is a poorly understood concept. The evolution of red wines in terms of certain sensorial characteristics as influenced by phenolic compounds is also not well known. The question remains whether winemakers can predict the ageing potential of a red wine and if these can be used to predict the sensory composition of that wine after a period of ageing. During red wine aging changes in the chemical composition could be translated into changes of the sensory profile. Wines with higher aging potential should keep better and/or even improve their characteristics and quality.

The pink colour of rose wines are sought after, but in some cases quickly change during ageing into an orange onion skin hue. Factors influencing this are not clear and need investigation.

### Motivation

Phenolic compounds, especially anthocyanins, play a crucial role in red wine colour and its stability. The combination of anthocyanins with other phenolic compounds leads to a large number of molecules with different colour properties and sometimes an increased colour stability. The establishment of faster and cheaper spectroscopic methods which can be used in combination with a large phenolic database could give wine producers an estimation of how their wines should develop during aging in terms of colour stability and phenolic development.

Moreover, in combination with the chemical analyses some specific mouthfeel attributes such as colour, astringency and bitterness, have been proposed as important markers of the wine aging process. The need thus exists for wine laboratories and perhaps participating cellars to understand the colour stability phenomena and to be able to rapidly estimate the ageability of young red wines. The need also exists for the calibration of SA wine makers in terms of astringency and bitterness and to assess whether this can be used to predict and follow the sensorial profiles of red wines during ageing. The general phenolic evaluation of red wines has also not been followed using a large number of commercial red wines.

Rose wines' growth have increased dramatically over the past 5 years, both in SA and internationally. SA wine producers make world class rose wines and the colour of rose is important to the consumer. However, rose wines' colour can change to a more unwanted orange form, but factors affecting this are not clear.

## 5. ACCUMULATED PROGRESS TABLE

| Objectives | Milestones (Significant event or stage in a project) | Date Achieved |
|---|---|---|
| Commercial wines monitoring | Follow commercial wines' sensory composition over time<br><br>Follow commercial wines' general phenolic evolution over time<br><br>Attempt to model red wines' ageability and colour stability | 2020-07-07 |

| | | |
|---|---|---|
| | from phenolic composition | |
| Experimental wines monitoring | Follow phenolic evolutions in red wines with different initial phenolic levels | 2019-12-07 |
| Rose wines' evolution | Factors affecting rose wines' colour over time | 2018-12-07 |

## 6. WORKPLAN (MATERIALS AND METHODS)

### Commercial Red wines

A large number of 2016 commercial red wines were analysed for their phenolic composition after malolactic fermentation, after 6 months and 12 months barrel ageing, as well as after one year (called 24 months) and two years (called 36 months) bottle ageing. Eighty-two commercial wine samples including a wide variety of cultivars ((Cabernet Sauvignon (23), Shiraz (19), Pinotage (13), Merlot (11), Ruby Cabernet (2), Cabernet Franc (4), Cinsault (1), Grenache noir (1), Malbec (1), Mourvèdre (1), Petit Verdot (4) and Pinot Noir (2)) were thus collected from collaborating cellars. Additionally, within the experimental wines (59) the cultivars forming part of the study were as follows: Cabernet Sauvignon (13), Cabernet Franc (1), Cinsault (3), Grenache Noir (2), Malbec (3), Merlot (8), Mourvèdre (1), Durif (1), Petit Verdot (4), Pinotage (2), Roobernet (1), Shiraz (19) and Tannat (1). However, a small number certain of these wines were lost during the trail due to winemakers blending them without informing us beforehand.

Phenolic analyses included the Somers assay parameters, colour density and hue, CIELab colour coordinates (luminosity (L), redness (a), yellowness (b), hue (h) and chroma/saturation (C)), methyl cellulose precipitable tannin assay (MCP) tannins, total phenols, high performance liquid chromatography (HPLC) for phenolic compounds (27 individual phenols corresponding to the four main phenolic families were quantified) and finally some new variables created from the phenolic data. The new variables include two tannin/anthocyanin ratios (MCP/total anthocyanins and polymeric phenols/monomeric anthocyanins), anthocyanins polymers/monomers ratio, total content of proanthocyanidins, phenolic acids, flavonols and anthocyanins from the HPLC analyses and finally the % of proanthocyanidins, % of anthocyanins, % of phenolic acids and % of flavanols.

Spectral properties in different regions of the electromagnetic spectrum including the UV-Visible, near-infrared, mid-infrared and a combination of near and mid infrared (FOSS instrument) were measured in the wines at the same stages when the phenolic analyses were performed. Partial least squares single calibrations were optimized using different software with cross validation of the models.

Phenolic level and evolution indexes were attempted with a combination of multivariate analytical techniques. After an initial exploration of the data, principal component analysis and the contributions plot were initially evaluated. Discriminant analysis was also performed with orthogonal partial least squares (OPLS-DA), making use of the S-plot for interpretation. A multi-block approach was also evaluated to test for the stability of indexes developed over time. OPLS was in this case used for the data analysis.

The sensorial evaluation of 20 selected commercial 2016 wines (consisting of Shiraz, Cabernet Sauvignon, Pinotage and Merlot) after MLF, after 6 months, 12 months and 1 and 2 years of bottle ageing consisted of a rating exercise of mouthfeel perceptions such as astringency, bitterness, fullness, body, sweetness and sourness and the wines' ageing potential/cellaring time (scale from 1 to 5). After 1 and 2 years of bottle ageing the winemakers also had to indicate how well the wines have aged. Around 30 winemakers were used each time for these blind tastings. The same tasting was also conducted in 2019 on a smaller selection of 2019 commercial red wines (n=10). This was done to prevent possible palate fatigue. Selections of the wines were mostly made according to their initial phenolic levels, which represented a range of total tannin and total phenolic levels.

**Experimental red wines**

Cabernet Sauvignon and Shiraz grapes from Faure and Elgin respectively were used to produce red wines and bottled with a screw cap. This was done to follow the phenolic evolution of these wines over time. Phenolic analyses done after malolactic fermentation and after 12 months of ageing included those listed on Phenolab (tannins, anthocyanins, colour density, total phenolics and polymeric pigments) as well as phloroglucinolysis with HPLC.

Cabernet Sauvignon grapes from Stellenbosch were also harvested at two ripeness levels, 23° Balling and 25° Balling. Each harvest lot was split into six biological replicates and three were vinified per normal experimental cellar procedure, and three were fermented with the manufacturer's recommended dose of pectolytic enzymes. The wines were fermented to dryness and sequentially inoculated for malolactic fermentation, and then cold stabilized. Wines were bottled under screw cap and aged at 15°C. Phenolic content (tannins, anthocyanins, colour density, total phenolics and polymeric pigments) was analyzed using a spectrophotometric method (PhenoLab) after six months of bottle age, and again after 18 months of bottle age.

**Rose wines**

The distinct pink colour is of utmost importance in rosé wines and an exploratory study was done in order to determine the variation that occurs in the colour of South African rosé wines as well as how this develops over time. Two different experiments were conducted. The first investigated the effects of oxygen at bottling and different bottle storage temperatures on Shiraz rosé colour development. Grapes were separated into six 45 kg batches and treated separately. Each batch was crushed and destemmed, and 30 mg/L sulphur dioxide was added. For three of the batches, one hour skin contact was allowed prior to pressing. For the other three batches, four hours skin contact were allowed prior to pressing. Two g/hL lafazym CL settling enzymes were added to all the containers, and settling was allowed overnight. Juices were racked into fermentation vessels and inoculated with 25 g/hL Laffort Zymaflore X5 yeast. Once fermentation was complete, 50 mg/L $SO_2$ as well as 50 g/hL bentonite was added. After three days, the wines were moved to a -4°C cold room and left for three weeks to undergo cold stabilization. After cold stabilization, wines were racked into clean containers, and the 20 L resultant wine was split into two batches of 10 L for further treatments. One batch was bottled immediately, while the other was first saturated with oxygen to around 8 mg/L and then bottled. The wines were bottled in standard green Consol bottles with Nampak screwtops. The bottled wines were stored at three different temperatures; 4°C, 15°C and

25°C. Spectrophotometric analyses were performed at 0, 6 and 12 months after bottling.

The second experiment involved the use of 39 different commercial rose wines which spanned a wide variety of vintages and were all stored at 15°C in the dark. Wines were analysed spectrophotometrically at collection, as well as after six months of storage.

Analyses on all these wines included UV-Visible spectrophotometry to determine anthocyanin content (mg/L), total phenolic content (AU), SO2 resistant pigments (AU) as well as CIELAB measurements. (luminosity (L), redness (a), yellowness (b), hue (h) and chroma/saturation (C).

## 7. RESULTS AND DISCUSSIONS

### Commercial red wines

### Sensory results of commercial red wines

In Fig 1 Multifactorial analyses plot (MFA) of all the 2016 wines over all the different intervals that can be seen. Around 24 000 individual quantitative sensory data points have been used to construct these graphs. It can be seen that certain wines developed more than others. In Fig 2A the Multifactorial analyses of the sensory data only of time 0 and after 36 months can be seen. Certain wines changed more in terms of the overall sensory data, with wine SOT60 changing very little, as well as CKW17Q39. Wine PKT57 for instance developed more on Fig. 1. If one considers the MFA's loadings plot (Fig. 2B) it is clear that a number of the sensory characteristics investigated associated better with ageing potential such as persistence, body and astringency to a lesser extent at time 0. Ageing perception (in other words how well the wines aged) was also associated with persistence, body and astringency at time 36 months. These sensory descriptors were thus associated with the concept of ageing potential by winemakers. Interestingly, acidity and especially sweetness did not associate very well with the concept of ageing potential.

Fig 1. MFA plot of all the wines tasted at 0, 6, 12, 24 and 36 months (legends of the wines have been removed to avoid too much cluttering of the graph).

