The effect of region, yeast strain and ascorbic acid on the development of a sulphur-like aroma and on Sauvignon blanc wine quality

by

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any other university for a degree.

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ABSTRACT

Highly valued Sauvignon blanc wines, with the distinctive cultivar-typical aromas, reminiscent of grassy, green pepper or asparagus-like, are produced in some South African regions. Quite often, however, neutral and sulphur-like, low quality Sauvignon blanc wines are produced and this phenomenon is of great concern to wine producers and consumers, and affects our competition on overseas markets, negatively.

The aim of this study was to investigate the effect of region, ascorbic acid/SO_2 treatments and yeast strain on Sauvignon blanc wine aroma and quality. Wines were produced from grapes obtained from the warmer Robertson and the relatively cooler Stellenbosch regions (1998 season). The juices were treated with different combinations of ascorbic acid/SO_2 treatments [commercially available ascorbic acid/meta preparate, SO_2 (control), pure ascorbic acid/SO_2] and *Saccharomyces cerevisiae* yeast strains (Vin 13, VL3C, NT 116). The wines were analysed for esters, higher alcohols, monoterpenes and 2-methoxy-3-isobutylpyrazine (ibMP). The wines were also sensorially evaluated for wine aroma intensities (fruity/ester, sulphur-like, grassy/green pepper) and overall quality. Additionally, the synergistic action of ibMP and the sulphur-containing component, 4-mercapto-4-methylpentan-2-one (MMP), considered to be the most important impact components of Sauvignon blanc, was studied. The two components were added, separately and in combinations at increasing concentrations, to different media. The nuances perceived, varied from dusty, grassy to green pepper for ibMP and from guava, sulphur-like to cat urine or "conifer" for MMP.

Significant differences were observed between the wines treated with the different combinations of ascorbic acid/SO_2 treatments and fermented with different yeast strains, irrespective of region. The highest quality, cultivar-typical Sauvignon blanc wines were produced from the pure ascorbic acid/SO_2 treatment in combination with yeast strains Vin 13 and NT 116. This coincided with high ester and low higher alcohol concentrations, which did not overpower the typical Sauvignon blanc character. The treatments had, in some cases, a significant effect on monoterpene levels, but it was concluded that these differences were not big enough to affect wine quality. Levels of ibMP were too low and could not be reliably measured. Low

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quality wines, with prominent, undesirable sulphur-like aromas, were produced from juices, treated with the commercially available ascorbic acid/meta preparate and the French yeast strain, VL3C.

Techniques, followed to identify the aroma components causing the sulphur-like off-flavours, as MMP or as other sulphur-containing components, were gas chromatography/mass spectrometry, solid phase microextraction and sniffing. However, these tests were not successful and studies to identify these off-flavours should be continued.

It was succeeded in this study to produce Sauvignon blanc wines without the undesirable, sulphur-like aromas. Although this investigation showed that a newly developed, commercially available ascorbic acid/meta preparate did not yield any sulphur-like off-flavours, quite often Sauvignon blanc wines with such off-flavours are still produced. Further research is needed to clarify this phenomenon.

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UITTREKSEL

Hoë kwaliteit Sauvignon blanc wyne, met kenmerkende kultivar-tipiese, gras-, groenrissie- of aspersie-agtige aromas, word in sekere streke van Suid Afrika geproduseer. Die gereelde produksie van neutrale, en swawelagtige lae kwaliteit Sauvignon blanc wyne, wek egter nie net groot kommer by wynprodusente en verbruikers nie, maar het ook 'n negatiewe impak op kompetisie met oorsese markte.

Die doel van hierdie studie was dus om die effek van streek, askorbiensuur/SO_2 behandelings en gisras op Sauvignon blanc wynaroma en kwaliteit vas te stel. Wyne is berei met druiwe wat verkry is van die warmer Roberston en relatief koeler Stellenbosch streke (1998 seisoen). Verskillende kombinasies askorbiensuur/SO_2 behandelings [kommersieel-beskikbare askorbiensuur/meta preparaat, SO_2 (kontrole), suiwer askorbiensuur/SO_2] en *Saccharomyces cerevisiae* gisrasse (Vin 13, VL3C, NT 116) is gebruik tydens die wynbereidingsproses. Spesifieke ester-, hoër alkohol-, monoterpeen- en 2-metoksi-3-isobutiel metoksipirasienkonsentrasies (ibMP) is in die wyne bepaal. Die wyne is ook sensories vir wynaroma intensiteite (vrugtig/ester, swawelagtig, gras/groenrissie-agtig) en algehele kwaliteit geëvalueer. Die sinergistiese aksie van ibMP en die swawelbevattende komponent, 4-merkapto-4-metielpenta-2-oon (MMP), wat beskou word as die belangrikste impakkomponente in Sauvignon blanc, is addisioneel bestudeer. Hierdie komponente is in toenemende konsentrasies, individueel en in kombinasies, tot verskillende media gevoeg. Die nuanses wat waargeneem is, het van stowwerig, gras-, groenrissie-agtig vir ibMP, tot koejawelagtig, swawelagtig, katurine, konifeer vir MMP, gevarieer.

Ongeag streke, is betekenisvolle verskille tussen die wyne wat berei is met die verskillende kombinasies van askorbiensuur/SO_2 behandelings en gisrasse, waargeneem. Hoër kwaliteit, kultivar-tipiese Sauvignon blanc wyne is berei met suiwer askorbiensuur/SO_2 in kombinasie met gisras Vin 13 of NT 116. Alhoewel die hoë ester- en lae hoër alkohol- konsentrasies, hierdie resultate bevestig het, is die tipiese Sauvignon blanc karakter nie hierdeur oorheers nie. Sommige behandelings het wel 'n betekenisvolle invloed of monoterpeenkonsentrasies gehad het, maar was te min om 'n effek op wynkwaliteit uit te oefen nie. Die konsentrasievlakke van ibMP was te laag om te meet. Lae kwaliteit wyne met prominente ongewensde,

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swawelagtige aromas, het egter voorgekom in wyne wat met die kommersieel-beskikbare askorbiensuur/meta preparaat en die Franse gisras, VL3C, berei is.

Verskeie tegnieke soos gaschromatografie/massa spektrometrie, soliede fase mikro-ekstraksie en "sniffing", is gebruik om die komponent, MMP, wat moontlik verantwoordelik vir hierdie ongewensde swawelagtige wangeure was, te identifiseer. Die identifikasie hiervan was egter onsuksesvol, en verdere studies is nodig om die komponent(e) verantwoordelik vir hierdie wangeure, te identifiseer.

Die suksesvolle produksie van Sauvignon blanc, sonder ongewensde swawelagtig aromas, was wel moontlik. Alhoewel hierdie studie ook duidelik getoon het dat daar geen swawelagtige wangeure in die wyne wat met die nuut ontwikkelde, kommersieel-beskikbare askorbiensuur/meta preparaat berei was, voorgekom het nie, vind die produksie van Sauvignon blanc wyne met van hierdie wangeure nog steeds plaas. Verdere navorsing rakende hierdie aspek is nodig.

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This thesis is dedicated to the memory of my friend

- **Elize le Roux**

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

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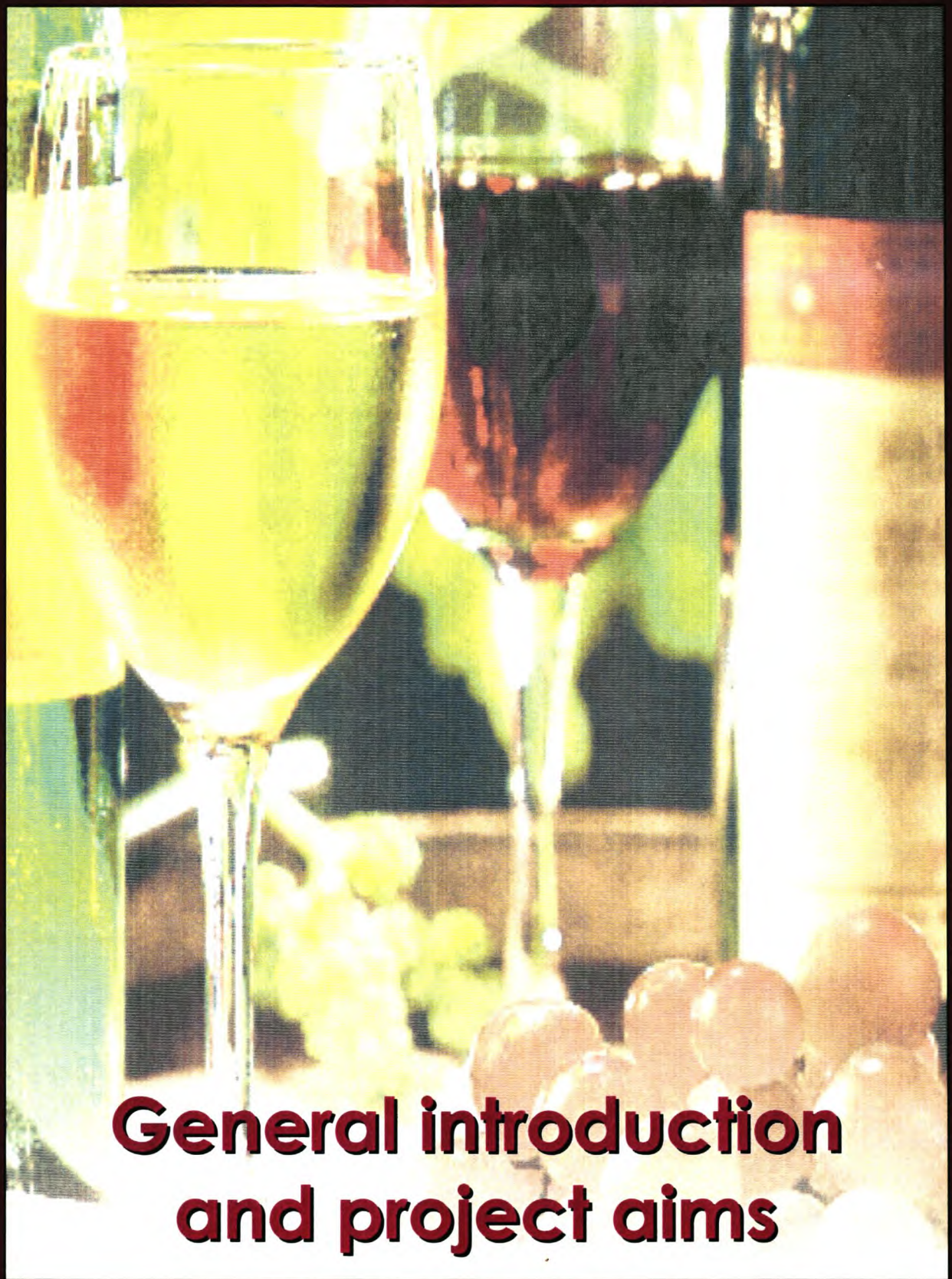
CHAPTER 1



General introduction and project aims

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CHAPTER 1

GENERAL INTRODUCTION AND PROJECT AIMS

1. 1 INTRODUCTION

Vitis vinifera L. cv. Sauvignon blanc is one of the cultivars which is extensively cultivated in many of the important wine producing countries of the world. The distinctive and easily distinguishable aroma of Sauvignon blanc makes the wines produced from these grapes popular and in demand with producers and consumers.

Sauvignon blanc wine cultivar, well-known for its dry and sweet wines originated in the Bordeaux region of France (Robinson, 1994). Although Sauvignon blanc grapes are mainly used to produce typical cultivar wines, it is also used in blends with other cultivars, like Semillon and Muscadelle (Lichine, 1976). The well-known and famous wines from the Bordeaux region, the sweet Sauterne and dry Graves, are produced from such blends. Besides the Bordeaux region, Sauvignon blanc is also grown in the Loire Valley in France, where typical cultivar wines like the Sancerre and the Pouilly-Fumé are produced.

With the emphasis on quality products and because of the distinctive aroma of Sauvignon blanc, it is considered to be one of the most important wine cultivars in the South African wine industry together with others, like Chenin blanc, Chardonnay, Pinotage, Shiraz, Merlot and Cabernet Sauvignon. As a result of the increasing popularity and competition with overseas wine producing areas like Italy, Argentine, California, Australia and New Zealand, the demand for Sauvignon blanc wine is still increasing. This is demonstrated by the increase from 0.4% in 1981 to 4.9% in 1999 of Sauvignon blanc planted of the total wineproducing areas in South Africa (Anon., 1999). Of the seven local wine producing areas in South Africa, five of these regions regard Sauvignon blanc as the most important quality white wine cultivar, with the highest percentage (11.6%) production in the Stellenbosch area. The production of Sauvignon blanc wine in South Africa is estimated to increase to twenty-nine million litres by the year 2000 (Booysen, 1996).

Extensive research has been done on the identification of aroma components of Sauvignon blanc wines and on various factors which can affect the development of the aroma of this cultivar. Wines often possess aromas characteristic of grass, green pepper, asparagus, blackcurrant and box tree, and are easily distinguished from

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other wine cultivars. Methoxypyrazines, especially 2-methoxy-3-isobutylpyrazine (ibMP), 2-methoxy-3-isopropylpyrazine (ipMP) and 2-methoxy-3-sec-butylpyrazine (sbMP) were identified as those chemical components, mainly responsible for the typical grassy, green pepper nuances in the cultivar character of Sauvignon blanc wine (Augustyn *et al.*, 1982; Allen & Lacey, 1993). However, other chemical components, like monoterpenes, C_{13}-norisoprenoids and a number of sulphur-containing components such as, 4-mercapto-4-methylpentan-2-one (MMP) also contribute to the varietal character and overall quality of Sauvignon blanc wine.

Typical Sauvignon blanc wines are produced locally, however, most of the rest lack in cultivar-typical character and often possess a neutral character. Changes in the quality and a decrease in cultivar character of Sauvignon blanc wines often occur during ageing and storing of wines. Apart from the lack in varietal aroma, South African Sauvignon blanc wines also often possess undesirable sulphur-like aromas. The presence of an important sulphur-containing component, MMP, which derives from a precursor in the grapes during fermentation, is mainly responsible for an aroma, which can manifest as box tree or cat urine nuances (Dubourdieu *et al.*, 1993; Darriet *et al.*, 1995). A number of other equally important sulphur-containing components may also be involved (Tominaga *et al.*, 1996; Tominaga *et al.*, 1998). Most of the local wine producers and consumers regard these aromas, when in too high concentrations, as negative and not typical of Sauvignon blanc wine. It is therefore of great importance to investigate the influence of, amongst others, MMP on the quality of South African Sauvignon blanc wines.

Investigations done on Sauvignon blanc grapes and wines, showed by the manipulation of different climatic conditions and viticultural practices, that more typical and complex Sauvignon blanc wines can be produced (Allen & Lacey, 1993; Marais *et al.*, 1999). The aim of this study was firstly to determine the effect of region, ascorbic acid and yeast strain on the composition and quality of locally produced Sauvignon blanc wines. A further aim was to apply this knowledge in order to produce more cultivar-typical Sauvignon blanc wines without negative sulphur-like aromas. Additionally, attempts were made to identify these sulphur-like aromas.

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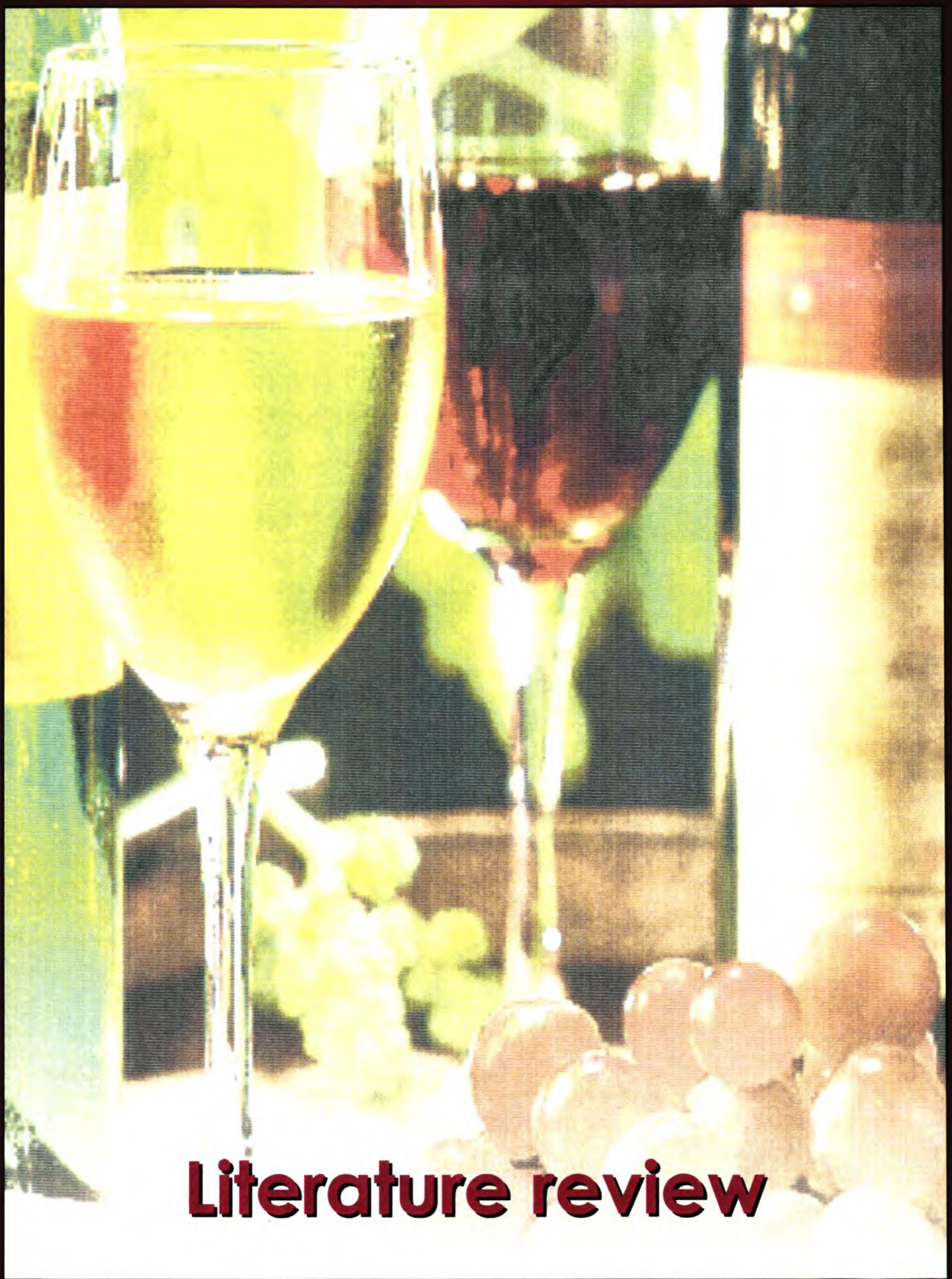
CHAPTER 2



Literature review

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CHAPTER 2

LITERATURE REVIEW

1. GENERAL ASPECTS OF FLAVOUR

1.1 General

Our senses of taste and smell enable us to enjoy food and drink. Flavour can be defined as, the sensation produced by a material taken in the mouth, and principally perceived by the senses of taste and smell, and also by the general pain, tactile and temperature receptors in the mouth (Hall, 1968). Based on oral contact and observation, flavour denotes the sum of the characteristics of the material that produces the sensation. Nature and experience enable us to associate "pleasurable, good or characteristically" tastes and odours of food. Any off-flavours or deviation of specific flavours in food and drinks, are regarded as not typical and are often stumbling blocks in the utilisation of these foods.

1.2 Nature of stimuli

Tastes and odours are stimuli and humans react to them. A stimulus can be defined as any chemical or physical activator, which causes a response in a receptor cell (American Society for Testing and Materials, 1968). The receptor for each of our senses is specialised to receive a specific stimulus. Odorous molecules adsorb on the surface of the olfactory receptors (Beidler, 1966). Nerve impulses travel from the receptors to the brain for interpretation of different dimensions of sensations, like quality, intensity, extension, duration, like and dislike.

The presence and development of certain flavours and aromas, and their impact on the final quality of Sauvignon blanc wines were investigated in this study. Flavours or aromas usually are derived as secondary stimuli from the simultaneous stimulation and interaction of senses like taste, smell, audition, etc., whereas the primary stimulus is usually visual. A specific concentration of odourant, above its theshold value, in the vapour phase is needed to initiate an odour response. This concentration at which the odour of a substance is noticeable or produces sensations like vision, smell, taste, etc., is called a threshold value (Jackson, 1994). The identity

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and intensity of aroma components vary according to the ability to detect them and explains therefore the wide range of olfactory thresholds and the different aromas, which a human can simultaneously perceived.

1.3 Chemical aspects of flavour

Flavour research provides the necessary knowledge and answers, not only to identify and determine the threshold values of aromas, but also helps in intensifying preference flavours or preventing the development of off-flavours. The isolation and identification of a large variety of volatile aroma components were made possible by the following aspects (Teranischi *et al.*, 1971):

i) Man's sensitivity in detecting odours.
ii) Diversity of compounds that may contribute to the aroma impact.
iii) Objective sensory evaluation methods.
iv) Variation in olfactory thresholds of aroma components.

The structure of an odorous molecule determines the chemical and physiological properties of an aroma. The attributes of aroma components are complementary to the size, shape and electronic status of the receptor site (Beidler, 1966). On the contrary, similar chemical structures may possess different aromas, or very different structures can possess similar aromas (Teranishi *et al.*, 1971). Of all the stereoisomers of menthol, it is only menthol itself which possesses the peppermint flavour, whereas the other iso-, neo- and isoneomenthol isomers all have musty nuances. All the volatile aroma components present in a complex spectrum of odours, despite of their low volatility, play an important role in the final aroma or sensation perceived. Advances in technology and instrumentation, like gas chromatography, mass spectrometry and nuclear magnetic resonance spectroscopy make it possible to identify new aromas, their chemical structures, and their developing mechanisms in the study of flavour of food and beverages.

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2. PROPERTIES OF SAUVIGNON BLANC WINE AROMA

The flavour and/or aroma of wine is/are one of the most important aspects of quality. Without any flavour or aroma, wine like other food and drink, would be tasteless. During the last decade or two, extensive investigations were done on the aroma of various wine cultivars. One of these cultivars, *Vitis vinifera* L. cv. Sauvignon blanc, has grown in popularity because of the characteristic aroma and high quality wines this cultivar is known for. However, different components affect this characteristic aroma and often lead to disagreement among wine producers and consumers in defining the typical characteristic aroma of Sauvignon blanc wine.

2.1 Origin and cultivation of Sauvignon blanc

Sauvignon blanc originated from the regions of Sauterne and Sancerre in Bordeaux, France (Robinson, 1994). Different kinds of dry and sweet wines, also used in blends with other cultivars, like Semillon and Muscadelle, are produced from grapes of this cultivar (Lichine, 1976). The well-known and very popular sweet Sauterne and dry Graves from France are also produced from Sauvignon blanc grapes. In the Loire Valley in France where Sauvignon Jaune, Surin, Blanc Fumè, synonyms for Sauvignon blanc, are still cultivated, typical cultivar wines like the Sancerre and the Pouilly-Fumé are produced. Other wine producing countries in the southern hemisphere, like Australia, New Zealand and South Africa, also cultivate Sauvignon blanc. Wines of this cultivar often display different aroma intensities, because of different terroir influences and oenological practices (Allen *et al.*, 1988; Marais *et al.*, 1999). More asparagus/green pepper-like Sauvignon blanc wines are produced in the cooler areas, like the Marlborough region of New Zealand, whereas the more tropical fruit-like wines are produced in the warmer regions of Australia.

