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University of Technology

Factors affecting port wine colour stability

by

Daylene Portia Mitchell

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Supervisor: Prof J. Van Wyk

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DECLARATION

I, Daylene Portia Mitchell declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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ABSTRACT

Port is a wine style that comes from Portugal. It is a sweet fortified dessert wine that is made in red and white styles. The taste is a balanced and complex combination of berry fruit, acidity, sweetness, alcohol and tannins. The taste should be sweet, smooth, complex, with some spiciness and a dry finish, but not astringent (Anon., 2009). There are a variety of port types in terms of flavour intensity, aroma and sweetness levels. Young immature ports can be fruity, simple, coarse, spicy and astringent. The sweetness results from the natural grape sugar in the wine, while wine spirits is added to fortify and ensure microbiological stability during aging (Anon., 2009). In this study the work was done on the ruby port style wine, ruby port wine is well known for its characteristic of being bright red in colour and therefore also very difficult to preserve in terms of colour stability in general. Colour is one of the principle parameters of the quality of not only port wine but also red wine in general, since it is the first characteristic to be perceived by the consumer in the glass. The colour of port wine also gives an indication of possible defects, the body, age and the evolution of the wine during storage. Colour, therefore, has an important influence on the overall acceptability of the product to the consumer. During aging, the wine colour changes, mainly due to progressive structural changes of anthocyanins. These changes are often perceived as undesirable by port consumers. As a result, the Cape Port Producers Association (CAPPA) requested this type of research to be done on port wine to improve the port wine making process in order to also give port wine a more stable colour. Therefore the objective of this study was to manipulate some of the parameters in port wine making, such as type of spirit used to fortify, storage temperature and also storage time in order to improve optimum stability of port wine colour.

From the first part of the study it was evident that the type of fortifying spirits, storage time and temperature had a significant effect on the colour of the port wine samples. The 96.5% (v.v^{-1}) fortifying spirits, shorter storage time and storage temperature below 25°C resulted in a more stable ruby port colour as well as the lowest change over time. As the study progressed the design variables differed in terms of two types of cultivars used the spirits used to fortify the port wine samples with, addition of a pectolytic enzyme to some of the port wine samples, as well as storage time of 12 months and only two storage temperatures. It could be concluded that at the end of this part of the study, that port wine colour stability was affected by the interaction of

the design variables in each treatment and less so by individual design variables in the study. It could also be concluded in this study that higher levels of acetaldehyde present in the spirits used to fortify port wine, did have a significant impact on ruby port wine and colour stability. The application of pectolytic enzyme preparation does not necessarily have a significant effect on its own but depends on the type of cultivar used. Storage time and temperature should also be kept to a minimum to ensure the desirable bright red colour of a ruby port wine.

ACKNOWLEDGEMENTS

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Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

1.1 Background to the research problem

Colour is one of the main sensorial properties of all red wine styles (including port) and is of crucial importance to the consumer since it is the first characteristic to be perceived in the glass. The mysteries of red wine and its pigments have interested many researchers over the last number of decades. Anthocyanins are mainly responsible for the colour of red wine in general (Oliveira *et al.*, 2015). The colour of port wine also gives an indication of possible defects, the body, age and the evolution of the wine during storage. Colour, therefore, has an important influence on the overall acceptability of the product to the consumer (Anon., 2010). The chemical changes in red wine composition are exceptionally complex, due to pigment molecules changing from the moment the grapes are brought to the winery, during the crushing, fermentation and thereafter in the barrel and during ageing (Waterhouse & Kennedy, 2004).

During aging, the wine colour changes, mainly due to progressive structural changes of anthocyanins. Moreover, colour density, total pigment colour, total sulphur dioxide (SO_2) and total and free aldehydes were monitored during aging of port containing varying amounts of aldehydes and SO_2. It was found that the initial colour increase in port is a result of interactions of free aldehydes and anthocyanins and other phenolics (McRae *et al.*, 2015). Free aldehydes are the residual concentration of aldehydes resulting from consumption of aldehydes in the reactions producing colour, formation of aldehyde by coupled oxidation of ethanol and the liberation of free aldehydes from aldehyde bisulphite adducts by oxidation of SO_2 (Anon, 2015b). A study by these authors on port containing a high concentration of SO_2, aldehyde content, showed that colour density (corrected for temporary bleaching by SO_2) remained unchanged, but total pigment colour became less, indicating aging by reactions not involving aldehydes. Hence, colour changes are interpreted in terms of two competitive reactions, *viz.* aldehyde-induced reactions superimposed upon direct condensation of anthocyanins and other phenolics. The total effect of these various changes are often perceived as undesirable by port consumers (Mori *et al.*, 2015).

When assessing wines, colour and appearance play an important role both in terms of consumer appeal, and also as a quality control parameter. In terms of the effect of colour on the sensorial assessment of ports, it was found that appearance attributes dominate the assessment of both aroma and flavour. Without any doubt, the colour of port wine is considered the most important component of quality (Pinho *et al.*, 2012).

1.2 Statement of research problem

In South Africa, the Cape Port Producers Association (CAPPA) specifically focuses on the quality of port wines. One of the more prominent issues regarding port wine flagged as an area where not much research had been done was the colour and colour stability of port wine. Therefore, this study was conducted to identify which factors affect the colour of port wine, both negatively and positively in order to determine how to optimize colour stability in port wine. The factors that were manipulated in this study were must treatment, storage time, storage temperature, aldehyde content and also using different grape cultivars to make the port wine with.

1.3 Objectives of the research

1.3.1 The broad objectives of this study were two-fold:

To measure CIELab colour, absorbance, aldehyde content, sensory profile, phenolic content and routine wine profile parameters of port wine as a function of wine must treatment (Thermovinification *vs* Conventional vinification), wine spirit, storage temperature and storage time with a view to identify the factors that will afford optimum port wine colour stability.

To measure CIELab colour, absorbance, aldehyde content, sensory profile, phenolic content and routine wine profile parameters of port wine as a function of grape cultivar, wine must treatment (pectolytic enzymes), different aldehyde levels, storage temperature and storage time with a view to identify the factors that will afford the best port wine colour stability.

1.3.2 The specific objectives of this study were:

To measure CIELab colour, absorbance (at 420, 520 and 620 nm), aldehyde content, sensory profile, phenols and routine wine profile parameters, firstly to determine the impact of thermovinified must *vs* conventionally treated must; secondly to determine

the influence of different wine spirits, namely at 74.0% (v.v^{-1}), 85.1% (v.v^{-1}) and 96.5% (v.v^{-1}) alcohol content to fortify the port wine with; and lastly as a function of using specific storage temperatures which include 4°C, 10°C, 25°C and 35°C as well as specific storage times of 0,1, 4 and 7 months.

To measure CIELab colour, absorbance (at 420, 520 and 620 nm), aldehyde content, sensory profile, phenols and routine wine profile parameters firstly to determine the impact of using two different grape cultivars (Pinotage and Tinta Barrocca) with which to make the port wine; Secondly to determine the influence of added pectolytic enzymes to the wine must prior to fermentation; thirdly to determine the influence of different levels of aldehydes in the wine spirits used to fortify the base wine with; and lastly as a function of using specific storage temperatures which is 10 and 25°C) as well as specific storage times of 0, 3, 6, 9 and 12 months.

1.4 Hypotheses

All of the hypotheses following below are non-statistical:

Two different grape cultivars will be used of which one is a typical port cultivar. It is, therefore, expected that the typical port cultivar, Tinta Barroca, would result in a more stable port wine colour and quality. It is expected that thermovinification, as a treatment of must *vs* conventionally treated must, when making the base wine for the port wine, will lead to a more intense colour in the port wine. Also it is expected that the use of a pectolytic enzyme preparation will improve the stability of port wine colour, since the use of pectolytic enzymes in the wine must will extract more colour from the grape skins.

Based on previous trials done at Nietvoorbij Cellar, it is expected that a lower alcohol concentration wine spirit used to fortify the port wine with will give an improved colour to port wine and, therefore, also improve stability in port wine colour. So based on previous trials done by Nietvoorbij Cellar with different aldehyde levels in the wine spirit, it is expected that a higher aldehyde level will result in a higher and more stable colour in the port wine.

Storing port wine at different temperatures will also affect the colour of the port wine, with higher temperatures hypothesised to result in a darker colour in the port wine. It is hypothesised that port wine stored at 10°C and 25°C will effect a more stable colour in port wine. It is hypothesised that, since the age of the port wine will also affect the colour of the port wine, increased storage time will lead to an increase

in browning. It is also expected that over time at different time intervals (time 0, 3, 6, 9 and 12 months) that the longer the port is stored the darker but more stable the port colour will be.

1.5 Delineation of the research

Due to not many wine cellars using this technique or nor having the equipment to do thermovinification, the thermovinified must and the control used in the study was from the same cultivar (Pinotage), but not from the same cellar. Due to limited availability, thermovinified must was fortified with only 74.0% $(v.v^{-1})$ wine spirits.

In the second part of the study, two wine cultivars (Tinta Barroca and Pinotage) were used to make the port wine. The port wine was made with grapes from one vintage and also grapes received from one geographical area, namely Stellenbosch.

In the first part of the study, three different wine spirits were used. These differed in terms of ethanol content, namely 74.0% $(v.v^{-1})$, 85.1% $(v.v^{-1})$ and 96.5% $(v.v^{-1})$. In the second part of the study one wine spirit was used with different added aldehyde levels (spirits on its own at 96.5% $(v.v^{-1})$, 96.5% $(v.v^{-1})$ spirits with 50 mg L^{-1} added acetaldehyde and 96.5% $(v.v^{-1})$ spirits with 450 mg L^{-1} added acetaldehyde). Hence, the spirits used in the second part of the study were of the same strength in alcohol content but varied in terms of acetaldehyde levels.

The storage period was limited to 9 months in the first part and to 12 months in the second part of the study.

1.6 Significance of the research

The Cape Port Producers Association (CAPPA) requested this research to be done to improve the port wine making process in order to identify parameters that will affect improved colour stability in port wine. Not much research has been done especially not in South Africa about port wine colour. Hence it is not known how factors interact that might have a significant impact on the colour of port wine.

Based on empirical knowledge, the average port producer is familiar with the fact that with time and age the colour of the wine changes from bright red to a more brownish hue, but there could be a scientific way of extending the period so that the wine retains the bright red colour, especially in the case of a ruby port since a bright red colour is required in this type of port. This study was then also conducted to be more specific about identifying which factors do, as well as how they directly

influence the colour of port wine. As stated, these factors include the type of grape cultivar used, the type of spirits (aldehyde content) and alcohol percentage of spirits used to fortify the port wine with, the storage time and storage temperature as well as the effect of using a pectolytic enzyme when making the port wine.

Therefore, this study was conducted to manipulate some of the normal practices of port wine making, such as the factors mentioned above, in order to improve optimum stability of port wine colour.

1.7 Expected outcomes of the research

It is expected that the results of this study will enable information sharing with the wine industry especially CAPPA, regarding improving practices of the port wine making process. The focus of improved practice will be enhanced or optimum port colour stability.

Particular aspects of the process where more clarity is required include the type of wine spirits to be used to fortify the port wine with and the ideal aldehyde concentration of the spirit and their impact on optimum colour stability in port wine.

The second aspect, the storage temperature during ageing will also have an influence and from this study the ideal storage temperature for optimum colour stability after storage after a certain period of time will be known. The storage period for port wines is normally very long, with storage in wood for a certain time, but based on this study the producer can determine how long, and depending on the temperature of the storage room, how it will affect the optimum colour of the final product.

The results of this study are also expected to confirm that a typical port cultivar such as Tinta Barroca will afford a more stable colour and to confirm better port character than any other cultivar, but from the taste panel results will provide a basis of comparison for Pinotage, which was also used to make the port wine with. Lastly the pectolytic enzyme used was expected to extract more colour from the grape skins and also effect optimum colour stability of the final product.

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CHAPTER 2

LITERATURE REVIEW

2.1 What is port?

Port is a wine style that comes from Portugal, more specifically the Douro region which is also the oldest port production area in the world. The varied microclimates in the Duoro region have resulted in 48 grape varieties being used in port production. These varieties include Tinta Francisca, Tinta Roriz (named Tempranillo in Spain), Touriga Francesca, Tinta Barroca and Touriga Nacional, all considered to produce the finest and most complex port wine (Christovan & Paterson, 2003). Port wine is a sweet fortified dessert wine that is made in red and white styles. The taste is a balanced and complex combination of berry fruit, acidity, sweetness, alcohol and tannins. The taste should be sweet, smooth, complex, with some spiciness and a dry finish, but not astringent (Joyce, 2009).

There are a variety of port types in terms of flavour intensity and sweetness levels. Young immature ports can be fruity, simple, coarse, spicy and astringent. The sweetness results from the natural grape sugar in the wine, while wine spirits is added to fortify and ensure microbiological stability during aging (Joyce, 2009). Port can be very sweet, sweet, semi-dry or extra dry. How sweet the wine will be in the final product is a choice made during production and depends on when the wine spirits is added to stop fermentation of the wine and the balancing before bottling. The latter entails the addition of more wine spirits or tartaric acid (Joyce, 2009).

As mentioned, port wine is also termed a fortified wine, where fortified wines are fermented or partly fermented wines to which distilled spirit of grape origin is added. Fortified wines include products as diverse as port and Madeira which originate from Portugal, sherry from Spain and various other products from Australia, South Africa or the USA. Many fortified wines are produced in such a way that they can be aged for a considerably long period, either in wooden barrels or in bottles. In the case of port wine, either way of ageing gives the wines their own character and style (Ho *et al.*, 1999).

In South Africa, the practice of fortifying wines, which is the addition of brandy or wine sprits to fermenting must, started in the Cape area to create a port "style"

wine. This practice can be traced back to the beginning of the 19th century, when there was a strong demand for these wines in Europe. At this time the wines of South Africa, more specifically the Cape, were well known in Europe, especially the sweet and fortified wines of Constantia (Anon, 2015a). Between the 1800's and 1980 the demand for a good quality port style wine grew even more, in South Africa and abroad. By the late 1980's dedicated producers of fortified wines started to align their style of port winemaking with that of the top Portuguese port producers. These producers then formed The Cape Port Producers Association (CAPPA) in 1992 (formerly known as the South African Port Producers Association (SAPPA)) and have continued to promote the crafting of fine Cape fortified wines from Portuguese varietals. Recently, both in the Douro region and locally, the focus has shifted to the crafting of exceptional varietal and blended table wines from the traditional port varietals (Anon, 2015a).

Since June 2012, according to South African legislation that stemmed from negotiations and eventually an agreement between the European Union and all other port wine producing countries, the term or class name "Port or Port wine" may not be used anywhere in the world except in Portugal. However, in South Africa, we are still allowed to produce the port style wine but have to classify it on the label as a fortified or as a dessert wine. These port style wines are still accepted and appreciated in Europe even though it cannot be identified as port (Anon, 2014).

2.2 Port wine characteristics

Unlike sherry, grape variety is central to the flavour of port. However, the method and length of maturation determine the final characteristics of the port, as with sherry styles. The total titratable acidity of port ranges from $3.45 - 5.86$ g L^{-1}, as tartaric acid, with volatile acidity (as acetic acid) less than 0.35 g L^{-1} (Anon, 2010).

Although most port wines spend some time in wooden barrels, port can be divided into two main categories: wood-aged ports and bottle-aged ports. Within these categories there are a number of styles, with tawny port and vintage port, the typical examples of each category, respectively. Tawny ports have little to no ageing potential, whereas vintage ports have good ageing potential (https://en.wikipedia.org/wiki/Aging). Predominantly, wood-aged ports are ready to drink right after they are bottled and put onto the market. They should be consumed within 2 – 3 years after bottling. These ports do not need to be decanted as most

have been filtered. They have a short cork and the bottle is not meant to be laid down (Joyce, 2009). Bottle-aged ports, on the other hand, start out in large casks for a brief period of time but then mature and age for a longer period than wood-aged ports, and sometimes a very long period, inside the bottle. These wines have a long cork, and are meant to lie on their sides and are not filtered. As a result, these ports usually produce a sediment. Hence, vintage port wines always need to be decanted (Joyce, 2009).

Ruby ports are usually darker in colour than tawny ports due to shorter periods of barrel maturation. Levels of phenolic material are higher in such styles as the ruby port wines, which impart slightly astringent flavours. Ruby port wines are also more extensively produced than the other types of port. After fermentation it is stored for shorter periods than the other port style and normally in stainless steel tanks rather than barrels. This is done to prevent oxidation and to preserve its distinct, rich claret colour (Pinho *et al.*, 2012). The colour palette of tawny ports is in the amber range, while the flavour displays complex dried fruit flavours that are less astringent than ruby ports, and often has oak-wood flavour characteristics. This is due to the extended contact of the wine with the wood, which reduces the levels of phenols and also extracts compounds which often make the port more spicy (Anon, 2010).

Vintage ports are more complex than both ruby and tawny ports, in part due to production from a single selected vintage and also due to minimal wood aging. As mentioned, while the best known style of wood-aged port is tawny port, the best known style of bottle-aged port is vintage port and one distinguishing characteristic of this port style is that the sugar content of the must varies with the particular vintage. This is due to the climate changes and weather conditions of the harvest of each particular year which, therefore, have a significant influence on final flavour. Port wine made in this style exhibit complex, fruity aroma and flavour characteristics, with a full-bodied mouth-feel and purple-red coloration (Anon, 2010).

Port wine is described as a "soft wine" which consists of different port wine styles and varieties depending on the time and place of consumption, as well as the consumer. "Soft wine" is a term used in Portugal to distinguish port wine from table wine (Oliveira & Clemente, 2002). The sensorial properties of port are appreciated throughout the world. Available in both white and red wine styles, and with some port varieties sweeter than others, the way ports are served is also important, namely chilled or at ambient temperature, depending on the time of day it is consumed and

also considering whether it is consumed after a meal or on its own (Oliveira & Clemente, 2002).

Quality evaluation is made in several ways: chemical analysis, sensory evaluation using human sensory organs and, more recently, also using electronic methods. Quality evaluation by human sensory organs is done by means of a taste panel which then evaluates the port wine in terms of colour, aroma, flavour and overall taste. Electronic methods would include spectrophotometric methods, the CIELab colorimeter method and the electronic tongue (Oliveira & Clemente, 2002).

2.3 Aspects of vinification techniques and fortification that influence wine colour, including port wine

2.3.1 Harvesting, fermentation and fortification

The Production of port wine can broadly be divided into a few stages and each of these stages as well as the sequence of steps, has a decisive influence on the style and quality of the final product. These stages include: selection of grape cultivar, treatment for example addition of pectolytic enzyme and crushing of grapes, alcoholic fermentation, addition of grape spirits and maturation (Tredoux & Silva Feirrera, 2012).

Port wine, especially the wine before fortification, is a complex matrix which contains volatile compounds, as minor components which play an important role in organoleptic quality. These flavours are produced through metabolic pathways during ripening and harvesting of grapes (primary aroma), during fermentation which determines secondary aroma, and also during storage of wines (post-fermentation). Factors such as grape variety, environmental conditions such as climates and soils, fermentation conditions including yeast, pH and temperature and storage or ageing conditions, will contribute to the final aroma and flavour of the wine (Noguerol-Pato *et al.*, 2009). In South Africa, the critical elements in the production of port wine, is the meticulous harvesting of ripe grapes, preferably Portuguese varietals (Anon, 2015b).

Grapes are hand harvested, at 23 – 28 °Brix (°B), in very traditional settings. Some are still foot-trodden in stone lagares, especially in Portugal, but mechanized crushers are now used most often. Sulphite is then added at 50 – 100 mg kg^{-1}, but

more recently this amount is typically increased to 100 – 200 mg kg^{-1}. Sulphites are added since it helps extract colour and delays fermentation (Joyce, 2009).

In traditional settings, fermentations take place in smaller farm houses which is called "quantas", while in larger companies it is done in the wine cellar. A commercial yeast starter culture at 1 – 2% is used to ensure a complete fermentation. Major port houses use the "Ducellier Autovinifiers" which work with a complicated system of pressure build-up and release, driven by carbon dioxide created during the fermentation. The violent agitation caused over a short period of time causes maximum extraction of colour and flavour. However, today less aggressive methods are used, such as the regular pump-over method. Fermentation temperatures are typically 26 – 29°C, depending on the climate and area of the winery (Joyce, 2009).

In South Africa, CAPPA members use either traditional open top cement fermenters (kuipe) with intensive pigeage, temperature-controlled stainless steel tanks with extensive pump-overs or a combination of both to obtain the desired colour and flavours (Anon, 2015).

Previously, at an alcohol level of 4 – 6%, the free run juice was run off or grapes were pressed to remove juice and then run into the spirits. Although this practice is still followed in parts of Portugal, today the fermentation is stopped at about 10 – 14 ^{o}B and the wine is fortified to about 18% alcohol. At the time when the must is fermented to the correct alcohol level, or when it reaches the required ^{o}Brix, the must is pressed or free run juice is separated from the must and added to the determined amount of brandy (spirit). The fermentation stops almost immediately due to the elevated alcohol levels (Joyce, 2009). The desired style of port, in terms of the desired sweetness level and alcohol content, will determine how much alcohol is added and at what stage. If a sweeter port wine is desired, the fortification with brandy spirit will be done where the juice is at a high sugar to alcohol level, for example 16 ^{o}B. If a more dry finish and higher alcohol level in the port is desired, the fortification will be done at a lower sugar to alcohol level of the base wine. Since the acid levels of port is critical, the pH of the must should range from 3.60 to 3.80 (Anon, 2010).

Typically, 77% spirits is used to fortify the base wine, with 100 L of 77% alcohol applied to 450 L must. The ratio of spirits to base wine is calculated by a formula called the Pearson's square. The volume of added ethanol depends on a few factors which include the alcohol of the must before fermentation, the alcohol level in

the wine after fermentation, the alcohol level of the spirit that will be used and also on the final alcohol level of the port wine. The lower the alcohol, the more flavour and aroma the spirits offer to the final character of the port. As mentioned previously, tannins and acid may be added later during balancing of the wine as well as adjusting the alcohol and sweetness of the final port according to the preferred style (Joyce, 2009).

The port in then stored at a very low temperature to settle and will be pumped over at least three times at this stage before it is then either placed in wooden casks or directly filtered and bottled, depending on the style of the port wine. Vintage ports will be the ones selected to mature in wooden casks for longer whilst some younger (or ruby) ports, will be filtered and bottled directly. Red ruby ports are fined and cold stabilized, filtered and bottled. This is done to sustain the young, fruity character which is synonymous with ruby port wines (Joyce, 2009).

On the other hand, many tawny port producers use a version of the *solera* system to give a uniform consistency. This means that fortification occurs by addition of the wine to the spirit and not the other way around. Sometimes younger port wines will be added to the older port wine at the time of final blending to add another dimension of taste to the port (Joyce, 2009).

In South Africa, each port style is defined and the production is regulated by the Liquor products Act 60 of 1989, and enforced by its officers. CAPPA and its members played an integral part in the compilation of these regulations (Anon, 2015a).

These styles include and are termed in the Act as: Cape ruby, Cape pink, Cape white, Cape tawny, Cape dated tawny (the product shall be a tawny of a single vintage year), Cape late bottled vintage and Cape vintage. All these classes still have to comply with the same characteristics of the traditional port wine classes, but have to use the names as stated above because of the amendment to legislation of the use of the name port wine only allowed in Portugal (Anon, 2014).

2.3.2   Cultivars and types of must used in port wine making

It is important to understand the role that viticultural practices play in the management of the colour of wine. The compounds that are responsible for wine colour are in large part grape-derived, so it is essential to understand where they are situated in the grape, when they are bio-synthesized, and how production practices

influence their extraction and concentration in the grape must (Kennedy, 2010). Over the years, many studies have been performed to determine the influence of grape variety on the colour of wines, especially red wines (Garcia-Marin *et al.*, 2013). In South Africa, a renaissance of sorts started in the late 1960's with producers in the Cape planting quality Portuguese varietals, especially Tinta Barrocca and Touriga Nacional to improve the quality of the local fortified wines,including port style wines, with these two cultivars still being very popular today. Hence, Tinta Barrocca was one of the varietals included in the present study (Anon, 2015a). However port wine is also produced from local cultivars such as Shiraz, Pinotage and other typical red wine varietals (Tredoux & Silva Feirrera, 2012).

While making a red table wine from grapes like Cabernet Sauvignon, Petite Syrah, Zinfandel or many other red grape varietals, juice can be removed from the fermentation at the appropriate time for fortification to make a port, while the original wine is left to ferment till the required dryness for the table wine is reached (Joyce, 2009). The remaining must will now have an increased skin to juice ratio which will definitely enhance the fruit and tannin concentration of the table wine. This process is similar to bleeding off juice for a rose wine derived from a red grape after very short skin contact with the must. When making port in this way, the juice removed should be added to the brandy (spirit) in terms of the traditional method, but in practice today the spirit is often added to the fermenting wine to stop the fermentation (Anon, 2010).

Another treatment used for red wine production is thermovinification which involves exposing grape berries to heat sources, typically steam or boiling water, to extract pigments. Thermovinification, where the must is typically heated at 70 – 80ºC in contact with the skins, has been shown as beneficial for increasing colour by increasing anthocyanin release. However, it also increases pectin levels and denatures endogenous grape pectinases, thereby hindering clarification and filtration.

Fortification may occur after fermenting the wine dry (as occurs with Sherry), while sweetness is added in the form of concentrated grape juice (Rogerson *et al.*, 2000). Some winemakers that have used thermovinified must in their port wine previously, reported that although it extracts a lot of colour from the grape skins it gives an unstable effect in the final port wine and it results in a lot of sediment in the port wine (Ms. Sian Nieuwhoudt, Winemaker, Swartland Cellar, Malmesbury, 2011, personal communication).

2.3.3   Processing of wine and port wines using pectolytic enzymes

Over the past few decades, increasing interest was shown in the application of enzymes in wine-making. Enzymatic treatments of grape must were found to give better wine clarification, juice yield, colour and aroma extraction as well as wine stability. Enzyme colour extraction has been proposed as alternative technology to be used either with or without thermovinification (Rogerson *et al.*, 2000). This has not been explored widely in terms of port wine, but since it enhances colour extraction there may be merit in exploring it.

The use of pectolytic enzymes was shown to be suitable to improve the extraction of the colour of red wines, aroma compounds and soluble polysaccharides. Hence, industrial pectolytic enzyme preparations have been used widely for many decades since it may be used to increase the grape must yield during pressing, to facilitate the settling of the must and improve clarification and filtration (Espejo & Armada, 2010). The principle enzyme groups used in winemaking are pectinase, cellulase, hemicellulase, oxidoreductase, protease and β-glycosidase (Espejo & Armada, 2010). They are mostly derived from cultures of *Aspergillus niger* which is an organism accepted as GRAS (Generally Recognised As Safe) by EFSA (Canal-Llauberes, 1993). Enzymes in wine and port production are controlled by Commission Regulation EC 606/09, 2009 (Espejo & Armada, 2010). Besides the main pectolytic activities, industrially used pectinase preparations also contain hemicellulytic, cellulytic and other activities, including glycosidic activities (Rogerson *et al.*, 2000).

In terms of port wine, previous studies compared the two most widely used maceration techniques for port wine production, namely open tank treading and static tanks with pumped-over juice. However, at the time not much attention was paid to employing pectolytic enzymes for alternative processing of port grapes, musts and wines (Rogerson *et al.*, 2000). Therefore, these authors investigated the influence of a pectolytic enzyme preparation on juice yield, colour release, colour stability and wine filterability, during the production of several single varietal port wines.

From a colour extraction point of view, pectolytic enzyme applications would facilitate the break-up of the grape cell wall enabling more rapid release of anthocyanins from the anthocyanoplast. It would also assist juice and wine clarification by breaking down the released grape pectins. These authors found that the application of the pectolytic enzyme preparations, during room temperature

maceration for port production, resulted in a more intense wine colour with increases of up to 40% than would normally be achieved without pectolytic application (Rogerson *et al.*, 2000). More recently, it was found that the extraction capacity of enzymes depends on the composition of the enzyme preparations and their activities, among other factors such as temperature or the conditions of the treatment. This then also confirms what the previous authors found, namely that in red grape varieties the colour extraction from skins are increased when the enzyme preparations have high cellulase and hemicellulase activities and also increase the polyphenol content and mainly the anthocyanin content of these wines (Espejo & Armada, 2010).

2.3.4 The role of aldehydes in wine spirit, used in port wine *(in terms of colour and then sensorially)*

As wine ages, whether in tanks or bottled, it is exposed to oxygen and the effects of oxidation. Acetaldehyde is also a product of oxidation. It has been observed that acetaldehyde formation leads to the modification of red colour. One of the major findings in red wine colour chemistry has been the identification of the acetaldehyde adduct of malvin-3-*O*-glucoside in wine (Kennedy, 2010).

In port winemaking, the wine spirits used to stop fermentation usually contains very high levels of different aldehydes, thus the condensation of anthocyanins with proanthocyanidins mediated by these aldehydes would be expected to play an important role in the achievement of a better and more stable port wine colour. The contribution of acetaldehyde to the formation of ethyl-linked pigments with cathechins and procyanidin dimers has been widely reported (Pissarra *et al.*, 2005). The presence of high levels of aldehydes deriving from the sprits, combined with high levels of polyphenols present in some port wines like "Vintage" and "Tawny" ports prepared for prolonged ageing, could play an important role in their colour evolution (Pissarra *et al.*, 2005).

The quality of the wine spirit is determined by its analytical and sensorial characteristics and for each vintage of port winemaking a wide range of wine spirits that is commercially available is chosen for that vintage by the port winemaker. The aroma of the spirit is related to its composition with respect to higher alcohols, esters and aldehydes. The entire process of distillation as well as the quality of the original base wine used, plays a huge role in the aldehyde content present in the resultant

wine spirit. Apart from benzaldehyde, which has a pleasant bitter almond aroma, the other aldehydes studied display unpleasant aromas (green leaves, bitter, unripe fruit) which could impact negatively on the port wine aroma. However, their chemical reaction with the polyphenols could ameliorate this negative effect on port wine aroma (Pissarra *et al.*, 2005).

2.3.5   Blending of port

Blending may occur during maturation or bottling, with wines from the same vintage or wines from a previous vintage matured at various ages. Blending is not always done, nor necessary, in modern port wine production. The type of wine blended and amounts utilized depend upon the required attribute for each port wine style, and also depend on the individual approach of the winery or the port winemaker (Anon, 2010).

Sweetening and colouring wines, which are fortified to 20% alcohol, may be added to ruby and tawny ports, if producing the Portuguese style of port, as opposed to the English style. Vintage ports are blends of the same vintage, which are mixed prior to bottling (Anon, 2010).

2.3.6   Stabilization and bottling

Ruby and tawny ports are clarified with fining agents such as bentonite, to remove colour and tannins before being stabilized for one week at cold temperatures, usually between 0 and –8°C. Filtration occurs subsequently, with diatomaceous earth as a filtration aid, followed by membrane filtration after which the wine is bottled (Anon, 2010).

Limited information is available regarding the white port styles, although similar procedures to those used for the stabilization and clarification of sherry are used. However, South African winemakers produce a number of white ports. These ports are often known as Cape white and are made from a variety of different grape cultivars such as Chenin Blanc, Chardonnay and Colombard. These white ports style wines are made in the same style as red port styles with only the resulting colour and flavour differences due to the different varietals used (Anon, 2015b). Vintage ports destined for bottle maturation are not cold stabilized or filtered, as the sediment is considered essential to the aging of the wine (Anon, 2010).

2.3.7   Port maturation

Young port wines have high levels of grape-derived phenolic material, particularly tannins which produce strong astringent flavours. These strong flavours can be mellowed by wood or bottle maturation for a period of at least three years. The young port is normally stored in wooden vats over a period of time, with the exception of ruby ports. Vintage port styles undergo 2 – 3 years of wood maturation before bottle aging, the latter often for lengthy periods of up to 50 years. Ruby ports are matured for 3 – 5 years in the wood, while tawny ports are matured in wood for 30 years and beyond (Oberholster, 2000). Anthocyanin pyruvates, which result from the reaction between anthocyanins and pyruvic acid, are the major wine pigments after 1 – 2 years of ageing whilst anthocyanins decrease significantly in amount, during the same period of time (Pissarra *et al.*, 2005).

Another important change that occurs during oxidative aging, is that increasing amounts of esters are formed to produce ethyl esters of lactic, malic, succinic and tartaric acids (the types of acids are influenced by the grape variety). These esters contribute minimally to the aroma of the wine, although they have a significant impact upon the wine character, such as enhancing mouth-feel and producing fuller tastes. Polymerization of aldehydes also occurs during maturation in wood, which leads to the nutty and wood flavours encountered in such styles (Oberholster, 2000).