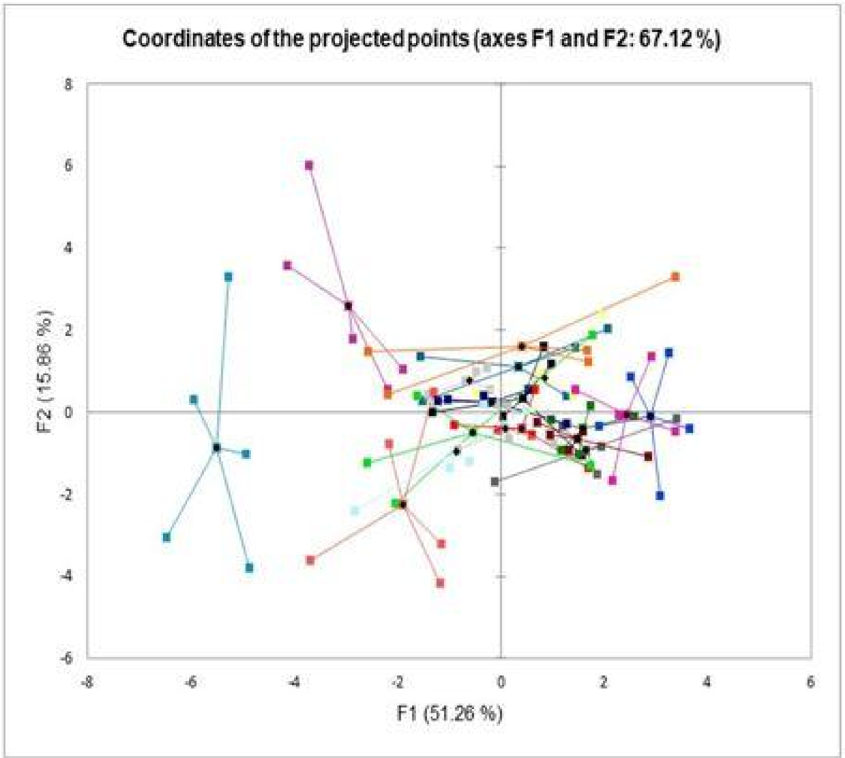



Fig 2A. MFA plot of the wines at 0 and 36 months.

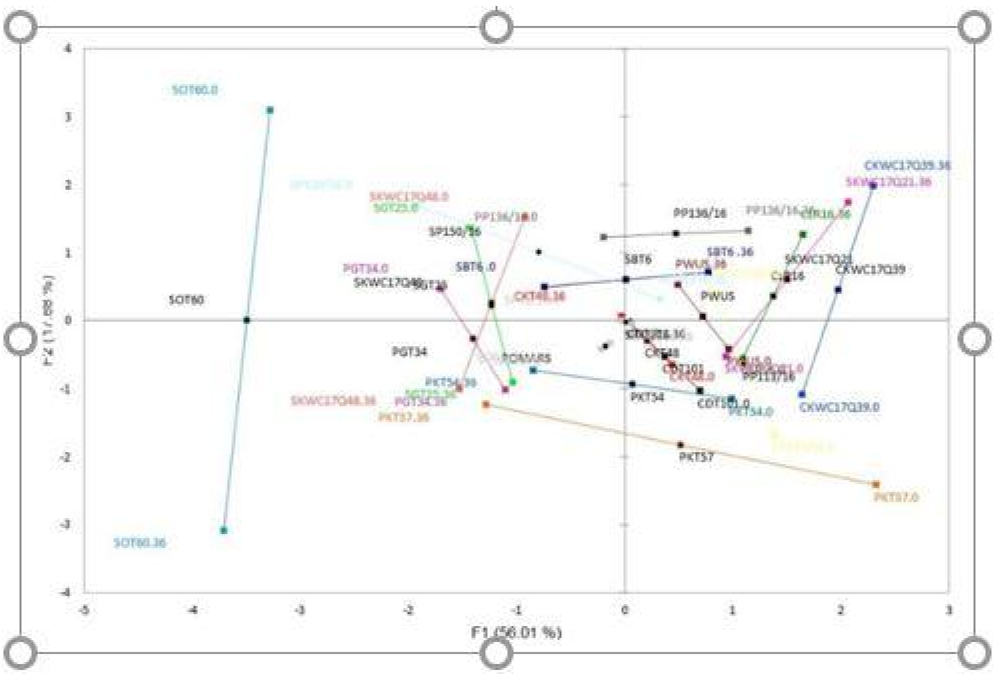
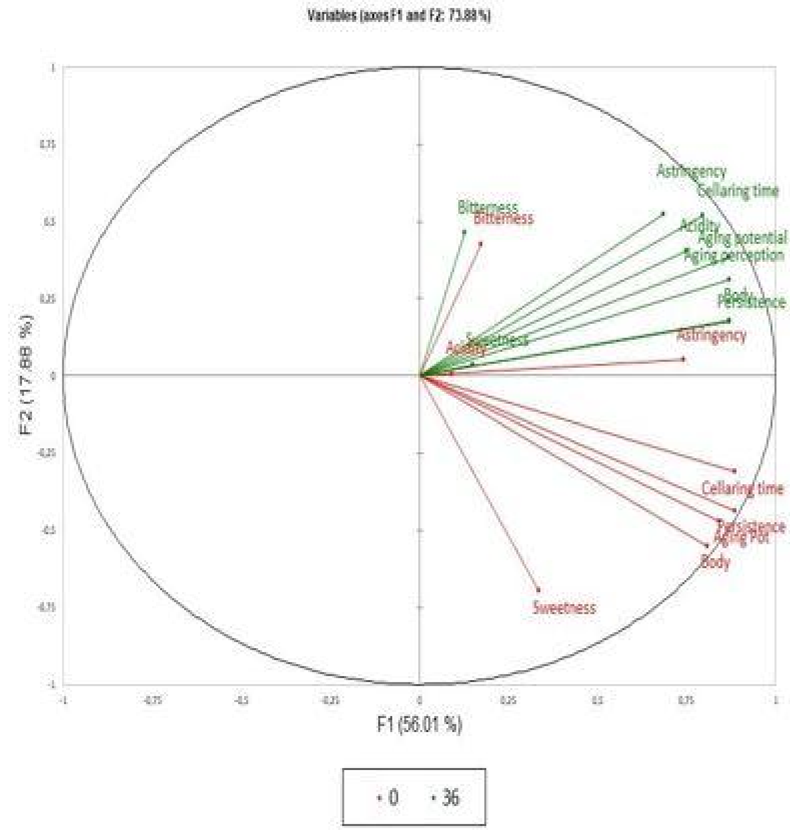
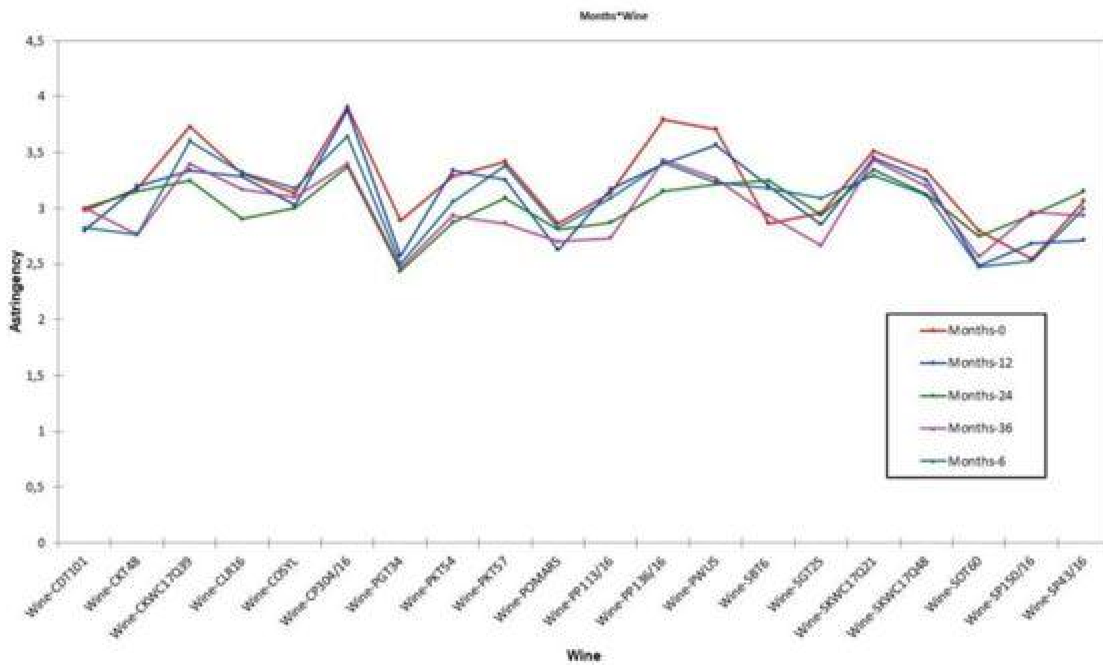


Fig 2.B. MFA loadings plot of the wines at 0 and 36 months.



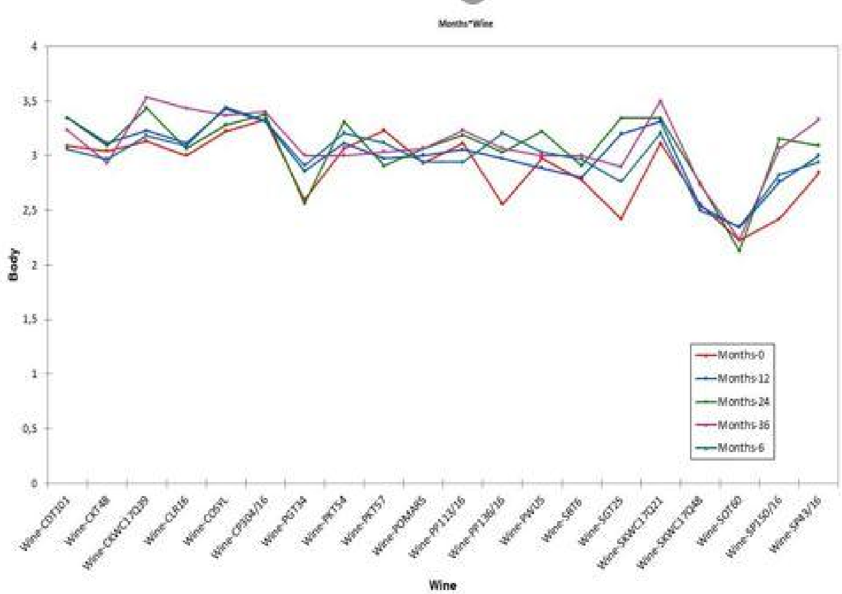

In Fig 3 the average astringency ratings of the wines over the different times tasted can be seen. Overall the astringency did not change significantly for most the wines, but this was not the case for all wines. Certain wines became less astringent over time, but the astringency ratios when comparing these wines stayed relatively the same.

