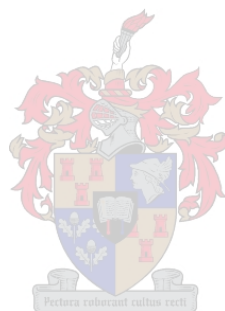


# **Chemical, sensory and consumer analysis of cork taint in South African wines**



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Thesis presented in partial fulfilment of the requirements for the degree of  
Master of Science in Food Science

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**March 2009**

## Declaration

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## Abstract

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This study focused on a serious quality-related problem in the global wine industry, including the South African Wine Industry, namely cork taint in wine. Annually, large financial losses are incurred by cork suppliers and wine producers, as a result of cork-tainted wine. Although contaminated new unused corks are frequently implicated as the origin of this taint, contaminated cellar equipment and water can also be the source of the problem.

An explorative investigation into the incidence of cork taint in South African wines showed that 3.8% of the 133 wines tested, contained 2,4,6-trichloroanisole (TCA) concentrations of 3.5 ng/L and higher, as determined by gas chromatography coupled with electron capture detection (GC-ECD). TCA concentrations higher than 1 ng/L were found in 18% of the wines tested. All affected wines were sealed with solid or agglomerate cork stoppers. These wines were sourced from various wineries in the Western Cape region, South Africa and were of different cultivars. None of the wines sealed with synthetic closures had any detectable TCA, 2,4,6-tribromoanisole (TBA) or pentachloroanisole (PCA) levels and only very low 2,3,4,6-tetrachloroanisole (TeCA) levels (1 ng/L or less). Another group of 28 wines that were rejected by the official South African wine regulatory body on the basis of the presence of mouldy taint during wine certification, was also included in this study. GC-ECD analysis showed that 30% of the wines in this group contained TCA at concentrations of 3.5 ng/L and higher. These results pointed to a relative high incidence of TCA in the wines investigated, especially those sealed with cork stoppers. Although no general conclusions should be made on the incidence of cork taint in the wider wine industry based on the results found within this explorative investigation, these findings confirmed the presence of cork taint in South African wines.

Detection threshold values were determined for TCA, TeCA, TBA and PCA in three wine cultivars using the standard ASTM method. Results indicate that factors relating to the wine cultivar seemed to affect threshold values considerably. Our research proposes a detection range rather than an average detection threshold. Detection ranges established for TCA, TeCA, TBA and PCA in Chenin blanc, Pinotage and Shiraz coincide with reported values in literature. This result can be regarded as a valuable expansion of the existing knowledge of detection threshold values.

Descriptive sensory analysis indicated significant ( $P \leq 0.05$ ) changes in the aroma profile of Chenin blanc, Pinotage and Shiraz after TCA, TeCA, TBA or PCA was added to the

respective base wines that contained no detectable levels of the haloanisoles. The mouldy taint induced by these haloanisoles were described as *mouldy*, *mouldy-chemical*, *mouldy-chlorine*, as well as *mouldy-acidic*. In Chenin blanc, additions of TCA, in the concentration range 1 to 17 ng/L, resulted in a marked increase in the mouldy aroma and was accompanied by an immediate decrease in fruitiness. This change was already evident at added TCA concentrations of 1 ng/L. Similar trends were observed in Pinotage, while the addition of low levels of TCA to Shiraz (2 ng/L) resulted in a significant ( $P \leq 0.05$ ) decrease in the herbaceous character of the wine. The aroma changes observed were prominent enough to render the wine totally unacceptable in comparison to its original character.

Consumers' degree of liking did not seem to be affected by very low concentration levels of TCA in Chenin blanc, Pinotage or Shiraz, but rejection increased as the concentration increased beyond detection threshold level. A slight gender effect was also noticed. Female consumers appeared to be more sensitive to increasing levels of TCA, whereas male consumers did not respond as negatively to higher concentration levels of TCA.

This study makes an important contribution towards understanding the sensory impact of especially TCA contamination in wine, through the establishment of concentration ranges at which these compounds exert a noticeable detrimental effect on the aroma profile of wine. Additional insight into cork taint in wine is provided by the consumer preference studies, where the effects of the taint on the product acceptance by consumers are demonstrated. The development of a *modus operandi* to ensure that sensory panels provide reliable data, can be regarded as an important contribution to wine-related research. This study is one of the first where advanced sensometric techniques were applied in sensory studies on cork tainted wines.

## Opsomming

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Hierdie studie het gefokus op 'n ernstige kwaliteitsverwante probleem in die globale wynindustrie, insluitende die Suid-Afrikaanse wynindustrie, naamlik kurkbederf in wyn. Jaarliks word groot finansiële verliese gely deur beide kurkprodusente, sowel as wynkelders as gevolg van wyn wat hierdie defek toon. Alhoewel gekontamineerde nuwe ongebruikte kurke dikwels geïmpliseer word as die bron van kontaminasie, kan gekontamineerde keldertoerusting en water ook die oorsprong van die probleem wees.

'n Loodsstudie is onderneem om die voorkoms van kurkbederf in Suid-Afrikaanse wyne te ondersoek. Resultate het gewys dat 3.8% van die 133 wyne wat getoets is, 2,4,6-trichloroanisool (TCA) konsentrasies van 3.5 ng/L en hoër getoon het, soos gemeet met gas chromatografie gekoppel met elektronseleksie deteksie (GC-ECD). TCA konsentrasies hoër as 1 ng/L is aangetref in 18% van die wyne wat ontleed is. Al die geaffekteerde wyne was met soliede of agglomoraat kurk verseël. Die wyne is verkry van verskillende kelders in the Wes-Kaap, Suid-Afrika en verskillende kultivars was verteenwoordig. Geen van die wyne wat met sintetiese bottel sluiters verseël was, het meetbare vlakke van TCA, 2,4,6-tribromoanisool (TBA) of pentachloroanisool (PCA) gehad nie en slegs baie lae vlakke van 2,3,4,6-tetrachloroanisool (TeCA) (1 ng/L of minder) is aangetref. Nog 'n groep van 28 wyne is ondersoek vir die voorkoms van kurkbederf. Die wyne is tydens sertifisering afgekeur deur die amptelike Suid-Afrikaanse wynreguleringsliggaaam, op grond van die teenwoordigheid van kurkbederf in die wyne. GC-ECD analises het getoon dat 30% van die wyne in hierdie groep TCA konsentrasies van 3.5 ng/L en hoër gehad het. Hierdie resultate het gedui op 'n relatiewe hoë insidensie van TCA in die wyne wat ondersoek is, veral dié wat met kurke verseël was. Alhoewel geen algemene afleidings gemaak kan word oor die insidensie van TCA in die wyer wynindustrie op grond van hierdie loodsstudie nie, het die resultate wel die voorkoms van kurkbederf in Suid-Afrikaanse wyne bevestig.

Die deteksiedrempelwaardes is bepaal vir TCA, TeCA, TBA en PCA in drie wyn kultivars deur gebruik te maak van die standaard ASTM metode. Resultate dui daarop dat faktore soos die wynkultivar die deteksiedrempelwaardes betekenisvol beïnvloed het. Ons navorsing stel voor dat 'n deteksiereeks in plaas van 'n gemiddelde deteksiewaarde gebruik word. Die deteksiereekse wat in hierdie studie bepaal is vir TCA, TeCA, TBA en PCA in Chenin blanc, Pinotage en Shiraz, stem ooreen met reeds gerapporteerde waardes in die

literatuur. Hierdie resultaat kan beskou word as 'n waardevolle uitbreiding van die bestaande teorie oor deteksiedrempelwaardes.

Beskrywende sensoriese analise het getoon dat statistiese beduidende veranderinge ( $P \leq 0.05$ ) in die aromaprofiel van Chenin blanc, Pinotage en Shiraz wyn plaasgevind het, nadat TCA, TeCA, TBA of PCA by die wyne, wat self geen meetbare vlakke van haloanisool komponente gehad het nie, gevoeg is. Die kurkbederf is gekarakteriseer as *muf*, *muf-chemies*, *muf-chlooragtig* en *muf-suuragtig*. In Chenin blanc, het TCA toevoegings, in die konsentrasiereeks 1 tot 17 ng/L, 'n merkbare toename in die kurkagtige aroma, maar ook 'n onmiddellike afname in vrugtigheid tot gevolg gehad. Die verandering was reeds merkbaar teen konsentrasievlakke van 1 ng/L. Soortgelyke tendense is waargeneem in Pinotage, terwyl die toevoeging van lae vlakke van TCA in Shiraz (2 ng/L) 'n beduidende afname ( $P \leq 0.05$ ) in die kruid-agtige karakter van die wyn veroorsaak het. Die veranderinge wat waargeneem is, was prominent genoeg om die wyn heeltemal onaanvaarbaar in vergelyking met die oorspronklike wyn te laat.

Verbruikers se aanvaarbaarheid van die wyne waarby haloanisool verbindings gevoeg is, was nie beïnvloed deur baie lae konsentrasie vlakke van TCA nie, alhoewel aanvaarbaarheid gedaal het soos die konsentrasie van TCA bo waarnemings-drempel waarde gestyg het. 'n Geringe verskil is ook tussen manlike en vroulike verbruikers aangedui. Vroulike verbruikers was meer sensitief vir kurkbederf namate die TCA-vlakke gestyg het, terwyl die manlike verbruikers minder negatief gereageer het teenoor kurkbederf.

Hierdie studie maak 'n belangrike bydrae tot die insig in die sensoriese impak van haloanisool kontaminasie, veral deur TCA, op die aroma van wyn. Belangrike bydraes is gemaak in die vasstelling van konsentrasie-intervalle waar veral TCA 'n merkbare negatiewe effek op die aromaprofiel van wyn het. Addisionele insig is ook verkry in die verbruikersvoorkeurstudies, waar die effekte van kurkbederf op die voorkeure van verbruikers aangetoon is. Die ontwikkeling van 'n *modus operandi* om te verseker dat betroubare data van die sensoriese analise verkry is, kan beskou word as 'n belangrike bydrae tot wyn-verwante navorsing. Hierdie studie is een van die eerstes waar gevorderde sensometriese tegnieke toegepas is in sensoriese studies op wyne met kurkbederf.

## Notes

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The language and style used in this thesis are in accordance with the requirements of the scientific journal, *International Journal of Food Science and Technology*.

This thesis represents a compilation of manuscripts where each chapter is an individual entity and therefore some repetition between chapters may occur.

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*“Those who hope in the Lord will renew their strength. They will soar on wings like eagles.” [Isaiah 40:31](#).*



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## Chapter 1

### Introduction

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The control of the sensory quality of wine is of paramount importance if the consumer is to be presented with a high-quality wine (Boutou & Chatonnet, 2007). However, a wine taint can destroy a wine and is regarded as a major factor in the determination of the quality of wine (Fuller, 1995). Sensory wine quality is dependent upon its aromas, which is again directly related to the presence of volatile chemical compounds. A wine taint can be defined as an aroma in wine caused by chemical compounds originating from the wine itself or from an outside source, causing the wine to become unacceptable.

Cork taint is regarded as one of the most important taints found in wine (Vlachos *et al.*, 2007). It is characterised by an unpleasant, musty, earthy, mouldy aroma, also referred to as a “wet basement” aroma (Prescott *et al.*, 2005; Ross, 2002). This defect has resulted in massive financial losses (10 billion US dollar a year) by wine producers all over the globe (Fuller, 1995). In 2000 it was estimated that it affected 0.5% to 2% of European bottled wines and 1% to 5.5% of Australian wines (Peña-Neira *et al.*, 2000).

The chemical compounds mainly responsible for causing cork taint are believed to be the haloanisoles and in wine these substances have extremely low perception threshold values of less than approximately 10 parts per trillion (ppt or ng/L) (Simpson, 1990). *Untainted* or *clean* wines are usually without traces of haloanisoles. Of these compounds, 2,4,6-trichloroanisole (TCA) is most frequently detected in wine, although 2,3,4,6-tetrachloroanisole (TeCA), 2,4,6-tribromoanisole (TBA) and pentachloroanisole (PCA) are also detected, but the prevalence of the latter three substances is somewhat lower than that of TCA (Casey, 1999; Coque *et al.*, 2003; Miki *et al.*, 2005). Other compounds have also been associated with this taint. Both geosmin (*trans*-1,10-dimethyl-*trans*-9-decanol) and 2-methylisoborneol (1,2,7,7-tetramethyl-*exo*-bicycloheptane-2-ol) can induce an earthy, musty, muddy aroma in wine, as well as in contaminated municipal water supply systems. The latter two compounds also have low sensory threshold values, 25 ng/L for geosmin and 30 ng/L for isoborneol in water (Darriet *et al.*, 2000; Salemi *et al.*, 2006). If these two substances are present in wine, it is usually as a result of the use of contaminated water during wine production (Darriet *et al.*, 2000).

Cork is a natural product produced from the bark of the cork oak *Quercus suber* L. (Juanola *et al.*, 2004). The treatment of cork with hypochlorite solutions during the processing of bottle closures may result in the formation of minute amounts of 2,4,6-trichlorophenol (TCP) in the raw product. A number of moulds (*Trichoderma* sp., *Cladosporium* sp., *Penicillium* sp., *Fusarium* sp., *Chrysonilia* sp., etc) are able to degrade these chlorophenols (Coque *et al.*, 2003; Prak *et al.*, 2007). Although cork is considered to be the major source of cork taint in wine (Casey, 1999), contaminated cellar equipment, winery surfaces such as wooden doors, as well as drainage systems have also been positively identified (Prescott *et al.*, 2005; Simpson, 1990).

A vast amount of research has been conducted on cork taint. In order to study the problem classical analytical methods are used for the analysis of haloanisoles in wine. The latter include the extraction of the compounds of interest from the sample matrix using headspace solid-phase extraction method (HS-SPME), solid-phase extraction method (SPE) followed by instrumental analysis of these compounds by gas chromatography (GC) coupled with either electron capture detection (ECD) or mass spectrometry (MS) (Cazes, 2005; Insa *et al.*, 2005).

To date, some research has been conducted on the sensory detection thresholds (DT's) of compounds responsible for cork taint in wine. However, reports indicated that there is a great deal of variation in reported DT values, especially for TCA in wines (Prescott *et al.*, 2005). For example, in a study by Suprenant and Butzeke (1997) the average DT level of TCA in Sauvignon blanc, determined by an experienced panel, was 17 ng/L. In other studies the DT level of TCA in white wine was found to be approximately 4 ng/L (Amon *et al.*, 1989; Sanvicens *et al.*, 2003). For TCA in red wine, DT levels of 2 to 5 ng/L (Liacopoulos *et al.*, 1999) and 22 ng/L (Alvarez-Rodriguez *et al.*, 2002) were reported. The latter variation in DT levels indicates that the DT level of a specific compound can differ considerably in different matrices. There is also a tendency for trained panelists to vary considerably in their ability to detect compounds at low concentration levels. In this regard Pollnitz *et al.* (1996) found that only 40% of a group of experienced wine assessors were able to identify TCA in a range of wines when the TCA concentration was 3 ng/L and higher. With inexperienced wine tasters the situation is much worse and Suprenant and Butzeke (1997) found that the average DT level of TCA in Sauvignon blanc was as high as 210 ng/L for inexperienced tasters. Although sensory analysis is regarded as a reliable research technique, it can be very difficult to detect cork taint at low concentrations due to the varying sensitivity of the panelists, tiring of the smell and taste senses and the temporal persistence of the taint.

These are important factors to consider when performing sensory analysis on cork tainted wines (Mazzoleni & Maggi, 2007).

Sensometrics applies mathematical and statistical methods to problems from sensory and consumer analysis. These techniques are widely used in food research and can also be applied with success in wine research. Multivariate techniques such as preference mapping are used for the determination of the drivers of liking, as well as sensory responses of consumers and trained panelists (Næs and Risvik, 1996). To date, there is limited information available on the consumer's response to cork taint in wine, and although some work has been done on consumer rejection threshold (CRT) levels for TCA in white wine, there are no formal CRT levels for the respective compounds associated with cork taint (Prescott *et al.*, 2005).

To investigate the prevalence of cork taint in South African wines and the resultant impact on the sensory characteristics of the affected wines, an in-depth investigation focusing on instrumental, sensory and consumer analyses is required. Furthermore, data obtained need to be analysed using advanced multivariate statistical techniques in order to interpret the correlations between the chemical, sensory and consumer data.

## **RESEARCH AIMS**

Limited information on the incidence of cork taint in South African wines is available. The first aim will be to determine the natural incidence of TCA, TeCA, TBA and PCA in a selection of South African wines by using GC-ECD analysis.

Although sensory threshold levels of haloanisoles have been studied widely, the findings vary considerably. Different cultivars, wine styles, as well as the experience of assessors can have a major impact on threshold levels. The second aim will be to determine the detection thresholds (DT's) of TCA, TeCA, TBA and PCA in three wine cultivars (Chenin blanc, Pinotage and Shiraz) using the ASTM method of ascending limits, as well as sensory panels differing in wine tasting experience.

The third aim will be to determine the sensory profile of Chenin blanc, Pinotage and Shiraz spiked with known levels of TCA, TeCA, TBA and PCA using descriptive sensory analysis, as well as the determination of the consumer rejection threshold (CRT) of the latter three wine cultivars spiked with different levels of TCA using the 9-point hedonic scale.

Appropriate sensometric techniques will be applied to investigate the relationship between sensory and consumer data.

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## Chapter 2

### Literature review: Cork taint in wine

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## INTRODUCTION

Historic records suggest the first wine being produced on a large scale was during the Neolithic period (ca. 5400 - 5000 B.C.) in the northern Zagros Mountains of Iran. Due to the complex nature of the product, one can be sure that wine faults also existed in those early times and without doubt had a significant influence on the quality of the wine (McGovern, 2003). Wine taints can transform the specific character of an excellent wine into an undesirable product and this can result in massive financial losses for the wine producer (Fuller, 1995).

Cork has been the most popular material for the production of wine bottle stoppers for centuries and is still regarded as the norm for quality wines. The usage of cork stoppers is, however, not entirely without problems. One of the most notorious of these problems is a musty/mouldy taint known as *cork taint* that is often attributed to chemical compounds frequently present in corks. This has led the wine industry to believe that by not using cork as a bottle closure, the chances of bottled wine being contaminated with cork taint will decrease. While this has been shown to be true in many instances, it may, however, not always be the case. In some instances cork taint may also originate from cellar equipment like barrels and wooden structures or from the atmosphere in the cellar (Juanola *et al.*, 2004).

Worldwide, the economic losses as a result of cork taint are substantial. A study by Fuller (1995) showed that cork taint led to total financial losses of up to 10 billion US dollars per annum in the worldwide wine and cork industries. Cork taint can furthermore have an immense negative impact on the wine industry and is regarded as one of the major causes of rejection of wines by consumers (Prescott *et al.*, 2005). As a result of this a substantial amount of research in the field of cork taint has been performed. This research process is ongoing, focussing on the compounds causing cork taint and their origin, the factors affecting their transfer from corks to wine (Sefton & Simpson, 2005), as well the relationship between the sensory and instrumental data (Juanola *et al.*, 2004).

This literature review will focus on cork taint in wine, the incidence and origin thereof, as well as the formation and chemistry of the compounds causing it. In addition, the relevant sensory and analytical methods used in the analysis of tainted wines will also be discussed.

## WINE BOTTLE CLOSURES

Cork is a natural product produced from the bark of the cork oak *Quercus suber* L. and is still considered the superior wine bottle closure. However, due to cork taint, the use of corks poses a significant risk (Juanola *et al.*, 2004). In order to eliminate this risk alternative wine bottle closures have become available, including synthetic and technical corks (also containing a synthetic component), aluminum screw caps with polymeric liners, glass stoppers, etc. Godden *et al.* (2001) showed in a study on 20 wines sealed with various stoppers (cork, synthetic moulded cork, synthetic extruded cork, technical cork, natural cork and screw cap) that no one closure could be considered entirely suitable for long-term storage of wine as assessed by various criteria. In the latter study there was concern with the incidence of cork taint (TCA-contamination of the wine) resulting from four of the closures tested (where two of the latter closures contained cork), as well as the development of other off-taints. One closure (synthetic) resulted in a styrene-like taint in the wine and after a period of storage another closure (also synthetic) resulted in a rubber-like taint in the wine.

When selecting closures for wines, winemakers should assess the impact and risks of the deficiencies associated with the respective closures. Other important factors such as the length of time that the wine will be stored, the nature of storage conditions and the physical characteristics of the closure should be taken into account when choosing the most effective closures for wine (Godden *et al.*, 2001).

## CORK TAINT

*Cork taint* is the name given to an off-aroma in wine which is primarily caused by a group of volatile compounds, namely haloanisoles which are formed from their respective halophenol precursors via a biomethylation process. The taint usually arises when organic plant material or some synthetic phenol-contaminated substrate, has been exposed to chlorine and in turn has been utilised as growth substrate by certain filamentous fungi to produce haloanisoles. These anisoles cause a mouldy, musty or earthy aroma that is highly undesirable in wine, even at very low concentrations (Prak *et al.*, 2007). The terms *cork taint* or *corked* are, however, misleading in implying that the taint originates from cork exclusively. As mentioned, cork taint was shown to arise from other sources beside cork such as wooden structures in wine cellars, wooden pallets, cellar walls, drainage systems in cellars, etc. (Whitfield *et al.*, 1997). In the past the use of hypochlorite solutions during the maintenance of cellar sanitation has led to cork tainted wine and was thus a major problem in the wine industry (Chatonnet *et al.*, 2004).

More than a hundred volatile compounds have been isolated from corks and of these, several contribute to the phenomenon of cork taint (Rocha *et al.*, 1996). In the wine and cork industry one of these compounds, 2,4,6-trichloroanisole (TCA) has become synonymous with cork taint. Three other compounds 2,3,4,6-tetrachloroanisole (TeCA), pentachloroanisole (PCA) and 2,4,6-tribromoanisole (TBA) are also associated with cork taint, however, to a lesser extent. TCA has a very low sensory threshold (1-5 ng/L) in wine and small amounts of releaseable TCA can migrate from spoiled corks to the wine itself during bottle-maturation (Juanola *et al.*, 2004). At low concentrations it becomes difficult in sensory assessments to distinguish between mouldy, musty and earthy attributes often associated with cork taint and frequently TCA is regarded as the only compound solely responsible for the taint and consequently the only compound being analysed for (Simpson & Sefton, 2007).

Other volatile compounds such as geosmin (*trans*-1,10-dimethyl-*trans*-9-decanol) and 2-methylisoborneol (1,2,7,7-tetramethyl-*exo*-bicycloheptane-2-ol) also have the potential to be associated with cork taint. These compounds also result in a musty/earthy character and its presence is commonly a result of using water containing these compounds (Salemi *et al.*, 2006). Geosmin and 2-methylisoborneol also have relatively low perception threshold values, 25 ng/L and 30 ng/L in water, respectively (Darriet *et al.*, 2000; Salami *et al.*, 2006). Similarly, the taint caused by a compound such as guaiacol (smoky, phenolic or medicinal character) is dissimilar to that of TCA. Guaiacol can act in synergism with haloanisoles to produce a prominent cork taint aroma even when the latter compounds are both present in wine at concentration levels lower than their respective detection threshold values (Prak *et al.*, 2007; Silva Pereira *et al.*, 2000).

### **Formation and chemistry of compounds causing cork taint**

Chlorophenols are not natural occurring compounds and are usually a result of contamination of phenol containing products (paints, resins, flame retardants, plant matter, etc) by chlorine. These phenolic compounds are chlorinated producing chlorophenols when they come in contact with chlorine and, once formed, various micro-organisms (*Penicillium* sp., *Trichoderma* sp., *Chrysonilia* sp., *Cladosporium* sp., *Fusarium* sp., etc) are able to degrade these chlorophenols (Prak *et al.*, 2007).

During the harvesting of cork bark and manufacturing of cork, two major cork processing steps can lead to the formation of chlorophenols. These are boiling of bark slabs with water containing chlorine and bleaching of cork cylinders with hypochlorite solutions, thus making

the product susceptible to haloanisole formation via biomethylation by certain fungi as described in the next section (Simpson & Sefton, 2007). Fortunately, in 1990 bleaching of corks was suspended due to the occurrence of cork taint. Bleaching is thus not a problem anymore but the use of chlorine in sanitation may be.

The four main phenolic precursors for the anisoles causing cork taint are 2,4,6-trichlorophenol (TCP), 2,3,4,6-tetrachlorophenol (TeCP), 2,4,6-tribromophenol (TBP) and pentachlorophenol (PCP). These halophenols are converted to the respective haloanisoles, namely TCA, TeCA, TBA and PCA through biomethylation (Casey, 1999; Coque *et al.*, 2003; Insa *et al.*, 2005). Biomethylation is carried out by the chlorophenol *O*-methyltransferase enzyme (CPOMT), that is able to methylate a wide array of phenols (including chlorophenols and bromophenols) to form the resultant haloanisoles (Coque *et al.*, 2003).

Whitfield *et al.* (1997) showed that TCP and TBP are able to be methylated, by the fungus *Paecilomyces variotii*. When the fungal cell comes in contact with TCP, it would typically produce a variety of oxidative enzymes (for example laccases and peroxidases) that are actively secreted from the cells to attack and degrade the chlorophenols outside the cells. As a result of this detoxification, most of the TCP would be degraded without any harm to the fungus (Coque *et al.*, 2003). Nevertheless, due to the fact that chlorophenols are liposoluble, there is always a small proportion that are able to cross the cell wall and cytoplasmic membrane reaching the content of the cell (cytoplasm and nucleus), where they can irreversibly damage important proteins, or even genetic material (DNA). To avoid this hazard, the fungal defense system immediately produces the enzyme CPOMT (Coque *et al.*, 2003). As mentioned above, this type of enzyme is responsible for the conversion of toxic TCP inside the cell into a harmless (for the fungus) compound TCA. The enzymatic step converts the phenolic precursor by removing an active hydrogen molecule and substituting it with a methyl group (CH<sub>3</sub>) as seen in Figures 2.1 a, b, c and d. TCA is then secreted from the fungal cells and it is rapidly absorbed by cork, wood, or any other material on which the filamentous fungi are growing. Such a defensive strategy is very common among these fungi, with the result that most fungi present in both cork or cellars can synthesize haloanisoles (Prak *et al.*, 2007).

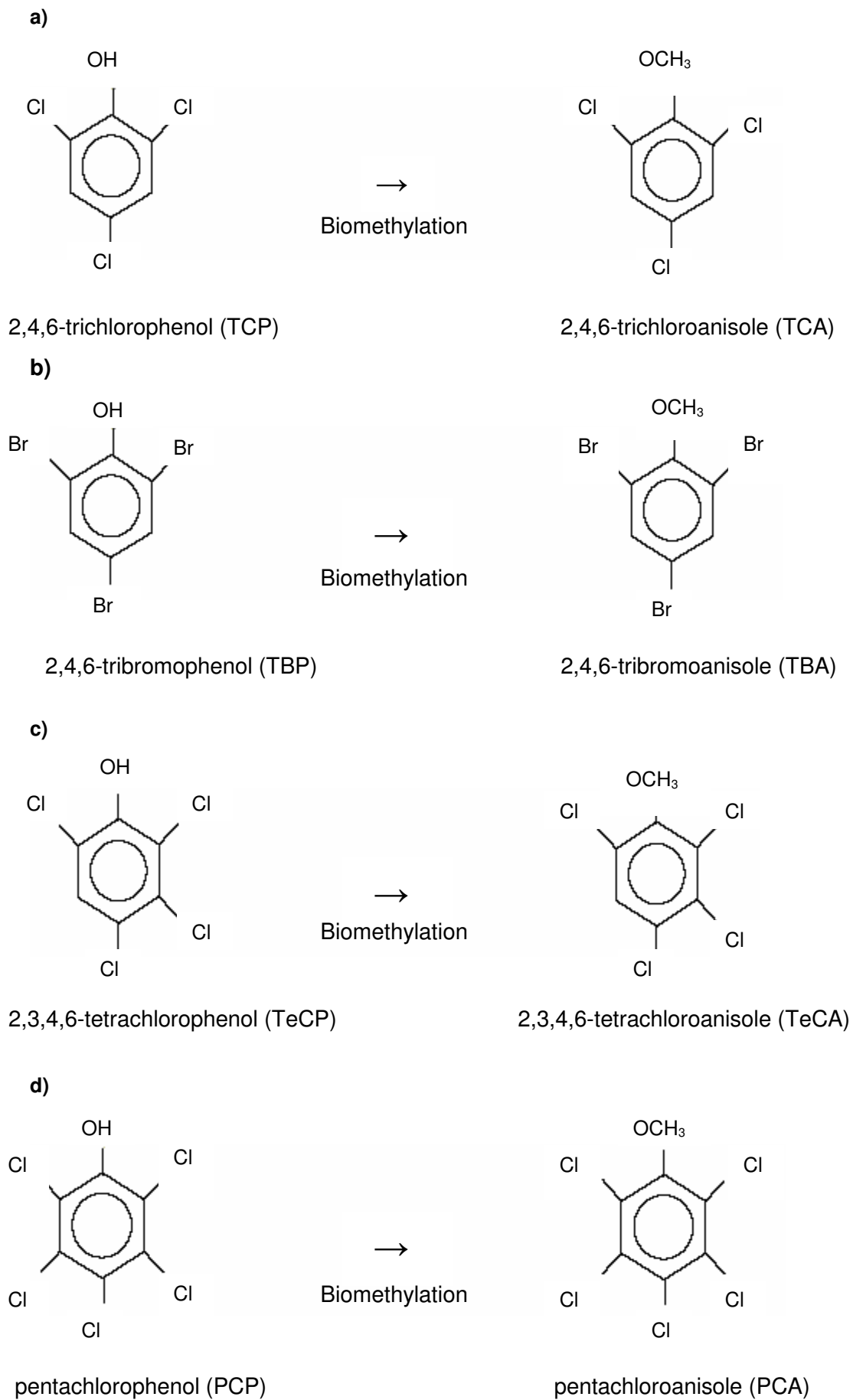
There is evidence that TCA can originate from several other sources. Burttschell *et al.* (1959) have shown that during the chemical formation of chlorophenols, two-and-a-half equivalents of chlorine reacted with phenol in an aqueous solution. Mixtures of TCP (major component) along with 2- and 4-chlorophenol and 2,4- and 2,6-dichlorophenol (minor components) were formed. The chlorine in cleaning products, sanitisers and city/town water

supply systems can enter water drainage systems and waterways where it can react with phenolic compounds thereby creating the ideal environment for the chemical formation of chlorophenols (Simpson & Sefton, 2007).

Another haloanisole, TBA, is formed via biomethylation of TBP. TBA is able to contaminate the cellar atmosphere and can absorb on many surfaces such as wooden barrels or structures, glass, Styrofoam ceilings, cellar walls, etc. In some cases even when initial sources of contamination have been removed, residual contamination absorbed on walls could be sufficient to render the building unsuitable for storage of materials which are destined to come into contact or in close proximity of wine at any point in time (Chatonnet *et al.*, 2004). According to Whitfield *et al.* (1997) TBA was also able to contaminate polyethylene film when it was brought into close contact with fiberboard that was previously contaminated with TBP and inoculated with *Paecilomyces variotii*. The latter is a known methylator of TBP (Whitfield *et al.*, 1997). Polyvinylchloride (PVC) is a plastic commonly used in linings of wine bottle screw caps, also creating an opportunity for cork taint developing in wines sealed in the latter manner.

Regulations for the treatment of cork and cork bark aimed at ensuring the quality of produced cork stoppers are in place for the manufacturing processes of corks. One such regulation is the International Code of Good Practices (SYSTECODE) that was established by C.E.LIÈGE (The European Cork Federation), and is a quality assurance system for the cork industry (International Code of Good Practices, C.E.Liege, 2008). As corks are recognised as the most preferred wine closure it is understandable that cork stopper production is monitored. However, in reality, this may not always be the case (Coque *et al.*, 2003). According to Simpson and Sefton (2007) small cork producers mostly lack the knowledge on what the chemical composition of the processing aids or cleaning agents should ideally be. This is a problem for the cork industry since these small cork producers usually do the initial processing of the bark slabs and often use calcium hypochlorite as a cork bleaching agent to enhance the appearance of the cork surface. Fortunately, in 1990 bleaching of corks was suspended due to the occurrence of cork taint.

From the above, it is clear that there are a large number of sources that can result in elevated levels of haloanisoles, and consequently contaminate bottled wine or even large batches resulting in cork taint spoilage. The use of corks can therefore have devastating consequences, mainly as a result of ignorance by cork producers as shown by Simpson and Sefton (2007). These consequences can have long-lasting effects on the wine and cork industry as a whole. It is thus important to inform wine and cork producers of new developments and strategies for the prevention of cork taint.



**Figure 2.1** Changes in chemical structure when a) TCP, b) TBP, c) TeCP and d) PCP are respectively converted to TCA, TBA, TeCA and PCA through biomethylation.

## **Incidence of cork taint in wine**

It is estimated that 2 to 7% of wines that use cork as their wine bottle stopper, do develop cork taint (Fuller, 1995). However, according to Sefton and Simpson (2005) approximately only 1% of all corks are classified as TCA-contaminated during quality assessments of corks by major Australian wineries. A possible explanation for this inconsistency might be due to the fact that corks are examined by smell and, only if cork taint is suspected it is chemically analysed in laboratories for haloanisoles. Due to the vast range of compounds that could exist in cork, some corks that are tainted with haloanisoles may easily remain undetected by smell alone.

It has been reported that TCA is responsible for about 80% of the cases of cork taint (Coque *et al.*, 2003; Juanola *et al.*, 2004). In a survey of commercial wines presented at a wine assessment course for experienced wine analysts, 18 of the 374 bottles (4.8%) were found to be affected by cork taint by at least 20% of the participants. TCA was subsequently detected by chemical analysis at concentrations of 1 ng/L and higher in each of the 18 bottles of wine (Pollnitz *et al.*, 1996). Hervè *et al.* (2004) also stated that during quality assurance screenings by trained sensory analysts, TCA has been found in 70% to 80% of corks rejected due to mouldiness.

In another recent study by Soleas *et al.* (2002) from the Liquor Control Board of Ontario, Canada, 2400 commercial wines were sensory and chemically analysed. These wines were tested by a panel of expert tasters who judged 145 of these wines as tainted by fungal aromas. After analysis by GC-MS only 71 wines (49%) had TCA levels higher than a detection threshold of 2 ng/L. This result clearly indicates that for 51% of the wines (N=74) the contamination was attributable to unknown compounds other than TCA. In this study, however, the levels of TeCA, TBA and PCA levels were not determined and therefore the ultimate cause of the taint in the 51% of the tainted wines remains uncertain.

Cork taint is not only a wine-related problem but also a general quality concern and creates problems in multiple food products (Coque *et al.*, 2003; Whitfield *et al.*, 1997). The occurrence of this taint is well documented in other foodstuffs such as eggs, poultry, pulp chips, dried fruits, Brazilian coffee, drinking water, as well as marine food products (Cserjesi & Johnson, 1972; Engel *et al.*, 1966; Spadone *et al.*, 1990).

It is evident that the results and conclusions drawn from the above-mentioned researchers vary widely, however, the incidence of cork taint, especially TCA, is well documented in

literature. Research on the incidence of haloanisoles in wine, especially in South African wines, is necessary. This will give an estimation of the situation in the South African wine industry and will potentially clarify some inconsistencies in previous findings.

## **SENSORY METHODS USED IN WINE ANALYSIS**

Sensory analysis has been defined as a scientific method used to evoke, measure, analyze and interpret people's reactions to products based on their senses (Stone & Sidel, 1993). Descriptive sensory analysis is one such method that facilitates the scientist to obtain complete sensory descriptions of the product in question and to acquire the underlying attributes which are essential to the acceptance thereof. In quality assurance of food or beverage products the use of descriptive analysis can be an invaluable tool when a problem must be defined and investigated (Lawless & Heymann, 1998).

During wine sensory analysis compounds such as alcohol, which contribute to a large portion of the chemical make-up of wine, could impact the way wine odorants are perceived. A study conducted by Fisher and Noble (1994) included 18 wines varying in ethanol content, pH and (+)-catechin level. Trained panelists assessed sourness and bitterness intensities in the wine and found that an increase in ethanol content raised bitterness and only had a slight effect on sourness. Similar effects of ethanol and bitterness were observed by Martin and Pangborn (1970) and Vidal *et al.* (2004). In a recent study, Grosch (2001) observed that the less ethanol present in a complex wine model mixture, the greater the intensity of the fruity and floral odours. This was ascribed to the increase in partial pressure of the odorants with reduced ethanol concentration. Ethanol thus displays the ability to modify the perception of wine aroma and volatile compounds, but it remains uncertain whether this impact is physico-chemical and/or perceptual. Le Berre *et al.* (2007) also showed for instance that high concentration of whiskey lactone (described as a *woody aroma*) had a significant masking effect on isoamyl acetate (*fruity aroma*) in a diluted alcohol solution.

A reasonably large amount of sensory research has been conducted on the determination of detection threshold levels of the compounds associated with cork taint (Amon *et al.*, 1989; Chatonnet *et al.*, 2004; Duerr, 1985; Liacopoulos *et al.*, 1999; Prescott *et al.*, 2005). Limited information is published on the application of descriptive sensory analysis on tainted wines where the aim is to profile the wines and indicate the spectrum of sensory attributes associated with the respective compounds resulting in cork taint (Pollnitz *et al.*, 1996). Although a number of consumer studies have been performed to determine the consumer rejection level of cork tainted wines, limited information is available on this subject (Prescott



*et al.*, 2005). The sensory methodologies generally used in researching the detection threshold, sensory profile and acceptability of compounds associated with cork taint will be discussed in this section.