During cask maturation colour is enhanced due to anthocyanin-aldehyde-tannin reactions. Anthocyanins are the main colour pigments of red wine and therefore, also in red port wine. Maceration, fermentation and aging conditions affect the composition of wine anthocyanins and, therefore, also influences the wine colour. A molecular interaction between grape anthocyanins and co-pigments occurs during maturation of red wines stored at relatively low temperatures. The colour changes are mainly due to condensation reactions between anthocyanins and other phenolic compounds, such as tannins (Romero & Bakker, 2000). The newly formed red pigments were first thought to result mainly from reactions between anthocyanins and flavanols, whether mediated by acetaldehyde or not. Nevertheless, reactions between anthocyanins and/or flavanols with other compounds, such as pyruvic acid, vinylphenol, vinylcatechol, α-ketoglutaric acid, acetone, 4-vinylguaiacol and glyoxylic acid, have been demonstrated to yield new families of anthocyanin-derived pigments, namely pyranoanthocyanins with spectroscopic features that may contribute to a more orange-red colour of wine (Pissarra *et al.*, 2005).

2.4 Chemistry and quality aspects of red- and port wine colour

2.4.1 Port winemaking techniques affecting colour and colour extraction

The hue or tint of a wine normally indicates its age or degree of oxidation and for many winemakers wine colour provides the first clue for wine evaluation as it is the first attribute to be seen in the glass. Scientific investigations looked at the influence of colour and wine preference and these studies show that there is a relationship between the quality of wine as judged by a panel and the colour. It is, therefore, also important to understand the relationship between production practices and wine colour in order to manage wine composition effectively. To understand wine colour, it is important to understand the chemical composition of wine from a colour point of view, the reactivity of these colour compounds and how production practices can be managed (Kennedy, 2010).

As with other red wines, the initial anthocyanin pigments of port wine undergo progressive co-pigmentation reactions with the other wine components, notably phenols, during maturation. Hence, after 2 years very little of the original anthocyanins remain. The result of the prolonged red wine maturation is a gradual shift of colour from purple (528 nm) through various hues of red to tawny, given enough time and the right conditions (Buglass, 2011). The colour differences observed in different types of port wine are attributed to changes in the phenolic compounds extracted from the grapes during vinification and maturation (Pinho *et al*., 2012).

Vitisins are the first group of pyroanthocyanins to be identified in red wines, also known as anthocyanin-pyruvic acid adducts. At wine pH 3, the vitisins are characterized by both higher colour intensity as well as lower intensity values than those of the original anthocyanins. Their colours are between red and purple but closer to purple. The visitins are less reactive and are relatively unaffected by SO_2 whereas the original anthocyanins are readily bleached by this widely used preservative. However, on further maturation of the wine, the vitisins gradually undergo polymerization reactions with flavan-3-ols and simple phenols, such as 4-vinylphenol. The products of the latter reaction are known as vinylpyranoanthocyanins, that are formed from vitisin A, with 4 vinylphenol having hues on the bluer side of purple (538 nm) than malvidin-3-glucoside (528 nm) at normal wine pH. The polymerization reactions that lead to the gradual change of

colour from ruby to brown occur more rapidly in the presence of oxygen (Buglass, 2011).

2.4.1.1   Colour extraction in red wine and in port wine

As mentioned, the principle components responsible for the colour of red grapes, red wine and red port styles are the anthocyanins which are thought to originate in anthocyanoplasts located in vacuoles present in the skins of the fruit (Harbourne, 2013). Moreover, for all red grape varieties of commercial significance, the anthocyanins are restricted to the skin tissue. The composition of anthocyanins in the grape varies considerably with the different varieties (Kennedy, 2010). Maceration gives red wine its essential colour and tannic structure, being particularly important for grape processing during the production of red wines intended for ageing (Rogerson *et al.*, 2000).

These pigments are crucial to wine quality, since when assessing wines, colour and appearance play an important role both in terms of consumer appeal, and also as a quality control parameter. In terms of the effect of colour on the sensorial assessment of ports, it was found that appearance attributes dominate the assessment of both aroma and flavour. Without any doubt, the colour of port wine is considered the most important component of quality (Rogerson *et al.*, 2000). During ageing, the initial red-purple colour of young red wines is progressively shifted towards more orange-like hues. The changes occurring in terms of the evolution of red wine colour during ageing, are generally attributed to the transformation of the original grape anthocyanins to new pigments and are the result of oxidation-reduction reactions and complexation with other compounds, such as carbohydrates, proteins, metals or flavanols (Rogerson *et al.*, 2000).

2.4.2   Sulfur dioxide and its influence on red wine and port colour

Sulfur dioxide is frequently added just after crushing and destemming at levels between 50 and 100 mg.L^{-1}, depending on the ripeness of the grape. The addition of sulphites stops the oxygen consumption in the must itself by the inhibition of the enzymes which catalyse the oxidation of phenolic compounds. One of these enzymes, tyrosinase that is normally present in healthy grapes is completely inactivated by a relatively low dose of sulphur dioxide, whilst another enzyme

produced by *Botrytis cinerea* and derived from rotten grapes is less sensitive to sulfur dioxide, hence requiring higher levels of SO_2 (Buglass, 2011).

Sulfur dioxide may also be added as a fundamental additive at various stages during the wine making process for its antimicrobial, antioxidant and reducing activity, but the maximum total concentration is 150 mg.L^{-1}. South African legislative restrictions limit SO_2 in the free form to a maximum of 60 mg.L^{-1} for the free sulfur content and 200 mg.L^{-1} maximum in its bound form (Buglass, 2011).

Sulfur dioxide behaves differently in red grape juice to how it behaves in white juice. In red juice, it becomes weakly bound to the anthocyanins causing them to lose colour. When the wine is tested for free sulfur dioxide by the aspiration method, these weak bonds are usually broken by the addition of acid to the wine. However, a high level of SO_2 should be avoided since it may result in a lower intensity of red colour in the juice (Buglass, 2011). Also SO_2, significantly affects port wine colour, decreasing and increasing as SO_2 increases, respectively (Van Jaarsveld & October, 2015).

2.4.3 Interactions of wine components with lees and its influence on wine colour.
The definition of wine lees given by the EEC regulation No. 337/79 states that "wine lees is the residue that forms at the bottom of receptacles containing wine, after fermentation, during storage or after authorized treatments, as well as the residue obtained following the filtration or centrifugation of this product" (Pérez-Serradilla & Luque de Castro, 2008).

It seems that ageing over lees is a relatively new method for red wine production. The wine ages over the cellular remains of the yeasts that fermented it and therefore becomes enriched in volatile aromatic compounds, and its density is increased through the release of high molecular weight polysaccharides from the cell walls of the dead yeasts (Pérez-Serradilla & Luque de Castro, 2008).

Previously, ageing over lees has been used in the manufacture of white wines fermented in barrels, natural sparkling wines and aged biological wines produced with yeast, for example sherry. Currently the technique is used in the making of red wines since it affords good quality products and better structure, aromatic profile and colour stability (Palomero *et al.*, 2007).

The importance of wine lees when it comes to its interaction with phenolic compounds, is that the lees can absorb phenolic compounds, and release into wine

both phenolic compounds and enzymes that can modify the phenolic fraction in the wine.

However, anthocyanins also interact with the lees and this is important since anthocyanins and their derivatives are the main pigments responsible for wine colour. The adsorption of anthocyanins to wine lees resulting in decreased anthocyanin levels after contact with lees (Pérez-Serradilla & Luque de Castro, 2008) indicates that this method would be undesirable when the objective is to maximize colour extraction, such as is the objective of the present study (Pérez-Serradilla & Luque de Castro, 2008).

2.5 Phenolic extraction during fermentation and red wine making

Red wines are traditionally fermented in open vats with a regular punching down or pumping over of the layer of red grape skins, also known as the cap of grape skins. More recently, red wines are fermented in a wide variety of different tanks and vats, with varying degrees of oxygen exposure and automation in the winemaking process. The manner in which the cap is managed will influence colour extraction, the degree of maceration of skins, ease of temperature control, draining efficiency, duration of maceration and control of oxidation and spoilage (Buglass, 2011).

When maceration takes place, temperature management becomes a critical part of the winemaking process. Temperature control and the sustained contact with the skins will enhance colour extraction from the grapes. The fermentation process is exothermic which means that heat is produced as the yeast metabolizes the sugars and if the heat generation is allowed to continue uncontrolled, a too high maceration temperature will result which will give a burned character to the grapes and to the wine. The ideal fermentation temperature is between 20 and 30ºC in order to ensure optimum colour and tannin extraction. Moreover, in this temperature range, the fruity flavours and aromas are extracted and not volatilized, which is desirable in red wine production. Maceration time is also critical for obtaining wines with good colour intensity and stability. Leaving the red wine juice in contact with the skins for a short maceration time (normally only for a few hours) will lead to a light pink colour which is sufficient for a rose wine, but up to a few days of skins contact is typically required for a "full bodied" red wine (Anon, 2015b).

Furthermore, the physical separation of the skins from the juice due to the buoyancy of the cap and the tendency of the cap to become hot leads to stratification

of temperature in the tank. Therefore, temperature should be monitored at several points in the tank, especially in closed systems. In this regard, an important aspect of conventional red wine fermentation is the surface area and depth of skins in the buoyant cap, which can influence the degree of juice/skin contact and the heat properties of the cap. For example, shallow, wide tanks with a large surface area have a large area of contact between the juice and the cap, which encourages extraction and heat transfer. Tall, narrow tanks, on the other hand, give rise to a much thicker and deeper cap of skins and are also able to dissipate heat more easily from the cap due to relatively large tank wall surface area in contact with the skins. Because of the heat generated and the need for a uniform temperature as explained earlier, the cap needs to be pumped over regularly in these tanks, to maintain the ideal temperature of between 20 - 30 $^{\circ}$C (Buglass, 2011).

In addition, "pumping over" ensures that the cap is submerged and mixed efficiently, in order for most of the colour and moderate quantities of tannin to be extracted from the cap of floating skins which will leach the phenolics, e.g. tannin, out of the skin cells. Increased must-skin contact also leads to the pressed juice from the cap having higher levels of colour pigment and phenolics, while reduced contact results in insufficient colour and tannin and the wine may be thin and lacking in flavour. In the case where such a base wine would be used in port wine making, it would result in a thin and less full-bodied and full-flavoured port wines (Buglass, 2011).

2.6 Flavour and Colour

Phenolic compounds include natural phenols and polyphenols in wine, which include a large group of hundreds of chemical compounds that affect the taste, colour and mouthfeel of wine. These compounds can be divided into major subclasses such as the phenolic acids, stilbenes, flavonoids and tannins. These are further divided in classes within the subclasses such as: flavanols, isoflavonoids, flavonols, flavones and anthocyanins. Phenolic compounds exhibit a wide range of structures.

They are divided into two categories, *viz*. flavonoids and non-flavonoids. The basic phenol group is an aromatic benzene ring with at least one hydroxyl group attached. In grapes, a number of compounds containing this highly reactive phenol group are produced, and these are extracted into the wines (Anon, 2010). Flavonoids would include the anthocyanins and tannins which mainly contribute to the colour and

taste of the wine and the non-flavonoids include the stilbenoids such as resveratrol, and phenolic acids which include caffeic and cinnamic acids (Anon, 2015c).

The concentration of phenolic compounds in grapes depends on the grape cultivar and is influenced by viticultural and environmental factors such as maturity stage, seasonal conditions, production area and fruit yield (Gómez-Alfonso *et al.*, 2007). These phenolic compounds are key components of wine and are directly related to its quality parameters. These compounds not only contribute to the organoleptic characteristics of wine such as bitterness and astringency, but they are also the main cause of colour changes in wine since phenolic compounds are also the major substrate for the consumption of oxygen in wine. For example, the light yellow colour, as well as the undesireable brown colour of white wines are both due to the phenolic content and the oxidation of these phenolics (Pérez-Serradilla & Luque de Castro, 2008).

The fact that phenolic compounds contribute directly or indirectly to colour, astringency, bitterness, aroma and mouthfeel led to more attention being paid to these substances because of their antioxidant properties. Determination of this group of compounds is important since they can characterize variations in wine types and styles and differences in winemaking and maturation processes (Matejícek *et al.*, 2005). Non-flavonoids are important phenols in the grape pulp and in oak wood, while flavonoid phenols do not exist in the pulp, but originate from skins, seeds and stems. However, these flavonoid compounds in wine are extracted mostly from the skins and seeds of grapes during fermentation of the wine must (Anon, 2010). For red grapes, 30 – 40% of the total phenolic material is located in the skins and 60 – 70% in the seeds. It is important to note that, even with prolonged skin contact and maceration, phenolic extraction from skins is less than 50% of the amount available, while close to 60% of the available seed phenolics are extracted during fermentation (Anon, 2010).

2.7 Anthocyanins

Anthocyanins are the largest and most important group of water-soluble pigments in nature. They are responsibe for a large variety of colours and therefore, are also responsible for the red colour in red grapes and wines (Czibulya *et al.*, 2015). Typical

anthocyanins responsible for the red colour in red wine are malvidin-3-*O*-glucoside Anthocyanins are derivatives of phenyl-2-benzopyrillium salts.

Their basic structure comprises the A-ring, which is a phloroglucinol derivative, linked to a pyrilium ring, which is linked to the B-phenolic ring. The anthocyanidin have no sugar groups attached and are not found free in grapes or wines as they are very unstable. The anthocyanins, however, have an O-sugar group at position 3 which confers significantly greater stability on them (Furtado *et al.*, 1993). There are predominant anthocyanin species in red grapes and, therefore, in red wine, the flavylium form and the hemiacetal forms. The flavylium form is the desirable form from a production point of view because it is the observed red form whilst the hemiacetal form is colourless. The other equilibrium forms are present in minor quanities (Kennedy, 2010).

However, anthocyanins are also highly unstable compounds and very susceptible to oxidative degradation through various processes in the winemaking process. Several parameters have been identified to affect copigmentation in red wine, such as temperature, pH, ionic strength and alcohol content (Czibulya *et al.*, 2015). Studies conducted by several authors have found that both forms of the anthocyanin take part in the formation of the copigmentation complex. In recent studies it was found that copigmentation in red wines were most pronounced at a pH of 3.3. The presence of different cations can have a high effect on the development of the sandwich type structure of anthocyanin polyphenol copigments, which are partially responsible for the deep colour of red wine (Czibulya *et al.*, 2015).

There are several forms of anthocyanins that exist in equilibrium at wine pH, with only 25% or less in the red, flavylium form. Sulphur dioxide also forms a colourless bisulphate addition compound with the flavylium ion (Anon, 2010). The extraction and management of anthocyanins in young wines is very important in red wine quality, as evidenced by the positive correlations between red wine colour and overall wine quality (Somers, 1978).

The red colour of young wine comes from the original grape anthocyanins, a class of flavonoids extracted from grape skins. Immediately after pressing the concentration of anthocyanins in red wine is maximal. Progressive decrease of grape anthocyanins during ageing occurs as a result of redox reactions, reactions with flavanols and other small molecules (Anon, 2010). This decrease in the concentration of anthocyanins occurs fairly quickly with an observed reduction in red and an

increase in yellow colour, normally occurring within the first two years. From a production point the major goal of colour management is stabilising the red colour and reducing the rate at which yellowness and eventually browning increases (Kennedy, 2010). To understand the transformation that occur in red wine it is important to understand the reactivity of anthocyanins since it is generally accepted that the red colour in aged red wines is largely due to the presence of anthocyanins that have become modified. As wine colour transitions from grape-based anthocyanin to modified anthocyanin, its appearance changes from blue-red to brick-red (Kennedy, 2010).

Moreover, factors that affect the expression or brightness of colour in a wine by anthocyanins include pH, SO_2, polymerisation and co-pigmentation. Of these factors, co-pigmentation as mentioned before, is also affected by pH, ethanol concentration, temperature and the amount and type of other compounds in the wine that may act as co-pigments, such as flavonoids and other phenols. The amount and type of other phenols in the wine will also affect the amount of polymeric pigments. Proteins and polysaccharides can also become involved in these reactions (Glories *et al.*, 1983). Polymerisation reactions involving anthocyanins are largely influenced by temperature, meaning higher polymerisation and higher concentration of anthocyanins in the grape must exist at lower temperatures (Somers, 1978). With increased oxygen contact (Glories *et al.*, 1983), and as a wine matures and ages, the polymeric pigments become increasingly responsible for red wine colour. The colour changes in red wines due to condensation reactions between anthocyanins and other phenolic compounds naturally occurring in wines, are well documented and exert the biggest influence on the colour changes in red wine during maturation (Romero & Bakker, 1999).

Moreover, anthocyanins are not only a major factor in red wine quality, but are also a widespread source of naturally occurring colorants of foods. Another major polyphenol characteristic include their radical-scavenging capacity, which is involved in antioxidant properties and their ability to interact with proteins.

2.7.1 *Flavonols*

Flavonols are polyphenols belonging to the class of flavonoids. Flavonols are also products of the flavonoid biosynthetic pathway, which also give rise to anthocyanins and tannins in grapes (Mattivi *et al.*, 2006). Flavonol aglycones are characterised by

an unsaturated bond between carbon two (C2) and carbon three (C3), and an oxygen double bond on carbon four (C4). They can exist in grapes as aglycones, but most commonly have a sugar group attached at C3. The most common flavonols in grapes are quercetin and kaempferol, and the most common glycosides are glucosides, galactosides and glucuronides. Hydrolysis can occur during winemaking to increase the proportion of the free aglycone (Anon, 2010).

The flavanols are found mostly in the skins and stems and leaves and form in response to exposure to UV radiation in sunlight. They are easily extracted into wine, but are not very soluble in water and some alcohol has to be present for extraction. They are bitter, very strong co-pigments, have low redox potential, and may become involved in phenol polymerisation reactions. They also have received attention for their possible protective role against coronary heart disease (Anon, 2010).

2.7.2 Carbonyls

Many carbonyls are formed in wine as normal by-products of microbial fermentation and chemical oxidation, or from oak barrels during winemaking and ageing. Their concentration can vary significantly from wine to wine, mainly due to variations in winemaking and storage conditions. In sweet wines, for example the content of carbonyl compounds surpasses that of dry table wines, due to sugar oxidation. Important carbonyl substances in wine include acetaldehyde, pyruvic acid and acetoin (Elias *et al.*, 2008).

Although analysis of these carbonyls in wine is complicated due to their low concentration, volatility, and their ability to form complexes with other wine components, their contribution to the chemistry of wine is a complex subject in which their effects on flavour and colour are the most noticeable. Carbonyls are also known to take part in wine ageing reactions, with potential benefits to the colour of red wines in particular (Elias *et al.*, 2008).

2.8 Sensory evaluation

Sensory evaluation is done on wines and port wines especially where both odour and flavour of the wines play an important role. The human olfactory system is able to distinguish between a large number of chemical compounds at very low concentrations. For the odourant to be effective it must possess certain molecular properties, such as to either partially or completely dissolve in water with sufficiently

high vapour pressure and low polarity, or to be fat soluble and with a molecular weight not greater than 300 Da (Genovese *et al.*, 2009).

A part of this study also involved sensory evaluation of the port style wines at the different time intervals. The sensory evaluation was done by a trained panel of 7 tasters which were familiar with this style of wine. The wines were assessed based on factors such as colour, port style character, oxidative character and overall quality of the wine. The taster had to rate each factor by means of appending a mark on a line scale during tasting. Sensory colour to the tasting panel would signify the best colour to be perceived by the taster who also represents the target market of a typical ruby port style wine, which is namely a young bright red and full-flavoured wine.

The first impression of food odour happens during inhalation, when the odours are released into the headspace through the external nostrils and stimulate the olfactory receptors in the nasal cavity (orthonasal route). However, food aroma is often perceived during eating when the odourants interact with receptors by travelling from the mouth to the nasal cavity (retronasal route). The sensations of orthonasal and retronasal odours differ in the level of perception, even though they involve the same mechanisms. Differences are in fact due to salivation, chewing and temperature which all are factors able to change the sensorial properties of food when it enters the mouth (Genovese *et al.*, 2009).

2.9 Colour measurement in red wines

As mentioned previously, colour is one of the main quality parameters of wines, especially red wines. The colour provides information about defects, the type and the conservation of wines during storage. This also has an important influence on the overall acceptability by consumers (Pérez-Magariňo & González-Sanjosé, 2003). Some more discerning consumers buy only wines with a high degree of clarity. If the wine shows some cloudiness, or contains some particle deposition at the bottom of the bottle, these consumers will not purchase such wines. This type of quality evaluation will also be done by means of sensory evaluation before the wine is bottled and marketed (Pérez-Magariňo & González-Sanjosé, 2003).

To objectively define and evaluate the colour of wines is not easy. In wineries the main objective of doing routine analysis of the colour of wines is to control and evaluate the wine quality, often using the Glories parameters. These chromatic indices are obtained by performing measurements at three wavelengths, namely 420,

520 and 620 nm, which make the indices easy to calculate and to interpret (Pérez-Magarińo & González-Sanjosé, 2003). The absorbance values measured for a typical South African ruby port style wine would be in the ranges of 1.0 – 1.5 (at A420 nm), 1.3 – 1.4 (at A520 nm) and 0.3 – 0.35 (at A620 nm).

The classical method to measure the colour of foods and beverages, including wines, was established by the "Commission Internationale de L'Eclairage" (CIE) based on the determination of tristimulus values. This method is based on a three-dimensional space, called the CIE-xyz space. Values are calculated from transmittance values measured at wavelengths over the whole of the visible spectrum under specific conditions (no light or movement interferences), using a spectrophotometer (Pérez-Magarińo & González-Sanjosé, 2003). Today, the CIELAB method is one of the most widely used and has been applied by several authors to determine the chromatic characteristics of different wines and to study their evolution, including colour stability. These studies confirm that the CIELAB method is the most precise to measure the colour and is the most useful in the differentiation and characterisation of wines based on colour (Pérez-Magarińo & González-Sanjosé, 2003). Therefore it is relevant to note that the optimum, CIELAB values for South African ruby port style wines as follows: between 40 – 51 (L), 42 – 50 (A) and 22 – 27 (B).

2.10 Shelf-life and colour changes in red wine

The shelf-life of wine is a primary concern of the wine industry, also taking into consideration the problems caused by different sources of yeast and bacteria leading to spoilage. However, the shelf-life of a young wine is directly related to its resistance to oxidation, especially since wine typically contains significant levels of natural antioxidants belonging to different families of phenolic compounds (Escudero *et al.*, 2002).

As the wine ages, these different classes of phenolic compounds undergo various oxygen-mediated condensations until they finally precipitate into brown polymeric pigments, which cause undesirable changes in the physical appearance of wine. However, it needs to be noted that wine quality is often lost before the colour changes in wine appear, and is sometimes also accompanied by oxygen-related off-flavours. These off-flavours have not been broadly described and different terms have been used to define the same off-flavour (Escudero *et al.*, 2002).

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CHAPTER 3

PORT WINE COLOUR STABILITY AS A FUNCTION OF WINE SPIRIT, GRAPE MUST TREATMENT, STORAGE TEMPERATURE AND TIME.

3.1 Abstract

The aims of this part of the study were to measure CIELab colour, absorbance (at 420, 520 and 620 nm), aldehyde content, sensory profile, phenolic content and routine wine profile parameters of port wine as a function of wine must treatment (Thermovinification vs Conventional vinification), wine spirit, temperature and storage time with a view to identify the factors that will afford optimum port wine colour stability. It was expected that the thermovinified must would result in a brighter red colour that the conventional wine must, however, the thermovinified must, proved to be unstable after only one month of storage and not suitable for use in port wine making. It was also found that port wine colour stability was affected by the interaction of the design variables in each treatment and less so by individual design variables in this study. Lower storage temperatures of 4°C and 10°C were more favourable to colour stability, whereas storage time gave inconclusive results in terms of colour stability over time and therefore the storage time was extended in the second part of the study. The 96.5% (v.v^{-1}) spirits, used as one of the fortifying spirits in this study, resulted in the second best objective colour and the overall best sensory colour in the port wine samples in this study and was therefore selected for use as the base spirit in the second part of this study.

3.2 Introduction

Port is the generic name for the fortified wines of the Upper River Douro, in Northeastern Portugal, close to the border with Spain. In these vineyards, the granite and fragile schist soils, the hot, dry climate and the numerous grape varieties all contribute to the basic wine. Different vinification and maturation methods are then responsible for producing the range of port wines, from white, through to tawny to red, with the latter two being the most abundant. The climate in South Africa, especially the Western Cape, is commonly described as Mediterranean. i.e. the climate is similar to that of Portugal. Hence this allows South Africa to produce high quality port wines (Buglass, 2011).

The red grape varieties traditionally used for port production include Tinta Amarella, Tinta Barroca, Touriga Francesca and Touriga Nacional, to name only a few. All these varieties contribute different aspects of character to the wine, but the general opinion is that Touriga Francesca and Touriga National generally produce the best wine (Buglass, 2011). In this study, Pinotage was used. In South Africa, Pinotage is also not a very typical port wine cultivar. However, Pinotage grapes were experimented with in the past as a port wine cultivar and the port producers association (CAPPA), requested that this study should be done with a typical South African red wine cultivar.

There are a variety of port types in terms of flavour intensity, aroma and sweetness levels. Young immature ports can be fruity, simple, coarse, spicy and astringent. The sweetness results from the natural grape sugar in the wine, while wine spirits is added to fortify and ensure microbiological stability during aging (Joyce, 2009). Port can be very sweet, sweet, semi-dry or extra dry. How sweet the wine will be in the final product is a choice made during production and depends on when the wine spirits is added to stop fermentation of the wine and the balancing before bottling. The latter entails the addition of more wine spirits or tartaric acid (Joyce, 2009). The wine aroma is dependent on factors such as the grape variety, production region, climatic conditions, winemaking practices and ageing process (Arcari *et al.*, 2013)

When assessing wines, colour and appearance play an important role both in terms of consumer appeal, and also as a quality control parameter. In terms of the effect of colour on the sensorial assessment of ports, it was found that appearance attributes dominate the assessment of both aroma and flavour. Without any doubt, the colour of port wine is considered the most important component of quality (Rogerson *et al.*, 2000). The principle components responsible for the colour of red grapes, red wine and red port styles are the anthocyanins. The skin of grape berries accumulates large amounts of anthocyanins which thus also contribute to the sensory attributes of red wine (Mori *et al.*, 2015). There is also a direct linear correlation between total anthocyanin and total polyphenol content in wine and their contribution to colour density and contribution to wine colour stability (Balcan *et al.*, 2015).

During ageing, the initial red-purple colour of young red wines is progressively shifted towards more orange-like hues. These changes are generally attributed to the

transformation of the original grape anthocyanins to new pigments through reactions such as oxidation-reduction reactions and complexation with other compounds, including carbohydrates, proteins, metals or flavanols (Rogerson *et al.*, 2000). Temperature is an important factor that affects anthocyanin biosynthesis as well as stability during storage. Elevated temperatures decrease the concentration of anthocyanins in grapes on the vine, as well as in red wines during storage (Mori *et al.*, 2015).

Moreover, the colour of red wines is strongly influenced by the phenolic content (including anthocyanins) of the grapes, as well as the oenological practices during the winemaking process and also by storage conditions. The total effect of these various changes are often perceived as undesirable by port consumers. As a result, the Cape Port Producers Association (CAPPA) requested this research to be done to improve the port wine making process in order to identify parameters that will affect colour stability in port wine.

Therefore, the objective of this study is to investigate the influence of practices (port wine making) such as type of spirit used to fortify, storage temperature and also storage time in order to improve optimum stability of port wine colour. The aims of this study are also to measure CIELab colour, absorbance (at 420, 520 and 620 nm), aldehyde content, sensory profile, phenolic content and routine wine profile parameters of port wine as a function of wine must treatment (thermovinification *vs* conventional vinification), wine spirit, temperature and storage time with a view to identify the factors that will afford optimum port wine colour stability. The CIELab space is used as a more accurate measurement of colour in wines, this three-dimensional colour space is a non- linear transformation of the CIE-xy system, defined by L*, a*, b* values which represent different chromatic characteristics. L*, C, and h* parameters are determined from the former and are related to the psychophysical attributes of colour (Pérez-Magariño & Gonzáles-San José, 2002).

3.3 Materials and methods

3.3.1 Port wine making

One part of the port wine were produced from 760 kilograms Pinotage grapes, followed by the normal red wine making process at the Nietvoorbij experimental cellar. These wines were then fortified with 96.5% (v.v^{-1}), 85.1% (v.v^{-1}), and 74% (v.v^{-1}) wine spirits, respectively. The winemaking procedure used to produce fortified

wines, involves stopping the fermentation of the must by adding a good quality wine spirit, when half of the concentration of sugar has been converted to alcohol (Arcari *et al.*, 2013). Another batch of port wine was made from thermovinified must, received as such from an outside cellar. During thermovinification, the grapes were de-stemmed, crushed and then cycled through a heat exchanger to $80^{\circ}C$ for a few hours as described by Spada (2013). The thermovinified must was then fortified with the 74% wine spirits only.

These port wines were then bottled and labeled for storage at different time intervals and different temperatures. Separate storage rooms with the temperature controlled at 4, 10, 25 and $35^{\circ}C$, respectively, were used for this specific purpose.

Samples of these wines were then analysed at four different time intervals namely time zero, 1 month, 4 months and 7 months. Free SO_2 content, total SO_2 content, pH, titratable acidity, CIELab and spectrophotometric absorbance for colour, as well as aldehyde, polyphenol and anthocyanin levels were measured. Sensory analysis was also done on these wines by a trained expert panel consisting of 7 sensory judges at the respective time intervals.

3.3.2 Chemical analysis

Determination of free sulphur dioxide content in port wine. Fifty mL of port sample was pipetted out into a 250 mL Erlenmeyer flask. Ten mL of 9 N H_2SO_4 was added to the sample. One mL starch solution was added and the solution titrated rapidly with 1/64 N KI/KIO_3 solution until the first darkening of the solution to a bluish colour appeared and persisted for at least 1 minute.

Determination of total sulphur dioxide content in port wine. Fifty mL port sample was pipetted into a 250 mL Erleynmeyer flask and 20 mL of 1 N NaOH was added and left for 15 minutes before adding 10 mL of 9 N H_2SO_4 and 1 mL of starch solution, followed by rapid titration as in the previous section.

 Determination of pH and TA (titratable acidity) in wine using an automatic titrator (Mettler). The instrument was calibrated according to the instrument instructions and then set up in advance for the determination of pH and titratable acidity. Twenty five mL of the sample was pipetted out into a 50 mL plastic beaker, the sample was then placed in a tray holder of the instrument and "run" activated on the instrument whereby the instrument then titrated the sample against a 1N NaOH solution and thereafter displayed a pH and TA reading.

Determination of colour (absorbance) by using the Cecil CE 2021 2000 series spectrophotometer. The instrument automatically calibrated after in-putting the command. Thereafter, the wavelengths were entered on the data system/PC at wavelengths of 420, 520 and 620 nm. Quartz cuvettes (4.5 mL, 1 cm x 1 cm) were used to hold the samples in the spectrophotometer. Water samples were used as a reference to zero the instrument.

Determination of anthocyanins in ruby port wine by using a spectrophotometer. The appropriate dilution factor was determined for the samples by diluting with potassium chloride buffer, pH 1.0, until the absorbance of the sample at the "Vis Max" at 700 nm was within the linear range of the spectrophotometer. The final volume of the diluted sample was divided by the initial volume of sample used to obtain the dilution factor. The spectrophotometer was zeroed with distilled water at all the wavelengths used. Two dilutions of the sample were prepared, one with potassium chloride buffer, pH 1.0, and the other with sodium acetate buffer at pH 4.5, diluting each by the previously determined dilution factor. The dilutions were left to equilibrate for 15 min. The absorbance of each dilution was measured at the "Vis Max" at 700 nm, against a blank reference sample (distilled water).

Determination of polyphenols by the use of the Folin Ciocalteau reagent with gallic acid as the standard to measure total polyphenols in a sample. The computer programme was followed to perform the plate counts. Standards were prepared and control wells were included. Twenty five µL of sample was pipetted in triplicate to the sample wells (C1-H12). Hundred and twenty five µL Folin reagent was added to each well using a multichannel pipette. Five minutes waiting time elapsed before adding 100 µL Na_2CO_3 to each well using a multichannel pipette. The plate was left for 2 hours at room temperature before taking a reading.

Determination of CIELab Colour using the Konica Minolta Spectrophotometer CM-5. The instrument was calibrated and then set for specific parameters, such as L* a* b* L* C* and h. The sample was pipetted into a 1 mm plastic cuvette which was placed inside the spectrophotometer to get a reading. All readings were transferred to a computer for retrieval of the data at a later stage for data analysis. However, the assessment of colour is more complex than a numeric expression of value. A more simple approach to compare the colour of samples is an assessment of the colour difference (delta) from a known standard. The difference between two colours can be described by the total distance between those two colours in the three–dimensional

CIELab colour space (ΔE*). The units of ΔE* were designed so that a value of 1 would be equal to the least difference likely to be visually noticeable. It is a commonly used value in quality control programmes as it encompasses differences in hue, chroma and lightness (Anon, 2013). Delta E in this study would be an expression of colour stability of the port wine over a period of time, where low ΔE* values would signify the highest colour stability in these port wine samples.

Determination of acetaldehyde using capillary gas chromatography (GC). The apparatus used was a HP 5890 Series II GC, HP 7673 Injector, HP 3396A integraters HP 6890 GC Series GC, HP 7683 Series auto sampler (FID) detector. A standard sample was prepared (port with pure aldehyde) and stored in a refrigerator at $4^{\circ}C$. Calibration was done with the standard sample. The autosampler carousel was loaded with vials containing the samples after which the method was initiated. The peaks were integrated by the HP 3396A integrator and the data trace recorded for each analysis. Quantification was based on the external standard method.