Fig 3. Average astringency ratings of the wines at different times.



In Fig 4 the average values of the body of the wines can be seen. Certain wines were evaluated as thin and low in body, such as SOT60, CKT48 and PGT34, while others such as COSYL, CKWC17Q39 and SKWC17Q21 were rated high. The wines showed the same trends for body, with similar wines being higher in body than those being higher in astringency. However, a trend for a general increase in body was observed.

Fig 4. Average body ratings of the wines at different times.



The persistence (after taste) of the wines can be seen in Fig 5. Interestingly, some of the wines that were deemed lower in body such as SOT60 and CP304 were also scored lower in this regard. Persistence also increased in some cases over time, but a similar trend when the wines were compared to each other were observed over time. In Fig 6 the average ageing potential of the wines can be seen before bottling. What is interesting is that even during the 12 month evolution, the ageing potential of the wines did not change dramatically, with the same trends being seen over this period of time for the different wines. This might indicate that a red wine can be sensorially evaluated for longer term ageing potential any time after MLF and 12 months.

Certain wines were rated as significantly lower in ageing potential, such as SOT60, PGT34 and SKWC17Q48 while wines such as COSYL, SKWC17Q21, CP304/16 and CKWC17Q39 were rated as significantly higher in ageing potential. This can also be seen in Table 1, where the letters of significance between the ageing potential for all the wines are shown. Interestingly, most of the wines that were rated as having a lower ageing potential aged the worst after 24 and 36 months, while those being indicated as having the highest ageing potential aged the best at these time periods (yellow in Table 1). However,it was clear that a large number of wines did not differ significantly in terms of ageing potential, this is due to the winemakers not agreeing if these wines had a low, medium or high ageing potential.

Fig 5. Average persistence ratings of the wines at different times.

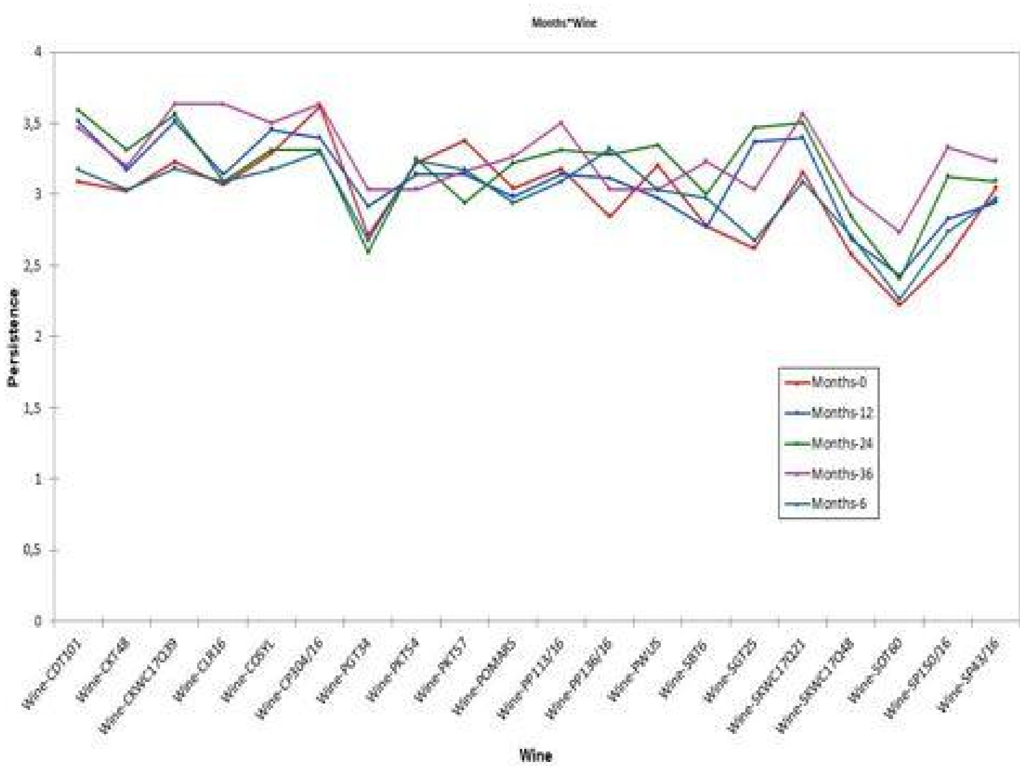
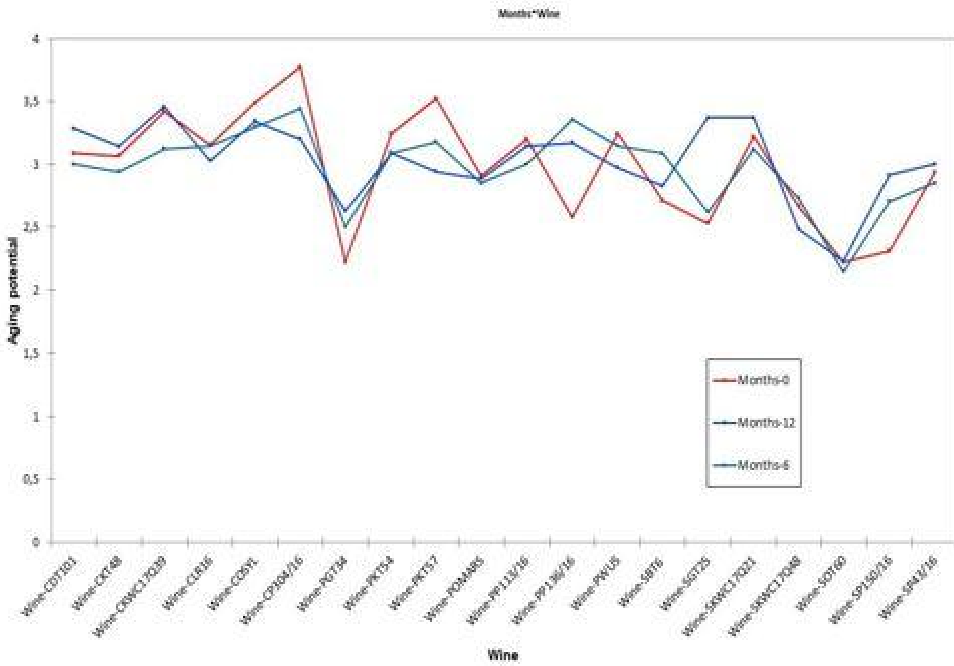


Fig 6. Average ageing potential ratings of the wines at times 0, 6 and 12 months.




We then performed the same tasting in 2019 on wines from the same vintage and then the combined results with those of the young wines from the 2016 vintage. We decided to use fewer wines this time to reduce possible palate fatigue. As can be seen in Fig 7 where the ageing potential sensory data of both 2016 and 2019 wines were combined, an association between ageing potential and persistence, body and to a lesser extent, astringency was again found. However, only a few 2019 wines were again significantly different in terms of ageing potential, as can be seen in Table 2. This again indicated that the winemakers agreed that wines at the extreme of low and high ageing potential differed in terms of ageing potential, but not for most of the wines tested. However, the winemakers only rated one wine as having higher ageing potential in terms of phenolic ageing potential (results not shown), which probably indicates that they also consider other factors when applying the concept of ageing potential to a red wine.

Table 1. Average values for ageing potential and how well these have aged (aged perception) after 24 and 36 months (ageing perception) of the 2016 wines. Different letters indicate significance.

| | | ageing potential | | | | | | | | | aged perception | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Category | S mean | Groups | | | | | | | Category | S mean | Groups | | | | | |
| Wine-SOT60 | 2,14 | A | | | | | | | Wine-SOT60 | 2,48 | A | | | | | |
| Wine-PGT34 | 2,45 | A | B | | | | | | Wine-PGT34 | 2,86 | A | B | | | | |
| Wine-SKWC17Q48 | 2,63 | | B | C | | | | | Wine-PKT57 | 2,95 | | B | C | | | |
| Wine-SP150/16 | 2,83 | | B | C | D | | | | Wine-SKWC17Q48 | 2,98 | | B | C | D | | |
| Wine-SGT25 | 2,89 | | | C | D | | | | Wine-PKT54 | 3,28 | | B | C | D | E | |
| Wine-SBT6 | 2,90 | | | C | D | | | | Wine-SP43/16 | 3,33 | | | C | D | E | |
| Wine-POMARS | 2,93 | | | C | D | | | | Wine-CKT48 | 3,37 | | | C | D | E | |
| Wine-SP43/16 | 3,02 | | | C | D | E | | | Wine-SP150/16 | 3,42 | | | | D | E | F |
| Wine-PKT57 | 3,02 | | | C | D | E | | | Wine-SBT6 | 3,42 | | | | D | E | F |
| Wine-PKT54 | 3,04 | | | | D | E | | | Wine-SGT25 | 3,43 | | | | D | E | F |
| Wine-CKT48 | 3,05 | | | | D | E | F | | Wine-POMARS | 3,49 | | | | | E | F |
| Wine-PP113/16 | 3,08 | | | | D | E | F | G | Wine-PP136/16 | 3,50 | | | | | E | F |
| Wine-PWUS | 3,08 | | | | D | E | F | G | Wine-PP113/16 | 3,51 | | | | | E | F |
| Wine-PP136/16 | 3,09 | | | | D | E | F | G | Wine-CLR16 | 3,53 | | | | | E | F |
| Wine-CLR16 | 3,15 | | | | D | E | F | G | Wine-PWUS | 3,54 | | | | | E | F |
| Wine-CDT101 | 3,21 | | | | D | E | F | G | Wine-CP304/16 | 3,58 | | | | | E | F |
| Wine-COSYL | 3,34 | | | | | E | F | G | Wine-CDT101 | 3,63 | | | | | E | F |
| Wine-SKWC17Q21 | 3,35 | | | | | E | F | G | Wine-COSYL | 3,66 | | | | | E | F |
| Wine-CP304/16 | 3,44 | | | | | | F | G | Wine-SKWC17Q21 | 3,83 | | | | | | F |
| Wine-CKWC17Q39 | 3,45 | | | | | | | G | Wine-CKWC17Q39 | 3,85 | | | | | | F |