### **Detection thresholds**

The method used in sensory analysis for discriminating between products or different concentration levels of any given compound in wine, is a forced choice method similar to the triangle test. This method is also often applied when determining threshold levels of compounds (Mazzoleni & Maggi, 2007). During this type of testing, three samples are presented simultaneously to the panelist. Two samples are the same (from the same formulation) and the third sample is an odd sample (from a different formulation). The null hypothesis states that the probability (P) of making a correct choice when there is no perceptible difference between the samples is one in three ( $H_0 : P_t = 1/3$ ). The alternative hypothesis states that the probability that the population will make the correct choice when they perceive something different between the samples will be larger than one in three ( $H_0 : P_t > 1/3$ ) (Lawless & Heymann, 1998). The modified forced choice method where a series of triangle tests in ascending concentration is presented to the judges for testing (ASTM E679-91 method), is frequently used to ascertain the detection threshold values of aroma compounds in wine (ASTM, 1997; Shareefdeen, 2005; Lim & Lawless, 2006; Mazzoleni & Maggi, 2007).

Triangle testing has been implemented successfully by various authors in the determination of detection thresholds of haloanisoles in wine (Mazzoleni & Maggi, 2007; Lawless & Heymann, 1998, Prescott *et al.*, 2005; Sefton & Simpson, 2005). Sefton and Simpson (2005) made a distinction between detection (a minimum value of a sensory stimulus needed to give rise to a sensation) and the recognition threshold (the minimum value of a sensory stimulus permitting identification of a sensation perceived). According to Sefton and Simpson (2005) the detection threshold of TCA in wine can range between 1.4 - 4.6 ng/L and that for recognition between 4.2 - 10 ng/L.

Various detection thresholds for TCA have been reported in literature by several authors. Amon (1989) and Sanvicens *et al.* (2003) respectively reported detection threshold values of 4 and 4 - 10 ng/L for TCA in dry white wine and white wine, respectively. Silva Pereira *et al.* (2000) reported a detection threshold value of 10 ng/L for TCA in white and red wine and Alvarez-Rodriguez *et al.* (2002) reported a high detection threshold value of 22 ng/L for TCA in red wine.

It is well known that the wine matrix, expertise of the panel and methodology used, can have a major impact on the detection threshold of a specific compound (Mazzoleni & Maggi, 2007; Sefton & Simpson, 2005). A study by Martineau *et al.* (1995) involving three (distinctly) different wine cultivars demonstrated that the detection threshold of diacetyl was up to 15 times higher in Cabernet Sauvignon than in Chardonnay. The effect of wine style on the detection limits of TCA was researched by Mazzoleni and Maggi (2007). Their research involved different cultivars of white and red wines with differences in vintage, grape composition and wine style. They found that for white wines, detection of TCA was easier in non-wooded than in wooded wines. In red wines the woody aroma only had a minor influence on the detection of TCA. The overall style of both red and white wines therefore had a significant influence on the panelist's ability to detect TCA successfully.

### **Descriptive sensory analysis**

According to Lawless (1999), descriptive sensory analysis is the primary sensory tool for analysing complex aromas, fragrances, flavours, etc. The use of a panel to specify the intensities of specific attributes is the foundation of descriptive sensory analysis. The task of the panelists is to provide an intensity rating for each of the attributes that reflect the perceived intensity of that specific characteristic in the product. This is based on a psychophysical model for subjective intensity. As a result of this model the sensory perception can be analyzed and reported using a set of independent descriptors. Independent indicates that the individual descriptors offer a different kind of experience when they are perceived. For instance a *fruity* note is unrelated to a *spicy* note (Engen & Pfaffmann, 1959, 1960).

Descriptive sensory analysis is therefore a generic research technique used by sensory scientists to produce objective descriptions of products in terms of perceived sensory characteristics. This technique usually involves 1) training of the judges to score the respective samples according to the specific sensory attributes on a line scale; 2) the determination of judge reproducibility; 3) analysis of the samples according to an experimental design, followed by analysis of variance or an appropriate multivariate statistical technique. This technique should never be used for consumers because in this method a panel of judges is trained to be consistent and reproducible (Lawless & Heymann, 1997).

Wine aroma is very complex, since the end product constitutes a large number of chemical compounds. The essence of a specific wine lies in the ratio and the combination of these

compounds (Juanola *et al.*, 2004). This makes research of wine aroma extremely challenging especially when profiling complex wine aromas. Studies carried out by Engen and Pfaffmann (1959, 1960) showed that humans are able to accurately identify only three levels of odour intensity, compared to other sense modalities where up to seven or even more can be accurately discerned. It can be extremely difficult to accurately distinguish between odour intensities at detection threshold level. To minimize noise in the data, it is therefore essential to use reliable judges with sufficient training and/or experience of the specific aromas.

It is well known that sensory analysis of odours is far more difficult than analysing visual, texture or taste modalities (Lawless & Heymann, 1998). Humans usually have difficulty to identify common odours even in the simplest of mixtures (Laing *et al.*, 1991). In a complex medium such as wine it is even more difficult. Lawless (1999) stated from his experience as a wine judge on the Beverage Testing Institute, USA that in any given panel of about seven wine judges, no two would have exactly the same description of aroma character and that any two people might agree on one or two of the odour notes present. This effect may be partly ascribed to the individual differences in the sensitivity to specific odour compounds, as observed in other olfactory methods such as evaluating gas chromatographic effluents by smell (Marin *et al.*, 1988). Furthermore, perceptual synergism is an effect observed when odours are still detected in products even when they exist in concentrations below their respective threshold values (Selfridge & Amerine, 1978).

### **Consumer sensory analysis**

Consumer sensory analysis should be performed at the end of product development or a reformulation cycle and is usually used to compare prototypes or market competitors. In food and beverage consumer products, two main approaches are usually being followed namely the measurement of preference and the measurement of acceptance (Jellinek, 1964). In the measurement of preference the consumer has a choice between competing products. The consumer has to choose one product over another. In the measurement of acceptance or liking, the consumer panelists rate their liking for the product on a scale. The 9-point hedonic scale is usually used when degree of liking, i.e. preference as well as acceptance are to be measured. On this scale 9 represents *like extremely* and 1 is *dislike extremely* where 5 represents *neither like nor dislike*. Acceptance tests only require one product, but in most cases acceptance scores are determined for multi-product tests and then preference can be determined indirectly from these scores (Lawless & Heymann, 1998).

Limited consumer studies on cork taint have been conducted. Prescott *et al.* (2005) indicated that some consumers reject a product containing TCA, but only at high concentrations. This study showed that the concentration at which TCA was rejected was much higher than a detection threshold level indicated by a trained panel. Prescott *et al.* (2005) also pointed out that red wine had a much lower rejection level than white wine due to the more natural earthy aromas of the red wines masking the cork taint.

## **Sensometrics**

The long-term success of a product is usually dependent of its performance when the product is being consumed. This is largely a result directly related to the ingredients and the manufacturing processes, which together determine the sensory characteristics of the products. Preference mapping is a technique that can be used to measure the *performance* of a product in terms of how it is liked or disliked by consumers (Helgesen *et al.*, 1997).

The preference mapping techniques refer to a range of multivariate statistical methods that are used to relate sensory to consumer data (McEwan *et al.*, 1998). Preference mapping may be divided into two categories, external analysis (PREFMAP) and internal analysis (MDPREF). MDPREF or internal preference mapping is derived from preference data where products and individual consumer's hedonic information are projected into the perceptual map (product space). This product space represents differences among the products and a set of directions, one for each consumer, that show the individual's direction of increasing preference (Kuhfeld, 1993; McEwan *et al.*, 1998). During PREFMAP, a perceptual product space is obtained from sensory (trained panel) or instrumental data. The consumer's hedonic response (preference scores) is then projected into the product space in order to obtain a preference map indicating the drivers for consumer preference (Lawless & Heymann, 1998; McEwan *et al.*, 1998).

## **ANALYTICAL METHODS USED IN WINE ANALYSIS**

There are a vast number of different instrumental methods available to determine the chemical profile of products such as wine. This section will focus on gas chromatography which is used frequently in aroma and flavour analysis. Gas chromatography (GC) is an analytical technique commonly used for the separation and quantification of volatile compounds (Grob, 1977). GC-analysis of volatile compounds in wine is a very important tool used for wine classification, quality control and understanding wine sensory properties (Ortega *et al.*, 2001). TCA causes a problem when it is present in wine at very low

concentration levels (as low as 2 ng/L to 3 ng/L) (Amon *et al.*, 1989; Liacopoulos *et al.*, 1999; Pollnitz *et al.*, 1996, Sanvicens *et al.*, 2003). Therefore, due to the high sensitivity of several GC-methods of detection, eg. mass spectrometry (MS) and electron capture detection (ECD), it is one of the most commonly used methods during analysis of TCA in wines (Riu *et al.*, 2007; Vlachos *et al.*, 2007). In most cases ECD is preferred due to lower cost and higher sensitivity (Vlachos *et al.*, 2007). Flame ionization detection (FID) is also a GC-method of detection which is mostly used in the study of important wine volatile compounds. It can also be used for haloanisole analysis, but it is generally not sensitive enough for analysis of low haloanisole concentrations (Casez, 2005).

### **Electron capture detector**

The electron capture detector (ECD) was a result of a series of developments by the Shell Company's Research and Development Laboratory in California during 1951. An ECD response is based on a decrease of beta-particles emitted by a radioactive source within the detector when electron-capturing species pass through it. The original design was based on a beta-ray ionization cross-section detector. From the limited success of the detector a new beta-ray argon detector was developed in 1958 (Grob, 1977).

The ECD is now probably the most sensitive of GC-detectors presently available. However, like most highly-sensitivity detectors, it is also very specific and will only detect substances with electron capturing properties such as halogens. The sensitivity of the ECD is as low as  $1 \times 10^{-9}$  g/L and is thus very commonly used in trace analysis of halogenated compounds (Alzaga *et al.*, 2003; Insa *et al.*, 2005; Riu *et al.*, 2007; Vlachos *et al.*, 2007).

### **Mass spectrometry**

Mass spectrometry (MS) is another detection method commonly used in combination with GC during the identification and quantification of compounds causing off-odourants in wine (Boutou & Chatonnet, 2007; Insa *et al.*, 2005; Vlachos *et al.*, 2007). MS was also used in comparative studies comparing the aroma profile of wines at different stages of ripening (Palomo *et al.*, 2007). The high sensitivity of MS has made it possible for the analysis of minor wine compounds, as well as low concentration levels of TCA (Insa *et al.*, 2005; Vlachos *et al.*, 2007).

## Flame ionization detector

The flame ionization detector (FID) has a very wide dynamic range but has less sensitivity than MS and ECD. FID will detect, with the exception of about half a dozen low-molecular-weight compounds, a range of substances that contain carbon with roughly the same sensitivity (Cazes, 2005). FID has not been commonly used in studies for the quantification of haloanisoles but has been extensively used for the analysis of wine volatiles, as well as differences in aroma composition at different stages in the wine making process (Ortega *et al.*, 2001).

## SUMMARY

Cork taint has caused a major upset in the global wine and cork industry during the past two decades, leading to substantial financial losses in this regard. 2,4,6-Trichloroanisole is reported to be the main cause of cork taint and is able to render wine undesirable by presenting a mouldy character at extremely low concentration levels. Cork has become the main focus of cork taint and various legislations have been laid in place for the prevention of cork taint in cork, as well as in the end-product. Subsequently many alternative closures have been made available to the wine industry, however, these closures are not flawless. In aged and/or high quality wines cork is still the most preferred wine bottle stopper regardless of the major risk of cork taint (Sefton & Simpson, 2005).

Chemical analytical methods have shown to be very well developed for haloanisole quantification in wine and usually include the application of GC-MS or GC-ECD combined with various methods of extraction. These analytical methods are mostly used in the quality control of cork or wine (Alzaga *et al.*, 2003; Boutou & Chatonnet, 2007; Insa *et al.*, 2005; Riu *et al.*, 2007; Vlachos *et al.*, 2007).

Certain aspects of cork taint in wine are still relatively unknown and research is needed to elucidate this. As seen in this literature review large variations in detection threshold values have been reported by various authors (Alvarez-Rodriguez *et al.*, 2002; Amon, 1989; Sanvicens *et al.*, 2003; Silva Pereira *et al.*, 2000). Consumer analysis has also shown that rejection of wines containing TCA occur at concentrations well above its detection threshold level (Prescott, *et al.*, 2005). The incidence of cork taint has been well researched in many countries (Coque *et al.*, 2003; Fuller, 1995; Juanola *et al.*, 2004; Pollnitz *et al.*, 1996; Sefton & Simpson, 2005; Soleas *et al.*, 2002), however, the incidence of cork taint in South African wines is relatively unknown. Furthermore, limited descriptive sensory and consumer studies

have been done on cork tainted wine (Prescott *et al.*, 2005). Scientific information in this field will be of great value to the South African, as well as the international wine industry.

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## Chapter 3

### Explorative investigation into the incidence of cork taint in commercial South African wines

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## ABSTRACT

It is estimated that 2 to 7% of all wines produced internationally develop a degree of cork taint. The aim of this study was to determine the incidence of TCA (2,4,6 trichloroanisole), TBA (2,4,6-tribromoanisole), TeCA (2,3,4,6-tetrachloroanisole) and PCA (pentachloroanisole) in South African wines. Wines were sourced at different wineries in the Western Cape region, South Africa and these wines varied in cultivar, as well as type of closure used to seal the finished product. Gas chromatography-electron capture detection (GC-ECD) was used to determine the presence of TCA, TBA, TeCA and PCA. The sampled wines sealed with solid or agglomerate cork stoppers illustrated TCA concentrations of >1 ng/L in 18% of the samples, and TCA concentrations >3.5 ng/L in 3.8% of the sampled wines. Wine sealed with synthetic closures (synthetic corks and screw caps) illustrated no TCA, TBA and PCA contamination and only low levels of TeCA contamination (1 ng/L or less). According to this study the incidence of this TCA is moderately high in a small sample set of South African wines sealed with corks.

## INTRODUCTION

It is estimated that about 2 to 7% of wines (mostly from Europe, Australia and United States) that use cork as bottle closures develop a degree of cork taint (Prescott *et al.*, 2005; Sefton & Simpson, 2005). This percentage is a significant one, especially in a competitive global wine market where wine producers cannot afford to harm their reputation by selling corked wine to loyal consumers, as well as potential customers. Due to the negative impact that cork taint can potentially have on the wine industry, much effort has gone into researching this problem (Chatonnet *et al.*, 2004; Fuller, 1995; Juanola *et al.*, 2004; Prak *et al.*, 2007; Sefton & Simpson, 2005).

Apart from haloanisoles, more than 100 volatile compounds (of which some can produce taints in wine) have been detected in finished corks (Rocha *et al.*, 1996). It has also been reported that TCA is indeed responsible for about 80 to 100% of the cases of cork taint (Coque *et al.*; 2003; Juanola *et al.*, 2004; Pollnitz *et al.*, 1996).

The incidence of cork taint in South African wines is not highly documented. No estimate for the incidence of cork taint in South African wines could be found in scientific literature. However, cork taint does exist in South African wines and has resulted in the establishment of 1) the South African Cork Council (SACC) with the responsibility of setting up quality guidelines to limit the occurrence of cork taint and 2) laboratories specialising in the testing of

releasable TCA (2,4,6-trichloroanisole), TBA (2,4,6-tribromoanisole), TeCA (2,3,4,6-tetrachloroanisole) and PCA (pentachloroanisole) in natural and agglomerate corks and/or the analysis of wines for the corresponding phenolic precursors TCP (2,4,6-trichlorophenol), TBP (2,4,6-tribromophenol), TeCP (2,3,4,6-tetrachlorophenol) and PCP (pentachlorophenol).

The aim of this study was to take the proverbial *snap-shot* of the incidence of TCA, TBA, TeCA and PCA in a selection of South-African wines. The study consisted of two separate analyses. In the first analysis (Sample set 1) the incidence of the haloanisoles TCA, TeCA, TBA and PCA in 133 bottled South African wines selected at random within the Western Cape wine region was investigated. The second analysis (Sample set 2) included an investigation on the incidence of TCA, TeCA, TBA and PCA in 28 bottled wines rejected during certification due to a mouldy taint. Both analyses implemented gas chromatography (GC) as an analytical technique and electron capture detection (ECD) as a method of detection.

## **MATERIALS AND METHODS**

### **Samples**

#### *Sample set 1*

One hundred and thirty three bottled wines (N=133) were obtained from wine farms in the Western Cape region, South Africa in 2007. This set of wine samples included different cultivars, vintages and wine styles. Ninety three (N=93) of these wines were sealed with conventional or agglomerate corks, 20 bottles contained synthetic corks and another 20 bottles were sealed with aluminum screw caps. Prior to analyses, these wine samples were stored in a cool environment and exposed corks were sealed off with aluminum foil to prevent any possible contamination.

#### *Sample set 2*

The second set of wine samples consisted of twenty eight (N=28) bottles of wine which were previously subjected to certification by the South African Wine and Spirits Board. Twenty (N=20) of the bottles were rejected during certification due to mouldy taint and eight (N=8) bottles passed the certification process. The latter eight wines served as control samples and were considered to be free of any taint.

## **Wine certification**

Sample set 2 was certified using a panel of judges consisting of five to seven experienced wine evaluators. The samples were presented to each judge in a randomised order. The tasting was conducted without the knowledge of winery, cultivar, wine style and vintage. The panel had to taste the samples for overall wine quality and the presence of specific foreign aromas. As soon as 60% of the judges indicated a specific foreign aroma (wine fault), a new bottle of the same wine was opened and the sample in question was re-evaluated to ascertain whether the wine should be certified or not. Should four out of the five participating judges indicate the same wine fault again, the wine was rejected without a possibility of revising the certification decision (Theron, 2008).

## **Chemical analysis and instrumentation**

All the samples were analyzed by Quantum Laboratories, Institute of Wine Biotechnology (IWBT), Stellenbosch University, South Africa. The above-mentioned four haloanisoles were extracted from the wine by headspace solid phase micro extraction (SPME) and quantified by dual column GC-ECD. All results were given in ng/L (parts per trillion). The limit of detection (LOD) for haloanisoles was 0.2 ng/L and the limit of quantification (LOQ) for haloanisoles was 0.5 ng/L. The analyses were conducted mid 2008.

## **RESULTS AND DISCUSSION**

TCA is undoubtedly the major chemical compound causing cork taint (Amon *et al.*, 1989; Duerr, 1985; Sanvicens *et al.*, 2003) and TCA contamination is usually perceived in wine by a sensory panel at a detection threshold of approximately 3.5 ng/L (Amon *et al.*, 1989; Liacopoulos *et al.*, 1999; Pollnitz *et al.*, 1996; Prescott *et al.*, 2005; Sanvicens *et al.*, 2003; Sefton & Simpson, 2005). In view of this, the results in Tables 3.1 and 3.3 illustrate haloanisole concentrations of 1 ng/L (*below detection threshold*) and higher, and the results in Tables 3.2 and 3.4 illustrate haloanisole concentrations of 3.5 ng/L (*at detection threshold*) and higher.

### **Sample set 1**

Results from the GC-ECD analysis showed that 18% of the 133 bottled wines in Sample set 1 contained TCA at concentration levels of 1 ng/L and higher (Table 3.1). These bottles were all sealed with solid or agglomerate corks. As evident in Table 3.1 the bottled wines



sealed with solid and agglomerate corks illustrated TeCA contamination in 2.2% of the bottles at concentration levels of 1 ng/L and higher. Table 3.2 illustrates that 3.8% of bottles sealed with corks contained TCA at concentration levels of 3.5 ng/L and higher. As mentioned, a concentration of 3.5 ng/L is high enough to produce a cork taint character in most wines (Amon *et al.*, 1989; Liacopoulos *et al.*, 1999; Pollnitz *et al.*, 1996; Prescott *et al.*, 2005; Sanvicens *et al.*, 2003; Sefton & Simpson, 2005). The incidence of 3.8% in this study corresponds well to the estimation that worldwide approximately 2% to 7% of all bottled wines are affected by cork taint (Amon *et al.*, 1989; Liacopoulos *et al.*, 1999; Pollnitz *et al.*, 1996; Prescott *et al.*, 2005; Sanvicens *et al.*, 2003; Sefton & Simpson, 2005).

**Table 3.1** The incidence of TCA, TeCA, TBA and PCA at concentrations of 1 ng/L and higher in Sample set 1

Closure type	TCA (%)	TeCA (%)	TBA (%)	PCA (%)
Synthetic corks (N=20)	0.0	15.0	0.0	0.0
Screw caps (N=20)	0.0	0.0	0.0	0.0
Solid and agglomerate corks (N=93)	18.0	2.2	0.0	0.0

**Table 3.2** The incidence of TCA, TeCA, TBA and PCA at concentrations of 3.5 ng/L and higher in Sample set 1

Closure type	TCA (%)	TeCA (%)	TBA (%)	PCA (%)
Synthetic corks (N=20)	0.0	0.0	0.0	0.0
Screw caps (N=20)	0.0	0.0	0.0	0.0
Solid and agglomerate corks (N=93)	3.8	0.0	0.0	0.0

Interesting to note is that according to Table 3.1 and Table 3.2, 15.2% (18% minus 3.8%) of this group of wines contained TCA at concentrations between 1 ng/L and 3.5 ng/L. In time these concentrations may increase, possibly due to the migration of TCA through the cork cell structure to the wine. This migration of TCA through the cell structure of cork is the result of a concentration gradient. This leads to higher concentrations of TCA on the outer layer of the cork. The TCA can then be transferred to the wine through a dynamic ongoing process (Casey, 1994; Pollnitz *et al.*, 1996) and result in an even higher TCA concentration in the wine.

Bottles sealed with screw caps showed no traces of haloanisole contamination (Table 3.1). However, the wines sealed with synthetic corks contained traces of TeCA at concentrations of 1 ng/L and higher (Table 3.1). None of the bottled wines contained TeCA, TBA or PCA at concentrations levels of 3.5 ng/L and higher. In literature the average detection threshold

levels reported for TeCA are 10 ng/L to 15 ng/L (Chatonnet *et al.*, 2006) and for PCA more than 50 ng/L (Chatonnet *et al.*, 2006). It can thus be assumed that TeCA and PCA would have no influence on the wines in terms of cork taint.

## Sample set 2

The results in Table 3.3 indicate that of the 20 bottles of wine that were rejected by the certification panel for mouldy taint, 45% contained TCA at concentration levels of 1 ng/L and higher. According to Table 3.4, 30% of the rejected bottles of wine contained TCA at concentration levels of 3.5 ng/L and higher. None of the non-rejected samples contained TCA (Tables 3.3 and 3.4). These results signify that TCA is the major contributor to cork taint (Tables 3.3 and 3.4) and played a major role in the rejection of these wines during certification (Coque *et al.*, 2003; Juanola *et al.*, 2004; Pollnitz *et al.*, 1996; Prak *et al.*, 2007).

Five percent of the rejected wines contained TeCA at concentration levels of 3.5 ng/L and higher (Table 3.4). However, TeCA is still well below its threshold value of 10 ng/L to 15 ng/L in all the samples (Table 3.5) (Chatonnet *et al.*, 2004). TeCA on its own would most probably not result in a mouldy taint, although in conjunction with TCA there could be a synergistic effect (Prak *et al.*, 2007; Silva-Pereira *et al.*, 2000). According to Table 3.4 the compound PCA was detected in 10% and 14% of rejected and non-rejected bottled wines respectively, at concentrations of 3.5 ng/L and higher. The fact that the incidence of PCA was higher in non-rejected wines indicates that the low levels of PCA were not of significant influence during the certification process, i.e. during the human assessment of wines. It should also be noted that PCA concentrations in the rejected wines (Table 3.5) were well below its reported detection value of > 50 ng/L (Chatonnet *et al.*, 2006).

**Table 3.3** The incidence of TCA, TeCA, TBA and PCA at concentrations of 1 ng/L and higher in 28 bottled wines, twenty samples were rejected and eight were not rejected by an expert panel of judges for mouldy taint during certification

Samples	TCA (%)	TeCA (%)	TBA (%)	PCA (%)
Rejected (N=20)	45.0	5.0	0.0	25.0
Not rejected (N=8)	0.0	7.0	0.0	28.0

**Table 3.4** The incidence of TCA, TeCA, TBA and PCA at concentrations of 3.5 ng/L and higher in 28 bottled wines, twenty samples were rejected and eight were not rejected by an expert panel of judges for mouldy taint during certification

Samples	TCA (%)	TeCA (%)	TBA (%)	PCA (%)
Rejected (N=20)	30.0	5.0	0.0	10.0
Not rejected (N=8)	0.0	0.0	0.0	14.0

**Table 3.5** Concentration of (ng/L) of TCA, TeCA, TBA and PCA in twenty rejected wine bottles during certification due to mouldy taint

Wine nr.	TCA	TeCA	TBA	PCA
1	31.2	0	0	0
2	5.1	0	0	0
3	4.99	0	0	0
4	42.31	0	< 0.5	0
5	0.6	0	0	0
6	< 0.5	3.8	0	4.5
7	26.51	0.59	0	3.35
8	9.8	0.85	0	1.2
9	2.35	0	0	0
10	3.2	0	0	0
11	0.57	0	0	0
12	0	0	0	0
13	0	0	0	0
14	1.91	0	0	0
15	0	0	0	0
16	0	0	0	0
17	0	0	0	0
18	0	0	0	0
19	0	0	0	0
20	0	0	0	0

Note that some of the rejected wines in Table 3.5 (Sample nr. 12, 13, 15-20) contained no haloanisoles. It is possible to deduce that *human error* could have played a role during the certification process or perhaps a component other than TCA, TeCA, TBA or PCA could have been responsible for the mouldy taint (Rocha *et al.*, 1996). The latter finding is consistent with a study done by Soleas *et al.* (2002) where expert tasters identified 145 bottles of wine as tainted by fungal aromas. Analysis by GC-MS indicated that only half of

the wines had TCA levels of 2 ng/L and more. Soleas *et al.* (2002) suggested that the samples containing no TCA most probably contained other compounds resulting in the taint similar to that induced by TCA. It should be noted that the results from Sample set 2 are limited by the number of samples, especially the control samples (N=8).

## CONCLUSIONS

In this study the occurrence of cork taint was investigated chemically in a selection of wines sourced at different wineries in the Western Cape region, South Africa, as well as in wines rejected during certification due to a mouldy taint.

The results (*Sample set 1*) showed that TCA was the most common cork taint compound present in South African wines. Furthermore, all the wines containing TCA were sealed with a solid or agglomerate corks. From this study it can be concluded that TCA contributes most towards cork taint and that cork is most probably the main factor involved in TCA contamination (Prescott *et al.*, 2005; Sefton & Simpson, 2005) in bottled South African wines. The synthetic corks and screw caps resulted in no TCA, TBA and PCA contamination and only sub-threshold concentrations of TeCA. These findings can be regarded as a positive outcome regarding synthetic wine closures.

Wines previously subjected to certification (*Sample set 2*) revealed interesting results. As one would expect, the rejected wines illustrated the presence of TCA above threshold level. The results again indicate that TCA is the main contributor towards cork tainted wines. The fact that some of the wines did not pass certification due to the presence of a mouldy taint, but had no haloanisole contamination according to GC-ECD analysis, indicates that compounds other than haloanisoles were most probably responsible for the perceived mouldy taint.

In summary, these findings suggest that the incidence of TCA in a relatively small sample set is in agreement with worldwide estimates. Although the percentage of cork tainted wines is relatively low, the problem cannot be ignored and the South African wine industry should focus on the reduction of TCA in wines, but also the identification of other compounds responsible for an aroma similar to that of TCA.

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## Chapter 4

### Detection threshold levels of TCA, TeCA, TBA and PCA in a selection of South African wines

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## ABSTRACT

Although the detection threshold level for 2,4,6-trichloroanisole (TCA) in wine has been researched widely, literature indicates that there is still a large variation in reported threshold values for TCA. Detection threshold information on 2,3,4,6-tetrachloroanisole (TeCA), 2,4,6-tribromoanisole (TBA), as well as pentachloroanisole (PCA) is more limited. The aim of this study was to determine the detection threshold of TCA, TeCa, TBA, as well as PCA in Chenin blanc, Pinotage and Shiraz using panels differing in wine tasting experience. The standard method for the determination of odour thresholds by a forced choice ascending concentration series method of limits was used (ASTM E679 – 04). The *average* Best Estimate Threshold (BET), as well as a variation of the latter, the *median* BET was calculated. In the former calculation (*average* BET) the censored data were excluded from the data set and in the latter calculation (*median* BET) the censored data were included in the final data set. The results showed that the removal of censored data resulted in a more stable threshold value in terms of confidence levels (95%). The median method resulted in a tendency to overestimate the respective detection threshold values.

The detection threshold values measured for TCA, TeCA, TBA and PCA are in line with that reported in literature. However, when indicating a detection threshold for a specific haloanisole, it is important to specify a range and not only an average threshold value.

The average detection threshold values of the panels differing in wine tasting expertise were comparable. There was not enough evidence to prove that the two groups differed significantly when determining the detection threshold levels for TCA in two red wines.

## INTRODUCTION

The compounds 2,4,6-trichloroanisole (TCA), 2,3,4,6-tetrachloroanisole (TeCA), 2,4,6-tribromoanisole (TBA) and pentachloroanisole (PCA), collectively known as the haloanisoles, can result in a wine with a characteristic, unpleasant mouldy aroma. This defect is called *cork taint* and affects approximately 2% to 7% of all bottled wines internationally (Mazzoleni & Maggi, 2007).

As compounds responsible for taints are usually detected by the human nose at very low concentrations, sensory analysis can be an important tool in detecting the presence of these compounds. Triangle testing has been implemented successfully by various authors in the determination of detection thresholds in wine (Mazzoleni & Maggi, 2007; Lawless &



Heymann, 1997, Prescott *et al.*, 2005; Sefton & Simpson, 2005). Sefton and Simpson (2005) made a distinction between detection (a minimum value of a sensory stimulus needed to give rise to a sensation) and the recognition threshold (the minimum value of a sensory stimulus permitting identification of a sensation perceived). According to the latter researchers the detection threshold of TCA in wine can range between 1.4 – 4.6 ng/L and that for recognition between 4.2 – 10 ng/L.

As illustrated in Table 4.1 there is some degree of variation in the findings of various researchers on the detection thresholds of TCA (cited by Mazzoleni & Maggi, 2007). It is well known that the wine matrix, expertise of the panel and methodology used, can have a major impact on the detection threshold of a specific compound (Mazzoleni & Maggi, 2007; Sefton & Simpson, 2005).

**Table 4.1** Spectrum of detection thresholds of TCA in wine

<b>Medium</b>	<b>DT level (ng/L)</b>	<b>References</b>
Dry white wine	4	Amon (1989)
White wine	4-10	Sanvicens <i>et al.</i> (2003)
Wine	10	Silva Pereira <i>et al.</i> (2000)
Red wine	22	Alvarez-Rodriguez <i>et al.</i> (2002)

The determination of odour thresholds requires the collective sensory response of a selected group of individuals, called panellists. However, the correct identification of a compound such as TCA at very low concentration levels in a specific wine can pose challenges such as the varying sensitivity of panel members (a factor affected by physiological differences or professional experience), tiredness of sense organs, temporal persistence of a characteristic aroma, as well as perceptual synergistic effects (Grosch, 2001; Le Berre *et al.*, 2007; Selfridge & Amerine, 1978). Furthermore, specific compounds such as alcohol or other aromatic wine compounds can have a significant effect on the perception of cork taint in various mediums (Lawless, 1999).

The purpose of this study was to determine the detection threshold levels for the haloanisoles known to cause cork taint (TCA, TeCA, TBA and PCA) in three South African wines (Chenin blanc, Pinotage and Shiraz) by using sensory panels differing in wine tasting expertise. The first panel, the so-called *novice* panel consisted of judges with limited or no previous exposure to these compounds and was thus trained extensively in detecting cork taint aroma. The second panel, the so-called *expert* panel, was a panel of expert wine

tasters with extensive exposure to wine tasting and/or to the compounds in question at the onset of the research project. The determination of the detection thresholds was conducted by making use of the triangle test method as described by the ASTM E679 – 04 standard method. The data were analysed according to ASTM E679 – 04 method which utilises the calculation of the *average* Best Estimate Threshold (BET), as well as a variation of the latter conventional method, i.e. calculating the *median* BET.

## **MATERIALS AND METHODS**

### **Samples and spiking**

Three wines were used for the determination of detection thresholds (Chenin blanc, Pinotage and Shiraz), all from the 2007 harvest. Chenin blanc had a distinctive fruity aroma and a slight guava aroma. The Pinotage was slightly wooded and had a strong berry aroma. The Shiraz had an herbaceous aroma with almost no fruity notes, but with a slight aroma of honey (Hughson & Boakes, 2002). See Chapter 6 for more details on the wines.

After the wines were bottled manually, samples were taken to determine whether the wines were free of haloanisoles. The latter analyses were conducted by a laboratory (Quantum Laboratories, South Africa) using a dual column GC-ECD (gas chromatography electron capture detection) method making use of volatile headspace extraction (Alzaga *et al.*, 2003; Vlachos *et al.*, 2007). The results indicated that all the samples were free of haloanisoles (below the limit of detection; < 0.5 ng/L), the samples were thus suitable for further analyses.

Each of the wines was spiked with the respective haloanisoles TCA, TeCA, TBA and PCA (Aldrich, South Africa) diluted in 99.5% ethanol (Merck, South Africa) to the concentrations (spiking solution) as depicted in Table 4.5 and then spiked in the different wines to achieve the end concentrations as illustrated in Tables 4.2, 4.3 and 4.4. The concentration of the respective compounds in each range increased with a constant factor of approximately 1.5. Spiking was performed in a fume cupboard to avoid atmospheric contamination of the surroundings due to the extremely volatile nature of haloanisoles. It was injected directly into the wine bottle after the wine was first measured in a metric cylinder. The spiking always took place 1 to 1.5 hours before the sensory thresholds were determined. This was to minimize the effect that oxidation could have on the results of the analyses (Escudero *et al.*, 2002).

The respective concentration ranges (Tables 4.2, 4.3 and 4.4) used for the determination of the detection thresholds of the various compounds were decided on after reviewing the literature (Juanola *et al.*, 2004; Mazzoleni & Maggi, 2007; Prescott *et al.*, 2005; Sanvicens *et al.*, 2003; Silva Pereira *et al.*, 2000), as well as in consultation with a company specialising in cellar hygiene and the detection of haloanisoles (Thales, South Africa).

**Table 4.2** Eight concentration levels of the respective haloanisoles in Chenin blanc for the determination of detection thresholds

<b>Compound</b>	<b>Concentration (ng/L)</b>							
TCA	1	1.5	2.5	3.5	5	7.5	11.5	17
TeCA	1	2	3	5	7	10	15	23
TBA	1	1.5	2.5	3.5	5	7.5	11.5	17
PCA	10	15	23	34	51	76	114	171

**Table 4.3** Eight concentration levels of the respective haloanisoles in Pinotage for the determination of detection thresholds

<b>Compound</b>	<b>Concentration (ng/L)</b>							
TCA	2	3	5	7	10	15	23	34
TeCA	2	3	5	7	10	15	23	34
TBA	1	2	3	5	7	10	15	23
PCA	15	23	34	51	76	114	171	256

**Table 4.4** Eight concentration levels of the respective haloanisoles in Shiraz for the determination of detection thresholds

<b>Compound</b>	<b>Concentration (ng/L)</b>							
TCA	2	3	5	7	10	15	23	34
TeCA	3	5	7	10	15	23	34	51
TBA	1	2	3	5	7	10	15	23
PCA	15	23	34	51	76	114	171	256

**Table 4.5** General formula for the spiking of wines with haloanisoles

<b>Spiking solution (pg/<math>\mu</math>L) in ethanol</b>	<b>Dilution factor</b>	<b>Spiked in wine (<math>\mu</math>L in 100 mL)</b>	<b>End concentration (ng/L)</b>
10	10000	10	1
10	6666.67	15	1.5
10	5000	20	2
10	4000	25	2.5
10	3333.33	30	3

10	2857.14	35	3.5
10	2000	50	5
10	1428.57	70	7
10	1333.33	75	7.5
10	1000	100	10
10	869.56	115	11.5
10	666.67	150	15
10	434.78	230	23
10	294.11	340	34
100	1960.78	51	51
100	1315.79	76	76
100	877.19	114	114
100	584.48	171	171
100	390.63	256	256

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### Determination of detection threshold levels

The determination of the detection threshold levels was carried out in accordance with the standard method of the American Society for Testing and Materials (ASTM E 679 – 04). This method is the standard practice for the determination of odour and taste thresholds by a forced choice ascending concentration series method of limits.

#### *Subjects and training*

Two panels, a *novice* and an *expert* panel, were used for the determination of detection thresholds of the four haloanisoles in each of the three wines (Chenin blanc, Pinotage, Shiraz). At the onset of the project the *novice* panel had no or limited experience in cork taint determination and the eight judges were thus trained extensively in the detection of TCA, TeCA, TBA and PCA in the respective wines. The *expert* panel consisted of nine wine experts with extensive wine tasting experience and/or wine tainted with haloanisoles and hence received no formal training in the detection of TCA, TeCA, TBA and PCA in the respective wines. The *expert* panel was used to verify the results of the *novice* panel.

The *novice* panel was trained in two phases. In *Phase 1* each judge received a control sample containing only the base wine, as well as a reference standard consisting of the base wine and the specific haloanisole at a concentration of 20 ng/L more than the highest concentration of that specific haloanisole (Tables 4.2, 4.3 & 4.4). The latter samples were used to characterise the aroma of the specific haloanisole and thus familiarise the judges of this panel with the particular aroma. In *Phase 2* of training each judge received eight sets of

samples, each set containing three samples. In each set, two of the three samples contained only the base wine (untainted wine) and a third sample in every set contained the base wine plus the added haloanisole. The concentration increased with a constant factor of approximately 1.5 from set 1 through to set 8 as illustrated in Tables 4.2, 4.3 and 4.4. The sets were presented in an order of ascending concentration with the samples in each set presented in a randomised order. The volume of each sample was 20 mL and all the samples were served in ISO wine tasting glasses at  $20 \pm 1$  °C. Each sample was numbered with a random three-digit code. The judges were instructed to smell the headspace of the samples in each set, i.e. in the order presented and then they had to indicate the *odd* or tainted sample. Each judge was required to rest for a total of 2 min between every set and 5 min between the fourth and fifth set. The latter rest period was regarded as the *half-way mark* for this procedure. The latter was cancelled strong carry-over effect of the specific taint in the wine, as well as to minimise tiredness of the sense organs. This procedure was repeated until consensus was reached by the group on the odd sample within each set. The *expert* panel received no formal training for this specific project and only received instruction on how to analyse the sample.