The characteristic aroma of Sauvignon blanc make the wines of this cultivar easily distinguishable from other white wine cultivars. Sauvignon blanc is regarded as one of the noble white wine cultivars of South Africa and is planted in most of the wine producing regions, like Robertson (region IV), Stellenbosch (region III) and Elgin (region II). Based on the Winkler (1974) system, the wine producing regions of South Africa were classified according to degree days by Le Roux (1974) (**Figure 1**).

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Sauvignon blanc is becoming an increasingly important cultivar in South Africa as is confirmed by the increase from 2.4% in 1985 to 4.9% in 1998 of area planted (Anon., 1999). Extensive plantings of Sauvignon blanc are still under way and estimations done on the wine production of the noble cultivars in South Africa by Booysen *et al.* (1996) and Truter (1998), shows an annual increase towards the year 2003. Sauvignon blanc wine production is expected to increase from 27 million litres in 1999 to 33.4 million litres in 2003 (Truter, 1998).

2.2 Varietal aroma of Sauvignon blanc

Sauvignon blanc wines offer characteristics of moderate acidity, near dryness and clarity of flavour. The relative high intensities and distinctive aromas of Sauvignon blanc are the main reasons for the popularity of this cultivar. Green, vegetative, grassy, herbaceous, peppery, green pepper, asparagus, dried fig or gooseberry, are aromas found in typical Sauvignon blanc (Allen *et al.*, 1991, Lacey *et al.*, 1991, Darriet *et al.*, 1991). However, the majority of Sauvignon blanc wines produced in South African are lacking in cultivar character and tend to have a more neutral character. Cat urine or sulphur-like aroma are also sometimes used to described Sauvignon blanc wine aroma, and often lead to controversy between local wine producers and consumers whether these characteristics are negative or not.

The development and presence of certain aroma components as the result of metabolic activities or pathways in grapes, plays an important role in the complexity of Sauvignon blanc aroma. The complex aroma of Sauvignon blanc mainly consists of aroma components, i.e. methoxypyrazines, norisoprenoids, monoterpenes, esters and higher alcohols, that are formed from these metabolic reactions, as well as activities of certain enzymes. The presence of these aroma components is determined by certain climatic, viticultural and oenological influences (Marais *et al.*, 1996).

These conditions, as well as chemical changes during wine making, and ageing of wine, contribute to the character of the wine. Although some aroma components are present in very low concentrations, together with a large variety of other aroma components, most contribute to the substantial aroma and overall quality of the wine. Sensory and chemical analyses are still increasingly used to better understand the distinctive flavour of Sauvignon blanc and the development thereof.

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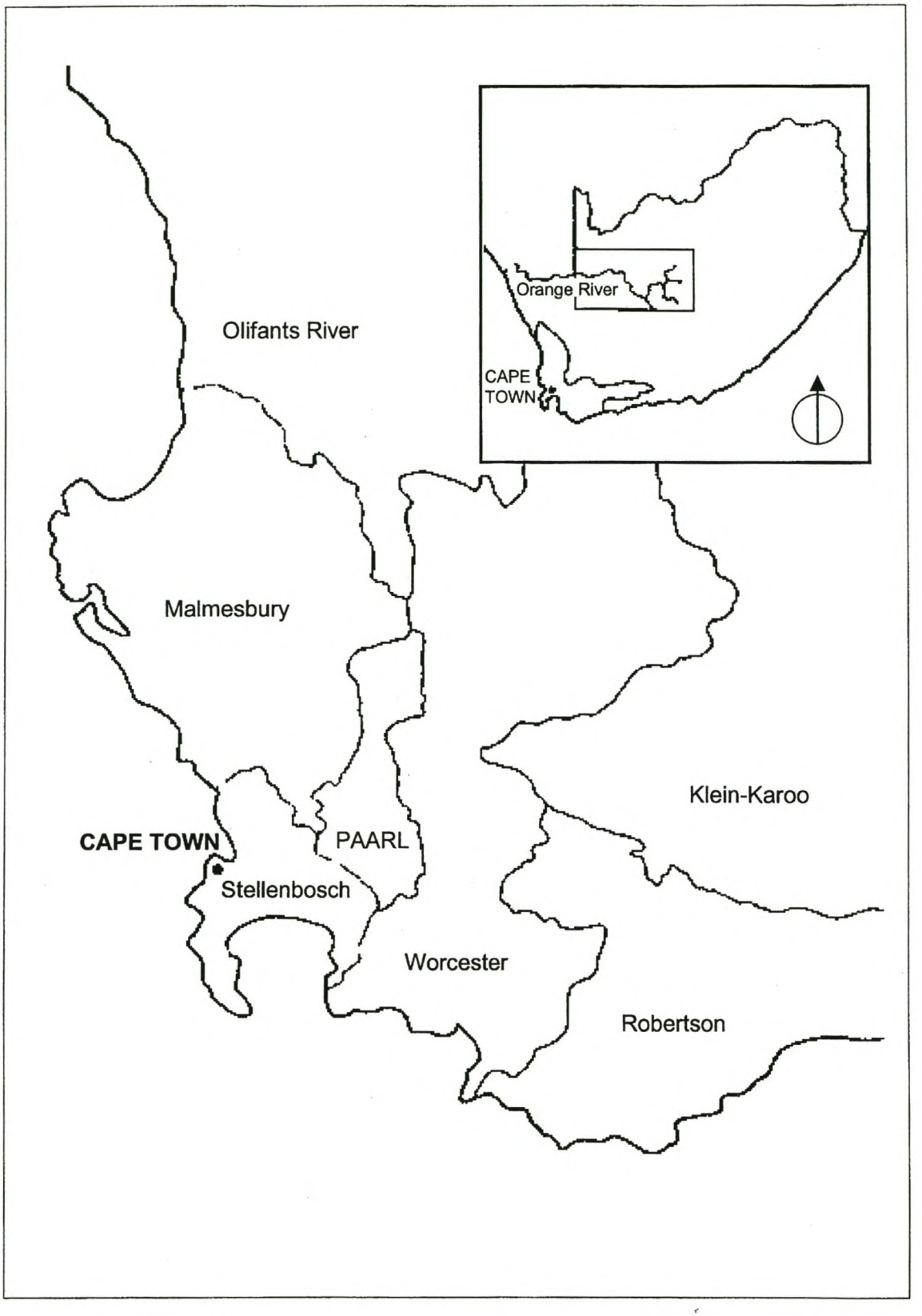

Figure 1. Wine regions of South Africa on which KWV statistics are based (Anon., 1999).

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Volatile metabolites are found in low concentrations in grapes, and are usually present in much higher concentrations than the flavourless glycoconjugates in the bound form and are therefore contribute to the typical aromas of Sauvignon blanc (Sefton *et al.*, 1994). The conjugates however, release additional volatile components by acid and/or glucosidase enzyme hydrolysis during vinification and storage of wines, and aromas like tea, toasty, honey, oak, pineapple, lime, floral and talc could develop.

3. AROMA COMPONENTS OF SAUVIGNON BLANC

3.1 Methoxypyrazines

The characteristic aroma of Sauvignon blanc can be described as grassy, green, vegetative, black currant, gooseberry, green pepper or asparagus-like (Lacey *et al.*, 1991; Allen & Lacey, 1993). These aromas are not unique to Sauvignon blanc, but are also used to describe aromas of other cultivars, like Cabernet Sauvignon and Semillon. Sauvignon blanc, classified as one of the aromatic cultivars, has a pronounced aroma profile. This aroma profile consists of various chemical components of which methoxypyrazines are considered to be the most important impact components (**Figure 2**) (Allen *et al.*, 1988; Allen *et al.*, 1991; Lacey *et al.*, 1991).

Methoxypyrazines are commonly found in vegetables, like green peas, green peppers, potatoes and beetroot, as well as in the skins of grapes. These aroma components are nitrogen-containing ring structures and the product of amino acid catabolism (Murray & Whitfield, 1975). The exact metabolic pathway(s) in the formation of these components in Sauvignon blanc grapes is unknown (Rizzi, 1988). Different methoxypyrazines, like 2-methoxy-3-isobutylpyrazine (ibMP), 2-methoxy-3-isopropylpyrazine (ipMP) and 2-methoxy-3-sec-butylpyrazine (sbMP) were identified, of which ibMP is more commonly found in the grapes of Sauvignon blanc (Allen *et al.*, 1988; Allen *et al.*, 1991; Lacey *et al.*, 1991). These methoxypyrazines differ in aroma nuances from the green pepper character of ibMP to the asparagus-like aroma of ipMP (Allen *et al.*, 1988; Lacey *et al.*, 1991). Other nuances, like tomato leaf, boxwood and black currant can also sometimes be detected, especially during the fermentation of Sauvignon blanc must (Buttery *et al.*, 1969; Sefton *et al.*, 1994). The

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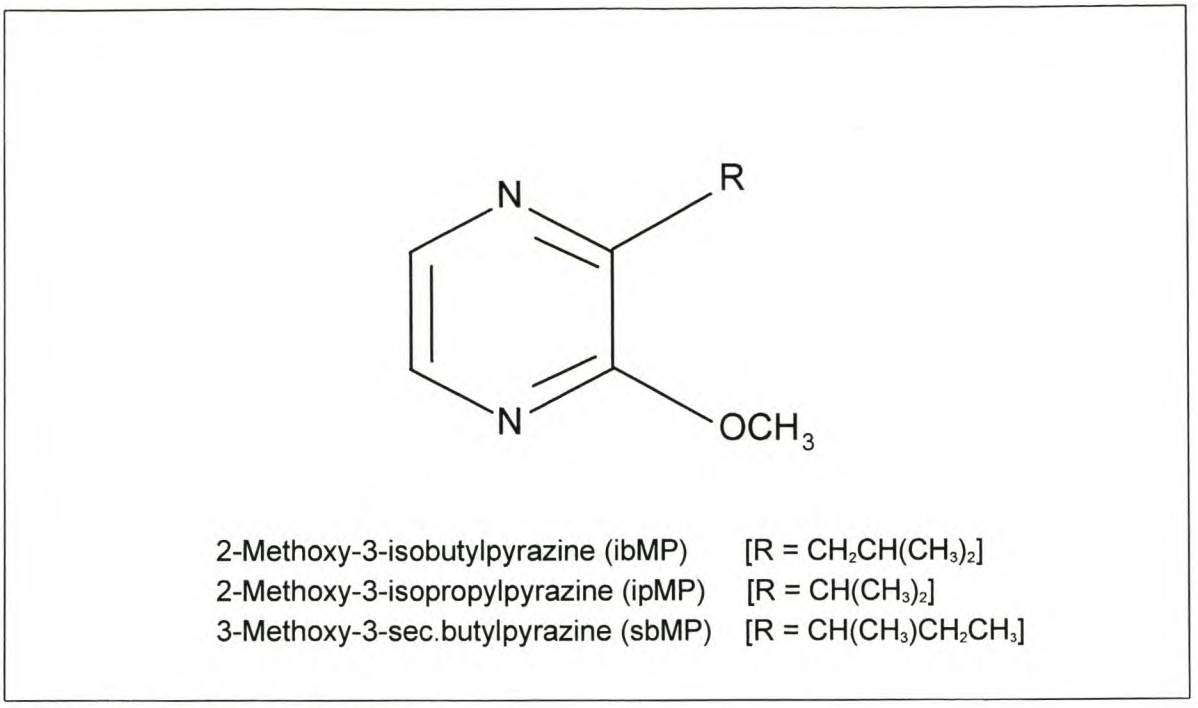

2-Methoxy-3-isobutylpyrazine (ibMP) [R = $CH_2CH(CH_3)_2$]
2-Methoxy-3-isopropylpyrazine (ipMP) [R = $CH(CH_3)_2$]
3-Methoxy-3-sec.butylpyrazine (sbMP) [R = $CH(CH_3)CH_2CH_3$]

Figure 2. Methoxypyrazines.

threshold level of ibMP in water is 2 ng. l^{-1}. The occurrence of methoxypyrazines in concentrations above their threshold levels is responsible for the distinctive aroma of Sauvignon blanc grapes and wine. However, the contribution of not only methoxypyrazines, but also that of other chemical components, like monoterpenes, norisoprenoids and sulphur-like components adds to the complexity of Sauvignon blanc aroma (Marais, 1983; Sefton *et al.*, 1994; Marais, 1996).

3.2 Monoterpenes

The grape-derived monoterpenes, in the free or bound form, are important constituents of grape and wine flavour (Williams *et al.*, 1982; Harris *et al.*, 1987). These volatile aroma components are used to differentiate between cultivar clones (Marais & Rapp, 1991). Of the more than 60 terpenes identified in grapes and wines, linalool and geraniol are considered to be some of the most important and commonly found constituents in aromatic wines (**Figure 3**) (Marais, 1983).

Monoterpenes have potent floral, rose-like (geraniol and nerol), coriander (linalool), green (nerol oxide) and herbaceous aromas, and are common constituents of flowers and fruit-like grapes. Free monoterpenes derive from glycosidic precursors by means of enzymatic reactions or from the hydrolyses of the free odourless bound terpenes (polyols) (Williams *et al.*, 1980).

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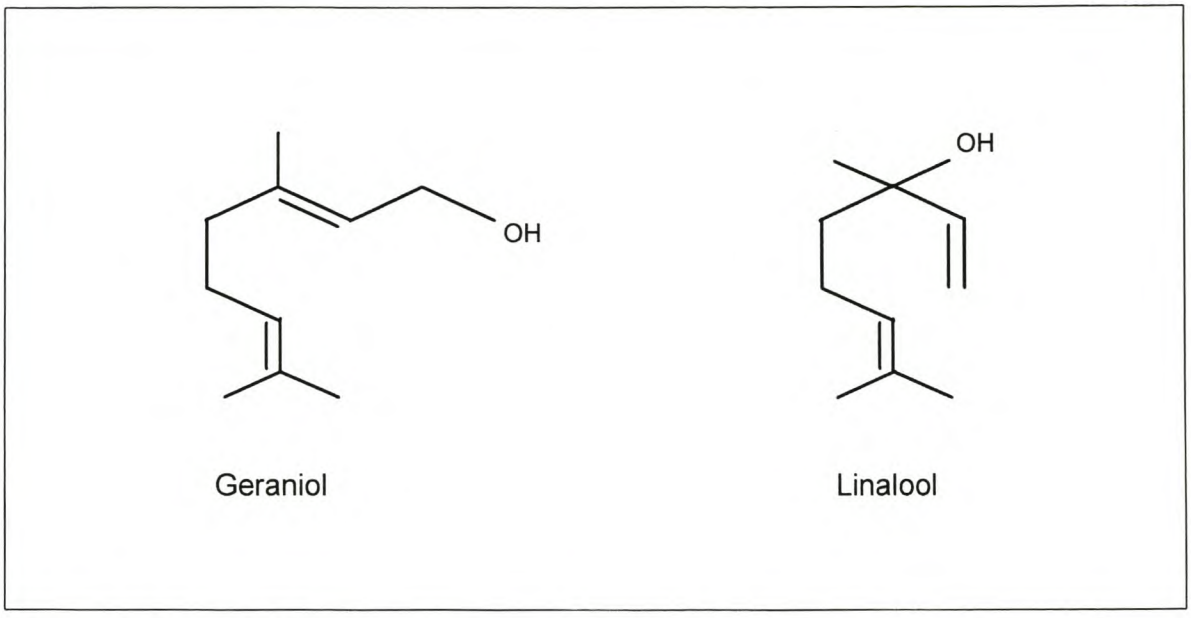

Figure 3. Chemical structures of some important monoterpenes.

3.3 Norisoprenoids

Norisoprenoids, like beta-damascenone, are regarded as important contributors to aroma (**Figure 4**). Numerous C_{13}-norisoprenoids have been identified, and develop via the degradation of carotenoids, like beta-carotene and lutein (Marais, 1992). These components are present as free volatiles or as glycosidically bound forms, which increase in concentration during ripening. Norisoprenoids are influenced by viticultural practices as shown in studies done by Marais *et al.* (1992). Higher concentrations of norisoprenoids such as TDN, beta-damascenone, trans-vitispirane, cis-vitispirane, actinidol 1, actinidol 2 and 9-hydroxymegastigm-7-en-3-one were found in the grapes exposed to sunlight of Weisser Riesling and Chenin blanc. By the application of specific viticultural practices, microclimatic conditions can be changed in order to optimise the development of norisoprenoids.

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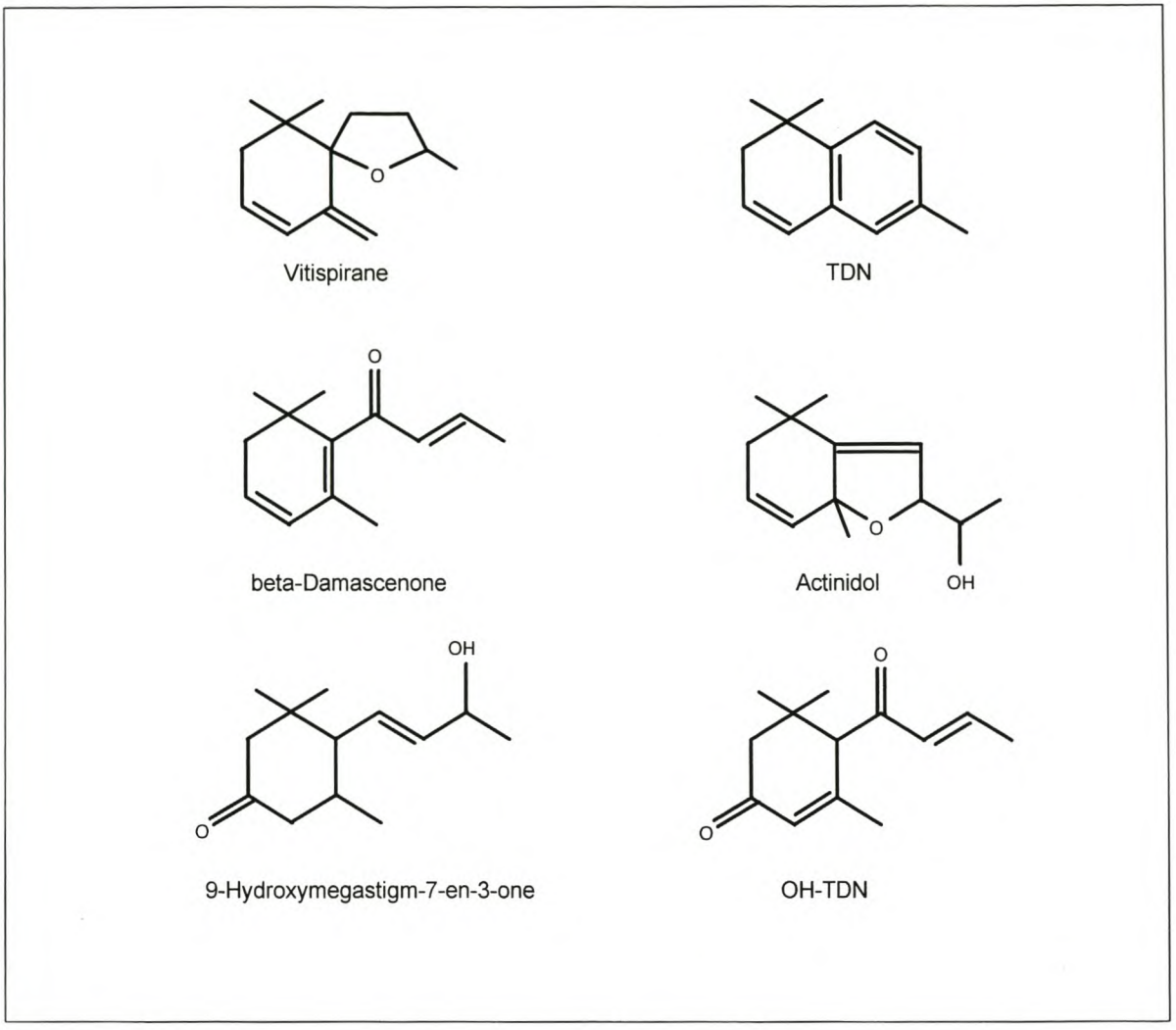

Figure 4. Chemical structures of some important C_{13}-norisoprenoids.

3.4 Sulphur-containing components

Small quantities of sulphur-containing components can have a substantial effect on the aroma of foods, drinks and wine, and may be the source of off-flavours.

3.4.1 Common sulphur-containing components

Hydrogen sulfide (H_2S), the most commonly found sulphur-containing component, derives from sulphate found in the tissues of grapes, sulphite from sulphur dioxide, amino acid degradation or sulphur fungicidal sprays (Jackson, 1994). Hydrogen sulfide generally appears as product of decomposition in various foods and is also formed during some cooking processes. Sulphur components develop during enzymatic and non-enzymatic reactions during the ripening of grapes and the wine

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making process (Rauhut *et al.*, 1993). However, various sulphur-containing components, like methylmercaptopropanol, ethanethiol, and dimethyl disulfide can develop from free radicals, especially during the production of wine (**Figure 5**). Wines containing high levels of sulphur-containing amino acids, like methionine, cysteine and the vitamin riboflavin, are those most likely to be at risk.

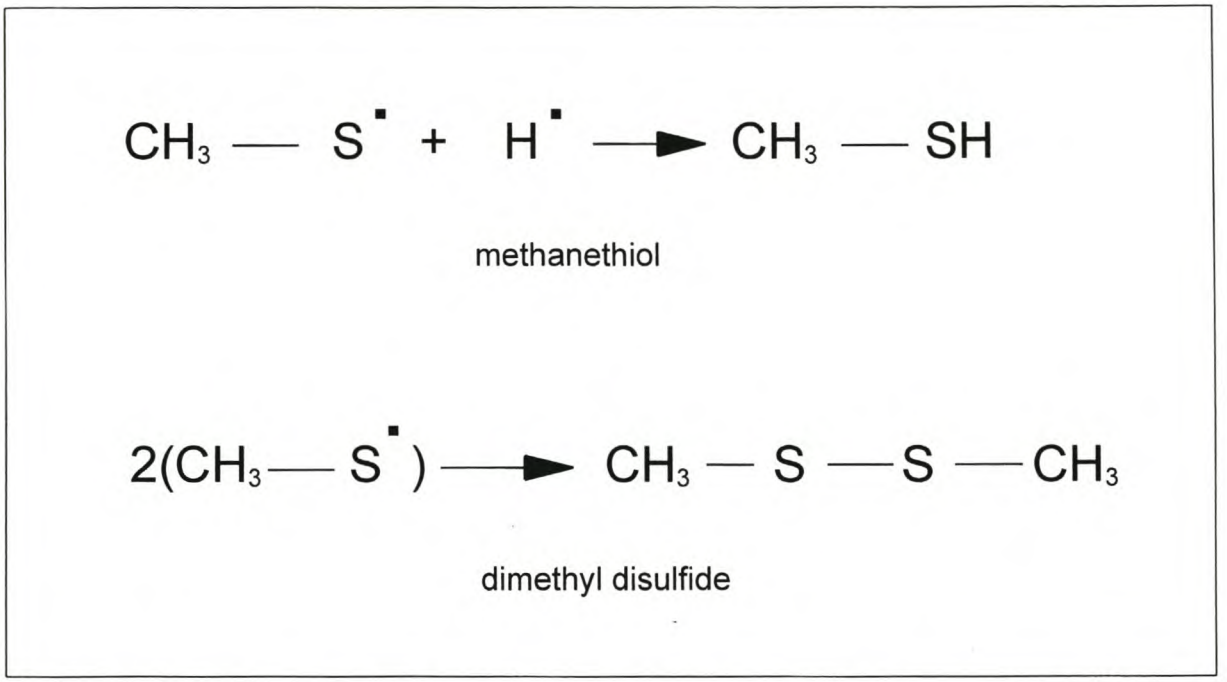

Figure 5. Reaction of free radicals to produce methanethiol and dimethyl disulfide. (Jackson, 1994).