Fig 7. Sensory results of both the 2016 and 2019 young wines combined. 2016 wines: green, 2019 wines: blue

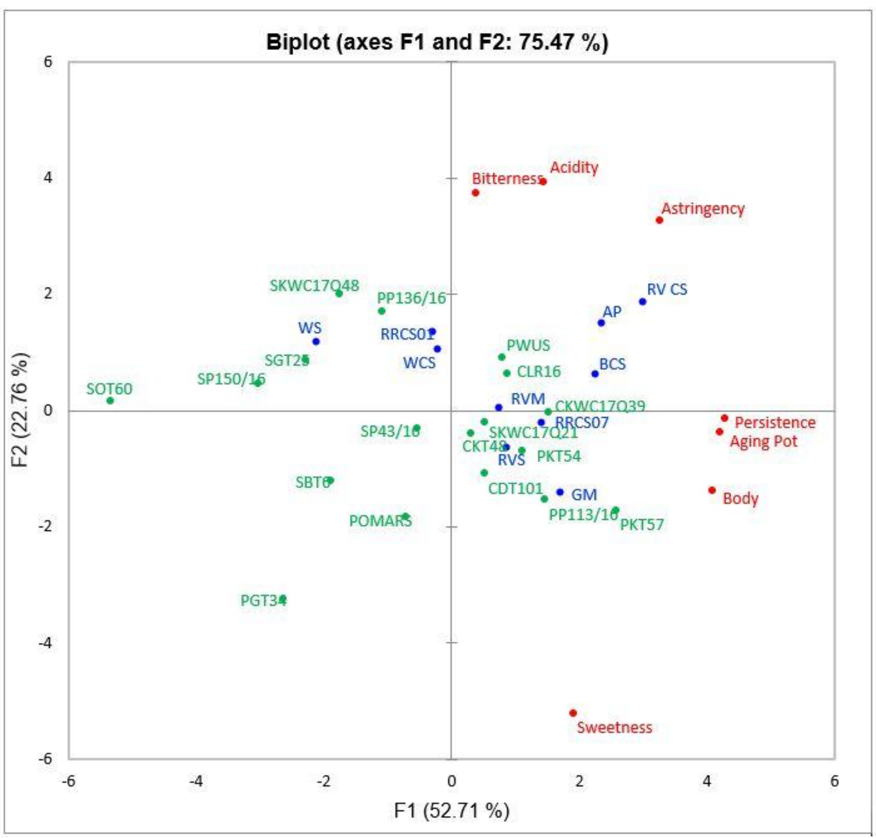


Table 2. Average values for ageing potential of the 2019 wines. Different letters indicate significance.

| Category | LS means | Groups | | |
|---|---|---|---|---|
| Wine-RV CS | 3,577 | A | | |
| Wine-GM | 3,538 | A | | |
| Wine-RRCS07 | 3,423 | A | B | |
| Wine-AP | 3,308 | A | B | |
| Wine-BCS | 3,269 | A | B | |
| Wine-RRCS01 | 3,231 | A | B | |
| Wine-RVM | 3,231 | A | B | |
| Wine-RVS | 3,231 | A | B | |
| Wine-WCS | 3,038 | | B | C |
| Wine-WS | 2,615 | | | C |

**General phenolic evolution of the commercial wines**

We also followed the general phenolic evolution of the 2016 wines over time. This was a very large dataset and only the general trends are shown in Tables 3. A larger decrease in the total phenolic content (TP) after barrel aging was seen, followed by further decreases during bottle aging, but to a lesser extent. This is indicating increased phenolic stability during the bottle aging period with wines, but less so during barrel ageing due to a more oxidative environment.

The total anthocyanin content showed significant decreasing levels during the aging process in our study. This is due to replacement of free anthocyanins with more stable pigments, which is reflected in the SO2 resistant pigment, Chemical age 1 and 2 increasing. However, some of these changes happened to a lesser extent during bottle ageing.

Colour measurements, such colour density and hue, provide a good indication of the status of certain phenolic structures in red wines. A significant change in colour was observed during the red wine aging process with bluish colourations being turned into more brownish hues. Interestingly, in our study, the yellow and blue tonalities showed maximum values after the barrel aging process with a subsequent slight decrease observed after extended bottle aging. Similar results were found for the red colouration; with a decrease after 36 months. An increase in the hue was also observed during the aging period with a greater increase occurring during barrel aging when compared to bottle aging, which again is probably due to the higher oxidative state of the wines in the barrels compared to bottles. Certain phenolic decreases during extended bottle ageing might be due to precipitation of certain phenolic compounds. This work shows that phenolic evolution in commercial wines takes place in both the barrel and the bottle, but that the former will probably lead to a larger extent of evolution. Tannin levels stayed relatively constant during this period of time, with only a significant difference between the young wine and after bottle ageing. This increase could be due to barrel derived tannins increasing in the wines or the conformation of the tannins changing, leading to a higher MCP tannin reading.

Table 3. Average and standard deviation values for the phenolic parameters and colour measurements of the wines included in the study. Significant differences are shown with letters.

| | 0 | | | | 12 months | | | | 24 months | | | | 36 months | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total phenolics | 56,54 | ± | 12,06 | a | 53,41 | ± | 9,50 | b | 52,97 | ± | 9,61 | b | 51,23 | ± | 8,03 | c |
| Tannins (mg/L) | 2065,38 | ± | 616,96 | c | 2393,69 | ± | 597,21 | bc | 2412,76 | ± | 585,05 | b | 2473,28 | ± | 611,72 | a |
| Anthocyanins (mg/L) | 471,06 | ± | 125,65 | a | 336,84 | ± | 72,58 | b | 188,20 | ± | 82,07 | c | 179,26 | ± | 44,12 | d |
| SO2 resistant pigments | 2,78 | ± | 0,90 | c | 3,25 | ± | 0,97 | b | 3,93 | ± | 1,21 | a | 4,15 | ± | 1,03 | a |
| Chemical Age 1 | 0,33 | ± | 0,05 | d | 0,47 | ± | 0,05 | c | 0,56 | ± | 0,06 | b | 0,67 | ± | 0,08 | a |
| Chemical Age 1 | 0,12 | ± | 0,03 | d | 0,19 | ± | 0,04 | c | 0,48 | ± | 0,24 | b | 0,46 | ± | 0,12 | a |
| 420%yellow/brown | 4,98 | ± | 1,51 | d | 7,26 | ± | 2,75 | a | 6,51 | ± | 1,79 | b | 5,75 | ± | 1,24 | c |
| 520%red | 8,50 | ± | 3,37 | b | 10,53 | ± | 3,83 | a | 9,07 | ± | 2,77 | b | 7,51 | ± | 1,85 | c |
| 620%blue | 1,86 | ± | 0,69 | c | 2,59 | ± | 1,05 | a | 2,22 | ± | 0,70 | b | 1,99 | ± | 0,53 | c |
| Colour density | 15,35 | ± | 5,50 | c | 20,38 | ± | 7,42 | a | 17,80 | ± | 5,21 | b | 15,26 | ± | 3,54 | c |
| Hue | 0,61 | ± | 0,09 | d | 0,70 | ± | 0,12 | c | 0,73 | ± | 0,06 | b | 0,77 | ± | 0,06 | a |

**Phenolic level and evolution indexes for commercial red wines (PLE)**

We attempted to also develop a phenolic level and evolution index (PLEC) of the eighty 2016 wines tested for phenolics over the period of this project. We considered a wine with a high PLEC index having high phenolic content, as well as stability of these compounds over time. The wines were thus ranked according to the phenolic content and stability of these phenolic compounds. Subsequently those wines meeting these criteria were used as high PLEC wines. Two strategies were used to ensure an accurate selection of these wines.

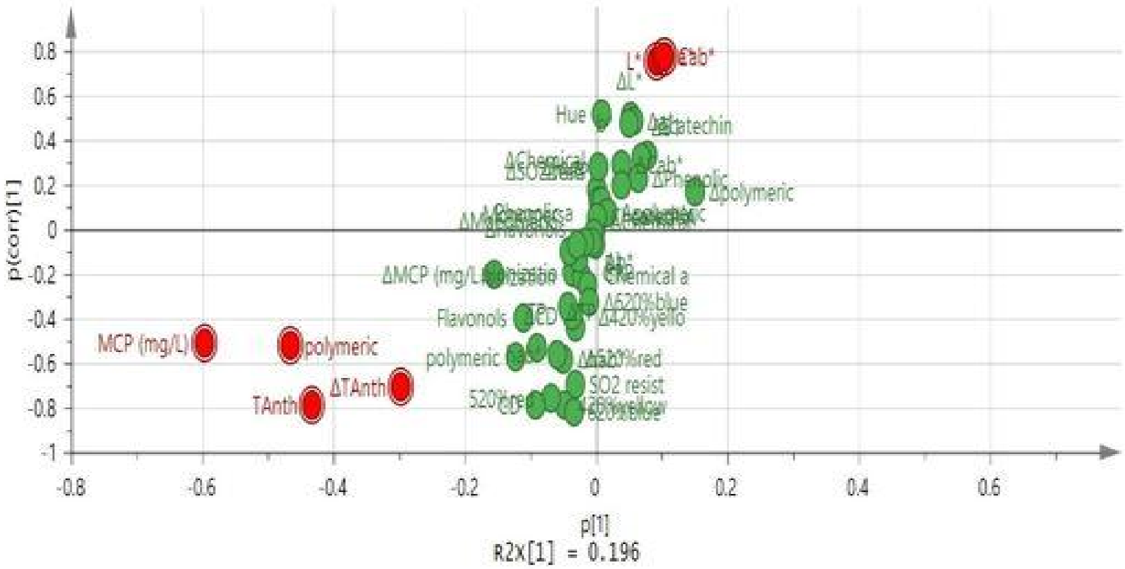
First, the change in the phenolic content during the barrel and bottle ageing period was calculated. As the phenolic measurements can increase or decrease the absolute value of that difference was calculated. The average of the variation was also calculated and used as a threshold to identify if a wine had stable phenolics (variation lower than the median, meaning stable phenolics for a specific phenolic measurement). This was done for all the phenolic measurements and was an estimation of the stability of the phenolic content over time. On the other hand, the phenolic content at time 0 was used to evaluate the high phenolic content wines. The average for a phenolic measurement was calculated and those wines with phenolic levels higher than the average were considered as higher phenolic content wines. The targeted wines were thus those that initially had a high phenolic content with minimal change over time.