#### *Determination of detection thresholds*

The detection thresholds of the respective haloanisoles were determined by both panels as described in *Phase 2* of the training. The *novice* panel determined the detection levels of all four haloanisoles (TCA, TeCA, TBA & PCA) in all three wine cultivars (Chenin blanc, Pinotage and Shiraz). The *expert* panel only determined the detection levels of TCA in Pinotage and Shiraz. Four replicates were completed per haloanisole per wine on consecutive days.

#### **Analysis of data**

The data were analysed using the procedure as described by the ASTM-method (E 679 – 04), so-called Method 1. The latter procedure was modified slightly, so-called Method 2, and applied to the data.

#### *Method 1*

In Method 1 the best estimate threshold (BET) value was calculated for the total group of judges on all four replicates as described by the ASTM-method (E 679 – 04). When a judge made three or more consecutive correct identifications, the BET was calculated from the onset of the last missed identification and the first correct identification. If all the samples were detected correctly the BET was calculated by using the lowest concentration divided by

the constant factor ( $\approx 1.5$ ) used for creating the concentration range. In Table 4.6 a miss is indicated by a zero (0) and a correct identification by a plus (+). Panellist 6 (Table 4.6) had three or more correct identifications from 15 ng/L onwards. The last missed identification was thus at 10 ng/L. The calculation for the BET should be as follows;  $\sqrt{10 \times 15} = 12.25$  ng/L. The *geometric mean* of the Group-BET is then calculated using the average of the total BET-values. When the panellist was not able to detect three consecutive samples correctly, this data were regarded as *censored data* and were thus omitted from the data set as it represented a false measurement. The data in Tables 4.7 and 4.9 were calculated according to *Method 1*.

#### Method 2

In Method 2 the principles of the first method were also applied, however, the censored data were included. Medians were calculated per judge using all data of all four replicates. The median is then calculated for the total group using the medians of the individual judges to achieve a total group median-BET value. A median is not sensitive for extreme values, however, if the replications are limited this method can easily lead to over-estimation.

**Table 4.6** Example of odour threshold determination for an added substance in wine

Panelist	Judgements								Best estimate threshold (BET)		
	Concentration increases (ng/L) →								Value	Log <sub>10</sub> of value	
	2	3	5	7	10	15	23	34			
1	+	+	+	+	+	+	+	+	1.63	0.21	
2	0	+	+	+	+	+	+	+	2.45	0.39	
3	0	+	+	+	+	+	+	+	2.45	0.39	
4	0	0	+	+	+	+	+	+	3.87	0.58	
5	0	0	+	0	+	+	0	+	27.96	1.45	
6	+	0	+	+	0	+	+	+	12.25	1.09	
7	0	+	+	+	0	+	0	0	2.45	0.39	
8	0	+	+	+	+	+	0	+	2.45	0.39	
										<b>4.89<sup>a</sup></b>	
										<b>4.09<sup>c</sup></b> ←	<b>0.61<sup>b</sup></b>

BET, Best-Estimate Threshold

<sup>a</sup> Sum of Log<sub>10</sub> value

<sup>b</sup> Average Log<sub>10</sub> value

<sup>c</sup> Converted geometric mean of the Group-BET

## RESULTS AND DISCUSSION

The detection thresholds illustrated for TCA, TeCA, TBA and PCA (Tables 4.7, 4.8 & 4.9) are in accordance with results reported in literature, i.e. 1.5 – 3 ng/L for TCA in wine (Duerr *et al.*, 1985); 10 – 15 ng/L for TeCA in white and red wines (Chatonnet *et al.*, 2006), 3.4 ng/L for TBA in wine (Chatonnet *et al.*, 2006) and > 50 ng/L for PCA (Chatonnet *et al.*, 2006).

A difference in threshold values for the three distinct cultivars, especially between the two red wines (Pinotage and Shiraz) and the white wine (Chenin blanc) are shown in Tables 4.7 and 4.8. It is documented in literature that differences in threshold values are found (Martineau *et al.*, 1995; Mazzoleni & Maggi, 2007). The results in our study indicate that a strong relationship exists between threshold value and wine cultivar.

Two methods, Method 1 and Method 2, respectively, were used to calculate the *average* and *median* BET threshold values as illustrated in Tables 4.7 and 4.8. As indicated in Tables 4.7 and 4.8, there is not a major difference between the thresholds levels of TCA, TeCA and TBA using the two methods of calculation. However, the detection threshold values of PCA differ notably when the two methods of calculation are used. The threshold value based on the *average-BET's* (Figure 4.7) is considerably lower than the threshold value based on the *median-BET's* (Figure 4.8) for all four replicates. Furthermore, slightly higher confidence levels (95%) are indicated for PCA in Table 4.8. The latter is an indication of a greater variation within the detection threshold for PCA as calculated by *Method 2*. By removing the censored data in *Method 1* (*average-BET's*) a more stable threshold value is created in terms of confidence levels (95%). By including the censored data in *Method 2* (*median-BET's*) there is a chance of an over-estimation of the detection threshold values, especially when the number of replicates is low. In this study there is thus an indication of a slight overestimation when calculating the BET from the median (*Method 2*) threshold value over four replicates for each judge.

The two panels (*novice* panel and *expert* panel) indicated that there was a slight difference in sensitivity between the *novice* panel with extensive training prior to the analyses (Table 4.7) and an *expert* panel (Table 4.9) with no training prior to the analyses but with extensive experience in wine evaluation. For Pinotage the detection threshold level for the *novice* panel was  $4.54 \pm 2.40$  ng/L and for the *expert* panel  $2.84 \pm 1.42$  ng/L, indicating a range of 2.14 – 6.90 ng/L for the *novice* panel and a range of 1.42 – 4.26 ng/L for the *expert* panel. For Shiraz the detection threshold levels were  $3.86 \pm 1.19$  ng/L for the *novice* panel and  $2.89 \pm 1.82$  ng/L for the *expert* panel, indicating a range of 2.64 – 5.05 ng/L for the *novice* panel

and a range of 1.07 – 4.71 ng/L for the *expert* panel. The latter results illustrate an overlap between the ranges. This indicates that there is not enough evidence to prove that the two groups differ significantly when determining the detection threshold levels of TCA in wine.

**Table 4.7** Detection thresholds (ng/L) for the novice panel calculated on the average best estimate threshold (BET) with confidence levels at 95% between four replicates (*Method 1*)

Compound	Chenin blanc		Pinotage		Shiraz	
	DT	Confidence Level (95%)	DT	Confidence Level (95%)	DT	Confidence Level (95%)
2,4,6-TCA	1.67	±0.46	4.54	±2.40	3.86	±1.19
2,3,4,6-TeCA	6.73	±2.28	8.67	±4.84	10.72	±4.33
2,4,6-TBA	2.05	±1.24	8.69	±3.48	4.12	±1.36
PCA	43.73	±20.26	51.18	±33.16	57.80	±28.27

DT, detection threshold (ng/L)

**Table 4.8** Detection thresholds (ng/L) for the novice panel calculated on the median best estimate threshold (BET) with confidence levels at 95% between four replicates (*Method 2*)

Compound	Chenin blanc		Pinotage		Shiraz	
	DT	Confidence Level (95%)	DT	Confidence Level (95%)	DT	Confidence Level (95%)
2,4,6-TCA	2.02	±0.46	4.75	±3.22	3.78	±1.19
2,3,4,6-TeCA	8.51	±2.52	10.30	±6.28	16.74	±4.33
2,4,6-TBA	1.56	±1.24	8.52	±4.18	5.92	±2.19
PCA	93.40	±27.74	139.67	±42.85	170.83	±43.99

DT, detection threshold (ng/L)

**Table 4.9** Detection thresholds (ng/L) for the expert panel calculated on the average best estimate threshold (BET) with confidence levels at 95% between all four replicates (*Method 1*)

Compound	Pinotage		Shiraz	
	DT	Confidence Level (95%)	DT	Confidence Level (95%)
2,4,6-TCA	2.84	±1.42	2.89	±1.82

DT, detection threshold (ng/L)

The confidence levels (95%) in Tables 4.7, 4.8 and 4.9 indicate a definite spectrum/range around the average BET threshold value and imply that detection threshold values for haloanisoles in wine should be indicated as a range and not just as an average. This could be largely due to the variation in sensitivity of subjects used for determining detection



threshold values of the haloanisole compounds (Marin *et al.*, 1988). Therefore, it could be totally misleading giving an average detection value for a specific compound in a specific wine, i.e. without indicating the valid range. Martineau *et al.* (1995) also mentioned that factors such as wine type can have a major effect on threshold levels, invalidating the use of a single threshold value for all wines.

## CONCLUSIONS

The detection threshold values measured in this study for TCA, TeCA, TBA and PCA are in line with what is reported in literature. However, when indicating a detection threshold for a specific haloanisole in quality control systems it is important to specify a range and not only an average threshold value. The latter can be influenced by a number of external factors such as the sensitivity of panel members, presence of other wine compounds and differences in cultivars and wine styles. Therefore, more research is needed to evaluate the specific effect of external factors such as the presence of other wine compounds, cultivars, wine styles, wines made from the different batches of grapes, etc.

Two methods were used to calculate *average BET* and *median BET* threshold values for the respective compounds. In the former method the censored data were excluded and in the latter method the censored data were included. In this study the results showed that the removal of censored data resulted in a more stable threshold value in terms of confidence levels. The median method resulted in a tendency to overestimate the respective detection threshold values, especially in this study where the number of replicates was low.

The average detection threshold values of the *novice* and *expert panels* indicated that there was a slight difference in sensitivity between the two panels. However, there was an overlap between the detection threshold ranges of TCA in Pinotage, as well as Shiraz indicating that there was not enough evidence to prove that the two groups differed significantly when determining the detection threshold levels of TCA in the two red wines.

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## Chapter 5

### Quality control of sensory panel data

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## **ABSTRACT**

A reliable panel is required in sensory analysis methods. The purpose of this study was to propose a *modus operandi* to tend to sensory panel data in order to improve residuals, as well as panel consensus. In order to improve panel reliability when analysing specific sensory attributes, sensory panel data were treated by evaluating residuals from a conventional ANOVA. Outlier data and odd judges were identified and discarded until residuals were normally distributed. SAS<sup>®</sup> line graphs indicated that a set pattern was followed by the majority of the judges after the outlier data and odd judges were removed from the data. Similarly, the PanelCheck Tucker1-plots indicated consensus was reached after the removal of outlier data and odd judges in the attribute analysed.

## **INTRODUCTION**

Sensory analysis uses humans as measuring instruments to study product sensory attributes. Sensory analysis thus requires trained judges performing the analyses, especially if the samples are tested in various sessions (Latreille *et al.*, 2006). Training of a sensory panel is crucial to the success of descriptive sensory analysis as research technique and ultimately also the validity and reliability of results (Lawless & Heymann, 1998). As with any instrument a sensory panel should be calibrated for analysing a specific attribute in a given product. In descriptive sensory analysis the latter can be achieved by determining judge reproducibility, i.e. by studying the significance levels of the interaction effects associated with the panel members, usually Judge\*Treatment and Judge\*Replication interactions (SAS<sup>®</sup>, 1995). Nowadays, a number of software tools are available for testing judge consistency and thus the improvement of the performance of sensory panels (Dahl *et al.*, 2008). The aim of this investigation was to explore judge reproducibility when performing descriptive sensory analysis by using standard statistical software (SAS<sup>®</sup>, Version 9; SAS Institute Inc, Cary, USA.) and a tool for visualising sensory data (PanelCheck, Version 1.3.1, Matforsk, Norway).

## **MATERIALS & METHODS**

### **Samples**

A panel of judges was trained to analyse specific aroma attributes of wine tainted with haloanisoles (Lawless & Heymann, 1998). For the purpose of this chapter the results of only two wines (Chenin blanc and Shiraz) spiked with 2,3,4,6-tetrachloroanisole (TeCA) and

2,4,6-trichloroanisole (TCA), respectively, will be analysed. The respective samples included the two wines spiked with eight concentration levels of TeCA and TCA, respectively (Table 5.1), as well as a control sample containing only the base wine. See Chapter 6 for details of sample preparation.

**Table 5.1** Concentration levels used in the descriptive analysis of TeCA spiked in Chenin blanc and TCA spiked in Shiraz

Compound	Concentration (ng/L)							
	1	2	3	5	7	10	15	23
TeCA in Chenin blanc	1	2	3	5	7	10	15	23
TCA in Shiraz	2	3	5	7	10	15	23	34

### Descriptive sensory analysis

Generic descriptive analysis was used for analysing the aroma attributes and a panel consisting of ten judges was trained in three consensus training sessions of 1 hour each (Lawless & Heymann, 1998). During each training session the panel members were exposed to the whole range of samples in ascending concentrations starting with the control sample. Descriptors were generated for the samples and discussed by the panel members until consensus was reached. The panel members were instructed to analyse the headspace aroma of the sample and give an intensity rating for each aroma descriptor on an unstructured line scale. Chenin blanc spiked with TeCA was analysed for fruity, mouldy and sweet aroma and Shiraz spiked with TCA was analysed for a herbaceous and mouldy-chlorine aroma. The results were discussed and consensus was reached upon minimum and maximum values for the intensity of each aroma attribute. The attributes were profiled on a 100 mm unstructured line scale with 0 = maximum low intensity and 100 = maximum high intensity. For Chenin blanc spiked with TeCA the descriptors were fruity aroma (0=No fruity aroma; 100=Prominent fruity aroma typical of Chenin blanc), mouldy aroma (0=No mouldy aroma; 100=Prominent mouldy aroma typical of TeCA) and sweet aroma (0=No sweet aroma; 100=Prominent sweet aroma similar to that of alcohol). For Shiraz spiked with TCA the descriptors were herbaceous aroma (0=No herbaceous aroma; 100=Prominent herbaceous aroma typical of Shiraz), mouldy-chlorine aroma (0=No mouldy-chlorine aroma; 100=Prominent mouldy-chlorine aroma typical of TCA).

The profiling was conducted by ten trained assessors in tasting booths with standard artificial daylight lighting and temperature control at 20°C ±1°C. The wine was analysed in standard ISO wine tasting glasses with a sample size of 20 ml at 20°C ±1°C. Each sample received a three digit code on the bottom of the glass. The judges received all treatments in a complete

randomised order, however, the control sample (base wine with no added haloanisole) and the sample with the highest concentration level within each range was always served in the first and last position, respectively. Each glass was covered by a Petri dish lid (Kimix, South Africa) and prior to the aroma analysis the judges were instructed to remove the Petri dish lid from the glass, swirl the wine and analyse the specific aroma attribute in the sample headspace by using a strong sniffing action. The analysis was replicated during four identical sessions for each assessor on four consecutive days.

### **Statistical analysis of data**

For the descriptive sensory analysis a randomized complete block design was used where each judge received a control sample containing only the base wine and eight spiked samples. The latter was replicated four times. Using SAS<sup>®</sup> software (Version 9; SAS Institute Inc, Cary, USA) the data were subjected to a test-retest analysis of variance (ANOVA) to test for reliability, i.e. temporal stability (Judge\*Replication interaction) and internal consistency (Judge\*Level interaction) (SAS<sup>®</sup>, 1995). The Shapiro-Wilk test was used to test for non-normality of the residuals (Shapiro & Wilk, 1965). If non-normality was significant ( $P \leq 0.05$ ) and caused by skewness, the outliers were identified and removed until the data were normal or symmetrically distributed (Glass *et al.*, 1972). Using line plots indicating temporal stability and internal consistency, single odd judges were identified and removed. To substantiate the latter, the same procedure was used to test for panel reliability using the software tool PanelCheck (Version 1.3.1, Matforsk, Norway). The final analysis of variance (ANOVA) was performed after the above-mentioned procedures have taken place. Student's t-least significant difference (LSD) was calculated at the 5% significance level to compare treatment means.

## RESULTS AND DISCUSSION

As already mentioned the aim of this investigation was to explore judge reproducibility when performing descriptive sensory analysis by using *SAS*<sup>®</sup> and *PanelCheck*. The results will be reported for TeCA in Chenin blanc and TCA in Shiraz, i.e. prior to the removal of odd judges, as well as after the removal of odd judges.

### Panel reliability of TeCA in Chenin blanc

The significance levels of the interaction effects associated with all the judges are given in Table 5.2 and this illustrates that some of the judges were not consistent. The Judge\*Level (internal consistency) interaction was significant ( $P < 0.01$ ) indicating that some of the judges were inconsistent in placing the respective samples in consecutive ascending order. The latter is visually demonstrated in the *SAS*<sup>®</sup> line graph (Figure 5.1a) indicating that Judges 8, 9 and 10 were not consistent with the rest of the judges, especially at the lower concentration levels of TeCA. The *PanelCheck* correlation loadings plot based on Tucker1 (Figure 5.1b) illustrates that all the assessors have more than 50% explained variance for this attribute in PC1 and PC2. Although the assessors are grouped together, it seems as though Judge 8 and Judge 10 are spread to the outer and inner edges of the plot, respectively. In view of the above, outliers were removed from the dataset until the residuals in the ANOVA were normally distributed. This automatically resulted in a positive Judge\*Level interaction with a P-value of 0.1578 (Table 5.3). Lawless and Heymann (1998) indicate that in a well trained panel interaction effects such as the Judge\*Level interaction should not be significant ( $P > 0.05$ ). As a result of the removal of odd judges for the attribute of mouldiness both the *SAS*<sup>®</sup> line graph (Figure 5.2a) and the *PanelCheck* correlation loadings plot based on Tucker1 (Figure 5.2b) illustrate an improved degree of internal consistency, as well as an improved agreement among the judges for the attribute of mouldiness.

### Panel reliability of TCA in Shiraz

The significant levels of the interaction effects associated with all the judges indicate that some of the judges were not consistent (Table 5.4). The significant Judge\*Level (internal consistency) interaction ( $P < 0.01$ ) indicates that some of the judges were inconsistent in placing the respective samples in a consecutive ascending order. The *SAS*<sup>®</sup> line graph (Figure 5.3a) confirms the latter finding by visually demonstrating that Judge 2, 6 and 7 were not consistent with the rest of the panel, especially at the low concentration levels. This can be ascribed to the fact that it is extremely difficult to distinguish between odour intensities at



or near detection threshold level (Engen & Pfaffmann, 1959, 1960; Lawless & Heymann, 1998). The PanelCheck correlation loadings plot based on Tucker1 (Figure 5.3b) illustrates that all the assessors have more than 50% explained variance for this attribute in PC1 and PC2. In contrast to the SAS<sup>®</sup> line graph (Figure 5.3a) which illustrates the variation of the judges over the eight concentration levels, the PanelCheck correlation loadings plot based on Tucker1 (Figure 5.3b) indicates the average variation. In this instance the results of the SAS<sup>®</sup> line graph was used as the main guideline to remove *odd* judges. Judges 2, 6 and 7 were therefore removed until the residuals in the ANOVA were normally distributed. This resulted in a positive Judge\*Level interaction (Table 5.5) with a P-value of 0.0505. As a result of removal of odd judges for the attribute of mouldiness for TCA in Shiraz the SAS<sup>®</sup> line graph (Figure 5.4a) and the PanelCheck correlation loadings plot based on Tucker1 (Figure 5.4b) respectively visually illustrate an improved degree of internal consistency, as well as an improved agreement among the judges for the sensory attribute of mouldiness.

## Tables & Figures: TeCA in Chenin blanc

**Table 5.2** ANOVA table of TeCA in Chenin blanc for testing for consistency of the judges prior to the removal of odd judges

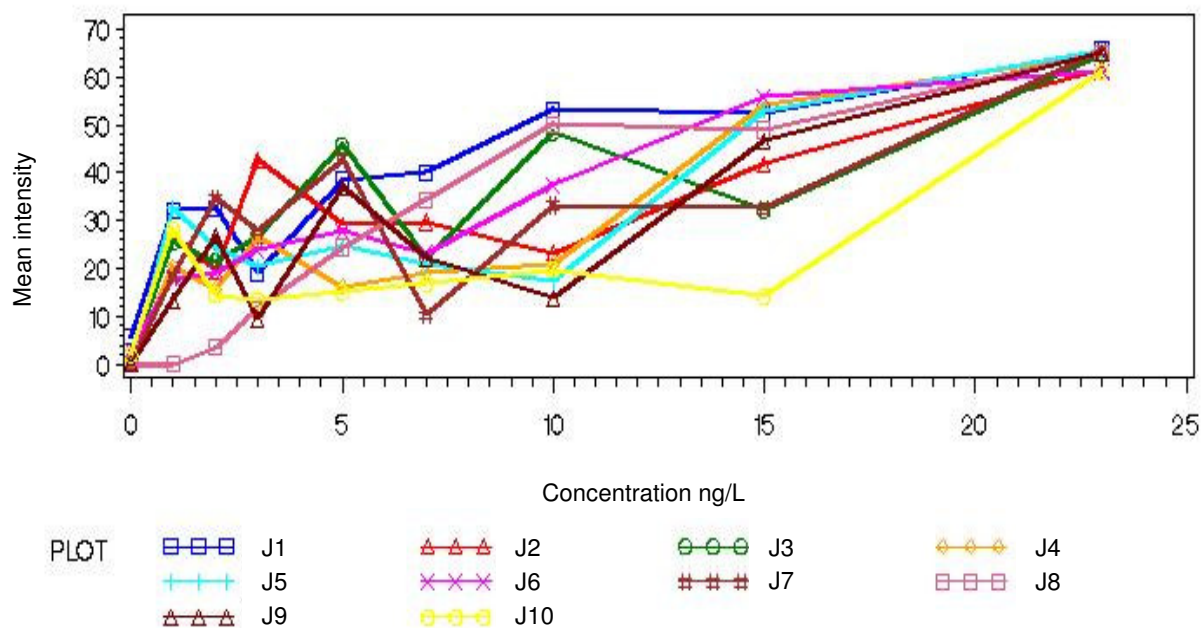
Source	DF <sup>a</sup>	Fruity aroma		DF	Mouldy aroma		DF	Sweet aroma	
		MS <sup>b</sup>	P <sup>c</sup>		MS	P		MS	P
REPL	3	131.17	0.4257	3	306.32	0.2875	3	32.73	0.3725
JUDGE	9	1570.37	<.0001	9	723.42	0.0022	9	295.93	<.0001
JUDGE*REPL	27	114.69	0.7303	27	106.41	0.9936	27	56.24	0.0113
LEVEL	8	8829.00	<.0001	8	11948.33	<.0001	8	359.82	<.0001
JUDGE*LEVEL	72	221.30	0.0061	72	343.04	0.0279	72	121.68	<.0001
Error	240	140.68		240	242.40		240	31.26	
Corrected Total	359	383.43		359	525.81		359	65.24	

<sup>a</sup> Degrees of freedom.

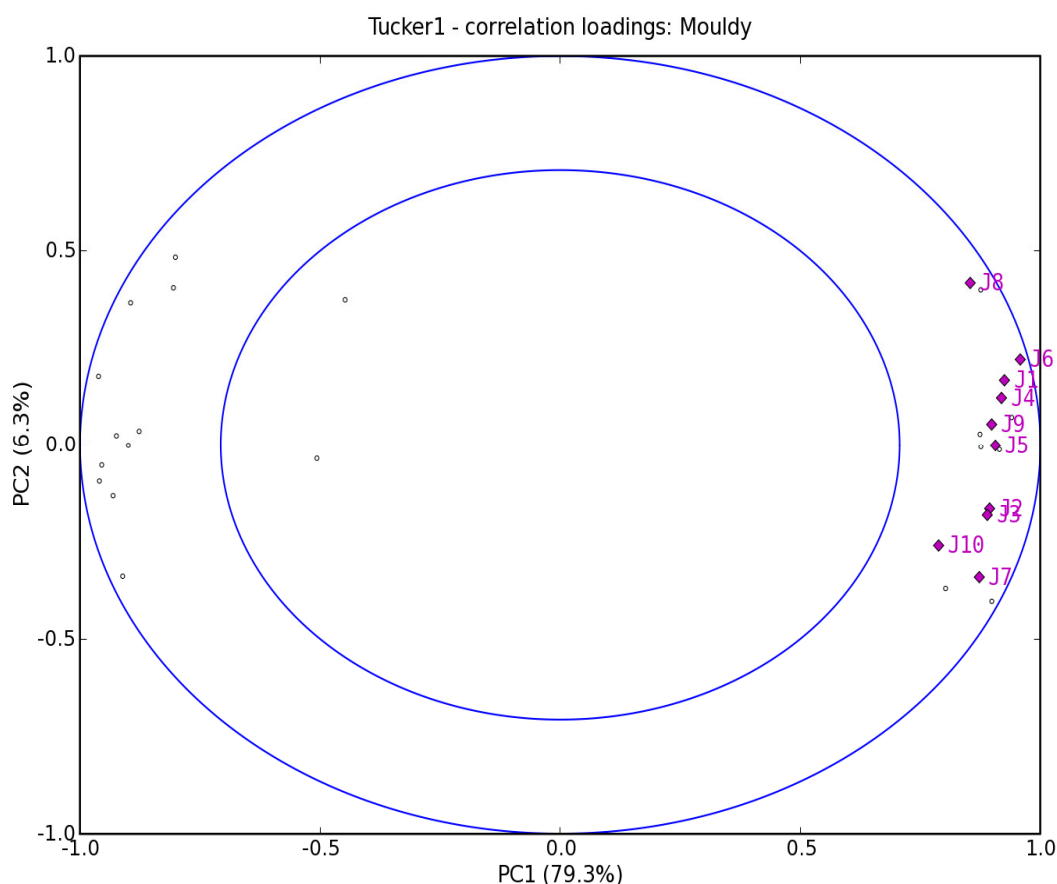
<sup>b</sup> Mean square.

<sup>c</sup> Probability.

### TeCA in Chenin blanc - Judge\*Level (Internal consistency) – Mouldy



**Figure 5.1a** SAS<sup>®</sup> line graph demonstrating internal consistency of the mouldy attribute in Chenin blanc spiked with increasing concentrations of TeCA for all judges prior to the removal of odd judges.



**Figure 5.1b** PanelCheck correlation loadings plot based on Tucker-1 of the mouldy attribute in Chenin blanc spiked with TeCA for all judges prior to the removal of odd judges. The inner and outer circles represent 50% and 100% explained variance, respectively.

**Table 5.3** ANOVA table of TeCA in Chenin blanc for testing for consistency of the judges after the removal of odd judges

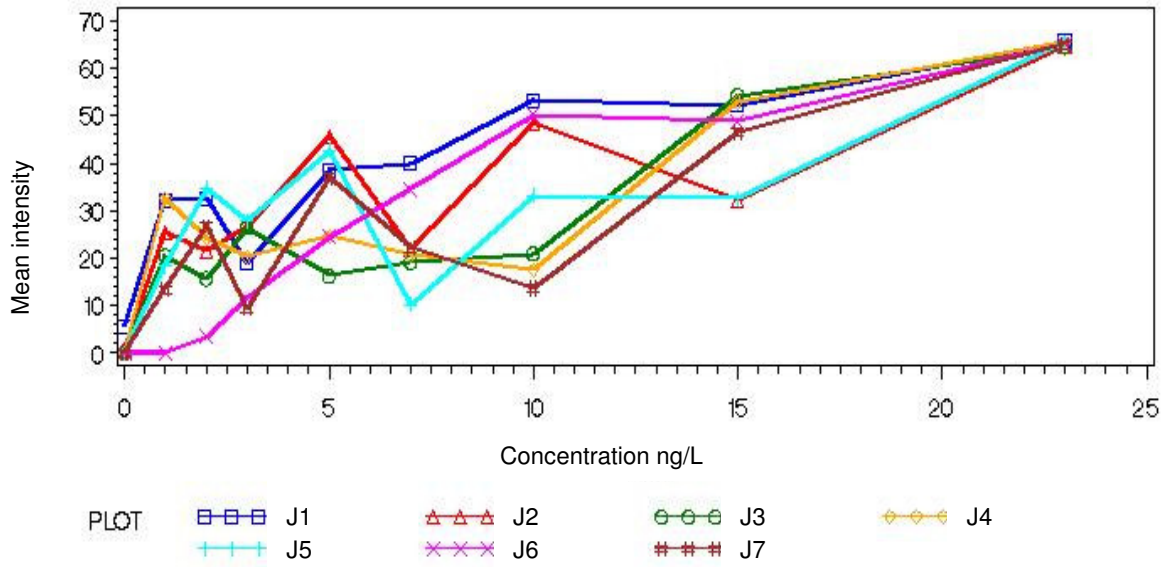
Source	DF <sup>a</sup>	Fruity aroma		DF	Mouldy aroma		DF	Sweet aroma	
		MS <sup>b</sup>	P <sup>c</sup>		MS	P		MS	P
REPL	3	122.60	0.5006	3	41.86	0.3023	3	112.14	0.7328
JUDGE	6	2039.64	<.0001	6	418.81	<.0001	6	627.94	0.0299
JUDGE*REPL	18	86.53	0.9247	18	27.49	0.6931	18	109.16	0.9829
LEVEL	8	6833.09	<.0001	8	836.10	<.0001	8	9178.04	<.0001
JUDGE*LEVEL	48	270.91	0.0052	48	42.52	0.1578	48	369.75	0.0572
Error	168	155.04		168	261.71		168	34.17	
Corrected Total	251	429.80		251	562.58		251	70.13	

<sup>a</sup> Degrees of freedom.

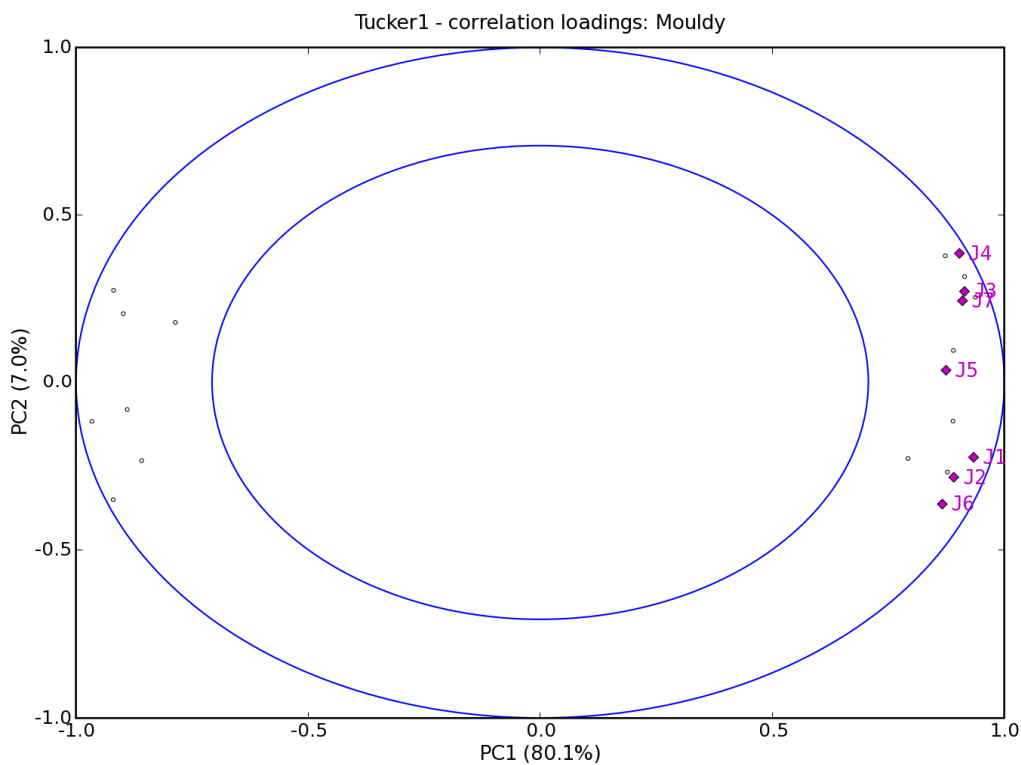
<sup>b</sup> Mean square.

<sup>c</sup> Probability.

| **TeCA in Chenin blanc - Judge\*Level (Internal consistency) - Mouldy**



**Figure 5.2a** SAS® line graph demonstrating internal consistency of the mouldy attribute in Chenin blanc spiked with increasing concentrations of TeCA after the removal of odd judges.



**Figure 5.2b** PanelCheck correlation loadings plot based on Tucker-1 of the mouldy attribute in Chenin blanc spiked with TeCA after the removal of odd judges. The inner and outer circles represent 50% and 100% explained variance, respectively.

## Tables & Figures: TCA in Shiraz

**Table 5.4** ANOVA table of TCA in Shiraz for testing for consistency of the judges prior to the removal of odd judges

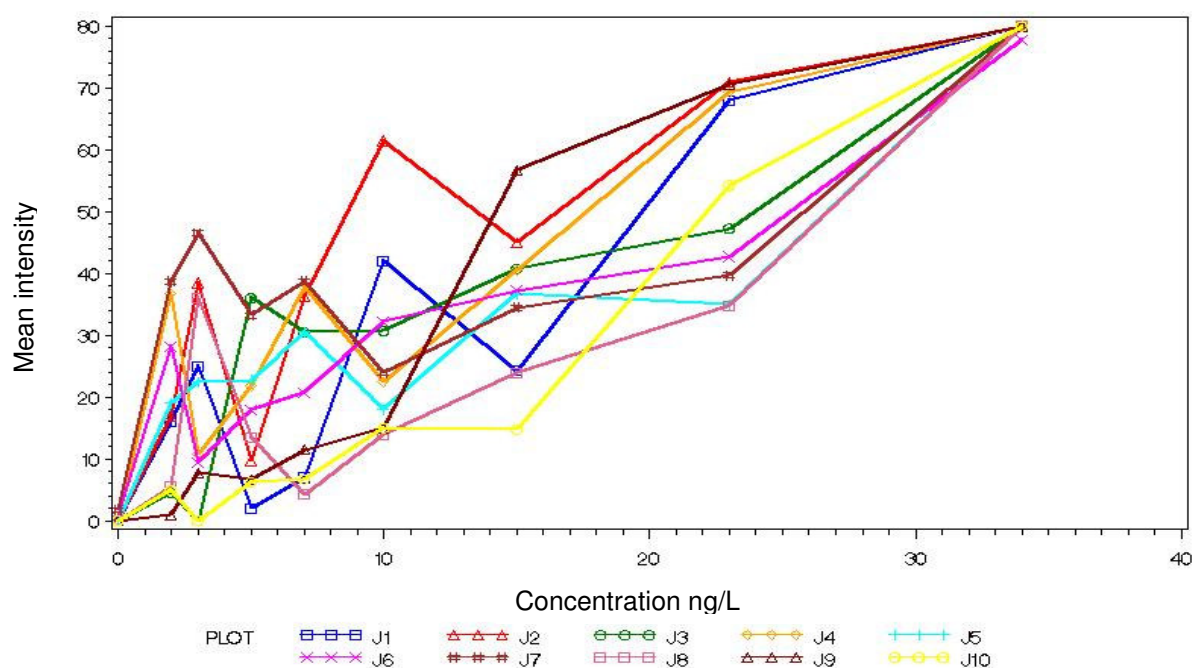
Source	DF <sup>a</sup>	Herbaceous aroma		DF	Mouldy-Chlorine aroma	
		MS <sup>b</sup>	P <sup>c</sup>		MS	P
REPL	3	19.3239	0.9773	3	25.5343	0.9765
JUDGE	9	4942.085	<.0001	9	1385.453	0.0002
JUDGE*REPL	27	125.9842	0.9937	27	86.5048	1
LEVEL	8	15325.04	<.0001	8	22176.67	<.0001
JUDGE*LEVEL	72	497.8675	0.0011	72	545.6493	0.0165
Error	239	287.5670		239	370.6716	
Corrected Total	358			358		

<sup>a</sup> Degrees of freedom.

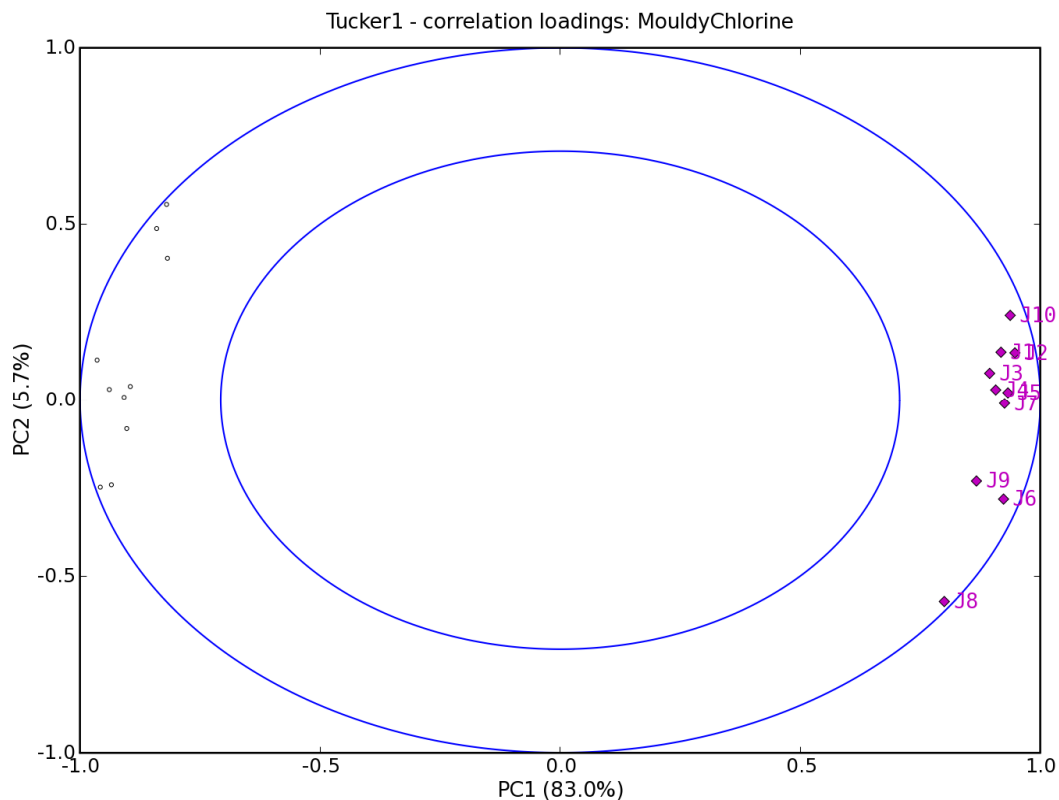
<sup>b</sup> Mean square.