Sensory analysis by a trained expert panel consisting of 7 sensory judges

The judges had to score each port wine sample at the different time intervals based on different characteristics on a 10 point line scale. These characteristics included, overall quality, port wine character, acetaldehyde character, overall colour and oxidative character.

3.3.3 Statistical analysis

A mixed design, changing one variable at a time was employed, with the design factors one batch of thermovinified must fortified with one spirit only and another batch of normal must, three different fortifying spirits, four storage temperatures, four time intervals at which samples were collected and analyses were conducted. Statistical analysis was performed using SPSS 19.0® for Windows.

Descriptive statistical analyses determined the mean and standard deviation of replicates. Univariate analyses of variance (ANOVA) were performed to ascertain which of the factors and interactions were significant in terms of the model. The highest order of effects that was statistically significant was explored to identify the combinations that performed the best with reference to the most stable colour in port wine. The effect that these design variables has on colour will also be discussed at a later stage. The level of confidence required for significance was selected at $p \leq 0.05$ Least significant difference (LSD) was the *post hoc* test performed to determine the

individual means per design variable (must treatment, fortifying spirits and temperature over time).

3.3.4 Results and Discussion

The influence of different spirits used for fortification (aldehyde contents and alcohol of these spirits differed) on anthocyanins, polyphenols, acetaldehyde and routine wine parameters were reported in Table 3.1. The different types of fortifying spirits had a significant effect ($p < 0.05$) on all parameters, with the p-values for the main effects being < 0.05 or < 0.0001 in most cases (Table 3.1). The 96.5% ($v.v^{-1}$) fortifying spirit resulted in the highest polyphenol, TA, alcohol, residual sugar and glycerol levels and pH and lowest level of total SO_2 ($p < 0.05$) (Table 3.1). Fortification with 85.1% ($v.v^{-1}$) resulted in the highest anthocyanins, total (TSO_2) and free SO_2 (FSO_2) ($p < 0.05$). From previous studies, it is expected that higher glycerol levels in wine would result in higher amounts of acetic acid as well as acetaldehyde. The average levels of glycerol in a dry red wine is 10.49 $g.L^{-1}$ and in a fortified or sweet wine 15.55 $g.L^{-1}$ (Hugh *et al.*, 2017). However, as seen in Table 3.1, the glycerol levels were considerably lower than the typical values for glycerol levels, particularly in fortified sweet wine. Furthermore, the relationship between glycerol levels and acetaldehyde and/or volatile acidity (acetic acid) reported by Hugh *et al.* (2017) was not observed in this study. Moreover, in spite of the reported higher acetaldehyde levels in response to higher glycerol level, glycerol does not affect colour stability, but its function is to contribute to the taste and mouth-feel of wine (Hugh *et al.*, 2017). The 74.0% ($v.v^{-1}$) fortifying spirits resulted in the highest acetaldehyde levels and volatile acidity and the lowest pH, TA, FSO_2, alcohol, residual sugar, glycerol, anthocyanin and polyphenol levels ($p < 0.05$) (Table 3.1). There is a direct relationship between SO_2, pH and TA as well as acetaldehyde levels present in the wine. The percentage of FSO_2 present in the wine is dependent on the pH of the wine, with low pH associated with high percentage FSO_2 that is present as molecular SO_2, therefore the effect (i.e protection against oxidation, microbial spoilage and enzymatic browning) of sulphites is more intense when the pH is low (Anon, 2015). However, in this study the samples with the lowest pH, those fortified with 74.0 % ($v.v^{-1}$), did not have the highest FSO_2 levels (Table 3.1). Fortification with 85.1% ($v.v^{-1}$) resulted in the second lowest pH and the highest FSO_2, whereas fortification with 96.5% ($v.v^{-1}$) resulted in the highest pH and the second lowest FSO_2 (Table 3.1).

Table 3.1 Influence of various port production and storage treatments on routine port wine parameters.

Treatment	Total antho-cyanins	Polyphenols	Total SO_2	Free SO_2	pH	TA	Volatile Acidity
Fortifying Spirits (% EtOH, v.v⁻¹)							
74.0	166.92 ± 70.06[c]	1860.92 ± 107.58[c]	54.33 ± 12.86[b]	1.79 ± 0.88[c]	3.73 ± 0.06[c]	4.57 ± 0.14[b]	0.30 ± 0.03[a]
85.1	210.46 ± 83.26[a]	1919.42 ± 119.42[b]	66.83 ± 15.83[a]	3.00 ± 0.78[a]	3.75 ± 0.07[b]	4.61 ± 0.16[a]	0.25 ± 0.02[b]
96.5	199.08 ± 80.57[b]	1979.58 ± 60.99[a]	45.54 ± 12.33[c]	2.00 ± 0.72[b]	3.79 ± 0.09[a]	4.68 ± 0.25[a]	0.23 ± 0.03[c]
Storage temperature							
4°C	251.11 ± 42.72[a]	1904.28 ± 146.83[a]	64.50 ± 10.5[a]	2.50 ± 1.04[a]	3.76 ± 0.08[a]	4.63 ± 0.25[b]	0.24 ± 0.05[b]
10°C	220.83 ± 55.04[b]	1920.61 ± 127.13[a]	62.06 ± 9.96[b]	2.33 ± 1.03[a]	3.75 ± 0.09[b]	4.74 ± 0.09[a]	0.25 ± 0.04[b]
Ageing period							
1 month	264.33 ± 49.38[a]	1908.71 ± 125.64[a]	63.42 ± 10.57[a]	2.08 ± 0.78[b]	3.67 ± 0.02[c]	4.67 ± 0.23[a]	0.27 ± 0.03[a]
4 months	170.13 ± 66.46[b]	1947.54 ± 82.40[a]	52.63 ± 15.80[b]	1.67 ± 0.87[c]	3.83 ± 0.04[a]	4.66 ± 0.13[a]	0.28 ± 0.03[a]
7 months	142.00 ± 63.03[c]	1903.67 ± 115.13[a]	50.67 ± 18.63[c]	3.04 ± 0.62[a]	3.77 ± 0.03[b]	4.53 ± 0.17[b]	0.24 ± 0.04[b]

Results are reported as mean ± standard deviation (SD) (n = 2). Analyses of variance (ANOVA) tested the significance of the main effects of all independent variables. Least significant difference (LSD) tested whether differences between individual means is significant (p ≤ 0.05). Different superscripts (a-d) per treatment indicate significant differences

Table 3.1(cont).

Main and higher order effects	Total antho-cyanins	Polyphenols	Total SO$_2$	Free SO$_2$	pH	TA	Volatile Acidity
				P-Values			
Alc	<.0001	0.0008	<.0001	0.0002	0.0031	<.0001	<.0001
Temp	<.0001	0.7687	<.0001	0.0003	<.0001	<.0001	0.0002
Alc x Temp	0.0678	0.6259	0.0037	<.0001	0.2598	0.8928	0.4182
Age	<.0001	0.2489	0.0040	<.0001	0.0004	<.0001	<.0001
Alc x Age	0.1613	0.3863	0.0003	<.0001	0.2325	0.4183	0.0704
Temp xAge	<.0001	0.2793	<.0001	<.0001	0.0003	<.0001	0.0009
Alc x Temp x Age	0.5558	0.2991	0.0006	<.0001	0.3285	0.7103	0.7157

Main and higher order effects: p-Values for port wine chemical parameters ($p \leq 0.05$). Alc = The different percentages fortification spirits; Temp = Storage temperature of port wine samples; Age = Storage time of port wine samples.

Table 3.1(cont).

Treatment	Acetaldehyde	Alcohol	Residual sugar	Glycerol
Fortifying Spirits (% EtOH, v.v[-1])				
74.0	96.61 ± 26.88[a]	20.96 ± 0.27[c]	104.62 ± 1.20[c]	8.43 ± 0.28[b]
85.1	56.78 ± 15.96[c]	21.21 ± 0.21[b]	108.24 ± 1.07[b]	8.70 ± 0.32[a]
96.5	69.66 ± 21.11[b]	21.43 ± 0.23[a]	111.06 ± 1.10[a]	8.71 ± 0.34[a]
Storage temperature				
4°C	86.72 ± 27.91[a]	21.26 ± 0.34[a]	108.83 ± 3.10[a]	8.63 ± 0.48[b]
10°C	78.87 ± 26.84[b]	21.23 ± 0.27[a]	108.69 ± 2.61[a]	8.59 ± 0.27[b]
25°C	68.50 ± 26.19[c]	21.17 ± 0.30[b]	107.03 ± 2.80[b]	8.46 ± 0.27[c]
35°C	63.30 ± 24.44[a]	21.14 ± 0.32[c]	107.34 ± 2.92[b]	8.78 ± 0.22a
Ageing period				
1 month	63.30 ± 24.44[d]	21.40 ± 0.18[a]	107.75 ± 2.80[b]	8.36 ± 0.20[b]
7 months	92.79 ± 22.86[a]	21.00 ± 0.27[b]	108.20 ± 3.01[a]	8.86 ± 0.24[a]

Results are reported as mean ± standard deviation (SD) (n = 2). Analyses of variance (ANOVA) tested the significance of the main effects of all independent variables. Least significant difference (LSD) tested whether differences between individual means is significant ($p \leq 0.05$). Different superscripts (a-d) per treatment indicate significant differences.

Table 3.1(cont).

Main and higher order effects	Acetaldehyde	Alcohol	Residual sugar	Glycerol
		p-Values		
Alc	<.0001	<.0001	<.0001	<.0001
Temp	<.0001	0.0115	<.0001	<.0001
Alc x Temp	0.0001	0.2336	0.8663	0.2484
Age	<.0001	<.0001	0.0460	<.0001
Alc x Age	<.0001	0.0994	0.3011	0.0456
Temp xAge	0.8754	0.0889	0.0725	<.0001
Alc x Temp x Age	0.7803	0.0356	0.4262	0.6584

Main and higher order effects: p-Values for port wine chemical parameters ($p \leq 0.05$). Alc = The different percentages fortification spirits; Temp = Storage temperature of port wine samples; Age = Storage time of port wine samples

Hence, these two fortification treatments (85.1 and 96.5% (v.v^{-1})) approximately followed the trend as expected for the relationship between pH and FSO$_2$.

With regards to the relationship between pH and TA, it may be expected that the lowest pH would result in the highest TA, but from Table 3.1 the opposite result is seen. However, the pH of wine measures the concentration of free hydrogen ions (H$^+$) in solution, while TA is a measure of the total amount of hydrogen ions. Hence, since the pH depends on the ability of acids to dissociate, in the presence of buffers, a wine with a high TA may have a higher pH than a wine with a lower TA, resulting in the fact that there is no direct predictable relationship between pH and TA (Anon, 2017). The results of this study hence fall within the norm (Table 3.1).

The alcohol concentration and the temperature also affect the equilibrium between bisulphite ions and molecular SO$_2$, however, acetaldehyde is the most important SO$_2$ binding compound in must and wine (Anon, 2015). Acetaldehyde also reacts directly with anthocyanins and tannins in red wine to form polymeric pigment and modified tannins, which would then have an effect on the taste and colour stability of the wine depending on the amount of acetaldehyde present in the wine (Sheridan & Elias, 2016). Hence, from the results reported in Table 3.1, fortification with 85.1% (v.v^{-1}) spirits resulted in lowest acetaldehyde and highest free and total SO$_2$ and anthocyanins. Fortification with 96.5% (v.v^{-1}) spirits resulted in the second lowest/highest acetaldehyde level and second lowest/highest FSO$_2$. Fortification with 74.0% (v.v^{-1}) spirits resulted in the highest level of acetaldehyde and the lowest FSO$_2$ (Table 3.1). Hence, the expected inverse relationship between acetaldehyde and FSO$_2$ was observed in this study (Table 3.1). Moreover, there is a direct relationship between acetaldehyde levels and the amount of SO$_2$ present in wine, since low acetaldehyde in the wine normally results in high amounts of SO$_2$ and anthocyanins, because lower acetaldehyde levels have less binding capacity for SO$_2$ and anthocyanins. Moreover, this would also ultimately have an effect on wine colour and colour stability (Anon, 2015).

The presence of SO$_2$ in wine, especially at the early winemaking stages, has the ability to bring about a greater extraction of anthocyanins and phenolics. Sulphur dioxide can denature some proteins located in the membranes of the grape skin cells, producing micro leaks and improving the extraction of colour. Moreover, SO$_2$ can bind anthocyanins, making them more soluble and extractable, especially in a water-alcohol medium (Zironi *et al.*, 2015). From Table 3.1 this direct relationship

between SO_2 and anthocyanins can be observed, the higher the amount of FSO_2 the higher the anthocyanin level in the wine. Fortification with 74% (v.v^{-1}) spirits resulted in the lowest FSO_2 as well as the lowest level of anthocyanins and polyphenols, whereas fortification with 85.1% (v.v^{-1}) spirits resulted in the highest FSO_2 and the highest anthocyanins as well as the second highest level of polyphenols (Table 3.1). Moreover, from Table 3.1 the relationship between anthocyanins, acetaldehyde, polyphenols and pH in port wine follows the expected trend. As discussed, the pH and acetaldehyde present in the wine influence the anthocyanins in the wine which is directly responsible for the red colour in wine and the colour stability over time. Moreover, the colour given by anthocyanins is a function of their concentration due to self-aggregation as well as the co-pigmentation with other wine components such as acetaldehyde and the pH of the wine, leading to the formation of anthocyanin-derived pigments with a colour different than red. Furthermore, in previous studies it was reported that anthocyanins react with flavan-3-ols, directly or mediated by aldehydes, contributing to the red/purple colour in young red wines (Pina *et al.*, 2015). However, the impact of the aforementioned relationships (pH, SO_2, acetaldehyde and anthocyanins) on colour will be discussed together with the data on colour and colour stability.

Storage temperature also impacted significantly ($p < 0.05$) on most of the dependent variables of these port wine samples (Table 3.1). Temperature is an important factor that not only affects anthocyanin biosynthesis in plants including grapes, but it was also shown that elevated temperature during storage decreases the concentration of anthocyanins (Mori *et al.*, 2007). Samples stored at 35°C showed a significant decrease in anthocyanins, compared to the samples stored at 4°C which confirms the aforementioned trend ($p < 0.05$) (Table 3.1). Anthocyanins, responsible for the purple red colour of young wines, which is also the ideal colour in a ruby port, participate in reactions with other phenolic compounds to generate other, more chemically stable molecules or pigments. These changes involve oxidation, polymerisation and other complex interactions, leading to changes in wine colour and improved colour stability.

In Table 3.1 the highest values for free and total SO_2 were observed at 4°C and the lowest value at storage temperature of 35°C ($p < 0.05$). In traditional wine making practices there is a direct relationship between sulphur dioxide levels, pH (normal pH levels 3.2 – 3.5) and temperature especially in terms of wine preservation

and colour stability (Anon, 2015). As mentioned, the percentage of FSO_2 in molecular form depends on the pH, meaning FSO_2 being higher when the pH is low. Thus the effects of the sulphites are more intense when the pH is low. Also, the percentage of alcohol in the wine, as well as storage temperature in particular, affect the equilibrium between bisulphite ions and molecular SO_2, with the molar fraction increasing at higher storage temperatures of the wine (Anon, 2015). The amount of FSO_2 was significantly lower for samples stored at 35°C than samples stored at the lower storage temperatures ($p < 0.05$) (Table 3.1)

Samples stored at 35°C resulted in significantly lower TA values ($p < 0.05$). Higher pH and lower TA values would have a negative effect on the overall quality and colour of port wine, indicating that storage at 35°C may result in poor quality wines. Storage at 4°C resulted in higher acetaldehyde levels ($p < 0.05$) in port wine samples than storage at 10°C and 25°C ($p < 0.05$) (Table 3.1). Storage at the highest temperature of 35°C resulted in lower acetaldehyde levels, even though not significantly different ($p > 0.05$) due to the high standard deviation (63.30 ± 24.44) (Table 3.1). High storage temperature also resulted in a significant decrease in residual sugar ($p < 0.05$) (Table 3.1).

Storage time also impacted significantly ($p < 0.05$) on most of the dependent variables of these port wine samples (Table 3.1). Aging is an enological technique usually employed with wines to improve and stabilize wine sensory attributes like red wine colour (Silva Lago-Vanzela *a.*, 2014). Samples stored after 7 months had significantly ($p < 0.05$) lower values for total anthocyanins as well as total SO_2 compared to port wine samples stored at 1 and 4 months (Table 3.1). Samples at 7 months of storage had a significantly higher value for FSO_2 ($p < 0.05$), while a significant decrease in pH and TA ($p < 0.05$) can be observed at 4 and 7 months of storage (Table 3.1). Anthocyanins are commonly associated with the respective flavylium cation that has a red colour. However, *in vitro*, the flavylium cation is stable only at very acidic pH values (pH < 1) that are very rare in natural environments. At moderately acid pH values, such as those found in wines, other species are present in solution, for example *in vitro* the red colour of the flavylium cation at pH 1 turns immediately purple/blue if the pH is increased to 4, but the blue colour fades in minutes (Pina *et al.*, 2015). Also, from previous studies it has been found that during wine ageing the intense red violet colour changes to more orange hues. These colour changes are due to the reaction of anthocyanins with other wine components such as

acetaldehydes, phenolic acids, pyruvic acids, as well as SO_2 including that deriving from yeast metabolism during fermentation in the wine. Those reactions lead to the formation of orange pyranoanthocyanin compounds (Pina *et al.*, 2015). These aforementioned changes were observed in this study as a significant decrease in total anthocyanins, and a significant increase in acetaldehyde at 7 months of storage ($p < 0.05$) (Table 3.1). The complete effect that these design variables of this study had on physical colour will be discussed in the next section.

In Table 3.2 port wine CIELAB (L* a* b*)/CIELCH and other colorimetric descriptives as influenced by treatment are depicted. Fortifying spirits had a significant influence on L*, a*, b*, C and h. The 85.1% (v.v^{-1}) fortifying spirits produced significantly higher colour values than fortification with the other two fortifying spirits in terms of a*, b*, C, while L* and h* values were significantly higher than those for the 96.5% (v.v^{-1}) spirits. From this it can be seen that although fortification with 85.1% (v.v^{-1}) spirits resulted in a lighter colour, it also resulted in a brighter red, i. e. the highest a* as well as h* (or hue) values ($p < 0.05$) (Table 3.2). Although this treatment also showed the highest ΔE* value, the difference was not significant ($p > 0.05$), hence this index of colour stability was not affected by fortifying spirits. This agrees with the results in Table 3.1 where fortification with 85.1% (v.v^{-1}) spirits resulted in lower acetaldehyde levels which lead to less binding activity with the SO_2 and anthocyanins, which could result in lower colour stability (Anon, 2015) even though higher a* and h* values was observed. The presence of acetaldehyde is responsible for many of the beneficial changes that occur in red wine, as a result of oxidation (Sheridan & Elias, 2016). However, since ΔE* did not differ significantly among spirits treatment, these results were contrary to the hypothesis that a higher percentage of alcohol used as fortifying spirits would result in a more stable colour in port wine.

The units of ΔE* are designated such that a value of 1 would be equal to the least difference likely to be visually noticeable. However, perceptual non-uniformities in the underlying CIELAB colour space has been redefined more recently, proposing ΔE* to have a Just Noticeable Difference (JDN) of 2.3 (Anon, 2013). Therefore, although the ΔE* values of the samples stored at 4, 10 and 20°C are very low and closer to 1 showing not much change in colour over time, it is clearly visible from the results in Table 3.2 that storage at 35°C had a much higher ΔE* value (4.38) when compared to the other storage temperatures, where the ΔE* value ranged from 1.11–

Table 3.2 Port wine CIELab (L* a* b*) / CIELCH and other colorimetric descriptives as influenced by treatment

Treatment	Colour descripitves					
	L*	a*	b*	C	h*	ΔE*
Fortifying Spirits (% EtOH,v.v⁻¹)						
74.0	1.12 ± 0.40[ab]	5.65 ± 2.52[b]	1.57 ± 0.67[b]	5.86 ± 2.61[b]	12.93 ± 3.46[a]	2.18 ± 1.64[a]
85.1	1.21 ± 0.54[a]	7.36 ± 3.02[a]	1.85 ± 0.78[a]	7.62 ± 3.14[a]	14.13 ± 1.41[a]	2.47 ± 1.97[a]
96.5	1.04 ± 0.35[b]	5.96 ± 2.39[b]	1.41 ± 0.77[b]	6.16 ± 2.46[b]	12.46 ± 3.09[b]	2.23 ± 1.26[a]
4	1.27 ± 0.35[a]	7.33 ± 2.02[a]	1.91 ± 0.60[a]	7.59 ± 2.08[a]	12.79 ± 3.68[a]	1.81 ± 1.02[b]
10	1.13 ± 0.20[a]	5.89 ± 1.07[b]	1.53 ± 0.36[b]	6.04 ± 1.18[b]	13.81 ± 2.39[a]	1.11 ± 0.80[c]
25	0.87 ± 0.24[b]	5.15 ± 1.56[c]	1.19 ± 0.52[c]	5.35 ± 1.58[c]	12.82 ± 2.58[a]	1.87 ± 1.22[b]
35	1.25 ± 0.70[a]	6.93 ± 4.53[a]	1.88 ± 1.30[a]	7.21 ± 4.68[a]	13.29 ± 2.75[a]	4.38 ± 1.13[a]
Ageing period						
1	0.89 ± 0.32[b]	4.53 ± 1.51[b]	1.06 ± 0.44[b]	4.69 ± 1.54[b]	11.23 ± 2.80[b]	2.52 ± 1.16[a]
7	1.36 ± 0.42[a]	8.12 ± 2.45[a]	2.20 ± 0.69[a]	8.41 ± 2.55[a]	15.13 ± 0.80[a]	2.07 ± 1.97[b]

Results are reported as mean ± standard deviation (SD) (n = 2). Analyses of variance (ANOVA) tested the significance of the main effects of all independent variables. Least significant difference (LSD) tested whether differences between individual means is significant ($p \leq 0.05$). Different superscripts (a-d) per treatment indicate significant differences.

Table 3.2 (Cont.)

Main and higher order effects	L*	a*	b*	C	h*	ΔE*
			P-Values			
Alc	0.0813	<.0001	0.0019	<.0001	0.0569	0.2181
Temp	0.0002	<.0001	<.0001	<.0001	0.5295	<.0001
Alc x Temp	0.1298	0.3201	0.6208	0.3398	0.7044	0.0017
Age	<.0001	<.0001	<.0001	<.0001	<.0001	0.0044
Alc x Age	0.0138	0.0265	0.0391	0.0269	0.0141	<.0001
Temp xAge	<.0001	<.0001	<.0001	<.0001	0.7588	<.0001
Alc x Temp x Age	0.1642	0.0045	0.0655	0.0032	0.4909	0.0040

Main and higher order effects: p-values for port wine chemical parameters ($p \leq 0.05$). Alc = The different percentages fortification spirits; Temp = Storage temperature of port wine samples; Age = Storage time of port wine samples.

1.87, confirming the hypotheses that the highest storage temperature would effect the lowest colour stability. The various storage temperatures had a significant effect on L* ($p < 0.0002$ (main effect)), whereas storage at 4°C had the highest values for a*, b* and C. However the actual L* values ranged from 0.87–1.27 indicating that the samples were all very dark as the values of the port wine samples stored at the different temperatures were very close to 1 which means it was very dark, close to black on the actual L* scale. The port wine samples stored at 4°C and 35°C had both significantly higher values for a* ($p < 0.05$), even though there is such a large difference in storage temperature. However, this was due to the high value for standard deviation among the 35°C samples which means that the lowest a* value was recorded for 35°C, namely 2.4 (6.93 minus 4.53) (Table 3.2), as well as the lowest colour stability, as mentioned. The best colour indices were observed at storage temperature 10°C with the samples stored at 4°C having the second lowest ΔE* value (Table 3.2).

A highly significant difference was seen for all the dependent variables (L*, a*, b*, C, h* and ΔE*) between storage at 1 and 7 months of these wine samples ($p < 0.0001$, main effect) (Table 3.2). With one exception, all the dependent variables showed significantly higher values at 7 months of storage, compared to storage after 1 month, with higher values in redness (a*), b* h* and chroma (C) observed (Table 3.2). A noticeable exception is, ΔE* which was lower for the samples stored at 7 months. Therefore, the colour change after 7 months of storage was less than the JND for ΔE*. Hence, while it was hypothesised, that the longer the ruby port samples are stored, the less acceptable the colour will be for a typical ruby port wine over time, in this study the hypotheses was refuted.

The values of the Glories values in Table 3.3, colour intensity (CI), redness, yellowness and blueness were calculated from the absorbance values measured at 420, 520 and 620 nm. As depicted in Table 3.3, 74. 0% (v.v^{-1}) fortifying spirits had a higher value for 420 nm, which is a browning indicator, colour intensity, blueness and a significantly lower value for redness. Fortification with 85.1% (v.v^{-1}) spirits had a significantly lower value at 420 nm and a significantly higher value for redness compared to the other two fortifying spirits ($p < 0.05$) (Table 3.3). These results correspond with the results as seen in Table 3.2, where the CIELab colour parameters a* and h* indicated superior redness for 85.1% fortifying spirits compared to the other two fortifying spirits.

Storage of samples at 4°C had a significantly lower (p < 0.05) value for 420 nm and a significantly higher (p < 0.05) value for redness compared to samples stored at 35°C. Samples stored at 35°C had a significantly higher value at 420 nm (p < 0.05), the second lowest value for colour intensity (CI), a significantly lower value for redness and the second lowest value for yellowness (p > 0.05) (Table 3.3). These are all indicators of an undesirable, unstable colour for ruby port wine and the storage temperatures also corresponds with the results in Table 3.2.

The best results were seen at storage temperature 10°C and secondly 4°C for CIELab parameters and in the case of storage temperature in Table 3.3 the more stable colour is observed at 4°C and secondly 10°C, confirming that 4°C and 10°C were the most favourable storage temperatures for ruby port wine samples.

After 1 month of storage the port wine samples showed a significantly lower value at 420 nm (p < 0.05) and yellowness, and a significantly higher value for redness (p < 0.05) (Table 3.3). Storage of port wine samples at 7 months, resulted in a significantly higher value at 420 nm as well as CI and a significantly lower value for redness (p < 0.05) (Table 3.3). These results are contrary to the results observed in Table 3.2, where storage at 7 months resulted in a more stable colour.

The sensory descriptors (colour, port character, oxidative character and overall quality) as influenced by treatment on port wine samples are depicted in Table 3.4. The 74.0% (v.v^{-1}) fortifying spirits resulted in the best port wine character and overall quality, although it was not significantly different to fortification with the other two fortifying spirits (p > 0.05) (Table 3.4). Fortification with 85.1% (v.v^{-1}) spirits resulted in the lowest port colour values, whereas fortification with 96.5% (v.v^{-1}) spirits, resulted in the highest colour score (p > 0.05) (Table 3.4). However, fortification with 96.5% (v.v^{-1}) spirits, also resulted in the highest oxidative character and second highest port character as well as second highest overall quality compared to fortification with the other two spirits, although all not significant (p > 0.05) (Table 3.4). From the results as seen in Table 3.2, fortification with 96.5% (v.v^{-1}) spirits, resulted in the second lowest value for ΔE* indicating the least change in colour with this fortification spirits and from Table 3.4 it is evident that fortification with 96.5% (v.v^{-1}) spirits, also resulted in the best sensory colour. This confirmed the hypothesis that the 96.5% (v.v^{-1}) spirits, would be the best choice for fortifying ruby port wine,

Table 3.3 Port wine Glories and other colorimetric descriptives as influenced by treatment

Treatment	Colour descriptives				
	A420nm	CI	Redness	Blueness	Yellowness
Fortifying Spirits (% EtOH,v.v⁻¹)					
74.0	0.42 ± 0.05[a]	1.36 ± 0.17[a]	52.43 ± 2.73[c]	16.59 ± 1.52[a]	30.98 ± 2.03[c]
85.1	0.39 ± 0.04[b]	1.23 ± 0.12[c]	53.62 ± 2.78[a]	14.58 ± 1.07[c]	31.80 ± 2.37[a]
96.5	0.41 ± 0.04[a]	1.31 ± 0.13[b]	53.00 ± 2.78[b]	15.56 ± 1.30[b]	31.44 ± 2.11[b]
Storage temperature					
4ºC	0.38 ± 0.05[c]	1.23 ± 0.14[b]	55.16 ± 1.15[a]	14.39 ± 1.09[d]	30.45 ± 0.39[c]
10ºC	0.41 ± 0.05[b]	1.34 ± 0.16[a]	54.23 ± 1.48[b]	15.44 ± 1.50[c]	30.32 ± 0.41[c]
25ºC	0.42 ± 0.04[a]	1.37 ± 0.11[a]	52.97 ± 1.87[c]	16.37 ± 1.67[a]	30.66 ±0.74[b]
35ºC	0.43 ± 0.04[a]	1.25 ± 0.14[b]	49.70 ± 2.62[d]	16.09 ± 1.07[b]	34.21 ± 2.76[a]
Ageing period					
1 month	0.37 ± 0.04[c]	1.23 ± 0.14[c]	55.12 ± 1.53[a]	14.47 ± 1.39[c]	30.41 ± 0.55[c]
4 months	0.39 ± 0.02[b]	1.26 ± 0.10[b]	52.76 ± 2.19[b]	15.97 ± 1.26[b]	31.27 ± 2.98[b]

| 7 months | 0.46 ± 0.03[a] | 1.41 ± 0.13[a] | 51.17 ± 2.87[c] | 16.28 ± 1.32[a] | 32.54 ± 2.98[a] |

Results are reported as mean ± standard deviation (SD) (n = 2). Analyses of variance (ANOVA) tested the significance of the main effects of all independent variables. Least significant difference (LSD) tested whether differences between individual means is significant ($p \leq 0.05$). Different superscripts (a-d) per treatment indicate significant differences

Main and higher order effects	A420nm	CI	Redness	Blueness	Yellowness
			P-Values		
Alc	<.0001	<.0001	<.0001	<.0001	<.0001
Temp	<.0001	<.0001	<.0001	<.0001	<.0001
Alc x Temp	0.5972	0.4489	0.1261	0.0011	0.0143
Age	<.0001	<.0001	<.0001	<.0001	<.0001
Alc x Age	<.0001	<.0001	<.0001	<.0001	<.0001
Temp xAge	<.0001	<.0001	<.0001	<.0001	<.0001
Alc x Temp x Age	0.8003	0.7052	0.1165	0.2538	0.0209

Main and higher order effects: p-Values for port wine chemical parameters ($p \leq 0.05$). Alc = The different percentages fortification spirits; Temp = Storage temperature of port wine samples; Age = Storage time of port wine samples

Table 3.4 Port wine sensory descriptors as influenced by treatment.

Treatment	Sensory descriptive (%)			
Fortifying Spirits (% EtOH,v.v^{-1})	**Colour**	**Port character**	**Oxidative character**	**Overall quality**
74.0	77.72 ± 5.64[a]	54.86 ± 8.55[a]	33.92 ± 10.12[b]	55.33 ± 8.24[a]
85.1	75.17 ± 5.29[b]	53.43 ± 6.22[a]	30.68 ± 8.72[c]	54.02 ± 5.04[a]
96.5	78.14 ± 5.61[a]	54.42 ± 8.83[a]	37.22 ± 10.90[a]	54.31 ± 6.82[a]
Storage temperature				
4	76.07 ± 6.45[bc]	55.58 ± 7.58[a]	34.60 ± 9.81[a]	55.42 ± 6.25[a]
10	77.84 ± 3.79[ab]	55.33 ± 7.17[a]	33.43 ± 10.06[a]	55.24 ± 5.17[a]
25	78.42 ± 4.21[a]	56.29 ± 7.14[a]	35.24 ± 10.87[a]	56.77 ± 5.97[a]
35	75.70 ± 7.17[c]	49.75 ± 8.33[b]	32.48 ± 10.59[a]	50.79 ± 8.22[b]
Ageing period				
1	80.89 ± 3.17[a]	62.01 ± 6.30[a]	23.92 ± 5.75[c]	60.88 ± 4.27[a]
4	78.89 ± 3.01[b]	51.93 ± 5.63[b]	34.03 ± 5.88[b]	52.08 ± 5.35[b]
7	71.24 ± 4.84[c]	48.77 ± 4.42[c]	43.86 ± 6.67[a]	50.71 ± 5.52[b]

and therefore informed the selection of this spirits for fortification in the second part of this study. The lowest sensory colour, port wine character, oxidative character as well as overall quality is observed for samples stored at 35°C (Table 3.4). The second lowest overall quality is observed at 4°C and the best observed colour, port character and overall quality is observed at 25°C of storage, although not significant ($p > 0.05$) (Table 3.4). Ports should therefore, not be kept at temperatures higher than 25°C.

After 1 month of storage for the port wine samples resulted in significantly higher sensory colour, port wines character and overall quality and the lowest oxidative character ($p < 0.05$) (table 3.4). Whereas, after 7 months of storage, the port wine samples had a significantly lower sensory colour, port character as well as overall quality ($p > 0.05$) (Table 3.4). These results correspond with the results depicted in Table 3.3 for storage time.