However, the development of certain sulphur components does not necessarily derive from high concentrations of amino acids. Heavy sulphur components (components with boiling points higher than 90°C) can have a detrimental effect on wine aroma, and nuances of garlic (trans-2-methylthiophanol, 4-methylthiobutan-1-ol), onions (2-methyltetrahydrothiophenone), burnt, cabbage (methionol) or cauliflower (2-[methylthio]-ethanol) can be present (Beloqui *et al.*, 1995). Although most of the sulphur-containing components have distinctive aromas, regarded in most cases as negative aromas, the sulphur component bis(2-hydroxyethyl)disulfide, has no specific odour, as found in investigations done by Beloqui *et al.* (1995) on wine. This component acts as a precursor for 2-mercaptoethanol and H_2S and prompts the development of the very unpleasant, distinctive poultry-like sulphur aromas these components are known for.

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3.4.2 4-Mercapto-4-methylpentan-2-one (MMP)

The highly potent sulphur-containing component, 4-mercapto-4-methylpentan-2-one (MMP), may occur in Sauvignon blanc wine and in a variety of other foods (Polak *et al*, 1988; Darriet *et al.*, 1991). Mercapto ketones and derivatives of mercaptans are very volatile and important impact aroma components with very low threshold values. Prominent aromas of garlic, cabbage, and off-flavours like rotten plant material, unpleasant bathroom odours, "catty" or cat urine-like odours are associated with these mercapto components (Moncrieff, 1970; Maarse & Tennoewer de Brauw, 1974; Cosser *et al.*, 1980). In most food and drinks, MMP is the product of the reaction between mesityl oxide and hydrogen sulphide (**Figure 6**) (Pearce *et al.*, 1967; Cosser *et al.*, 1980). As the result of this reaction in a variety of products like cheese, beer, pork and fruit juices, MMP manifests as different nuances as seen in **Figure 6**:

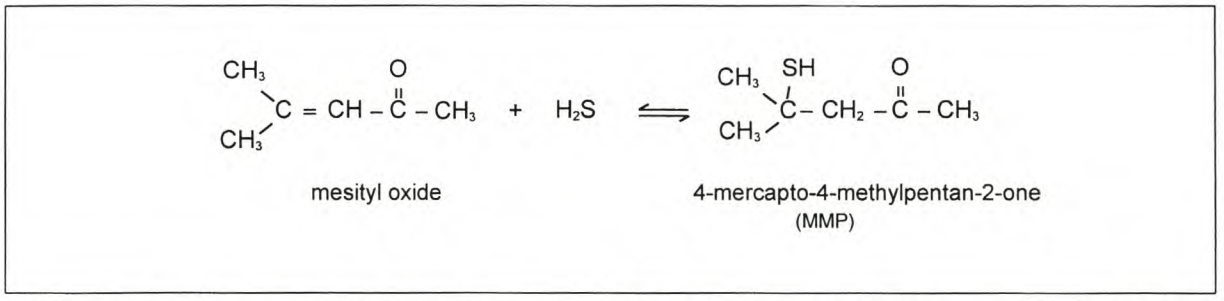

Figure 6. The reaction of mesityl oxide and H_2S to form MMP (Cosser *et al.*, 1980).

3.4.2.1 MMP, a product of contamination

1. MMP in cheese and ham

The same distinctive aroma of MMP was reported in cheese, beer and polluted river water (Maarse & Tennoever de Brauw, 1974; Steinholz & Stensen, 1979; Cosser *et al.*, 1980). Mesityl oxide, which was present as a contaminant in the packaging material, reacted with hydrogen sulphide (H_2S) to form MMP, and catty or cat urine-like aromas were perceived. The same phenomenon was found in ham. The distinctive aroma of MMP derived from the chemical reaction between the wrapping of the ham and the meat itself (Franz *et al.*, 1990). This reaction normally takes place easily at room temperature.

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2. MMP in pork

Investigations of volatile sulphur-containing components in salted and salted-boiled pork were done by Golovnya *et al.* (1982). Since relatively small quantities of these components were found in the meat using gas chromatographic analyses, it was difficult to detect the organoleptic effects of these components.

The heat-treated salted pork showed noticeable amounts of MMP and was suspected that it could be the result of the decrease in the lower fatty acid content, like acetic, propionic and butyric acid during the heating process. Chemical interaction between these fatty acids and mercaptans normally takes place and thio-esters are formed. The Maillard reaction could, however, also play a significant role in the increase in concentration of MMP in the salted boiled pork (Golovnya *et al.*, 1982).

3. Aroma components similar to MMP

In an investigation done by Polak *et al.* (1988), aroma components similar to MMP were introduced to a panel of judges. The purpose of this exercise was to investigate structure-odour relationship. The component, MMP, was correctly identified 190 out of 220 times, however, in some cases, components like trans-8-mercapto-p-menthan-3-one, t-amyl-mercaptane and 3-mercapto-3-methyl-2-pentanone were identified instead. From these investigations it was evident that a tertiary mercapto-amyl structure [$(C)_2$CSHCC] in the chemical structure was important to induce the cat urine character. The keto-group on the other hand was of no importance.

3.4.2.1 Naturally occurring MMP

4-Mercapto-4-methylpentan-2-one also occurs naturally and was identified in the following:

1. MMP in the Carob bean (*Ceratonia siliqua* L.)

The carob bean tree (*Ceratonia siliqua* L.) belongs to the family of the Leguminosae and is widely cultivated in many parts of the Mediterranean coast, like in Spain, Portugal, Turkey, Italy and Greece (Macleod & Forcen, 1992). The beans of this tree change from green to a dark brown colour when they are fully matured. The ripe beans have then a shrivelled appearance, because of the reduction of moisture (from 75% to 20%), while the sugar content increases (from 20% to 50%). The beans consist of several hard seeds embedded in pulp. When the beans are harvested, the

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seeds are used in the manufacturing of locust bean gum, while the pulp is processed and used as emulsifiers or stabilisers in the food industry. Carob powder is produced from the pulp, and is used in food as a substitute for cocoa, as well as in a wide range of caffeine-free products like cakes, biscuits and beverages. Unpleasant, sulphurous aromas were sometimes detected in products where this carob powder was used as an ingredient. Extracts of the volatile components of these products were prepared and analysed by Macleod and Forcen (1992). Some of these extracts possessed aroma characteristics of sweet, buttery, caramel, ester-like, oily/fatty, as well as unpleasant, sulphur-like and rancid/sweaty odours. The major contributors to some of these aromas were identified as aliphatic acids (77.5%). Eight aliphatic sulphur components were identified in the carob bean extract of which dimethyl disulphide (in combination with aldehydes, especially 3-methylbutanal) produced a cocoa-like aroma. 4-Mercapto-4-methylpentan-2-one was present in relatively higher concentrations than the other sulphur components and can therefore probably explain the presence of unpleasant sulphurous aromas.

2. MMP in Japanese Green Tea

A very popular beverage in Japan, is a green tea called Sen-cha (Kumazawa & Masuda, 1999). The specific odour of this tea makes it popular and widely consumed. Research done on Sen-cha, identified MMP as the aroma component responsible for the distinctive aroma of this tea.

3. MMP in grapefruit juice

The consumer's demand for citrus juices throughout the year led to the development of sophisticated technologies of juice processing (Buettner & Schieberle, 1999). The flavour of manufactured juice generally differs from that of the freshly hand-squeezed juice. In order to overcome this problem, studies were done on the volatile constituents in grapefruit juice to gain the necessary knowledge. 4-Mercapto-4-methylpentan-2-one was identified, together with 35 other aroma components, to be one of the more potent components present in fresh grapefruit juice (Buettner & Schieberle, 1999).

4. MMP in box tree and broom leaves

The aroma of Sauvignon blanc wines is often described as box tree or broom-like (Darriet *et al.*, 1995). Chemical analyses on the aroma constituent of the box tree

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and broom plants were done in order to correlate with the sensory descriptions commonly used by wine tasters during the evaluations of Sauvignon blanc wine. The box tree (*Buxus sempervirens* L.), a shrub, is commonly found in forests, parks and gardens. The characteristic aroma is released by the leaves of the plant. Broom (*Sarothamnus scorparius* (L.) Koch.) leaves possess the same aroma as that of the box tree. 4-Mercapto-4-methylpentan-2-one was identified by gas chromatography and mass spectrometry as the component to be responsible for the distinctive aroma found in these plants (Tominaga & Dubourdieu, 1997).

5. MMP and related sulphur-containing components in wine

Chenin blanc and Colombar wine often possess a characteristic guava-like aroma. In investigations done by Du Plessis & Augustyn (1981), different concentrations of MMP were added to a neutral wine. Most of the wines were identified by a panel of judges as Chenin blanc and Colombar, because of the guava aroma they possessed. The aroma of some of the wines, however, was described as "catty" or "sweaty". This illustrated the ability of MMP to present different nuances in wine, depending on the concentration thereof, which consequently affects the wine aroma and quality (Du Plessis & Augustyn, 1981; Marais & Swart, 1999).

A number of sulphur-containing components were recently identified in Sauvignon blanc wines. All of these components play a decisive role in their contribution to aroma and the overall quality of Sauvignon blanc wine.

During the alcoholic fermentation of Sauvignon blanc must, aromatic volatile thiols are released and aromas reminiscent of box tree, broom, black currant, citrus zest, grapefruit, passion fruit, cooked leeks or cat urine can be perceived in the wine (Dubourdieu *et al.*, 1993; Darriet *et al.*, 1995; Tominaga *et al.*, 1996; Tominaga *et al.*, 1998a). Gas chromatography and mass spectrometry enabled the identification of the sulphur-containing components, 4-mercapto-4-methylpentan-2-ol (**A**), 3-mercapto-hexan-1-ol (**B**), 3-mercaptohexyl acetate (**C**), 3-mercapto-3-methylbutan-1-ol (**D**), and 4-mercapto-4-methylpentan-2-one (**E**), which are responsible for these aromas (**Figure 7**) (Darriet *et al.*, 1991; Darriet *et al.*, 1995; Tominaga *et al.*, 1996; Tominaga *et al.*, 1998a). The different perception threshold values of these components under the same conditions lead to the development of different aromas according to the presence of the relevant sulphur component. 4-Mercapto-4-methylpentan-2-one was identified as the most important impact, sulphur-containing component present in Sauvignon blanc wine (Darriet *et al.* 1991).

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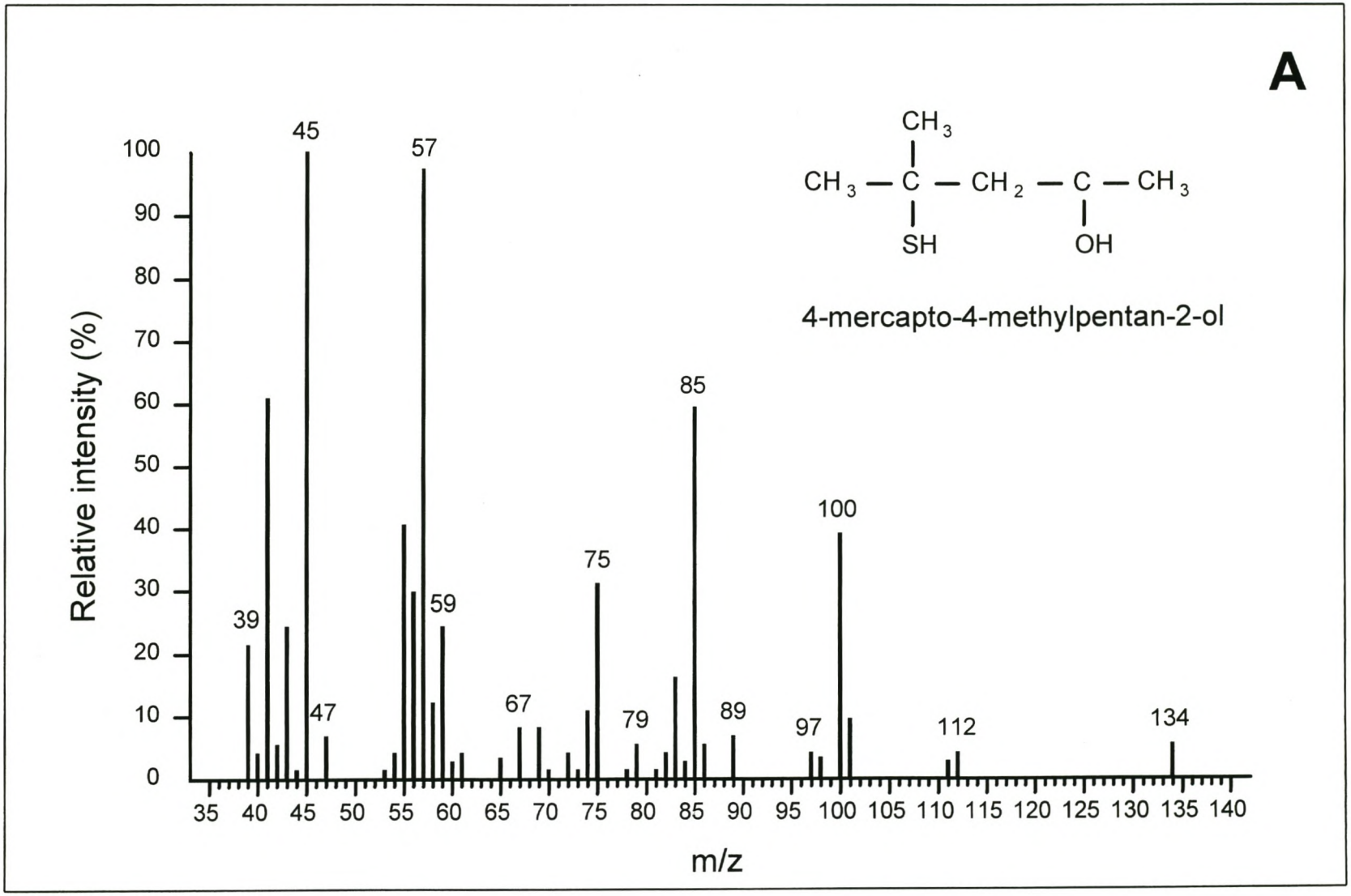

Figure 7. Mass spectra of 4-mercapto-4-methylpentan-2-ol (Tominaga *et al.*, 1998a).

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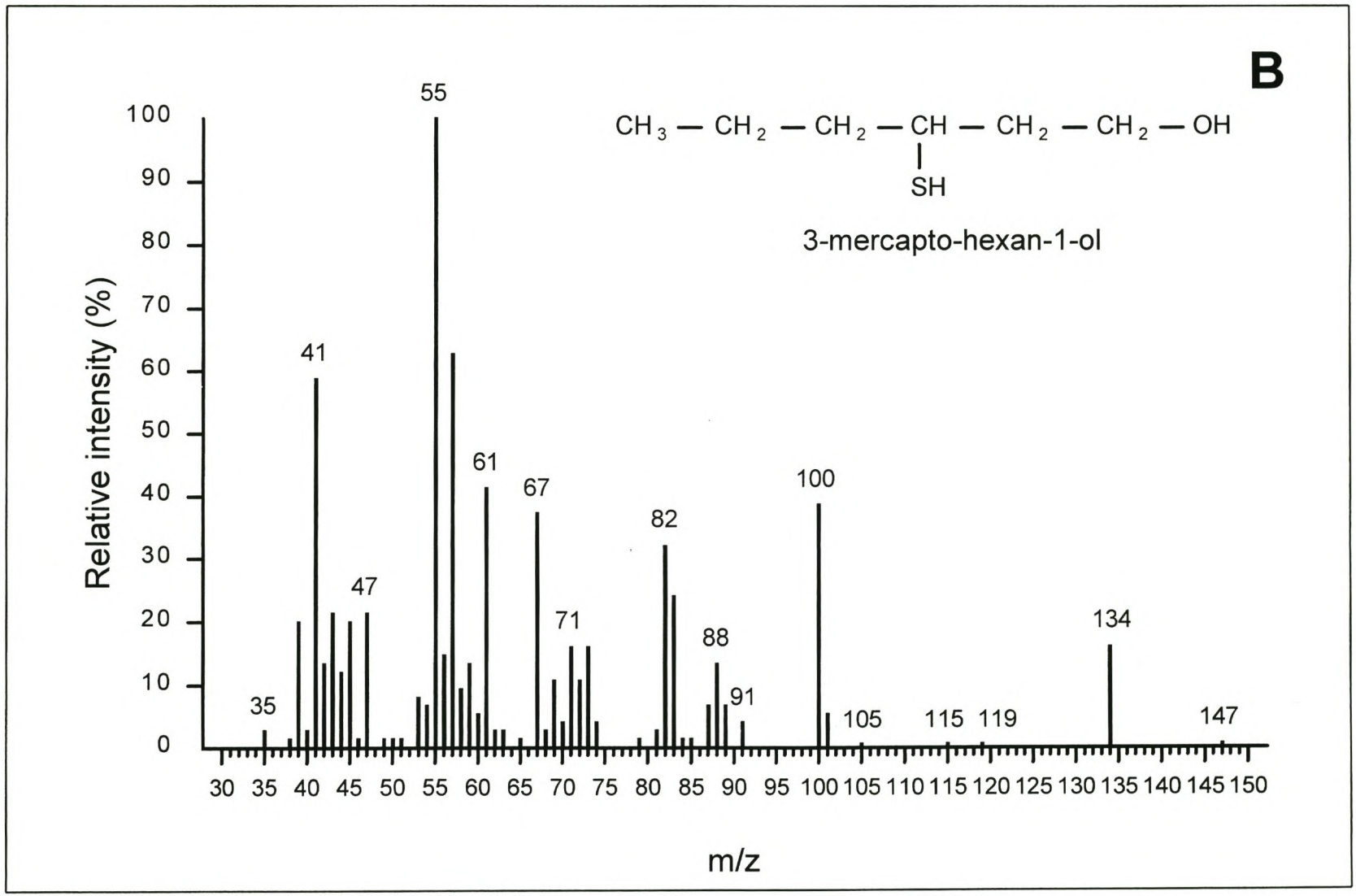

Figure 7. Mass spectra of 3-mercapto-hexan-1-ol (Tominaga *et al.*, 1998b).

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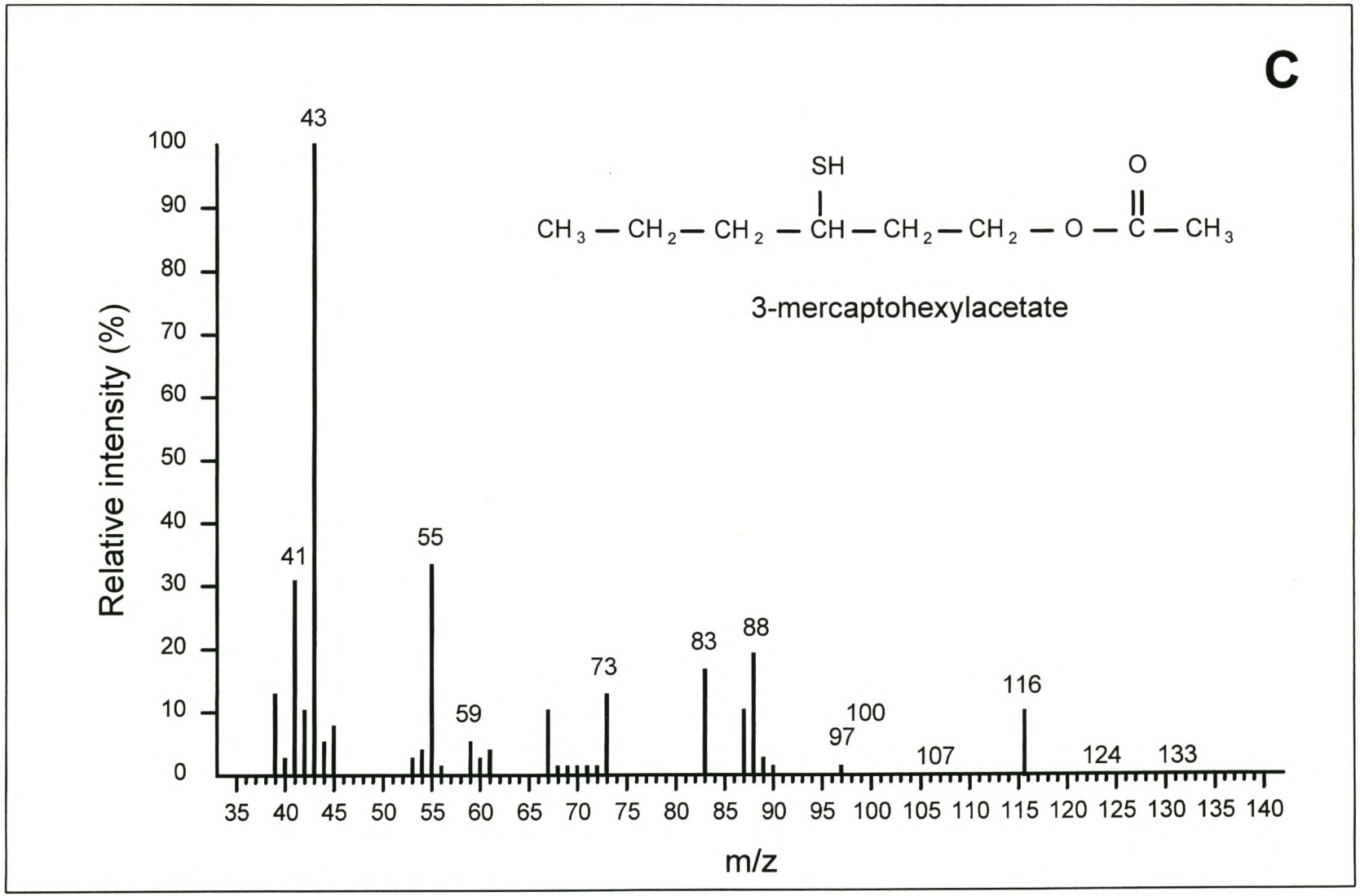

Figure 7. Mass spectra of 3-mercaptohexylacetate (Tominaga *et al.*, 1998b).