<sup>c</sup> Probability.

**TCA-Shiraz - Judge\*Level (Internal consistency) - Mouldy/Chlorine**



**Figure 5.3a** SAS<sup>®</sup> line graph demonstrating internal consistency of the mouldy/chlorine attribute in Shiraz spiked with increasing concentrations of TCA for all judges prior to the removal of odd judges.



**Figure 5.3b** PanelCheck correlation loadings plot based on Tucker-1 of the mouldy/chlorine attribute in Shiraz spiked with TCA for all judges prior to the removal of odd judges. The inner and outer circles represent 50% and 100% explained variance, respectively.

**Table 5.5** ANOVA table of TCA in Shiraz for testing for consistency of the judges after the removal of odd judges

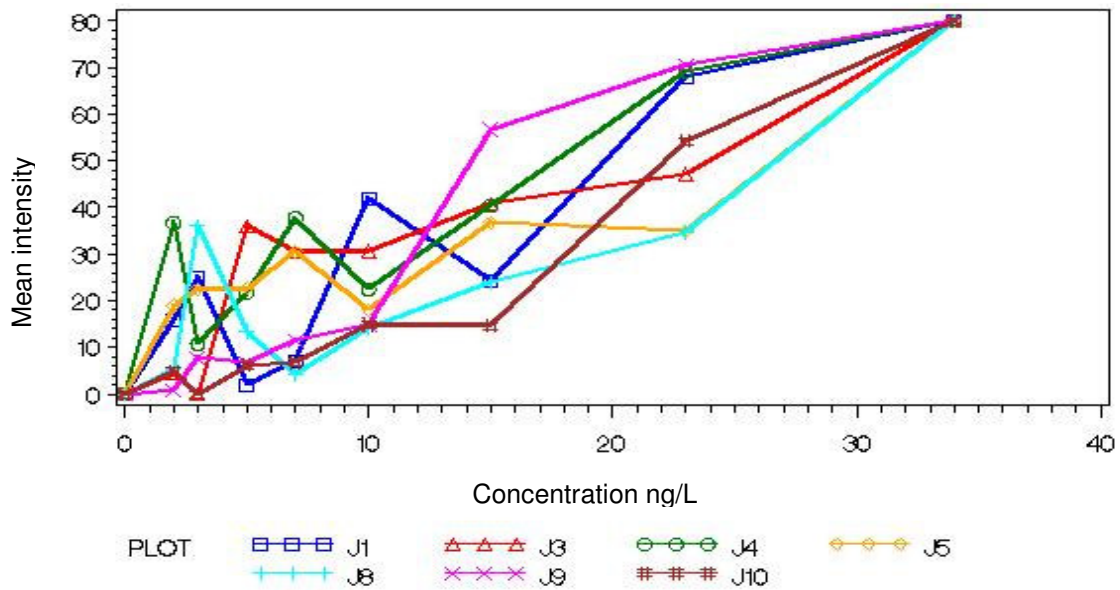
Source	DF <sup>a</sup>	Herbaceous aroma		DF	Mouldy-Chlorine aroma	
		MS <sup>b</sup>	P <sup>c</sup>		MS	P
REPL	3	15.8163	0.9843	3	125.0476	0.7891
JUDGE	6	2754.282	<.0001	6	965.077	0.0157
JUDGE*REPL	18	140.5641	0.9705	18	74.1397	0.9998
LEVEL	8	13384.32	<.0001	8	17058.23	<.0001
JUDGE*LEVEL	48	514.5394	0.0082	48	511.336	0.0505
Error	83	1817.78		83	2030.242	
Corrected Total	167	304.8035		168	357.0912	

<sup>a</sup> Degrees of freedom.

<sup>b</sup> Mean square.

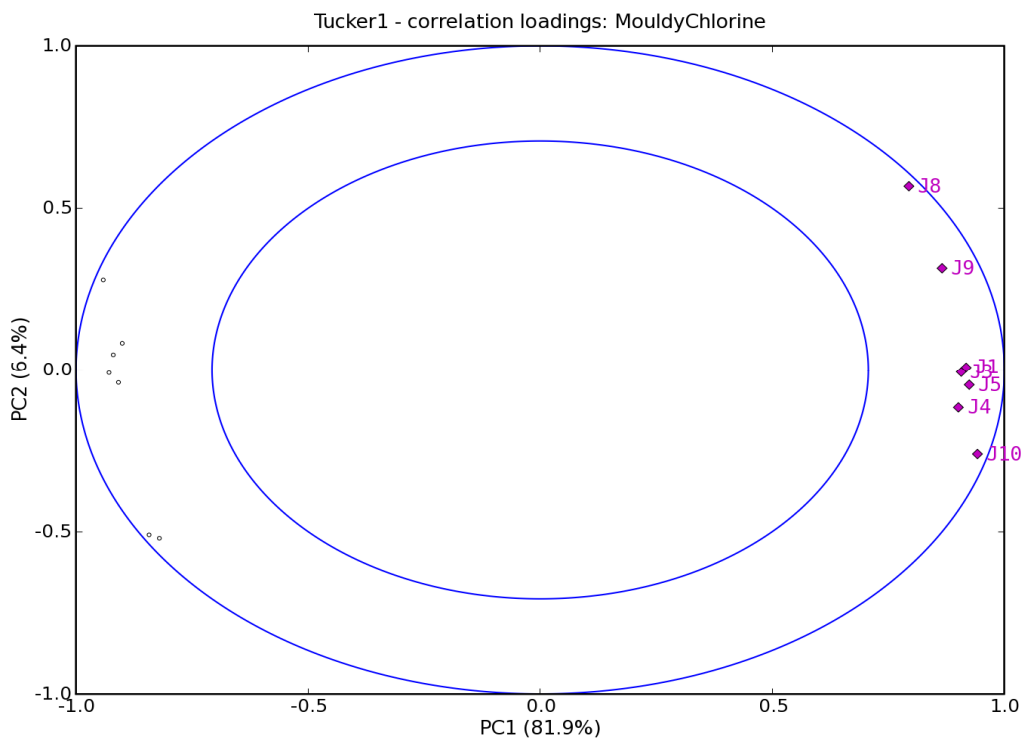
<sup>c</sup> Probability.

TCA-Shiraz - Judge\*Level (Internal consistency) - Mouldy/Chlorine



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**Figure 5.4a** SAS® line graph demonstrating internal consistency of the mouldy/chlorine attribute in Shiraz spiked with increasing concentrations of TCA after the removal of odd judges.



**Figure 5.4b** PanelCheck correlation loadings plot based on Tucker-1 of the mouldy/chlorine attribute in Shiraz spiked with TCA after the removal of odd judges. The inner and outer circles represent 50% and 100% explained variance, respectively.

## CONCLUSIONS

The proposed procedures enable to test, within a conventional ANOVA, the reliability of the sensory panel over the course of a number of replications (sessions) according to the overall performance of the panel and the individual performance of each judge. The residuals as indicated by the ANOVA show to be an effective means to identify outliers and eventually odd judges. The SAS<sup>®</sup> line graph shows to be an effective visualisation tool to indicate whether a set pattern is followed by the panel of judges and which judges does not follow the pattern indicated by the majority of the judges. The latter, however, is dependent on the training of the judges which is necessary to achieve the anticipated pattern required. The SAS<sup>®</sup> line graph, in conjunction with the ANOVA, is therefore an effective means to identify and discard outlier measurements and odd judges from the data. The PanelCheck Tucker1-plots indicate the spread of the judges within an attribute and thus show whether consensus was reached within an attribute. It is therefore clearly indicated by the Tucker1-plots that consensus was reached within the attribute of mouldiness after the odd judges were discarded. These tools can therefore be used separately or in conjunction with one another to improve residuals in the ANOVA and to indicate consensus among judges.

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## Chapter 6

### Sensory characterisation of Chenin blanc, Pinotage and Shiraz spiked with eight concentration levels of 2,4,6-TCA, 2,3,4,6-TeCA, 2,4,6-TBA and PCA

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## ABSTRACT

Limited information on the sensory characterisation of cork tainted wines is available. The aim of this study was to characterise three wines (Chenin blanc, Pinotage and Shiraz) spiked with eight concentration levels of 2,4,6-trichloroanisole (TCA), 2,3,4,6-tetrachloroanisole (TeCA), 2,4,6-tribromoanisole (TBA), pentachloroanisole (PCA), respectively. Descriptive sensory analysis was used to characterise the aroma profile of each of the individual wines spiked with the different concentration levels of the latter compounds. The effect of TCA on degree of liking was further tested in all three the wines. Preference mapping was conducted to establish the main drivers for liking or disliking of wines containing TCA. It was found that low concentration levels of the TCA, TeCA, TBA or PCA resulted in a substantial and immediate decrease of the natural aroma attributes of the respective wines, namely fruitiness of Chenin blanc and Pinotage and the natural herbaceous character of Shiraz ( $P \leq 0.05$ ). Conversely, the mouldy aroma associated with cork taint increased as the concentration levels of the haloanisoles increased ( $P \leq 0.05$ ). Discriminant analysis plots indicated that the trained panelists could discriminate effectively between the lowest and the highest levels of haloanisoles. However, at the low and mid concentration levels the panel members illustrated difficulty in discriminating effectively. The consumer tests for TCA in Chenin blanc, Pinotage and Shiraz indicated that consumers were not sensitive to low concentrations of TCA and only rejected the TCA tainted wines at concentration levels higher than the respective detection thresholds.

## INTRODUCTION

It has been estimated that approximately 5% of all bottled wine are affected by *cork taint* rendering the wine spoilt (Coque *et al.*, 2003; Fuller, 1995; Juanola *et al.*, 2004; Prak *et al.*, 2007; Prescott *et al.*, 2005; Sefton & Simpson, 2005). This mouldy-like quality results from contamination of the wine by 2,4,6-trichloroanisole (TCA), 2,3,4,6-tetrachloroanisole (TeCA), pentachloroanisole (PCA), 2,4,6-tribromoanisole (TBA) (Sefton & Simpson, 2005), but also by geosmin and 2-methyl isoborneol (Salemi *et al.*, 2006). Among these, TCA has been blamed as the most contributory compound because of its frequent occurrence in tainted wines (Insa *et al.*, 2005). The latter trend is also confirmed by this study as indicated in Chapter 3.

Cork taint usually arises when organic plant material or any phenol containing substrate has been exposed to chlorine and in turn has been utilised as growth substrate by certain

filamentous fungi. Cork taint is hence known as a fungal aroma although the name *cork taint* or *corked* can imply that the taint originates from cork exclusively. Cork taint does, however, arise from sources other than cork such as wooden structures in wine cellars, wooden pallets, cellar walls, drainage systems in cellars, etc. (Juanola *et al.*, 2004). When these products come into physical contact or in close proximity of wine or any product that will eventually come into contact with wine, the wine can become tainted and eventually acquire a mouldy aroma (Chatonnet *et al.*, 2004; Simpson & Sefton, 2007; Whitfield *et al.*, 1997).

To date, the majority of sensory studies on cork taint have been concerned with determining the threshold at which the respective haloanisoles can be detected (Mazzoleni & Maggi, 2007; Prescott *et al.*, 2005). Furthermore, some work has also been carried out on determining the consumer rejection level of cork taint in wines (Prescott *et al.*, 2005). A number of studies indicate that these compounds only have to be present at very low concentrations in wine (parts per trillion or ng/L), with TCA having a detection threshold value of less than 10 ng/L in wine (Insa *et al.*, 2005; Mazzoleni & Maggi, 2007).

Wine is a very complex medium which contains of a large number of volatiles existing in specific ratios. The unique character of individual wines is a result of the specific ratios (Juanola *et al.*, 2004). Certain wine constituents, such as alcohol, can have a considerable effect on the perceived aroma of wine (Fisher & Noble, 1994; Grosch, 2001; Pangborn, 1970; Vidal *et al.*, 2004). Due to this and many other reasons, the sensory analysis of wine odorants is extremely difficult. Engen and Pfaffmann (1959, 1960) have shown that humans are able to accurately identify only a limited number of odour intensities at low concentration levels. To minimize noise in sensory data, it is vital to use reliable judges with sufficient training and/or experience of specific aromas.

Descriptive sensory analysis is a tool frequently used in the area of sensory research to obtain a complete sensory description of products. It is also useful in situations where a detailed specification of the sensory attributes of a product or a comparison among several products is desired (Lawless & Heymann, 1998; Stone & Sidel, 1993). This technique usually involves 1) training of the judges to score the respective samples according to the specific sensory attributes on a line scale; 2) the determination of judge reproducibility; 3) analysis of the samples according to an experimental design, followed by analysis of variance or appropriate multivariate statistical techniques (Lawless & Heymann, 1998).

In consumer sensory analysis preference and/or acceptability can be measured. In preference measurement consumers indicate whether one product is to be chosen over one

or more products, whereas in the measurement of acceptance consumers rate their liking for a range of products on a scale (Jellinek, 1964; Lawless & Heymann, 1998). The hedonic scale is generally used when acceptance, as well as preference is to be measured. Consumers have to indicate which one of nine terms ranging from *Dislike extremely* (1) to *Like extremely* (9) best describes their attitude towards the product being tested (Lawless & Heymann, 1998).

Preference mapping is a technique used for measuring the *performance* of a product in terms of liking and also shows the specific drivers for the liking of a product (Helgesen *et al.*, 1997). Preference mapping refers to a range of multivariate statistical techniques and the latter are generally used to relate sensory data to consumer data (McEwan *et al.*, 1998).

The purpose of this study was to characterise three wines (Chenin blanc, Pinotage and Shiraz) spiked with eight concentration levels of TCA, TeCA, TBA and PCA, respectively. Descriptive sensory analysis was used to characterise the aroma profile of each of the individual wines spiked with the different concentration levels of the latter compounds, as well as the base wine containing no TCA. The effect of TCA on degree of liking was further tested in all three the wines by using the 9-point hedonic scale. Preference mapping was conducted on the latter data sets to establish the main drivers for liking or disliking of wines containing various concentration levels of TCA.

## **MATERIALS AND METHODS**

### **Wine samples**

Three hundred liters of each of 2007 Chenin blanc, Pinotage and Shiraz were supplied by a local producer of wines (Distell Group Ltd, Stellenbosch, South Africa). The young wines (approximately eight months old) were transferred from industrial scale wine storage tanks (on site at the local producer) into smaller tanks to make up the received end volume of wine. Thereafter the wines were bottled manually at the Institute of Wine Biotechnology (IWBT), Stellenbosch University, South Africa. After bottling, samples were taken to determine whether the wines were free of haloanisoles. The latter analyses were conducted by a laboratory (Quantum Laboratories, South Africa) using a dual column GC-ECD (gas chromatography electron capture detection) method making use of volatile headspace extraction (Alzaga *et al.*, 2003; Vlachos *et al.*, 2007). The results indicated that all the samples were free of haloanisoles (below the limit of detection; <0.5 ng/L), the samples were thus suitable for further sensory analyses.

## Chemicals and spiking

Solutions of TCA, TeCA, TBA and PCA (Aldrich, South Africa) were made up in 99.5% ethanol (Merck Chemicals, South Africa) to ultimately achieve wine samples spiked with the respective compounds, with eight concentration levels per compound per wine. The concentration range of the spiked samples used in the descriptive analysis and consumer sensory analysis can be seen in Tables 6.1, 6.2 and 6.3, respectively. A ninth sample which contained only the respective base wine was used as a control sample. To avoid any contamination in the laboratory the spiking was confined to a fume cupboard. The TCA, TeCA, TBA and PCA standard solutions had a concentration of 1 mg/mL and were used during the spiking procedure to produce the spiking solution at concentrations shown in Table 6.5. All the final additions of TCA, TeCA, TBA or PCA were done from the end spiking solution as seen in Table 6.5. The concentration ranges in Tables 6.1 to 6.3 were decided on after reviewing literature (Juanola *et al.*, 2004; Mazzoleni & Maggi, 2007; Prescott *et al.*, 2005) and in consultation with an accredited French company (Thales, South Africa) specialising in cellar hygiene. The latter research and development initiatives included making up various series of spiked solutions with water, cork soaking solution (12% v/v alcohol solution used for the extract haloanisoles from cork), as well as with Chenin blanc, Pinotage and Shiraz. After nosing the headspace of the latter tainted samples, it was decided that wine would be the most appropriate base solution for the purpose of this project.

**Table 6.1** Concentration levels of the four haloanisoles used for spiking Chenin blanc for conducting descriptive sensory analysis

Compound	Concentration (ng/L)							
TCA	1	1.5	2.5	3.5	5	7.5	11.5	17
TeCA	1	2	3	5	7	10	15	23
TBA	1	1.5	2.5	3.5	5	7.5	11.5	17
PCA	10	15	23	34	51	76	114	171

**Table 6.2** Concentration levels of the four haloanisoles used for spiking Pinotage for conducting descriptive sensory analysis

Compound	Concentration (ng/L)							
TCA	2	3	5	7	10	15	23	34
TeCA	2	3	5	7	10	15	23	34
TBA	1	2	3	5	7	10	15	23
PCA	15	23	34	51	76	114	171	256

**Table 6.3** Concentration levels of the four haloanisoles used for spiking Shiraz for conducting descriptive sensory analysis

<b>Compound</b>	<b>Concentration (ng/L)</b>							
TCA	2	3	5	7	10	15	23	34
TeCA	3	5	7	10	15	23	34	51
TBA	1	2	3	5	7	10	15	23
PCA	15	23	34	51	76	114	171	256

**Table 6.4** Concentration levels of 2,4,6-TCA used for spiking Chenin blanc, Pinotage and Shiraz respectively for conducting consumer sensory analysis using the hedonic scale

<b>Compound</b>	<b>Concentration (ng/L)</b>							
TCA in Chenin blanc	1	1.5	2.5	3.5	5	7.5	11.5	17
TCA in Pinotage	2	3	5	7	10	15	23	34
TCA in Shiraz	2	3	5	7	10	15	23	34

**Table 6.5** Formula for spiking of haloanisoles in wine to achieve a specific end concentrations (ng/L)

<b>Spiking solution (pg/<math>\mu</math>L) in ethanol</b>	<b>Dilution factor</b>	<b>Spiked in wine (<math>\mu</math>L in 100 mL)</b>	<b>End concentration (ng/L)</b>
10	10000	10	1
10	6666.67	15	1.5
10	5000	20	2
10	4000	25	2.5
10	3333.33	30	3
10	2857.14	35	3.5
10	2000	50	5
10	1428.57	70	7
10	1333.33	75	7.5
10	1000	100	10
10	869.56	115	11.5
10	666.67	150	15
10	434.78	230	23
10	294.11	340	34
100	1960.78	51	51
100	1315.79	76	76
100	877.19	114	114
100	584.48	171	171
100	390.63	256	256

A volume of 750 mL of wine was measured in a one-liter metric cylinder and poured back into the bottle before the wine was spiked with the respective compounds. The spiking always took place an hour, but not longer than two hours, before sensory and consumer analysis commenced. This was to minimize the affect that oxidation could have on the results of the analyses (Escudero *et al.*, 2002).

### **Descriptive sensory analysis**

A panel of 10 judges was trained to analyse the specific aroma attributes of each wine (Chenin blanc, Pinotage and Shiraz) spiked individually with different levels of the respective compounds (TCA, TeCA, TBA and PCA). The Chenin blanc base wine had a prominent fruity character with a slight guava note. The Pinotage base wine was slightly wooded with a strong fruity (mostly berry) aroma and the Shiraz base wine had a natural herbaceous aroma with almost no fruity notes. Therefore the wines in this study respectively illustrated a typical Cheni blanc, Pinotage and Shiraz character (Hughson & Boakes, 2002). When TCA, TeCA, TBA or PCA were added to the respective wines, the following sensory aroma attributes came to the fore: mouldy; mouldy-chemical; mouldy-acidic; sweet aroma associated with excessively sweet wine.

Generic descriptive analysis was used as research technique and for each wine x compound combination the panel had three training sessions of 1 hour each in order to reach consensus on the sensory aroma attributes of the respective tainted samples (Lawless & Heymann, 1998). During each training session the panel members were exposed to the full range of samples (nine samples including the base wine) in ascending concentrations starting with the control sample. Descriptors were generated and discussed by the panel members. The panel members were instructed to analyse the headspace aroma of the samples and give an intensity rating for the specific aroma on an unstructured line scale (Lawless & Heymann, 1998). The results were discussed and consensus was reached on the minimum and maximum values of each aroma attribute. The attributes, as shown in Table 6.6, were assigned a “0” on the 100 mm line when no aroma was detected and a “100” when a prominent aroma was detected. For instance: Fruity aroma (0 = No fruity aroma; 100 = Prominent fruity aroma typical of Chenin blanc), Mouldy aroma (0 = No mouldy aroma; 100 = Prominent mouldy aroma typical of TCA) and sweet aroma (0 = No sweet aroma; 100 = Prominent sweet aroma associated with excessively sweet wine).

The final profiling analyses were conducted by 10 trained assessors in booths with standard artificial daylight lighting and temperature control at 20°C ±1°C. The wine was analysed in



standard ISO wine tasting glasses, sample size was 20 mL and samples were served at 20°C ±1°C. Each sample was coded with a three-digit code at the bottom of the glass. The judges received all treatments in a random order, however, the control sample (0 ng/L) and the sample with the highest concentration level in each range were always served in the first and last position, respectively. Each glass was covered by a Petri dish lid (Kimix, South Africa) and prior to analysis the judges were instructed to remove the Petri dish lid from the glass, swirl the wine and analyse the specific aroma attributes in the sample headspace using a strong sniffing action. Each wine x compound combination was replicated four times on four consecutive days. Thereafter the training and testing procedure of the next wine x compound combination commenced.

**Table 6.6** Aroma attributes associated with the tainted wines

Compounds added to the base wines*	Aroma attributes		
	Chenin blanc	Pinotage	Shiraz
TCA	Fruity	Fruity	Herbaceous
	Mouldy Sweet	Mouldy Sweet	Mouldy-Chlorine
TeCA	Fruity	Fruity	Herbaceous
	Mouldy Sweet	Mouldy Sweet	Mouldy-Chlorine
TBA	Fruity	Fruity	Herbaceous
	Mouldy Sweet	Mouldy Sweet	Mouldy
PCA	Fruity	Fruity	Herbaceous
	Mouldy	Mouldy-Chemical	Mouldy-Acidic

\*Both the Chenin blanc and Pinotage base wines had a strong fruity aroma, the Shiraz base wine illustrated a strong herbaceous aroma.

### Consumer sensory analysis

Three consumer tests were conducted on three separate occasions where the above-mentioned wines (Chenin blanc, Pinotage and Shiraz) were spiked with different levels of TCA, respectively. Eight samples (eight concentration levels of TCA) were split in two by creating two standard subsets of four samples each. Each subset contained every second concentration level as illustrated in Table 6.7. A control sample (containing only the base wine in question) was assigned to each subset enabling the data from both subsets to be pooled for statistical analysis. Each subset thus contained five samples. All five samples were assigned a three-digit code and samples were presented to the consumers in a complete randomised order. Per wine a hundred target consumers were sourced.

Biographical data (gender, age and rate of consumption of wines) were obtained for each individual consumer.

The consumers were instructed to smell, as well as taste the wine samples. Each consumer received a water biscuit (Carr, UK) and water to clean their pallet before and after tasting each sample. The consumers had to indicate their degree of liking of the samples on a standard nine-point hedonic scale where 1 represents *Dislike extremely* and 9 represents *Like extremely* (Lawless & Heymann, 1998). The tests were conducted in a sensory laboratory with standard artificial daylight lighting and temperature control at 20°C ±1 °C.

**Table 6.7** Concentration levels of 2,4,6-TCA used for spiking Chenin blanc, Pinotage and Shiraz respectively for consumer analysis using the hedonic scale

Compound	Concentration (ng/L)				
TCA In Chenin blanc - Subset 1	0	1	2.5	5	11.5
TCA In Chenin blanc - Subset 2	0	1.5	3.5	7.5	17
TCA in Pinotage - Subset 1	0	2	5	10	23
TCA in Pinotage - Subset 2	0	3	7	15	34
TCA in Shiraz - Subset 1	0	2	5	10	23
TCA in Shiraz - Subset 2	0	3	7	15	34

## Statistical procedures

### *Descriptive sensory analysis*

For the descriptive sensory analysis of the three wines spiked with the respective haloanisoles (TCA, TeCA, TBA PCA, respectively) a randomized complete block design was used for each wine x compound combination. Each judge received a control sample containing the base wine and eight spiked samples (Tables 6.1, 6.2 and 6.3). In all the wine x compound combinations eight spiked samples were served, except for TCA in Chenin blanc where the 7.5 and 11.5 ng/L concentrations were omitted from the descriptive analysis. Prior to analysis of the data, the latter missing values in the TCA x Chenin blanc data-set were achieved by linear interpolation of the data. All data were subjected to test-retest analyses of variance (ANOVA) using SAS<sup>®</sup> software (Version 9; SAS<sup>®</sup> Institute Inc, Cary, USA) to test for reliability, i.e. temporal stability (Judge\*Replication interaction) and internal consistency (Judge\*Level interaction) (SAS<sup>®</sup>, 1995). The Shapiro-Wilk test was used to test

for non-normality (Shapiro & Wilk, 1965). If non-normality was significant ( $P \leq 0.05$ ) and caused by skewness, the outliers were identified and removed until the data were normal or symmetrically distributed (Glass *et al.*, 1972). Using SAS<sup>®</sup> line plots indicating temporal stability and internal consistency, single odd judges were identified and removed. PanelCheck software (Version 1.3.1, Matforsk, Norway) was used to substantiate the latter results, therefore testing for panel reliability. The final analysis of variance (ANOVA) was performed after the above-mentioned procedures have taken place. Student's t-least significant difference (LSD) was calculated at the 5% significance level to compare treatment means.

#### *Consumer sensory analysis*

During the consumer sensory analysis five samples were analyzed by each consumer. One sample was the control sample containing only the base wine (Chenin blanc, Pinotage or Shiraz) and the four remaining samples were spiked with TCA (See concentrations listed in Table 6.7). Every second consumer, thus 50% of the consumers, received four spiked samples at concentration levels according to Subset 1 (Table 6.7) plus the control sample containing the base wine and the other 50% of the consumers received the concentration levels according to Subset 2 (Table 6.7) and a control containing the base wine in question. The control sample was considered as a *standard sample* for both groups of consumers therefore the data were pooled for analysis of variance (ANOVA) (SAS<sup>®</sup>, Version 9; SAS<sup>®</sup> Institute Inc, Cary, USA.). The Shapiro-Wilk test was used to test for non-normality in the data (Shapiro & Wilk, 1965). If skewness appeared to be the result of outliers these outliers were identified and discarded until the data were considered normal or symmetrically distributed (Glass *et al.*, 1972). This procedure was repeated for the three respective wines.

#### *Multivariate statistical techniques*

Several multivariate statistical techniques were performed using the XLSTAT software (Version 7.5.2, Addinsoft, New York, USA). Principal component analysis (PCA)\* was conducted in order to discover the relationship between attributes of the spiked wine and also to investigate sample patterns (Guchu *et al.*, 2006). Discriminant analysis (DA) was used to perceptually map the means of the sensory attributes at different concentration levels to ascertain whether the panelists were able to distinguish between different concentration levels (Lawless & Heymann, 1998). External preference mapping using Partial Least Squares (PLS) was performed by regressing consumer preference scores (y-space) onto the trained panel data (x-space) to establish relationships between sensory attributes and consumer degree of liking (Berna *et al.*, 2005; Carroll *et al.*, 1970; Dailliant-Spinnler *et al.*, 1996; Tenenhaus *et al.*, 2005).

\*Note that **Principal Component Analysis (PCA)** and **Pentachloroanisole (PCA)** both utilise the same abbreviation.

## RESULTS

### Sensory attributes of Chenin blanc spiked with TCA, TeCA, TBA and PCA

The Chenin blanc base wine was characterised as an extremely fruity wine (Table 6.6). When the Chenin blanc was spiked with the respective haloanisoles, high concentrations at/or above detection threshold resulted in a mouldy aroma, as well as a sweet aroma typical of excessively sweet wine.

#### *Chenin blanc spiked with TCA*

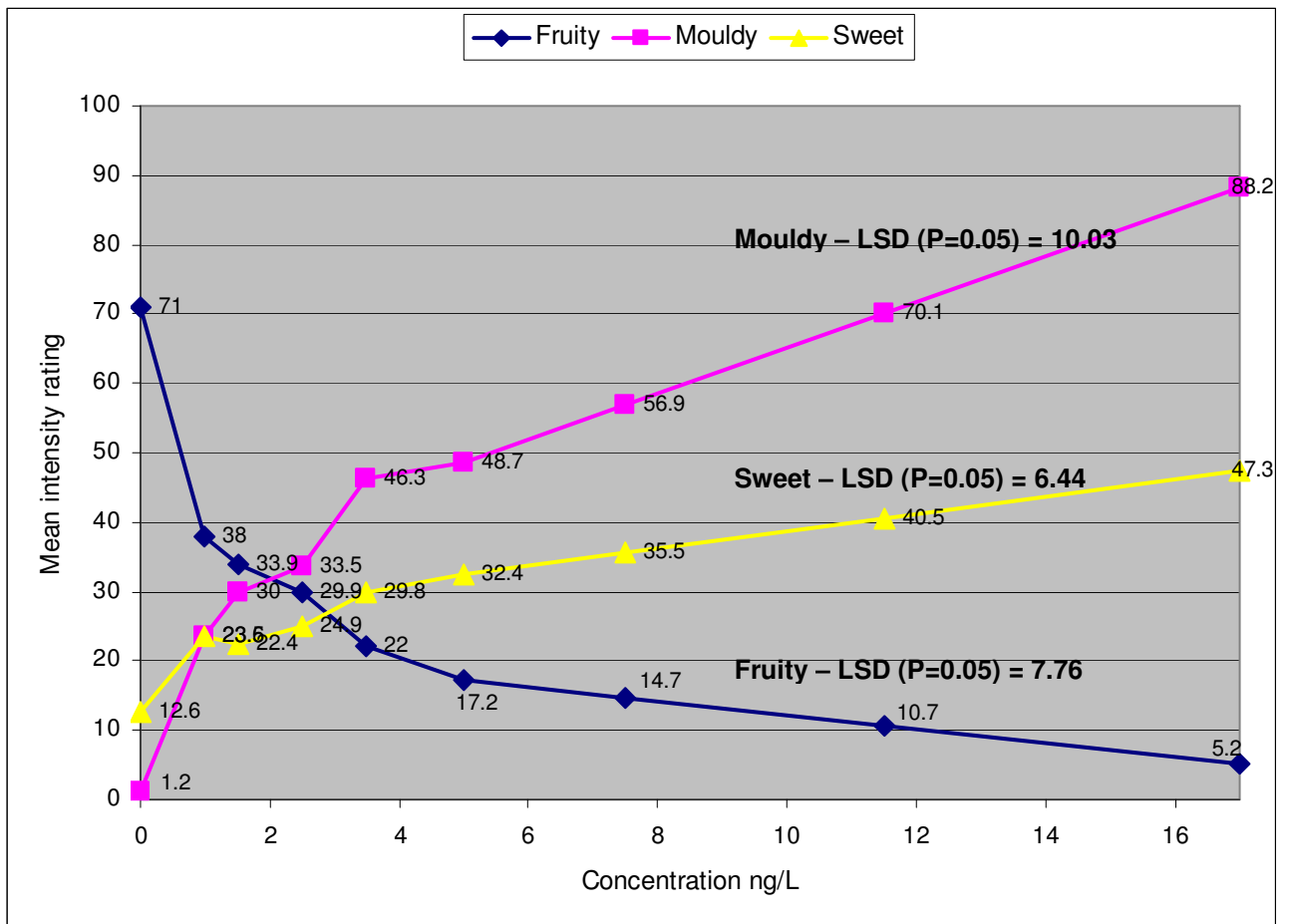
The line plot in Figure 6.1a indicates a substantial decrease in the intensity of fruity aroma and increase of mouldy aroma as soon as TCA is added to Chenin blanc, even at 1 ng/L. The mouldy aroma at a concentration level of 17 ng/L is extremely high with a mean intensity value of 88.2; similarly the degree of fruitiness is virtually non-existent at 17 ng/L with a mean intensity value of 5.2. The sweet aroma in Figure 6.1a also shows a sizeable increase, however, the angle of the line plot is slightly smaller than that of mouldy aroma.

According to Chapter 4 (Table 4.7) the detection threshold for TCA in Chenin blanc is 1.67 ng/L. At this point (Figure 6.1a) the intensity of the fruity, as well as the mouldy aroma was detectable with an approximate mean intensity value of 30.0 for both attributes.

In Table 6.8 significant differences ( $P \leq 0.05$ ) are indicated between the base wine (0 ng/L and 1 ng/L) for fruity, mouldy and sweet aroma. Thereafter the intensity of the fruity and mouldy aromas (Table 6.8) stays constant ( $P > 0.05$ ) until a concentration of 2.5 ng/L is reached. Beyond 2.5 ng/L, the next significant ( $P \leq 0.05$ ) decrease in fruitiness (Table 6.8) is at 3.5 ng/L and 17 ng/L, respectively. After 2.5 ng/L the mouldy aroma (Table 6.8) increases substantially from 3.5 ng/L ( $P \leq 0.05$ ). The sweet aroma (Table 6.8) tends to increase less sharp in intensity from 5 ng/L and onwards.

On Factor 1 (F1) the DA plot (Figure 6.1b) indicates that a good distinction was made between 0 ng/L (L0) and 17 ng/L (L17). As for the mid concentration range the panel could not discriminate clearly between different samples.

In the PCA bi-plot 94.75% of the variance is explained by Factor 1 (F1) and F2 (Figure 6.1c). Figure 6.1c confirms the findings in Figure 6.1b on the degree of discrimination between the different concentrations. The scores for the base wine with no TCA added (0 ng/L; L0), as well as the scores with the highest level of spiking with TCA (17 ng/L; L17) associated strongly within concentration level, however, they also lie on opposite sides of the plot on F1 indicating a strong discrimination between the two extreme concentration levels. As expected, fruity aroma correlates strongly with 0 ng/L; and mouldy and sweet aroma correlate strongly with 17 ng/L. The respective scores of the mid concentration range lie scattered in the middle of the plot (Figure 6.1c). However, the lower end of the mid concentration range (1 ng/L, 1.5 ng/L and 2.5 ng/L) relates better to the fruity attribute and the higher end of the mid concentration range (3.5 ng/L and 5 ng/L) relates better to the attributes mouldy and sweet.