Therefore, as hypothesised, fortification with 96.5 % spirits, as well as lower storage temperature and a shorter storage time resulted in a more stable ruby port colour. Hence these parameters were used to guide the selection of the parameters implemented as design factors in the second part of this port wine study. These design factors were namely, fortification with 96.5 6 % fortification spirits (spiked with commercial acetaldehyde), storage temperature of only 4 and 10°C and a longer storage time (up to 12 months) to extend the storage time since the typical shelf-life of a ruby port wine is plus minus 3 years (Tredoux & Silva Ferrreira, 2012).

Figure 3.1A depicted the influence of storage time on the port samples. Very clear groupings were observed at the three different storage times, with each one of the respective storage times grouping together. Figure 3.1B depicts ports fortified with different spirits (74.0, 85.1 and 96.5% (v.v⁻¹)) and stored at the four different storage temperatures (4. 10, 25 and 35°C). From this Figure groupings are visible, however, groupings were observed for the fortifying spirits and less so for temperature or for the combinations of treatments (fortification spirits and storage temperature). Figure 3.1C depicts the effect of different fortification spirits at the different storage times. More overlapping were observed for the different fortification spirits but a trend is observed with the different storage times grouping together at 1, 4 and 7 months, respectively. Figure 3.1D depicts the effect of the combination of storage time and temperature. Clear groupings were observed for ports stored at high temperatures for longer periods of time. These results correspond with the results in Table 3.4 where the best sensory colour was observed at the lower storage temperatures of 4 and 10°C, as well as storage time of 1 and 4 months.
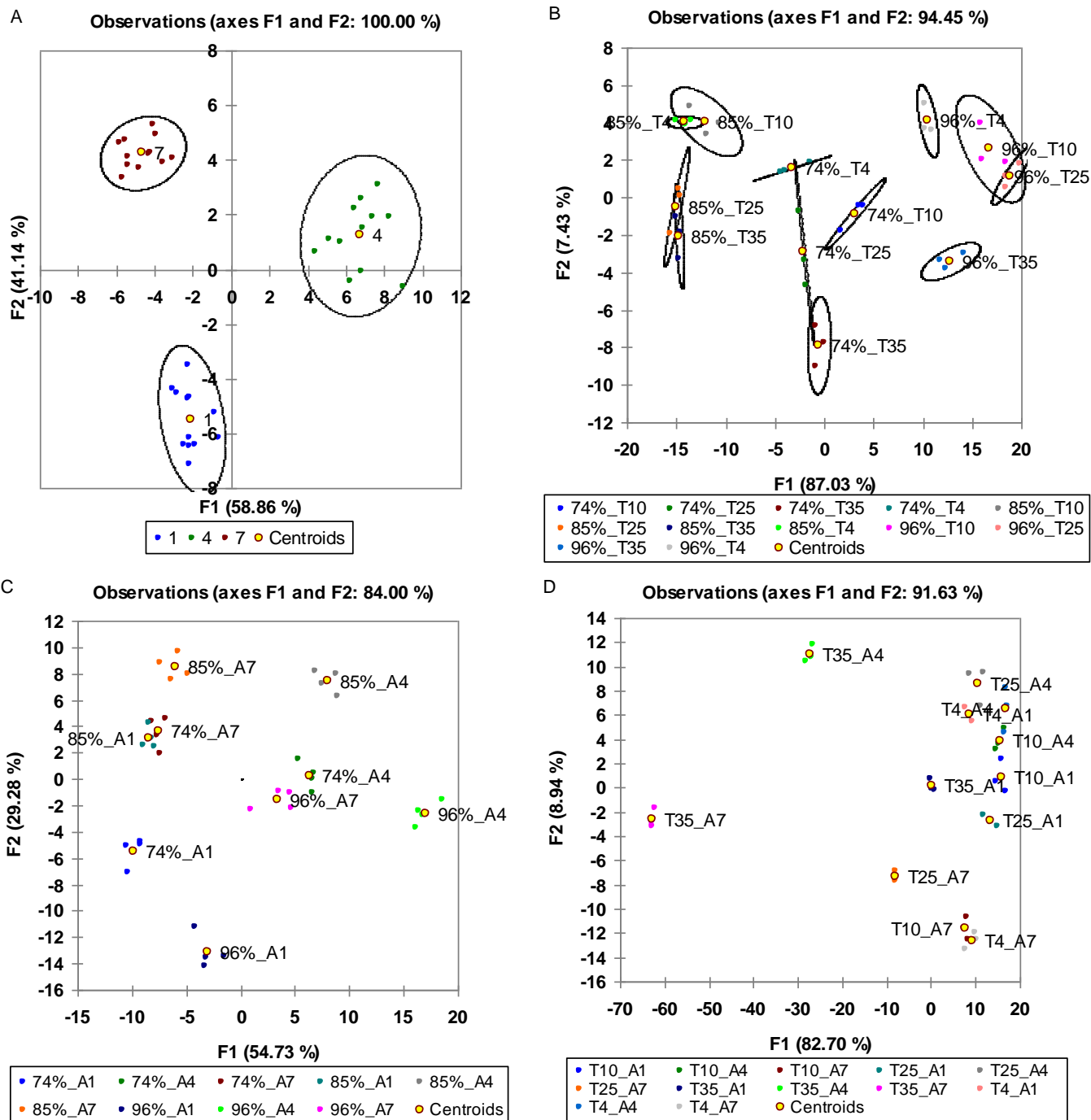
Figure 3.1 Plot of discriminant scores of the first two discriminant factors (F1, F2) of port wines fortified with different spirit types, stored at different temperatures and times. Treatment classes were spirit type, storage temperature and storage time. Variables: Colour, port character, oxidative character, overall quality, A_{420nm}, A_{520nm}, A_{620nm}, total anthocyanins, total polyphenols, pH, total acid (TA), free SO_2, total SO_2. Fig. 3.1A: 1, 4, 7 signifies storage age in months; Fig. 3.1B: 74_T10 signifies fortified with 74.0% (v.v^{-1}) spirits and stored at 10ºC; Fig. 3.1C: 74.0% _A1 signifies fortified with 74.0% (v.v^{-1}) spirits and aged for 1 month. Fig. 3.1D: T10_A1 signifies storage at 10ºC and aged for 1 month.

3.4 Conclusions

The thermovinified port wine samples became unstable after 1 month of storage and could not be used to read colour values in this study. After some careful consideration and correspondence with the wine industry and winemakers that are familiar with the use of thermovinified must, it was decided to exclude these samples from the study. The head winemaker of Swartland Winery in Malmesbury (Mr. Stian Van Zyl, personal communication), confirmed that port wine produced from thermovinified must becomes unstable after a while and leaves a residue in the port wine samples, resulting in port wine of unacceptable quality.

Port wine colour stability was affected by the interaction of the design variables in each treatment and less so by individual design variables in this study. However, to make an informed choice for the design of the second part of this study, the effects of the main variables on port wine parameters, CIELab and Glories colour parameters as well as the sensory descriptors of the port wine samples, were considered. Therefore, as mentioned before, 96.5% (v.v^{-1}) spirits resulted in the second best objective colour and the overall best sensory colour in the port wine samples in this study and was therefore selected for use as the base spirit in the second part of this study. Storage at 4 and 10°C resulted in the highest values for a*, redness, acetaldehyde as well as the best sensory colour, therefore these parameters (4°C and 10°C) were used as design variables for the second part of the study (Chapter 4). Storage after 7 months resulted in higher values for a*(redness) and lower values for ΔE*(stability) (Table 3.2) whereas the results from Table 3.3 were the opposite after storage of 7 months and therefore the storage time for the next part of the study was extended to 12 months. In previous studies, also determining colour stability in red wine, it was also found that ageing decreased the colour intensity while the hue is increased (Babincev *et al.*, 2016).

3.5   References

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CHAPTER 4

PORT WINE COLOUR STABILITY AS A FUNCTION OF ALDEHYDE LEVEL, GRAPE CULTIVAR, STORAGE TEMPERATURE AND TIME INTERVAL.

4.1 Abstract

The aim of this part of the study was to measure CIELab colour, absorbance (at 420, 520 and 620 nm), aldehyde content, sensory profile, phenolic content and routine wine profile parameters of port wine as a function of grape cultivar, wine must treatment (pectolytic enzymes), different aldehyde levels, temperature and storage time with a view to identify the factors that will afford the best port wine colour stability. In terms of cultivar, Tinta Barrocca was expected to give the better colour stability, however, Tinta Barrocca with enzyme treatment showed less colour stability, indicating that a single factor such as type of cultivar does not clearly give higher colour stability. Acetaldehyde levels also plays a major role in terms of colour stability and the colour intensity of port wine, especially in a ruby port wine. In this study the spirits spiked with intermediate and high levels of commercial aldehyde used to fortify the port wine samples with, gave better colour stability and higher colour intensity and redness. Shorter storage times as well as low storage temperature at which these samples were stored also afforded more stability in this study.

4.2   Introduction

Colour is one of the principal parameters of the quality of wines, since this is the first characteristic to be perceived in the glass. The colour also gives information about possible defects, body, age and evolution of the wine during storage. During storage, whether in a barrel or steel tanks the wine is exposed to oxygen which could also dramatically alter the colour and texture of the wine (McRae *et al*., 2015). Therefore, colour has an important influence on the overall acceptability by the consumer (Pérez-Magariño & González-San José, 2002). However, the objective definition and evaluation of the colour is not always easy. The colour of wine has been measured to control and evaluate the wine quality by applying the CIELab space and Glories parameters. Glories parameters (absorbance 420, 520 and 620 nm) are measured in

routine wine laboratories but the information provided is very limited if compared with colour that the eye perceives, which is over a much wider visible spectrum of between 380 – 770 nm (Pérez-Magariño & González-San José, 2002). Together with instrumental measurement of colour, another important measure of colour from a consumer perspective is to measure sensory colour. Sensory analysis is widely applied in wine research to describe the effect of factors such as grape variety or processing on properties of wine, and to study the relationship between chemical and sensory characteristics (Monteleone, 2012).

The most classical method to measure the colour in foodstuffs and beverages, including wines, was established by the "Commission Internationale de L'Eclairage" (CIE) based on the determination of tristimulus values, which is based on a three-dimensional space, called the CIE-xy^2 space. These values are calculated from transmittance values measured at wavelengths over the whole visible spectrum under specific conditions, using a spectrophotometer. In 1986 this same body has adopted a new colour space called the CIELAB space, as a more representative measurement of colour (Pérez-Magariño & González-San José, 2003). This three-dimensional colour space is a non-linear transformation of the CIE XYZ tristimulus values and each colour is defined by its coordinates on three axes L*, a* and b*. C (Metric chroma) and h (Hue angle) parameters are calculated from the former. L*, C and h are cylindrical coordinates, which represents the psychophysical correlation of the basic attributes of colour. L* is explained as a measure of lightness, from completely opaque (0) to completely transparent (100); a*, a measure of redness (+a*) or greenness (-a*); +b* a measure of yellowness or –b* a measure of blueness; C, the chroma or saturation; and h, the hue angle (Pérez-Magariño & Gozález-San José, 2002).

 Both chroma and hue relate to human colour perception. When we refer to a named colour we are usually referring to its hue (Almela *et al.*, 2013). Hue angle (h) is defined as starting at the +*a* axis and is expressed in degrees; 0º would be +a* (red), 90º would be +b* (yellow), 180º would be –a* (green), and 270º would be –b* (blue) (Almela *et al.*, 2013).

However, the assessment of colour is more complex than a numeric expression of value. A more simple approach to compare the colour of samples is an assessment of the colour difference (delta) from a known standard. The difference between two colours can be described by the total distance between those two colours in the three–dimensional CIELab colour space (ΔE*). The units of ΔE* were

designed so that a value of 1 would be equal to the least difference likely to be visually noticeable. It is a commonly used value in quality control programmes as it encompasses differences in hue, chroma and lightness (Anon, 2013). Delta E in this study would be an expression of colour stability of the port wine over a period of time, where low ΔE^* values would signify the highest colour stability in these port wine samples.

The other colour measurements used in the study are the Glories values and they also relate to the CIELab colour space. The Glories values are based on measuring the absorbance of the sample at the wavelengths of 420, 520 and 620 nm. The 420:520 hue ratio is based on the absorbance at two wavelengths, A_{420} indicating yellow and A_{520} indicating red. In context, the CIE hue angle is based on the entire wine spectrum which means the entire range of the hue spectrum from the high to the low end of the colour spectrum. As a result, the CIE hue angle is more sensitive to subtle changes in wine chemistry and is a more accurate representation of colour present. However, the 420:520 ratio is more appropriate for red wines, whereas the CIE hue angle can describe any colour (Anon, 2013). The chromatic Glories parameters are colour intensity (CI), tonality (To), percentage of yellow (%Ye), percentage of red (%red) and percentage of blue (%blue). They are widely known and are used more frequently by oenologists than other colour notation systems (Pérez-Magariño & González-San José, 2006). In this study the Glories parameters and sensory evaluation of the colour was used, in addition to CIELab.

In terms of colour, red ports vary from deep purple to deep gold, and can be designated as ruby or tawny. Ruby port is the most extensively produced and widely available style of port wine and for many people serves as an introduction to fortified wines in general. After fermentation it is normally stored in stainless steel tanks to prevent oxidative aging and also to preserve its rich claret colour (Pinho et al., 2012). The description "Ruby" was neither officially nor legally regulated until the 1960's. However, the port trade reached consensus on the ruby port style. The red berry fruit aromas which characterise this style match the bright red colour which gave rise to the name (Anon, 2013). Wines chosen to produce a ruby port usually possess a deep colour, fruity aromas, full-bodied wine character, and rich tannins in the mouth (Moreira & Guedes de Pinho, 2011). A common denominator in the production of most fortified wines is that they are produced under oxidative conditions. Therefore, oxidation reactions are some of the most important processes for determining the

typical colour and flavour profile of a fortified wine. These attributes are most often identified during sensory analysis (Tredoux, 2012).

Generally, ruby blends are composed of wines from several vintages aged for up to three years and bottled young. Ruby port wines are wines in which the winemaker seeks to restrain changes in its deep red colour, while maintaining the fruitiness and strength of a young wine. It is the simplest version of port and can be one of the most satisfying styles of port wine. The colour or rather the loss of colour stability and paucity of knowledge on how to improve colour stability has a financial implication for the port wine makers and huge economic implication for the port wine industry (Moreira & Guedes de Pinho, 2011). In South Africa, for the same reasons as stated above, the fact that ruby port wine should retain its bright red colour regardless of its age, even though many factors have an effect on the colour and colour stability and the consumer acceptability of this port wine style, the Cape Port Producers Association (CAPPA) requested research to be done regarding the colour stability of port wines, especially ruby port wine.

Fungal enzymes, usually from *Aspergillus niger,* are used in winemaking because of their pectinolytic activities while the addition of enzymes may also contribute to aroma and colour enhancement (Dziadas & Jeleń, 2016). This research included the investigation of the effects of grape cultivar, addition of pectolytic enzyme, the type of alcohol used to fortify the base wine, as well as storage time and temperature on port colour stability and quality. There is evidence that the impact of viti-vinicultural practices and terroir on chemical and sensorial differences observed in wines is more pronounced than that of cultivar. However, since cultivar was a design factor in this study, inferences will be made concerning the effect of cultivar on colour and colour stability. Although there is some information about the influence of these factors available, not much research has been done on the colour stability of port and more specifically that of ruby port, and how these aforementioned factors affect the colour stability (Moreira & Guedes de Pinho, 2011). Therefore, the aim of this study was to measure CIELab colour, absorbance (at 420, 520 and 620 nm), aldehyde content, sensory profile, phenolic content and routine wine profile parameters of port wine as a function of grape cultivar, wine must treatment (pectolytic enzymes), different aldehyde levels, temperature and storage time with a view to identify the factors that will impact the most on port wine colour stability.

4.3 Materials and methods

4.3.1 Port wine making

The port wine samples were made from one batch of 700 kg Pinotage grapes and another batch of 700 kg Tinta Barroca grapes, both from the Stellenbosch district. Each batch of grapes was divided into two sub-batches, with one sub-batch receiving no enzyme, while the other was treated with an enzyme preparation after crushing the grapes. The enzyme preparation (Rapidase Vino Super R 3500D in liquid form (Food Specialities Beverages, France)) was obtained by diluting 20 mL of the concentrated enzyme in water, and was then added to the grape must and mixed thoroughly.

The grape must was then fermented on the skins in blue plastic (HDPE) drums in a cellar room at 25°C until the must reached a balling of 11°B after approximately three days. To facilitate colour extraction from the skins, the must and skins were pressed down every four hours. Each sub-batch of both Tinta Barrocca and Pinotage musts were then divided into three parts, each of which was then fortified with a different spirit, followed by basket pressing. The different spirit types were obtained by adding three different levels of commercial acetaldehyde, namely 0, 50 mg.L^{-1} and 450 mg.L^{-1}, respectively, to one selected base wine spirit type (i.e. wine spirits) with an alcohol percentage of 95% alcohol by volume. The aldehyde levels selected were based on the results of the first part of the study with the specific objective to ascertain the type and percentage alcohol base spirit to use.

These 6 batches of each cultivar were then filtered (0.45 μm filter sheets) and bottled in 250 mL screw-capped clear glass bottles (Consol, SA), labelled and stored at 10°C and 25°C, respectively. These port wines samples were analysed at five different time intervals, namely 0, 3, 6, 9 and 12 months, for free and total SO$_2$ content, pH, titratable acidity, CIELab and spectrophotometric absorbance for colour, as well as aldehyde, polyphenol and anthocyanin levels.

Sensory analysis was also performed on these wines by an experienced panel consisting of 7 sensory judges at the respective time intervals. Chemical terminology may be invoked when they are industry standards, familiar to the panellists, or when domestic descriptors are nonexistent. Examples such as oxidative character, acetaldehyde character and colour intensity were some of these chemical terminology used in this study, similar to a study by Jackson (2012).

4.3.2 Chemical analysis

<u>Determination of free sulphur dioxide content in port wine</u>. Fifty mL of port sample was pipetted out into a 250 mL Erlenmeyer flask. Ten mL of 9 N H_2SO_4 was added to the sample. One mL starch solution was added and the solution titrated rapidly with 1/64 N KI/KIO_3 solution until the first darkening of the solution to a bluish colour appeared and persisted for at least 1 minute.

<u>Determination of total sulphur dioxide content in port wine</u>. Fifty mL port sample was pipetted into a 250 mL Erleynmeyer flask and 20 mL of 1 N NaOH was added and left for 15 minutes before adding 10 mL of 9 N H_2SO_4 and 1 mL of starch solution, followed by rapid titration as in the previous section.

<u>Determination of pH and TA (titratable acidity) in wine using an automatic titrator (Mettler)</u>. The instrument was calibrated according to the instrument instructions and then set up in advance for the determination of pH and titratable acidity. Twenty five mL of the sample was pipetted out into a 50 mL plastic beaker, the sample was then placed in a tray holder of the instrument and "run" activated on the instrument whereby the instrument then titrated the sample against a 1 N NaOH solution and thereafter displayed a pH and TA reading.

<u>Determination of colour (absorbance) by using the Cecil CE 2021 2000 series spectrophotometer</u>. The instrument automatically calibrated after in-putting the command. Thereafter, the wavelengths were entered on the data system/PC at wavelengths of 420, 520 and 620 nm. Quartz cuvettes (4.5 mL, 1 cm x 1 cm) were used to hold the samples in the spectrophotometer. Water samples were used as a reference to zero the instrument.

<u>Determination of anthocyanins in ruby port wine by using a spectrophotometer</u>. The appropriate dilution factor was determined for the samples by diluting with potassium chloride buffer, pH 1.0, until the absorbance of the sample at the "Vis Max" at 700 nm was within the linear range of the spectrophotometer. The final volume of the diluted sample was divided by the initial volume of sample used to obtain the dilution factor. The spectrophotometer was zeroed with distilled water at all the wavelengths used. Two dilutions of the sample were prepared, one with potassium chloride buffer, pH 1.0, and the other with sodium acetate buffer at pH 4.5, diluting each by the previously determined dilution factor. The dilutions were left to equilibrate for 15 min. The absorbance of each dilution was measured at the "Vis Max" at 700 nm, against a blank reference sample (distilled water).

Determination of polyphenols by the use of the Folin Ciocalteau reagent with gallic acid as the standard to measure total polyphenols in a sample. The computer programme was followed to perform the plate counts. Standards were prepared and control wells were included. Twenty five µL of sample was pipetted in triplicate to the sample wells (C1-H12). Hundred and twenty five µL Folin reagent was added to each well using a multichannel pipette. Five minutes waiting time elapsed before adding 100 µL Na_2CO_3 to each well using a multichannel pipette. The plate was left for 2 hours at room temperature before taking a reading.

Determination of CIELab colour using the Konica Minolta Spectrophotometer CM-5. The instrument was calibrated and then set for specific parameters, such as L* a* b* L* C* and h. The sample was pipetted into a 1 mm plastic cuvette which was placed inside the spectrophotometer to get a reading. All readings were transferred to a computer for retrieval of the data at a later stage for data analysis.

Determination of acetaldehyde using capillary gas chromatography (GC). The apparatus used was a HP 5890 Series II GC, HP 7673 Injector, HP 3396A Intergrator, HP 6890 GC Series GC, HP 7683 Series auto sampler (FID) detector. A standard sample was prepared (port with pure aldehyde) and stored in a refrigerator at 4°C. Calibration was done with the standard sample. The autosampler carousel was loaded with vials containing the samples after which the method was initiated. The peaks were integrated by the HP 3396A integrator and the data trace recorded for each analysis. Quantification was based on the external standard method.

4.3.3 Statistical analysis

A mixed design, changing one variable at a time was employed, with the design factors two different grape cultivars, three acetaldehyde levels, two storage temperatures, five time intervals at which samples were collected and analyses were conducted. Statistical analysis was performed using SPSS 19.0® for Windows.

Descriptive statistical analyses determined the mean and standard deviation of replicates. Univariate analyses of variance (ANOVA) were performed to ascertain which of the factors and interactions were significant in terms of the model. The highest order of effects that was statistically significant was explored to identify the combinations that performed the best with reference to the most stable colour in port wine. The level of confidence required for significance was selected at $p \leq 0.05$ Duncan's new multiple range test (MRT) was the post hoc test performed to determine the individual means per design variable (cultivar, enzyme, aldehyde level and temperature over time).

4.4 Results and Discussion

Colour intensity, Tonality, Glories values, CIELab, LCh, ΔE^*, sensory colour, total anthocyanins and total polyphenol values (response variables) in response to the treatments are depicted in Table 4.1. The results of the ANOVA, followed by Duncan's new multiple range test (MRT) were used to indicate treatment differences resulting from the design variables. Hence the significant differences indicated in Table 4.1 signify that the main effects of the design variables on the response variables were significant.

Cultivar had a significant influence ($p < 0.05$) on all the response variables with the exception of a* (redness) and ΔE^* (Table 4.1). In this study two different cultivars were used, Tinta Barrocca and Pinotage. Both of these cultivars are used in making port style wine in South Africa, however, the Tinta Barroca is more commonly used than Pinotage, a cultivar that was first grown in South Africa (Arcari *et al.*, 2013). Since Tinta Barrocca is a typical port wine cultivar it was expected that the colour stability of Tinta Barrocca would be significantly higher than that of the Pinotage port wine style samples. In terms of colour intensity alone, the values for Tinta Barrocca were significantly higher ($p < 0.05$) without enzyme treatment (Table 4.1) than in the Pinotage ruby port samples. However, the opposite was true when

Table 4.1 Main effects of design variables on colour and related parameters (response variables) of port wine samples [1].

Cultivar[2]	En[3]	A[4]	p (°C)[5]	T[6] (h)	Colour Intensity	Tonality	% yellow	% red	% blue	L*	a*	b*	C	h	Sensory colour	ΔE*	Total Anthocyanins	Total Polyphenols
Pinotage	No	L	10	0	2.37 ± 0.33[a,ii]	0.58 ± 0.01[i]	31.94 ± 0.88[i]	54.58 ± 0.18[b,ii]	13.47 ± 0.69[a,ii]	49.62 ± 0.05[b,ii]	45.19 ± 0.04[a,ii]	4.66 ± 0.10[i]	42.50 ± 0.04[a,i]	57.86 ± 0.06[iii]	69.42 ± 1.81[a,]	0.00 ± 0.00[a,i]	238.46 ± 1.89[iv]	*1755.27 ± 6.90[iii]*
Pinotage	No	I	10	0	2.26 ± 0.01[a,ii]	0.59 ± 0.00[i]	32.31 ± 0.25[i]	54.37 ± 0.00[a,ii]	13.31 ± 0.01[b,ii]	50.56 ± 0.61[b,ii]	44.41 ± 0.43[a,ii]	4.04 ± 0.18[i]	41.72 ± 0.42[a,i]	56.71 ± 0.72[ii]	73.78 ± 1.11[a,]	0.00 ± 0.00[c,i]	237.96 ± 0.24[iv]	*1772.34 ± 106.98[iii]*
Pinotage	No	H	10	0	2.43 ± 0.02[b,iv]	0.58 ± 0.00[i]	31.60 ± 0.20[i]	54.50 ± 0.29[a,ii]	13.89 ± 0.08[c,i]	48.48 ± 0.00[a,ii]	45.81 ± 0.01[b,ii]	5.04 ± 0.08[i]	43.11 ± 0.01[b,i]	59.03 ± 0.00[ii]	73.07 ± 5.14[b,]	0.00 ± 0.00[b,i]	224.43 ± 2.83[iv]	*1939.05 ± 15.33[iii]*
Pinotage	No	L	25	0	2.37 ± 0.33[a,iv]	0.58 ± 0.01i	31.94 ± 0.88[i]	54.58 ± 0.18[b,ii]	13.47 ± 0.69[a,ii]	49.62 ± 0.05[b,ii]	45.19 ± 0.04[a,ii]	4.66 ± 0.10[i]	42.50 ± 0.04[a,i]	57.86 ± 0.06[i]	69.42 ± 1.81[a,]	0.00 ± 0.00[a,i]	238.46 ± 1.89[iv]	*1755.27 ± 6.90[iii]*
Pinotage	No	I	25	0	2.26 ± 0.01[a,iv]	0.59 ± 0.00i	32.31 ± 0.25[i]	54.37 ± 0.00[a,ii]	13.31 ± 0.01[b,ii]	50.56 ± 0.61[b,ii]	44.41 ± 0.43[a,ii]	4.04 ± 0.18[i]	41.72 ± 0.42[a,i]	56.71 ± 0.72[ii]	73.78 ± 1.11[a,]	0.00 ± 0.00[c,i]	237.96 ± 0.24[iv]	*1772.34 ± 106.98[iii]*
Pinotage	No	H	25	0	2.43 ± 0.02[b,iv]	0.58 ± 0.01i	31.60 ± 0.20[i]	54.50 ± 0.29[a,ii]	13.89 ± 0.08c,	48.48 ± 0.00[a,ii]	45.81 ± 0.01[b,ii]	5.04 ± 0.08[i]	43.11 ± 0.01[b,i]	59.03 ± 0.00[ii]	73.07 ± 1.81[b,]	0.00 ± 0.00[b,i]	224.43 ± 2.83[iv]	*1939.05 ± 15.33[iii]*
Pinotage	No	L	10	3	2.37 ± 0.27[a,ii]	0.61 ± 0.00[iii]	32.80 ± 0.55[ii]	53.08 ± 0.04[b,ii]	14.10 ± 0.00[a,ii]	49.87 ± 0.41[b,ii]	45.15 ± 0.12[a,ii]	3.15 ± 0.13[i]	45.26 ± 0.13[a,ii]	3.99 ± 0.16[i]	59.71 ± 0.20[a,]	1.55 ± 0.08[a,ii]	71.00 ± 2.44[iii]	*1694.46 ± 47.11[i]*
Pinotage	No	I	10	3	2.26 ± 0.01[a,iii]	0.61 ± 0.00[iii]	32.64 ± 0.05[ii]	52.75 ± 0.20[a,ii]	14.60 ± 0.26[b,i]	49.92 ± 2.22[b,ii]	44.96 ± 1.17[a,ii]	3.10 ± 0.35[i]	45.07 ± 1.20[a,ii]	3.94 ± 0.38[i]	60.64 ± 1.31[a,]	2.59 ± 0.82[c,ii]	70.38 ± 7.77[iii]	*1468.72 ± 2.10[i]*
Pinotage	No	H	10	3	2.43 ± 0.16[b,ii]	0.61 ± 0.00[ii]	32.27 ± 0.02[ii]	52.27 ± 0.49[a,ii]	15.45 ± 0.47[c,ii]	45.88 ± 0.36[b,ii]	45.89 ± 0.09[a,ii]	3.14 ± 0.04[i]	46.00 ± 0.09[b,ii]	3.92 ± 0.05[i]	65.50 ± 5.75[b,]	3.23 ± 0.32[b,ii]	74.40 ± 6.90[iii]	*1616.56 ± 23.68[i]*
Pinotage	No	L	25	3	1.23 ± 0.00[a,ii]	0.67 ± 0.01[iii]	34.02 ± 0.20[ii]	50.68 ± 0.17[b,ii]	15.29 ± 0.02[a,ii]	48.54 ± 0.14[b,ii]	42.22 ± 0.07[a,ii]	3.58 ± 0.07[i]	42.38 ± 0.07[a,ii]	4.84 ± 0.10[i]	59.92 ± 1.71[a,]	3.34 ± 0.02[a,ii]	74.80 ± 18.67[iii]	*1746.92 ± 98.68[i]*
Pinotage	No	I	25	3	1.30 ± 0.03[a,ii]	0.65 ± 0.00[iii]	33.47 ± 0.09[ii]	50.72 ± 0.02[a,ii]	15.79 ± 0.06[b,ii]	46.30 ± 0.40[b,ii]	42.84 ± 0.08[a,ii]	3.47 ± 1.16[i]	42.99 ± 0.07[a,ii]	4.64 ± 0.22[i]	58.14 ± 4.44[b,]	4.59 ± 0.03[c,ii]	68.46 ± 2.01[iii]	*1688.99 ± 11.25[i]*
Pinotage	No	H	25	3	1.31 ± 0.11[b,ii]	0.64 ± 0.00[iii]	32.64 ± 0.20[ii]	50.46 ± 0.08[a,ii]	16.88 ± 0.06[c,ii]	43.31 ± 0.09[a,ii]	42.61 ± 0.03[b,ii]	2.27 ± 0.07[i]	42.67 ± 0.03[b,ii]	3.05 ± 0.11[i]	68.64 ± 4.14[b,]	6.68 ± 0.14[b,ii]	71.30 ± 8.48[iii]	*1519.44 ± 30.23[i]*
Pinotage	No	L	10	6	1.20 ± 0.01[a,ii]	0.60 ± 0.00[ii]	32.24 ± 0.24[ii]	52.99 ± 0.11[b,ii]	14.75 ± 0.16[a,ii]	47.44 ± 0.07[b,ii]	45.59 ± 0.13[a,ii]	3.65 ± 0.09[ii]	45.74 ± 0.13[a,ii]	4.58 ± 0.10[i]	65.78 ± 1.71[a,]	2.44 ± 0.06[a,ii]	52.32 ± 1.22[ii]	*1917.86 ± 21.58[ii]*
Pinotage	No	I	10	6	1.25 ± 0.00[a,ii]	0.60 ± 0.00[ii]	31.92 ± 0.00[ii]	53.03 ± 0.19[a,ii]	15.04 ± 0.18[b,ii]	46.24 ± 0.29[b,i]	45.65 ± 1.16[a,ii]	3.25 ± 0.01[ii]	47.27 ± 2.28[a,ii]	4.07 ± 0.01[i]	70.93 ± 6.36[a,]	4.58 ± 0.98[c,ii]	49.60 ± 1.92[ii]	*1636.02 ± 7.19[ii]*
Pinotage	No	H	10	6	1.33 ± 0.13[b,ii]	0.60 ± 0.00[ii]	31.54 ± 0.10[ii]	52.38 ± 0.05[a,ii]	16.07 ± 0.05[c,ii]	43.20 ± 0.20[a,ii]	45.55 ± 0.07[b,ii]	2.77 ± 0.16[ii]	45.64 ± 0.09[b,ii]	3.48 ± 0.20[i]	73.43 ± 4.85[b,]	5.75 ± 0.16[b,ii]	61.93 ± 5.72[ii]	*1690.97 ± 23.33[ii]*
Pinotage	No	L	25	6	1.16 ± 0.00[a,ii]	0.67 ± 0.00[iii]	33.94 ± 0.20[ii]	50.38 ± 0.05[b,ii]	15.66 ± 0.03[c,ii]	47.87 ± 0.14[b,ii]	41.17 ± 0.14[a,ii]	4.46 ± 0.10[ii]	41.41 ± 0.13[a,ii]	6.05 ± 0.02[i]	67.92 ± 8.18[a,]	4.39 ± 0.04[a,ii]	91.22 ± 0.78[ii]	*1848.16 ± 15.11[ii]*
Pinotage	No	I	25	6	1.19 ± 0.03[a,ii]	0.67 ± 0.00[iii]	33.67 ± 0.24[ii]	50.16 ± 0.05[a,ii]	16.15 ± 0.25[b,ii]	46.78 ± 0.43[b,ii]	41.38 ± 0.05[a,ii]	4.27 ± 0.04[ii]	41.60 ± 0.05[a,ii]	5.88 ± 0.04[i]	73.21 ± 0.30[a,]	4.90 ± 0.58[c,ii]	87.08 ± 4.36[ii]	*1653.32 ± 56.11[ii]*
Pinotage	No	H	25	6	1.28 ± 0.00[b,ii]	0.65 ± 0.00[iii]	32.42 ± 0.07[ii]	49.72 ± 0.05[a,ii]	17.84 ± 0.01[c,ii]	42.98 ± 0.31[a,ii]	41.22 ± 0.10[b,ii]	2.66 ± 0.12[ii]	41.31 ± 0.09[b,ii]	3.69 ± 0.17[i]	76.28 ± 2.01[b,]	7.55 ± 0.24[b,ii]	99.01 ± 11.86[ii]	*1724.54 ± 28.77[ii]*
Pinotage	No	L	10	9	1.37 ± 0.23[a,ii]	0.49 ± 0.11[ii]	28.33 ± 3.82[i]	57.84 ± 5.23[b,ii]	13.81 ± 1.41[a,ii]	46.02 ± 0.00[b,ii]	45.20 ± 0.00[a,ii]	3.66 ± 0.00[iii]	45.35 ± 0.00[a,ii]	4.62 ± 0.00[ii]	64.78 ± 0.50[a,]	1.86 ± 2.63[a,ii]	99.32 ± 1.22[i]	*1578.36 ± 16.58[i]*
Pinotage	No	I	10	9	1.21 ± 0.15[a,ii]	0.45 ± 0.19[i]	25.23 ± 8.28[i]	56.98 ± 5.87[a,ii]	17.77 ± 2.41[b,ii]	44.97 ± 0.45[a,ii]	45.17 ± 0.01[a,ii]	3.06 ± 0.00[iii]	45.27 ± 0.02[a,ii]	3.88 ± 0.01[ii]	65.14 ± 3.43[a,]	5.74 ± 0.18[c,ii]	93.02 ± 1.92[i]	*1598.25 ± 2.88[i]*
Pinotage	No	H	10	9	1.47 ± 0.03[b,ii]	0.56 ± 0.04[ii]	29.39 ± 1.78[i]	52.10 ± 1.36[a,ii]	18.50 ± 0.41[c,ii]	42.65 ± 0.15[a,ii]	44.63 ± 0.01[b,ii]	1.99 ± 0.09[iii]	44.67 ± 0.00[b,ii]	2.56 ± 0.12[ii]	65.64 ± 1.91[a,]	6.69 ± 0.14[b,ii]	75.51 ± 1.66[i]	*1602.84 ± 5.04[i]*
Pinotage	No	L	25	9	1.05 ± 0.02[a,ii]	0.66 ± 0.00[i]	33.80 ± 0.01[i]	50.49 ± 0.11[b,ii]	15.69 ± 0.12[a,ii]	48.12 ± 1.87[b,i]	42.31 ± 4.17[a,i]	4.78 ± 2.09[i]	42.56 ± 3.98[a,i]	6.61 ± 3.45[i]	64.64 ± 0.91[a,]	4.58 ± 2.05[a,ii]	46.69 ± 0.26[i]	*1605.90 ± 33.89[i]*
Pinotage	No	I	25	9	1.11 ± 0.04[a,ii]	0.65 ± 0.00[i]	33.25 ± 0.01[i]	50.69 ± 0.12[a,ii]	16.04 ± 0.67[b,ii]	47.76 ± 0.63[b,ii]	39.55 ± 0.14[a,ii]	6.45 ± 0.01[iii]	40.07 ± 0.14[a,ii]	9.26 ± 0.05[ii]	67.57 ± 0.60[a,]	6.17 ± 0.41[c,ii]	46.14 ± 2.27[i]	*1625.28 ± 58.40[i]*
Pinotage	No	H	25	9	1.18 ± 0.02[b,ii]	0.64 ± 0.00[i]	32.54 ± 0.05[i]	50.23 ± 0.80[a,ii]	17.22 ± 1.41[c,ii]	44.59 ± 0.67[a,ii]	39.44 ± 0.35[b,ii]	4.04 ± 0.14[iii]	39.65 ± 0.33[b,ii]	5.85 ± 0.25[ii]	64.86 ± 2.22[b,]	7.55 ± 0.09[b,ii]	43.78 ± 0.17[i]	*1604.37 ± 56.25[i]*
Pinotage	No	L	10	12	0.11 ± 0.00[a,i]	1.09 ± 0.05[iv]	35.74 ± 0.98[iii]	32.76 ± 0.79[b,i]	31.49 ± 0.18[a,iv]	45.41 ± 0.34[b,ii]	44.96 ± 0.00[a,ii]	3.85 ± 0.22[iv]	45.13 ± 0.01[a,ii]	4.89 ± 0.28[ii]	70.67 ± 1.41[a,]	4.29 ± 0.26[a,iv]	71.43 ± 3.41[i]	*1494.89 ± 37.79[i]*
Pinotage	No	I	10	12	0.10 ± 0.00[a,i]	1.19 ± 0.05[iv]	38.25 ± 1.41[iii]	32.06 ± 0.40[a,i]	29.68 ± 1.00[b,iv]	44.17 ± 0.49[b,ii]	45.01 ± 0.21[a,i]	3.43 ± 0.00[iv]	45.14 ± 0.21[a,ii]	4.36 ± 0.02[i]	65.66 ± 9.66[a,]	6.46 ± 0.60[c,iv]	66.98 ± 3.23[i]	*1472.89 ± 3.70[i]*
Pinotage	No	H	10	12	0.11 ± 0.00[b,i]	1.10 ± 0.00[iv]	36.05 ± 0.21[iii]	32.61 ± 0.19[a,i]	31.32 ± 0.41[c,i]	42.23 ± 0.19[a,ii]	44.09 ± 0.21[b,ii]	2.35 ± 0.00[iv]	44.15 ± 0.21[b,ii]	3.06 ± 0.02[ii]	74.83 ± 0.70[b,]	7.01 ± 0.07[b,iv]	58.07 ± 4.98[i]	*1447.21 ± 13.33[i]*
Pinotage	No	L	25	12	0.11 ± 0.00[a,i]	1.10 ± 0.00,[iv]	35.89 ± 0.00[iii]	32.47 ± 0.00[b,i]	31.62 ± 0.00[a,iv]	49.87 ± 0.00[b,ii]	38.36 ± 0.00[a,ii]	7.46 ± 0.00[i]	39.07 ± 0.00[a,ii]	11.01 ± 0.00[i]	76.67 ± 0.00[b,]	7.38 ± 0.00[a,iv]	34.51 ± 0.00[i]	*1512.19 ± 0.00[i]*
Pinotage	No	I	25	12	0.10 ± 0.00[a,i]	1.11 ± 0.00[iv]	36.89 ± 0.00[iii]	33.01 ± 0.00[a,i]	30.09 ± 0.00[b,iv]	47.94 ± 0.98[b,ii]	39.16 ± 0.23[a,ii]	6.90 ± 0.14[iv]	39.76 ± 0.20[a,ii]	9.98 ± 0.26[ii]	72.83 ± 1.41[a,]	6.54 ± 0.19[c,iv]	33.27 ± 1.05[i]	*1523.71 ± 25.19[i]*
Pinotage	No	H	25	12	0.11 ± 0.00[b,i]	1.10 ± 0.00[iv]	36.05 ± 0.21[iii]	32.61 ± 0.19[a,i]	31.32 ± 0.41[c,i]	44.79 ± 0.28[a,ii]	39.07 ± 0.16[b,ii]	4.78 ± 0.21[iv]	39.36 ± 0.14[b,ii]	6.98 ± 0.33[ii]	77.25 ± 1.30[b,]	7.69 ± 0.02[b,iv]	33.95 ± 1.66[i]	*1494.37 ± 22.23[i]*