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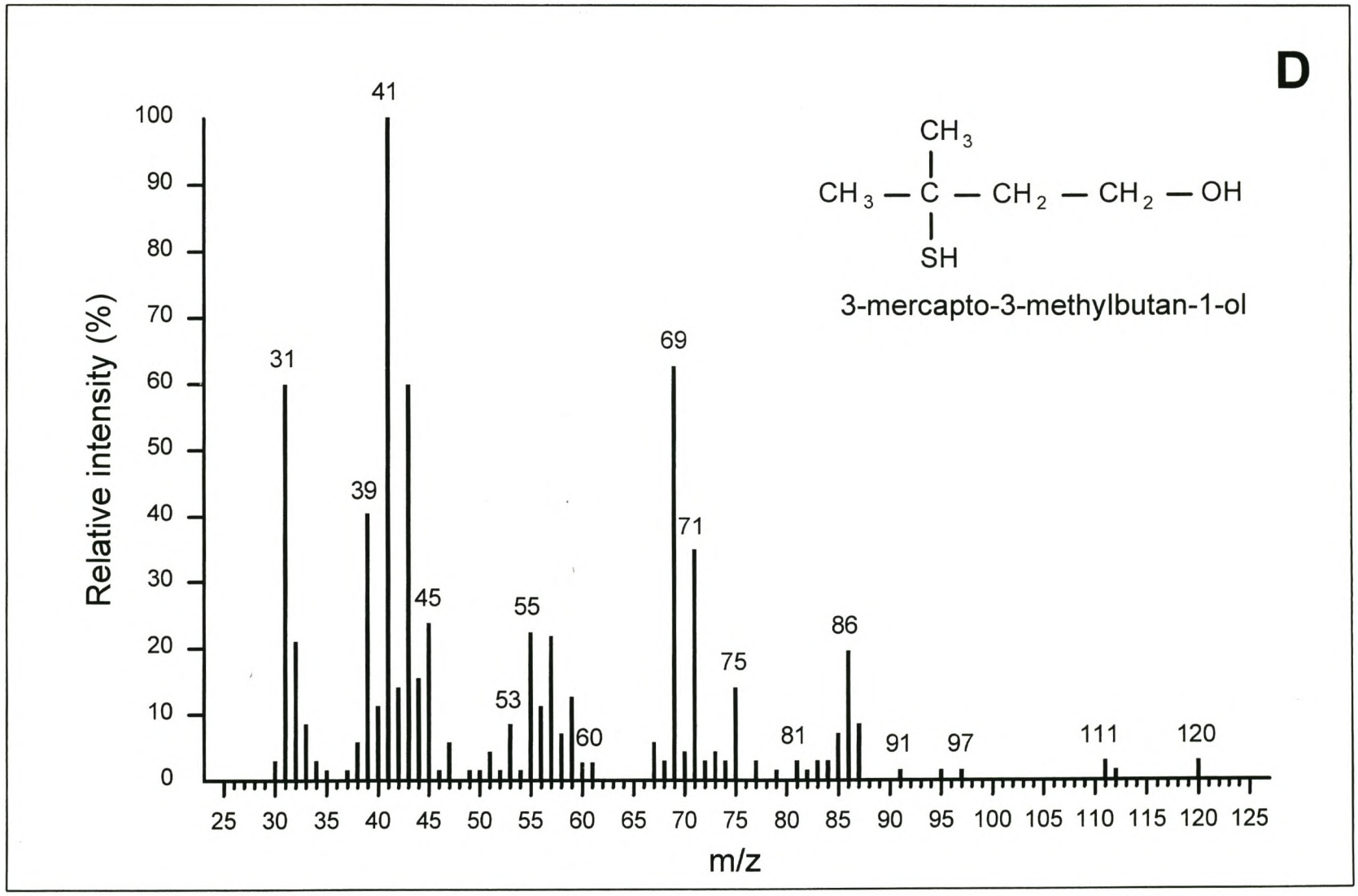

Figure 7. Mass spectra of 3-mercapto-3-methylbutan-1-ol (Tominaga *et al.*, 1998a).

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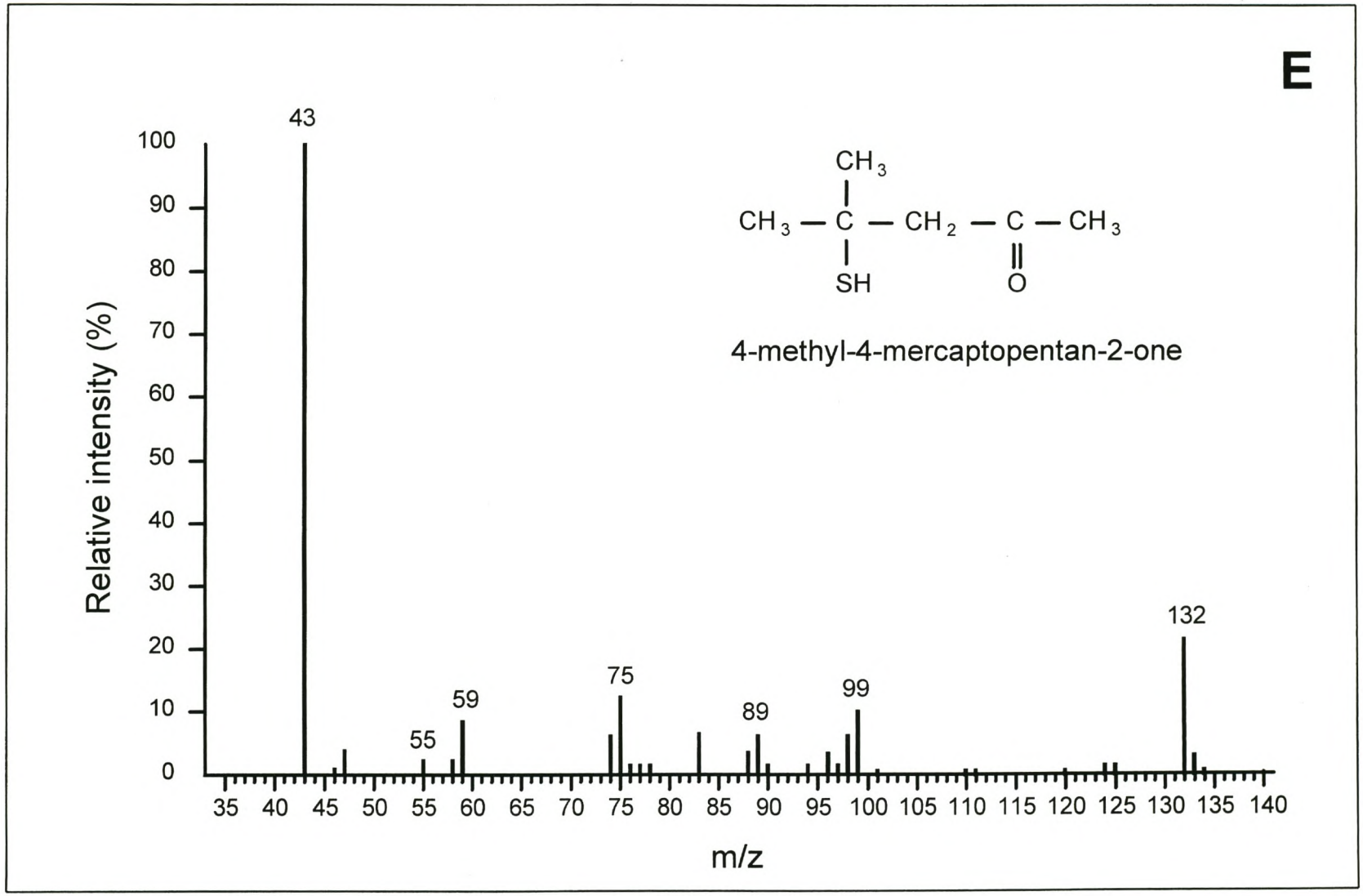

Figure 7. Mass spectra of 4-methyl-4-mercaptopentan-2-one (Darriet *et al.*, 1995; Tominaga *et al.*, 1998a).

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During fermentation, MMP is formed from the corresponding S-cysteine conjugates, a precursor in the grapes (Bouchilloux *et al.*, 1996; Tominaga *et al.*, 1998c). This component which sometimes can have a prominent effect on the typical character of Sauvignon blanc wine has a very low threshold value of 0.1 $ng.l^{-1}$ in water and 3 $ng.l^{-1}$ in wine (Dubourdieu *et al.*, 1993). 4-Mercapto-4-methylpentan-2-one manifests in high concentrations as cat urine-like or box tree aromas (Dubourdieu *et al.*, 1993; Darriet *et al.*, 1995). These aromas are often perceived by wine tasters as not typical and negative of Sauvignon blanc wine. Low concentrations of MMP, however, together with other aroma components, may contribute positively to the overall quality and complexity of wine.

In order to identify the volatile sulphur-containing components responsible for the varietal aroma of Sauvignon blanc wines, Darriet *et al.* (1995) analysed organic extracts using gas chromatography and an olfactometric mode of detection. Results of these aromagrams showed two odourant zones ZO1 and ZO2. The aromas of "box tree" and black currant bud, characteristic of the varietal aroma of Sauvignon blanc were detected at these two zones. These odourant zones seemed to be specific for Sauvignon blanc wines and have not been detected in wines of other cultivars (Darriet *et al.*, 1995).

3.4.2.2 Methods for analysing MMP in wine

Since MMP is such an important aroma component in Sauvignon blanc wine, methods to analyse this component have to be considered. The following techniques are available:

1. Gas chromatography (Darriet *et al.*, 1995)

The pH of 100 ml of Sauvignon blanc was adjusted to 7.5 with 10 M NaOH in order to prepare an organic extract. A mixture of 1:9 (v/v) of diethyl ether and pentane was used to extract the volatile components three times. The organic phases were pooled and concentrated under nitrogen to 500 µl. A volume of 3 µl was injected splitless onto a gas chromatograph coupled to an olfactometric detector. The intensity of the aromas detected at zones ZO1 and ZO2, and the time the "box tree" aroma lasted, were measured.

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2. p-Hydroxymercuribenzoic acid (pHMB) (Darriet *et al.*, 1995)

When p-hydroxymercuribenzoic acid (pHMB) was used to prepare an extract from Sauvignon blanc wine, the odourant zones ZO1 and ZO2, which were normally detected, disappeared. In a reaction with pHMB, a thiol normally looses its properties and is deactivated (Dubourdieu *et al.*, 1993b). The reverse reaction occurred when cysteine or glutathione was added at a level of 20 times higher than the molarity of pHMB. After incubation, the odourant zones were detected again. It was thus evident that thiols are responsible for the odours detected at zones ZO1 and ZO2.

3. Purge and trap technique (Darriet *et al.*, 1995)

A "purge and trap" injector was used in this technique. A sample of wine (8 ml) was heated to 70°C by an envelope of circulating water. The sample was purged by hydrogen for 30 minutes and cryogenically trapped. At the end of the purge, thermic desorption was carried out at 200°C for 10 minutes. A flame photometric detector (FPD) was used to detect the sulphur components.

4. Gas chromatography-mass spectrometry (Darriet *et al.*, 1995)

In this method a volume of 750 ml Sauvignon blanc wine was adjusted to pH 8. This was followed by extraction of 1:1 (v/v) diethyl ether and pentane. The organic phase was extracted with volumes of 10 ml, 5 ml and 5 ml of pHMB solution. The aqueous phase was concentrated to a volume of 10 ml to which 350 mg of glutathione was added. This was followed by another extraction, using a 1:1 (v/v) diethyl ether/pentane mixture. The organic phase obtained, was concentrated to 500 µl under nitrogen flow. The analysis was performed on a gas chromatograph coupled to a mass spectrometer. A commercially available MMP solution was used of which the exact quantity was determined. Glutathione and cysteine were used to compile calibration curves to determine the concentration of MMP in these analyses (Darriet *et al.*, 1995).

5. Sensory evaluation (Darriet *et al.*, 1995)

This technique was used to identify the perception thresholds of MMP. Different concentrations of a commercially available MMP were added to distilled water, 10% ethanol solution, ethanol solution containing 100g.l^{-1} of sucrose, a neutral wine, a red wine and a prepared ethanol (99.5%) solution [containing glycerol (8 g), calcium chloride (0.3 g), magnesium chloride (0.1 g), potassium hydrogen tartrate (2.5 g),

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potassium sulphate (1 g), sodium chloride (0.5 g), citric acid (0.4 g), succinic acid (1 g), lactic acid (2 g)]. These different solutions were presented to a panel of judges. The olfactory perception threshold of MMP in the different solutions was determined according to the ability of the judges, detecting the specific aroma of MMP.

6. "*Sniffing*" technique (Darriet *et al.*, 1995)

The same method as described in the gas chromatography technique (**1**), was used to prepare an organic extract. The extract was concentrated under nitrogen flow to 500 µl of which a volume of 1 µl was injected onto a gas chromatograph and sniffed (Darriet *et al.*, 1991). Different odourant zones were recorded at different retention times. The duration in the detection of the aromas was used to determine the concentration of MMP.

7. *Gas chromatography mass-spectrometry* (Tominaga *et al.*, 1998b)

A combination of previous described techniques was used to prepare an organic extract. Using 10 N NaOH, the pH of a volume of 500 ml of wine was adjusted to 7. The volatile components were extracted two times with 100 ml of dichloromethane. The organic phases were combined, centrifuged and treated twice with 10 ml p-hydroxymercuribenzoic acid (pHMB). The combined aqueous phase was maintained at pH > 7, using 5% HCl. This was loaded onto a strong basic anion exchanger column, which was previously activated by 0.1 M HCl and washed with 100 ml of deionised water. After percolation of the sample, the column was washed with potassium phosphate buffer (2mM, pH 7.2) and sodium acetate buffer (0.1 M, pH 6, 0.1 M NaCl). By using a cysteine solution (640 mg/60 ml, pH 7) the volatile thiols were then released from the column. The eluate was collected and extracted with dichloromethane (4 ml, 2.5 ml and 2.5 ml). The organic phases of three extractions were collected and concentrated under nitrogen flow to 25 µl. A volume of 2 µl was injected onto a gas chromatograph coupled to a mass spectrometer. 4-Methoxy-2-methyl-2-mercaptobutane was used as an internal standard.

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4. CLIMATICAL, VITICULTURAL AND OENOLOGICAL EFFECTS ON THE DEVELOPMENT OF MMP

4.1 Climate and viticulture

The wine regions of South Africa are divided into eight regions according to their climatic conditions and geographic location (Anon., 1999). These regions are classified according to degree days by Le Roux (1974). Climatic conditions, i.e. mesoclimatic (differences between vineyards) and microclimatic (differences in canopies in the same vineyard) have a profound effect on the aroma composition, wine quality and style of a wine (Smart *et al.*, 1985; Smart *et al.*, 1990; Iland, 1989a; 1989b; Marais *et al.*, 1996; Marais *et al.*, 1999).

Conditions within the canopy can be changed to a certain extent by means of canopy management practices. Grape composition is largely influenced by this aspect and different styles of wine can thus be produced from the same vineyard (Morrison & Noble, 1983; Hunter *et al.*, 1991; Marais *et al.*, 1999).

Research done on methoxypyrazines, the most important impact components responsible for the cultivar-typical aroma of Sauvignon blanc wine, revealed that grape and wine composition is mainly influenced by climate (Allen & Lacey, 1993; Marais, 1994a; 1996; Marais *et al.*, 1996; 1999). The concentration of ibMP decreases with ripeness and sunlight exposure of the grapes. Different regions produce different styles of Sauvignon blanc wines, which varies from the more typical green pepper/asparagus to the tropical-like kind of aromas (Marais *et al.*, 1999). Studies showed, however, that cooler climates are more favourable to cultivate typical Sauvignon blanc wines.

Since methoxypyrazines are not the only aroma components, contributing to Sauvignon blanc wine aroma, other aroma components, like norisoprenoids, monoterpenes, esters, higher alcohols and sulphur-containing components also play an important role in the overall quality and complexity of wine. The development, presence and concentrations of these components are undoubtedly also influenced by climatic conditions, and is thus suspected that climate may, therefore, also plays a role in the development of the sulphur-containing component, MMP, from an odourless precursor in the grapes.

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4.2 Yeast strain

The effect of fermentation on wine composition and quality is well-known. The metabolism of yeasts yields besides ethanol and CO_2, numerous byproducts, like glycerol, acids, esters, higher alcohols, other volatile substances and sulphur-containing components (Giudici & Zambonelli, 1992; Giudici *et al.*, 1993; Bertolini *et al.*, 1996; Lema *et al.*, 1996). Different yeast strains produce different combinations of volatile components (Soles *et al.*, 1982; Herriaz *et al.*, 1990). *Saccharomyces cerevisiae* strains, normally used for alcoholic fermentation during wine production, are easily recognised and classified according to their phenotypic characteristics and their ability to produce certain volatile components (Rozes *et al.*, 1992; Van Vuuren & Jacobs, 1992; Vezinhet *et al.*, 1992).

During the first two to four hours of fermentation, enzymatic activities increase, and thiols, secondary plant metabolites, are released due to the degradation of the corresponding S-cysteine conjugates (Tominaga *et al.*, 1998c). The precise metabolic pathway of this reaction is still unknown (Darriet *et al.*, 1991). Some yeasts possess the ability to metabolise different concentrations of MMP (Dubourdieu *et al.*, 1993b). The kind of yeast used and conditions during fermentation therefore, play an important role in MMP development (Celotti *et al.*, 1997).

Many sulphur-containing compounds, like certain amino acids, proteins, inorganic sulphates and residue from pesticides are present in wine (Rauhut *et al.*, 1993). These components are metabolised by yeasts to form mercaptans, H_2S and CS_2 (Cantarelli *et al.*, 1964).

The different yeasts present in Sauvignon blanc must were identified and small scale fermentations were performed, using some of these yeasts (Masneuf & Trione, 1995). The aim was to isolate a yeast strain specifically for Sauvignon blanc which would produce higher concentrations of MMP during fermentation. A *Saccharomyces cerevisiae* yeast strain, Zymaflore VL3C (Producers, Laffort), was isolated and genetically identified. More complex wines, with distinctive aromas of black currant or box tree were produced from must fermented by this yeast strain. When this is compared to South African Sauvignon blanc wines, which are less complex, with more grassy, asparagus or green pepper-like aromas, these characters often manifest as cat urine when too intensive, and are described as not typical of this cultivar.

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4.3 Ascorbic acid

Antioxidants, like ascorbic acid, are widely used to delay oxidative flavour deterioration and browning of food products. Ascorbic acid (vitamin C) occurs naturally in small quantities in fruit and vegetables (Albrecht *et al.*, 1991). The concentration of ascorbic acid in these products is influenced by cultivar, maturity, growing practice, climate, post harvest handling (Osuna-Garcia *et al.*, 1998). Ascorbic acid occurs naturally at concentration levels of 5 – 15 $mg.kg^{-1}$ in grapes (Van Wyk, 1995).

Ascorbic acid has been and is still added to wine to prevent oxidation. The pro- and antioxidative abilities of ascorbic acid enable it to react with the oxygen in wine, and forms a strong oxidising agent, H_2O_2 (**Figure 8**) (Van Wyk, 1995; Marais, 1995).

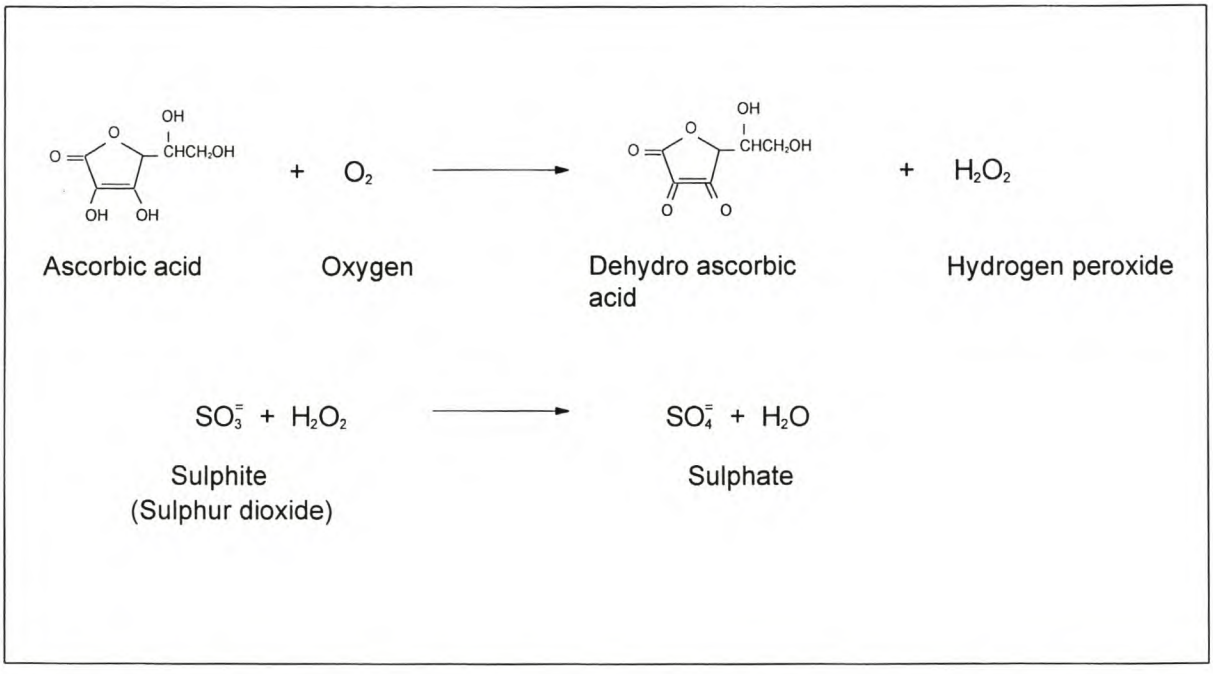

Figure 8. The pro- and antioxidative properties of ascorbic acid (Van Wyk, 1995).

During alcoholic fermentation, juice is normally oxidised to some extent and browning occurs due to enzymatic reactions. Oxidation of polyphenols takes place and quinones are formed with the simultaneous loss of some aroma components (**Figure 9a**) (Kanner & Mendel, 1977; Van Wyk, 1995). The quinones can be converted back to the original polyphenols in the presence of excess amounts of SO_2 or ascorbic acid (50 – 100 $mg.l^{-1}$) (**Figure 9b and 9c**).

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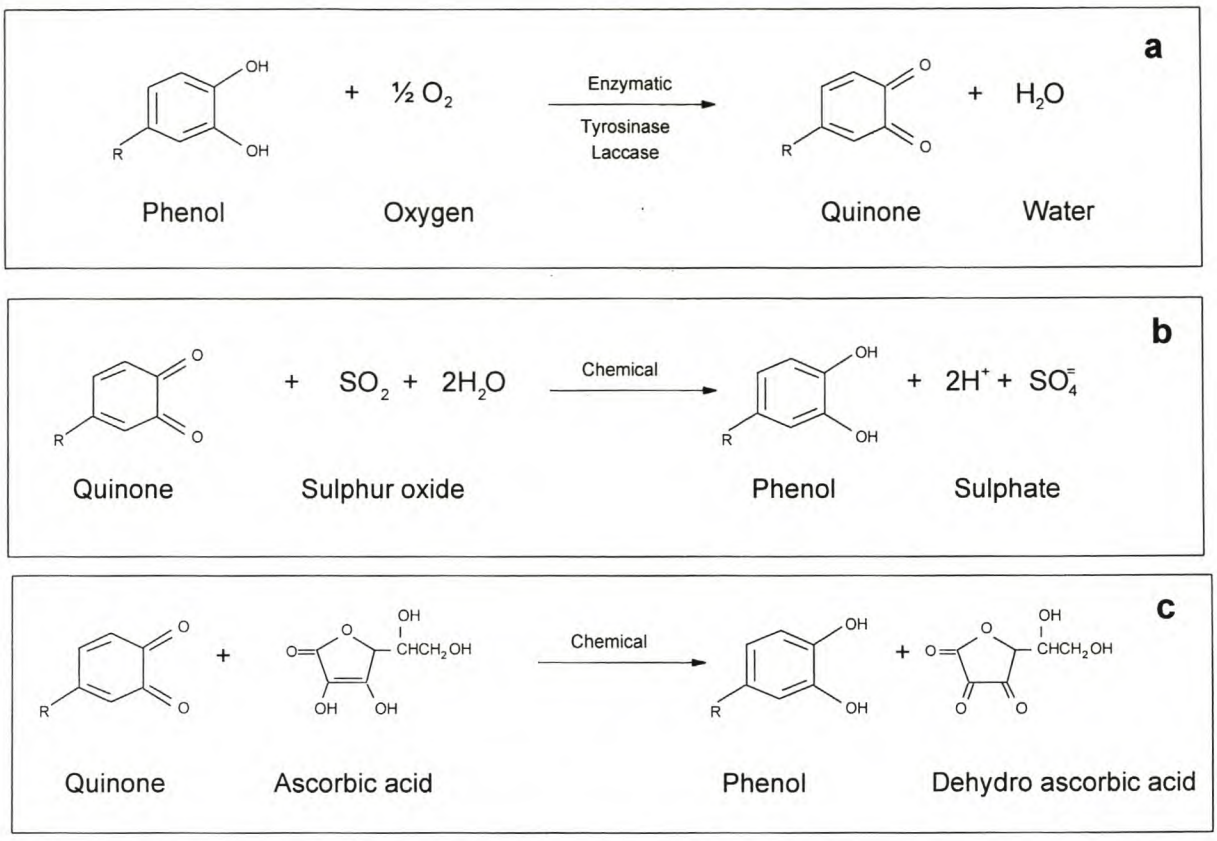

Figure 9. Oxidation and reduction reaction in must in the presence of SO_2 and ascorbic acid (Van Wyk, 1995).