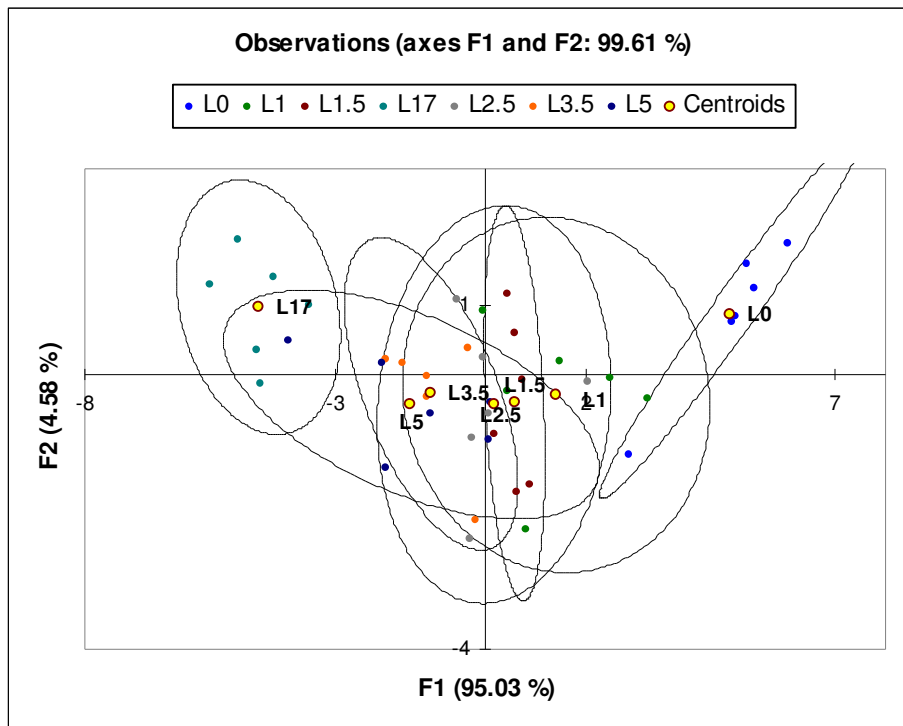


**Figure 6.1a** Mean intensity ratings of aroma attributes at increasing concentrations of TCA in Chenin blanc.

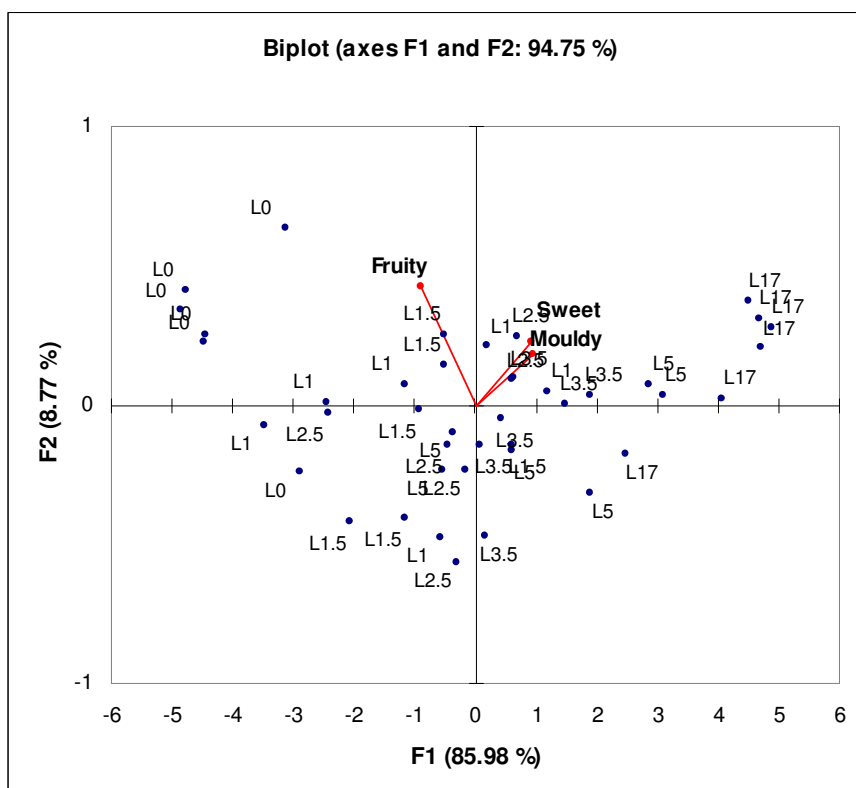
**Table 6.8** Significant differences between concentration levels of TCA in Chenin blanc as well as the LSD-values (P=0.05) for individual sensory attributes

Concentration (ng/L)	Fruity aroma	Mouldy aroma	Sweet aroma
0	71.0 <sup>a</sup>	1.20 <sup>f</sup>	12.6 <sup>f</sup>
1	38.0 <sup>b</sup>	23.5 <sup>e</sup>	23.6 <sup>de</sup>
1.5	33.9 <sup>bc</sup>	30.0 <sup>e</sup>	22.4 <sup>e</sup>
2.5	29.9 <sup>c</sup>	33.5 <sup>e</sup>	24.9 <sup>de</sup>
3.5	22.0 <sup>d</sup>	46.3 <sup>d</sup>	29.8 <sup>cd</sup>
5	17.2 <sup>d</sup>	48.7 <sup>cd</sup>	32.4 <sup>c</sup>
7.5	14.7 <sup>d</sup>	56.9 <sup>c</sup>	35.5 <sup>cb</sup>
11.5	10.7 <sup>de</sup>	70.1 <sup>b</sup>	40.5 <sup>b</sup>
17	5.20 <sup>e</sup>	88.2 <sup>a</sup>	47.3 <sup>a</sup>
	LSD = 7.76	LSD = 10.03	LSD = 6.44

LSD = Least significant difference; Means with different superscripts in the same column, differ significantly at the 5% level.



**Figure 6.1b** Discriminant analysis (DA) plot for TCA in Chenin blanc with ellipses around the centroids of the distinguishing groups.



**Figure 6.1c** Principal component analysis (PCA) bi-plot of TCA in Chenin blanc with level (scores) and sensory attributes (loadings). Each level (specific concentration of TCA) is represented by six judges. Factor 1 and 2 explain 94.7% of the variance.

### *Chenin blanc spiked with TeCA*

Figure 6.2a shows an immediate decrease in fruity aroma between the lowest concentration of TeCA (1 ng/L) and the base wine (0 ng/L), as well as an increase in mouldy and sweet aroma. The mouldy aroma at a concentration level of 23 ng/L is moderately high with a mean intensity value of 65.14; similarly the degree of fruitiness at 23 ng/L is extremely low with a mean intensity value of 16.96.

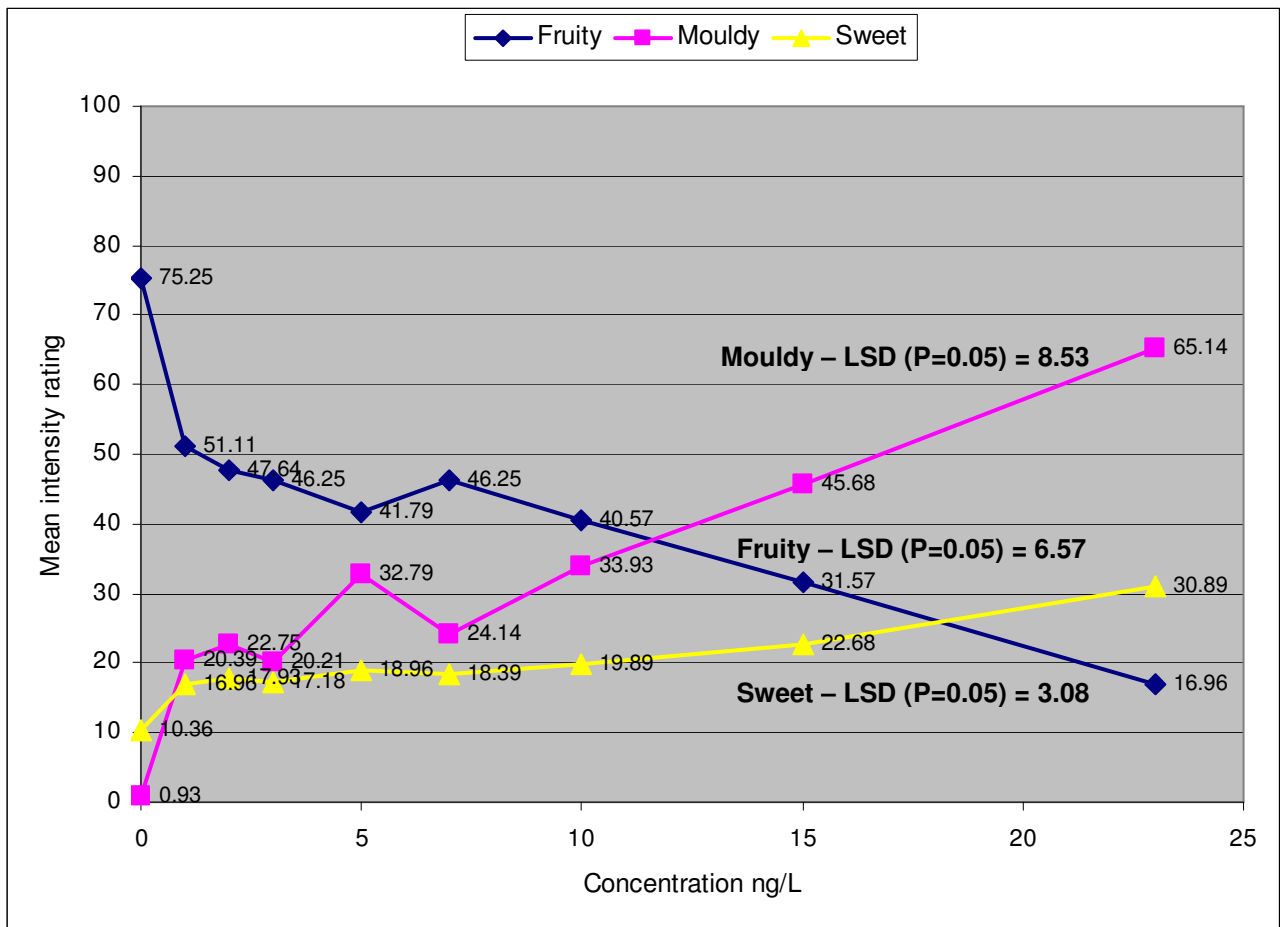
According to Chapter 4 (Table 4.7) the detection threshold for TeCA in Chenin blanc is 6.73 ng/L. At this point (Figure 6.2a) the intensity of the fruity aroma is slightly higher than that of the mouldy aroma, approximately 45 and 25 respectively.

Table 6.9 illustrates a significant ( $P \leq 0.05$ ) difference for the fruity and mouldy aroma between 0 ng/L and 1 ng/L. However, between 1 ng/L and 10 ng/L the increase in mouldiness and decrease in fruitiness is not linear and the respective consecutive concentration levels do not differ significantly ( $P > 0.05$ ). The intensity values of both fruitiness and mouldiness differ significantly ( $P \leq 0.05$ ) between 15 ng/L and 23 ng/L (Table 6.9). The only significant difference ( $P \leq 0.05$ ) for sweet aroma was noticed between 0 ng/L and 1 ng/L, between 7 ng/L and 15 ng/L, as well as between 15 ng/L and 23 ng/L (Table 6.9).

The DA plot (Figure 6.2b) indicates that the panel was able to distinguish clearly between the two highest concentrations, i.e. 15 ng/L (L15) and 23 ng/L (L23), as well as the base wine (0 ng/L; L0) and the remaining tainted wines. The panel could not discriminate clearly between the aroma intensities in the mid concentration range as their centroids (Figure 6.2b) are all positioned in the middle of the plot.

In the PCA bi-plot F1 and F2 explain 97.68% of the variance (Figure 6.2c). Figure 6.2c indicates a clear distinction between the scores of the judges for the highest concentration levels (L23), as well as the scores for the base wine (L0). The respective scores of the mid concentration range lie scattered in the middle of the plot indicating less distinction. On F1 the attribute fruitiness associates with the lower concentration levels and the attributes mouldiness and sweetness with the higher concentration levels. On F2 the attributes mouldiness and sweetness correlate negatively. This result is not clear. From Fig 6.2a and Table 6.9 it is evident that sweetness did not increase significantly ( $P > 0.05$ ) with increasing concentration of TeCA. This tendency is possibly responsible for the latter negative association in F2.



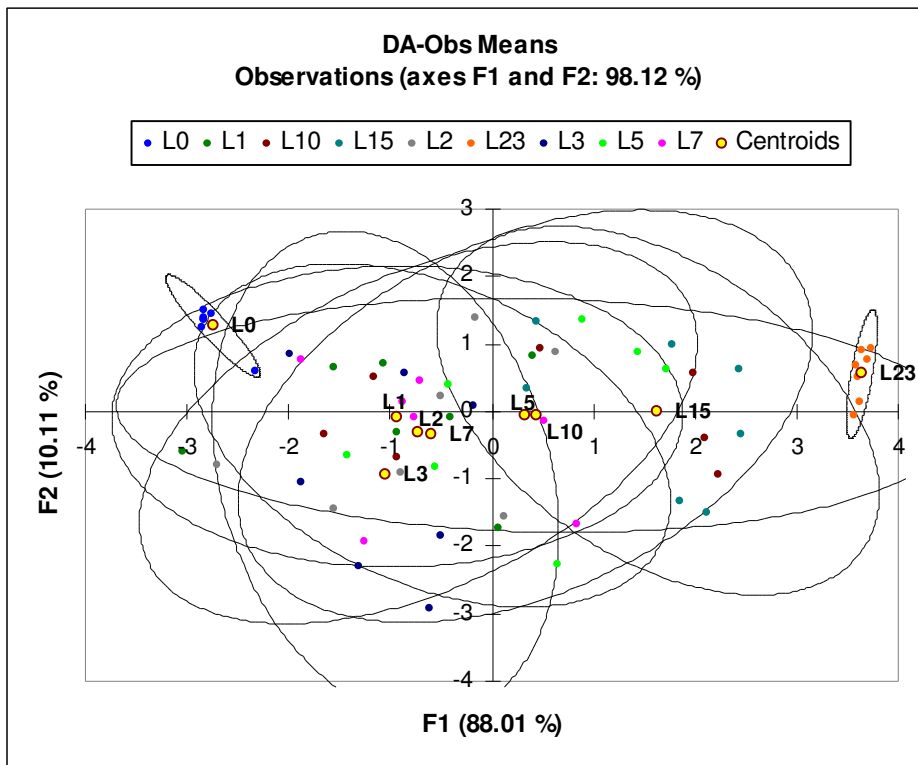


**Figure 6.2a** Mean intensity ratings of aroma attributes at increasing concentrations of TeCA in Chenin blanc.

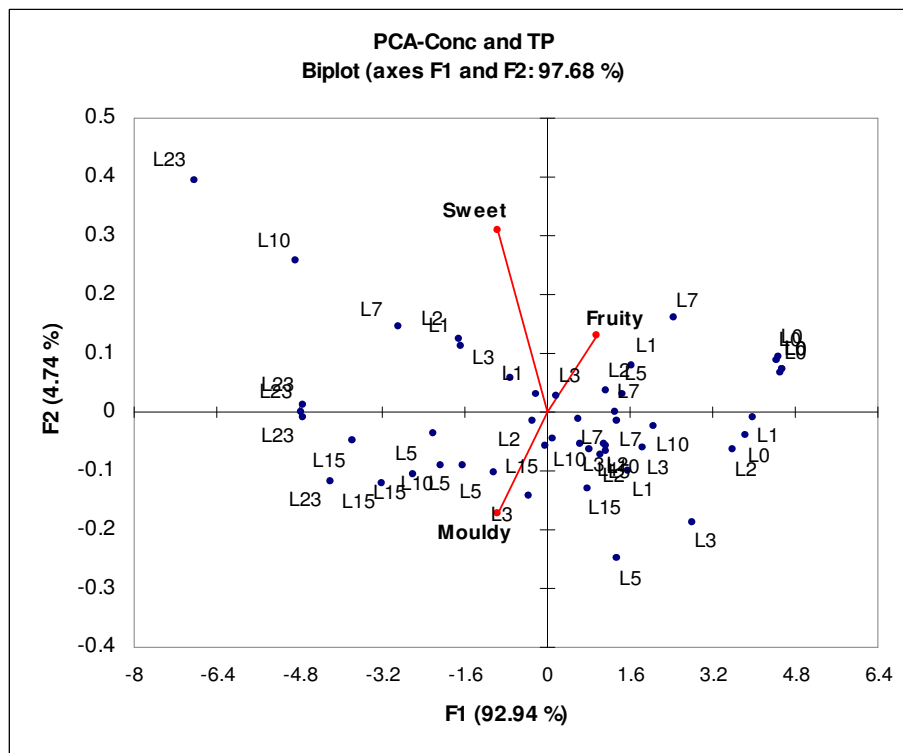
**Table 6.9** Significant differences between concentration levels of TeCA in Chenin blanc as well as the LSD-values (P=0.05) for individual sensory attributes

Concentration (ng/L)	Fruity aroma	Mouldy aroma	Sweet aroma
0	75.25 <sup>a</sup>	0.93 <sup>e</sup>	10.36 <sup>d</sup>
1	51.11 <sup>b</sup>	20.39 <sup>d</sup>	16.96 <sup>c</sup>
2	47.64 <sup>bc</sup>	22.75 <sup>d</sup>	17.93 <sup>c</sup>
3	46.25 <sup>bcd</sup>	20.21 <sup>d</sup>	17.18 <sup>c</sup>
5	41.79 <sup>cd</sup>	32.79 <sup>c</sup>	18.96 <sup>c</sup>
7	46.25 <sup>cbd</sup>	24.14 <sup>d</sup>	18.39 <sup>c</sup>
10	40.57 <sup>d</sup>	33.93 <sup>c</sup>	19.89 <sup>bc</sup>
15	31.57 <sup>e</sup>	45.68 <sup>b</sup>	22.68 <sup>b</sup>
23	16.96 <sup>f</sup>	65.14 <sup>a</sup>	30.89 <sup>a</sup>
	LSD = 6.57	LSD = 8.53	LSD = 3.08

LSD = Least significant difference; Means with different superscripts in the same column, differ significantly at the 5% level.



**Figure 6.2b** Discriminant analysis (DA) plot for TeCA in Chenin blanc with ellipses around the centroids of the distinguishing groups.



**Figure 6.2c** Principal component analysis (PCA) bi-plot of TeCA in Chenin blanc with levels (scores) and sensory attributes (loadings). Each level (specific concentration of TeCA) is represented by six judges. Factor 1 and 2 explain 97.6% of the variance.

### *Chenin blanc spiked with TBA*

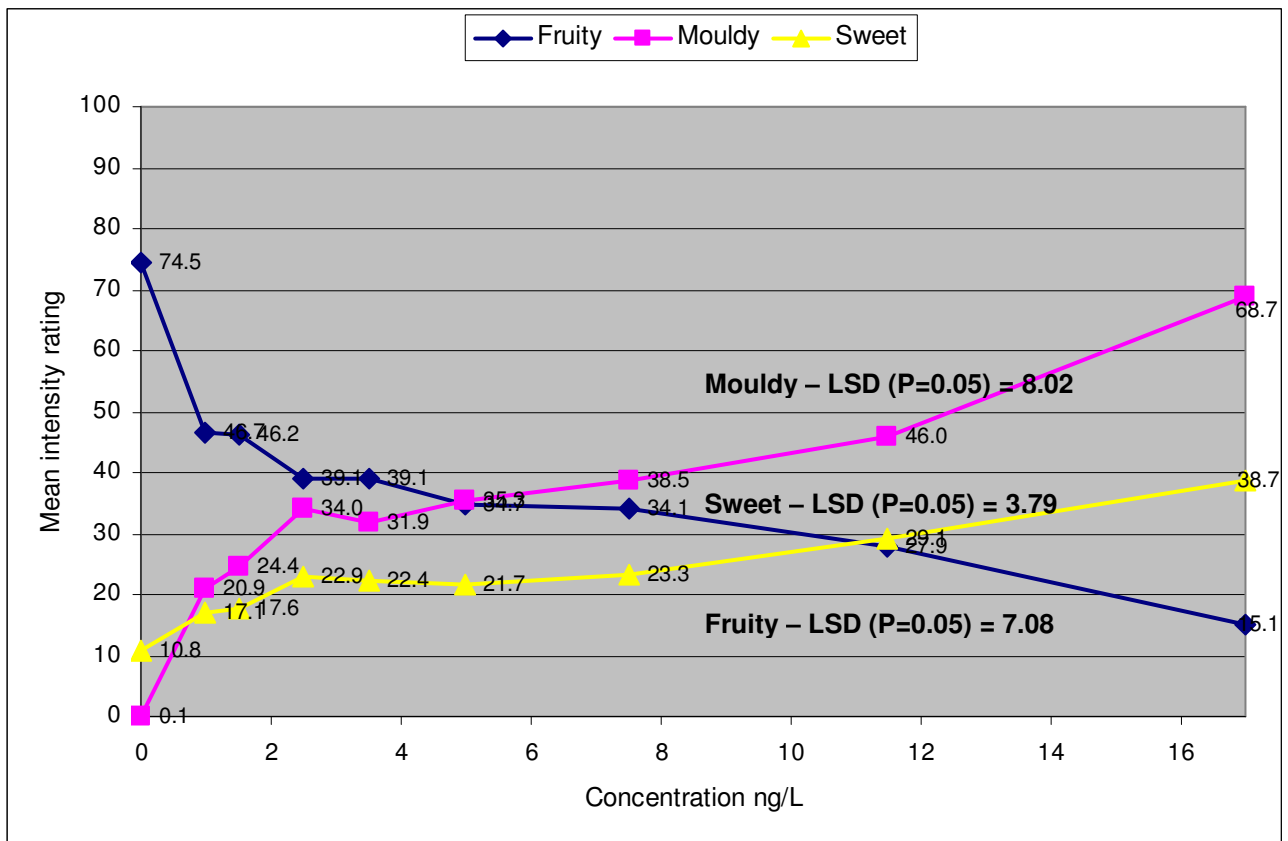
The line plot in Figure 6.3a for TBA is similar to that of TCA (Figure 6.1a), however, the mean intensity value for mouldy aroma at a concentration level of 17 ng/L for TBA is not as high as that of TCA.

According to Chapter 4 (Table 4.7) the detection threshold for TBA in Chenin blanc is 2.05 ng/L. At this point (Figure 6.3a) the mean intensity value of the fruity, as well as the mouldy aroma were both moderately low with approximate mean intensity values of 40 and 30, respectively.

Significant differences ( $P \leq 0.05$ ) in fruitiness, mouldiness and sweetness are noted in Table 6.10 between the base wine and the sample with a concentration of 1 ng/L. A further significant difference ( $P \leq 0.05$ ; Table 6.10) is observed between concentrations 1.5 ng/L and 2.5 ng/L for all attributes. However, for both fruitiness and mouldiness no significant differences are observed between concentrations 2.5 ng/L and 7.5 ng/L. Beyond a concentration of 7.5 ng/L the intensity of the fruity aroma decreases substantially ( $P \leq 0.05$ ), and that of mouldiness and sweetness increases significantly ( $P \leq 0.05$ ).

The DA plot (Figure 6.3b) indicates that a good distinction was made between 0 ng/L (L0) and 17 ng/L (L17). As for the mid concentration ranges, the panel could not discriminate clearly between different samples.

In the PCA bi-plot 97.72% of the variance is explained by F1 and F2 (Figure 6.3c). Figure 6.3c confirms the findings in Figure 6.3b on the degree of discrimination between the different concentration levels. The scores for the base wine with no TBA added (0 ng/L; L0), as well as the scores with the highest level of spiking with TBA (17 ng/L; L17) associated strongly within concentration level. The latter two concentration levels lie on opposite sides of the plot on F1 indicating a strong discrimination of extreme concentration levels. As expected, fruity aroma correlates strongly with 0 ng/L; and mouldy aroma correlates strongly with 17 ng/L. The respective scores of the mid concentration range lie scattered in the middle of the plot (Figure 6.3c).

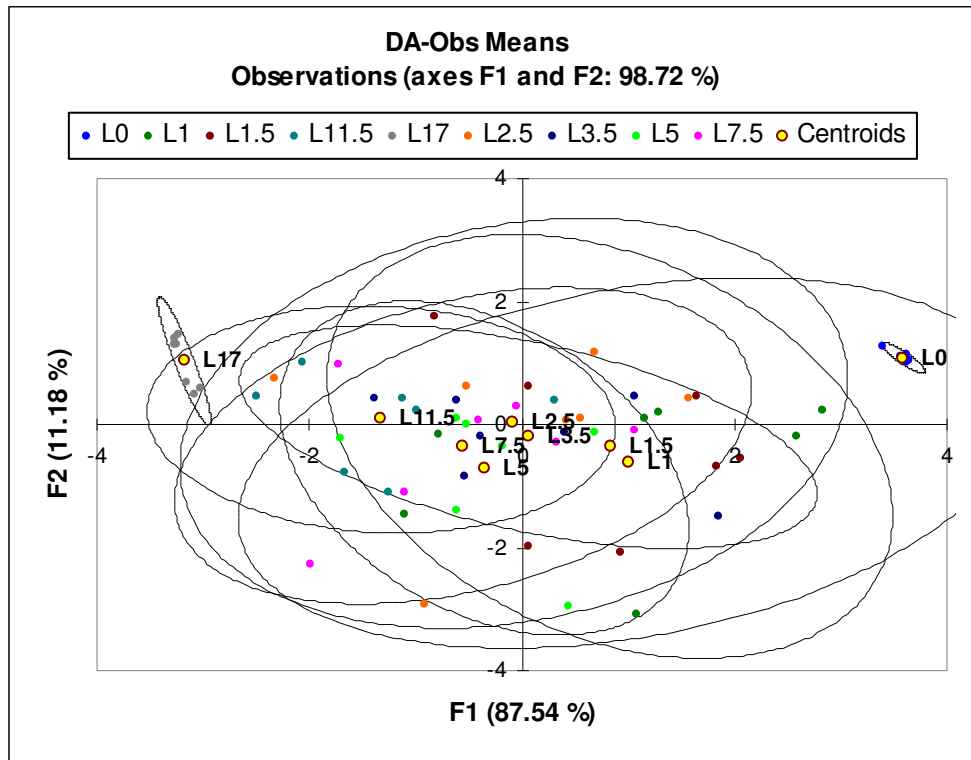


**Figure 6.3a** Mean intensity ratings of aroma attributes at increasing concentrations of TBA in Chenin blanc.

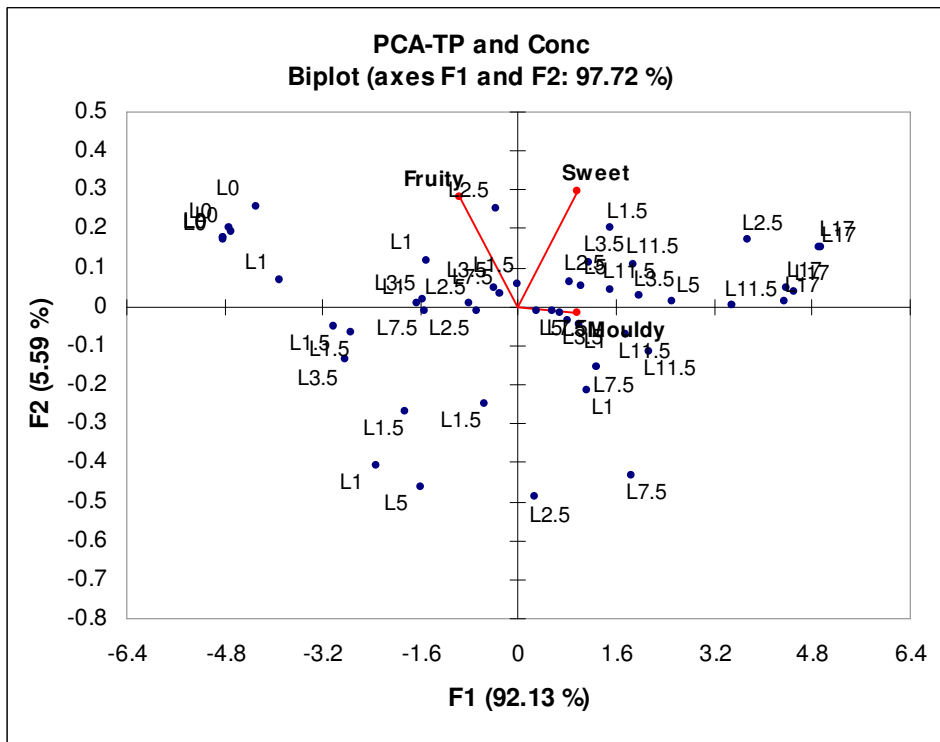
**Table 6.10** Significant differences between concentration levels of TBA in Chenin blanc as well as the LSD-values (P=0.05) for individual sensory attributes

Concentration (ng/L)	Fruity aroma	Mouldy aroma	Sweet aroma
0	74.50 <sup>a</sup>	0.07 <sup>f</sup>	10.75 <sup>e</sup>
1	46.70 <sup>b</sup>	20.89 <sup>e</sup>	17.07 <sup>d</sup>
1.5	46.21 <sup>b</sup>	24.43 <sup>de</sup>	17.57 <sup>d</sup>
2.5	39.07 <sup>c</sup>	33.96 <sup>c</sup>	22.93 <sup>c</sup>
3.5	39.11 <sup>c</sup>	31.93 <sup>bc</sup>	22.36 <sup>c</sup>
5	34.71 <sup>cd</sup>	35.32 <sup>c</sup>	21.67 <sup>c</sup>
7.5	34.14 <sup>cd</sup>	38.54 <sup>cb</sup>	23.32 <sup>c</sup>
11.5	27.93 <sup>d</sup>	45.96 <sup>b</sup>	29.11 <sup>b</sup>
17	15.07 <sup>e</sup>	68.74 <sup>a</sup>	38.70 <sup>a</sup>
	LSD = 7.08	LSD = 8.02	LSD = 3.79

LSD = Least significant difference; Means with different superscripts in the same column, differ significantly at the 5% level.



**Figure 6.3b** Discriminant analysis (DA) plot for TBA in Chenin blanc with ellipses around the centroids of the distinguishing groups.



**Figure 6.3c** Principal component analysis (PCA) bi-plot of TBA in Chenin blanc with levels (scores) and sensory attributes (loadings). Each level (specific concentration of TBA) is represented by six judges. Factor 1 and 2 explain 97.7% of the variance.

### *Chenin blanc spiked with PCA*

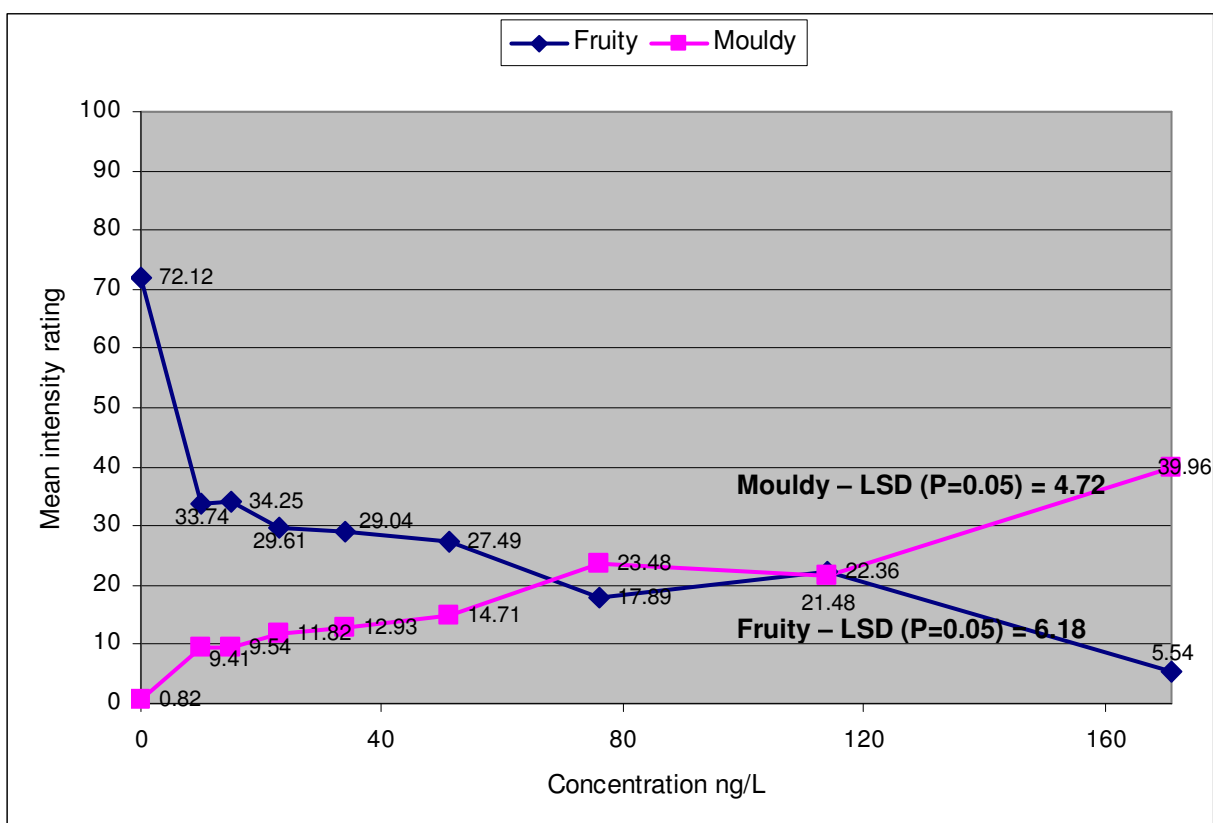
The PCA-induced line plot in Figure 6.4a for fruitiness is similar to that of TCA illustrated in Figure 6.1a, however, the PCA-induced line plot for mouldiness rises slowly with a maximum value of 39.96 at the highest concentration level of 171 ng/L. This indicates that the mouldy aroma induced by a high concentration level of PCA is not very strong.

According to Chapter 4 (Table 4.7) the detection threshold for PCA in Chenin blanc is 43.73 ng/L. At this point (Figure 6.4a) the intensity of the fruity aroma is moderately low, but the mouldy aroma is very low, just barely detectable.

Significant differences ( $P \leq 0.05$ ) in fruitiness and mouldiness are noted in Table 6.11 between the base wine and the sample with a concentration of 10 ng/L. Thereafter the trend in both attributes is not significant, only at 114 ng/L and 171 ng/L significant differences ( $P \leq 0.05$ ) in both attributes are illustrated.

The DA plot (Figure 6.4b) indicates that a good distinction was made between 0 ng/L (L0) and 171 ng/L (L17). As for the mid concentration ranges, the panel could not discriminate clearly between different samples.

In the principal component analysis (PCA) bi-plot a hundred percent of the variance is explained by F1 and F2 (Figure 6.4c). Figure 6.4c confirms the findings in Figure 6.4b on the degree of discrimination between the different concentrations, as well as the correlation of the respective attributes, fruitiness and mouldiness, with the 0 ng/L and 171 ng/L concentration levels, respectively.

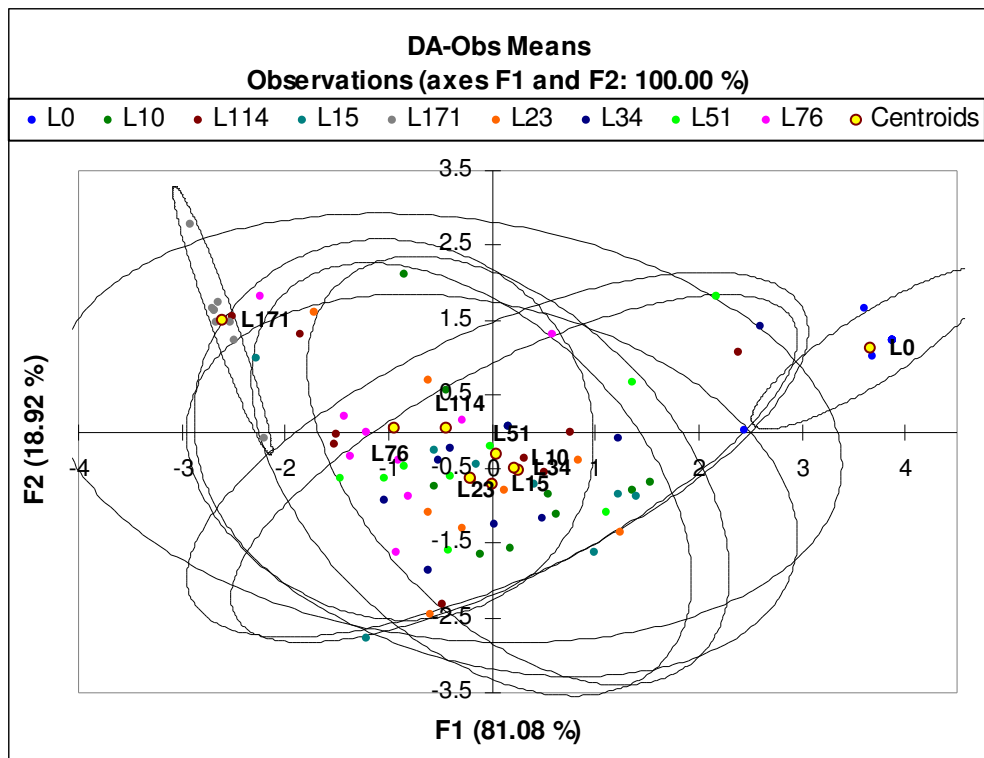


**Figure 6.4a** Mean intensity ratings of aroma attributes at increasing concentrations of PCA in Chenin blanc.

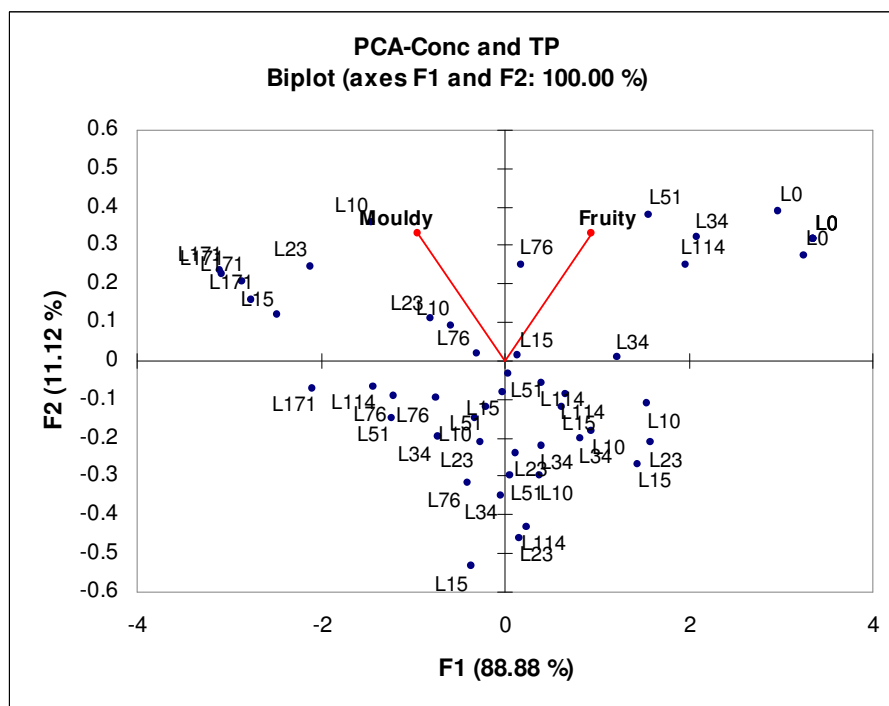
**Table 6.11** Significant differences between concentration levels of PCA in Chenin blanc as well as the LSD-values (P=0.05) for individual sensory attributes

Concentration (ng/L)	Fruity aroma	Mouldy aroma
0	72.12 <sup>a</sup>	0.82 <sup>e</sup>
10	33.74 <sup>b</sup>	9.41 <sup>d</sup>
15	34.25 <sup>b</sup>	9.54 <sup>d</sup>
23	29.61 <sup>bc</sup>	11.82 <sup>cd</sup>
34	29.04 <sup>bc</sup>	12.93 <sup>cd</sup>
51	27.49 <sup>cd</sup>	14.71 <sup>c</sup>
76	17.89 <sup>e</sup>	23.48 <sup>b</sup>
114	22.36 <sup>de</sup>	21.48 <sup>b</sup>
171	5.54 <sup>f</sup>	39.96 <sup>a</sup>
	LSD = 6.18	LSD = 4.72

LSD = Least significant difference; Means with different superscripts in the same column, differ significantly at the 5% level.