[1] Results are reported as the mean ± standard deviation (SD) (n = 2). Analyses of variance (ANOVAs) tested the influence of the main effects. Duncan's new multiple range test (MRT) tested the differences between individual means (p ≤ 0.05 is significant).

[2] Tinta B = Tinta Barrocca. Different shaded blocks in each column (background solid colour) indicate significance between cultivars with respect to the response variables.

[3] En = Enzyme. Different colour font indicates significance in terms of the effect enzyme treatment on the response variable. Yes = Addition of enzyme treatment; No = without enzyme treatment.

[4] A = Aldehyde with L = Low aldehyde level (50 mg.L-1); I = Intermediate aldehyde level (No commercial acetaldehyde added); H = High aldehyde level (Normal wine spirits spiked with 450 mg/L-1 . a-c The different superscripts in the same column indicate significant differences (p ≤ 0.05).

[5] Temp = Storage Temperature : 10 and 25°C. Font in Italics indicate no significant difference (p < 0.05). Temperature had a significant effect all response variables except for **Sensory Colour** and **Total Polyphenols**.

[6] T = Time intervals of tests: 0, 3, 6, 9 and 12 months. i-iv The different superscripts indicate significant differences due to different storage time intervals (p < 0.05).

Table 4.1 continued.

Cultivar[2]	En[3]	A[4]	Temp (°C)[5]	T[6] (h)	Colour Intensity	Tonality	% yellow	% red	% blue	L*	a*	b*	C	h	Sensory colour	ΔE*	Total Anthocyanins	Total Polyphenols
Pinotage	Yes	L	10	0	$2.71 \pm 0.02^{a,i}$	0.57 ± 0.00^{i}	31.53 ± 0.05^{i}	$54.76 \pm 0.06^{b,ii}$	$13.70 \pm 0.00^{a,i}$	$46.12 \pm 0.63^{b,ii}$	$47.29 \pm 0.12^{a,ii}$	5.58 ± 0.16^{i}	$44.60 \pm 0.12^{a,i}$	61.68 ± 0.11^{ii}	$70.50 \pm 3.73^{a,i}$	$0.00 \pm 0.00^{a,i}$	241.63 ± 1.65^{iv}	1927.12 ± 105.80^{iii}
Pinotage	Yes	I	10	0	$2.78 \pm 0.05^{a,i}$	0.57 ± 0.00^{i}	31.31 ± 0.02^{i}	$54.62 \pm 0.04^{a,ii}$	$14.05 \pm 0.00^{b,ii}$	$47.58 \pm 0.04^{b,ii}$	$46.48 \pm 0.04^{a,ii}$	4.75 ± 0.11^{i}	$43.78 \pm 0.03^{a,i}$	60.16 ± 0.04^{ii}	$69.00 \pm 5.45^{a,i}$	$0.00 \pm 0.00^{c,i}$	237.46 ± 3.77^{iv}	1905.17 ± 187.45^{iii}
Pinotage	Yes	H	10	0	$2.87 \pm 0.00^{b,i}$	0.57 ± 0.00^{i}	31.19 ± 0.01^{i}	$54.61 \pm 0.01^{a,ii}$	$14.19 \pm 0.00^{c,ii}$	$48.31 \pm 0.14^{a,ii}$	$45.92 \pm 0.10^{b,ii}$	4.62 ± 0.21^{i}	$43.23 \pm 0.09^{b,i}$	59.27 ± 0.14^{ii}	$71.35 \pm 1.71^{b,i}$	$0.00 \pm 0.00^{b,i}$	230.28 ± 0.70^{iv}	2111.44 ± 15.33^{iii}
Pinotage	Yes	L	25	0	$2.71 \pm 0.02^{a,i}$	0.57 ± 0.00^{i}	31.53 ± 0.05^{i}	$54.76 \pm 0.06^{b,ii}$	$13.70 \pm 0.00^{a,ii}$	$46.12 \pm 0.63^{b,ii}$	$47.29 \pm 0.12^{a,ii}$	5.58 ± 0.16^{i}	$44.60 \pm 0.12^{a,i}$	61.68 ± 0.11^{ii}	$70.50 \pm 3.73^{a,i}$	$0.00 \pm 0.00^{a,i}$	241.63 ± 1.65^{iv}	1927.12 ± 05.80^{iii}
Pinotage	Yes	I	25	0	$2.78 \pm 0.05^{a,i}$	0.57 ± 0.00^{i}	31.31 ± 0.02^{i}	$54.62 \pm 0.04^{a,ii}$	$14.05 \pm 0.00^{b,ii}$	$47.58 \pm 0.04^{b,ii}$	$46.48 \pm 0.04^{a,ii}$	4.75 ± 0.11^{i}	$43.78 \pm 0.03^{a,i}$	60.16 ± 0.04^{ii}	$69.00 \pm 5.45^{a,i}$	$0.00 \pm 0.00^{c,i}$	237.46 ± 3.77^{iv}	1905.17 ± 187.45^{iii}
Pinotage	Yes	H	25	0	$2.87 \pm 0.00^{b,i}$	0.57 ± 0.00^{i}	31.19 ± 0.01^{i}	$54.61 \pm 0.01^{a,ii}$	$14.19 \pm 0.00^{c,ii}$	$48.31 \pm 0.14^{a,ii}$	$45.92 \pm 0.10^{b,ii}$	4.62 ± 0.21^{i}	$43.23 \pm 0.09^{b,i}$	59.27 ± 0.14^{ii}	$71.35 \pm 1.71^{b,i}$	$0.00 \pm 0.00^{b,i}$	230.28 ± 0.70^{iv}	2111.44 ± 15.33^{iii}
Pinotage	Yes	L	10	3	$1.43 \pm 0.07^{a,ii}$	0.60 ± 0.00^{iii}	32.10 ± 0.19^{ii}	$53.02 \pm 0.24^{b,ii}$	$14.87 \pm 0.05^{a,i}$	$42.77 \pm 0.01^{b,ii}$	$48.71 \pm 0.00^{a,ii}$	3.98 ± 0.08^{i}	$48.87 \pm 0.00^{a,ii}$	4.67 ± 0.10^{i}	$65.07 \pm 2.92^{a,i}$	$3.98 \pm 0.05^{a,ii}$	115.22 ± 11.98^{iii}	1730.76 ± 63.29^{i}
Pinotage	Yes	I	10	3	$1.53 \pm 0.00^{a,ii}$	0.59 ± 0.00^{iii}	31.53 ± 0.06^{i}	$52.84 \pm 0.16^{a,i}$	$15.62 \pm 0.10^{b,i}$	$41.56 \pm 1.59^{b,ii}$	$48.38 \pm 1.01^{a,ii}$	4.09 ± 1.42^{i}	$48.56 \pm 1.13^{a,ii}$	4.81 ± 3.57^{i}	$67.00 \pm 2.82^{a,i}$	$6.45 \pm 1.59^{c,ii}$	111.17 ± 17.27^{iii}	1500.54 ± 118.84^{i}
Pinotage	Yes	H	10	3	$1.53 \pm 0.00^{b,ii}$	0.59 ± 0.00^{iii}	31.40 ± 0.09^{i}	$52.82 \pm 0.19^{a,ii}$	$15.76 \pm 0.09^{c,i}$	$40.77 \pm 0.02^{a,ii}$	$48.75 \pm 0.01^{b,ii}$	4.05 ± 0.16^{i}	$48.92 \pm 0.02^{b,ii}$	4.75 ± 0.18^{i}	$70.50 \pm 0.70^{b,i}$	$8.07 \pm 0.17^{b,ii}$	117.97 ± 24.27^{iii}	1670.10 ± 189.85^{i}
Pinotage	Yes	L	25	3	$1.44 \pm 0.03^{a,ii}$	0.64 ± 0.01^{iii}	32.78 ± 0.19^{ii}	$50.95 \pm 0.49^{b,ii}$	$16.25 \pm 0.29^{a,i}$	$41.94 \pm 0.26^{b,ii}$	$45.15 \pm 0.00^{a,ii}$	3.38 ± 0.08^{i}	$45.28 \pm 0.00^{a,ii}$	4.28 ± 0.08^{i}	$71.64 \pm 4.94^{a,i}$	$5.19 \pm 0.31^{a,ii}$	114.79 ± 17.66^{iii}	1440.87 ± 66.80^{i}
Pinotage	Yes	I	25	3	$1.46 \pm 0.00^{a,ii}$	0.63 ± 0.00^{iii}	32.33 ± 0.02^{ii}	$50.69 \pm 0.07^{a,ii}$	$16.96 \pm 0.05^{b,i}$	$40.48 \pm 0.66^{b,ii}$	$44.53 \pm 0.00^{a,ii}$	2.44 ± 0.22^{i}	$44.60 \pm 0.01^{a,ii}$	3.13 ± 0.28^{i}	$73.78 \pm 1.52^{a,i}$	$7.73 \pm 0.55^{c,ii}$	119.24 ± 17.14^{iii}	1708.39 ± 40.07^{i}
Pinotage	Yes	H	25	3	$1.49 \pm 0.00^{b,ii}$	0.63 ± 0.00^{iii}	32.28 ± 0.00^{ii}	$50.63 \pm 0.00^{b,ii}$	$17.08 \pm 0.00^{c,i}$	$39.34 \pm 0.15^{a,i}$	$44.92 \pm 0.12^{b,ii}$	2.56 ± 0.22^{i}	$45.00 \pm 0.14^{b,ii}$	3.26 ± 0.28^{i}	$70.50 \pm 4.34^{b,i}$	$9.26 \pm 0.00^{b,ii}$	107.61 ± 9.27^{iii}	1486.62 ± 78.05^{i}
Pinotage	Yes	L	10	6	$1.42 \pm 0.01^{a,ii}$	0.58 ± 0.00^{iii}	31.14 ± 0.09^{i}	$53.37 \pm 0.08^{b,ii}$	$15.48 \pm 0.17^{a,i}$	$40.84 \pm 0.63^{b,i}$	$48.16 \pm 0.04^{a,ii}$	3.77 ± 0.10^{ii}	$48.31 \pm 0.10^{a,ii}$	4.48 ± 0.11^{i}	$76.78 \pm 0.50^{a,i}$	$5.65 \pm 0.06^{a,ii}$	58.01 ± 4.54^{ii}	1805.94 ± 10.06^{ii}
Pinotage	Yes	I	10	6	$1.48 \pm 0.00^{a,ii}$	0.58 ± 0.00^{iii}	30.79 ± 0.03^{ii}	$52.93 \pm 0.10^{a,ii}$	$16.27 \pm 0.05^{b,i}$	$38.90 \pm 0.02^{b,ii}$	$47.99 \pm 0.07^{a,ii}$	3.80 ± 0.08^{ii}	$48.14 \pm 0.08^{a,ii}$	4.52 ± 0.09^{i}	$75.92 \pm 1.71^{a,i}$	$8.86 \pm 0.06^{c,ii}$	55.97 ± 3.23^{ii}	1919.39 ± 36.69^{ii}
Pinotage	Yes	H	10	6	$1.47 \pm 0.00^{b,ii}$	0.58 ± 0.00^{iii}	30.81 ± 0.00^{ii}	$52.98 \pm 0.11^{a,ii}$	$16.20 \pm 0.10^{c,i}$	$38.76 \pm 0.20^{a,i}$	$47.88 \pm 0.22^{b,ii}$	3.42 ± 0.31^{ii}	$48.00 \pm 0.24^{b,ii}$	4.09 ± 0.35^{i}	$78.07 \pm 2.12^{b,i}$	$9.82 \pm 0.07^{b,ii}$	55.16 ± 1.22^{ii}	1771.34 ± 43.16^{ii}
Pinotage	Yes	L	25	6	$1.35 \pm 0.00^{a,ii}$	0.64 ± 0.00^{iii}	32.72 ± 0.15^{ii}	$50.62 \pm 0.31^{b,ii}$	$16.64 \pm 0.15^{a,i}$	$41.69 \pm 0.17^{b,ii}$	$43.76 \pm 0.09^{a,ii}$	4.02 ± 0.07^{ii}	$43.95 \pm 0.08^{a,ii}$	5.23 ± 0.12^{i}	$76.92 \pm 1.71^{a,i}$	$5.88 \pm 0.02^{a,ii}$	95.86 ± 6.12^{ii}	1901.08 ± 39.56^{ii}
Pinotage	Yes	I	25	6	$1.40 \pm 0.00^{a,ii}$	0.64 ± 0.00^{iii}	32.17 ± 0.12^{ii}	$50.05 \pm 0.22^{a,ii}$	$17.76 \pm 0.10^{b,i}$	$39.92 \pm 0.02^{b,ii}$	$43.28 \pm 0.17^{a,ii}$	2.95 ± 0.14^{ii}	$43.39 \pm 0.16^{a,ii}$	3.89 ± 0.20^{i}	$75.50 \pm 0.90^{a,i}$	$8.50 \pm 0.01^{c,ii}$	97.04 ± 2.88^{ii}	1882.25 ± 33.09^{ii}
Pinotage	Yes	H	25	6	$1.39 \pm 0.01^{b,ii}$	0.64 ± 0.00^{iii}	32.30 ± 0.00^{ii}	$50.12 \pm 0.02^{a,ii}$	$17.56 \pm 0.05^{c,i}$	$39.31 \pm 0.07^{a,i}$	$43.46 \pm 0.09^{b,ii}$	2.93 ± 0.16^{ii}	$43.56 \pm 0.10^{b,ii}$	3.86 ± 0.20^{i}	$77.57 \pm 0.40^{b,i}$	$9.48 \pm 0.06^{b,ii}$	105.45 ± 4.11^{ii}	1767.79 ± 5.03^{ii}
Pinotage	Yes	L	10	9	$1.44 \pm 0.07^{a,ii}$	0.57 ± 0.00^{ii}	30.52 ± 0.02^{i}	$52.90 \pm 0.23^{b,ii}$	$16.56 \pm 0.26^{a,i}$	$40.41 \pm 0.06^{b,ii}$	$47.36 \pm 0.13^{a,i}$	3.19 ± 0.24^{iii}	$47.47 \pm 0.14^{a,ii}$	3.85 ± 0.27^{ii}	$67.50 \pm 0.50^{a,i}$	$6.20 \pm 0.03^{a,ii}$	89.37 ± 1.31^{i}	1812.93 ± 64.18^{i}
Pinotage	Yes	I	10	9	$1.78 \pm 0.13^{a,ii}$	0.62 ± 0.02^{ii}	31.08 ± 0.41^{i}	$49.48 \pm 1.64^{a,ii}$	$19.42 \pm 1.23^{b,i}$	$38.82 \pm 0.06^{b,ii}$	$46.84 \pm 0.09^{a,i}$	3.15 ± 0.31^{iii}	$46.95 \pm 0.07^{a,ii}$	3.85 ± 0.39^{ii}	$72.42 \pm 2.42^{a,i}$	$8.91 \pm 0.15^{c,ii}$	80.40 ± 0.70^{i}	1823.64 ± 50.48^{i}
Pinotage	Yes	H	10	9	$1.66 \pm 0.02^{b,ii}$	0.60 ± 0.01^{ii}	30.61 ± 0.23^{i}	$50.43 \pm 0.60^{a,ii}$	$18.95 \pm 0.37^{c,i}$	$39.11 \pm 0.21^{a,ii}$	$46.64 \pm 0.13^{b,i}$	2.37 ± 0.02^{iii}	$46.70 \pm 0.13^{b,ii}$	2.91 ± 0.02^{ii}	$67.00 \pm 0.80^{b,i}$	$9.50 \pm 0.10^{b,ii}$	83.99 ± 1.40^{i}	1789.48 ± 2.10^{i}
Pinotage	Yes	L	25	9	$1.30 \pm 0.00^{a,ii}$	0.66 ± 0.00^{ii}	33.09 ± 0.01^{i}	$50.09 \pm 0.29^{b,ii}$	$16.81 \pm 1.27^{a,i}$	$42.80 \pm 0.31^{b,ii}$	$42.09 \pm 0.31^{a,i}$	5.95 ± 0.16^{iii}	$42.51 \pm 0.28^{a,ii}$	8.04 ± 0.28^{i}	$68.42 \pm 1.01^{a,i}$	$6.20 \pm 0.12^{a,ii}$	52.94 ± 0.52^{i}	1771.12 ± 18.03^{i}
Pinotage	Yes	I	25	9	$1.34 \pm 0.03^{a,ii}$	0.65 ± 0.00^{ii}	32.60 ± 0.00^{i}	$49.72 \pm 0.08^{a,ii}$	$17.67 \pm 0.87^{b,i}$	$41.28 \pm 0.03^{b,ii}$	$41.62 \pm 0.24^{a,i}$	4.52 ± 0.31^{iii}	$41.87 \pm 0.21^{a,ii}$	6.19 ± 0.47^{i}	$69.57 \pm 3.83^{a,i}$	$7.96 \pm 0.15^{c,ii}$	47.80 ± 0.26^{i}	1677.80 ± 147.11^{i}
Pinotage	Yes	H	25	9	$1.36 \pm 0.00^{b,ii}$	0.65 ± 0.00^{ii}	32.42 ± 0.13^{i}	$49.83 \pm 0.54^{a,ii}$	$17.73 \pm 0.40^{c,i}$	$41.01 \pm 0.02^{a,ii}$	$41.78 \pm 0.10^{b,i}$	4.51 ± 0.27^{iii}	$42.03 \pm 0.08^{b,ii}$	6.17 ± 0.39^{ii}	$71.78 \pm 0.91^{b,i}$	$8.39 \pm 0.00^{b,ii}$	50.28 ± 4.98^{i}	1775.71 ± 4.32^{i}
Pinotage	Yes	L	10	12	$0.10 \pm 0.01^{a,i}$	1.13 ± 0.07^{iv}	36.60 ± 1.78^{iii}	$32.39 \pm 0.50^{b,i}$	$30.99 \pm 1.27^{a,iv}$	$39.79 \pm 0.28^{b,ii}$	$47.13 \pm 0.01^{a,i}$	3.12 ± 0.01^{iv}	$47.23 \pm 0.14^{a,ii}$	3.78 ± 0.02^{ii}	$74.66 \pm 0.91^{a,i}$	$6.79 \pm 0.26^{a,ii}$	69.33 ± 2.53^{i}	1602.57 ± 128.57^{i}
Pinotage	Yes	I	10	12	$0.11 \pm 0.00^{a,i}$	1.10 ± 0.00^{iv}	35.86 ± 0.04^{iii}	$32.48 \pm 0.01^{a,i}$	$31.64 \pm 0.03^{b,iv}$	$38.84 \pm 0.20^{b,ii}$	$46.20 \pm 0.06^{a,i}$	2.63 ± 0.01^{iv}	$46.28 \pm 0.06^{a,ii}$	3.25 ± 0.01^{ii}	$72.50 \pm 1.17^{a,i}$	$9.00 \pm 0.21^{c,ii}$	59.49 ± 0.17^{i}	1696.11 ± 3.70^{i}
Pinotage	Yes	H	10	12	$0.11 \pm 0.01^{b,i}$	1.11 ± 0.00^{iv}	36.21 ± 0.45^{iii}	$32.58 \pm 0.15^{a,ii}$	$31.19 \pm 0.60^{c,iv}$	$38.98 \pm 0.18^{a,ii}$	$46.00 \pm 0.19^{b,i}$	2.35 ± 0.17^{iv}	$46.06 \pm 0.20^{b,ii}$	2.93 ± 0.21^{ii}	$74.25 \pm 0.11^{b,i}$	$9.61 \pm 0.04^{b,ii}$	61.54 ± 0.43^{i}	1665.19 ± 5.93^{i}
Pinotage	Yes	L	25	12	$0.11 \pm 0.00^{a,i}$	1.13 ± 0.00^{iv}	36.20 ± 0.00^{iii}	$31.89 \pm 0.00^{b,i}$	$31.89 \pm 0.00^{a,iv}$	$43.19 \pm 0.26^{b,ii}$	$41.70 \pm 0.29^{a,i}$	6.42 ± 0.12^{iv}	$42.19 \pm 0.28^{a,ii}$	8.75 ± 0.24^{ii}	$76.75 \pm 2.00^{a,i}$	$6.38 \pm 0.21^{a,ii}$	36.24 ± 2.27^{i}	1730.17 ± 5.92^{i}
Pinotage	Yes	I	25	12	$0.11 \pm 0.00^{a,i}$	1.13 ± 0.00^{iv}	36.40 ± 0.37^{iii}	$32.21 \pm 0.40^{a,ii}$	$31.38 \pm 0.77^{b,i}$	$42.08 \pm 0.71^{b,ii}$	$41.02 \pm 0.48^{a,i}$	5.12 ± 0.26^{iv}	$41.35 \pm 0.45^{a,ii}$	7.12 ± 0.45^{ii}	$75.25 \pm 4.35^{a,i}$	$7.78 \pm 0.14^{c,ii}$	26.22 ± 5.42^{i}	1704.49 ± 22.97^{i}
Pinotage	Yes	H	25	12	$0.10 \pm 0.00^{b,i}$	1.11 ± 0.00^{iv}	36.53 ± 0.01^{iii}	$32.86 \pm 0.24^{a,i}$	$30.60 \pm 0.23^{c,i}$	$41.84 \pm 0.19^{a,ii}$	$41.27 \pm 0.19^{b,i}$	5.06 ± 0.36^{iv}	$41.58 \pm 0.14^{b,ii}$	6.99 ± 0.52^{ii}	$77.25 \pm 2.47^{b,i}$	$7.99 \pm 0.09^{b,ii}$	33.71 ± 0.96^{i}	1712.88 ± 5.19^{i}

[1]Results are reported as the mean ± standard deviation (SD) (n = 2). Analyses of variance (ANOVAs) tested the influence of the main effects. Duncan's new multiple range test (MRT) tested the differences beween individual means (p ≤ 0.05 is significant).

[2]Tinta B = Tinta Barrocca. Different shaded blocks in each column (background filled colour) indicate significance between cultivars with respect to the response variables.

[3]En = Enzyme. Different colour in font indicates significance in terms of the effect enzyme treatment on the response variable. Yes = Addition of enzyme treatment; No = without enzyme treatment.

[4]A = Aldehyde with L = Low aldehyde level (50 mg.L^{-1}); I = Intermediate aldehyde level (No commercial acetaldehyde added); H = High aldehyde level (Normal wine spirits spiked with 450 mg/L^{-1} acetaldehyde). [a-c]The different superscripts in the same column indicate significant differences (p ≤ 0.05).

[5]Temp = Storage Temperature : 10 and 25°C. Font in Italics indicates no significant difference (p < 0.05). Temperature had a significant effect on all response variable except for **Sensory Colour** and **Total Polyphenols**.

[6]T = Time intervals of tests: 0, 3, 6, 9 and 12 months. [i-iv]The different superscripts indicate significant differences due to different storage time intervals (p < 0.05).

Table 4.1 continued.