The ability to lower the redox potential of must during fermentation is considered to be the most important attribute of ascorbic acid. However, the following properties of ascorbic acid, i.e strong antioxidant ability, protects SO_2 from oxidation, does not affect the taste of wine when added in low concentration, does not promote the extraction of phenols during skin-contact, it is easy to use and there is no health risk attached in using it, are also regarded as important (Van Wyk, 1995).

Ascorbic acid is able to reduce Fe^{3+} to Fe^{2+}. A complex between ascorbic acid and the reduced form of iron can be formed, which catalyzes the breakdown of hydroperoxides and forms reactive free radicals (Kanner & Mendel, 1977). Reactions with these radicals can result in the production of secondary oxidation products (aldehydes, ketones, acids) that can cause undesirable off-flavour development (Jacobsen, 1999).

Investigations on the use of pure ascorbic acid (a) derivatives of ascorbic acid, like dihydroxy fumarin acid (b) and the oxidised form of ascorbic acid, dehydro ascorbate (c) in the wine making process showed that MMP was present in all the wines produced. (**Figure 10**) (Dubourdieu, 1993b). The presence of high

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concentrations of MMP, as already stated, leads to off-flavours in the wine, and ascorbic acid could therefore initiate the development of this thiol.

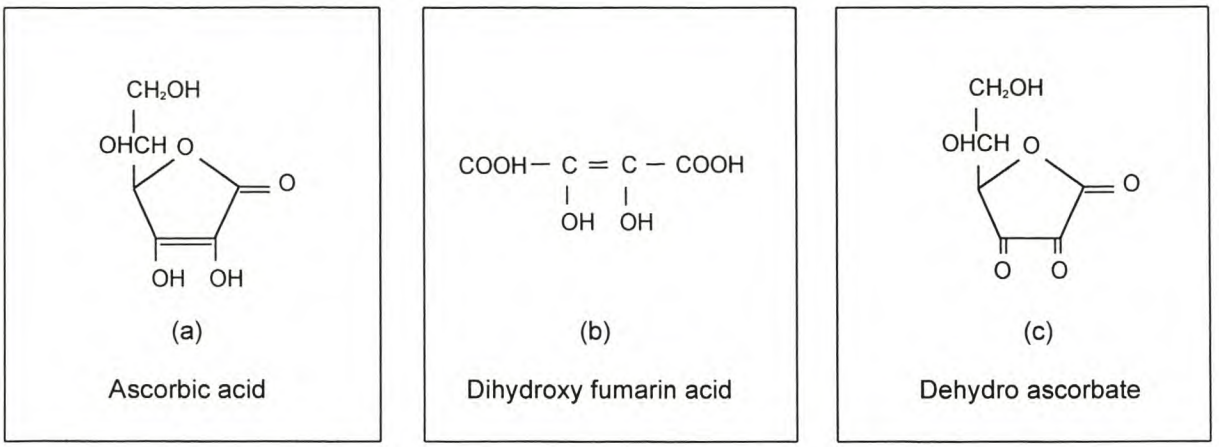

Figure 10. Ascorbic acid and derivatives of ascorbic acid (Dubourdieu, 1993b).

5. CONCLUSIONS

Sauvignon blanc is an important white cultivar for the South African wine industry. Competition with other wine producing countries, necessitates investigations on the effects of climatic, viticultural and oenological factors on Sauvignon blanc grape and wine composition and quality. It is evident that complex aromas determine Sauvignon blanc wine quality, and knowledge about them and the factors affecting their concentration levels, is essential.

The effects of methoxypyrazines on Sauvignon blanc cultivar-typical character were well studied. However, sulphur components also have prominent effects on the aroma and quality of Sauvignon blanc wines. The sulphur-containing component, MMP, occuring widely in various food products, is considered with methoxypyrazines, the most important impact aroma components present in Sauvignon blanc wine. Depending on the concentration of this mercapto component, mostly negative nuances are perceived by local wine tasters and consumers.

Developments in flavour research and improvements in analytical techniques provided the necessary knowledge to identify this component, normally present in very low concentrations. However, more information is necessary to monitor the development of MMP during fermentation and to gain the necessary knowledge to

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prevent the development of too high concentrations of thus and other mercapto components. Therefore it is important to develope techniques that will produce Sauvignon blanc wines without these sulphur-like aromas, and it appears that the effects of climate/region, ascorbic acid and yeast strain are of special significance in this regard.

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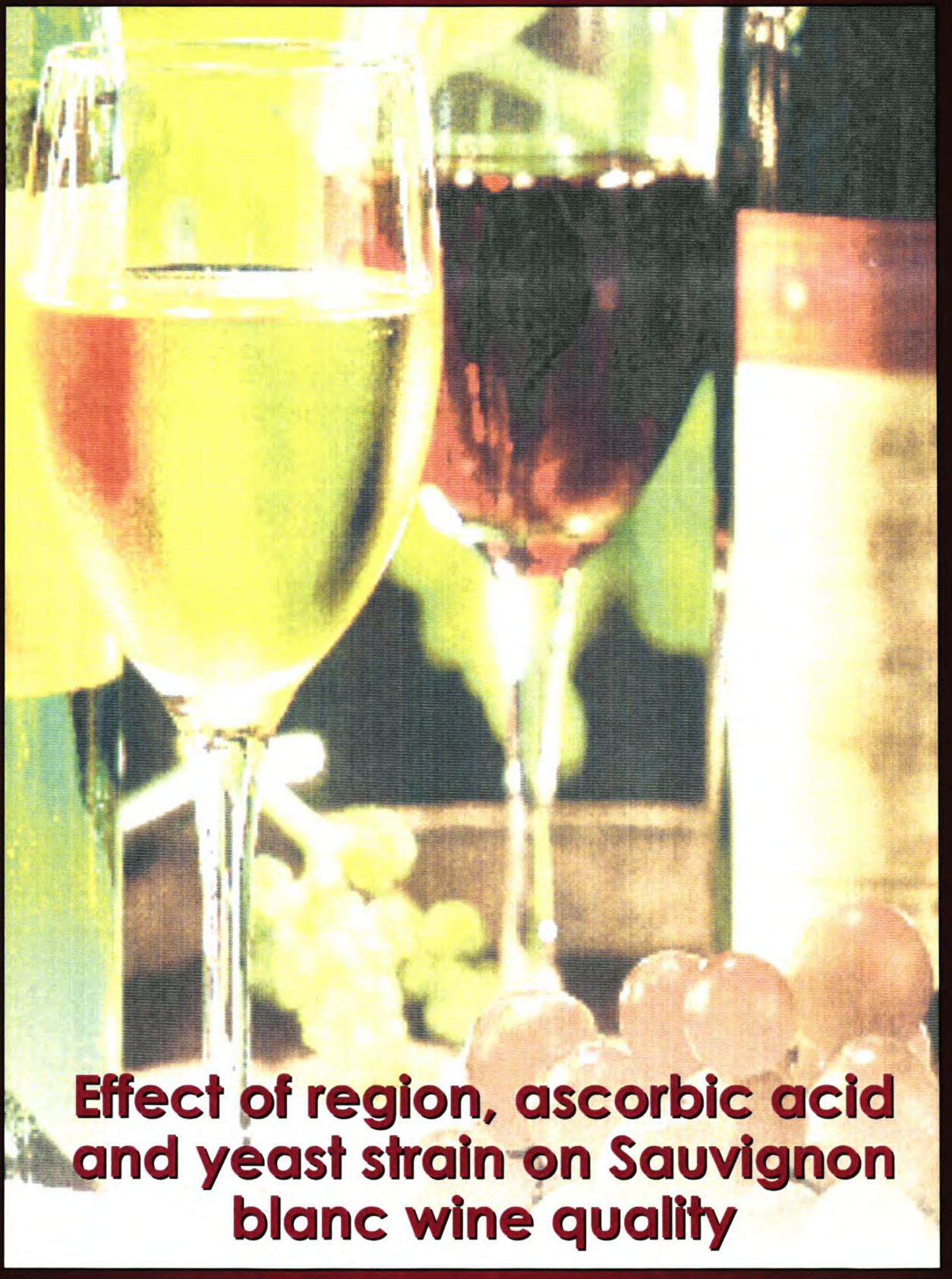
CHAPTER 3



Effect of region, ascorbic acid and yeast strain on Sauvignon blanc wine quality

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CHAPTER 3

EFFECT OF REGION, ASCORBIC ACID AND YEAST STRAIN ON SAUVIGNON BLANC WINE QUALITY

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Summary

Sauvignon blanc cultivar-typical aroma is affected by different components of which 2-methoxy-3-isobutylpyrazine and 4-mercapto-4-methylpentan-2-one are probably the most important. Climatic, viticultural and oenological conditions may have a prominent effect on the levels at which these impact aroma components occur in wine. Sauvignon blanc wines were produced from grapes from two climatically different regions. Different ascorbic acid/SO_2 combinations and different *Saccharomyces cerevisiae* yeast strains were used during the production of the wines. The wines were sensorially evaluated for specific wine characteristics, namely fruity/ester aroma intensity, grassy/green pepper aroma intensity, sulphur-like aroma intensity and overall wine quality. Significant differences were observed between treatments. A commercially available preparate (ascorbic acid/meta) and yeast strain VL3C produced sulphur-like, low quality wines under the conditions of this investigation. The highest quality wines were produced from pure ascorbic acid/SO_2 treatments and fermentation by the yeast strain Vin 13.

Introduction

The study of wine and the sensory evaluation thereof is a complex science. Certain aromas can be related to the chemical composition of wines, and are only sensorially detectable when the responsible chemical aroma components occur above their threshold levels (Cavazza *et al.*, 1993). The aroma of *Vitis vinifera* L. cv. Sauvignon blanc can easily be distinguished from that of other white wine cultivars. Yet, disagreement about the typical aroma of Sauvignon blanc wine, still exists. The

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cultivar-typical aroma of Sauvignon blanc wines can be described as asparagus, green, grassy, green pepper and pyrazine-like (Allen *et al.*, 1988; Allen & Lacey, 1993; Lacey *et al.*, 1991). Fruity/floral-like aromas are also important in Sauvignon blanc wine and are probably caused by, i.e. certain monoterpenes and norisoprenoids (Marais, 1994a; Marais *et al.*, 1996; Marais *et al.*, 1999). The most common methoxypyrazines present in Sauvignon blanc, were identified as 2-methoxy-3-isobutylpyrazine (ibMP) (green pepper-like aroma), 2-methoxy-3-isopropylpyrazine (ipMP) (asparagus-like aroma) and 2-methoxy-3-sec-butylpyrazine (sbMP) (Augustyn *et al.*, 1982; Allen *et al.*, 1988; Lacey *et al.*, 1991).

Sulphur-containing components also have a prominent effect on the quality of wine (Rapp *et al.*, 1985; Park & Noble, 1993). Low concentrations of sulphur components can enhance the complexity of aroma and quality, but too high concentrations often lead to unpleasant off-flavours. Darriet *et al.* (1995) identified an important volatile sulphur component, 4-mercapto-4-methylpentan-2-one (MMP) in wine. This is the same mercapto ketone that is responsible for the characteristic cat urine, box tree or broom tree odour (Polak *et al.*, 1988; Darriet *et al.*, 1995; Tominaga & Dubourdieu, 1997). Because it often occurs at levels above its threshold value, MMP may have a marked effect on the complex aroma of Sauvignon blanc wine. When present at too high concentrations, it is regarded as negative and not typical for Sauvignon blanc. Besides MMP, some other mercapto components were also identified in Sauvignon blanc wine, i.e. 4-mercapto-4-methylpentan-2-ol, 3-mercaptohexan-1-ol (similar to citrus, grapefruit and passionfruit aromas) and 3-mercapto-3-methylbutan-1-ol (cooked leeks aroma) (Tominaga *et al.*, 1998a). The concentration levels and contribution of these volatile thiols to quality has been clarified (Tominaga *et al.*, 1998b).

Studies showed that cooler climates are favourable to the production of more typical Sauvignon blanc wines (Allen & Lacey, 1993; Lacey *et al.*, 1991; Marais 1994b; Marais *et al.*, 1996; Marais *et al.*, 1999). Furthermore, viticultural and oenological practices play a prominent role in the composition and quality of wine. Oenological practices, like skin-contact, extract higher concentrations of phenolic compounds, monoterpenes and methoxypyrazines from the skins and give wine a more complex character and higher quality (Marais, 1998). Yeasts and yeast autolysates are rich in free amino acids and can generate sulphur-containing components from precursors by enzymatic and non-enzymatic reactions (Münch & Schieberle, 1998). During fermentation, yeast and enzyme activities increase and

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metabolise sulphur-containing amino acids and proteins, as well as, inorganic sulphates to form H_2S, CS_2 and mercaptans (Rauhut *et al.*, 1993). Some of these impact volatile thiols present in Sauvignon blanc wine, e.g. MMP, are released by yeasts through the degradation of S-cysteine conjugates (Tominaga *et al.*, 1998c). Not all yeast strains, however, are involved in the production of mercapto ketones, like MMP.

Local Sauvignon blanc wines often possess a neutral character and a lack of typical aromas like green pepper, vegetative, grassy or asparagus. They also often present undesirable sulphur-like aromas. With the increase in popularity of Sauvignon blanc world-wide, it is extremely important to produce higher quality Sauvignon blanc wines with typical aromas and to eliminate faults such as sulphur-like off-odours. It is claimed that some oenological practices, like the use of certain yeast strains and ascorbic acid, produce more typical, fresh/fruity Sauvignon blanc wines. The purpose of this investigation was therefore to determine the effect of specific treatments, like the use of ascorbic acid, SO_2 and different yeast strains on Sauvignon blanc wine quality. The knowledge gained will be applied to identify those techniques that will enhance the quality of local Sauvignon blanc wine.

Material and methods

1. Wine production

Sauvignon blanc wines were produced during the 1998 season from grapes obtained from a warmer Robertson region and a relatively cooler Stellenbosch region. Grapes were harvested at approximately 20.5°B and divided into nine equally representative samples (60 kg per sample). The Sauvignon blanc wine production process is illustrated in **Figure 1**.

Each sample was crushed and different concentrations of ascorbic acid and SO_2 added: Samples 1 - 3 (20 $g.hl^{-1}$ ascorbic acid/meta preparate [supplied by AEB Africa (Pty) Ltd]), samples 4 - 6 (standard + 30 $mg.l^{-1}$ free SO_2) (control) and samples 7 - 9 (10$g.hl^{-1}$ pure ascorbic acid [Univar product no. 118 10 20] + 30 $mg.l^{-1}$ free SO_2). The ascorbic acid/meta preparate is commercially available on the local market. The standard treatment did not include the use of ascorbic acid.

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All juices were subjected to skin contact for six h at 15°C and then pressed at 50 kPa. Pectolytic enzyme (2 $ml.l^{-1}$ juice) was added and the juice stored at 15°C for settling overnight. The clear juice of each sample was divided into three 20 l cannisters, each containing 18 l juice. The three cannisters of each sample were innoculated with *Saccharomyces cerevisiae* yeast strains Vin 13, NT 116 (South African yeast strains, supplied by Anchor Yeast) and VL3C (French yeast strain, supplied by Vintec (Pty) Ltd.), respectively. Rehydration and innoculation were performed according to standard Nietvoorbij practices for small-scale white wine production. Fermentation was performed at 15°C until the wines were dry. The sugar content of the wines was determined by using the Lane and Eynon procedure (Amerine & Ough, 1974). The wines were then bottled and kept at 15°C until sensory evaluation. All treatments were done in triplicate.

2. Sensory evaluation

Wines were sensorially evaluated by an experienced panel of six judges. The panel was previously trained to evaluate the individual characteristics of Sauvignon blanc wine. Wines were evaluated eight months after bottling for fruity/ester aroma intensity, grassy/green pepper aroma intensity, sulphur-like aroma intensity and overall wine quality. A line-method, as illustrated in **Figure 2**, was used, i.e. evaluating the intensity of each characteristic or the quality by marking an unstructured, straight 10 cm line. The wines were also subjected to ranking, using the same sensory characteristics as above. The strongest intensity and highest quality were ranked first and the weakest or lowest, last.

3. Statistical analysis

The standard analysis of variance method and the Friedman two-way analysis of variance method were applied to determine the statistical differences on the results of the sensory evaluation (intensity of each characteristic and overall quality) and ranking of wines, respectively (Siegel, 1956; Snedecor & Cochran, 1980). Least significant differences (LSD) were used to separate treatment means.

Results and discussion

The sensory evaluation results for wines from the Robertson and Stellenbosch regions, are given in **Figures 3** and **4**, respectively. Data are the means of treatments done in triplicate. The ascorbic acid/meta preparate treatment, using

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overall wine quality as parameter, resulted in the lowest quality wines in all cases, irrespective of yeast strain and origin of grapes. This was the result of the high intensities of sulphur-like aromas, which had a masking effect on the fruity/ester and green pepper-like aromas of the wines. The high intensity of sulphur-like nuances might have been caused by contaminating substances in the ascorbic acid/meta preparate and not by the known components themselves. The anti-oxidative properties of both ascorbic acid and meta bisulphite are well-known, and should have had no detrimental effect on wine quality when used correctly (Van Wyk, 1995). However, recent results illustrated that the use of ascorbic acid, with or without SO_2, may also lead to serious oxidation problems in bottled wines (Peng *et al.*, 1999). Treatment with pure ascorbic acid/ SO_2 produced the highest quality wines, due to low sulphur-like and relatively high fruity/ester and grassy/green pepper aroma intensities (**Figures 3** and **4**).

When the data from this study, using the different yeast strains, are compared, it is clear that Vin 13 produced the highest quality wines, irrespective of ascorbic acid and SO_2 treatments, and the origin of grapes. The best treatment combination for the production of quality Sauvignon blanc wines, was found to be the combination of ascorbic acid/SO_2 and Vin 13. The locally developed yeast strain NT 116, however, also performed well. Under the conditions of this investigation, the French yeast strain VL3C did not perform well, mainly due to the formation of relatively high intensities of sulphur-like aromas. When regions are compared, no marked differences were observed on the basis of yeast strain or the ascorbic acid and SO_2 treatments.

Differences between treatments and between yeast strains were also evaluated statistically (**Tables 1** and **2**). These results confirmed those observed individually in **Figures 3** and **4**. Non-significance may be ascribed to interaction between treatments. These results were also confirmed by those of the ranking evaluations (**Tables 3** and **4**). Again, the wines produced from the pure ascorbic acid/SO_2 treatment were in most cases preferred to those of the other two treatments. Yeast strains were not statistically compared in the ranking evaluations.

Conclusions

Yeast strain and the use of ascorbic acid were found to have a significant effect on wine aroma characteristics and wine quality. The combination of pure ascorbic

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acid/SO_2, and the yeast strain Vin 13, as well as NT 116, is recommended for the production of quality cultivar-typical Sauvignon blanc wines under South African conditions. However, ascorbic acid should always be used judiciously.

The question arises whether the sulphur-like aroma, which mainly occurred with the use of the locally available ascorbic acid/meta preparate, was caused by MMP and/or other sulphur-containing components, i.e. do chemical analyses support the sensory data. In the meantime, a new commercially available ascorbic acid/meta preparate was developed, which does not cause the observed undesirable sulphur-like aroma. However, considering the fact that locally produced Sauvignon blanc wines still often present sulphur-like off-odours, studies on the occurrence of these components should be conducted.

Acknowledgements

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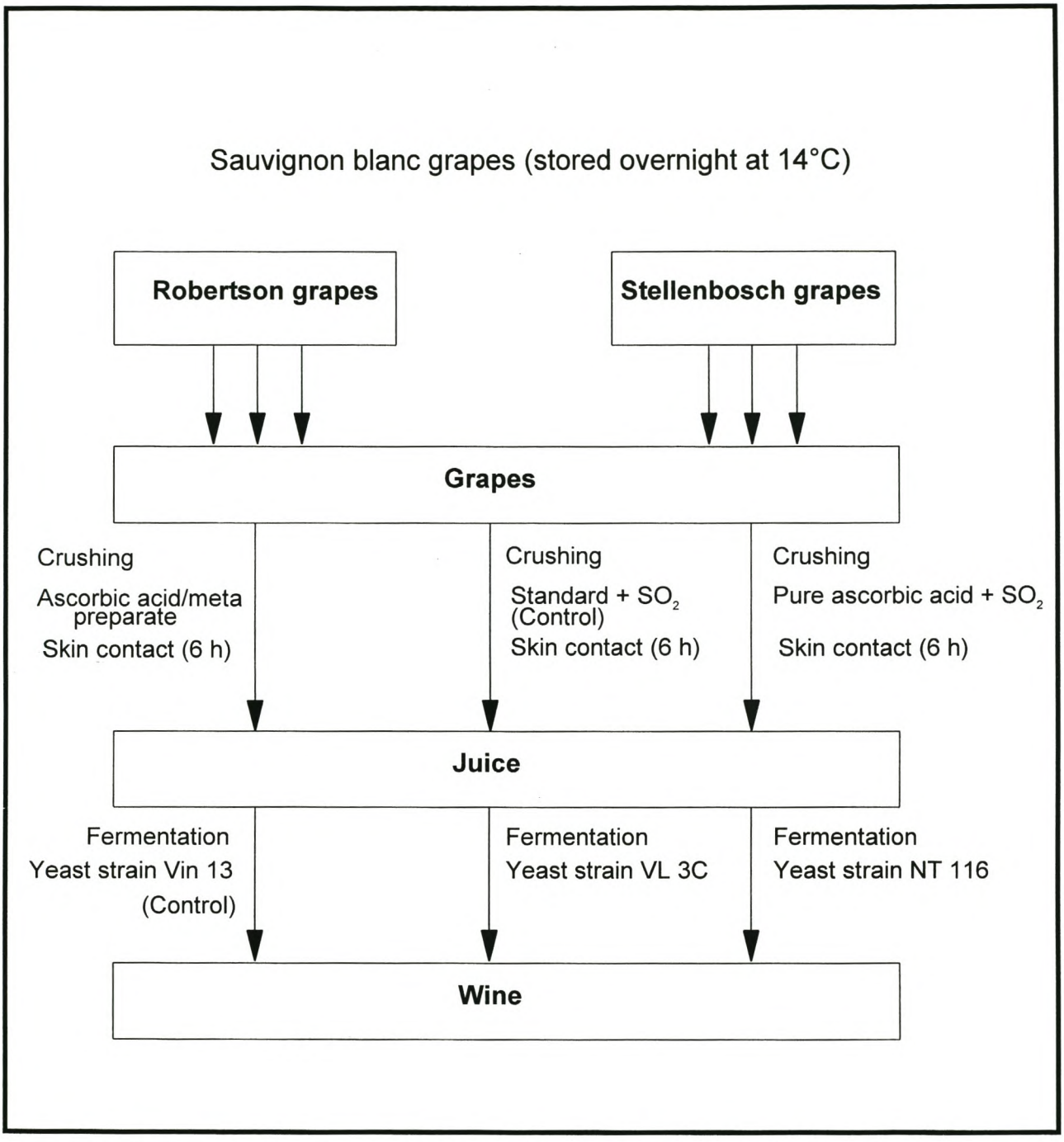

Figure 1. Schematic illustration of the Sauvignon blanc wine production process (1998 season).