A scoring system was then attempted to provide a PLEC index score for each wine. Two different approaches were used in this case. In the first approach a score of 0, 1 or 2 was given per phenolic measurement. If the variation in phenolic concentrations in the beginning and the end was higher than the median and the levels for that phenolic measurement were lower than the median a 0 score was given. If one of the two conditions (stability or content) was met, a score of 1 was allocated. Finally, if the wine was stable and the phenolic content was high a score of 2 was given. The addition of the scores provided a ranking in terms of PLEC values for each wine, with higher scores leading to a high PLEC value.

In the second approach instead of the 0 or 1 or 2 system a ranking was given to the wines in terms of lower to higher change in phenolic changes or higher to lower concentration. The wine with the lowest change was ranked with a 1 and the wine with the largest change was allocated a 50 (number of wines followed through the whole ageing process). The same was used for the concentration, with a 1 being allocated for the wine with the highest phenolic content and a 50 to the wine with the lowest. The scores for the measured parameters were added and those wines with the lower total scores were selected as high PLEC wines.

After the scoring process those wines that were found as having high phenolic content and having stable phenolics in both approaches were selected. The next step was to profile those wines that were characterized as high PLEC wines. First of all, a group to average comparison was performed and a contribution plot generated, while a second approach to profile the wines was attempted through a discriminant analysis (Fig 8). Wines high in MCP tannins, total anthocyanins, polymeric phenols, change in anthocyanins and lower in L*, a* and cab* seems to have separated the high PLEC wines from the rest. A high anthocyanin content and a large variation in anthocyanin content (possibly incorporated into more stable structures with tannins) is thus required in combination with high tannin content. This profile lead to wines with higher colour properties as well as lower hue (browning/oxidation tonalities). A PCA plot using the phenolic characteristics of high PLEC wines was then modelled and used for the establishment of the PLEC index.

Fig 8. Discriminant analysis comparing the high PLEC group. S-plot showing the variables that are driving the differences between wines.




The PLEC index was calculated as follows

PLEC index (Wine1) = (((t1wine1+1)/2)*0.665)*10

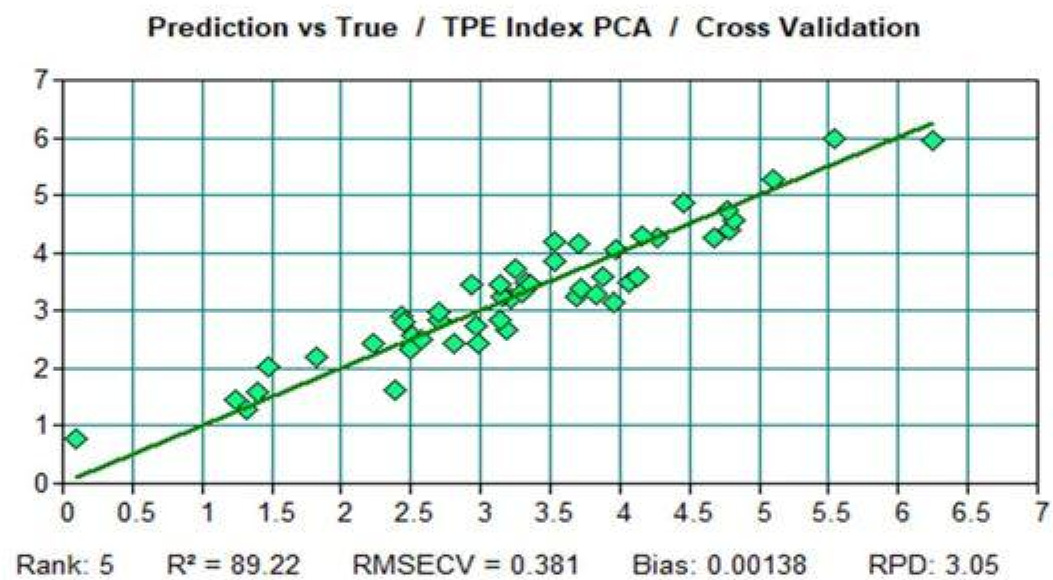
The score values t were made positive by adding 1 and dividing by 2. To account for the explained variance, the index was multiplied by 0.665 (accounting for the explained variance of principal component 1). Finally, the 10 is used to fit the index into 0-10 scale.

Alternatively, the wines were also classified according to three equal ranges giving rise to a low, medium and high PLEC index categories. A discriminant analysis with three classes was therefore performed. A classification exercise was then explored to evaluate if the discriminant model could be used to classify further wines into one of these three ranges according to its phenolic profile. Ninety eight % of the samples were accurately classified with only 1 sample being misclassified.

Finally, we evaluated the use of only the UV-visible spectra of the wine samples for this purpose. This is important because an accurate calibration could be used to predict the PLEC index of the wines by just measuring the UV-visible spectral properties. A relatively good prediction accuracy was obtained with a RPD value of 2.53, indicating that the model could be used for the prediction of the PLEC index of new wines. The only requirement will thus be the measurement of the UV-Visible spectral properties. Fig 9 shows another attempt but in this case only using 5 selected wavelengths. The results showed that this will be possible as the RPD values were again over 2.5 (3.05), making it suitable to determine the PLEC index in a much more simplified manner.

An aging index based on phenolic concentration and evolution (stability) was thus successfully obtained. This aging index correlates well with some of the most relevant phenolic measurements. Wines with high aging index values are therefore generally wines with initial high levels of tannins, anthocyanins, flavonols and therefore phenols and polymeric pigments. The proposed phenolic concentration and evolution index provides values in the range of 0 to 7.5. Values of the index from 0- 2.5 were obtained for LOW aging potential wines, from 2.5- 4,5 for MEDIUM aging potential and finally from 4,5- 7.5 for HIGH aging potential wines. An accurate spectroscopy calibration using five key wavelengths (270 nm, 290 nm, 500 nm, 520 nm, 540 nm) can thus be used to obtain this index.

Fig 9. Observed vs predicted values and validation statistics for PLEC index attempted with a few selected wavelengths.

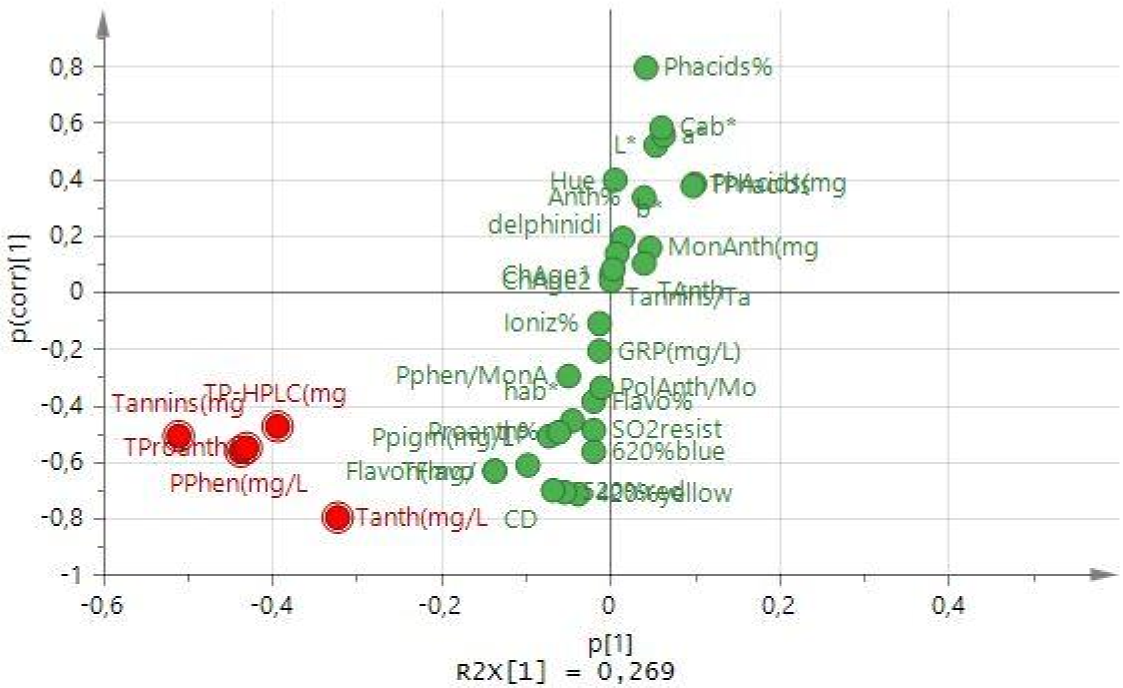



**Aging index combining phenolic and sensorial results of commercial wines**

An aging index from phenolic data, using the wines included in the sensory studies was then attempted. In this case we decided to investigate the phenolic profile of those wines that were rated with high scores by the tasters. The profile of the high aging potential wines was then compared against the remaining wines. The most explanatory variables of the variables that were characteristic of the high aging potential wines were the total tannins, polymeric phenols, total polymeric phenols, total anthocyanin content and the total phenols (highlighted in red) (Fig 10).

The high aging potential wines were selected based on previous results as shown below. This was used as a starting point as wines that were rated high initially which were also perceived high in aged perception at 24 and 36 months of aging.