Cultivar[2]	En[3]	A[4]	Temp (°C)[5]	T[6] (h)	Colour Intensity	Tonality	% yellow	% red	% blue	L*	a*	b*	C	h	Sensory colour	ΔE*	Total Anthocyanins	Total Polyphenols
Tinta B	No	L	10	0	2.49 ± 0.05[a,iv]	0.66 ± 0.00[i]	34.81 ± 0.09[i]	52.74 ± 0.00[b,iii]	12.44 ± 0.10[a,i]	52.42 ± 0.38[b,iii]	43.80 ± 0.22[a,ii]	3.15 ± 0.41[i]	41.11 ± 0.22[a,i]	55.15 ± 0.36[ii]	72.64 ± 1.11[a]	0.00 ± 0.00[a,i]	230.11 ± 3.30[iv]	2471.41 ± 10.72[iii]
Tinta B	No	I	10	0	2.50 ± 0.00[a,iv]	0.65 ± 0.00[i]	34.73 ± 0.12[i]	52.79 ± 0.13[a,ii]	12.47 ± 0.00[b,i]	57.19 ± 3.59[b,ii]	40.57 ± 2.55[a,ii]	1.73 ± 0.83[i]	37.88 ± 2.55[a,i]	49.92 ± 4.04[i]	73.93 ± 0.70[a]	0.00 ± 0.00[c,i]	230.94 ± 1.18[iv]	2481.17 ± 0.00[iii]
Tinta B	No	H	10	0	2.66 ± 0.00[b,ii]	0.64 ± 0.00[i]	34.34 ± 0.01[i]	52.95 ± 0.04[a,i]	12.70 ± 0.05[c,i]	48.76 ± 0.48[a,ii]	45.32 ± 0.28[b,i]	3.91 ± 0.42[i]	42.62 ± 0.28[b,i]	58.61 ± 0.48[ii]	75.21 ± 1.30[b]	0.00 ± 0.00[b,i]	221.09 ± 2.83[v]	2576.59 ± 13.80[iii]
Tinta B	No	L	25	0	2.49 ± 0.00[a,iv]	0.66 ± 0.00[i]	34.81 ± 0.09[i]	52.74 ± 0.00[b,iii]	12.44 ± 0.10[a,i]	52.42 ± 0.38[a,iii]	43.80 ± 0.22[a,ii]	3.15 ± 0.41[i]	41.11 ± 0.22[a,i]	55.15 ± 0.36[ii]	72.64 ± 1.11[a]	0.00 ± 0.00[a,i]	230.11 ± 3.30[iv]	2471.41 ± 10.72[iii]
Tinta B	No	I	25	0	2.50 ± 0.00[a,iv]	0.65 ± 0.00[i]	34.73 ± 0.12[i]	52.79 ± 0.13[a,ii]	12.47 ± 0.00[b,i]	57.19 ± 3.59[b,ii]	40.57 ± 2.55[a,iii]	1.73 ± 0.83[i]	37.88 ± 2.55[a,i]	49.92 ± 4.04[i]	73.93 ± 0.70[a]	0.00 ± 0.00[c,i]	230.94 ± 1.18[iv]	2481.17 ± 0.00[iii]
Tinta B	No	H	25	0	2.66 ± 0.00[b,ii]	0.64 ± 0.00[i]	34.34 ± 0.01[i]	52.95 ± 0.04[a,ii]	12.70 ± 0.05[c,i]	48.76 ± 0.48[a,iii]	45.32 ± 0.28[b,ii]	3.91 ± 0.42[i]	42.62 ± 0.28[b,i]	58.61 ± 0.48[ii]	75.21 ± 1.30[b]	0.00 ± 0.00[b,i]	221.09 ± 2.83[iv]	2576.59 ± 13.80[iii]
Tinta B	No	L	10	3	1.17 ± 0.14[a,ii]	0.69 ± 0.00[iii]	35.96 ± 0.40[ii]	51.80 ± 0.04[b,ii]	12.23 ± 0.35[a,i]	49.87 ± 0.41[b,ii]	43.62 ± 1.18[a,ii]	3.71 ± 0.48[i]	43.77 ± 1.22[a,ii]	4.86 ± 0.50[i]	60.21 ± 7.77[a]	1.95 ± 0.98[b,i]	133.71 ± 0.70,[iii]	2262.14 ± 35.64[i]
Tinta B	No	I	10	3	1.28 ± 0.04[a,ii]	0.69 ± 0.00[iii]	35.76 ± 0.05[ii]	51.73 ± 0.13[a,ii]	12.49 ± 0.18[b,i]	49.92 ± 2.22[b,ii]	44.63 ± 1.15[a,ii]	4.38 ± 0.68[i]	44.85 ± 1.20[a,ii]	5.60 ± 0.72[i]	61.00 ± 1.82[a]	8.35 ± 2.29[c,ii]	138.35 ± 5.68[iii]	2887.56 ± 553.69[i]
Tinta B	No	H	10	3	1.36 ± 0.01[b,ii]	0.67 ± 0.00[iii]	35.01 ± 0.05[ii]	51.94 ± 0.02[a,ii]	13.04 ± 0.07[c,i]	45.88 ± 0.36[a,ii]	46.74 ± 0.10[b,i]	5.50 ± 0.59[i]	47.07 ± 0.17[b,ii]	6.71 ± 0.70[i]	67.14 ± 1.20[b]	3.04 ± 1.26[b,i]	148.37 ± 28.24[iii]	2339.27 ± 100.52[i]
Tinta B	No	L	25	3	1.28 ± 0.00[a,ii]	0.76 ± 0.00[iii]	37.76 ± 0.10[ii]	49.14 ± 0.27[b,ii]	13.09 ± 0.16[a,i]	48.54 ± 0.01[b,ii]	43.55 ± 0.18[a,ii]	6.13 ± 0.43[i]	43.98 ± 0.24[a,ii]	8.01 ± 0.52[i]	68.00 ± 3.63[b]	5.03 ± 1.62[c,i]	138.78 ± 15.22[iii]	2311.04 ± 67.72[i]
Tinta B	No	I	25	3	1.24 ± 0.01[a,ii]	0.76 ± 0.00[iii]	37.71 ± 0.02[ii]	49.60 ± 0.05[a,ii]	12.69 ± 0.02[b,i]	46.30 ± 0.40[a,ii]	43.39 ± 0.63[a,ii]	6.32 ± 0.10[i]	43.85 ± 0.08[a,ii]	8.28 ± 0.12[i]	60.78 ± 1.11[a]	10.17 ± 3.85[c]	138.79 ± 17.49[iii]	2357.42 ± 89.10[i]
Tinta B	No	H	25	3	1.32 ± 0.01[b,ii]	0.74 ± 0.00[iii]	36.87 ± 0.10[ii]	49.66 ± 0.05[a,ii]	13.46 ± 0.15[c,i]	43.31 ± 0.09[a,ii]	44.24 ± 0.63[b,ii]	6.50 ± 0.16[i]	44.82 ± 0.22[b,ii]	8.35 ± 0.16[i]	68.43 ± 5.04[b]	3.66 ± 0.21[b,i]	143.30 ± 2.53[iii]	2332.71 ± 5.70[i]
Tinta B	No	L	10	6	1.18 ± 0.00[a,ii]	0.68 ± 0.00[iii]	35.15 ± 0.12[ii]	51.63 ± 0.16[b,ii]	13.21 ± 0.33[a,i]	47.44 ± 0.07[b,i]	44.71 ± 0.07[a,ii]	4.59 ± 0.00[ii]	44.95 ± 0.14[a,ii]	5.86 ± 0.00[i]	71.50 ± 2.31[a]	3.34 ± 0.35[a,ii]	68.90 ± 0.52[ii]	2666.05 ± 49.46[ii]
Tinta B	No	I	10	6	1.15 ± 0.06[a,ii]	0.68 ± 0.01[iii]	35.45 ± 0.40[ii]	51.51 ± 0.28[a,ii]	13.02 ± 0.12[b,i]	46.24 ± 0.29[b,i]	45.48 ± 0.33[a,ii]	5.12 ± 0.54[ii]	45.77 ± 0.38[a,ii]	6.42 ± 0.64[i]	69.71 ± 2.42[a]	10.63 ± 5.31[c]	76.07 ± 0.17[ii]	2547.35 ± 20.97[ii]
Tinta B	No	H	10	6	1.32 ± 0.04[b,ii]	0.65 ± 0.02[iii]	33.90 ± 0.70[ii]	52.04 ± 0.65[a,i]	14.04 ± 0.05[c,i]	43.20 ± 0.20[a,i]	46.57 ± 0.07[b,i]	5.71 ± 0.34[ii]	46.92 ± 0.11[b,ii]	6.99 ± 0.41[i]	70.85 ± 0.40[b]	3.76 ± 1.32[b,ii]	81.12 ± 8.88[ii]	2582.86 ± 92.17[ii]
Tinta B	No	L	25	6	1.20 ± 0.01[a,ii]	0.74 ± 0.00[ii]	36.72 ± 0.32[ii]	49.37 ± 0.06[b,ii]	13.89 ± 0.26[a,i]	47.87 ± 0.14[b,ii]	42.03 ± 0.91[a,ii]	7.65 ± 0.00[ii]	42.73 ± 0.89[a,ii]	10.32 ± 0.22[i]	71.14 ± 2.22[a]	5.67 ± 0.04[a,ii]	115.96 ± 6.38[ii]	2614.12 ± 2.99[ii]
Tinta B	No	I	25	6	1.09 ± 0.11[a,ii]	0.68 ± 0.11[ii]	34.51 ± 4.00[ii]	50.86 ± 2.61[a,ii]	14.61 ± 1.43[a,i]	46.78 ± 0.43[b,ii]	41.61 ± 2.24[a,ii]	7.68 ± 0.04[ii]	42.31 ± 0.23[a,ii]	10.46 ± 0.12[i]	68.71 ± 4.44[b]	9.45 ± 3.64[c,ii]	123.07 ± 10.14[ii]	2590.62 ± 7.76[ii]
Tinta B	No	H	25	6	1.27 ± 0.02[b,ii]	0.73 ± 0.00[ii]	36.30 ± 0.10[ii]	49.11 ± 0.01[a,ii]	14.57 ± 0.12[c,i]	42.98 ± 0.31[a,i]	43.30 ± 0.28[b,ii]	7.86 ± 0.16[ii]	44.01 ± 0.31[b,ii]	10.28 ± 0.14[i]	72.00 ± 1.00[b]	5.12 ± 0.55[b,ii]	162.04 ± 4.24[ii]	2587.10 ± 66.19[ii]
Tinta B	No	L	10	9	1.04 ± 0.05[a,ii]	0.62 ± 0.00[i]	33.80 ± 0.23[i]	53.69 ± 0.26[b,ii]	12.50 ± 0.03[a,i]	46.02 ± 0.00[b,i]	44.31 ± 0.00[a,i]	4.94 ± 0.00[iii]	44.58 ± 0.00[a,i]	6.36 ± 0.00[ii]	67.07 ± 0.90[a]	2.95 ± 0.00[a,i]	106.99 ± 1.22[i]	2301.14 ± 2.20[i]
Tinta B	No	I	10	9	1.11 ± 0.04[a,ii]	0.62 ± 0.00[i]	33.52 ± 0.02[i]	53.48 ± 0.17[a,ii]	12.98 ± 0.14[b,i]	44.97 ± 0.45[b,i]	45.79 ± 0.56[a,i]	6.21 ± 0.13[iii]	46.20 ± 0.57[a,i]	7.72 ± 0.07[ii]	63.64 ± 1.11[a]	12.15 ± 3.81[c]	119.06 ± 5.16[i]	2305.30 ± 13.15[i]
Tinta B	No	H	10	9	1.21 ± 0.06[b,ii]	0.63 ± 0.02[ii]	33.26 ± 0.31[i]	52.75 ± 1.14[a,ii]	13.97 ± 0.83[c,i]	42.65 ± 0.15[a,ii]	46.24 ± 0.45[b,i]	6.06 ± 0.02[iii]	46.64 ± 0.45[b,i]	7.46 ± 0.10[ii]	65.35 ± 3.74[b]	4.61 ± 0.09[b,ii]	100.32 ± 7.17[i]	2316.22 ± 7.35[i]
Tinta B	No	L	25	9	1.02 ± 0.10[a,i]	0.74 ± 0.02[ii]	37.19 ± 0.77[i]	49.82 ± 0.51[b,ii]	12.98 ± 0.71[a,i]	48.12 ± 1.87[b,ii]	41.58 ± 4.60[a,i]	7.65 ± 3.40[iii]	42.38 ± 3.91[a,ii]	10.68 ± 5.67[i]	64.50 ± 0.50[a]	6.43 ± 3.81[c,ii]	50.83 ± 5.95[i]	2400.46 ± 70.59[i]
Tinta B	No	I	25	9	1.08 ± 0.09[a,i]	0.74 ± 0.00[i]	36.81 ± 0.00[i]	49.53 ± 0.38[a,ii]	13.65 ± 0.38[b,i]	47.76 ± 0.63[b,ii]	40.11 ± 0.77[a,i]	10.21 ± 0.36[ii]	41.13 ± 0.35[a,ii]	14.38 ± 0.65[ii]	64.93 ± 1.31[a]	11.07 ± 2.53[c]	50.22 ± 1.23[i]	2304.90 ± 4.24[i]
Tinta B	No	H	25	9	1.20 ± 0.05[b,ii]	0.74 ± 0.00[i]	36.65 ± 0.06[i]	49.07 ± 0.39[a,ii]	14.27 ± 0.33[c,i]	44.59 ± 0.67[a,ii]	41.17 ± 0.23[b,i]	9.84 ± 0.23[iii]	42.34 ± 0.16[b,ii]	13.45 ± 0.38[ii]	68.21 ± 1.52[b]	7.37 ± 0.02[b,ii]	46.94 ± 4.63[i]	2339.62 ± 37.50[i]
Tinta B	No	L	10	12	0.11 ± 0.00[a,i]	1.12 ± 0.02[iv]	36.32 ± 0.17[ii]	32.26 ± 0.69[b,i]	31.40 ± 0.52[a,i]	45.41 ± 0.34[b,i]	44.78 ± 0.43[a,i]	6.22 ± 0.10[iv]	45.22 ± 0.41[a,i]	7.91 ± 0.21[ii]	78.91 ± 2.00[a]	5.01 ± 0.89[a,i]	106.19 ± 12.68[i]	2300.27 ± 16.29[i]
Tinta B	No	I	10	12	0.11 ± 0.00[a,i]	1.07 ± 0.00[iv]	35.77 ± 0.17[ii]	33.18 ± 0.20[a,i]	31.03 ± 0.37[b,i]	44.17 ± 0.04[b,i]	44.78 ± 0.70[a,i]	6.51 ± 0.13[iv]	45.26 ± 0.70[a,i]	8.27 ± 0.35[ii]	80.50 ± 3.29[a]	11.03 ± 2.97[c]	106.50 ± 4.20[i]	2340.10 ± 26.67[i]
Tinta B	No	H	10	12	0.11 ± 0.00[b,i]	1.10 ± 0.00[iv]	36.20 ± 0.44[ii]	32.76 ± 0.39[a,i]	31.02 ± 0.84[c,i]	42.23 ± 0.19[a,i]	46.02 ± 0.24[b,i]	6.93 ± 0.27[iv]	46.54 ± 0.19[b,i]	8.57 ± 0.38[ii]	79.33 ± 0.70[b]	5.21 ± 0.80[b,i]	91.92 ± 0.14[i]	2306.56 ± 16.29[i]
Tinta B	No	L	25	12	0.10 ± 0.00[a,i]	1.12 ± 0.01[iv]	36.52 ± 0.11[ii]	32.42 ± 0.40[b,i]	31.04 ± 0.28[a,i]	49.87 ± 0.00[b,ii]	39.34 ± 0.29[a,i]	11.31 ± 0.20[i]	40.93 ± 0.20[a,i]	16.03 ± 0.51[i]	74.50 ± 3.77[a]	9.36 ± 0.70[a,ii]	61.53 ± 0.26[i]	2355.82 ± 14.82[i]
Tinta B	No	I	25	12	0.11 ± 0.00[a,i]	1.13 ± 0.00[iv]	36.68 ± 0.22[ii]	32.31 ± 0.19[a,i]	31.00 ± 0.42[b,i]	47.94 ± 0.09[b,ii]	39.57 ± 0.28[a,i]	11.48 ± 0.37[i]	41.20 ± 0.16[a,i]	16.19 ± 0.60[i]	76.50 ± 0.94[a]	12.15 ± 2.18[c]	49.11 ± 0.87[i]	2350.05 ± 17.04[i]
Tinta B	No	H	25	12	0.10 ± 0.00[b,i]	1.12 ± 0.01[iv]	36.52 ± 0.11[ii]	32.42 ± 0.40[a,i]	31.04 ± 0.28[c,i]	44.79 ± 0.28[a,i]	40.75 ± 0.12[b,i]	10.87 ± 0.03[i]	42.17 ± 0.12[b,ii]	14.94 ± 0.00[i]	77.75 ± 3.42[b]	8.40 ± 0.23[b,ii]	41.38 ± 2.36[i]	2323.90 ± 14.14[i]

[1] Results are reported as the mean ± standard deviation (SD) (n = 2). Analyses of variance (ANOVAs) tested the influence of the main effects. Duncan's new multiple range test (MRT) tested the differences bewleen individual means (p ≤ 0.05 is significant).

[2] Tinta B = Tinta Barrocca. Different shaded blocks in each column (background filled colour) indicate significance between cultivars with respect to the response variables.

[3] En = Enzyme. Different colour in font indicates significance in terms of the effect enzyme treatment on the response variable. Yes = Addition of enzyme treatment; No = without enzyme treatment.

[4] A = Aldehyde with L = Low aldehyde level (50 mg.L^{-1}); I = Intermediate aldehyde level (No commercial acetaldehyde added); H = High aldehyde level (Normal wine spirits spiked with 450 mg/L^{-1} acetaldehyde). [a-c] The differer in the same column indicate significant differences (p ≤ 0.05).

[5] Temp = Storage Temperature : *10* and *25*°C. Font in Italics indicates no significant difference (p < 0.05). Temperature had a significant effect on all response variable except for **Sensory Colour** and **Total Polyphenols**.

[6] T = Time intervals of tests: 0, 3, 6, 9 and 12 months. [i-iv] The different superscripts indicate significant differences due to different storage time intervals (p < 0.05).

Table 4.1 continued.

Cultivar[2]	En[3]	A[4]	Temp (°C)[5]	T[6] (h)	Colour Intensity	Tonality	% yellow	% red	% blue	L*	a*	b*	C	h	Sensory colour	ΔE*	Total Anthocyanins	Total Polyphenols
Tinta B	Yes	L	10	0	2.47 ± 0.22^{a,iv}	0.54 ± 0.15^i	30.37 ± 6.02^i	56.47 ± 4.86^{b,ii}	13.14 ± 0.15^{a,i}	46.12 ± 0.06^{b,ii}	47.81 ± 0.19^{a,ii}	3.82 ± 0.32^i	45.12 ± 0.18^{a,i}	62.21 ± 0.29^{ii}	74.35 ± 2.32^{a,}	0.00 ± 0.00^{ai}	231.11 ± 0.00^{iv}	0,0000
Tinta B	Yes	I	10	0	2.59 ± 0.06^{a,iv}	0.64 ± 0.00^i	34.37 ± 0.81^i	53.24 ± 0.12^{a,ii}	12.37 ± 0.04^{b,i}	47.58 ± 0.04^{b,ii}	48.18 ± 0.03^{a,ii}	4.83 ± 0.14^i	45.49 ± 0.04^{a,i}	63.49 ± 0.07^{ii}	73.00 ± 3.63^{a,}	0.00 ± 0.00^{ci}	235.12 ± 1.89^{iv}	2518.85 ± 94.68^{iii}
Tinta B	Yes	H	10	0	2.78 ± 0.00^{b,ii}	0.63 ± 0.00^i	34.01 ± 0.01^i	53.36 ± 0.30^{a,ii}	12.62 ± 0.15^{c,i}	48.31 ± 0.14^{a,ii}	48.32 ± 0.03^{a,ii}	4.63 ± 0.28^i	45.62 ± 0.03^{b,i}	64.05 ± 0.05^{ii}	77.85 ± 0.40^{b,}	0.00 ± 0.00^{bi}	222.26 ± 5.43^{iv}	2542.97 ± 56.73^{iii}
Tinta B	Yes	L	25	0	2.47 ± 0.22^{a,i}	0.54 ± 0.15^i	30.37 ± 6.02^i	56.47 ± 4.86^{b,ii}	13.14 ± 0.15^{a,i}	46.12 ± 0.06^{b,ii}	47.81 ± 0.19^{a,ii}	3.82 ± 0.32^i	45.12 ± 0.18^{a,i}	62.21 ± 0.29^{ii}	74.35 ± 2.32^{a,}	0.00 ± 0.00^{ai}	231.11 ± 0.00^{iv}	2534.30 ± 15.33^{iii}
Tinta B	Yes	I	25	0	2.59 ± 0.06^{a,i}	0.64 ± 0.00^i	34.37 ± 0.81^i	53.24 ± 0.12^{a,ii}	12.37 ± 0.04^{b,i}	47.58 ± 0.04^{b,ii}	48.18 ± 0.03^{a,ii}	4.83 ± 0.14^i	45.49 ± 0.04^{a,i}	63.49 ± 0.07^{ii}	73.00 ± 3.63^{a,}	0.00 ± 0.00^{ci}	235.12 ± 1.89^{iv}	2518.85 ± 94.68^{iii}
Tinta B	Yes	H	25	0	2.78 ± 0.00^{b,i}	0.63 ± 0.00^i	34.01 ± 0.01^i	53.36 ± 0.33^{a,ii}	12.62 ± 0.15^{c,i}	48.31 ± 0.14^{a,ii}	48.32 ± 0.03^{b,ii}	4.63 ± 0.28^i	45.62 ± 0.03^{b,i}	64.05 ± 0.05^{iii}	77.85 ± 0.40^{b,}	0.00 ± 0.00^{bi}	222.26 ± 5.43^{iv}	2542.97 ± 56.73^{iii}
Tinta B	Yes	L	10	3	1.40 ± 0.01^{a,iii}	0.68 ± 0.00^{iii}	35.46 ± 0.04^{ii}	51.56 ± 0.16^{b,ii}	12.97 ± 0.12^{a,}	42.77 ± 0.01^{b,ii}	46.82 ± 0.21^{a,ii}	4.64 ± 0.14^i	47.06 ± 0.24^{a,ii}	5.66 ± 0.19^i	63.64 ± 1.51^{a,}	1.34 ± 0.12^{aii}	100.62 ± 10.23^{iii}	2260.63 ± 70.57^i
Tinta B	Yes	I	10	3	1.41 ± 0.00^{a,ii}	0.67 ± 0.00^{iii}	35.24 ± 0.03^{ii}	51.89 ± 0.12^{a,ii}	12.86 ± 0.09^{b,}	41.56 ± 1.59^{b,ii}	47.06 ± 0.31^{a,ii}	4.93 ± 0.38^i	47.32 ± 0.35^{a,ii}	5.97 ± 0.41^i	66.35 ± 3.74^{a,}	2.35 ± 0.17^{cii}	105.63 ± 9.10^{iii}	2293.40 ± 95.53^i
Tinta B	Yes	H	10	3	1.47 ± 0.01^{b,ii}	0.67 ± 0.00^{iii}	34.86 ± 0.15^{ii}	51.76 ± 0.08^{a,ii}	13.37 ± 0.07^{c,i}	40.77 ± 0.02^{a,ii}	47.78 ± 0.05^{b,ii}	5.38 ± 0.22^i	48.08 ± 0.02^{b,ii}	6.42 ± 0.27^i	73.92 ± 1.11^{b,}	1.07 ± 0.02^{bi}	95.06 ± 12.50^{iii}	2313.05 ± 188.91^i
Tinta B	Yes	L	25	3	1.31 ± 0.02^{a,ii}	0.74 ± 0.00^{iii}	37.21 ± 0.03^{ii}	49.75 ± 0.13^{b,ii}	13.02 ± 0.09^{a,}	41.94 ± 0.26^{a,ii}	44.70 ± 0.16^{a,ii}	6.55 ± 0.27^i	45.18 ± 0.21^{a,ii}	8.34 ± 0.31^i	66.00 ± 0.80^{a,}	4.21 ± 0.04^{ai}	88.63 ± 10.45^{iii}	2154.27 ± 37.06^i
Tinta B	Yes	I	25	3	1.35 ± 0.01^{a,ii}	0.74 ± 0.00^{iii}	37.20 ± 0.02^{ii}	49.83 ± 0.07^{b,ii}	12.96 ± 0.04^{b,}	40.48 ± 0.66^{b,ii}	44.97 ± 0.08^{a,ii}	6.71 ± 0.00^i	45.47 ± 0.08^{b,ii}	8.49 ± 0.00^i	65.78 ± 4.54^{a,}	4.08 ± 0.09^{ci}	95.92 ± 10.75^{iii}	2250.04 ± 208.16^i
Tinta B	Yes	H	25	3	1.33 ± 011^{b,iii}	0.72 ± 0.01^{ii}	36.42 ± 0.24^{ii}	49.98 ± 0.47^{a,ii}	13.59 ± 0.22^{c,i}	39.34 ± 0.15^{a,ii}	45.20 ± 0.60^{b,ii}	6.41 ± 0.51^i	45.65 ± 0.28^{b,ii}	8.07 ± 0.53^i	68.50 ± 0.29^{b,}	3.74 ± 0.60^{bi}	101.55 ± 0.86^{iii}	2354.39 ± 59.17^i
Tinta B	Yes	L	10	6	1.26 ± 0.02^{a,ii}	0.68 ± 0.01^{ii}	35.23 ± 0.30^{ii}	51.28 ± 0.22^{b,ii}	13.48 ± 0.08^{a,}	40.84 ± 0.06^{b,i}	46.96 ± 0.04^{a,ii}	5.57 ± 0.07^{ii}	47.29 ± 0.03^{a,ii}	6.77 ± 0.09^i	71.43 ± 0.80^{a,}	2.17 ± 0.31^{aiii}	78.23 ± 1.13^{ii}	2486.94 ± 61.45^{ii}
Tinta B	Yes	I	10	6	1.31 ± 0.00^{a,ii}	0.67 ± 0.00^{ii}	34.81 ± 0.10^{ii}	51.56 ± 0.11^{a,ii}	13.62 ± 0.21^{b,}	38.90 ± 0.02^{b,i}	47.00 ± 0.09^{a,ii}	5.45 ± 0.14^{ii}	47.31 ± 0.10^{a,ii}	6.62 ± 0.16^i	73.21 ± 1.11^{a,}	1.50 ± 0.15^{cii}	78.42 ± 1.92^{ii}	2452.50 ± 35.22^{ii}
Tinta B	Yes	H	10	6	1.35 ± 0.01^{b,ii}	0.66 ± 0.00^{ii}	34.46 ± 0.08^{ii}	51.82 ± 0.05^{a,ii}	13.71 ± 0.03^{c,i}	38.76 ± 0.20^{a,i}	47.86 ± 0.22^{b,ii}	6.00 ± 0.17^{ii}	48.23 ± 0.24^{b,ii}	7.15 ± 0.16^i	74.71 ± 0.20^{b,}	1.77 ± 0.57^{biii}	81.64 ± 3.49^{ii}	2280.28 ± 173.86^{ii}
Tinta B	Yes	L	25	6	1.24 ± 0.00^{a,ii}	0.75 ± 0.00^{iii}	36.96 ± 0.04^{ii}	49.25 ± 0.13^{b,ii}	13.78 ± 0.18^{a,i}	41.69 ± 0.17^{b,ii}	43.05 ± 0.65^{a,ii}	7.79 ± 0.51^i	43.75 ± 0.78^{a,ii}	10.25 ± 0.51^i	71.14 ± 0.00^{a,}	6.54 ± 0.48^{aiii}	136.87 ± 2.88^{ii}	2392.09 ± 105.67^{ii}
Tinta B	Yes	I	25	6	1.30 ± 0.03^{a,ii}	0.73 ± 0.03^{ii}	36.36 ± 0.82,^{i}	49.77 ± 1.00^{a,ii}	13.85 ± 0.18^{b,}	39.92 ± 0.02^{b,i}	44.14 ± 0.02^{a,ii}	8.34 ± 0.00^i	44.92 ± 0.02^{a,ii}	10.70 ± 0.00^i	69.71 ± 0.81^{a,}	5.50 ± 0.09^{cii}	129.57 ± 4.63^{ii}	2502.84 ± 203.83^{ii}
Tinta B	Yes	H	25	6	1.34 ± 0.00^{b,ii}	0.72 ± 0.00^{iii}	35.94 ± 0.14^{ii}	49.44 ± 0.15^{a,ii}	14.61 ± 0.12^{c,i}	39.31 ± 0.07^{a,i}	44.44 ± 0.29^{b,ii}	7.90 ± 0.18^i	45.14 ± 0.03^{b,ii}	10.08 ± 0.16^i	76.00 ± 0.19^{b,}	5.08 ± 0.31^{bii}	148.62 ± 6.56^{ii}	2441.90 ± 95.17^{ii}
Tinta B	Yes	L	10	9	1.20 ± 0.08^{a,ii}	0.63 ± 0.00^{ii}	33.60 ± 0.15^i	52.99 ± 0.19^{b,ii}	13.40 ± 0.35^{a,}	40.41 ± 0.26^{a,i}	46.05 ± 1.01^{a,i}	5.55 ± 0.53^{ii}	46.38 ± 1.08^{a,i}	6.87 ± 0.49^i	67.00 ± 1.61^{a,}	2.83 ± 0.62^{a,iv}	97.84 ± 3.84^i	2430.10 ± 147.18^i
Tinta B	Yes	I	10	9	1.25 ± 0.04^{a,ii}	0.62 ± 0.00^{ii}	33.39 ± 0.12^i	53.09 ± 0.74^{a,ii}	13.51 ± 0.05^{b,}	38.82 ± 0.26^{b,i}	46.56 ± 0.45^{a,i}	6.04 ± 0.23^{iii}	47.25 ± 0.00^{a,i}	7.34 ± 0.28^i	69.50 ± 2.31^{a,}	2.02 ± 0.57^{c,iv}	97.35 ± 0.87^i	2435.82 ± 14.70,^{i}
Tinta B	Yes	H	10	9	1.43 ± 0.13^{b,ii}	0.63 ± 0.01^{ii}	32.93 ± 0.67^i	51.95 ± 0.11^{a,ii}	15.11 ± 0.55^{c,i}	39.11 ± 0.21^{a,ii}	47.38 ± 0.07^{b,i}	6.20 ± 0.40^{iii}	47.78 ± 0.01^{b,i}	7.46 ± 0.48^i	70.86 ± 0.60^{b,}	2.24 ± 0.11^{b,iv}	79.35 ± 5.68^i	2307.90 ± 2.94,^{i}
Tinta B	Yes	L	25	9	1.15 ± 0.02^{a,ii}	0.73 ± 0.00^{ii}	36.64 ± 0.02^i	49.73 ± 0.00^{b,ii}	13.61 ± 0.01^{a,i}	42.80 ± 0.31^{b,ii}	42.14 ± 0.82^{a,i}	10.91 ± 0.50^{ii}	43.54 ± 0.89^{a,ii}	14.58 ± 0.28^{ii}	67.07 ± 0.90^{a,}	9.22 ± 0.11^{a,iv}	38.84 ± 4.20^i	2376.54 ± 13.23^i
Tinta B	Yes	I	25	9	1.17 ± 0.01^{a,ii}	0.73 ± 0.00^{ii}	36.61 ± 0.09^i	49.84 ± 0.21^{a,ii}	13.54 ± 0.30^{b,}	41.28 ± 0.03^{b,ii}	41.38 ± 1.50^{a,i}	10.56 ± 0.00^{ii}	42.71 ± 1.44^{a,ii}	14.32 ± 0.50^{ii}	67.50 ± 5.75^{a,}	10.11 ± 2.41^{c}	37.04 ± 7.78^i	2384.34 ± 3.67^i
Tinta B	Yes	H	25	9	1.24 ± 0.01^{b,ii}	0.72 ± 0.00^{ii}	36.03 ± 0.05^i	49.89 ± 0.02^{a,ii}	14.06 ± 0.03^{c,i}	41.01 ± 0.02^{a,ii}	42.62 ± 0.28^{b,i}	9.89 ± 0.18^{ii}	43.75 ± 0.26^{b,ii}	13.07 ± 0.24^{ii}	67.36 ± 2.12^{b,}	7.82 ± 0.52^{b,iv}	31.72 ± 5.50^i	2335.98 ± 69.12^i
Tinta B	Yes	L	10	12	0.11 ± 0.00^{a,i}	1.12 ± 0.02^{iv}	36.20 ± 0.44^{iii}	32.32 ± 0.21^{b,i}	31.46 ± 0.22^{a,i}	39.79 ± 0.28^{b,i}	45.36 ± 1.10^{a,i}	5.84 ± 0.85,^{v}	45.74 ± 1.80^{a,i}	7.33 ± 0.79^i	79.33 ± 1.18^{a,}	4.07 ± 1.59^{a,iv}	97.16 ± 4.63^i	2389.23 ± 0.17^i
Tinta B	Yes	I	10	12	0.11 ± 0.00^{a,i}	1.12 ± 0.02^{iv}	36.57 ± 0.51^{iii}	32.59 ± 0.23^{a,i}	30.83 ± 0.28^{b,i}	38.84 ± 0.21^{b,i}	46.40 ± 0.72^{a,i}	6.68 ± 0.61^{iv}	46.89 ± 0.08^{a,i}	8.19 ± 0.61^{ii}	77.75 ± 2.00^{a,}	2.93 ± 0.16^{c,iv}	100.93 ± 0.34^i	2383.07 ± 118.56^i
Tinta B	Yes	H	10	12	0.11 ± 0.00^{b,i}	1.10 ± 0.03^{iv}	36.04 ± 0.99^{iii}	32.61 ± 0.19^{a,i}	31.33 ± 0.79^{c,i}	38.98 ± 0.18^{a,i}	47.14 ± 0.24^{a,i}	7.16 ± 0.45^{iv}	47.69 ± 0.16^{b,i}	8.63 ± 0.58^{ii}	80.91 ± 1.53^{b,}	3.35 ± 0.12^{b,i}	88.01 ± 4.28^i	2408.22 ± 14.82^i
Tinta B	Yes	L	25	12	0.11 ± 0.00^{a,i}	1.12 ± 0.01^{iv}	36.40 ± 0.16^{iii}	32.45 ± 0.44^{b,i}	31.13 ± 0.23^{a,i}	43.19 ± 0.26^{a,i}	41.68 ± 0.73^{a,i}	11.73 ± 0.21^{iv}	43.30 ± 0.76^{a,ii}	15.72 ± 0.00^i	78.83 ± 0.47^{a,}	10.13 ± 0.27^a	43.42 ± 0.52^i	2427.61 ± 18.52^i
Tinta B	Yes	I	25	12	0.10 ± 0.00^{a,i}	1.14 ± 0.04^{iv}	36.96 ± 0.59^{iii}	32.23 ± 0.64^{a,i}	30.80 ± 0.05^{b,i}	42.08 ± 0.71^{b,i}	41.87 ± 0.16^{a,i}	11.60 ± 0.02^{iv}	43.45 ± 0.16^{a,i}	15.48 ± 0.02^{i}	77.41 ± 1.29^{a,}	9.52 ± 0.24^{c,iv}	47.43 ± 3.06^i	2378.35 ± 12.60^i
Tinta B	Yes	H	25	12	0.11 ± 0.00^{b,i}	1.10 ± 0.04^{iv}	36.20 ± 0.44^{iii}	32.76 ± 0.39^{a,i}	31.02 ± 0.84^{c,i}	41.84 ± 0.19^{a,i}	42.19 ± 0.35^{b,i}	10.61 ± 0.31^{i}	43.51 ± 0.26^{b,ii}	14.11 ± 0.50^i	78.88 ± 2.42^{b,}	8.69 ± 0.72^{b,iv}	41.31 ± 0.70^i	2378.88 ± 29.64^i

[1]Results are reported as the mean ± standard deviation (SD) (n = 2). Analyses of variance (ANOVAs) tested the influence of the main effects. Duncan's new multiple range test (MRT) tested the differences beween individual means (p ≤ 0.05 is significant).