**Figure 6.4b** Discriminant analysis (DA) plot for PCA in Chenin blanc with ellipses around the centroids of the distinguishing groups.



**Figure 6.4c** Principal component analysis (PCA) bi-plot of PCA in Chenin blanc with levels (scores) and sensory attributes (loadings). Each level (specific concentration of PCA) is represented by six judges. Factor 1 and 2 explain 100% of the variance.



## Sensory attributes of Pinotage spiked with TCA, TeCA, TBA and PCA

The Pinotage base wine was characterised as being strong in *fruitiness* (Table 6.6). Pinotage spiked with TCA, TeCA and TBA at concentrations at/or above detection threshold resulted in a *mouldy* aroma, as well as a *sweet* aroma typical of excessively sweet wine. The panel characterised Pinotage spiked with PCA as not having a typical mouldy aroma, but a *mouldy-chemical* aroma.

The line plots in Figure 6.5a for TCA, Figure 6.6a for TeCA, Figure 6.7a for TBA and Figure 6.8a for PCA indicate that the base wine (0 ng/L) has a reasonably strong fruity aroma (mean value of approximately 60) and that there is virtually no fruitiness left when the respective haloanisoles are at their highest concentrations. Conversely, the degree of mouldiness rises rapidly from 0 ng/L until it reaches a mean intensity rating of approximately 20 for all four compounds (TCA, TECA, TBA and PCA). Thereafter, the TCA line plot for mouldiness (Figure 6.5a) has a steep gradient until it reaches an extremely high intensity value of 90. Both the TeCA (Figure 6.6a) and TBA line plots (Figure 6.7a) for mouldiness indicate a mean intensity value of approximately 70 when the respective concentrations of TeCA and TBA are at their highest level. The PCA line plot (Figure 6.8a), by contrast, demonstrates a slow rise in mouldiness as the concentration of PCA increases. When the concentration of PCA is at its maximum concentration (256 ng/L) the mean intensity rating for mouldiness is only 60, illustrating a moderate degree of mouldiness. The addition of TCA, TeCA and TBA also resulted in a slight sweet aroma. This sweet aroma was more pronounced at the higher levels of the respective haloanisoles, especially when the Pinotage wine was spiked with TCA.

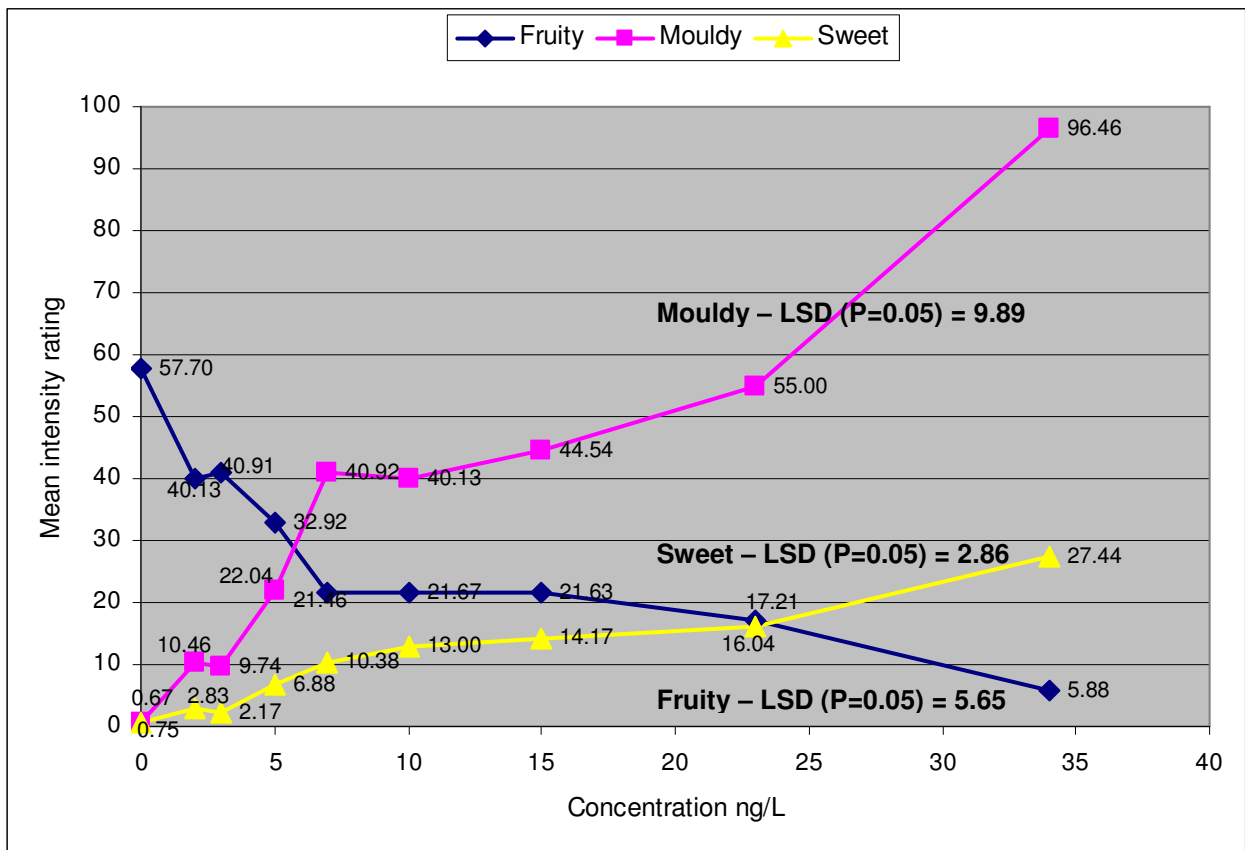
According to Chapter 4 (Table 4.7) the detection threshold levels for the respective haloanisoles in Pinotage are 4.54 ng/L for TCA; 8.67 ng/L for TeCA, 8.69 ng/L for TBA and 51.18 ng/L for PCA. At this point the mouldy aroma was detectable and all the compounds illustrated an approximate mean intensity value of 20 for mouldiness (Figure 6.5a for TCA; Figure 6.6a for TeCA; Figure 6.7a for TBA and Figure 6.8a for PCA). At the latter mean intensity values the fruity aroma decreased dramatically and was to a certain extent masked by mouldiness.

The significant differences ( $P \leq 0.05$ ) between samples are illustrated in Tables 6.12 to 6.15. In Table 6.12 the fruity aroma drops significantly ( $P \leq 0.05$ ) between the base wine (0 ng/L) and the concentration level of 2 ng/L for TCA. Thereafter the intensity of the fruity aroma of Pinotage with TCA (Table 6.12) decreases significantly ( $P \leq 0.05$ ) until it reaches a level of 5

ng/L; from then on the low level of fruity aroma stays the same for the highest four concentration levels of TCA ( $P>0.05$ ). The significant differences for mouldiness are reversed as the concentration of TCA increases (Table 6.12). With the addition of TeCA (Table 6.13); TBA (Table 6.14) and PCA (Table 6.15) the drop in fruity aroma with a corresponding increase of haloanisole concentration is similar: The fruity aroma drops significantly ( $P\leq 0.05$ ) between the base wine (0 ng/L) and lowest concentration level of the respective haloanisoles, thereafter the fruity aroma does not decrease significantly ( $P>0.05$ ) as the mid-concentration levels of the respective haloanisoles increase. Again, the significant differences ( $P\leq 0.05$ ) for mouldiness are reversed as the concentration levels of TeCA (Table 6.13), TBA (Table 6.14) and PCA (Table 6.15) are increased: The mouldy aroma increases significantly ( $P\leq 0.05$ ) between the base wine (0 ng/L) and lowest concentration level of the respective haloanisoles, thereafter the mouldy aroma does not increase significantly ( $P>0.05$ ) as the mid-concentration levels of the respective haloanisoles increase.

The DA plots for TCA (Figure 6.5b), TeCA (Figure 6.6b), TBA (Figure 6.7b) and PCA (Figure 6.8b) indicate that a good distinction was made between 0 ng/L (L0) and the highest concentration of the respective haloanisoles. Although the centroids illustrate some degree of order in the low, mid and high concentrations, the panel had difficulty in discriminating between the low concentrations.

In the principal component analysis (PCA) bi-plots more than 98% of the variance is explained by F1 and F2 (Figure 6.5c for TCA; Figure 6.6c for TeCA; Figure 6.7c for TBA and Figure 6.8c for PCA). These bi-plots confirm the findings on the degree of discrimination between the different concentrations illustrated in Figures 6.5b to 6.8b. The scores for the base wine with no haloanisole added (0 ng/L; L0), as well as the scores for the highest level of spiking associated reasonably strongly within concentration level (Figures 6.5c - 6.8c). They also lie on opposite sides of the plot on F1 indicating a strong discrimination between the two extreme concentration levels. As expected, fruity aroma correlates strongly with 0 ng/L (L0); and mouldy aroma correlates strongly with the highest concentration level. The respective scores of the mid concentration range lie scattered in the middle of the plot for all four haloanisoles. The association between mouldy and sweet aroma is stronger in Pinotage with TCA, than in Pinotage with added TeCA and TBA. The former stronger association between mouldy and sweetness (Figure 6.5c) is possibly as a result of line plots for mouldy and sweet aroma (Figure 6.5a) illustrating a reasonably similar gradient with increasing concentrations of TCA. In Figures 6.6a and 6.7a the gradients of the mouldy and sweet aroma line plots differ as the haloanisole concentration increases resulting a less strong association between sweet and mouldy aroma in Figures 6.6c and 6.7c.

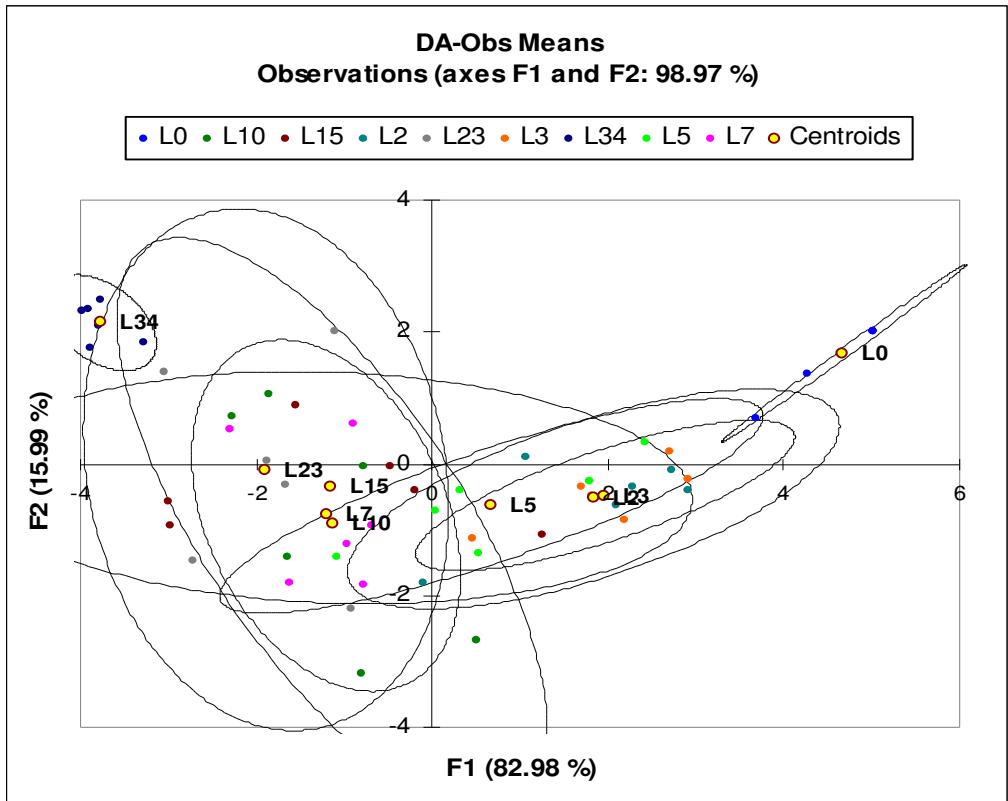


**Figure 6.5a** Mean intensity ratings of aroma attributes at increasing concentrations of TCA in Pinotage.

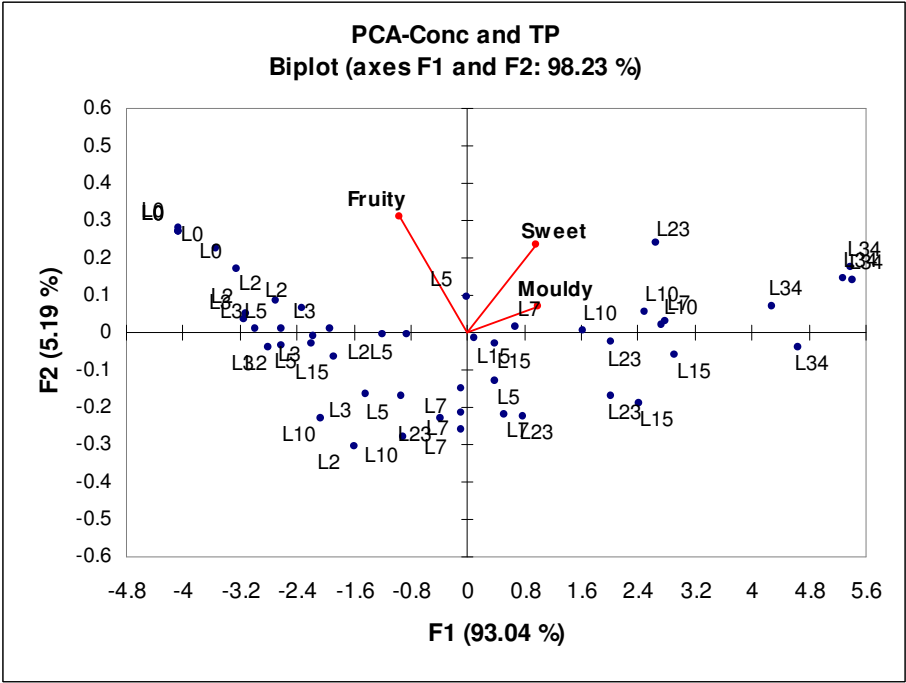
**Table 6.12** Significant differences between concentration levels of TCA in Pinotage as well as the LSD-values (P=0.05) for individual sensory attributes

Concentration (ng/L)	Fruity aroma	Mouldy aroma	Sweet aroma
0	57.70 <sup>a</sup>	0.75 <sup>f</sup>	0.75 <sup>f</sup>
2	40.13 <sup>b</sup>	10.46 <sup>e</sup>	2.83 <sup>f</sup>
3	40.91 <sup>b</sup>	9.74 <sup>e</sup>	2.17 <sup>f</sup>
5	32.92 <sup>c</sup>	22.04 <sup>d</sup>	6.88 <sup>e</sup>
7	21.46 <sup>d</sup>	40.92 <sup>c</sup>	10.38 <sup>d</sup>
10	21.67 <sup>d</sup>	40.13 <sup>c</sup>	13.00 <sup>cd</sup>
15	21.63 <sup>d</sup>	44.54 <sup>c</sup>	14.17 <sup>bc</sup>
23	17.21 <sup>d</sup>	55.00 <sup>b</sup>	16.04 <sup>b</sup>
34	5.9 <sup>e</sup>	96.46 <sup>a</sup>	27.44 <sup>a</sup>
	LSD = 5.65	LSD = 9.89	LSD = 2.86

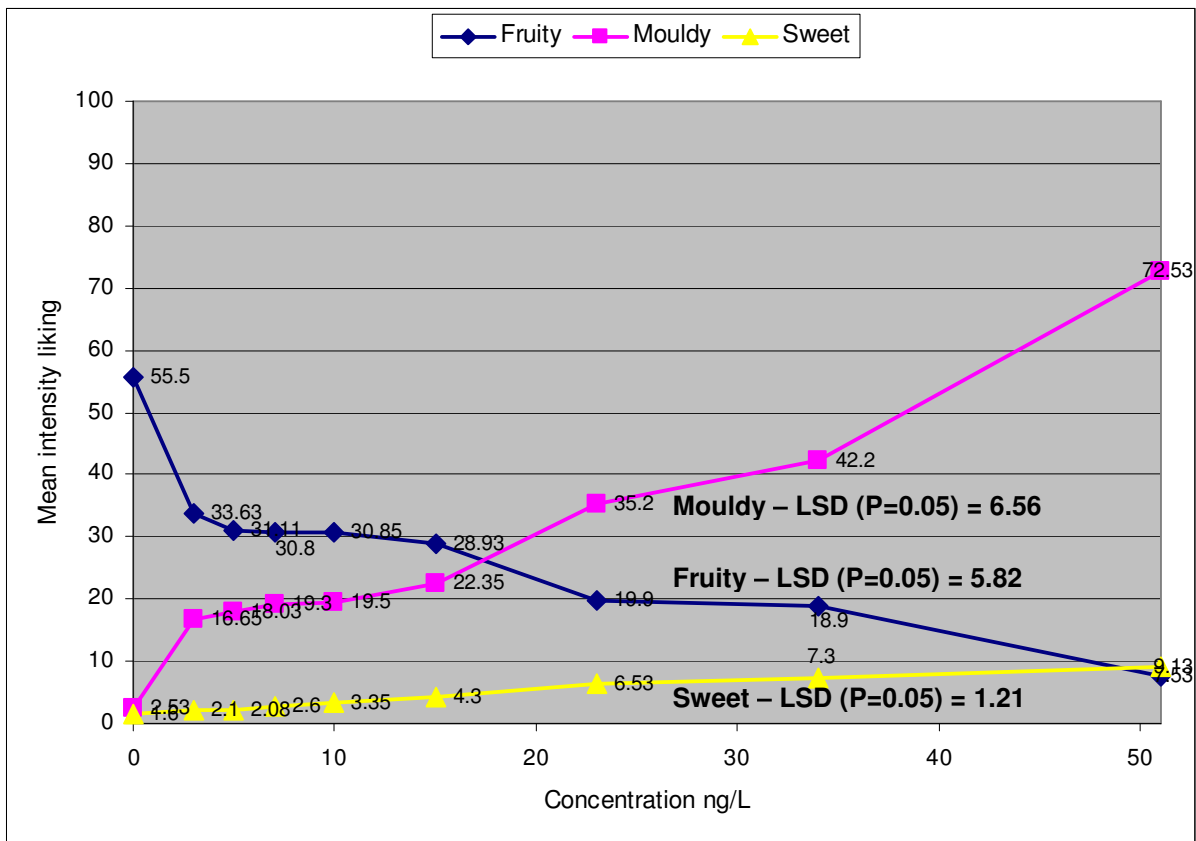
LSD = Least significant difference; Means with different superscripts in the same column, differ significantly at the 5% level.



**Figure 6.5b** Discriminant analysis (DA) plot for TCA in Pinotage with ellipses around the centroids of the distinguishing groups.



**Figure 6.5c** Principal component analysis (PCA) bi-plot of TCA in Pinotage with levels (scores) and sensory attributes (loadings). Each level (specific concentration of TCA) is represented by six judges. Factor 1 and 2 explain 98.2% of the variance.

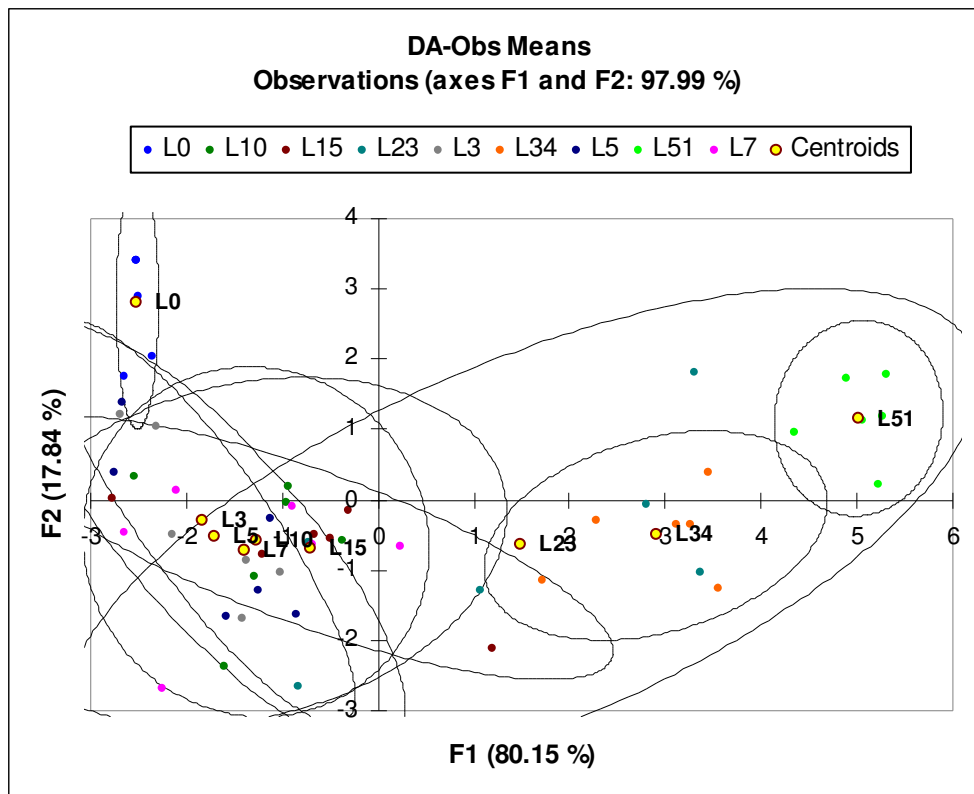


**Figure 6.6a** Mean intensity ratings of aroma attributes at increasing concentrations of TeCA in Pinotage.

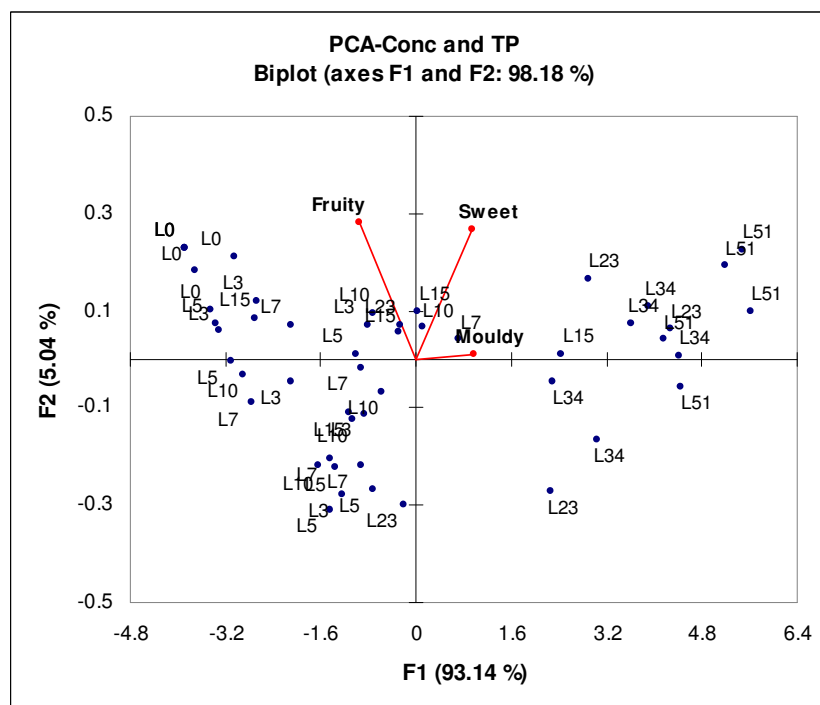
**Table 6.13** Significant differences between concentration levels of TeCA in Pinotage as well as the LSD-values (P=0.05) for individual sensory attributes

Concentration (ng/L)	Fruity aroma	Mouldy aroma	Sweet aroma
0	55.5 <sup>a</sup>	2.53 <sup>e</sup>	1.60 <sup>e</sup>
3	33.63 <sup>b</sup>	16.65 <sup>d</sup>	2.10 <sup>e</sup>
5	31.11 <sup>b</sup>	18.03 <sup>d</sup>	2.08 <sup>e</sup>
7	30.8 <sup>b</sup>	19.30 <sup>d</sup>	2.60 <sup>de</sup>
10	30.85 <sup>b</sup>	19.50 <sup>d</sup>	3.35 <sup>cd</sup>
15	28.93 <sup>b</sup>	22.35 <sup>d</sup>	4.30 <sup>c</sup>
23	19.90 <sup>b</sup>	35.20 <sup>c</sup>	6.53 <sup>b</sup>
34	18.90 <sup>c</sup>	42.20 <sup>b</sup>	7.30 <sup>b</sup>
51	7.53 <sup>c</sup>	72.53 <sup>a</sup>	9.13 <sup>a</sup>
	LSD = 5.82	LSD = 6.56	LSD = 1.21

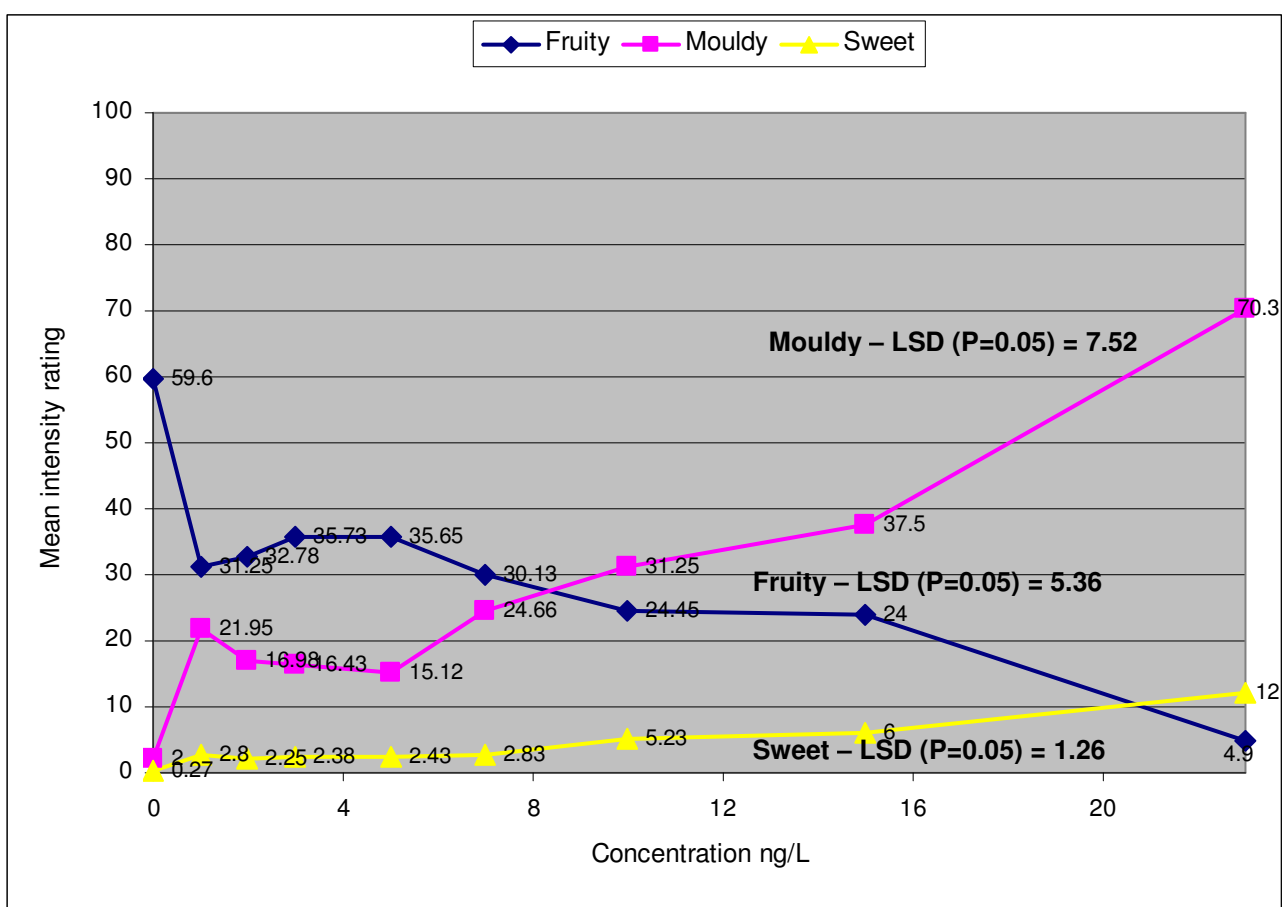
LSD = Least significant difference; Means with different superscripts in the same column, differ significantly at the 5% level.



**Figure 6.6b** Discriminant analysis (DA) plot for TeCA in Pinotage with ellipses around the centroids of the distinguishing groups.



**Figure 6.6c** Principal component analysis (PCA) bi-plot of TeCA in Pinotage with levels (scores) and sensory attributes (loadings). Each level (specific concentration of TeCA) is represented by six judges. Factor 1 and 2 explain 98.1% of the variance.

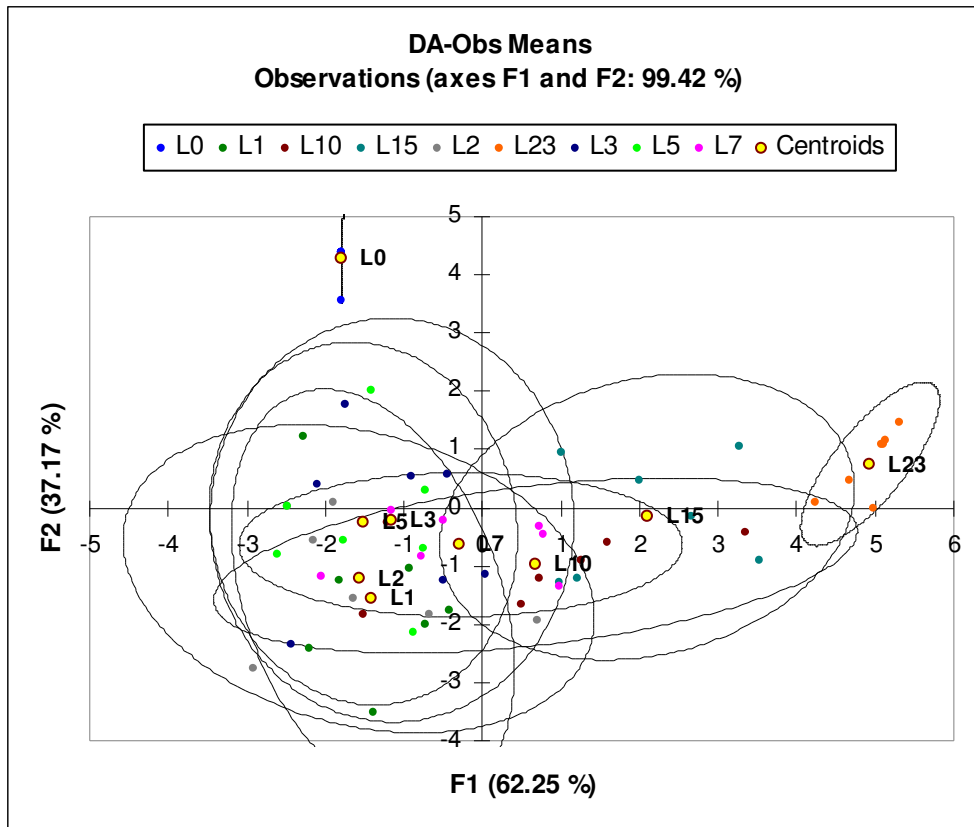


**Figure 6.7a** Mean intensity ratings of aroma attributes at increasing concentrations of TBA in Pinotage.

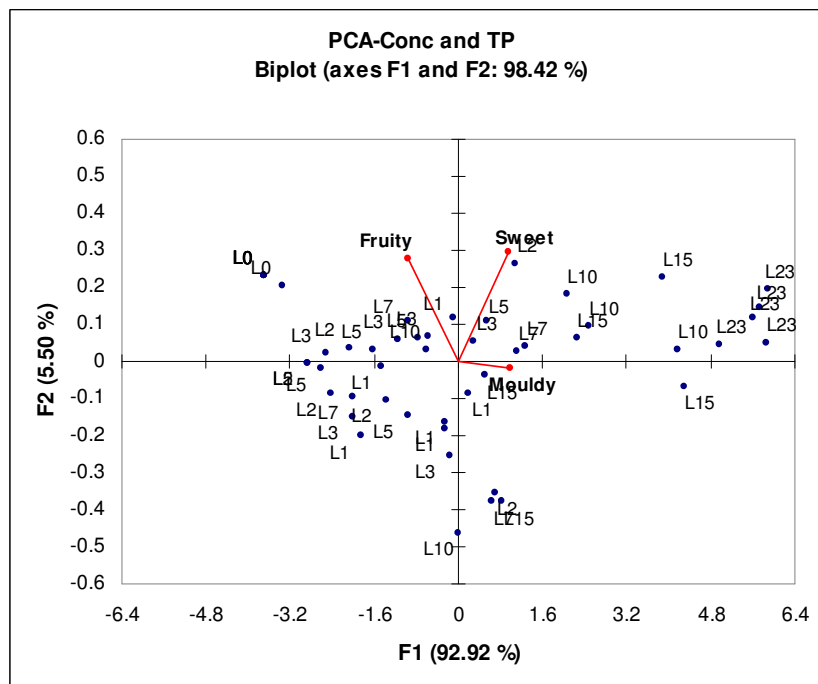
**Table 6.14** Significant differences between concentration levels of TBA in Pinotage as well as the LSD-values (P=0.05) for individual sensory attributes

Concentration (ng/L)	Fruity aroma	Mouldy aroma	Sweet aroma
0	59.60 <sup>a</sup>	2.00 <sup>f</sup>	0.27 <sup>d</sup>
1	31.25 <sup>bc</sup>	21.95 <sup>de</sup>	2.80 <sup>c</sup>
2	32.78 <sup>bc</sup>	16.98 <sup>e</sup>	2.25 <sup>c</sup>
3	35.73 <sup>b</sup>	16.43 <sup>e</sup>	2.38 <sup>c</sup>
5	35.65 <sup>b</sup>	15.12 <sup>e</sup>	2.43 <sup>c</sup>
7	30.13 <sup>c</sup>	24.66 <sup>cd</sup>	2.83 <sup>c</sup>
10	24.45 <sup>d</sup>	31.25 <sup>bc</sup>	5.23 <sup>b</sup>
15	24.00 <sup>d</sup>	37.50 <sup>b</sup>	6.00 <sup>b</sup>
23	4.90 <sup>e</sup>	70.30 <sup>a</sup>	12.00 <sup>a</sup>
	LSD = 5.36	LSD = 7.52	LSD = 1.26

LSD = Least significant difference; Means with different superscripts in the same column, differ significantly at the 5% level.

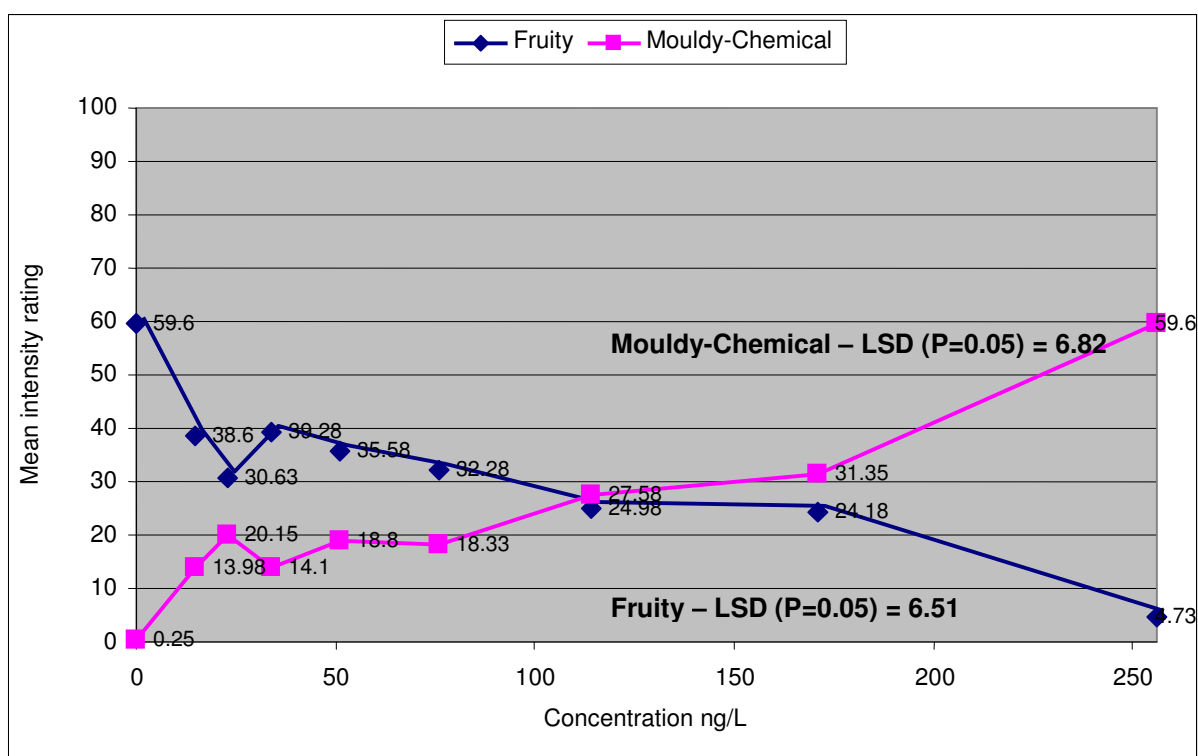


**Figure 6.7b** Discriminant analysis (DA) plot for TBA in Pinotage with ellipses around the centroids of the distinguishing groups.



**Figure 6.7c** Principal component analysis (PCA) bi-plot of TBA in Pinotage with levels (scores) and sensory attributes (loadings). Each level (specific concentration of TBA) is represented by six judges. Factor 1 and 2 explain 98.4% of the variance.