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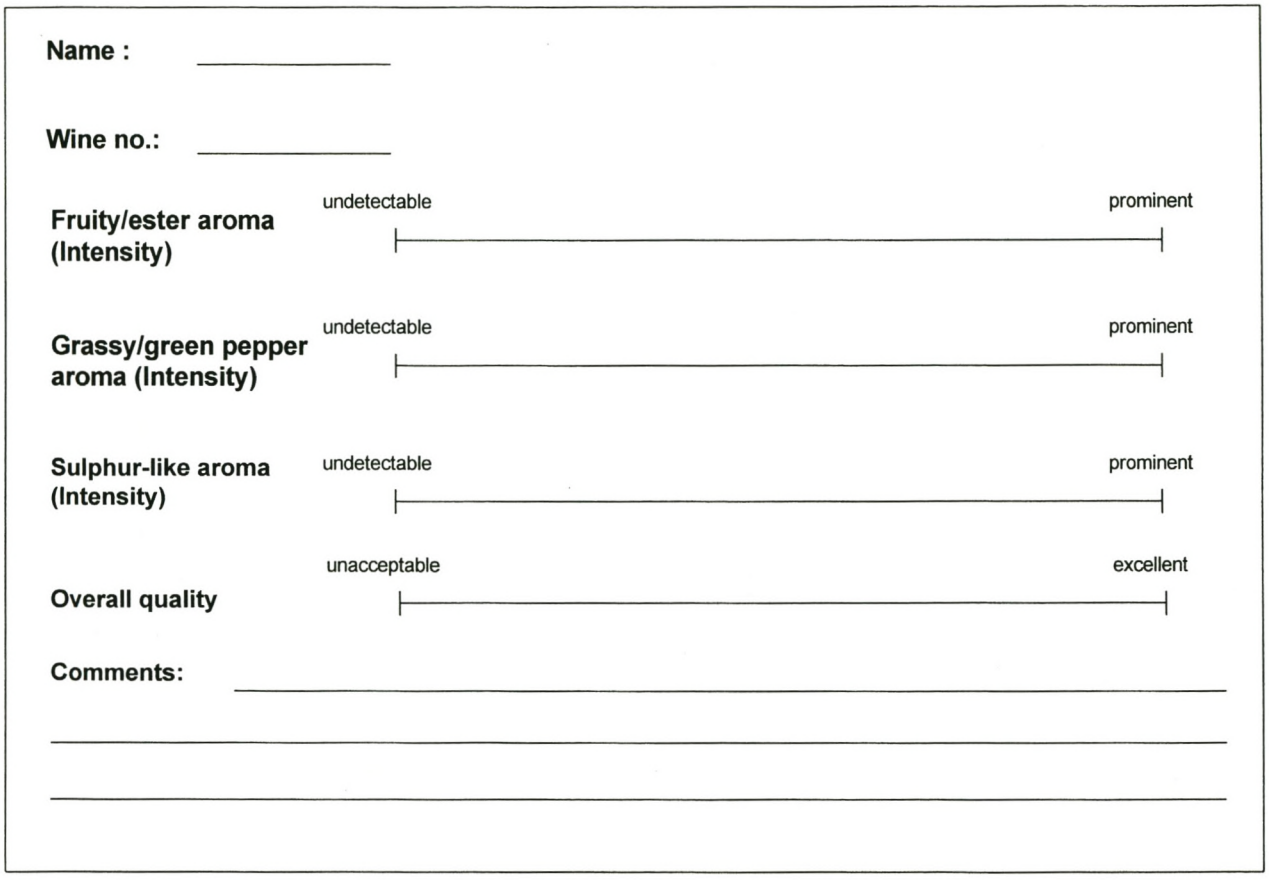

Figure 2. Sauvignon blanc evaluation form.

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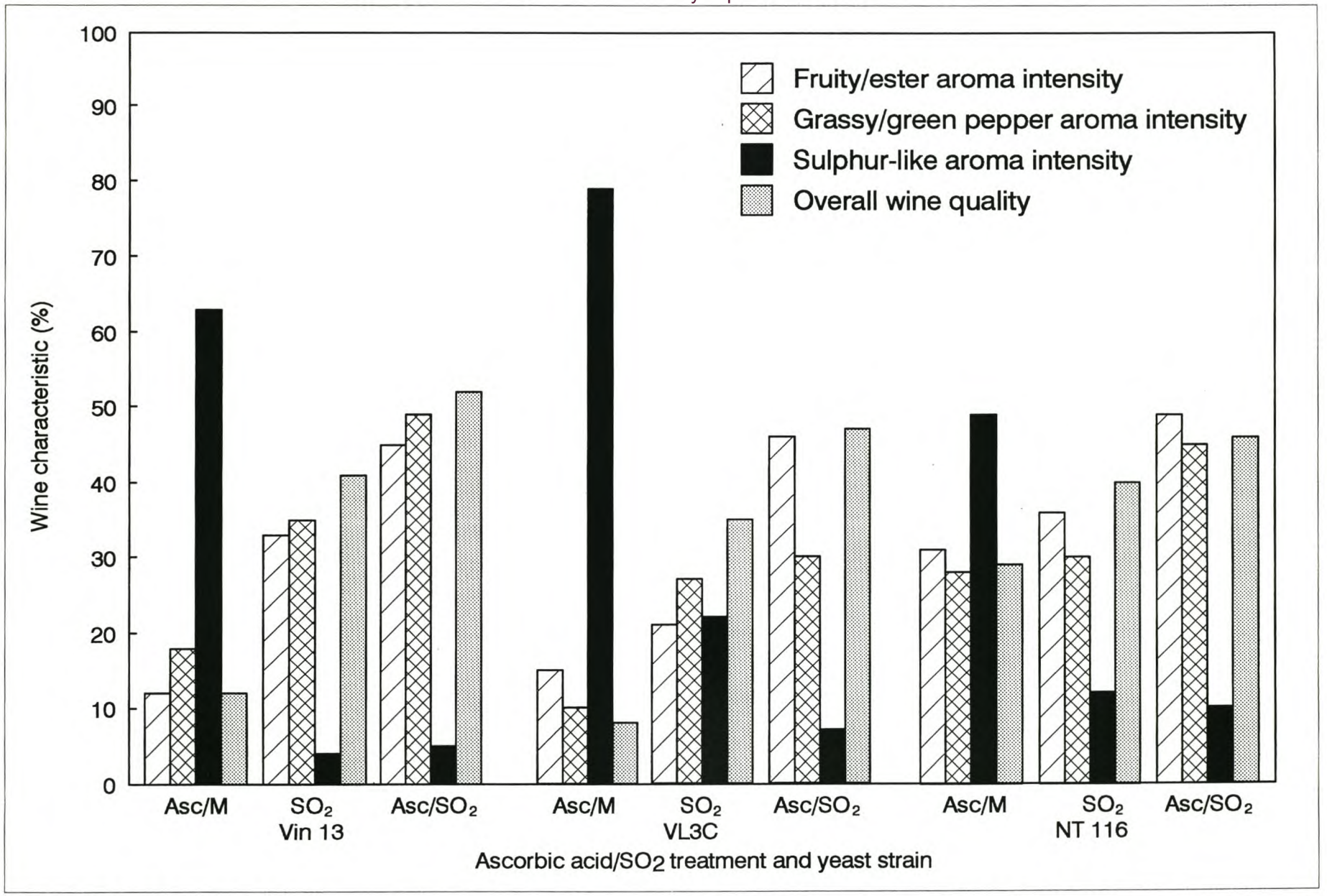

Figure 3. The effect of ascorbic acid/meta preparate (Asc/M), SO_2 and pure ascorbic acid/SO_2 (Asc/SO_2) treatment and yeast strain on Sauvignon blanc wine quality from the Robertson region (1998 season).

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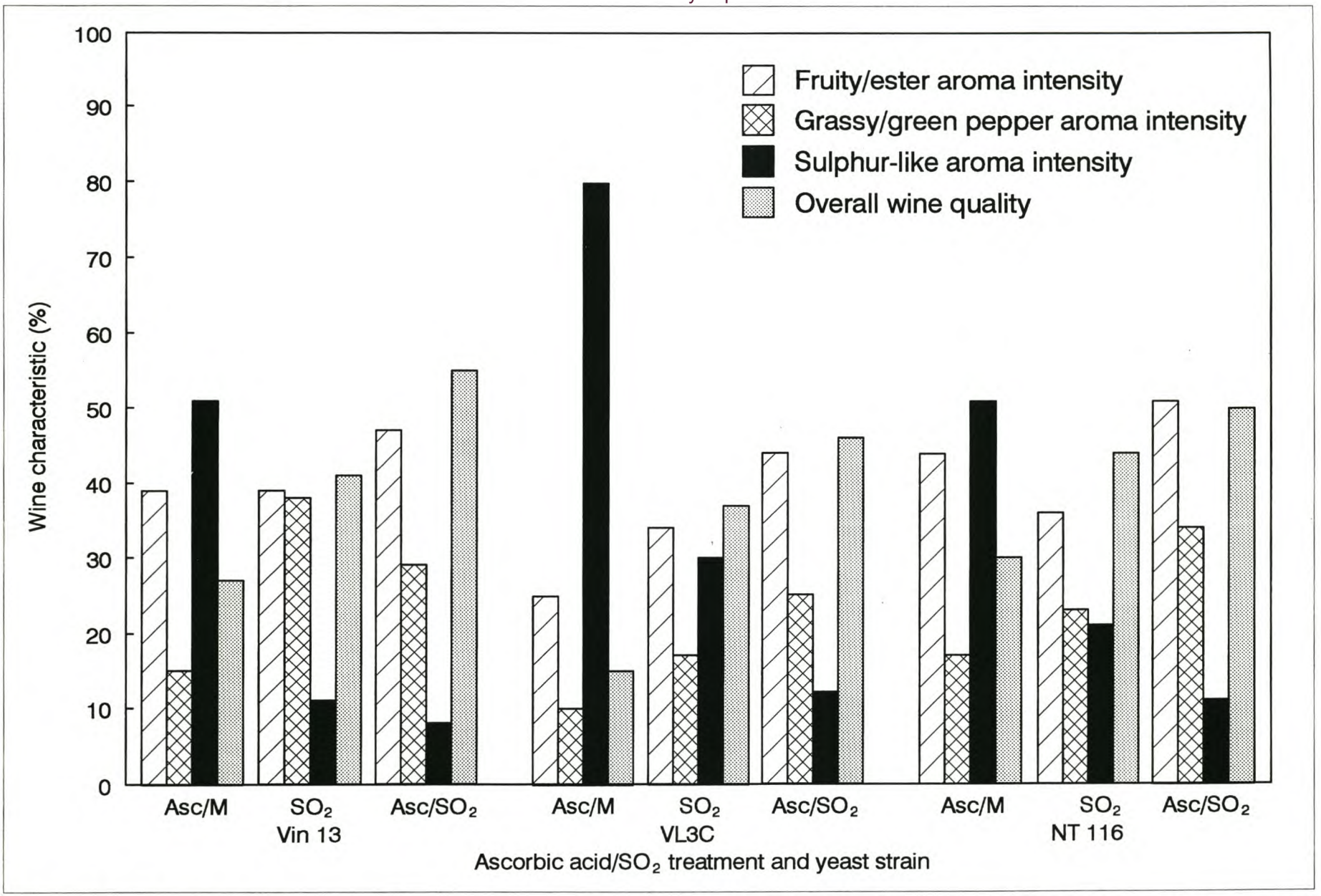

Figure 4. The effect of ascorbic acid/meta preparate (Asc/M), SO_2 and pure ascorbic acid/SO_2 (Asc/SO_2) treatment and yeast strain on Sauvignon blanc wine quality from the Stellenbosch region (1998 season).

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Table 1. The effect of ascorbic acid/meta preparate, SO_2 and pure ascorbic acid/SO_2 treatments on Sauvignon blanc wine quality produced from grapes from the Robertson and Stellenbosch regions (1998 season).

TREATMENT	ROBERTSON REGION				STELLENBOSCH REGION			
	Wine characteristic *							
	Fruity/ester aroma intensity	Grassy/ green pepper aroma intensity	Sulphur-like aroma intensity	Overall wine quality	Fruity/ester aroma intensity	Grassy/ green pepper aroma intensity	Sulphur-like aroma intensity	Overall wine quality
Ascorbic acid/meta preparate	19.222c	18.111c	64.000a	16.000c	36.222a	13.889b	57.500a	25.625b
Standard + SO_2 (Control)	33.333b	30.556b	12.556b	38.333b	36.556a	26.444a	7.000b	52.714a
Pure ascorbic acid/SO_2	47.000a	41.778a	7.444b	49.222a	47.222a	29.222a	10.444b	50.333a

Treatments designated by the same letter do not differ significantly ($p \leq 0.05$).
*Each value represents the average of three yeast strain (Vin 13, VL3C and NT 116) treatments.

Table 2. The effect of yeast strains Vin 13, VL3C and NT 116 on Sauvignon blanc wine quality produced from grapes from the Robertson and Stellenbosch regions (1998 season).

TREATMENT (Yeast strain)	ROBERTSON REGION				STELLENBOSCH REGION			
	Wine characteristic *							
	Fruity/ester aroma intensity	Grassy/ green pepper aroma intensity	Sulphur-like aroma intensity	Overall wine quality	Fruity/ester aroma intensity	Grassy/ green pepper aroma intensity	Sulphur-like aroma intensity	Overall wine quality
Vin 13	30.111a	33.778a	24.000b	35.222ab	41.889a	27.333a	15.625b	48.625a
VL3C	30.889a	22.667b	36.111a	30.222b	34.889a	17.444a	36.000a	35.125b
NT 116	38.556a	34.000a	23.889b	38.111a	43.222a	24.778a	23.750b	44.625a

Treatments designated by the same letter do not differ significantly ($p \leq 0.05$).
*Each value represents the average of three treatments (ascorbic acid/meta preparate, SO_2 and pure ascorbic acid/SO_2).

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Table 3. Ranking evaluation of Sauvignon blanc wines from the Robertson region, produced by different ascorbic acid/SO_2 treatments and three yeast strains (1998 season).

Treatment/Yeast	Wine characteristic*			
	Fruity/ester aroma intensity	Grassy/ green pepper aroma intensity	Sulphur-like aroma intensity	Overall wine quality
Ascorbic acid/meta preparate / Vin 13	41	45	23	42
Standard + SO_2 (Control) / Vin 13	36	36	41	35
Pure ascorbic acid/SO_2 / Vin 13	31	27	44	31
Significance	NS	**	**	NS

Treatment/Yeast	Wine characteristic*			
	Fruity/ester aroma intensity	Grassy/ green pepper aroma intensity	Sulphur-like aroma intensity	Overall wine quality
Ascorbic acid/meta preparate / VL3C	49	46	19	42
Standard + SO_2 (Control) / VL3C	36	35	44	35
Pure ascorbic acid/SO_2 / VL3C	23	27	45	31
Significance	**	**	**	NS

Treatment/Yeast	Wine characteristic*			
	Fruity/ester aroma intensity	Grassy/ green pepper aroma intensity	Sulphur-like aroma intensity	Overall wine quality
Ascorbic acid/meta preparate / NT 116	44	45	20	41
Standard + SO_2 (Control) / NT 116	37	40	43	39
Pure ascorbic acid/SO_2 / NT 116	27	23	45	28
Significance	**	**	**	**

*Each value represents the total score of three replicates by six judges. Lowest values = strongest intensity and highest quality.
** = Significant ($p \leq 0.05$).
NS = Not significant.

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Table 4. Ranking evaluation of Sauvignon blanc wines from the Stellenbosch region, produced by different ascorbic acid/SO_2 treatments and three yeast strains (1998 season).

Treatment/Yeast	Wine characteristic*			
	Fruity/ester aroma intensity	Grassy/ green pepper aroma intensity	Sulphur-like aroma intensity	Overall wine quality
Ascorbic acid/meta preparate / Vin 13	43	41	15	44
Standard + SO_2 (Control) / Vin 13	25	25	35	23
Pure ascorbic acid/SO_2 / Vin 13	22	24	40	23
Significance	**	**	**	**

Treatment/Yeast	Wine characteristic*			
	Fruity/ester aroma intensity	Grassy/ green pepper aroma intensity	Sulphur-like aroma intensity	Overall wine quality
Ascorbic acid/meta preparate / VL3C	41	37	19	33
Standard + SO_2 (Control) / VL3C	27	28	31	29
Pure ascorbic acid/SO_2 / VL3C	22	25	40	28
Significance	**	NS	**	NS

Treatment/Yeast	Wine characteristic*			
	Fruity/ester aroma intensity	Grassy/ green pepper aroma intensity	Sulphur-like aroma intensity	Overall wine quality
Ascorbic acid/meta preparate / NT 116	42	39	21	40
Standard + SO_2 (Control) / NT 116	25	29	31	26
Pure ascorbic acid/SO_2 / NT 116	23	22	38	24
Significance	**	**	**	**

*Each value represents the total score of three replicates by six judges. Lowest values = strongest intensity and highest quality.
** = Significant ($p \leq 0.05$).
NS = Not significant.

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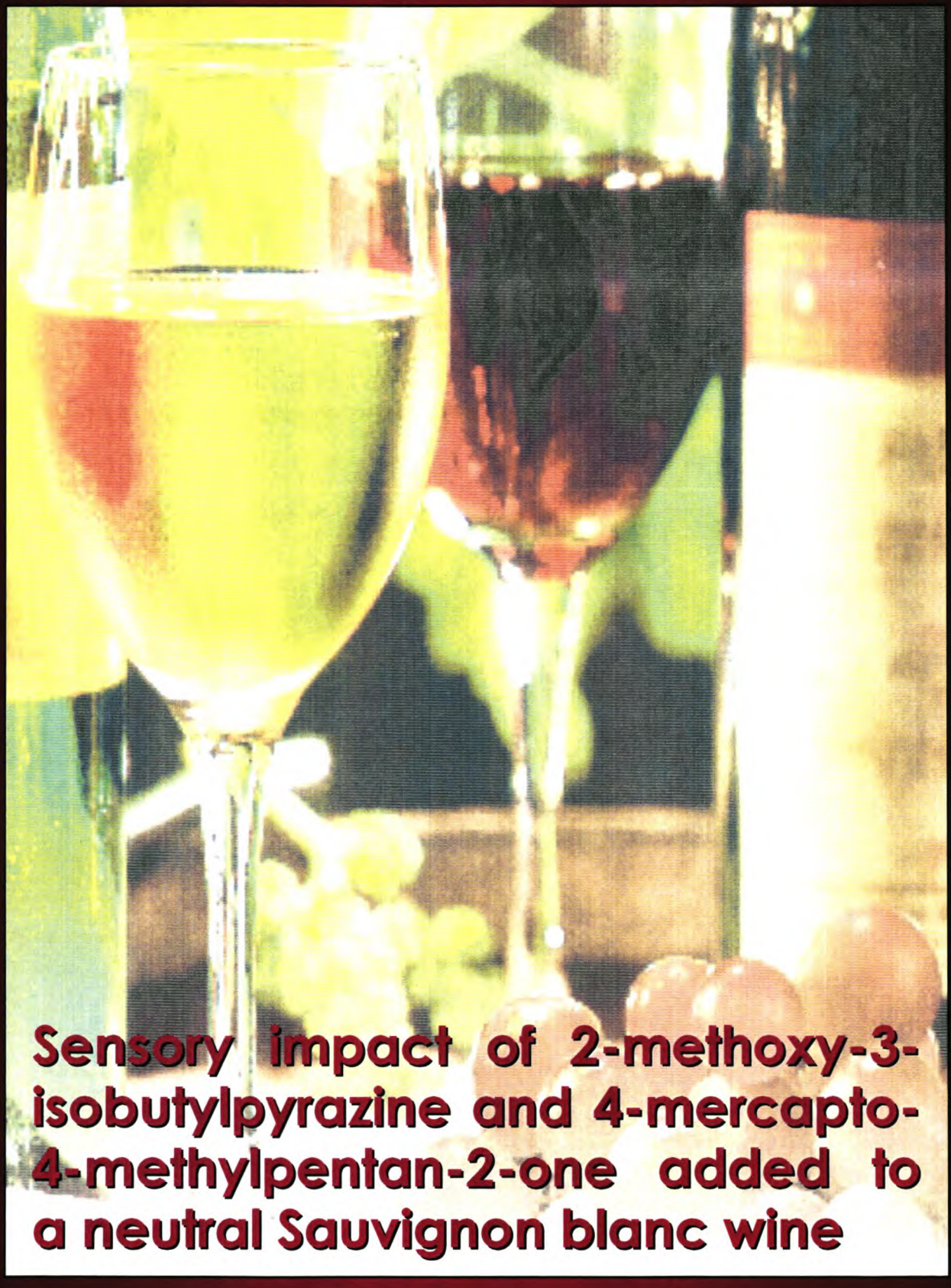
CHAPTER 4



Sensory impact of 2-methoxy-3-isobutylpyrazine and 4-mercapto-4-methylpentan-2-one added to a neutral Sauvignon blanc wine

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CHAPTER 4

SENSORY IMPACT OF 2-METHOXY-3-ISOBUTYLPYRAZINE AND 4-MERCAPTO-4-METHYLPENTAN-2-ONE ADDED TO A NEUTRAL SAUVIGNON BLANC WINE

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Summary

2-Methoxy-3-isobutylpyrazine (ibMP) and 4-mercapto-4-methylpentan-2-one (MMP) are considered amongst the most important Sauvignon blanc impact aroma components. These two components were added, separately and in combination at increasing concentrations, to different media, i.e. deionised water, a neutral non-Sauvignon blanc white wine and a neutral Sauvignon blanc wine. The media were then sensorially evaluated. The nuances perceived, varied from dusty, grassy to green pepper for ibMP and from guava, sulphur-like to cat urine or "conifer" for MMP. It was confirmed that ibMP and MMP are important impact components and that their contribution, either positive or negative, varied according to medium type and composition, as well as to the concentration levels of the components. This may be ascribed to the combined synergistic action of these two components. Other typical Sauvignon blanc aroma nuances, such as asparagus, gooseberry, passion fruit and fig were not detected and the relationship between these aromas and other Sauvignon blanc aroma impact components needs to be further investigated.

Introduction

The typical cultivar aroma of *Vitis vinifera* L. cv. Sauvignon blanc is described as vegetative, grassy, herbaceous, gooseberry, asparagus and green pepper-like. These nuances are mainly caused by a specific group of chemical components, namely methoxypyrazines. The most important contributor appears to be 2-methoxy-3-isobutylpyrazine (ibMP), which normally occurs in much higher concentrations in Sauvignon blanc grapes and wines than other methoxypyrazines, such as 2-methoxy-3-isopropylpyrazine and 2-methoxy-3-sec-butylpyrazine (Allen *et al.*, 1988;

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Allen *et al.*, 1991; Lacey *et al.*, 1991). Other components of special importance to the typical Sauvignon blanc aroma are a group of sulphur-containing components, namely 4-mercapto-4-methylpentan-2-one (MMP), 3-mercaptohexyl acetate, 4-mercapto-4-methylpentan-2-ol and 3-mercaptohexan-1-ol (Dubourdieu *et al.*, 1993; Darriet *et al.*, 1995; Tominaga *et al.*, 1996; Tominaga *et al.*, 1998b). Depending on the concentration level of MMP, it can present a box tree or cat urine aroma (Darriet *et al.*, 1995). This component is formed during fermentation from a cysteine precursor (Tominaga *et al.*, 1998a) and does therefore not occur in grapes and juice. Some of the above-mentioned components have extremely low threshold values, i.e. ibMP: 2 $ng.l^{-1}$ in water and 1 $ng.l^{-1}$ in wine and MMP: 0.1 $ng.l^{-1}$ in water and 3 $ng.l^{-1}$ in wine (Buttery *et al.*, 1969; Allen *et al.*, 1988; Dubourdieu *et al.*, 1993).

In previous studies a correlation between ibMP levels and the perceived typical Sauvignon blanc aroma was not always found (Marais *et al.*,1999). Naturally, the complexity and nuances of Sauvignon blanc aroma will depend on the presence of all impact and other components, and whether they occur at levels above their threshold values. Furthermore, synergism probably plays an important role and the same component can manifest differently in different media. Maga (1989) demonstrated that methoxypyrazine nuances varied according to concentration level and medium. Since ibMP and MMP are widely considered as the most important contributors to Sauvignon blanc aroma, it was decided to investigate the combined synergistic action of these two components. The purpose of this investigation was therefore to determine the effect of added ibMP and MMP, separately and in combination, on the aroma of a neutral Sauvignon blanc wine. In an effort to detect synergistic effects, a neutral non-Sauvignon blanc white wine and water were included as additional evaluation media.