[2]Tinta B = Tinta Barrocca. Different shaded blocks in each column (background filled colour) indicate significance between cultivars with respect to the response variables.

[3]En = Enzyme. Different colour in font indicates significance in terms of the effect enzyme treatment on the response variable. Yes = Addition of enzyme treatment; No = without enzyme treatment.

[4]A = Aldehyde with L = Low aldehyde level (50 mg.L⁻¹); I = Intermediate aldehyde level (normal wine spirits); H = High aldehyde level (Normal wine spirits spiked with 450 mg/L⁻¹ acetaldehyde). $^{a-c}$The different superscripts in the same column indicate significant differences (p ≤ 0.05).

[5]Temp = Storage Temperature : 10 and 25°C. Font in Italics indicate no significant difference (p < 0.05). Temperature had a significant effect on all response variable except for **Sensory Colour** and **Total Polyphenols**.

[6]T = Time intervals of tests: 0, 3, 6, 9 and 12 months. $^{i-iv}$The different superscripts indicate significant differences due to different storage time intervals (p < 0.05).

the must was treated with an enzyme, as discussed in more detail in the following paragraph.

Pectolytic enzyme treatment had a significant influence ($p < 0.05$) on colour intensity, %blue, L*, a*, C and h values (Table 4.1). The addition of pectolytic enzyme had a significant effect ($p < 0.05$) on colour intensity with the values ranging from 0.10 ± 0.00 to 2.43 ± 0.00 for Pinotage (no enzyme added) and 0.10 ± 0.00 to 2.87 ± 0.00 for Pinotage (enzyme added). The values for Tinta Barrocca were 0.10 ± 0.00 to 2.66 ± 0.00 (no enzyme added) and 0.10 ± 0.00 to 2.78 ± 0.00 (enzyme added) (Table 4.1). Hence, contrary to the aforementioned expectations, the highest colour intensity values were observed for Pinotage with enzyme added and not for Tinta Barrocca. Since %blue was the only other component of the Glories values that was significantly influenced by enzyme treatment (Table 4.1), it is difficult to extrapolate this to observed colour or to colour intensity.

The addition of pectolytic enzyme had a decreasing effect on the CIELab lightness value (L*) ($p < 0.05$; Table 4.1), with values ranging from 42.23 ± 0.19 to 50.56 ± 0.61 for Pinotage (no enzyme added) and in the range of 38.76 ± 0.20 to 48.31 ± 0.14 Pinotage (enzyme added). The values for Tinta Barrocca was 42.23 ± 0.19 to 57.19 ± 3.59 (no enzyme added) and 38.76 ± 0.20 to 48.31 ± 0.14 (enzyme added). Hence, contrary to the observation for colour intensity, the L*-value for Tinta Barrocca (enzyme added) was not higher than that for Pinotage (enzyme added) (Table 4.1). Hence, Tinta Barrocca samples (no enzyme added) were lighter in colour than the Pinotage samples (no enzyme added).

Apart from the aforementioned luminance or lightness component (L* value, ranging from 0 to 100), the L*a*b* colour also consists of two chromatic components (ranging from -120 to + 120), the two components being a* (from green to red) and b* (from blue to yellow) (Yam & Papadakis, 2004). Addition of pectolytic enzyme had an increasing effect ($p < 0.05$) on the red component (a*) ranging from 38.36 ± 0.00 to 45.81 ± 0.01 for Pinotage (no enzyme added) and in the range of 41.02 ± 0.48 to 48.75 ± 0.01 (enzyme added). For Tinta Barrocca the values are as follows: 39.34 ± 0.29 to 46.74 ± 0.07 (no enzyme added) and 41.38 ± 1.50 to 48.18 ± 0.03 (enzyme added). As can be seen from the aforementioned values, the addition of enzyme had a significantly increasing effect on redness (a*) ($p < 0.05$; Table 4.1) for both Tinta Barrocca and Pinotage. Albeit that the cultivar did not influence a* significantly ($p > 0.05$), the a* values for Tinta Barroca were numerically higher than for Pinotage, contributing to increased redness.

The addition of pectolytic enzyme had an increasing effect on chroma (C). The values ranged from 39.07 ± 0.00 to 46.00 ± 0.09 for Pinotage (no enzyme added) and 41.35 ± 0.45 to 48.92 ± 0.02 for Pinotage (enzyme added). The Tinta Barrocca values for (C) where 37.88 ± 2.55 to 47.07 ± 0.17 (no enzyme added) and 42.71 ± 1.44 to 48.23 ± 0.24 (enzyme added) (Table 4.1). The addition of pectolytic enzyme also had an increasing effect on hue (h) with values ranging from 2.56 ± 0.12 to 59.03 ± 0.00 for Pinotage (no enzyme added) and 2.91 ± 0.02 to 61.68 ± 0.11 Pinotage (enzyme added). Tinta Barroca values ranging from 4.86 ± 0.50 to 58.61 ± 0.48 (no enzyme added) and 5.66 ± 0.19 to 64.05 ± 0.05 (enzyme added). Hue is the attribute of colour that is related to the perceived colours: red, yellow, green and blue or a combination of two of them (Pérez-Magariño & González-San José, 2002). L*, C and h parameters are related to the psycophysical attributes of colour, i.e. perceived or visual colour. Interpreting the hue scale on this basis, one unit (the unit being degrees) is more to the red side of the scale, whereas 60 is more to the yellow side of the hue scale. Hence, the addition of pectolytic enzyme resulted in mean values ranging between 2 and 65 (Table 4.1), indicating a less bright red colour but rather a red colour more to the orange-yellow side on the hue scale.

Commercially available enzymes have been widely used in the oenological industry in wine producing countries to improve the important characteristics of wines, such as aroma and colour (Espejo & Armada, 2010). Hence, these findings were in line with the common expectation in the wine industry that the addition of enzyme treatment will improve the characteristics of wine, including wine colour.

Different aldehyde levels had a significant influence ($p < 0.05$) on colour intensity (CI), %red, %blue, L*, a*, C, sensory colour and ΔE^* (Table 4.1). The addition of the higher level of acetaldehyde resulted in significantly higher ($p < 0.05$) CI, L*, a*, C and sensory colour measurements than the lower and intermediate level of acetaldehyde level. However, the lower level of acetaldehyde resulted in significantly ($p < 0.05$) higher levels of %red than intermediate and higher levels of acetaldehyde. Aldehydes, especially acetaldehyde, interact with anthocyanins and flavanols, changing the red wine colour from red towards tawny during ageing and storage which means it also increases the colour intensity causing the shift from bright red to reddish brown hues due to the progressive displacement of anthocyanins by more stable pigments (Pissara *et al.*, 2005). As seen from Table 4.1, colour intensity is increased as well as C which are in agreement, since an increase in C visually results in a more intense colour. The addition of high and intermediate

level of acetaldehyde also had a significantly (p < 0.05) increasing effect on sensory colour and a decreasing effect on ΔE^*, whereas the addition of lower level of acetaldehyde, had a decreasing effect on sensory colour and an increasing effect on ΔE^* (Fig. 4.1). This then indicates more stability in colour in response to intermediate and high aldehyde levels, since the least change in ΔE^* equals greater colour stability. Moreover, the trends in sensory colour and ΔE^* agree in this instance (Fig. 4.1 and Fig 4.2).

Temperature has a significant influence (p < 0.05) on most of the response variables (Table 4.1) with the exception of sensory colour as well as total polyphenols. It is expected that port wine stored at a lower temperature would have more stable colour and ageing potential than a wine stored at or above 25°C. This was confirmed in the first part of this study where 35°C was included as a storage temperature and it affected the port wine samples negatively. The storage at 10°C compared to storage at 25°C led to significantly higher CI, %red, L*, a* and ΔE^* (Table 4.1). Storage at 10°C effected the least change over time for most of the response variables indicating greater colour stability at a lower storage temperature.

Storage time had a significant influence (p < 0.05) on all response variables (Table 4.1). Colour intensity and L* were significantly decreased (p < 0.05) when storage time was increased between 6 – 12 months, whereas %blue and tonality were significantly increased (p < 0.05) at 3 – 6 months and again at 12 months of storage. In terms of the other Glories parameters, %yellow where significantly decreased (p < 0.05) at 9 months of storage and %red showed a significant decrease at 12 months of storage. Redness (a*), b* and h significantly decreased (p < 0.05) between 3 – 12 months of storage time (Table 4.1). C* significantly decreased at 12 months of storage and Total anthocyanins significantly decreased (p < 0.05) at 12 months of storage.

Figure 4.1 depicts ΔE^* for all the treatments in (overall) port wine colour over the 12-month storage period. ΔE^* at time zero had a value of zero. This is due to the fact that at time zero no colour change occurred as yet. Colour differences (ΔE^*) between two colour points in the CIELab space are calculated as the Euclidean distance between their locations in the three-dimentional space defined by L*, a* and
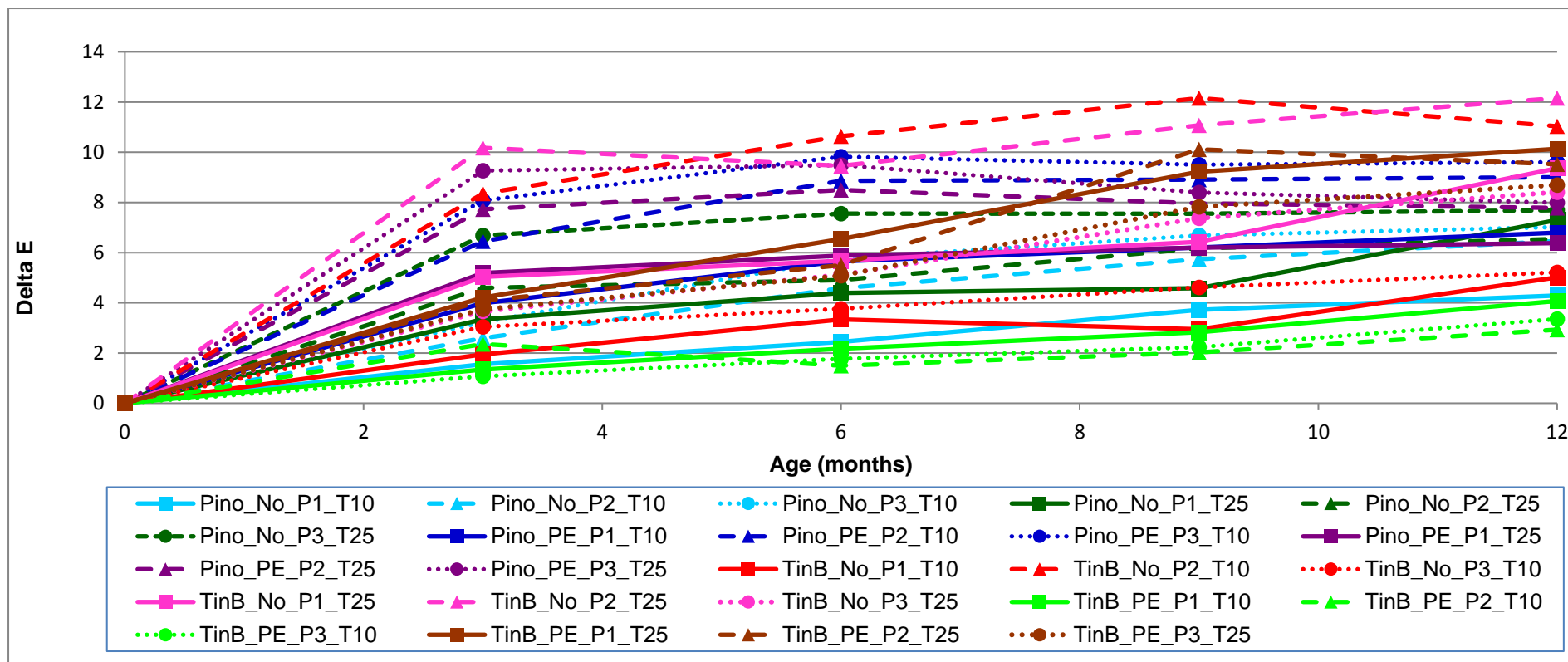
Figure 4.1   A representation of the effect of different treatments over storage time for Delta E.

Pino, Pinotage grapes; TinB, Tinta Barrocca grapes; PE, Pectolytic Enzyme; No, No Pectolytic Enzyme; T10, Storage temperature at 10 ^{0}C; T25, Storage temperature at 25^{0}C, P1, P2, P3, Aldehyde levels.

b*. ΔE* values above 2.7 CIELab units represent chromatic changes that can be perceived by the human eye (García-Marino *et al.*, 2013). Hence, after 12 months of storage time, from this point of view, Tinta Barrocca (TinB) with pectolytic enzyme (PE), intermediate aldehyde level (P2) and at storage temperature of 10°C (T10) (TinB_PE_P2_T10), showed the highest colour stability, followed by TinB_PE_P3_T10 (P3 = high aldehyde level) and TinB_PE_P1_T10 (P1 = low aldehyde level) (Fig 4.1) These results are also congruent with the results depicted in Table 4.1 where the ΔE* values become higher over time, indicating that storage time effects the biggest change in colour. Although most of the more stable treatments identified above are with Tinta Barrocca as a cultivar, cultivar did not affect ΔE* significantly (p > 0.05; Table 4.1).

The effect of the different treatments over storage time on sensory colour is depicted in Figure 4.2. In general terms, the plots show a decrease in sensory colour scores between 0 and 3 months of storage and from there an increase in sensory colour scores at 6 months, followed by a decrease at 9 months of storage and then another increase in sensory colour scores at 12 months storage time. These anomalies could be due to the fact that it was not possible to supply reference samples of the same port wine to the panellists to compare it with time zero at each tasting time interval. However, the panellists were highly trained and port wine experts and could be relied on to use their experience to rate each sample on merit. Moreover, in agreement with the results observed in Fig. 4.1, Tinta Barrocca with pectolytic enzyme added, addition of aldehyde at the highest level (P3) and at storage 10°C (TinB_PE_P3_T10) as well as TinB_No_P2_T10, TinB_PE_P1_T10 and Tin B_PE_P2_T10 resulted in the highest scores for sensory colour after 12 months of storage. A similarity in the responses to the treatments is observed when comparing the plots for (Fig. 4.2) sensory colour scores and the ΔE* values over time (Fig 4.1). The treatments that resulted in the least change over time in Figure 4.1 (ΔE*) namely TinB_PE_P3_T10, TinB_PE_P2_T10 as well as TinB_PE_P1_T10 and Pino_No_P1_T10 correspond with the treatments that were the best four in terms of observed sensory colour (Fig. 4.2).

The effect of the various treatments on redness (a*) over time is depicted in Figure 4.3. Redness is one of the most important characteristics of ruby port style wines and therefore also a very important factor in this study.

Figure 4.2 A representation of the different treatments over storage time for Sensory Colour.

Pino, Pinotage grapes; TinB, Tinta Barrocca grapes; PE, Pectolytic Enzyme; No, No Pectolytic Enzyme; T10, Storage temperature at 10 ^{0}C; T25, Storage temperature at 25^{0}C; P1, P2, P3, Aldehyde levels.
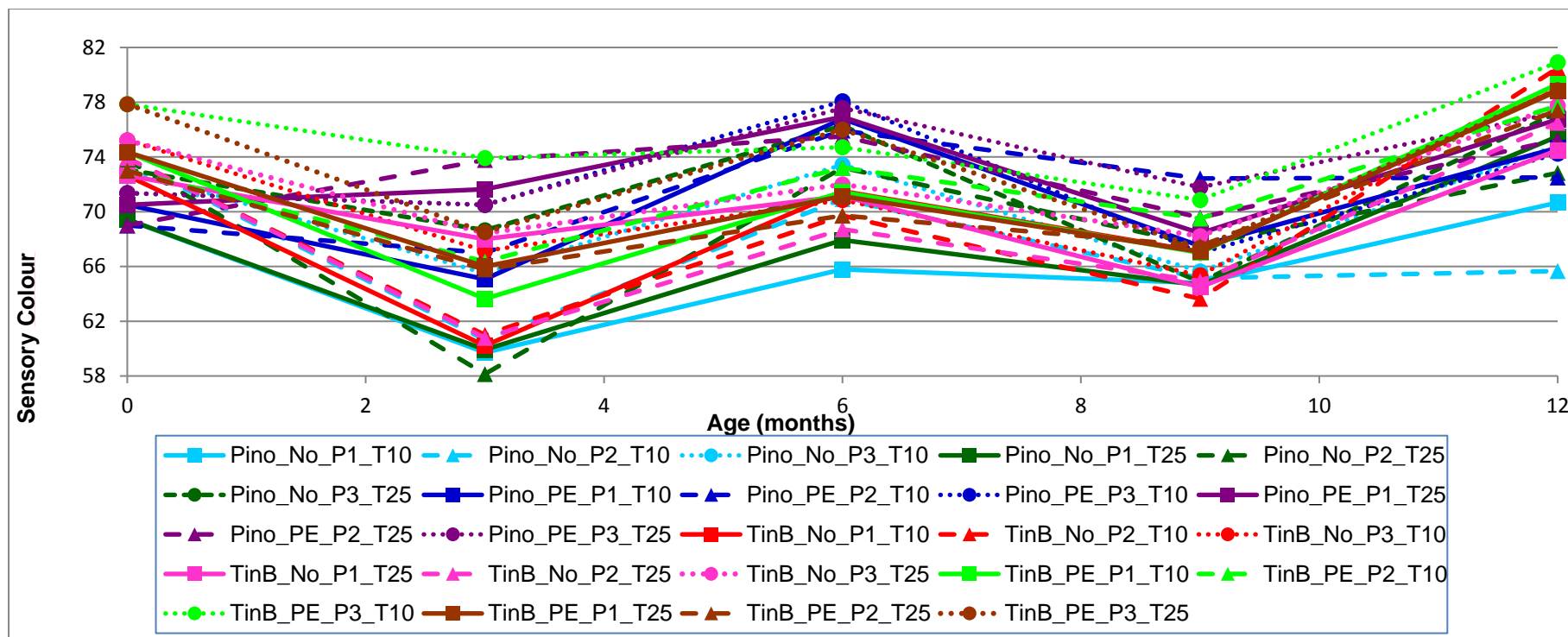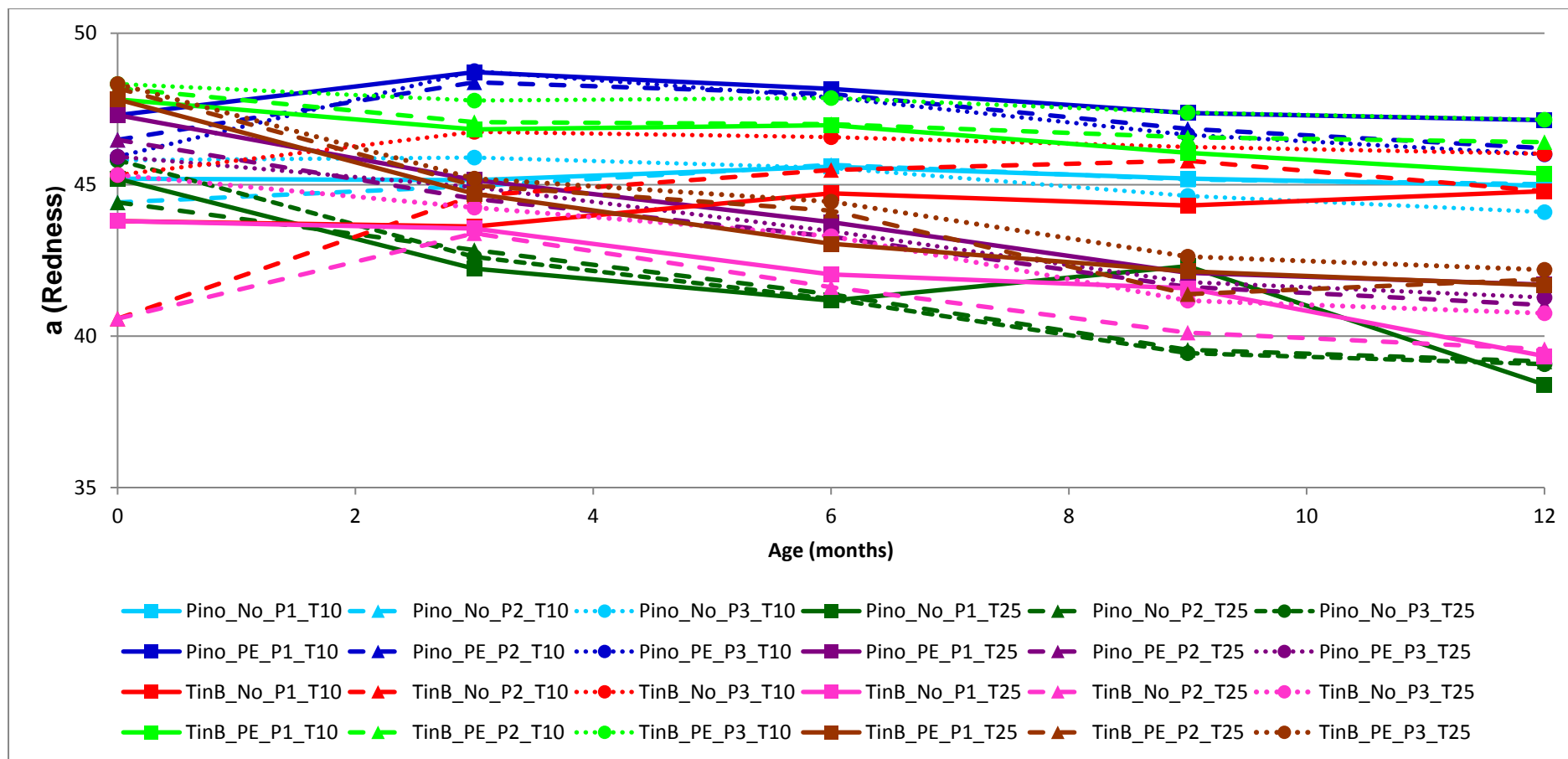
Figure 4.3   A representation of the effect of different treatments over storage time for Redness (a*).

Pino, Pinotage grapes; TinB, Tinta Barrocca grapes; PE, Pectolytic Enzyme; No, No Pectolytic Enzyme; T10, Storage temperature at 10 ^{0}C; T25, Storage temperature at 25^{0}C, P1, P2, P3, Aldehyde levels.

As evident in Table 4.1, the addition of enzyme had a significantly increasing effect on a* (p < 0.05), while the same trend is seen in Fig 4.3. Some of the treatments that resulted in the least change in redness over time included the addition of enzyme. These were: Tinta Barrocca with pectolytic enzyme added, intermediate aldehyde level (P2) and storage temperature 10°C (TinB_PE_P2_T10) and Pino_PE_P1_T10. In terms of colour stability the treatments indicating the lowest ΔE^* values are also treatments with enzyme addition (Fig 4.1) which is also in agreement with the aforementioned trend concerning a*. These treatments include TinB_PE_P2_T10, TinB_PE_P3_T10 and TinB_PE_P1_T10, indicating that enzyme treatment resulted in the least change in colour over time, therefore the highest colour stability.

When the data was combined to establish the relative influence of enzyme, age and cultivar, the DA plot (Fig. 4.4) indicates that age affected the groupings more than cultivar and enzyme. Time intervals zero and 12 months are clearly separate groupings, whereas the other time intervals are grouped together. However, it is important to consider the information depicted in the trend plots (Figures 4.1 – 4.3) since those made it clear that enzyme as well as age had an effect on colour stability.

Cultivar as well as temperature affected most of the groupings at all ages, especially at age 12 months in terms of their influence on colour stability and overall quality of the port wine, as depicted in the DA plot depicting the influence of cultivar and temperature at 12 months of storage (Fig. 4.5). The samples stored at 10°C and 25°C are separated for both cultivars (Fig. 4.5). This agrees with the ANOVA results that temperature had a significant influence on most of the response variables with the exception of sensory colour and polyphenols (Table 4.1). The combination of storage time and the temperature used for port wine storage also affects the anthocyanin content. In a recent study it was observed that after 6 months of storage of young red wines, the content of anthocyanins was lower in wines stored at 25°C compared to wines stored at 15°C (Oliveira *et al.*, 2015). Moreover, the trend plots (Figure 4.1 to Figure 4.3) showed that the more stable redness (a*), ΔE^* and sensory colour was seen at storage temperature of 10°C.
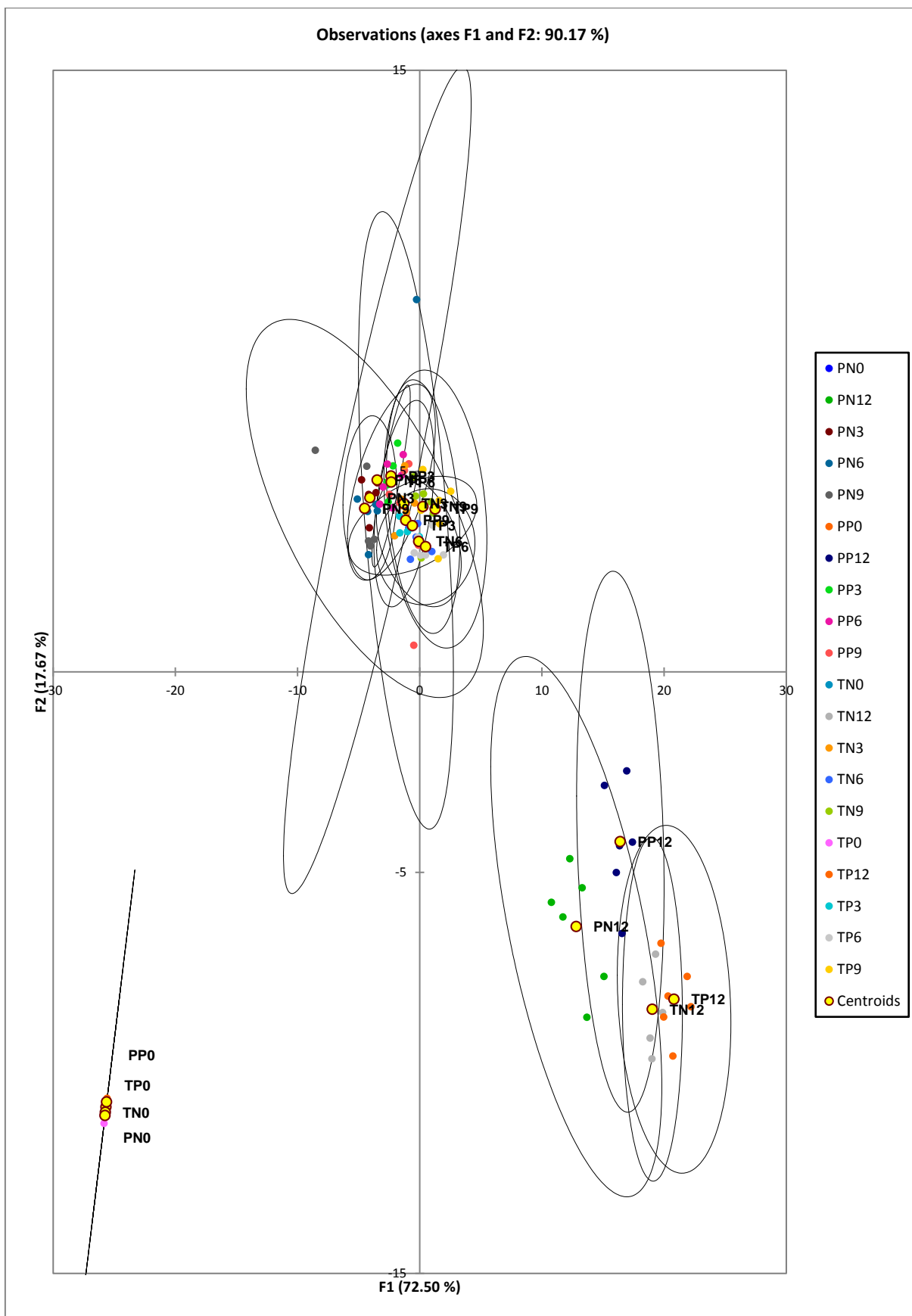
Figure 4.4: Discriminant Analysis plot depicting the influence of cultivar, enzyme and storage over time on redness (a*), ΔE* and sensory colour.
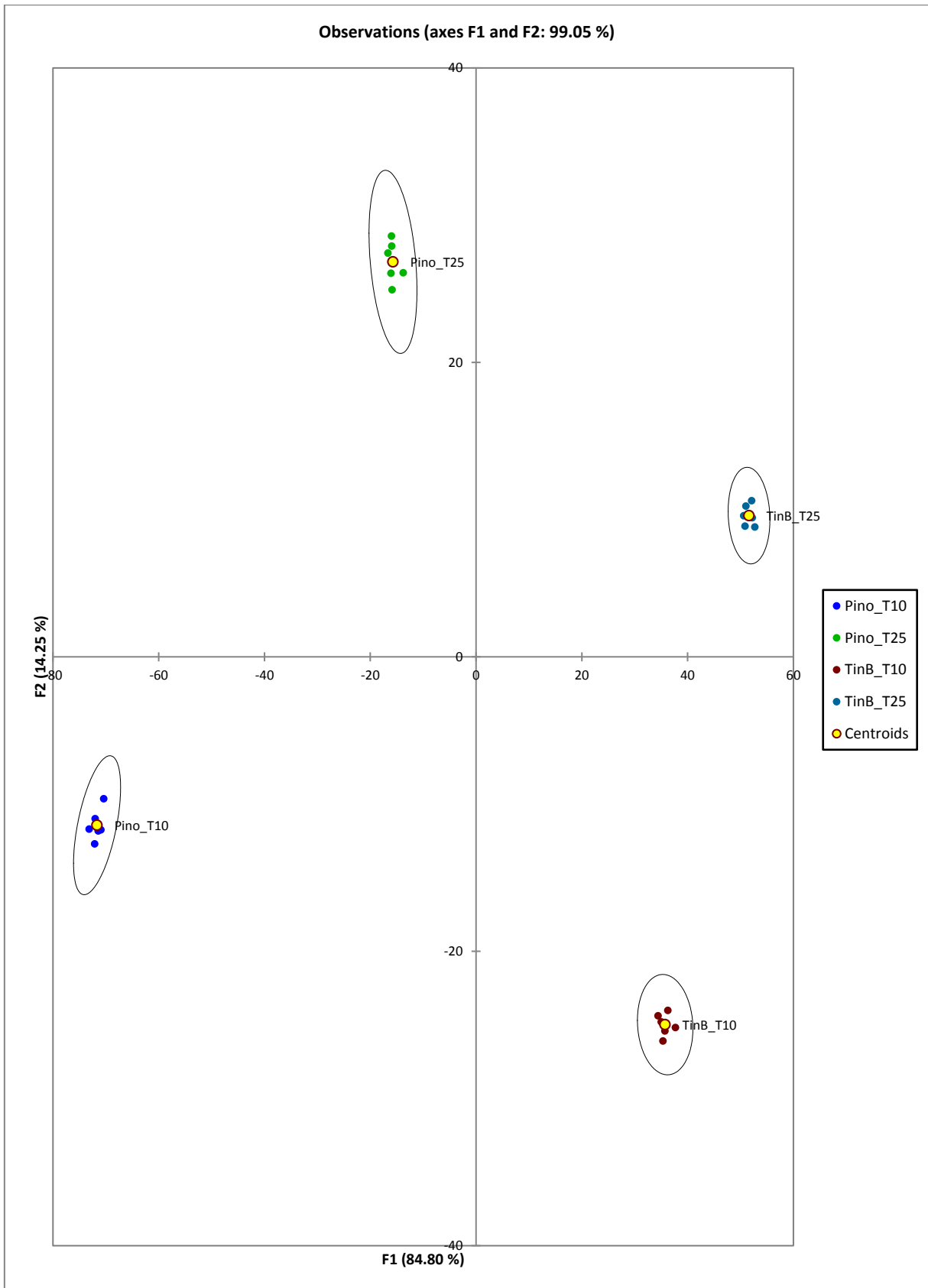
Figure 4.5: Discriminant Analysis plot depicting the effect of cultivar and temperature at time 12 months.

At this point it can be concluded that, in terms of the four main effects, the results showed that the design variable with the most pronounced effect on all response variables with the exception of redness (a*) and ΔE^*, was cultivar. Cultivar had a significant effect ($p < 0.05$) on most of the response variables, Tinta Barrocca had an effect on overall colour stability especially in combination with the other response variables as a treatment over time. However, Tinta Barrocca without enzyme treatment and storage temperature at 10°C had a significantly increasing effect on colour intensity, whereas Pinotage with enzyme treatment at storage temperature at 10°C also had a significantly increasing effect on colour intensity. From this it can be concluded that although port wines are typically produced from Portuguese cultivars such as Tinta Barrocca, it was also seen from this study that the South African cultivar Pinotage resulted in significant results on colour, more specifically colour intensity as well as the sensory colour of the port.