**Figure 6.8a** Mean intensity ratings of aroma attributes at increasing concentrations of PCA in Pinotage.

**Table 6.15** Significant differences between concentration levels of PCA in Pinotage as well as the LSD-values (P=0.05) for individual sensory attributes

Concentration (ng/L)	Fruity aroma	Mouldy-Chemical aroma
0	59.60 <sup>a</sup>	0.25 <sup>d</sup>
15	38.60 <sup>bc</sup>	13.98 <sup>c</sup>
23	30.63 <sup>de</sup>	20.15 <sup>c</sup>
34	39.28 <sup>b</sup>	14.10 <sup>c</sup>
51	35.58 <sup>bcd</sup>	18.80 <sup>c</sup>
76	32.28 <sup>cd</sup>	18.33 <sup>c</sup>
114	24.98 <sup>e</sup>	27.58 <sup>b</sup>
171	24.18 <sup>e</sup>	31.35 <sup>b</sup>
256	4.73 <sup>f</sup>	59.60 <sup>a</sup>
	LSD = 6.51	LSD = 6.82

LSD = Least significant difference; Means with different superscripts in the same column, differ significantly at the 5% level.



## Sensory attributes of Shiraz spiked with TCA, TeCA, TBA and PCA

The Shiraz base wine had a strong herbaceous aroma (Table 6.6). Shiraz spiked with TCA, and TeCA at concentrations at/or above detection threshold resulted in an aroma described as *mouldy-chlorine*. Shiraz spiked with TBA illustrated a typical *mouldy* aroma. Spiking with PCA at concentrations at/or above detection threshold resulted in an aroma described as *mouldy-acidic*.

The line plots in Figure 6.9a for TCA, Figure 6.10a for TeCA, Figure 6.11a for TBA and Figure 6.12a for PCA indicate that the base wine (0 ng/L) has a strong herbaceous aroma (mean value of approximately 80) and that there is virtually no herbaceous aroma left when the respective haloanisoles were at their highest concentrations. Equally, the degree of mouldy-like aroma rises rapidly from 0 ng/L until it reaches a mean intensity rating of approximately 15 for TCA, TECA, TBA and PCA (Figures 6.9a; 6.10a; 6.11a and 6.12a, respectively). Thereafter, the TCA and TeCA line plots for the mouldy-chlorine aroma (Figures 6.9a and 6.10a, respectively) both have a steep gradient until it reaches an intensity value of 80. The line plot of mouldiness for TBA (Figure 6.11a) indicates a mean intensity value of approximately 60 when the concentration of TBA is at its highest level. The PCA line plot (Figure 6.12a) demonstrates a slow rise in the mouldy-like attribute as the concentration of PCA increases. When the concentration of PCA is at its maximum concentration (256 ng/L) the mean intensity rating for mouldy-acidic is 41.7, illustrating a reasonably low intensity for this specific mouldy-like attribute.

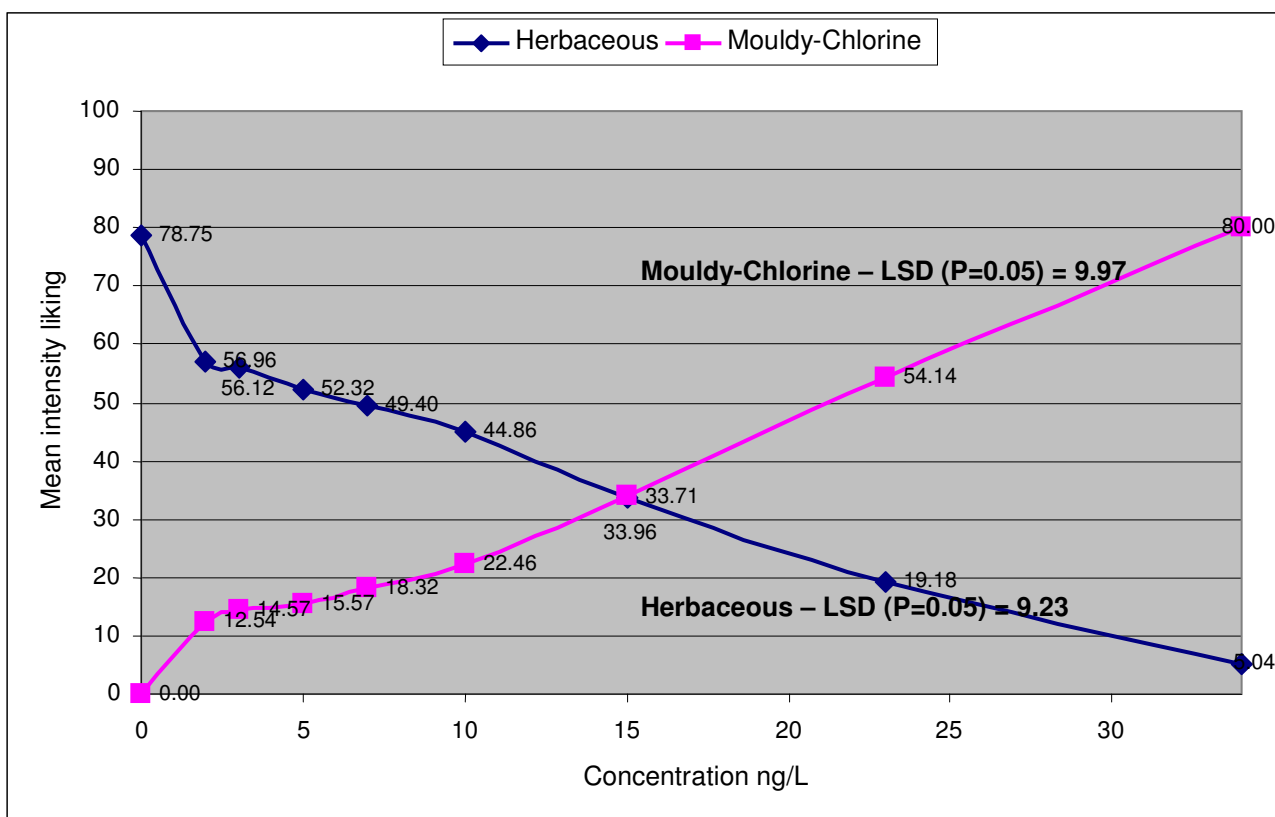
According to Chapter 4 (Table 4.7) the detection threshold levels for the respective haloanisoles in Shiraz are 3.86 ng/L for TCA, 10.72 ng/L for TeCA, 4.12 ng/L for TBA and 57.8 ng/L for PCA. At this point the herbaceous aroma has dropped but is still moderately strong with an intensity value of approximately 50, however, the mouldy-like taint is just perceivable with an approximate mean intensity value of 15 (Figure 6.9a for TCA; Figure 6.10a for TeCA; Figure 6.11a for TBA and Figure 6.12a for PCA).

The significant differences ( $P \leq 0.05$ ) between samples are illustrated in Tables 6.16 to 6.19. According to Tables 6.16 to 6.19 the herbaceous aroma drops significantly ( $P \leq 0.05$ ) between the base wine (0 ng/L) and the lowest concentration level of the respective haloanisoles; thereafter the herbaceous aroma does not decrease significantly ( $P > 0.05$ ) as the mid concentration levels of the respective haloanisoles increase. Again, the mouldy-like aroma increases significantly ( $P \leq 0.05$ ) between the base wine (0 ng/L) and lowest concentration

level of the respective haloanisoles, thereafter the mouldy-like aroma does not increase significantly ( $P>0.05$ ) as the mid concentration levels of the respective haloanisoles increase.

The DA plots for TCA (Figure 6.9b), TeCA (Figure 6.10b), TBA (Figure 6.11b) and PCA (Figure 6.12b) indicate that a good distinction was made between 0 ng/L (L0) and the highest concentration levels of the respective haloanisoles. Although the centroids illustrate some degree of order for the highest three concentrations, the panel had difficulty in discriminating between the low and mid concentrations.

In the principal component analysis (PCA) bi-plots a 100% of the variance is explained by F1 and F2, and more than 90% by F1 (Figure 6.9c for TCA; Figure 6.10c for TeCA; Figure 6.11c for TBA and Figure 6.12c for PCA). The scores for the base wine with no haloanisole added (0 ng/L; L0), as well as the scores for the highest level of spiking associated reasonably strongly within concentration level. They also lie on opposite sides of the bi-plot on F1 indicating a strong discrimination between the two extreme concentration levels. Herbaceous aroma correlates strongly with 0 ng/L (L0); and the mouldy-like aroma correlates strongly with the highest concentration level. Except for the latter two concentration ranges (L0 and highest concentration level), the respective scores of the mid concentration range lie scattered in the middle of the bi-plot for all four haloanisoles.

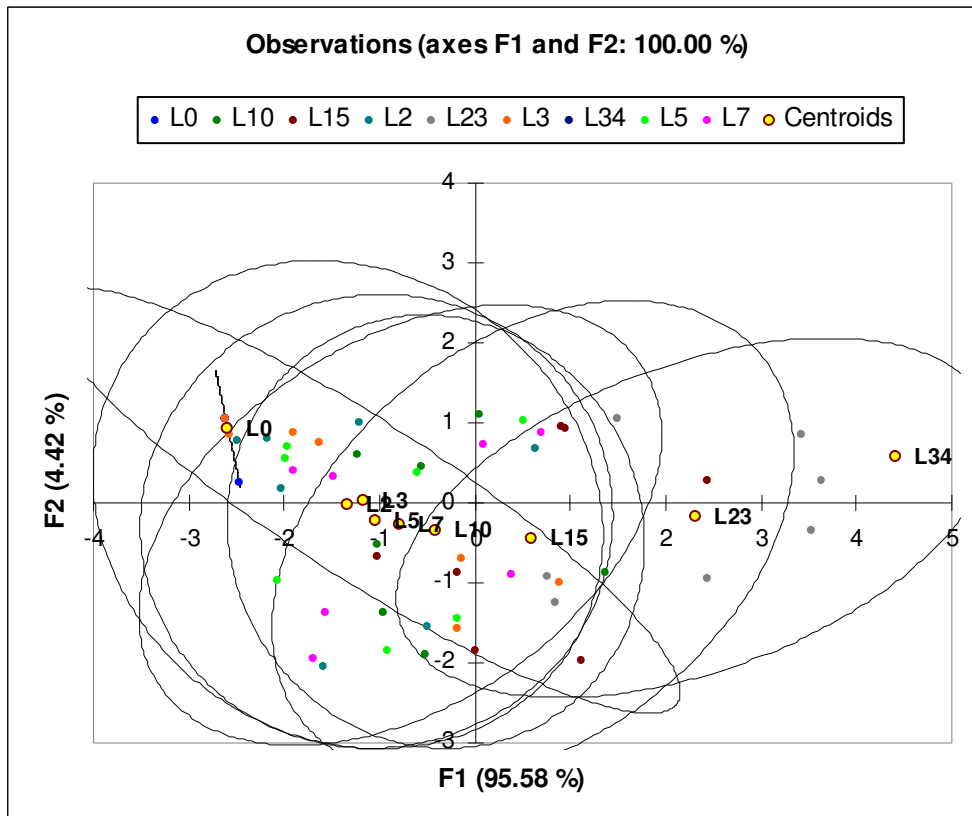


**Figure 6.9a** Mean intensity ratings of aroma attributes at increasing concentrations of TCA in Shiraz.

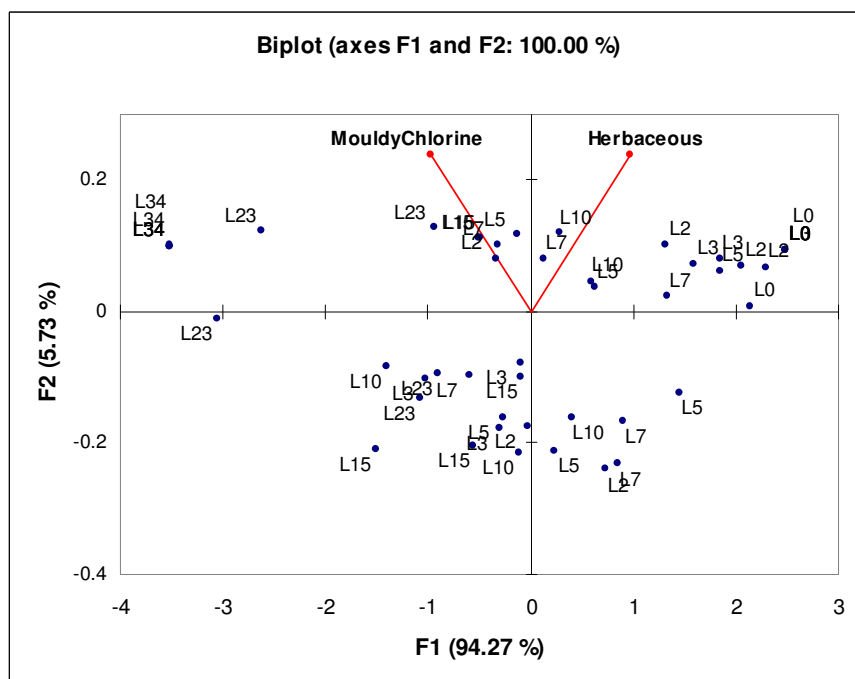
**Table 6.16** Significant differences between concentration levels of TCA in Shiraz as well as the LSD-values (P=0.05) for individual sensory attributes

Concentration (ng/L)	Herbacous aroma	Mouldy-Chlorine aroma
0	78.75 <sup>a</sup>	0.00 <sup>e</sup>
2	56.96 <sup>b</sup>	12.54 <sup>d</sup>
3	56.12 <sup>b</sup>	14.57 <sup>d</sup>
5	52.32 <sup>bc</sup>	15.57 <sup>d</sup>
7	49.40 <sup>bc</sup>	18.32 <sup>d</sup>
10	44.86 <sup>c</sup>	22.46 <sup>d</sup>
15	33.71 <sup>d</sup>	33.96 <sup>c</sup>
23	19.18 <sup>e</sup>	54.14 <sup>b</sup>
34	5.04 <sup>f</sup>	80.00 <sup>a</sup>
	LSD = 9.23	LSD = 9.97

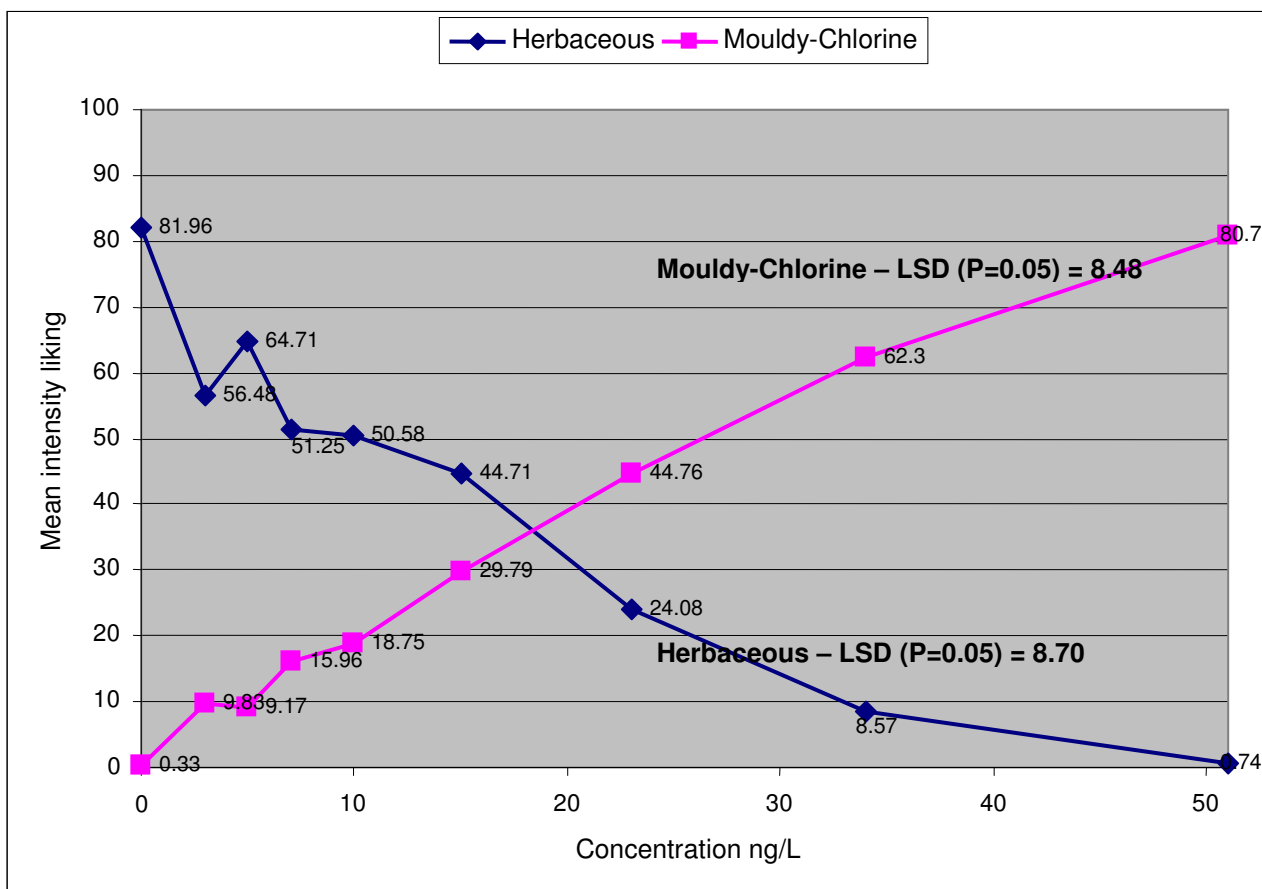
LSD = Least significant difference; Means with different superscripts in the same column, differ significantly at the 5% level.



**Figure 6.9b** Discriminant analysis (DA) plot for TCA in Shiraz ellipses around the centroids of the distinguishing groups.



**Figure 6.9c** Principal component analysis (PCA) bi-plot of TCA in Shiraz with levels (scores) and sensory attributes (loadings). Each level (specific concentration of TCA) is represented by six judges. Factor 1 and 2 explain 100% of the variance.

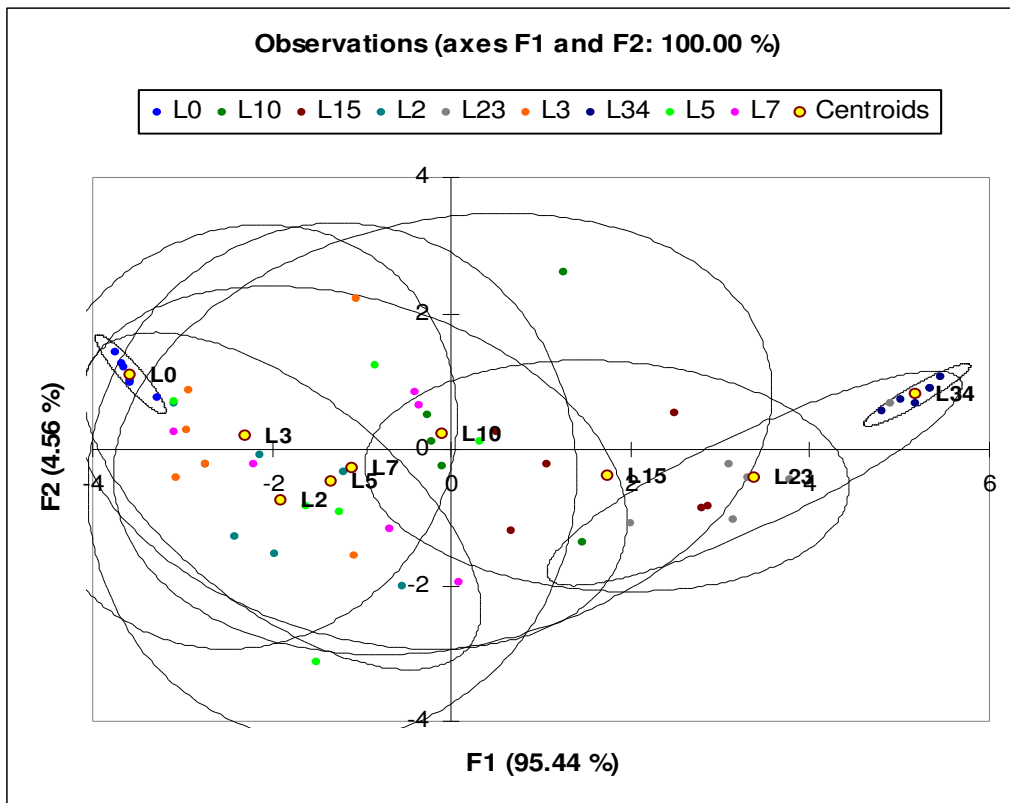


**Figure 6.10a** Mean intensity ratings of aroma attributes at increasing concentrations of TeCA in Shiraz.

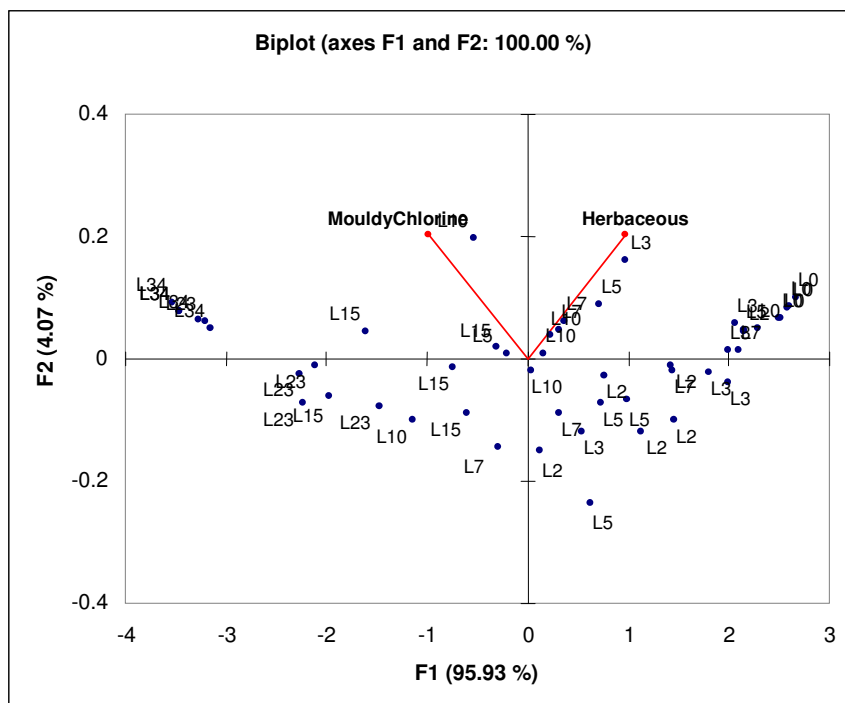
**Table 6.17** Significant differences between concentration levels of TeCA in Shiraz as well as the LSD-values (P=0.05) for individual sensory attributes

Concentration (ng/L)	Herbacous aroma	Mouldy-Chlorine aroma
0	81.96 <sup>a</sup>	0.33 <sup>g</sup>
3	56.48 <sup>bc</sup>	9.83 <sup>f</sup>
5	64.71 <sup>b</sup>	9.17 <sup>f</sup>
7	51.25 <sup>cd</sup>	15.96 <sup>ef</sup>
10	50.58 <sup>cd</sup>	18.75 <sup>e</sup>
15	44.71 <sup>d</sup>	29.79 <sup>d</sup>
23	24.08 <sup>e</sup>	44.76 <sup>d</sup>
34	8.57 <sup>f</sup>	62.30 <sup>b</sup>
51	0.74 <sup>f</sup>	80.70 <sup>a</sup>
	LSD = 8.70	LSD =8.48

LSD = Least significant difference; Means with different superscripts in the same column, differ significantly at the 5% level.

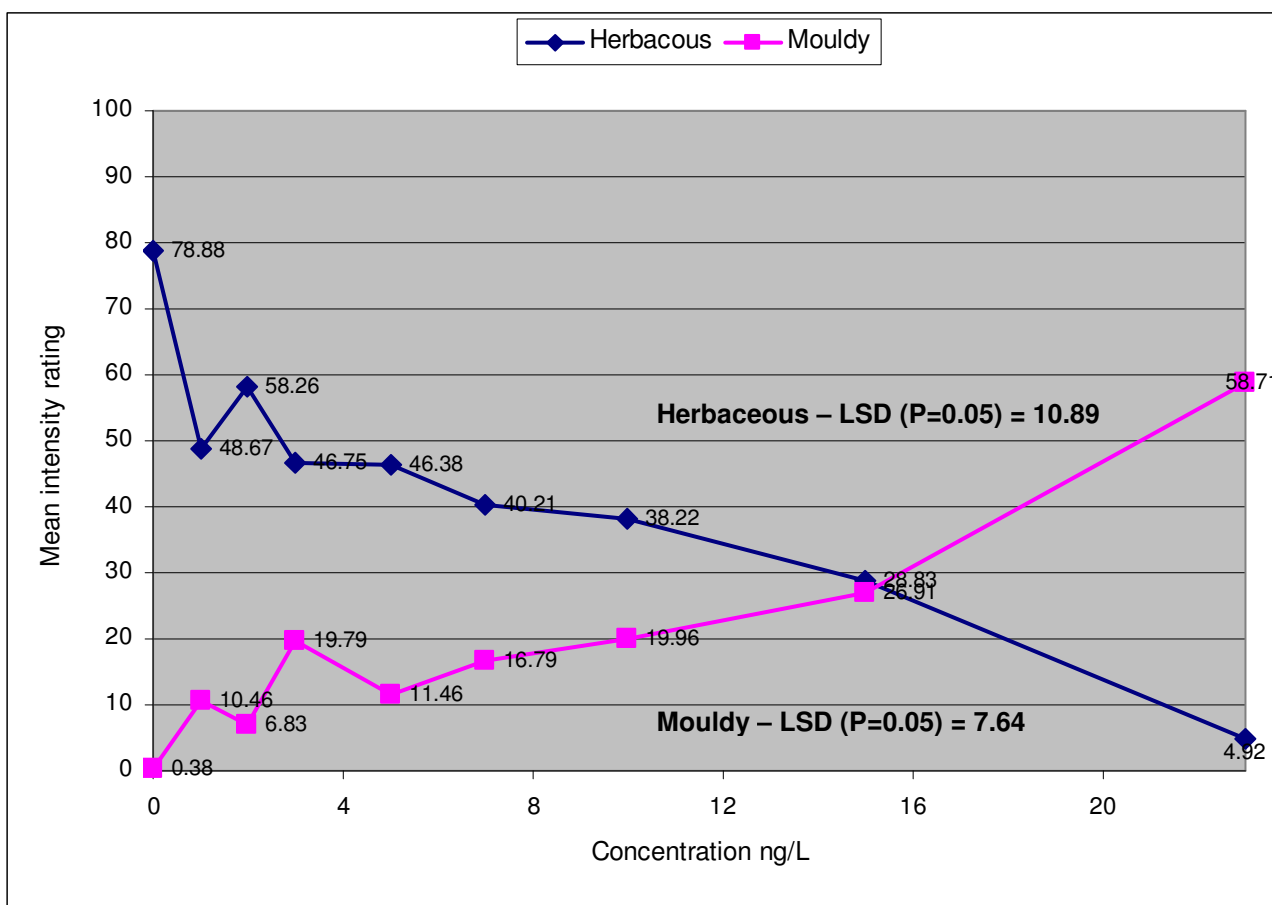


**Figure 6.10b** Discriminant analysis (DA) plot for TeCA in Shiraz with ellipses around the centroids of the distinguishing groups.



**Figure 6.10c** Principal component analysis (PCA) bi-plot of TeCA in Shiraz with levels (scores) and sensory attributes (loadings). Each level (specific concentration of TeCA) is represented by six judges. Factor 1 and 2 explain 100% of the variance.



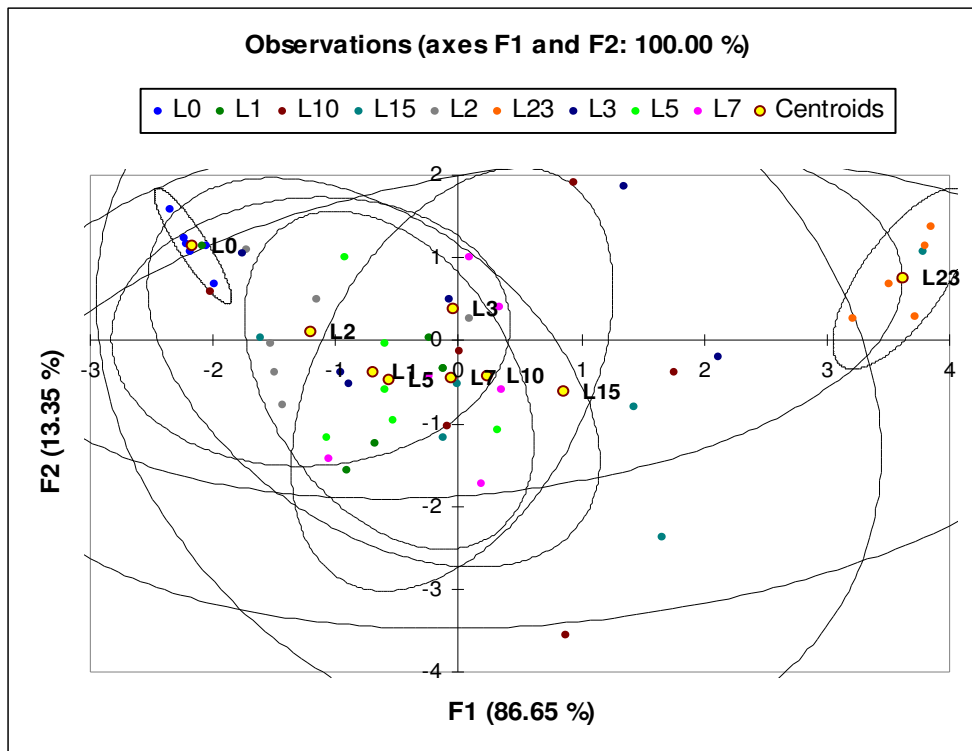


**Figure 6.11a** Mean intensity ratings of aroma attributes at increasing concentrations of TBA in Shiraz.

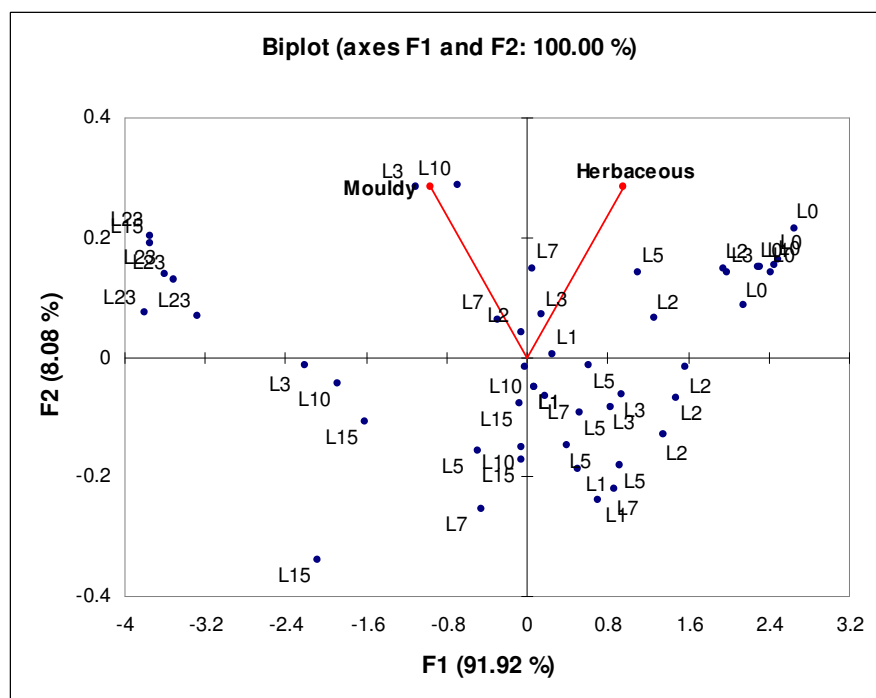
**Table 6.18** Significant differences between concentration levels of TBA in Shiraz as well as the LSD-values (P=0.05) for individual sensory attributes

Concentration (ng/L)	Herbaceous aroma	Mouldy aroma
0	78.88 <sup>a</sup>	0.38 <sup>f</sup>
1	48.67 <sup>bc</sup>	10.46 <sup>de</sup>
2	58.26 <sup>b</sup>	6.83 <sup>ef</sup>
3	46.75 <sup>c</sup>	19.79 <sup>bc</sup>
5	46.38 <sup>c</sup>	11.46 <sup>de</sup>
7	40.21 <sup>c</sup>	16.79 <sup>cd</sup>
10	38.22 <sup>cd</sup>	19.96 <sup>bc</sup>
15	28.83 <sup>cd</sup>	26.91 <sup>b</sup>
23	4.92 <sup>e</sup>	58.71 <sup>a</sup>
	LSD = 10.89	LSD = 7.64

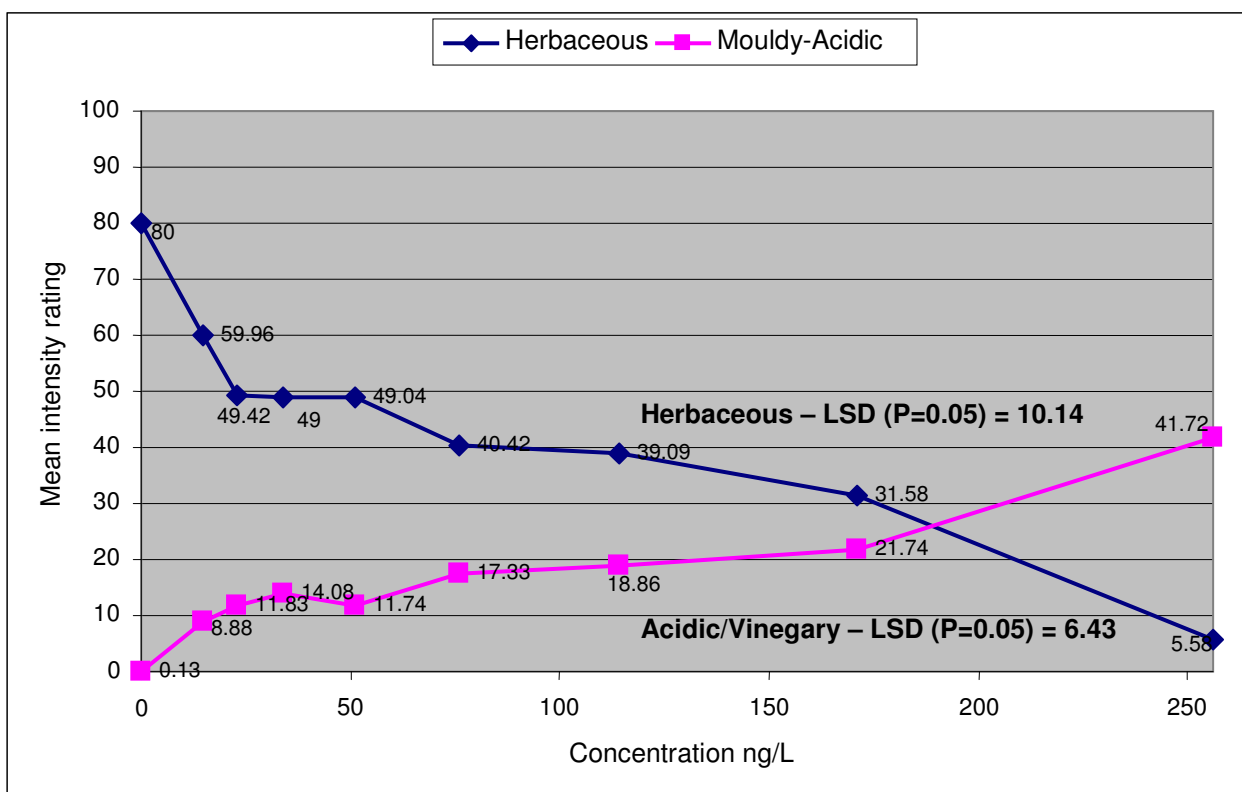
LSD = Least significant difference; Means with different superscripts in the same column, differ significantly at the 5% level.



**Figure 6.11b** Discriminant analysis (DA) plot for TBA in Shiraz with ellipses around the centroids of the distinguishing groups.



**Figure 6.11c** Principal component analysis (PCA) bi-plot of TBA in Shiraz with levels (scores) and sensory attributes (loadings). Each level (specific concentration of TBA) is represented by six judges. Factor 1 and 2 explain 100% of the variance.



**Figure 6.12a** Mean intensity ratings of aroma attributes at increasing concentrations of PCA in Shiraz.

**Table 6.19** Significant differences between concentration levels of PCA in Shiraz as well as the LSD-values (P=0.05) for individual sensory attributes

Concentration (ng/L)	Herbaceous aroma	Mouldy-Acidic aroma
0	80.00 <sup>a</sup>	0.13 <sup>f</sup>
15	59.96 <sup>b</sup>	8.88 <sup>e</sup>
23	49.42 <sup>c</sup>	11.83 <sup>de</sup>
34	49.00 <sup>cd</sup>	14.08 <sup>cde</sup>
51	49.04 <sup>cd</sup>	11.74 <sup>de</sup>
76	40.42 <sup>cde</sup>	17.33 <sup>bcd</sup>
114	39.09 <sup>de</sup>	18.86 <sup>cb</sup>
171	31.58 <sup>e</sup>	21.74 <sup>b</sup>
256	5.58 <sup>f</sup>	41.72 <sup>a</sup>
	LSD = 10.14	LSD = 6.43

LSD = Least significant difference; Means with different superscripts in the same column, differ significantly at the 5% level.