Materials and methods

Material: Two components, ibMP and MMP, were added to 250 ml lots of deionised water, a neutral non-Sauvignon blanc white wine and a neutral Sauvignon blanc wine. Both wines were from the 1998 vintage and locally produced. The components were added at the following concentrations: 0, 2, 4, 8, 16, 24 and 30 $ng.l^{-1}$ for ibMP, and 0, 0.2, 1, 5, 10, 15 and 20 $ng.l^{-1}$ for MMP. The ibMP was prepared by making a stock solution in ethanol from the pure substance, and MMP by making a stock solution in ethanol from a 0.4% MMP in polyethyleneglycol

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solution. This exact percentage was confirmed before use by gas chromatographically-determined peak areas. The concentration levels selected, correspond to actual wine values reported in the literature (Lacey *et al.*, 1991; Allen *et al.*, 1994; Tominaga *et al.*, 1998c). The two components were also added in different combinations (see **Table 1**). The samples were stored in the dark at 18°C under N_2 for one day prior to sensory evaluation.

Sensory evaluation: A panel of eight judges evaluated the samples. Five members of the panel were well-experienced in the evaluation of Sauvignon blanc aroma, while the rest was fairly experienced. No training was done and the judges were allowed to express their own opinions of the aroma nuances perceived. The common, well-known aroma descriptions, such as grassy, green pepper, guava and cat urine were, however mentioned to the judges. The occurrence of the aroma of a conifer shrub (probably *Juniperus sabina*) (Bloom, 1994), which resembles that of cat urine when the foliage is crushed or when it is exposed to full sunlight, was demonstrated. This particular aroma will be referred to as "conifer". Each description of each aroma was firstly individually given on a blank evaluation form without any indicated wine terms. The three media were presented to the judges in sequence of increasing concentrations of the added components (see **Table 1**). Each sample was then discussed afterwards to obtain a uniform description. Written comments were also studied later to confirm the outcome of the discussions.

Results and discussion

The aroma descriptions of the three media to which ibMP and MMP were added separately and in combination, are presented in **Table 1**. Although a neutral Sauvignon blanc wine was chosen, it can be assumed that it contained minute amounts of at least ibMP, which could affected the outcome of the results. Generally, ibMP caused dusty, grassy, herbaceous and green pepper nuances and MMP, "conifer", guava, sulphur-like and cat urine nuances. Prior to additions, the aroma of each wine was mainly detected as fruity nuances, which complemented the perceived ibMP and MMP aromas. Aroma descriptions varied between media, as well as with increasing concentrations. Aroma nuances also became stronger with increasing concentrations. When aroma descriptions between media were compared, some interesting differences became apparent. For example, the dusty

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nuances of ibMP at low levels occurred in water only. When MMP was added separately, guava and cat urine nuances were apparent in the water and neutral non-Sauvignon blanc wine, while sulphur-like/fruity nuances manifested stronger in the neutral Sauvignon blanc wine. Guava aroma was previously reported when MMP was added to Chenin blanc and Colombar wines (Du Plessis & Augustyn, 1981).

Generally, both ibMP and MMP, depending on their concentration levels, presented aromas, which could be perceived as positive or negative. In combination with ibMP, MMP added to the complexity of the wine, which is in agreement with findings of French researchers, namely that MMP in low concentrations plays a positive role in the characteristic aroma of Sauvignon blanc wine (Darriet *et al.*, 1995). Other typical Sauvignon blanc nuances, such as asparagus, gooseberry, tomato leaf, passionfruit and fig were not detected and can therefore not be ascribed to either ibMP or MMP. Asparagus aroma may be ascribed to 2-methoxy-3-isopropylpyrazine, and passionfruit aroma to 3-mercaptohexyl acetate, 4-mercapto-4-methylpentan-2-ol and 3-mercaptohexan-1-ol (Allen *et al.*, 1988; Tominaga *et al.*, 1996; Tominaga *et al.*, 1998b). The above-mentioned components have to be further investigated. In addition, other possible contributors to the typical Sauvignon blanc aroma have to be identified and evaluated.

Conclusions

Specific impact components, namely ibMP and MMP affect Sauvignon blanc aroma. Depending on the nature and composition of the medium, as well as the concentration levels of ibMP and MMP added, different aroma nuances are perceived, which may be ascribed to the combined synergistic action of these two components. Synergism is an important aspect in the evaluation of aroma and further investigations are needed to determine the relationship between typical Sauvignon blanc impact components and aroma.

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Table1. Aroma descriptions of 2-methoxy-3-isobutylpyrazine (ibMP) and 4-mercapto-4-methylpentan-2-one (MMP) added to different media.

Component	Concentration ($ng.l^{-1}$)	Aroma description and medium		
		Deionised water	**Neutral non-Sauvignon blanc wine**	**Neutral Sauvignon blanc wine**
Control	0	None	Only wine aroma/fruity	Only wine aroma/fruity
ibMP	2	Dusty	Only wine aroma/fruity	Only wine aroma/fruity
	4	Dusty/grassy	Grassy/green pepper/fruity	Grassy/fruity
	8	Dusty/grassy/green beans/herbaceous	Grassy /green pepper/fruity	Grassy/fruity
	16	Grassy/green beans/herbaceous	Grassy /green pepper/fruity	Green pepper/grassy
	24	Green pepper/green beans/herbaceous	Green pepper/fruity	Green pepper/grassy
	30	Green pepper/herbaceous	Green pepper/fruity	Green pepper/grassy
MMP	0.2	None	Only wine aroma/fruity	Sulphur-like/fruity
	1	Guava/cat urine/sulphur-like	Only wine aroma/fruity	Sulphur-like/fruity
	5	Cat urine/"conifer"	Guava/fruity/cat urine	Sulphur-like/fruity
	10	Cat urine/"conifer"	Fruity/cat urine	Sulphur-like/fruity
	15	Cat urine/"conifer"	Cat urine/guava	Sulphur-like/fruity/"conifer"
	20	Cat urine/"conifer"	Cat urine/guava/fruity	Sulphur-like/"conifer"
ibMP/MMP	2/0.2	Dusty/grassy/herbaceous	Fruity	Fruity
	4/1	Dusty/grassy/herbaceous	Fruity/grassy	Fruity/herbaceous
	8/5	Cat urine/grassy/herbaceous	Grassy/green pepper	Grassy/herbaceous
	16/10	Grassy/herbaceous/sulphur-like/green pepper	Grassy/green pepper/guava	Green pepper/grassy/fruity
	24/15	Grassy/herbaceous/sulphur-like/green pepper	Grassy/green pepper/guava	Green pepper/grassy/fruity
	30/20	Herbaceous/green pepper/cat urine	Grassy/guava/cat urine	Green pepper/grassy/fruity
	2/20	Cat urine/"conifer"	Grassy/guava/cat urine/sulphur-like	Sulphur-like/fruity/grassy
	4/15	Cat urine/guava/fruity	Grassy/fruity/guava/sulphur-like	Sulphur-like/fruity/grassy
	8/10	Grassy/green pepper	Grassy/green pepper/sulphur-like	Grassy/green pepper/fruity
	16/5	Grassy/herbaceous/dusty	Grassy/green pepper/sulphur-like	Grassy/green pepper
	24/1	Grassy/dusty	Grassy/green pepper	Grassy/green pepper
	30/0.2	Grassy/dusty	Grassy/green pepper	Grassy/green pepper

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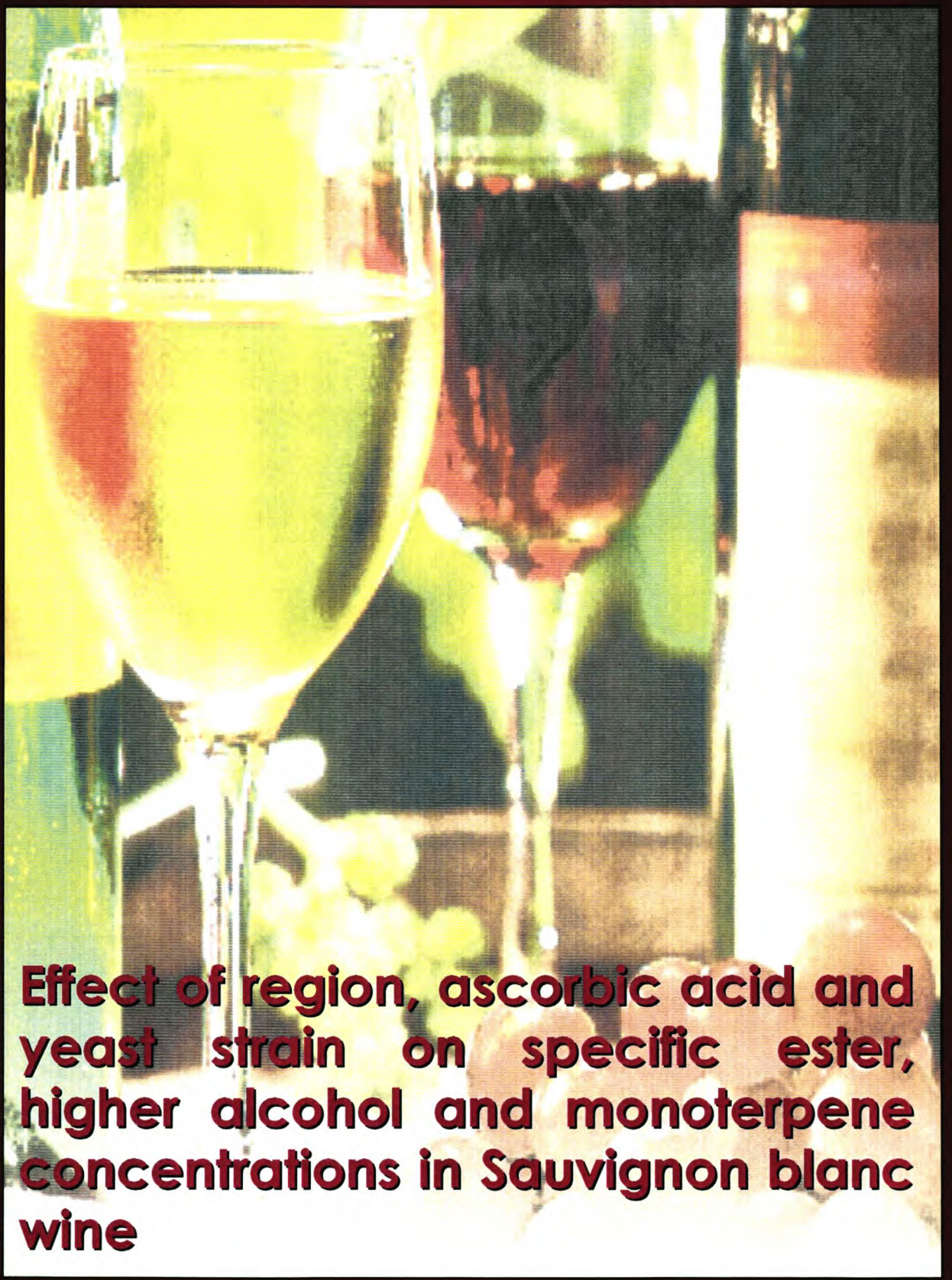
CHAPTER 5



Effect of region, ascorbic acid and yeast strain on specific ester, higher alcohol and monoterpene concentrations in Sauvignon blanc wine

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CHAPTER 5

EFFECT OF REGION, ASCORBIC ACID AND YEAST STRAIN ON SPECIFIC ESTER, HIGHER ALCOHOL AND MONOTERPENE CONCENTRATIONS IN SAUVIGNON BLANC WINE

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Summary

Climatic, viticultural and oenological effects play a significant role in the development of aroma components in grapes and wines. The effect of region, yeast strain and ascorbic acid/SO_2 treatments and yeast strain on ester, higher alcohol and monoterpene concentrations, potentially contributors to fruity/ester/floral-like aromas, were investigated in Sauvignon blanc wines. The wines were analysed by gas chromatography and sensorially evaluated. Significant differences in ester concentrations, higher alcohol concentrations, fruity/ester aroma intensity and overall wine quality were observed in the wines, treated with the different ascorbic acid/SO_2 treatments and fermented with different yeast strains. Generally, the pure ascorbic acid/SO_2 treatment and yeast strains, Vin 13 and NT 116, produced the highest ester, the lowest higher alcohol concentrations, and the highest quality wines. Although, the Sauvignon blanc in this study lacked the cultivar-typical aromas to some extent, ester concentrations were not too high to overpower these aromas, and actually contributed to the complexity thereof. The judicious use of ascorbic acid, together with SO_2, and yeast strains Vin 13 and NT 116 are recommended for the production of high quality Sauvignon banc wines.

Introduction

Aroma and quality are the most important attributes of wine and are the result of the occurrence of specific chemical constituents in grapes and wine. These chemical components can be useful indicators of the flavour potential and quality of wine. The

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distinctive aroma of Sauvignon blanc makes this cultivar highly valued amongst white wine cultivars in South Africa (Anon., 1999). Considerable research has been done to characterise the volatile profile of Sauvignon blanc wine (Marais, 1994). Aroma components of this cultivar can basically be divided into two groups, i.e. grape-related components (methoxypyrazines, monoterpenes and norisoprenoids) and components formed during fermentation (mercapto components, esters and higher alcohols) (Marais, 2000).

Methoxypyrazines are regarded as the most important impact aroma components present in Sauvignon blanc. 2-Methoxy-3-isobutylpyrazine (ibMP), 2-methoxy-3-isopropylpyrazine (ipMP) and 2-methoxy-3-sec-butylpyrazine are normally responsible for the characteristic vegetative, grassy, green pepper or asparagus-like aromas of this cultivar (Allen *et al.*, 1988; Allen *et al.*, 1991; Lacey *et al.*, 1991). Together with methoxypyrazines, sulphur-containing components, such as 4-mercapto-4-methylpentan-2-one (MMP), 4-mercapto-4-methylpentan-2-ol, 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate also contribute to the varietal aroma of Sauvignon blanc wine (Dubourdieu *et al.*, 1993; Darriet *et al.*, 1995; Tominaga *et al.*, 1996; Tominaga *et al.*, 1998). However, high concentrations of some of these mercapto components, especially MMP, often, depending on the concentration, may lead to box tree or cat urine off-flavours, which are regarded as negative and not typical of Sauvignon blanc wine aroma (Marais & Swart, 1999).

Apart from the above mentioned, cultivar-typical impact components, grape-derived monoterpenes and norisoprenoids, as well as fermentation formed esters and higher alcohols can also contribute to the overall aroma, quality and complexity of Sauvignon blanc wine (Swart *et al.*, 2000). This is especially relevant in the case of Sauvignon blanc wines, as in this study, which tend to be relatively neutral. Typical descriptions for aromas of some of the monoterpenes and norisoprenoids are floral and spice-like, while esters and higher alcohols are responsible for the fruity nuances in wines. Besides ethyl acetate, which is considered to be the most commonly occurring ester in wine, other ethyl esters (ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate) and acetate esters of higher alcohols (iso-butyl acetate, iso-amyl acetate, hexyl acetate, 2-phenylethyl acetate) are also present in wine. The main higher alcohols occurring in wine, are iso-butanol, iso-amyl alcohol, hexanol and 2-phenyl ethanol.

The presence of esters and higher alcohols in concentrations higher than their threshold values in wine, implicates that they may have a significant effect on the

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fruity, ester-like aromas of wines. Treatments like skin-contact, pressing and reductive wine making techniques by means of the use of ascorbic acid/SO_2 treatments, can enhance these aromas (Houtman *et al.*, 1980; Williams *et al.*, 1983; Marais, 1985; Van Wyk, 1995). Generally, viticultural and oenological practices can be manipulated in order to optimise the development of most aroma components (Marais & Van Wyk, 1986; Marais & Rapp, 1988; Marais, 1990; Marais *et al.*, 1992a; Marais *et al.*, 1992b; Marais *et al.*, 1996; Marais, 1998; Marais *et al.*, 1999; Swart *et al.*, 2000).

The aim of this study was to evaluate the effect of region, the use of ascorbic acid/SO_2 treatments and yeast strain on the specific ester, higher alcohol, monoterpene and methoxypyrazine contents and their role in the aroma and quality of Sauvignon blanc wines.

Materials and methods

1. Wine production

Sauvignon blanc wines were produced from grapes from the warmer Robertson and relatively cooler Stellenbosch regions during the 1998 harvest season (Swart *et al.*, 2000). Grapes were harvested at approximately 20.5°B and divided into nine equally representative samples (60 kg per sample). Each sample was crushed and treated with different concentrations of ascorbic acid and SO_2. Samples 1-3 (20g.l^{-1} ascorbic acid/meta preparate [supplied by AEB Africa (Pty) Ltd]), samples 4-6 (standard + 30 mg.l^{-1} free SO_2) (control) and samples 7-9 (10 g.hl^{-1} pure ascorbic acid [Univar product no. 118 10 20] + 30 mg.l^{-1} SO_2). The ascorbic acid/meta preparate is commercially available on the local market. Ascorbic acid was not used with the standard treatment (control).

All juices were subjected to skin-contact for six hours at 15°C and afterwards pressed at 50 kPa. After the addition of pectolytic enzyme (2 ml.l^{-1} juice), the juice was stored at 15°C for settling overnight. The clear juice of each sample was divided into three cannisters, each containing 18 l juice. The three cannisters of each sample were innoculated with *Saccharomyces cerevisiae* yeast strains, Vin 13, NT 116 (South African yeast strains, supplied by Anchor Yeast) and VL3C (French yeast strain, supplied by Vintec (Pty) Ltd.), respectively. The yeasts were rehydrated according to prescribed instructions for 20 minutes in a waterbath at 38°C and of

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which 3 ml.l^{-1} juice were innoculated. Fermentation was performed at 15°C until the wines were dry. The wines were then bottled and kept at 15°C until analyses and sensory evaluation. All treatments were done in triplicate.

2. Ester, higher alcohols and monoterpene analyses

Extraction of wine

A volume of 250 ml wine of each treatment was subjected to liquid-liquid extraction by Freon 11, according to the technique described by Marais (1986). The freon extracts were concentrated to 100 µl.

Gas chromatography

Gas chromatographic analyses were performed using a Hewlett Packard 5880 gas chromatograph with an automatic peak integrator. The capillary column and gas chromatographic conditions used, were:

Column:	Supelcowax 10 fused silica
	(60 m X 0.32 mm i.d. X 0.25 µm)
Injection temperature:	200°C
FID temperature:	250°C
Temperature programme:	10 min at 60°C
	1°C.min^{-1} up to 190°C
	30 min. at 190°C
Carrier gas:	Helium
Column flow rate:	1.5 ml.min^{-1}
Split ratio:	60:1
Injection volume:	0.8 µl

The esters and higher alcohols analysed, were ethyl butyrate (EtC4), ethyl hexanoate (EtC6), ethyl octanoate (EtC8), ethyl decanoate (EtC10), iso-butyl acetate (iBuAc), iso-amyl acetate (iAmAc), hexyl acetate (HexAc), 2-phenyl ethyl acetate (2FEA), iso-butanol (iBuOH), iso-amyl alcohol (iAmOH), hexanol (HexOH) and 2-phenyl ethanol (2FE). The monoterpenes analysed, were trans-furan linalool oxide, linalool, alpha-terpineol, citronellol, nerol and diendiol-1. The ester, higher alcohol and monoterpene concentrations were expressed as relative concentrations in relation to

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an internal standard (0.5 ml 2-ethyl hexanol at 80 $\mu g.l^{-1}$). The identities of the above-mentioned components were confirmed by the retention times of standards analysed under similar conditions.

3. Methoxypyrazine analyses

The techniques for the determination of ibMP, specifically, were as given by Harris *et al.* (1987) and Lacey *et al.* (1991).

4. Sensory evaluation

Wines were sensorially evaluated for fruity/ester intensity and overall wine quality by an experienced panel of six judges, eight months after bottling. A line-method was used by marking on an unstructured, straight 10 cm line (Swart *et al.*, 2000). The terms "undetectable" and "prominent" were used to qualify the left and right hand ends, respectively, for the fruity/ester intensity, and "unacceptable" and "excellent", respectively, for wine quality.

5. Statistical analyses

The significance of the effect of region, ascorbic acid treatments and yeast strain on ester, higher alcohol and monoterpene concentrations and on sensory data was statistically evaluated, according to the standard analysis of variance method (Snedecor & Cochran, 1980). Least significant differences (LSD) were used to separate treatment means of the relative concentrations of esters, higher alcohols and monoterpenes (SAS, 1990).

Results and discussion

The total relative ester concentrations were determined in the Sauvignon blanc wines from both Robertson and Stellenbosch regions are given in **Figure 1**. Generally, the highest ester concentrations were produced by the ascorbic acid/meta treatment, and the lowest by the control (only SO_2) treatment. However, in the Robertson region, nearly similar ester levels were produced by both ascorbic acid/SO_2 treatments. It appears that NT 116, and in some cases also Vin 13, produced the highest ester concentrations and VL3C the lowest, irrespective of the ascorbic acid/SO_2 treatments. Furthermore, the ester levels were markedly higher in the Robertson than in the Stellenbosch wines. These findings were statistically confirmed when the

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individual esters were evaluated (**Tables 1 and 2**).

The total relative higher alcohol concentrations in the wines, from the Robertson and Stellenbosch regions, are given in **Figure 2**. Generally, lower higher alcohol concentrations were produced by both ascorbic acid/SO_2 treatments, compared to the control (SO_2 treatment). The higher alcohol levels were markedly higher in the wines from the Stellenbosch than from the Robertson region. When the yeast strains were compared, tendencies differed between regions. Generally, the highest levels were produced by the French yeast strain in the Stellenbosch wines. These trends were statistically confirmed where the individual higher alcohols were concerned (**Tables 3 and 4**).

It appears that the different ascorbic acid/SO_2 treatments and yeast strains did not have a pronounced effect on monoterpene levels (**Figure 3**). This was expected, since grape-derived monoterpenes are not usually affected by these parameters. Apart from that, monoterpene concentrations were clearly higher in the cooler Stellenbosch than in the warmer Robertson wines. Although statistical differences in monoterpene levels were observed between treatments and between yeast strains, these values were generally of the same magnitude for each individual monoterpene (**Tables 5 and 6**). Therefore, it is doubtful if differences in aroma intensity or quality of the wines were the result of the effect of monoterpenes.

Both ascorbic acid/SO_2 treatments gave relatively good results, regarding higher ester and lower higher alcohol concentrations. However, apart from the undesirable, sulphur-like aroma, produced by the ascorbic acid/meta preparate in this study (Swart *et al.*, 2000), recent results showed that the use of ascorbic acid in general may also lead to serious oxidation problems in wine after bottling (Peng *et al.*, 1998). Therefore it can be deduced that the control (only SO_2) treatment should not be rejected on account of the possible production of lower ester and higher higher alcohol concentrations, and that pure ascorbic acid should be used with utmost care. It should also be stated that a new ascorbic acid/meta preparate is now commercially available, which apparently does not produce the negative sulphur-like aroma that was observed in this investigation (Swart *et al.*, 2000).

The Sauvignon blanc wines, used in this study, were relatively low in the cultivar-typical, grassy/green pepper character. This corresponds to the ibMP levels below the threshold value, which could not be reliably analysed. Therefore results on this component were not given.