Temperature was identified as the second design variable which had a significant effect on all response variables with the exception of sensory colour and polyphenols. At 10°C storage temperature the values for the response variables were higher than at 25°C, whereas the ΔE^* values at 10°C were lower, indicating more stable colour at lower storage temperature. This is followed by enzyme treatment which had a significant effect on colour intensity, %blue, L*, a*, b*, C and h. However, enzyme treatment did not have a significant effect on ΔE^*, which indicates that over time the enzyme treatment did not have a significant effect on the colour stability of ruby ports.

Storage time also had an effect on overall port wine colour stability, storage at time 0 – 3 months had the highest values indicating that ruby port wine has more intense redness if stored for a shorter period of time at lower temperatures. This is in agreement with previous studies on port and other fortified wines, especially in terms of storage temperature and time, where the results proved that storage at lower temperatures as well as consumption of ruby ports before three years of age yielded better colour, aroma and flavour profiles (Tredoux, 2012; Pinho *et al.*, 2012). Even though enzyme treatment had a significant effect on colour intensity and redness at time zero in this study, it did not improve overall colour stability in the port wine over time. Previous studies on red wine, showed that pectolytic enzyme treatment contributed to aroma and colour enhancement in the wine (Dziadas & Jelén, 2016).

Table 4.2 Routine profile parameters of port wine samples [1].

Design Variables					Response variables			
Cultivar[2]	Enzyme[3]	A*(acetaldehyde)[4]	Temperature (ºC)	Storage time (months)	Free SO_2	Total SO_2	pH	TA
					LSD^5 = 2.48	LSD = 7.95	LSD = 0.05	LSD = 0.25
Pinotage	No	P1	10	0	19.50 ± 0.71	73.50 ± 2.12	3.78 ± 0.02	3.97 ± 0.08
Pinotage	No	P2	10	0	17.50 ± 0.71	71.00 ± 4.24	3.76 ± 0.01	3.87 ± 0.04
Pinotage	No	P3	10	0	16.50 ± 0.71	74.50 ± 0.71	3.80 ± 0.00	3.85 ± 0.04
Pinotage	No	P1	25	0	19.50 ± 0.71	73.50 ± 2.12	3.78 ± 0.02	3.97 ± 0.08
Pinotage	No	P2	25	0	17.50 ± 0.71	71.00 ± 4.24	3.76 ± 0.01	3.87 ± 0.04
Pinotage	No	P3	25	0	16.50 ± 0.71	74.50 ± 0.71	3.80 ± 0.00	3.85 ± 0.04
Pinotage	No	P1	10	3	16.50 ± 0.71	70.00 ± 0.00	3.98 ± 0.00	3.40 ± 0.28
Pinotage	No	P2	10	3	18.00 ± 0.00	69.00 ± 4.24	3.97 ± 0.00	3.45 ± 0.35
Pinotage	No	P3	10	3	16.00 ± 0.14	75.50 ± 0.71	3.99 ± 0.01	3.40 ± 0.28
Pinotage	No	P1	25	3	16.50 ± 2.12	65.00 ± 0.00	3.98 ± 0.01	3.35 ± 0.21
Pinotage	No	P2	25	3	16.50 ± 0.71	65.00 ± 1.41	3.98 ± 0.00	3.50 ± 0.00
Pinotage	No	P3	25	3	15.00 ± 1.41	69.50 ± 0.71	4.00 ± 0.01	3.30 ± 0.28

[1]Results are reported as the mean ± standard deviation (SD) (n = 2). Analyses of variance (ANOVAs) tested the influence of the main effects. Duncan's new multiple range test (MRT) tested the differences beween individual means (p ≤ 0.05 is significant).

[2]Cultivars: Pinotage and Tinta B = (Tinta Barrocca).

[3]Enzyme: Yes = Addition of enzyme treatment; No = without enzyme treatment.

[4]Aldehyde: P1 = Low aldehyde level (50 mg.L^{-1}); P2 = Intermediate aldehyde level (No commercial acetaldehyde added); P3 = High aldehyde level (Normal wine spirits spiked with 450 mg.L^{-1} .

[5]LSD : Least significant difference.

Table 4.2
Continued.

Cultivar[2]	Enzyme[3]	A*(acetaldehyde)[4]	Temperature (°C)	Storage time (months)	Free SO$_2$	Total SO$_2$	pH	TA
					LSD[5] = 2.48	LSD = 7.95	LSD = 0.05	LSD = 0.25
Pinotage	No	P1	10	6	14.50 ± 0.71	68.50 ± 0.71	4.04 ± 0.00	3.70 ± 0.00
Pinotage	No	P2	10	6	14.00 ± 0.00	67.50 ± 3.54	4.04 ± 0.00	3.65 ±0.07
Pinotage	No	P3	10	6	13.00 ± 0.00	72.50 ± 2.12	4.06 ± 0.00	3.70 ± 0.00
Pinotage	No	P1	25	6	13.50 ± 0.71	59.00 ± 1.41	4.06 ± 0.00	3.60 ± 0.00
Pinotage	No	P2	25	6	13.50 ± 0.71	63.00 ± 5.66	4.07 ± 0.01	3.53 ± 0.04
Pinotage	No	P3	25	6	13.00 ± 0.00	66.00 ± 4.24	4.08 ± 0.01	3.50 ± 0.00
Pinotage	No	P1	10	9	14.00 ± 1.14	63.00 ± 2.83	3.88 ± 0.02	3.68 ± 0.04
Pinotage	No	P2	10	9	13.50 ± 0.71	63.50 ± 3.54	3.89 ± 0.00	3.65 ± 0.07
Pinotage	No	P3	10	9	13.50 ± 0.71	68.00 ± 2.83	3.92 ± 0.01	3.60 ± 0.00
Pinotage	No	P1	25	9	11.50 ± 0.71	55.50 ± 2.12	3.92 ± 0.01	3.50 ± 0.00
Pinotage	No	P2	25	9	13.00 ± 0.00	50.00 ± 1.41	3.89 ± 0.01	3.50 ± 0.00
Pinotage	No	P3	25	9	12.50 ± 0.71	58.50 ± 0.71	3.93 ± 0.00	3.40 ± 0.00

Table 4.2 Continued

Cultivar[2]	Enzyme[3]	A*(acetaldehyde)[4]	Temp (ºC)[5]	Time (months)[6]	FreeSO$_2$	Tot SO$_2$	pH	TA
					LSD[7] = 2.48	LSD = 7.95	LSD = 0.05	LSD = 0.25
Pinotage	No	P1	10	12	15.50 ± 0.71	63.00 ± 1.41	4.01 ± 0.00	3.60 ± 0.00
Pinotage	No	P2	10	12	14.00 ± 1.41	66.50 ± 2.12	3.96 ± 0.06	3.60 ± 0.00
Pinotage	No	P3	10	12	11.50 ± 0.71	68.50 ± 2.12	4.11 ± 0.13	3.55 ± 0.07
Pinotage	No	P1	25	12	14.00 ± 0.00	59.50 ± 7.78	4.06 ± 0.03	3.43 ± 0.04
Pinotage	No	P2	25	12	11.50 ± 0.71	53.00 ± 1.41	4.03 ± 0.01	3.40 ± 0.00
Pinotage	No	P3	25	12	9.50 ± 0.71	58.00 ± 0.00	4.04 ± 0.00	3.40 ± 0.00
Pinotage	Yes	P1	10	0	18.00 ± 2.83	76.00 ± 1.41	3.75 ± 0.00	4.05 ± 0.01
Pinotage	Yes	P2	10	0	16.00 ± 1.41	69.00 ± 2.83	3.75 ± 0.00	4.01 ± 0.00
Pinotage	Yes	P3	10	0	18.50 ± 0.71	73.50 ± 0.71	3.75 ± 0.01	4.07 ± 0.01
Pinotage	Yes	P1	25	0	18.00 ± 2.83	76.00 ± 1.41	3.75 ± 0.00	4.05 ± 0.01
Pinotage	Yes	P2	25	0	16.00 ± 1.41	69.00 ± 2.83	3.75 ± 0.00	4.01 ± 0.02
Pinotage	Yes	P3	25	0	18.50 ± 0.71	73.50 ± 0.71	3.74 ± 0.01	4.07 ± 0.01

Table 4.2

Continued.

Cultivar[2]	Enzyme[3]	A*(acetaldehyde)[4]	Temp (ºC)[5]	Time (months)[6]	FreeSO$_2$	Tot SO$_2$	pH	TA
					LSD[7] = 2.48	LSD = 7.95	LSD = 0.05	LSD = 0.25
Pinotage	Yes	P1	10	3	19.00 ± 0.00	69.00 ± 2.83	4.02 ± 0.00	3.60 ± 0.04
Pinotage	Yes	P2	10	3	15.50 ± 0.71	70.00 ± 1.41	3.97 ± 0.00	3.68 ± 0.04
Pinotage	Yes	P3	10	3	20.00 ± 0.00	69.50 ± 0.71	3.95 ± 0.00	3.70 ± 0.28
Pinotage	Yes	P1	25	3	17.00 ± 2.83	66.00 ± 1.41	3.99 ± 0.01	3.50 ± 0.14
Pinotage	Yes	P2	25	3	14.00 ± 1.41	64.50 ± 0.71	3.97 ± 0.00	3.55 ± 0.21
Pinotage	Yes	P3	25	3	14.50 ± 0.71	69.00± 1.41	3.96 ± 0.00	3.60 ± 0.04
Pinotage	Yes	P1	10	6	17.00 ± 0.00	66.00 ± 1.41	4.00 ± 0.01	3.80 ± 0.00
Pinotage	Yes	P2	10	6	13.00 ± 0.00	65.00 ± 1.41	4.02 ± 0.01	3.80 ± 0.00
Pinotage	Yes	P3	10	6	13.50 ± 0.71	68.50 ± 0.71	4.01 ± 0.00	3.83 ± 0.04
Pinotage	Yes	P1	25	6	14.50 ± 0.71	63.00 ± 1.41	4.02 ± 0.00	3.70 ± 0.00
Pinotage	Yes	P2	25	6	13.50 ± 0.71	61.50 ± 0.71	4.03 ± 0.00	3.70 ± 0.00
Pinotage	Yes	P3	25	6	13.50 ± 0.71	63.50 ± 0.71	4.03 ± 0.00	3.70 ± 0.00

Table 4.2

Continued.

Cultivar[2]	Enzyme[3]	A*(acetaldehyde)[4]	Temp (°C)[5]	Time (months)[6]	FreeSO$_2$	Tot SO$_2$	pH	TA
					LSD[7] = 2.48	LSD = 7.95	LSD = 0.05	LSD = 0.25
Pinotage	Yes	P1	10	9	14.50 ± 0.71	65.50 ± 2.12	3.88 ± 0.00	3.80 ± 0.00
Pinotage	Yes	P2	10	9	12.50 ± 0.71	60.00 ± 0.00	3.86 ± 0.02	3.73 ± 0.04
Pinotage	Yes	P3	10	9	12.50 ± 0.71	66.00 ± 1.41	3.87 ± 0.01	3.80 ± 0.00
Pinotage	Yes	P1	25	9	12.50 ± 0.71	54.50 ± 0.71	3.89 ± 0.00	3.63 ± 0.04
Pinotage	Yes	P2	25	9	12.50 ± 0.21	55.50 ± 0.71	3.88 ± 0.01	3.60 ± 0.00
Pinotage	Yes	P3	25	9	13.00 ± 0.00	58.00 ± 4.24	3.89 ± 0.01	3.60 ± 0.00
Pinotage	Yes	P1	10	12	15.50 ± 0.71	68.50 ± 2.12	4.01 ± 0.01	3.70 ± 0.00
Pinotage	Yes	P2	10	12	15.00 ± 1.41	66.00 ± 0.00	3.99 ± 0.00	3.63 ± 0.04
Pinotage	Yes	P3	10	12	12.50 ± 0.71	68.50 ± 0.71	3.98 ± 0.01	3.70 ± 0.00
Pinotage	Yes	P1	25	12	14.50 ± 0.71	50.00 ± 0.00	3.99 ± 0.01	3.60 ± 0.00
Pinotage	Yes	P2	25	12	13.00 ± 1.41	52.50 ± 0.71	4.01 ± 0.00	3.50 ± 0.00
Pinotage	Yes	P3	25	12	13.50 ± 0.71	56.00 ± 2.83	4.05 ± 0.05	3.55 ± 0.07

Table 4.2

Continued.

Cultivar[2]	Enzyme[3]	A*(acetaldehyde)[4]	Temp (ºC)[5]	Time (months)[6]	FreeSO$_2$	Tot SO$_2$	pH	TA
					LSD[7] = 2.48	LSD = 7.95	LSD = 0.05	LSD = 0.25
Tinta B.	No	P1	10	0	21.00 ± 2.83	73.50 ± 7.78	3.89 ± 0.00	4.44 ± 0.01
Tinta B.	No	P1	25	0	21.00 ± 2.83	73.50 ± 7.78	3.89 ± 0.00	4.44 ± 0.01
Tinta B.	No	P2	10	0	20.00 ± 1.41	82.00 ± 1.41	3.88 ± 0.01	4.46 ± 0.04
Tinta B.	No	P2	25	0	20.00 ± 2.83	82.00 ± 1.41	3.88 ± 0.01	4.46 ± 0.04
Tinta B.	No	P3	10	0	17.00 ± 1.41	79.50 ± 0.71	3.75 ± 0.13	4.51 ± 0.00
Tinta B.	No	P3	25	0	17.00 ± 1.41	79.50 ± 0.71	3.75 ± 0.13	4.51 ± 0.00
Tinta B.	No	P1	10	3	19.00 ± 1.41	98.00 ± 14.14	4.00 ± 0.01	4.20 ± 0.14
Tinta B.	No	P1	25	3	17.00 ± 4.24	66.50 ± 6.36	4.00 ± 0.00	3.90 ± 0.28
Tinta B.	No	P2	10	3	21.50 ± 2.12	90.00 ± 9.90	4.00 ± 0.00	4.13 ± 0.60
Tinta B.	No	P2	25	3	16.50 ± 0.71	77.50 ± 2.12	4.00 ± 0.00	3.95 ± 0.21
Tinta B.	No	P3	10	3	16.50 ± 0.71	83.50 ± 2.12	3.99 ± 0.00	4.15 ± 0.21
Tinta B.	No	P3	25	3	15.00 ± 0.00	79.00 ± 3.83	4.00 ± 0.01	4.10 ± 0.21

Table 4.2

Continued.

Cultivar[2]	Enzyme[3]	A*(acetaldehyde)[4]	Temp (ºC)[5]	Time (months)[6]	FreeSO$_2$	Tot SO$_2$	pH	TA
					LSD[7] = 2.48	LSD = 7.95	LSD = 0.05	LSD = 0.25
Tinta B.	No	P1	10	6	18.00 ± 0.00	81.00 ± 1.41	4.07 ± 0.00	4.25 ± 0.07
Tinta B.	No	P1	25	6	15.50 ± 0.71	66.00 ± 1.41	4.08 ± 0.00	4.10 ± 0.00
Tinta B.	No	P2	10	6	20.00 ± 1.41	91.50 ± 14.85	4.08 ± 0.01	4.25 ± 0.07
Tinta B.	No	P2	25	6	15.50 ± 0.71	70.00 ± 2.83	4.08 ± 0.00	4.10 ± 0.00
Tinta B.	No	P3	10	6	16.00 ± 0.00	81.00 ± 0.00	4.07 ± 0.01	4.23 ± 0.04
Tinta B.	No	P3	25	6	14.00 ± 0.00	74.00 ± 0.00	4.08 ± 0.00	4.10 ± 0.00
Tinta B.	No	P1	10	9	16.00 ± 0.00	81.50 ± 3.54	4.05 ± 0.00	4.18 ± 0.04
Tinta B.	No	P1	25	9	12.50 ± 0.71	66.00 ± 1.41	4.10 ± 0.00	4.00 ± 0.00
Tinta B.	No	P2	10	9	14.50 ± 0.71	77.00 ± 1.41	4.08 ± 0.00	4.20 ± 0.00
Tinta B.	No	P2	25	9	14.50 ± 0.71	60.00 ± 8.49	4.07 ± 0.01	4.00 ± 0.00
Tinta B.	No	P3	10	9	14.50 ± 0.71	81.00 ± 0.00	4.04 ± 0.01	4.15 ± 0.07
Tinta B.	No	P3	25	9	14.00 ± 0.00	63.50 ± 3.54	4.08 ± 0.01	4.03 ± 0.04

Table 4.2

Continued.

Cultivar[2]	Enzyme[3]	A*(acetaldehyde)[4]	Temp (ºC)[5]	Time (months)[6]	FreeSO$_2$	Tot SO$_2$	pH	TA
					LSD[7] = 2.48	LSD = 7.95	LSD = 0.05	LSD = 0.25
Tinta B.	No	P1	10	12	15.00 ± 1.41	72.00 ± 4.95	4.04 ± 0.01	4.10 ± 0.00
Tinta B.	No	P1	25	12	13.50 ± 0.71	63.50 ± 0.71	4.07 ± 0.01	3.95 ± 0.07
Tinta B.	No	P2	10	12	15.50 ± 0.71	85.00 ± 9.90	4.03 ± 0.02	4.10 ± 0.00
Tinta B.	No	P2	25	12	12.00 ± 0.00	61.00 ± 0.00	4.07 ± 0.01	3.95 ± 0.07
Tinta B.	No	P3	10	12	13.50 ± 3.54	76.50 ± 3.54	4.02 ± 0.01	4.15 ± 0.07
Tinta B.	No	P3	25	12	13.00 ± 0.00	58.50 ± 2.12	4.05 ± 0.01	3.90 ± 0.00
Tinta B.	Yes	P1	10	0	17.50 ± 0.71	74.50 ± 0.71	3.82 ± 0.00	4.60 ± 0.01
Tinta B.	Yes	P1	25	0	17.50 ± 0.71	74.50 ± 0.71	3.82 ± 0.00	4.60 ± 0.01
Tinta B.	Yes	P2	10	0	17.50 ± 0.71	78.00 ± 1.41	3.81 ± 0.00	4.65 ± 0.00
Tinta B.	Yes	P2	25	0	17.500 ± 0.71	78.00 ± 1.41	3.81 ± 0.00	4.65 ± 0.00
Tinta B.	Yes	P3	10	0	18.00 ± 0.00	74.50 ± 0.71	3.80 ± 0.00	4.60 ± 0.28
Tinta B.	Yes	P3	25	0	18.00 ± 0.00	74.50 ± 0.71	3.80 ± 0.00	4.60 ± 0.03

Table 4.2

Continued.

Cultivar[2]	Enzyme[3]	A*(acetaldehyde)[4]	Temp (°C)[5]	Time (months)[6]	FreeSO$_2$	Tot SO$_2$	pH	TA
					LSD[7] = 2.48	LSD = 7.95	LSD = 0.05	LSD = 0.25
Tinta B.	Yes	P1	10	3	18.50 ± 1.71	68.00 ± 2.83	3.99 ± 0.00	4.13 ± 0.25
Tinta B.	Yes	P1	25	3	19.00 ± 0.00	69.50 ± 2.12	3.99 ± 0.00	4.20 ± 0.00
Tinta B.	Yes	P2	10	3	18.00 ± 0.00	73.00 ± 1.41	3.97 ± 0.01	4.40 ± 0.14
Tinta B.	Yes	P2	25	3	15.50 ± 2.12	67.50 ± 2.12	3.98 ± 0.00	4.20 ± 0.28
Tinta B.	Yes	P3	10	3	15.50 ± 0.71	76.50 ± 2.12	3.97 ± 0.01	4.20 ± 0.14
Tinta B.	Yes	P3	25	3	14.50 ± 0.71	72.50 ± 0.71	3.97 ± 0.00	4.20 ± 0.00
Tinta B.	Yes	P1	10	6	20.00 ± 0.00	72.50 ± 4.95	4.04 ± 0.00	4.40 ± 0.00
Tinta B.	Yes	P1	25	6	17.50 ± 0.71	65.50 ± 0.71	4.05 ± 0.00	4.25 ± 0.07
Tinta B.	Yes	P2	10	6	18.00 ± 0.00	74.50 ± 0.71	4.04 ± 0.00	4.40 ± 0.00
Tinta B.	Yes	P2	25	6	17.00 ± 1.41	67.50 ± 2.12	4.05 ± 0.00	4.30 ± 0.00
Tinta B.	Yes	P3	10	6	17.50 ± 2.12	76.00 ± 1.41	4.04 ± 0.01	4.40 ± 0.00
Tinta B.	Yes	P3	25	6	17.00 ± 2.83	65.50 ± 0.71	4.05 ± 0.00	4.35 ± 0.21

Table 4.2

Continued.

Cultivar[2]	Enzyme[3]	A*(acetaldehyde)[4]	Temp (ºC)[5]	Time (months)[6]	FreeSO$_2$	Tot SO$_2$	pH	TA
					LSD[7] = 2.48	LSD = 7.95	LSD = 0.05	LSD = 0.25
Tinta B.	Yes	P1	10	9	14.50 ± 0.71	67.00 ± 0.00	4.08 ± 0.01	4.25 ± 0.07
Tinta B.	Yes	P1	25	9	13.00 ± 1.41	53.50 ± 0.71	4.08 ± 0.00	4.10 ± 0.00
Tinta B.	Yes	P2	10	9	14.00 ± 1.41	67.00 ± 4.24	4.05 ± 0.02	4.30 ± 0.00
Tinta B.	Yes	P2	25	9	13.00 ± 0.00	58.00 ± 0.00	4.06 ± 0.02	4.15 ± 0.07
Tinta B.	Yes	P3	10	9	14.00 ± 0.00	71.50 ± 4.95	4.05 ± 0.01	4.30 ± 0.00
Tinta B.	Yes	P3	25	9	12.50 ± 0.71	58.50 ± 6.36	4.06 ± 0.00	4.10 ± 0.00
Tinta B.	Yes	P1	10	12	15.50 ± 2.12	64.50 ± 4.95	4.04 ± 0.01	4.20 ± 0.00
Tinta B.	Yes	P1	25	12	12.00 ± 0.00	50.50 ± 0.71	4.05 ± 0.00	4.03 ± 0.04
Tinta B.	Yes	P2	10	12	16.00 ± 1.41	72.50 ± 2.12	4.02 ± 0.00	4.25 ± 0.07
Tinta B.	Yes	P2	25	12	13.00 ± 0.00	51.00 ± 1.41	4.02 ± 0.02	4.08 ± 0.04
Tinta B.	Yes	P3	10	12	14.50 ± 0.71	69.00 ± 4.00	4.01 ± 0.01	4.20 ± 0.00
Tinta B.	Yes	P3	25	12	14.00± 0.00	54.00 ± 4.24	4.02 ± 0.01	4.00 ± 0.00

[1]Results are reported as the mean ± standard deviation (SD) (n = 2). Analyses of variance (ANOVAs) tested the influence of the main effects. Duncan's new multiple range test (MRT) tested the differences beween individual means (p ≤ 0.05 is significant).

[2]Cultivars: Pinotage and Tinta B = (Tinta Barrocca).

[3]Enzyme: Yes = Addition of enzyme treatment; No = without enzyme treatment.

[4]Aldehyde: P1 = Low aldehyde level (50 mg.L^{-1}); P2 = Intermediate aldehyde level (No commercial acetaldehyde added); P3 = High aldehyde level (Normal wine spirits spiked with 450 mg.L^{-1} .

[5]LSD : Least significant difference.

Table 4.2 depicts the routine parameters of port wine samples in this study. The routine parameters (TA, pH, free SO_2 and total SO_2) are known to affect the organoleptic quality characteristics of wine, including colour and taste attributes as well as antioxidant and antimicrobial stability and polyphenol including anthocyanic chemistry. In most cases SO_2 has a binding effect on acetaldehyde and it protect wine aromas and makes the flat character disappear. SO_2 can also bind with phenolic compounds of red wine and the reaction is directly visible by the decolouration produced. The European Union wine regulation limits total SO_2 in the end product and every country has their own maximum allowable content depending on the type of fortified wines as well as the residual sugar content (Anon, 2015). pH and TA of the base wine before fortification plays and important role in overall port wine quality. Typical values for pH in port wine range from 3.2 – 4.0 (Anon, 2016). pH and TA shows opposite trends, i.e. increases and decreases, respectively over time of ageing. Decreases in aldehyde content over time, is accompanied by decreases in anthocyanins and FSO_2 and TSO_2, binding of SO_2 and anthocyanins to acetaldehyde. Total polyphenol levels stay relatively constant over a period of time, with a slight decrease from original levels over 12 months of ageing.

4.5 Conclusions

Port wine colour stability is affected by a combination of the design variables as a treatment and not necessarily by a single variable in this study. Tinta Barrocca as a cultivar on its own had higher colour intensity than Pinotage. However, as stated before, the combination of Tinta Barrocca with enzyme treatment showed less colour stability, indicating that a single factor such as type of cultivar does not clearly give higher colour stability. Intermediate and high levels of acetaldehyde had a significant effect on colour intensity as well as redness and a decreasing effect on ΔE^*, indicating better colour intensity and colour stability over time with these aldehyde levels. However, lower storage temperature as well as shorter storage times influenced the colour stability of the port wine samples in this study positively. It could also be concluded that the response variables colour intensity, redness and ΔE^* gave the best indication of colour stability in port wines over time.

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CHAPTER 5

GENERAL SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction/Summary

Fortified wines, including port wine, are wines to which grape spirits had been added. The production of port wines encompasses different processes, including selection of grape cultivar, treatment with either a pectolytic enzyme or not during crushing of grapes and fermentation, addition of grape spirits and maturation. Each of these steps has a decisive influence on the style and quality of the final product. Colour is one of the principal parameters of the quality of, not only port wine, but also red wine in general, since it is the first characteristic to be perceived by the consumer in the glass (Pinho *et al.*, 2012). The colour of port wine also gives an indication of possible defects concerning the body, age and the maturation shelf life of the wine during storage. Colour, therefore, has an important influence on the overall acceptability of the product to the consumer (Anon, 2015).

In this study some of the conventional port wine making practices were manipulated, such as type of spirit used to fortify, storage temperature and also storage time in order to improve stability or even achieve optimum stability of port wine colour. Since colour changes are often perceived as undesirable by port consumers, the Cape Port Producers Association (CAPPA) requested this type of research to be done on port wine with a view to improve the port wine making process in an effort to achieve a more stable port wine colour. (Anon, 2015).

5.2 Conclusions

In the first part of the study it was evident that the type of fortifying spirits, storage time and temperature had a significant effect on the colour of the port wine samples. The 96.5% (v.v^{-1}) fortifying spirits, shorter storage time and a storage temperature below 25°C resulted in a more stable ruby port colour as well as lowest change in colour over time (ΔE^*), thus confirming the hypothesis that longer storage times and higher storage temperatures will lead to a darker, brown, undesireable colour in ruby port wine (Mori *et al.*, 2015). Sensorially, the 96.5% (v.v^{-1}) fortifying spirits, shorter storage time and a storage temperature below 25°C afforded the best colour, port wine character and overall quality in the port wine samples.

As the study progressed the design variables differed in Chapter 4 in terms of cultivar (two types of cultivars were used), spirits used for fortification, pectolytic enzyme added to some of the port wine samples, storage time and temperature. In this part of the study it, port wine colour stability was affected by the interaction of the design variables in each treatment and less so by individual design variables in this study. Tinta Barroca as a cultivar without pectolytic enzyme treatment resulted in better colour intensity and colour stability in the port wine samples than Pinotage. However, application of the pectolytic enzyme preparation resulted in significantly lower colour intensity in Tinta Barroca than in Pinotage. This refuted the hypothesis that the addition of pectolytic enzyme to the port wine must will result in a brighter, more stable port wine colour. Moreover, although the application of a pectolytic enzyme preparation during the vinification of the port wine samples had a significant effect on most of the response variables in this study (Chapter 4), it had no effect on ΔE^* (stability over time). It can be concluded that pectolytic enzyme treatment does not contribute to colour stability on its own but it contributes to colour and flavour profiles in combination with a specific cultivar (in this study Pinotage) as a treatment of ruby port wine (Dziadas & Jelén, 2016). Moreover, although it was expected that a typical port wine cultivar like Tinta Barroca would result in the most stable port wine colour, the typical South African cultivar, Pinotage, resulted in significantly higher colour intensity as well as the sensory colour scores of the port. Therefore, it can be concluded that optimal colour in port wine is not limited to typical Portuguese port wine cultivars. This conclusion is based on the results of this study, considering the fact that grape origin, viti-vinicultural practices and terroir also affect final colour.

As hypothesized, intermediate and higher levels of aldehyde, in the fortifying spirits used for the port wine samples, resulted in a significantly higher colour intensity and redness and a significant decrease in ΔE^*. Hence, the observed higher colour stability confirmed that higher levels of acetaldehyde in the fortifying spirits will result in a brighter red and more stable colour in ruby port wine.

Temperature had a significant effect on all the response variables with the exception of sensory colour and polyphenols in this study especially in the second part of this study (Chapter 4). Lower storage temperatures and shorter storage times influenced the colour stability of port wines positively in this study overall (Oliveira *et al.*, 2015). The conclusion from both chapters regarding temperature is that the most stable port wine colour over time was observed at the lowest storage temperatures, namely at 4°C and 10°C. Storage time had a significant effect on all response

variables in both Chapters 3 and 4. Storage at time 0 – 3 months resulted in the highest redness and colour stability values, indicating that ruby port wine has more intense redness if stored for a shorter period of time at lower temperatures. This is in agreement with previous studies on port and other fortified wines, especially in terms of storage temperature and time, where the results proved that storage at lower temperatures as well as consumption of ruby ports before three years of age yielded better colour, aroma and flavour profiles (Tredoux, 2012; Pinho *et al.*, 2012).

The best overall treatments in this study could be ascribed to the effects of a combination of variables, rather than to the effect of individual variables. The more reliable indicators of colour and colour stability in this study were redness, colour stability as well as colour intensity and sensory colour. Redness (a*) is one of the most important characteristics of ruby port style wines and therefore also was a very important factor in this study. Some of the treatments that resulted in the least change in redness over time included the addition of enzyme (Espejo & Armada, 2010). These were: Tinta Barrocca with pectolytic enzyme added, intermediate aldehyde level (P2) and storage temperature 10°C (i.e. TinB_PE_P2_T10) and Pinotage with pectolytic enzyme added, high aldehyde level (P1) and storage temperature 10°C (i.e. Pino_PE_P1_T10). In terms of colour stability, the treatments indicating the lowest ΔE^* values were also treatments with enzyme addition, which was also in agreement with the aforementioned trend concerning a*. These treatments include TinB_PE_P2_T10, TinB_PE_P3_T10 and TinB_PE_P1_T10, indicating that enzyme treatment resulted in the least change in colour over time, therefore the highest colour stability. Tinta Barrocca with pectolytic enzyme added, addition of aldehyde at the highest level (P3) and at storage 10°C (TinB_PE_P3_T10) as well as TinB_No_P2_T10, TinB_PE_P1_T10 and Tin B_PE_P2_T10 resulted in the highest scores for sensory colour after 12 months of storage. A similarity in the responses to the treatments was observed when comparing the plots for (Fig. 4.2) (Chapter 4) sensory colour scores and the ΔE^* values over time (Fig 4.1) (Chapter 4). The treatments that resulted in the least change over time in Figure 4.1 (ΔE^*) namely TinB_PE_P3_T10, TinB_PE_P2_T10 as well as TinB_PE_P1_T10 and Pino_No_P1_T10 corresponded with the treatments that were the best four in terms of observed sensory colour. Although most of the more stable treatments identified above are with Tinta Barrocca as a cultivar, cultivar did not affect ΔE^* significantly ($p > 0.05$; Table 4.1).

5.3 Recommendations

From these aforementioned results, it could be concluded that higher levels of acetaldehyde present in the spirits used to fortify port wine, did have a significant impact on ruby port wine colour and colour stability over time and therefore it is recommended that the highest permissible level of acetaldehyde in the fortifying spirits should be used. It can also be concluded that application of a pectolytic enzyme preparation does not necessarily have a significant effect on port wine colour stability on its own, but since its effect depends on the cultivar used to produce the port wine, the decision to use it or not should be based on trials to determine its effect.

Storage temperature is also a critical variable in the colour and colour stability of ruby port wine. Hence, based on the results of this study, it is recommended that port wine should be stored at, or below, 10°C for optimal ruby port wine colour. Storage time should also be kept to a minimum to ensure the desirable bright red colour of a ruby port, preferably storage should not exceed 6 months before consumption (McRae *et al.*, 2015). For future application in terms of storage, the effect of the type of vessel that the ruby port wine is stored in could be assessed. For example, it is possible that wood contact in barrels for a limited amount of time would enhance the colour stability of the ruby port wine, if followed by further storage in glass bottles. This effect would be possible due to the stabilising effect of polyphenol-aldehyde complexes (Pina *et al.*, 2015).

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