## Demographics of consumers testing wines spiked with TCA

Consumers drinking wine at least once a week were sourced to analyse the acceptability of Chenin blanc, Pinotage and Shiraz spiked with different levels of TCA. Each consumer received a subset of the respective concentrations (Table 6.7), therefore 50% of the consumers analysed each subset. In the analysis of Chenin blanc 23 male participants and 97 female participants partook in this experiment; in the analysis of Pinotage 29 of the participants were male and 81 were female and in the analysis of Shiraz 34 males and 83 females participated in the experiment (Table 6.20).

**Table 6.20** Number of consumers participating in the consumer analysis of the products

	Chenin blanc	Pinotage	Shiraz
Male (N)	23	29	34
Female (N)	97	81	83
Total (N)	120	110	117

## Consumer acceptability of Chenin blanc spiked with TCA

For the total group of consumers (N=120) there was a tendency for the acceptability to decrease as the concentration of TCA increased (Table 6.21; Figure 6.13). For this group of consumers only samples with a concentration level of 7.5 ng/L TCA and higher differed significantly ( $P \leq 0.05$ ) from samples containing 1.5, 3.5 and 5 ng/L of TCA (Table 6.21). This indicates that the samples containing 0 – 5 ng/L TCA are equally acceptable as a result of extremely low levels of TCA. There was a slight gender effect (Table 6.21). The mean scores of the female consumers (N=97) illustrated a similar pattern when compared to that of the total group of consumers. However, most of the mean acceptability scores for the male consumers (N=23) were higher when compared to that of the female consumers. Furthermore the mean scores of the male consumers followed no significant pattern with increasing levels of TCA (Table 6.21).

The three samples with 15 ng/L and more TCA had mean values of less than 5 indicating that they were regarded as unacceptable (Total group; Table 6.21). The rest of the samples (0 – 10 ng/L TCA) had mean scores resembling a reasonably acceptable product. The detection threshold (DT) level for TCA in Chenin blanc was 1.67 ng/L (Table 4.7; Chapter 4).

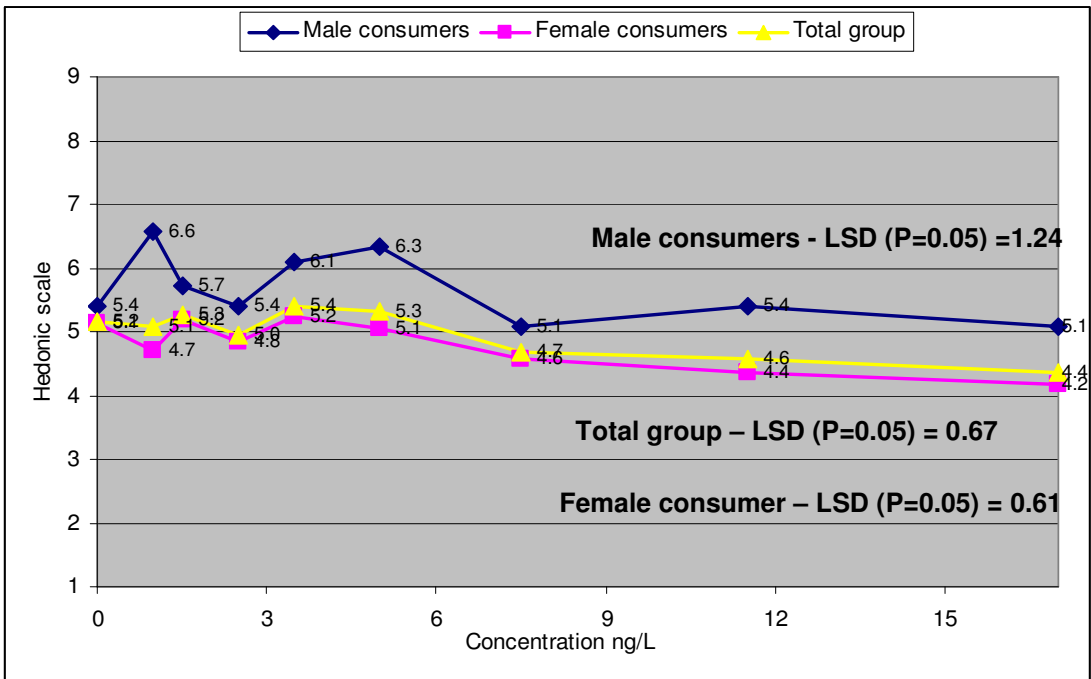
This illustrates that the DT level is much lower than the level where samples are regarded as not acceptable by a group of consumers.

The preference map in Figure 6.14 indicates the drivers of liking. This map shows that consumer liking of the total group (CP Total), as well as the female consumers (CP Female) tend to gravitate toward the lower concentrations of the TCA in the Chenin blanc which in turn are associated with the fruity aroma of Chenin blanc. Degree of liking in Chenin blanc is therefore directed away from the mouldy and sweet aroma which in turn are associated with the high concentrations of TCA. The males (CP Male) show a similar pattern but tend to gravitate more towards the lower, as well as the mid concentration levels of TCA.

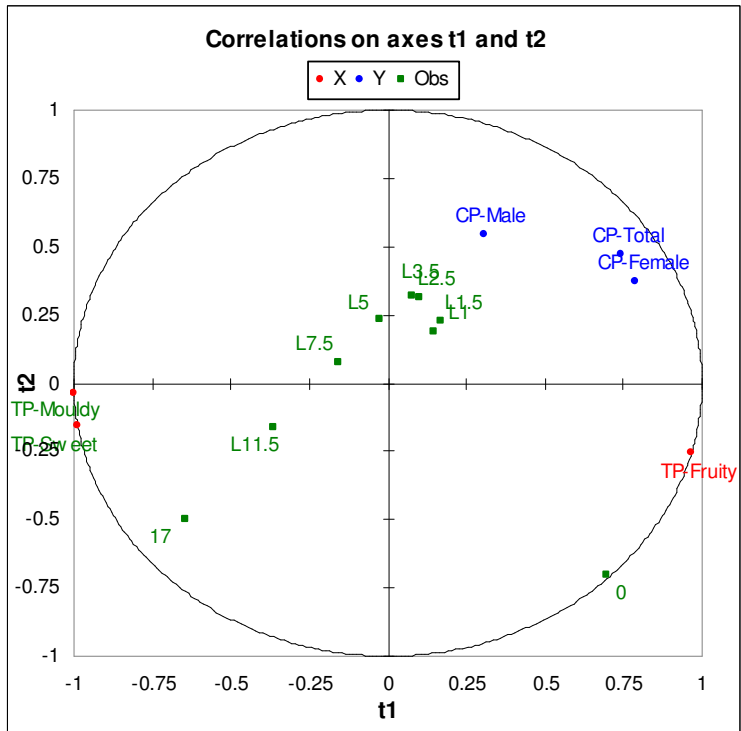
**Table 6.21** Means and LSD-values (P=0.05) for the degree of liking of Chenin blanc spiked with eight different concentration levels of TCA

<b>Concentration (ng/L)</b>	<b>Male consumers (N=23)</b>	<b>Female consumers (N=97)</b>	<b>Total group (N=120)</b>
0	5.4 <sup>ab</sup>	5.1 <sup>ab</sup>	5.2 <sup>ab</sup>
1	6.6 <sup>a</sup>	4.7 <sup>acd</sup>	5.1 <sup>abc</sup>
1.5	5.7 <sup>ab</sup>	5.2 <sup>a</sup>	5.3 <sup>a</sup>
2.5	5.4 <sup>ab</sup>	4.8 <sup>abc</sup>	5.0 <sup>abc</sup>
3.5	6.1 <sup>ab</sup>	5.2 <sup>a</sup>	5.4 <sup>a</sup>
5	6.3 <sup>a</sup>	5.1 <sup>a</sup>	5.3 <sup>a</sup>
7.5	5.1 <sup>b</sup>	4.6 <sup>bcd</sup>	4.7 <sup>bcd</sup>
11.5	5.4 <sup>ab</sup>	4.4 <sup>cd</sup>	4.6 <sup>cd</sup>
17	5.1 <sup>b</sup>	4.2 <sup>d</sup>	4.4 <sup>d</sup>
	LSD=1.24	LSD=0.61	LSD=0.67

LSD = Least significant difference; Means with different superscripts in the same column, differ significantly at the 5% level.



**Figure 6.13** Means for degree of liking of Chenin blanc spiked with different levels of TCA for the male, female and the total group of consumers (LSD at P=0.05 for Male consumers = 1.24; Female consumers = 0.61; Total Group = 0.67).



**Figure 6.14** External preference map indicating the degree of liking of the consumers in relation to the eight Chenin blanc samples spiked with TCA and the three sensory attributes (fruity, mouldy and sweet). The samples were spiked with 1 ng/L, 1.5 ng/L, 2.5 ng/L, 3.5 ng/L, 5 ng/L, 7.5 ng/L, 11.5 ng/L and 17 ng/L of TCA respectively. The map was obtained by using partial least square regression (PLS), where the consumer degree of liking (y-space) was regressed onto the sensory attributes (x-space).  $t_1$  indicates the first component and  $t_2$  indicates the second component (TP = Sensory attributes; CP Total, Female, Males = Consumer groups).

## Consumer acceptability of Pinotage spiked with TCA

For the total group of consumers (N=110) there was a tendency for the acceptability to decrease as the concentration of TCA in Pinotage increased (Table 6.22; Figure 6.15). For the total group of consumers the samples with concentration levels of 0, 2, 3, 5 and 7 ng/L TCA differed significantly ( $P \leq 0.05$ ) from the sample with 15 ng/L TCA (Table 6.22). Only the samples containing 15 and 34 ng/L of TCA had mean values of less than 5 indicating that they were regarded as unacceptable. The results of the female consumers (N=81) were reasonably similar to that of the total group. For the male consumers (N=29) there were no significant differences in degree of liking ( $P > 0.05$ ) and their mean scores for all the samples were slightly higher than that of the female consumers, especially for 10 and 15 ng/L (Figure 6.15). Only two samples had mean values of less than 5 indicating that they were regarded as unacceptable (Total group, Table 6.22). The rest of the samples spiked with TCA had mean values resembling a reasonably acceptable product. The DT level for TCA in Pinotage was 4.54 ng/L (Table 4.7; Chapter 4). This again illustrates that the DT level is much lower than the level where samples are regarded as not acceptable by a group of consumers.

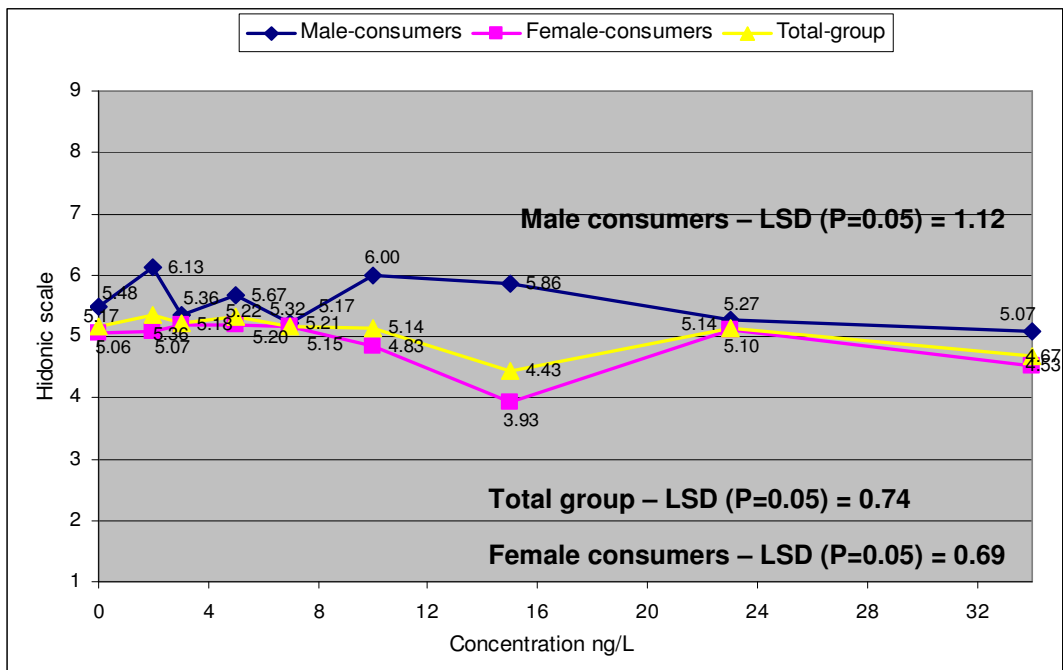
The preference map in Figure 6.16 indicates the drivers of liking for Pinotage spiked with TCA. This map shows that consumer liking of the total group (CP Total) gravitates toward the lower concentrations of the TCA in Pinotage which in turn are associated with the fruity aroma of Pinotage. Degree of liking in Pinotage is therefore directed away from the mouldy and sweet aroma of TCA which are again associated with the high concentrations of TCA.

**Table 6.22** Means and LSD-values ( $P=0.05$ ) for degree of liking of Pinotage spiked with eight different concentration levels of TCA

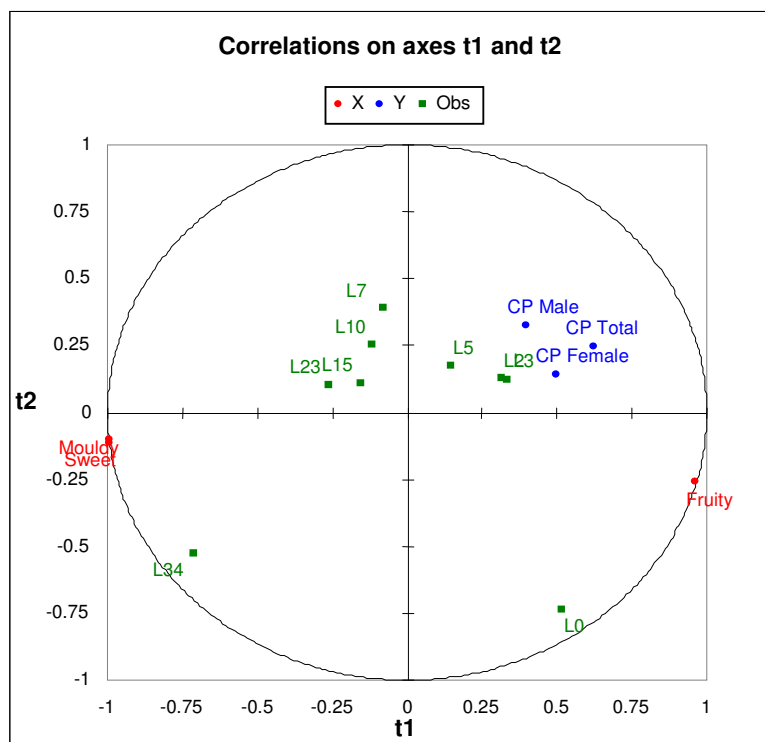
Concentration (ng/L)	Male consumers (N=29)	Female consumers (N=81)	Total group (N=110)
0	5.5 <sup>a</sup>	5.1 <sup>a</sup>	5.2 <sup>a</sup>
2	6.1 <sup>a</sup>	5.1 <sup>a</sup>	5.4 <sup>a</sup>
3	5.4 <sup>a</sup>	5.2 <sup>a</sup>	5.2 <sup>a</sup>
5	5.7 <sup>a</sup>	5.2 <sup>a</sup>	5.3 <sup>a</sup>
7	5.2 <sup>a</sup>	5.2 <sup>a</sup>	5.2 <sup>a</sup>
10	6.0 <sup>a</sup>	4.8 <sup>a</sup>	5.1 <sup>ab</sup>
15	5.9 <sup>a</sup>	3.9 <sup>b</sup>	4.4 <sup>b</sup>
23	5.3 <sup>a</sup>	5.1 <sup>a</sup>	5.1 <sup>ab</sup>
34	5.1 <sup>a</sup>	4.5 <sup>ab</sup>	4.7 <sup>ab</sup>
	LSD=1.12	LSD=0.69	LSD=0.74

LSD = Least significant difference; Means with different superscripts in the same column, differ significantly at the 5% level.





**Figure 6.15** Means for degree of liking of Pinotage spiked with different levels of TCA for the male, female and the total group of consumers (LSD at P=0.05 for Male consumers = 1.12; Female consumers = 0.69; Total Group = 0.74).



**Figure 6.16** External preference map indicating the degree of liking of the consumers in relation to the eight Pinotage samples spiked with TCA and the three sensory attributes (fruity, mouldy and sweet). The samples were spiked with with 2 ng/L, 3 ng/L, 5 ng/L, 7 ng/L, 10 ng/L, 15 ng/L, 23 ng/L and 34 ng/L of TCA respectively. The map was obtained by using partial least square regression (PLS), where the consumer degree of liking (y-space) was regressed onto the sensory attributes (x-space).  $t_1$  indicates the first component and  $t_2$  indicates the second component (TP = Sensory attributes; CP Total, Female, Males = Consumer groups).

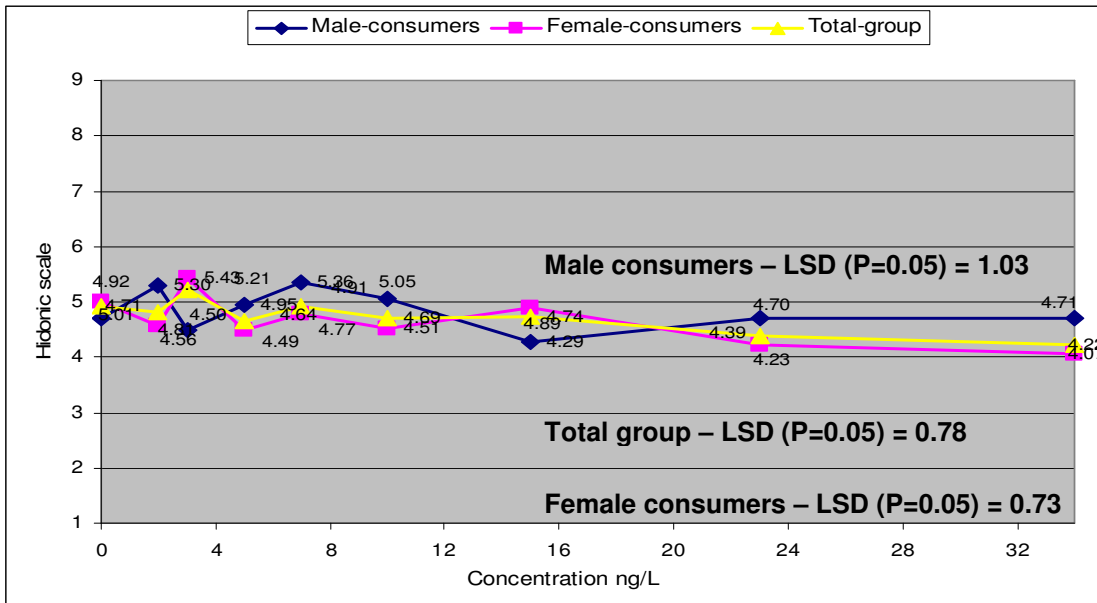
## Consumer acceptability of Shiraz spiked with TCA

For the total group of consumers (N=117) there was a tendency for the acceptability to decrease slightly as the concentration of TCA in Shiraz increased (Table 6.23; Figure 6.17). For the total group of consumers the sample with a concentration level 3 ng/L TCA differed significantly ( $P \leq 0.05$ ) from the samples with 23 and 34 ng/L of TCA (Table 6.23). The total group (Table 6.23) scored only one sample higher than 5, the rest of the samples all had mean acceptability scores of less than 5 indicating that they tend to be unacceptable. The latter results indicate that the consumers did not like any of the Shiraz samples, whether they were tainted or not. The fact that this Shiraz wine was slightly wooded and was only matured for 8 months could have impacted negatively on the acceptability of all the samples. The preference map in Figure 6.18 indicates the drivers of liking for Shiraz spiked with TCA. This map shows that consumer liking of the total group (CP Total) gravitates toward the lower concentrations of the TCA in Shiraz which in turn is associated with the herbaceous aroma of Shiraz. Degree of liking in Shiraz is therefore directed away from the mouldy-chlorine aroma of TCA which is associated with the high concentrations of TCA. Figure 6.18 indicates that the overall degree of liking of the female consumers (CP Female) for the samples was reasonably similar to that of the total group. The degree of liking of the male consumers (CP Male) gravitated more to the samples with low levels of TCA, specifically the sample with 7 ng/L of TCA (Figure 6.17).

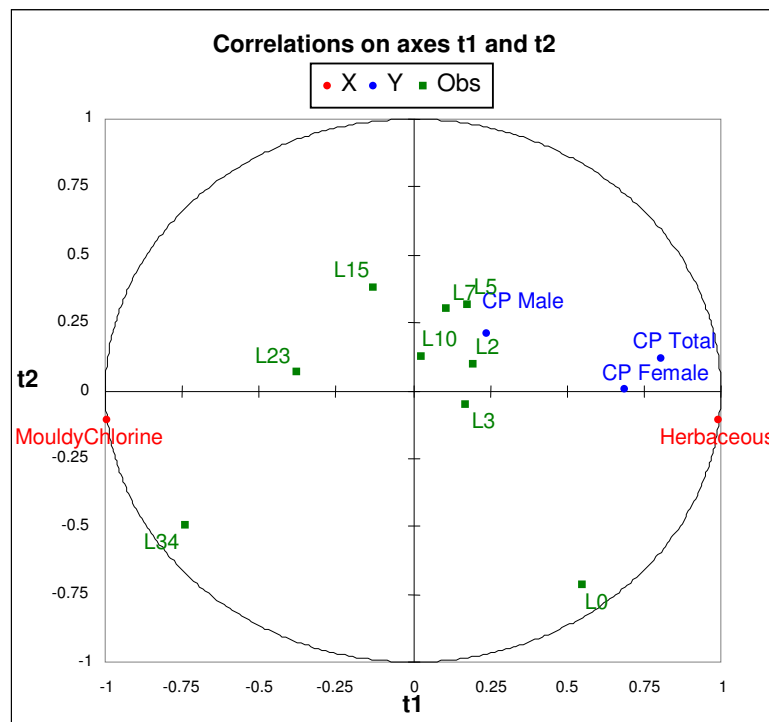
**Table 6.23** Means and LSD-values ( $P=0.05$ ) for degree of liking of Shiraz spiked with eight different concentration levels of TCA

Concentration (ng/L)	Male consumers (N=34)	Female consumers (N=83)	Total group (N=117)
0	4.71 <sup>ab</sup>	5.01 <sup>ab</sup>	4.92 <sup>ab</sup>
2	5.30 <sup>ab</sup>	4.56 <sup>bcd</sup>	4.81 <sup>ab</sup>
3	4.50 <sup>ab</sup>	5.43 <sup>a</sup>	5.21 <sup>a</sup>
5	4.95 <sup>ab</sup>	4.49 <sup>bcd</sup>	4.64 <sup>ab</sup>
7	5.36 <sup>a</sup>	4.77 <sup>abcd</sup>	4.91 <sup>ab</sup>
10	5.05 <sup>ab</sup>	4.51 <sup>bcd</sup>	4.69 <sup>ab</sup>
15	4.29 <sup>b</sup>	4.89 <sup>abc</sup>	4.74 <sup>ba</sup>
23	4.7 <sup>ab</sup>	4.23 <sup>cd</sup>	4.39 <sup>b</sup>
34	4.71 <sup>ab</sup>	4.07 <sup>d</sup>	4.22 <sup>b</sup>
	LSD=1.03	LSD=0.73	LSD=0.78

LSD = Least significant difference; Means with different superscripts in the same column, differ significantly at the 5% level.



**Figure 6.17** Means for degree of liking of Shiraz spiked with different levels of TCA for the male, female and the total group of consumers (LSD at P=0.05 for Male consumers = 1.03 ; Female consumers = 0.73; Total Group = 0.78).



**Figure 6.18** External preference map indicating the degree of liking of the consumers in relation to the eight Shiraz samples spiked with TCA and the three sensory attributes (herbaceous, mouldy-chlorine). The samples were spiked with 2 ng/L, 3 ng/L, 5 ng/L, 7 ng/L, 10 ng/L, 15 ng/L, 23 ng/L and 34 ng/L of TCA respectively. The map was obtained by using partial least square regression (PLS), where the consumer degree of liking (y-space) was regressed onto the sensory attributes (x-space).  $t_1$  indicates the first component and  $t_2$  indicates the second component (TP = Sensory attributes; CP Total, Female, Males = Consumer groups).

## DISCUSSION OF RESULTS

### Descriptive sensory analysis of wines spiked with TCA, TeCA, TBA and PCA

All the wines were profiled using descriptive sensory analysis and line plots were used to illustrate the change in aroma with increasing concentration levels of the respective haloanisoles, namely TCA, TeCA, TBA and PCA (Figures 6.1a - 6.4a for Chenin blanc; Figures 6.5a - 6.8a for Pinotage; Figures 6.9a - 6.12a for Shiraz). The Chenin blanc and Pinotage base wines were profiled as having an extremely fruity aroma and the Shiraz base wine had a strong herbaceous character. According to Jackson (2008) the latter sensory attributes are typical of the respective wines.

When spiked with the lowest concentration levels of the four haloanisoles, the fruitiness of the Chenin blanc and Pinotage and the herbaceous character of the Shiraz diminished significantly ( $P \leq 0.05$ ) (Sefton & Simpson, 2005). At the DT level of the respective haloanisoles the fruitiness of the Chenin blanc was approximately 50 to 60% less, and that of Pinotage and Shiraz approximately 40 to 50% less. At the highest concentration level the fruitiness was almost non-existent in all the wine x compound combinations. Our results are substantiated by Sefton and Simpson (2005), as well as Chatonnet *et al.* (2004), Prak *et al.* (2007) and Silva Pereira *et al.* (2000).

When spiked with the four haloanisoles a mouldy-like character developed in all the wines (Figures 6.1a - 6.4a for Chenin blanc; Figures 6.5a - 6.8a for Pinotage; Figures 6.9a - 6.12a for Shiraz). In the Chenin blanc and Pinotage it was profiled as mouldy, however, the Pinotage x PCA combination was characterised as having a mouldy-chemical aroma. The Shiraz spiked with TCA or TeCA developed a mouldy-chlorine aroma, with TBA a mouldy aroma and with PCA a mouldy-acidic aroma (Sefton & Simpson, 2005; Juanola *et al.*, 2004). The mouldy-like aroma increased significantly ( $P \leq 0.05$ ) in all three wines when spiked with the lowest concentration levels of the respective haloanisoles. At the DT level of all the compounds the mouldy-like aroma of Chenin blanc was 40 to 50% higher, and that of Pinotage and Shiraz increased by 20 to 30% (Prak *et al.*, 2007). At the highest concentration level the mouldy-like aroma was extremely high for all the wine x TCA combinations (Juanola *et al.*, 2004), fairly high for the wine x TeCA/TBA combinations and moderately high for the wine x PCA combinations (Insa *et al.*, 2005; Sefton & Simpson, 2005; Silva Pereira *et al.*, 2000; Whitfield *et al.*, 1997). Therefore, in the mid concentration ranges (Levels 3-5) the mouldy-like aroma increased marginally, but at the high concentration levels (Levels 6 – 8) the increase was substantial and more often than significant ( $P \leq 0.05$ ).

When spiking Chenin blanc and Pinotage with TCA, TeCA and TBA a strong sweet-associated aroma also developed with increasing concentrations of these compounds. This sweet-associated aroma is definitely not the result of a chemical reaction. Possibly the fruity aroma was being masked by the mouldy-like aroma and consequently the natural sweet aroma of the wine became more prominent (Le Berre *et al.*, 2007; Selfridge & Amerine, 1978).

The DA plots (Figures 6.1b - 6.4b for Chenin blanc; Figures 6.5b - 6.8b for Pinotage; Figures 6.9b - 6.12b for Shiraz) indicated that a good distinction was made between 0 ng/L (L0) and the highest concentration level of all the compounds. The panel could, however, not discriminate clearly between different samples in the low and mid concentration ranges (especially between the 3<sup>rd</sup> and 5<sup>th</sup> concentration levels). The principal component analysis (PCA) bi-plots (Figures 6.1c - 6.4c for Chenin blanc; Figures 6.5c - 6.8c for Pinotage; Figures 6.9c - 6.12c for Shiraz) confirmed the findings of the DA plots on the degree of discrimination between the different concentrations. The PCA bi-plots also indicated that the fruity aroma of the Chenin blanc and Pinotage, as well as the herbaceous aroma of Shiraz correlated strongly with the lowest concentration level (0 ng/L); and that the mouldy-like aroma correlated strongly with the highest concentration level the respective compounds (Juanola *et al.*, 2004). These two main aroma attributes were therefore inversely correlated on first factor (F1), the latter factor explaining more than 90% of the variance (Johansson *et al.*, 1999; Rødbotten *et al.*, 2004).

### **Consumer sensory analysis of wines spiked with TCA**

For the total group of consumers there was a tendency for the acceptability of the tainted wines to decrease as the concentration of TCA increased (Figure 6.13 for Chenin blanc; Figure 6.15 for Pinotage; Figure 6.17 for Shiraz).

The preference maps (Figure 6.14 for Chenin blanc; Figure 6.16 for Pinotage; Figure 6.18 for Shiraz) indicate the drivers of liking of the tainted samples. These maps showed that consumer liking of the total group (CP Total) gravitated toward the lower concentrations of the TCA in Chenin blanc, Pinotage and Shiraz which in turn are associated with the fruity aroma of Chenin blanc (Figure 6.14) and Pinotage (Figure 6.16) and the herbaceous aroma of Shiraz (Figure 6.18). Degree of liking of these wines was therefore directed away from the mouldy-like aroma which is associated with the high concentrations of TCA (Mazzoleni & Maggi, 2007). It is therefore clear that this group of consumers found the wine samples

tainted with low levels of haloanisoles not totally unacceptable. Prescott *et al.* (2005) and Teixeira *et al.* (2006) both found that the consumer rejection levels of tainted wines by consumers were marginally higher than the detection threshold values of corresponding taint compounds. In our study a slight gender effect was observed. Figures 6.14 and 6.18 indicated that the overall degree of liking of the female consumers (CP Female) was reasonably similar to that of the total group. The degree of liking of the male consumers (CP Male) gravitated more towards the samples tainted with low levels of TCA. Male consumers appear to like wines to be strong and robust and are therefore possibly not put off by low levels of TCA. Frewer and Van Trijp (2007) indicated similar results for various products, i.e. that male consumers tend to favour products with stronger flavours.

## **CONCLUSIONS**

The original sensory character of Chenin blanc, Pinotage and Shiraz was changed significantly when either TCA, TeCA, TBA or PCA was added to the base wine. The lowest concentration level of the latter compounds (near detection threshold level; Chapter 4) resulted in a substantial and immediate decrease of the natural aroma attributes of the respective wines, namely fruitiness of Chenin blanc and Pinotage and the natural herbaceous character of Shiraz. The most drastic effects were noticed for the compounds TCA, TeCA and TBA and to a much lesser extent for PCA. In the mid concentration ranges (Concentration levels 3-5) the decrease in characteristic aroma was less dramatic, but at high concentrations (Concentration levels 6-8) a more dramatic effect in the latter aroma profiles were noticed. The opposite is true for the characteristic aromas associated with cork taint.

The DA-plots indicated that the trained panelists could discriminate effectively between the lowest and the highest concentration levels of haloanisoles. However, at the lower and mid concentration levels the panel members illustrated difficulty in discriminating effectively, especially between the third and fifth concentration levels.

The consumer tests for TCA in Chenin blanc and Pinotage revealed similar results. The latter being, the consumers were not sensitive to low concentrations of TCA and rejected the TCA tainted Chenin blanc and Pinotage wines at concentration levels higher than the corresponding detection thresholds. However, at higher concentrations most of the consumers rejected the TCA tainted Chenin blanc and Pinotage point-blank. In the case of the Shiraz consumers were not sensitive to the increasing concentration levels of TCA, and therefore the wines were not rejected progressively more as the concentration of TCA

increased. In comparison to the females, the male consumers illustrated a lesser rejection of the tainted wines. The reason for this is not clear, however, the assumption can be made that male consumers possibly favour wines with a strong flavour profile and that they were therefore not influenced by the addition of increasing, but low, levels of TCA.

The preference maps demonstrate the above-mentioned tendencies effectively. Multivariate analysis of sensory and consumer data is effective in illustrating the main drivers of liking in the respective wines, and more specifically the rejection of the tainted wine samples.

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## Chapter 7

### General discussion and conclusions

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Worldwide it has been shown that cork taint not only led to considerable financial losses in the wine industry but also tarnished the reputation of several wines and producers. This has most likely been the driving force behind major research efforts in this field. The aims of most of these research efforts have been primarily focused on the determination of threshold levels, mainly for 2,4,6-trichloroanisole (TCA), as well as the incidence of cork taint in wine. The latter studies were mainly directed at the wine industries of Australia and the United States of America (Juanola *et al.*, 2004; Pollnitz *et al.*, 1996). Limited research has furthermore been conducted on the consumer perception of cork tainted wines, as well as on sensory profiling of such wines (Prescott *et al.*, 2005).

The occurrence of cork taint in the South African wine industry has for some time been identified as a significant concern but, despite this, scientifically not highly documented. The need for a study focusing on the incidence of cork taint in South African wines has therefore been recognised. Similarly, as a result of highly contradicting reports in literature, it was necessary to determine detection threshold values of the relevant haloanisoles, i.e. TCA, 2,3,4,6-tetrachloroanisole (TeCA), 2,4,6-tribromoanisole (TBA) and pentachloroanisole (PCA) in different wines. Lastly the sensory aroma profile of Chenin blanc, Pinotage and Shiraz spiked with different known levels of TCA, TeCA, TBA and PCA was determined together with the consumer rejection level of these three wines.

For the determination of the incidence of cork taint, wines from different wineries in the Western Cape region, South Africa, were analysed for anisole content using gas chromatography (GC) coupled to electron capture detection (ECD). TCA was found to be the most prevalent cork taint compound present in 3.8% of the wines at concentrations higher than detection threshold values. As all these wines were sealed with corks it was concluded that TCA contamination is very likely to originate from the usage of corks. These results are confirmed by findings of Sefton and Simpson (2005). In our study, the use of synthetic closures did not seem to have any significant effect on haloanisole contamination. It should be noted, however, that the wines sealed with corks were significantly more in number than those sealed with synthetic closures and therefore this finding cannot be considered conclusive. A small group of wines were subjected to certification prior to this

investigation and the certification panel rejected several of the wines on the basis of having a perceivable mouldy taint. Our results revealed that most of the latter wines were indeed tainted with noteworthy levels of TCA as determined by GC-ECD analysis. Interestingly, a small number of the rejected wines contained no haloanisoles. This indicates that compounds other than haloanisoles must have been responsible for the perceived mouldy taint. It can thus be concluded that while TCA can be recognised as the main contributor to cork taint in wines, a mouldy wine character is not always exclusively due to the presence of haloanisoles. This indicates the need for further research to establish the cause of such taints.

As discussed in the Literature Review (Chapter 2), there is large variability in reported detection threshold values for TCA, TeCA, TBA and PCA in wine. The factors known to have a substantial effect on detection threshold values of haloanisoles in a wine matrix are the presence of other wine compounds, cultivars, wine styles, wines made from the different batches of grapes, etc. (Sefton & Simpson, 2005). The detection threshold values determined for TCA, TeCA, TBA and PCA in this investigation (Chapter 4) coincide with that reported in literature (Amon, 1989; Sanvicens *et al.*, 2003; Sefton & Simpson, 2005). Due to the fact that factors such as cultivar play a vital role in the determination of threshold levels, our research has suggested that a range of detection for a specific haloanisole, and not only an average detection threshold value, need to be established. Martineau *et al.* (1995) also concluded that different factors have an important effect on threshold levels, invalidating the use of a single threshold. To obtain valid and reliable results in determining threshold levels in this study we ensured the use of a reliable panel with extensive training in cork taint-related aromas (Chapter 5), as well as an established method such as the standard ASTM-method for the determination of odour thresholds (ASTM, 1997).

Descriptive sensory analysis using trained panels (Chapter 6) revealed that the original sensory character and profile of Chenin blanc, Pinotage and Shiraz changed significantly when either TCA, TeCA, TBA or PCA were added to the base wines. It was shown that low concentration levels (even below threshold level) influenced the natural character of the wine and this became, as expected, more pronounced as the concentrations increased. The most drastic effects were noticed for the compounds TCA, TeCA and TBA and to a lesser extent for PCA. The presence of TCA, TeCA and TBA resulted in a *mouldy* aroma in Chenin blanc and Pinotage. In Shiraz TCA and TeCA resulted in a *mouldy-chlorine* aroma; and TBA in a *mouldy* aroma. PCA, however, had a *mouldy, sweet* aroma in Chenin blanc, a *mouldy-chemical* aroma in Pinotage and a *mouldy-acidic* aroma in Shiraz. These results highlight the

large influence that the background matrix of the wine can have on the sensory profile and is a valuable extension to the existing scientific knowledge.

Consumer rejection levels of TCA-tainted wines were also determined (Chapter 6). In the case of Chenin blanc and Pinotage consumers were not sensitive to low concentrations of TCA and rejected the tainted wines only at concentrations higher than the detection threshold. However, in Shiraz consumers were affected to a lesser extent by increasing concentrations of TCA and only at levels well above the detection level, consumer rejection was indicated. A slight gender effect was also observed. Most of the male consumers rejected the TCA-tainted wines at higher concentrations than the female consumers. This could be as a result of a gender difference in sensitivity for TCA, or the assumption can be made that male consumers possibly favour wines with a stronger flavour profile and are therefore not easily influenced by above-threshold levels of TCA.

Although descriptive sensory analysis is a powerful tool in describing the sensory profile of a product, especially when the data are analysed with multivariate techniques, it is suggested that more descriptors need to be formulated when profiling cork tainted wines. This will give rise to a more complete sensory profile and result in closer and more significant associations in PCA bi-plots (principal component analysis), as well as in external preference maps.

In conclusion, the findings of this study add to the understanding of the problem of cork taint, as well as to the existing knowledge-base. As there are still many questions to be answered this field, ongoing research is of the utmost importance.

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