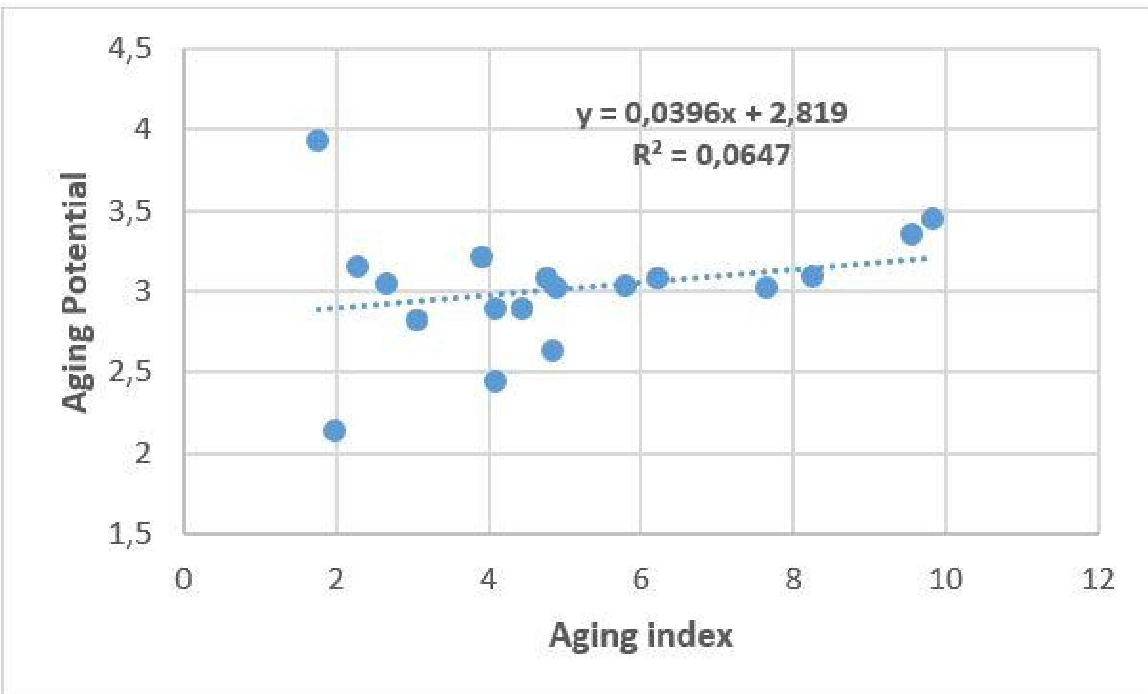
Fig 10. S-plot comparing the variables that are characteristic of high aging potential wines according to sensory scores.




A PCA model was fitted using only the most discriminatory variables as observed in the S-plot. The PCA bi-plot shows both the wines and the variables (phenolic measurements selected). As only the most discriminatory variables were included, it was expected that the samples will be distributed in the score space alongside PC1. However, when the samples were coloured according to the aging scores, it is observed that this is not the case, with the samples appearing randomly distributed through the scores space. This is a clear indication that the phenolic chemistry (or at least those we monitored in this study) of the wines alone could not model the aging scores given by the panel members.

The previous results can be confirmed with the direct correlation plotted between an aging index obtained using the position in the component 1 of the PC1 bi-plot (aging index = (t1+1)/2*10) and the aging potential scores given by the sensory panel. An index value was thus obtained for each wine and compared with the aging score. As expected, no correlation was observed (Fig 11).

Fig 11. Correlation plot between aging index from chemistry data and aging potential scores by the sensory panel




**Spectral data to predict aging scores and sensory attributes of commercial wines**

PLS regression was also attempted using UV-Visible and IR data to predict the aging potential scores from the sensory evaluation. UV-Visible, NIR and MIR spectra were evaluated. As observed in Fig 12 poor correlations were obtained with low prediction accuracies for the UV-Visible as well as NIR and MIR spectra (results not shown).

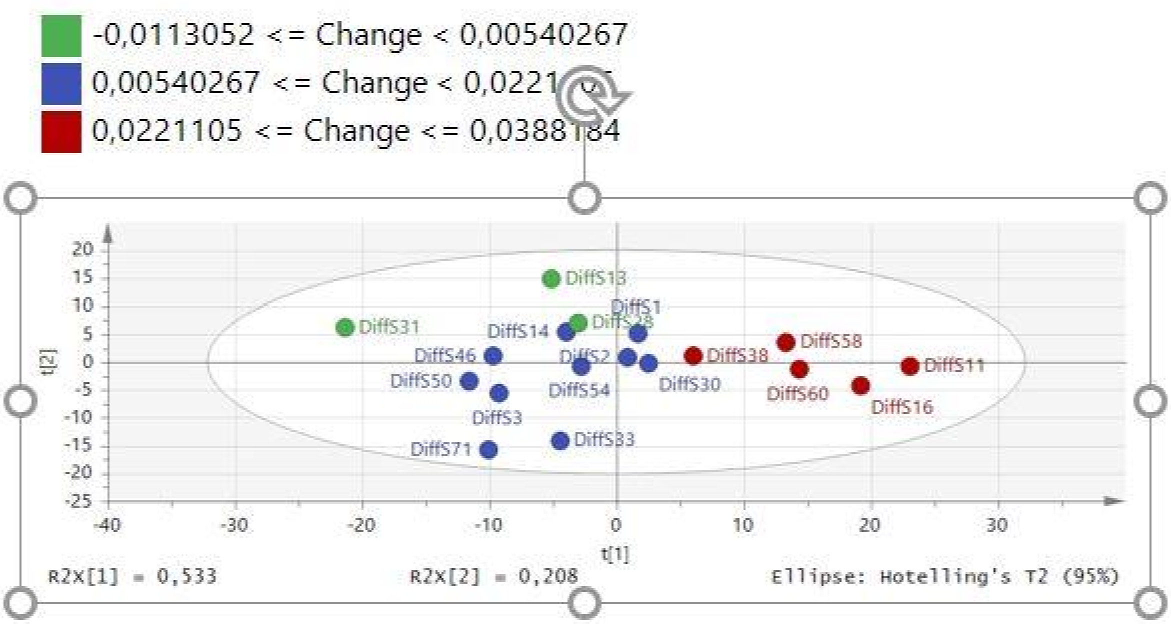
Fig 12 PLS regression of aging potential scores using UV-Visible spectra


Rank: 2 $R^2 = 45.27$ RMSEP = 0.466 Bias: -0.0229 RPD: 1.35
Validation No 2 UVVis AgingPot with raw spectra.q2

**Spectral differences to predict sensory scores of commercial wines**

The spectral differences during the aging process were also considered as a possible way to quantify the aging ability of the wines. In this case, the reasoning was with lower change in spectral properties the more stable a wine will be and the higher the aging potential. This strategy was evaluated for UV-Visible, NIR and MIR spectra data. Spectral differences were calculated as the spectra after bottle aging minus that at time 0. As can be observed in Fig. 13 the samples are separated and distributed in the scores space with also some overlapping being observed. The samples were coloured according to the overall change in spectral properties during the period evaluated. Three classes were created with three equal ranges as low, medium, and high.

Fig 13 PCA scores space for the UV-Vis spectral differences for those 2016 wines evaluated sensorially between the starting point and after bottle ageing, labelled according with overall spectral change.




For the ageing potential samples divided in three equal ranges and classes low, medium, and high were allocated. Ideally, one should observe a similar pattern as the one provided in Fig 13, which will indicate that a correlation exists between spectral differences and aging potential scores. However, the three aging potential classes appeared randomly distributed in the score space (results not shown), again indicating poor correlation between spectral change and aging potential scores. Identical results were observed when NIR and MIR spectral data was used to evaluate spectral change over time (results not shown).

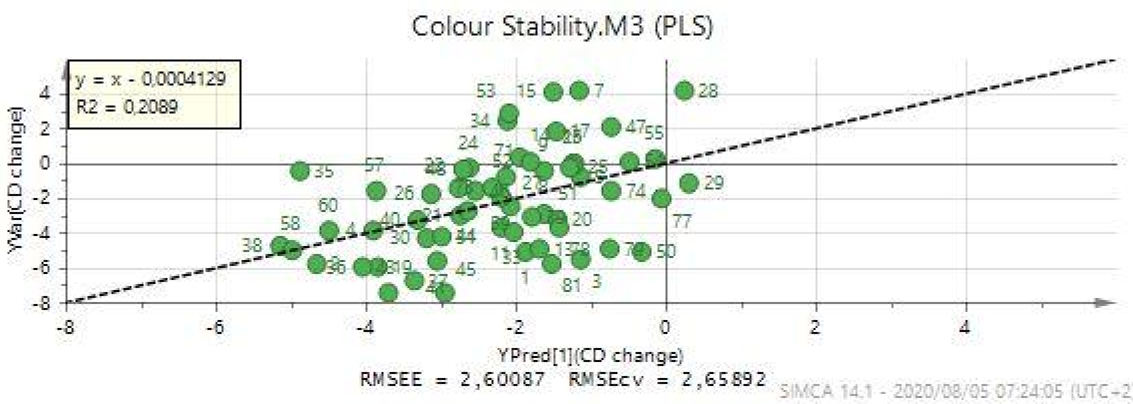
We also made all the above-mentioned data available to a final year student at the Industrial engineering department at Stellenbosch University. He did his final year project under the tutelage of professor Andries Engelbrecth (Voigt Chair in Data Science) at the department of

Industrial engineering. Extensive data analyses included SOM and K-means modelling, as well as random forest prediction modelling on the phenolic and sensory data. Unfortunately, no clear pattern between the sensory ageing potential and the phenolic composition of the wines could again be found. One of the deductions from these analyses was that the tasters could not agree sufficiently on low, medium and high ageing potential.

**Colour stability prediction of commercial wines**

We also attempted to predict the change in colour over time. For this we used the colour density parameter and calculated the colour change over the 24-month period of aging. We assumed that a smaller change in the colour indicates a higher colour stability of the wines over time. First, a PLS regression task was attempted with the phenolic measurements at initial point (Time 0). The ability of the chemical phenol data to predict colour change (or colour stability) is observed in Fig 14, which was not good.

Fig 14 PLS regression with the phenolic measurements as predictor variables and colour change over 24 months as predicted variable




Finally, the ability of spectral data to predict colour change over time was also assessed. UV-Vis, NIR and MIR spectral data was evaluated. A slightly better prediction ability was observed with R2 values of 0.57 for UV-Vis as best prediction accuracy of the three spectroscopy techniques (Fig 15). .

Fig 15 PLS regression with UV-Visible data to predict colour change (colour stability) over 36 months of aging


Rank: 4 $R^2 = 56.83$ RMSECV = 2.42 Bias: 0.123 RPD: 1.52
Validation No 7 UV-Vis colour stability.q2

**Experimental scale red wines**

An experiment conducted was to follow the phenolic evolution of Shiraz and Cabernet Sauvignon wines over time. In general the colour density increased in the Shiraz, but not to the same extent in the Cabernet Sauvignon, with a concurrent decrease in free anthocyanins. Tannin levels increased slightly in the Shiraz, but stayed relatively constant in the Cabernet Sauvignon. Total polymeric pigments increased in both wines made from these cultivars. Phloroglucinolysis results however, were not clear, with epi-catechin gallate increasing in the Shiraz. Small increases in the mean degree of polymerisation were also seen, possibly indicating tannin polymerisation (results not shown).

Another experiment assessed the effects of ripeness on phenolic evolution. Ripeness in red wine grapes drives phenolic extraction during maceration and alcoholic fermentation. Differences in ripeness as measured by sugar accumulation is often used to determine harvest dates and to make cellar decisions. This section investigated if more colour and phenolic material extraction took place from riper grapes and how these evolve during ageing.

In this set of data (Table 4), the extraction levels of anthocyanins, tannins and the total phenolic index was not significantly different between the ripeness levels. Cabernet Sauvignon as a rule gives wines with good anthocyanins and tannins, so it was not surprising that even slightly less ripe berries still extract good amounts of phenols. Enzyme use increased the concentration of every category, which was also expected, as pectolytic enzymes have been shown to open the cell wall structure of berries, allowing increased metabolite extraction. The rate of increase was similar for all measurements, with tannins showing a tendency to extract slightly more from 25° Balling, enzyme treated grapes.

A second part of this work was to explore if this increased extraction was still relevant after a year of aging. It is expected that anthocyanins will either form co-pigments with other molecules, or take on colourless forms as wine ages, and this effect was seen in both ripeness levels of wine. The rate of loss was similar for both control and enzyme treated wines. The total phenol index increased as well, possibly capturing the anthocyanin as it formed more stable co-pigments. Tannin levels decreased slightly with age for 23° Balling wines, but did not significantly change for 25° Balling wines. Colour density followed a similar pattern. Total phenolic index again increased as the wine aged, possibly reflecting a shift from anthocyanin to more stable co-pigments, but that is not collaborated by the change in polymeric pigments, which appear to have dropped slightly.

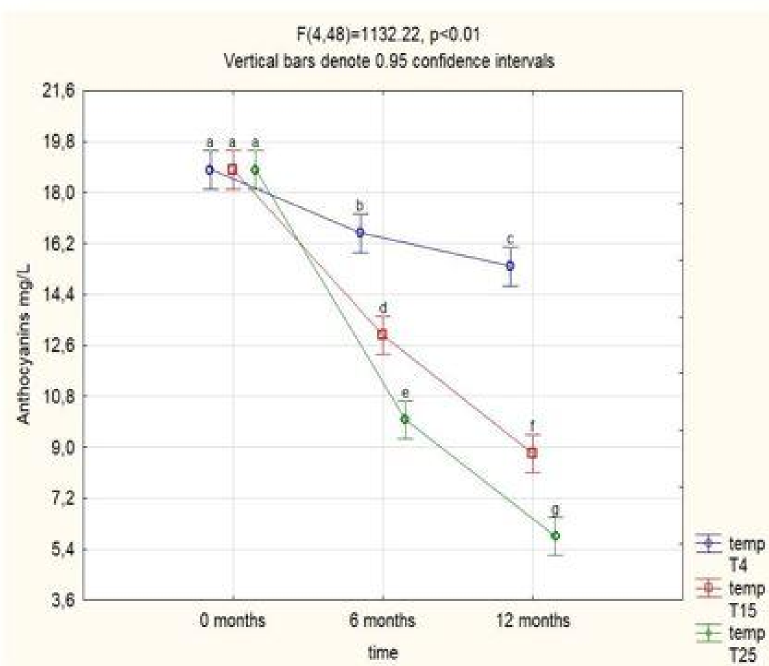
Table 4: Phenolic levels and evolution of Cabernet Sauvignon wines made with different grape ripening levels and enzyme treatments over times. SE: standard error

| Treatment | time (months) | Anthocyanins (mg/L) | SE | Polymeric pigments (mg/L) | SE | Tannins (mg/L) | SE | Total phenolics | SE | Colour density | SE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| control | 0 | 505,0 | 27,1 | 55,4 | 5,0 | 1460,8 | 120,8 | 49,2 | 3,1 | 19,1 | 1,6 |
| enzyme | 0 | 625,5 | 18,4 | 61,3 | 2,5 | 2101,1 | 75,3 | 61,8 | 1,9 | 21,2 | 0,8 |
| control | 0 | 508,2 | 32,6 | 44,9 | 4,0 | 1480,6 | 124,4 | 47,4 | 3,2 | 15,9 | 1,3 |
| enzyme | 0 | 621,6 | 30,2 | 54,4 | 2,8 | 2218,2 | 137,2 | 61,9 | 3,2 | 18,8 | 1,0 |
| control | 18 | 344,0 | 6,2 | 46,2 | 1,9 | 1285,1 | 24,5 | 52,2 | 0,8 | 14,9 | 0,5 |
| enzyme | 18 | 356,1 | 4,6 | 59,3 | 1,6 | 1737,8 | 36,0 | 60,9 | 0,8 | 15,1 | 0,2 |
| control | 18 | 365,6 | 11,2 | 43,0 | 2,0 | 1507,3 | 41,3 | 56,4 | 1,3 | 13,8 | 0,6 |
| enzyme | 18 | 394,2 | 31,9 | 51,7 | 5,1 | 1761,2 | 177,3 | 63,1 | 5,2 | 15,1 | 1,6 |

**Rose wines**

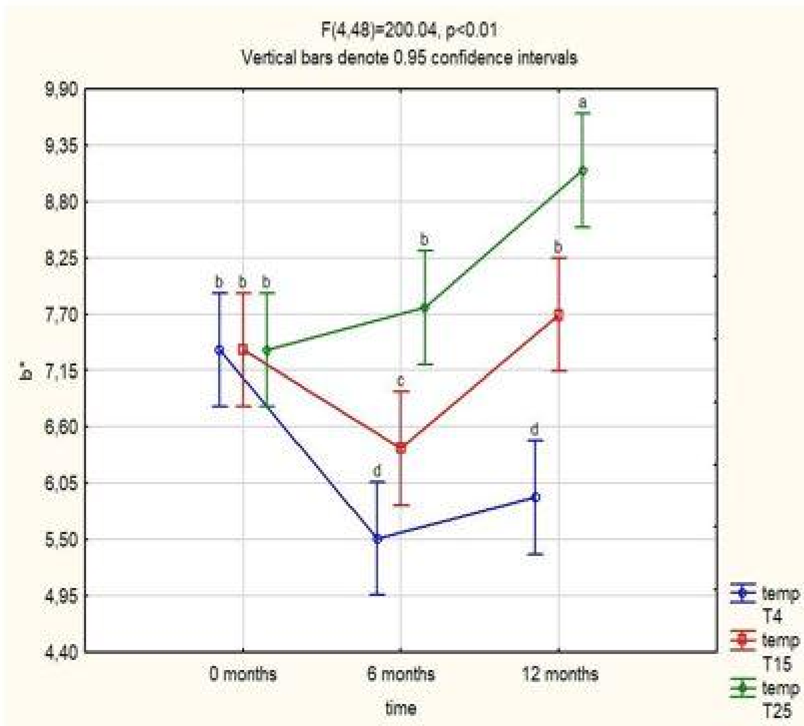
Longer skin contact led to more anthocyanins in the rose wines, as expected. Fig 16 shows that the storage temperature had a large impact on the anthocyanin levels in the wine. The higher the storage temperature, the quicker the anthocyanin levels decreased. 4°C appears to be the best storage temperature for the preservation of pink colour.

Fig 16. Impact of different storage temperatures on the anthocyanin concentration (mg/L) of rose wines at six month intervals. Different letters indicate significance.



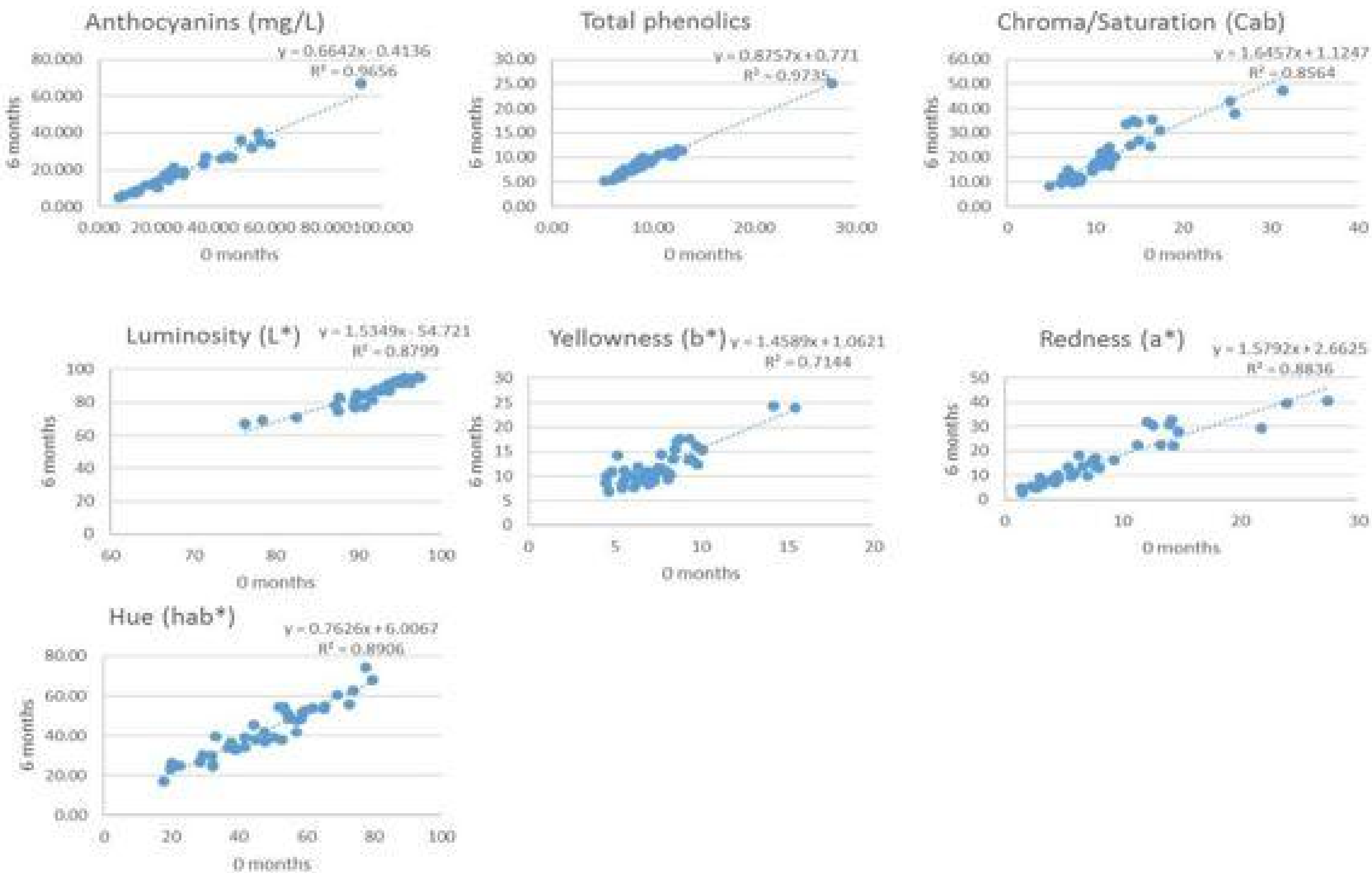
The higher the ‘b’ value, the more yellow the colour of the wine is. Thus, Fig 17 indicates that the higher the storage temperature, the more likely the wine is to develop a yellow hue over time. Again it is observed that a lower storage temperature is optimal for colour preservation. However, oxygen level at bottling did not influence the colour drastically (results not shown).

Fig 17 Line graph showing the effect of different storage temperatures on the 'b' CIElab co-ordinate in the experimental wines. Different letters indicate significance.




In Fig 18 the correlations between the various measured parameters in the commercial rose wines at zero months and six months are shown. As can be observed, good correlations were found between time 0 and after 6 months bottle ageing. Interestingly, luminosity, redness, yellowness and saturation all appear to increase slightly over the six month period. A possible explanation for this could be that the bleaching effect of sulphur dioxide has decreased and thus drives the colour changes. Sulphur resistant pigments were also analysed (data not shown) and were found to be in extremely low concentrations.

Fig 18 Correlations between the various parameters measured at bottling and at six months.




**8. CONCLUSIONS AND RECOMMENDATIONS**

It seems that winemakers can relatively accurately predict ageing potential for red wines that are on the extremes of the spectrum of ageing potential over a few years, in other words those that have very low and very high ageing potential. However, the ageing potential and how the wines eventually aged were often rated over a continuum, due to winemakers often not agreeing on how a wine that did not have an extremely low or high ageing potential should be rated.

We also attempted to compose a phenolic level and evolution index based on phenolic chemistry alone (PLEC), which was developed from the assumption that wines with a high initial phenolic content and lower change in terms of phenolics over time should have a high PLEC index values. We then investigated the phenolic composition of these wines and composed such an index. This index was also relatively well predicted using only five wavelengths in the UV-Vis spectra.

However, the phenolic composition could not explain the ageing capacity of the wines to a satisfactory extent. This could be due to the fact that our phenolic measurements did not account for all the phenolics in the wine, winemakers use other parameters than only phenolics to evaluate aging potential in the wines or the fact that the winemakers did not agree on the

ageability of a large percentage of the wines tested. However, in terms of chemical phenolic composition red wines with a high initial content will probably have a similar profile after ageing of a few years.
Preliminary results indicate that the colour change of rose wines from pink to yellow is more affected by storage temperature than the presence of oxygen at bottling.

## 9. PLANNED OUTPUTS

### a) TECHNOLOGY DEVELOPMENT, PRODUCTS AND PATENTS

None at this stage, but new knowledge has come to the fore on which factors winemakers keep in mind when assessing a red wine's capacity to age.
However, this very large dataset also forced us to investigate other types of advanced statistical analyses, which could be very helpful for future projects based on large datasets.
We can also incorporate the phenolic data obtained in this project into the PhenoLAB platform, which will increase it's wine phenolic database significantly.

### b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

Results emanating from this work can be presented at seminars or scientific conferences.

### c) HUMAN RESOURCES DEVELOPMENT / TRAINING (STUDENTS)

| Student Name and Surname | Student Nationality | Degree (eg Hons, MSc) | Level of studies in final year of project | Total Bursary Cost for Industry for entire project |
|---|---|---|---|---|
| **Honours** | | | | |
| **Masters** | | | | |
| Brock Kuhlman | USA | MSc | upgraded to PhD | R 235000 |
| Elzaan Fourie | South African | MSc | completed | R 120000 |
| **PhD** | | | | |
| **Postdocs** | | | | |
| Mihaela Mihnea | Romanian | | | R 50000 |

### d) LIKELY PUBLICATIONS (POPULAR, PRESS RELEASES, SCIENTIFIC)

Jose Luis Aleixandre-Tudo , Wessel du Toit. 2020. A chemometric approach to the evaluation of the ageing ability of red wines.Chemometrics and intelligent laboratory systems, 104067, 1-9.

J. L. Aleixandre-Tudo, W. J. du Toit. 2020. Evolution of phenolic composition during barrel and bottle aging, submitted to the South African Journal of Enology and Viticulture.

We foresee at least two more popular articles being generated from this work.

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

Mihaela Mihnea, José Luis Aleixandre-Tudó, Stephan Lamy and Wessel du Toit. 2017. Can red wine aging potential be explained through the sensory characteristics of the product? In Vino Analytica Scientia (IVAS 2017). Salamaca, Spain.

Mihaela Mihnea, José Luis Aleixandre-Tudó, Stephan Lamy and Wessel du Toit. 2017. Red wine aging potential as defined by South African winemakers. In Vino Analytica Scientia (IVAS 2017)

J.L. Aleixandre-Tudo, M. Minhea, W. du Toit .Understanding the wine aging potential. A multivariate approach. In Vino Analytica Scientia (IVAS 2017). Salamaca, Spain, 17-20 July 2017.

J.L. Aleixandre-Tudo, M. Minhea, W. du Toit. Ageing potential of SA red wines. Presented at the annual technical day of the SA Shiraz association, November 2017, Paarl.

W. du Toit., J.L. Aleixandre-Tudo, J. Brand, M. Minhea. How do winemakers predict the ageability of red wines? Presented at the annual technical day of the SA Shiraz association, November 2019, Paarl.

## 10. PROJECT OUTCOME AND IMPACT

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

**Other is:**

### The Value of the project to industry

This project brought new findings to the fore in terms of which mouthfeel factors are important for winemakers when assessing a wine's ageability. It also shows the stability of most phenolics in red wine over time, which can be interesting for wine producers when deciding on a wine destined for ageing. The importance of storing rose wines at a cool temperature in terms of its pink colour can also be of benefit to the industry.

## 11. PERSONS PARTICIPATING IN THE PROJECT:

| INITIALS AND SURNAME | HIGHEST QUALIFICATION | RACE (M,W) | GENDER (M,F) | INSTITUTE DEPARTM | POSITION | TOTAL COST TO PROJECT |
|---|---|---|---|---|---|---|
| **RESEARCH PERSONNEL** | | | | | | R 566000 |
| Dr Jose Luis Aleixandre Tudo | PhD | W | M | DVO | Researcher | R 566000 |
| **SUPPORT PERSONNEL** | | | | | | R 120000 |
| Jeanne Brand | PhD | W | F | DVO | Technical officer | R 0 |
| Jacomi van der Merwe | | W | F | DVO | Financial officer | R 20000 |
| Helene Nieuwoudt | PhD | W | F | IWBT | Researcher | R 50000 |
| Elsa Terblanche | MSc | W | F | DVO | Technical officer | R 50000 |

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

## 12. TOTAL COST OF PROJECT

| TOTAL ANNUAL COSTS (ALL YEARS) | CFPA | Raisin SA | HORTGRO | SATI | WINETECH | ARC | OTHER | TOTAL |
|---|---|---|---|---|---|---|---|---|
| **2015** | R 0 | R 0 | R 0 | R 0 | R 0 | R 0 | R 0 | R 0 |
| **2016** | R 0 | R 0 | R 0 | R 0 | R 0 | R 0 | R 0 | R 0 |
| **2017** | R 0 | R 0 | R 0 | R 0 | R 429000 | R 0 | R 0 | R 429000 |
| **2018** | R 0 | R 0 | R 0 | R 0 | R 470000 | R 0 | R 0 | R 470000 |
| **2019** | R 0 | R 0 | R 0 | R 0 | R 507000 | R 0 | R 0 | R 507000 |
| **2020** | R 0 | R 0 | R 0 | R 0 | R 0 | R 0 | R 0 | R 0 |
| **TOTAL** | R 0 | R 0 | R 0 | R 0 | R 1406000 | R 0 | R 0 | R 1406000 |