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With regard to the sensory evaluation of fruitiness intensity and overall wine quality, it was evident that the pure ascorbic acid/SO_2 treatment and yeast strains, Vin 13 and NT 116, produced the highest quality wines in both Robertson and Stellenbosch regions (**Tables 7 and 8**). This coincided with relatively high ester and low higher alcohol concentrations. Clearly, the ester concentrations were not as high as to overpower the cultivar-typical Sauvignon blanc character. Apart from this, differences in wine quality between treatments and between yeast strains were strongly affected by the undesirable sulphur-like aromas caused by the ascorbic acid/meta and VL3C yeast strain combination (Swart *et al.*, 2000).

Conclusions

Aroma components like esters, higher alcohols and monoterpenes are essential to the complexity and quality of Sauvignon blanc wine. The levels in which these components occur, are dependent on various factors, such as grape composition as affected by region/climate, yeast strain and the use of ascorbic acid. They become especially important when Sauvignon blanc wine lack cultivar-typical aroma. However, these fruity floral-like aromas should never overpower the typical Sauvignon blanc character, but only contribute to the complexity thereof. The use of ascorbic acid in a judicious manner together with SO_2, and yeast strains Vin 13 and NT 116 are recommended for the production of high quality Sauvignon blanc wines.

Acknowledgements

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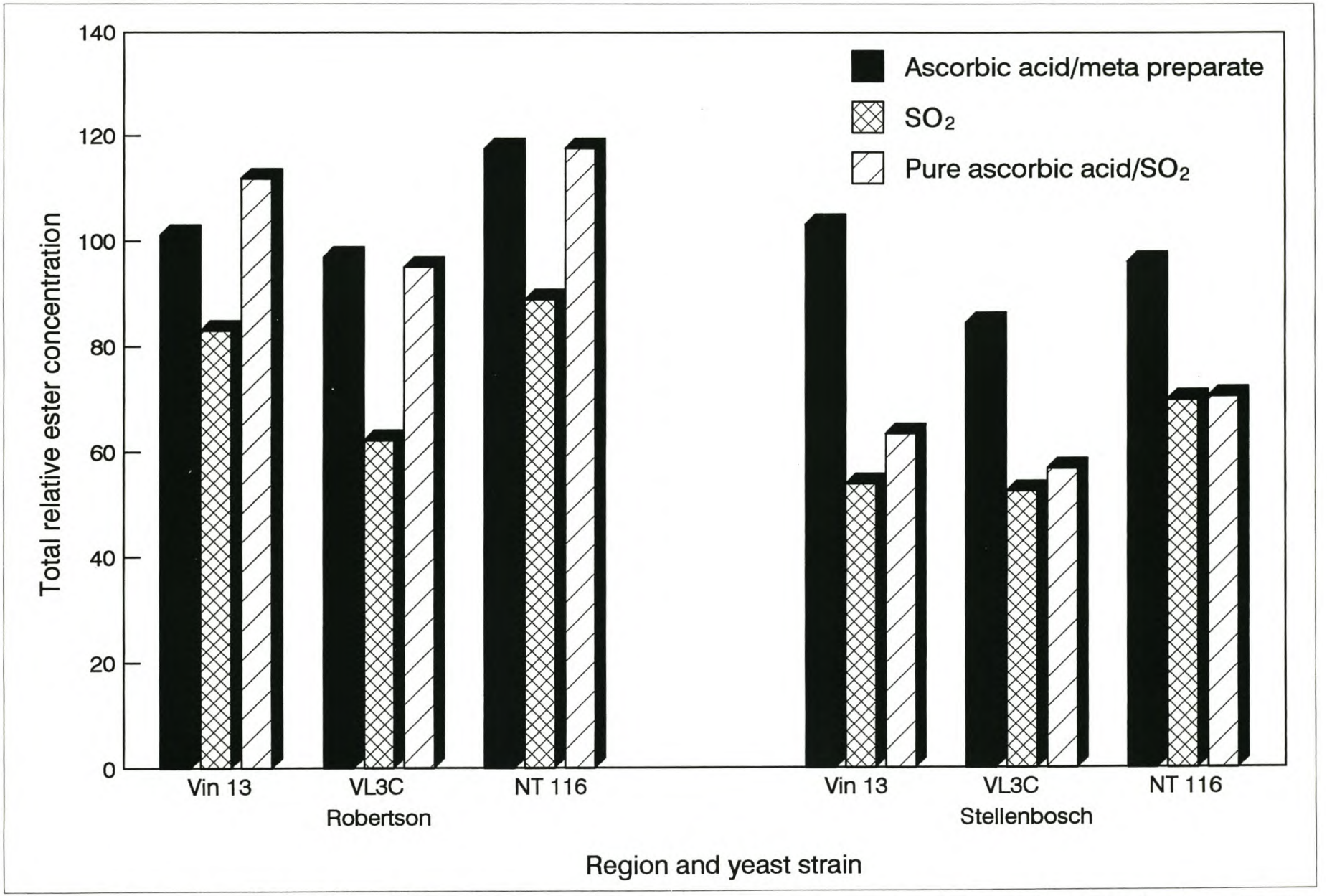

Figure 1. The effect of ascorbic acid/meta preparate (Asc/M), SO_2 (Control) and pure ascorbic acid/SO_2 (Asc/SO_2) treatment and yeast strain on the total ester concentration in Sauvignon blanc wines from the Robertson and Stellenbosch regions (1998 season).

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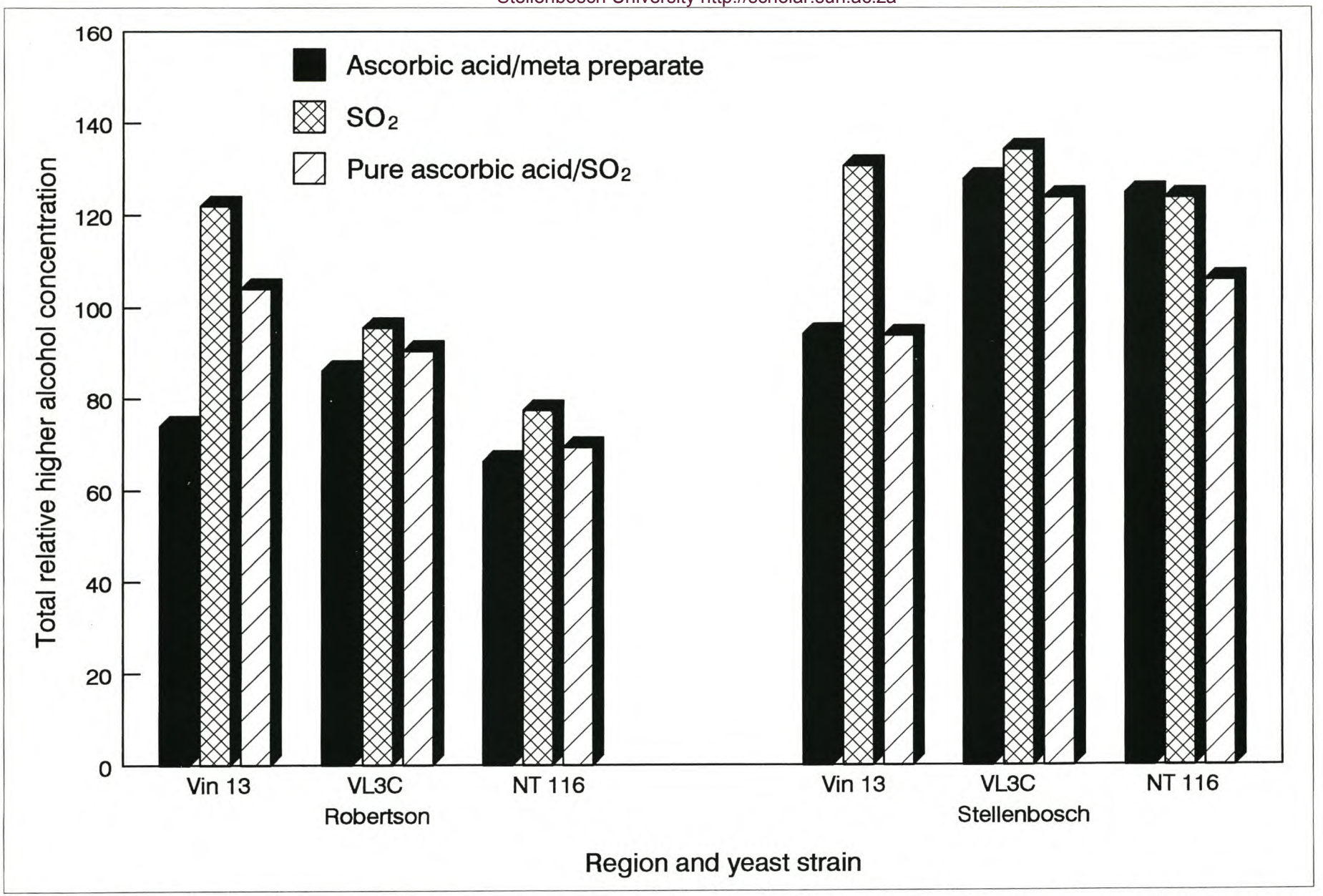

Figure 2. The effect of ascorbic acid/meta preparate (Asc/M), SO_2 (Control) and pure ascorbic acid/SO_2 (Asc/SO_2) treatment and yeast strain on the total higher alcohol concentration in Sauvignon blanc wines from the Robertson and Stellenbosch regions (1998 season).

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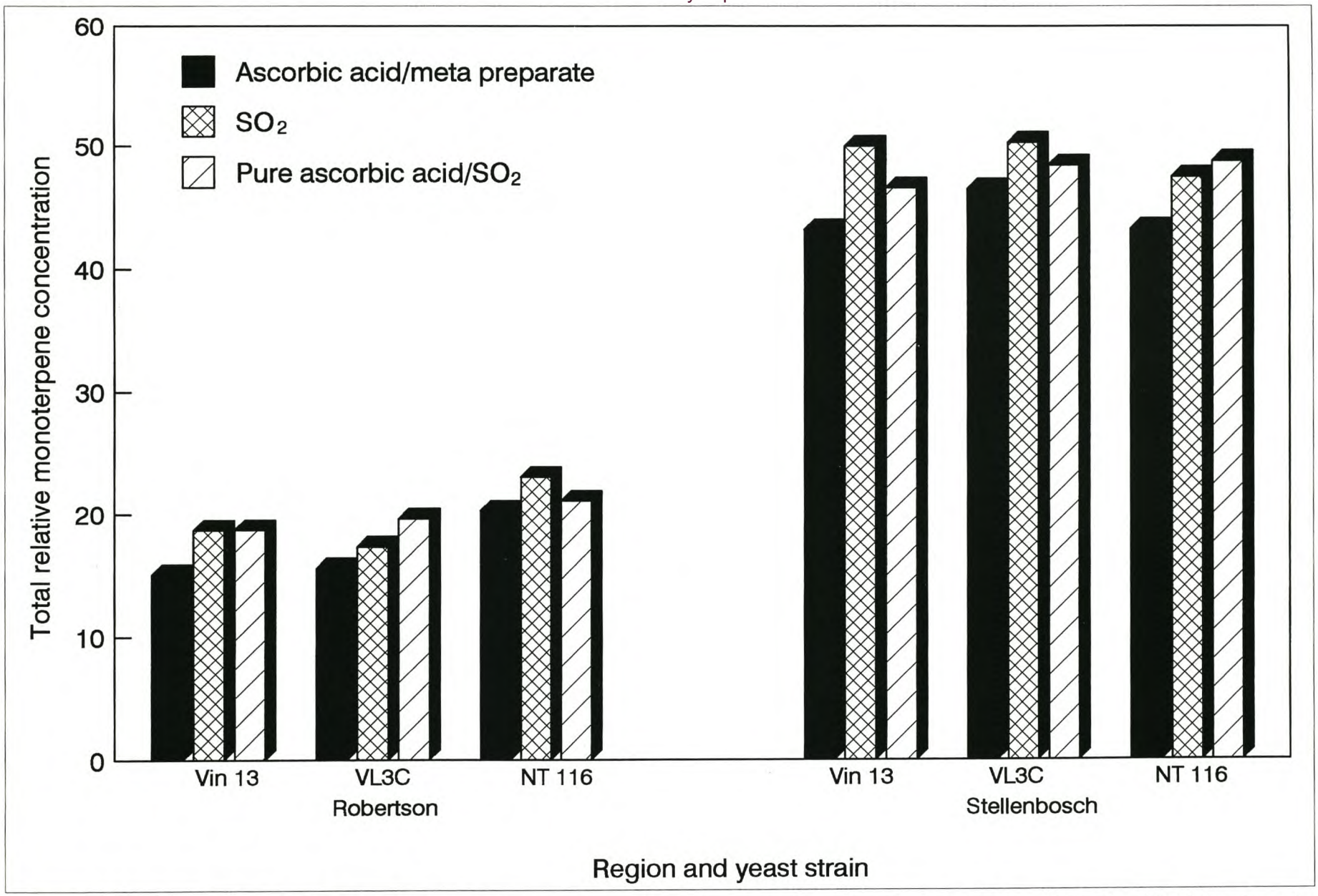

Figure 3. The effect of ascorbic acid/meta preparate (Asc/M), SO_2 (Control) and pure ascorbic acid/SO_2 (Asc/SO_2) treatment and yeast strain on the total monoterpene concentration in Sauvignon blanc wines from the Robertson and Stellenbosch regions (1998 season).

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Table 1. The effect of ascorbic acid/meta preparate, SO_2 and pure ascorbic acid/SO_2 treatments on individual relative ester concentrations in Sauvignon blanc wines of the Robertson and Stellenbosch regions (1998 season).

Treatment	Robertson							
	Relative ester concentration							
	EtC4	EtC6	EtC8	EtC10	iBuAc	iAmAc	HexAc	2FEA
Ascorbic acid/meta preparate	1.081a	6.907a	3.031a	10.452a	7.207a	42.942a	31.408a	2.065a
Standard + SO_2 (Control)	1.022a	6.092b	2.676b	8.977b	5.588b	28.508b	23.660b	1.509b
Pure ascorbic acid/SO_2	1.060a	7.097a	3.177a	10.737a	7.134a	37.802a	35.958a	1.649ab
Treatment	Stellenbosch							
	Relative ester concentration							
	EtC4	EtC6	EtC8	EtC10	iBuAc	iAmAc	HexAc	2FEA
Ascorbic acid/meta preparate	1.583a	7.654a	12.056a	3.327a	8.044a	41.617a	16.699a	3.484a
Standard + SO_2 (Control)	1.121b	6.151b	9.831b	2.790b	4.825b	29.775ab	11.898b	2.564b
Pure ascorbic acid/SO_2	1.022b	6.666b	10.314b	2.849ab	4.875b	23.680b	11.409b	2.918ab

Treatments designated by the same letter do not differ signifcantly ($p \leq 0.05$).
Each value represents the average of the three yeast strains (Vin 13, VL3C and NT 116) treatments. EtC4 = ethyl butyrate; EtC6 = ethyl hexanoate; EtC8 = ethyl octanoate; EtC10 = ethyl decanoate; iBuAc = iso-butyl acetate; iAmAc = iso-amyl acetate; HexAc = hexyl acetate; 2FEA = 2-phenylethyl acetate.

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Table 2. The effect of yeast strain on individual relative ester concentrations in Sauvignon blanc wines from the Robertson and Stellenbosch regions (1998 season).

Treatment (Yeast strain)	Robertson							
	Relative ester concentration							
	EtC4	EtC6	EtC8	EtC10	iBuAc	iAmAc	HexAc	2FEA
Vin 13	1.028b	7.599a	10.751a	3.054a	6.275b	39.962a	28.316b	1.499b
VL3C	0.775c	7.285a	10.779a	2.947a	5.756b	26.701b	25.891b	1.326b
NT 116	1.359a	5.211b	8.636b	2.883a	7.898a	42.590a	36.819a	2.397a
Treatment (Yeast strain)	Stellenbosch							
	Relative ester concentration							
	EtC4	EtC6	EtC8	EtC10	iBuAc	iAmAc	HexAc	2FEA
Vin 13	1.057b	7.324a	11.382a	3.147a	8.044a	39.073a	12.099b	2.609b
VL3C	0.964b	7.571b	11.871a	3.136a	4.825b	22.671b	11.525b	2.352b
NT 116	1.705a	5.576b	8.948b	2.656b	4.875b	33.328ab	16.382a	4.005a

Treatments designated by the same letter do not differ signifcantly ($p \leq 0.05$).
Each value represents the average of the ascorbic acid/SO_2 treatments (ascorbic acid/meta preparate, SO_2, and pure ascorbic acid/SO_2). EtC4 = ethyl butyrate; EtC6 = ethyl hexanoate; EtC8 = ethyl octanoate; EtC10 = ethyl decanoate; iBuAc = iso-butyl acetate; iAmAc = iso-amyl acetate; HexAc = hexyl acetate; 2FEA = 2-phenylethyl acetate.

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Table 3. The effect of ascorbic acid/meta preparate, SO_2 and pure ascorbic acid/SO_2 treatments on individual relative higher alcohol concentrations in Sauvignon blanc wines from the Robertson and Stellenbosch regions (1998 season).

Treatment	Robertson			
	Relative higher alcohol concentrations			
	iBuOH	iAmOH	HexOH	2FE
Ascorbic acid/meta preparate	8.655a	53.083a	12.114c	7.436b
Standard + SO_2 (Control)	10.722a	60.716a	17.477a	8.784a
Pure ascorbic acid/SO_2	8.121a	55.467a	13.759b	7.403b
Treatment	**Stellenbosch**			
	Relative higher alcohol concentrations			
	iBuOH	iAmOH	HexOH	2FE
Ascorbic acid/meta preparate	20.116a	74.731a	6.983b	14.184b
Standard + SO_2 (Control)	19.197a	81.395a	10.028a	19.360a
Pure ascorbic acid/SO_2	15.036a	66.123a	9.154a	19.149a

Treatments designated by the same letter do not differ signifcantly ($p \leq 0.05$).
Each value represents the average of the three yeast strain (Vin 13, VL3C, NT 116) treatments. iBuOH = iso-butanol; iAmOH = iso-amyl alcohol; HexOH = hexanol; 2FEA = 2-phenylethanol.

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Table 4. The effect of yeast strain on individual higher alcohol concentrations in Sauvignon blanc wines from the Robertson and Stellenbosch regions (1998 season).

Treatment (Yeast strain)	Robertson			
	Relative higher alcohols			
	iBuOH	iAmOH	HexOH	2FE
Vin 13	10.506a	67.950a	14.240b	7.336b
VL3C	10.676a	58.104a	16.817a	6.855b
NT 116	6.315b	43.212b	12.292c	9.433a
Treatment (Yeast strain)	**Stellenbosch**			
	Relative higher alcohols			
	iBuOH	iAmOH	HexOH	2FE
Vin 13	15.526b	65.769a	8.2422b	18.272a
VL3C	23.003a	81.432a	9.852a	14.772b
NT 116	15.820b	75.048a	8.071b	19.649a

Treatments designated by the same letter do not differ signifcantly ($p \leq 0.05$).
Each value represents the average of the three ascorbic acid/SO_2 treatments (ascorbic acid/meta, SO_2, pure ascorbic acid/SO_2). iBuOH = iso-butanol; iAmOH = iso-amyl alcohol; HexOH = hexanol; 2FEA = 2-phenylethanol.

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Table 5. The effect of ascorbic acid/meta preparate, SO_2 and pure ascorbic acid/SO_2 treatments on individual monoterpene concentrations in Sauvignon blanc wines from the Robertson and Stellenbosch regions (1998 season).

Treatment	Robertson					
	Relative monoterpene concentrations					
	trans-FLO	Lin	alpha-Terp	Citr	Nerol	Diendiol-1
Ascorbic acid/meta preparate	2.088a	5.251b	3.187b	3.180b	1.716a	1.644b
Standard + SO_2 (Control)	1.562b	5.393b	4.162a	4.174a	1.769a	2.752a
Pure ascorbic acid/SO_2	2.151a	6.195a	3.353b	3.248b	1.822a	2.667a
Treatment	Stellenbosch					
	Relative monoterpene concentrations					
	trans-FLO	Lin	alpha-Terp	Citr	Nerol	Diendiol-1
Ascorbic acid/meta preparate	3.001a	11.206b	5.097b	4.795b	3.119a	17.307a
Standard + SO_2 (Control)	2.882a	11.604ab	5.996a	7.024a	3.217a	17.820a
Pure ascorbic acid/SO_2	2.860a	12.448a	5.755ab	6.151a	3.959a	16.609a

Treatments designated by the same letter do not differ signifcantly ($p \leq 0.05$).
Each value represents the average of the three yeast strain (Vin 13, VL3C, NT 116) treatments. trans-FLO = trans-furan linalool oxide; Lin = linalool; alpha-Terp = alpha-terpineol; Citr = Citronellol; nerol = nerol; diendiol-1 = diendiol-1.

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Table 6. The effect of yeast strain on individual monoterpene concentrations in Sauvignon blanc wines from the Robertson and Stellenbosch regions (1998 season).

Treatment (Yeast strain)	Robertson					
	Relative monoterpene concentration					
	cis-FLO	Lin	alpha-Terp	Citr	Nerol	Diendiol-1
Vin 13	2.310a	4.506b	3.202b	2.403c	1.816a	2.948a
VL3C	1.759b	4.243b	3.025b	4.905a	1.734a	1.950b
NT 116	1.732b	8.090a	4.476a	3.294b	1.756a	2.166ab
Treatment (Yeast strain)	**Stellenbosch**					
	Relative monoterpene concentration					
	cis-FLO	Lin	alpha-Terp	Citr	Nerol	Diendiol-1
Vin 13	2.957a	12.569a	5.420a	4.415b	2.487b	18.504a
VL3C	2.845a	10.731b	5.648a	8.835a	3.985a	16.296b
NT 116	2.941a	11.958a	5.781a	4.720a	3.332ab	16.937ab

Treatments designated by the same letter do not differ signifcantly ($p \leq 0,05$).
Each value represents the average of the three ascorbic acid/SO_2 treatments (ascorbic/meta preparate, SO_2, pure ascorbic acid/SO_2). trans-FLO = trans-furan linalool oxide; Lin = linalool; alpha-Terp = alpha-terpineol; Citr = Citronellol; nerol = nerol; diendiol-1 = diendiol-1.

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Table 7. The effect of ascorbic acid/meta preparate, SO_2 and pure ascorbic acid/SO_2 treatments on Sauvignon blanc wine quality prepared with grapes from the Robertson and Stellenbosch regions (1998 season).

TREATMENT	ROBERTSON REGION		STELLENBOSCH REGION	
	Wine characteristics *			
	Fruity/ester aroma intensity	Overall wine quality	Fruity/ester aroma intensity	Overall wine quality
Ascorbic acid/meta preparate	19.222c	16.000c	36.222a	25.625b
Standard + SO_2 (Control)	33.333b	38.333b	36.556a	52.714a
Pure ascorbic acid/SO_2	47.000a	49.222a	47.222a	50.333a

Treatments designated by the same letter do not differ significantly ($p \leq 0.05$).
*Each value represents the average of the three yeast strain (Vin 13, VL3C and NT 116) treatments.

Table 8. The effect of yeast strains Vin 13, VL3C and NT 116 on Sauvignon blanc wine quality prepared with grapes from the Robertson and Stellenbosch regions (1998 season).

TREATMENT (Yeast strain)	ROBERTSON REGION		STELLENBOSCH REGION	
	Wine characteristic *			
	Fruity/ester aroma intensity	Overall wine quality	Fruity/ester aroma intensity	Overall wine quality
Vin 13	30.111a	35.222ab	41.889a	48.625a
VL3C	30.889a	30.222b	34.889a	35.125b
NT 116	38.556a	38.111a	43.222a	44.625a

Treatments designated by the same letter do not differ significantly ($p \leq 0.05$).
*Each value represents the average of the three ascorbic acid/SO_2 treatments (ascorbic acid/meta preperate, SO_2 and pure ascorbic acid/SO_2).

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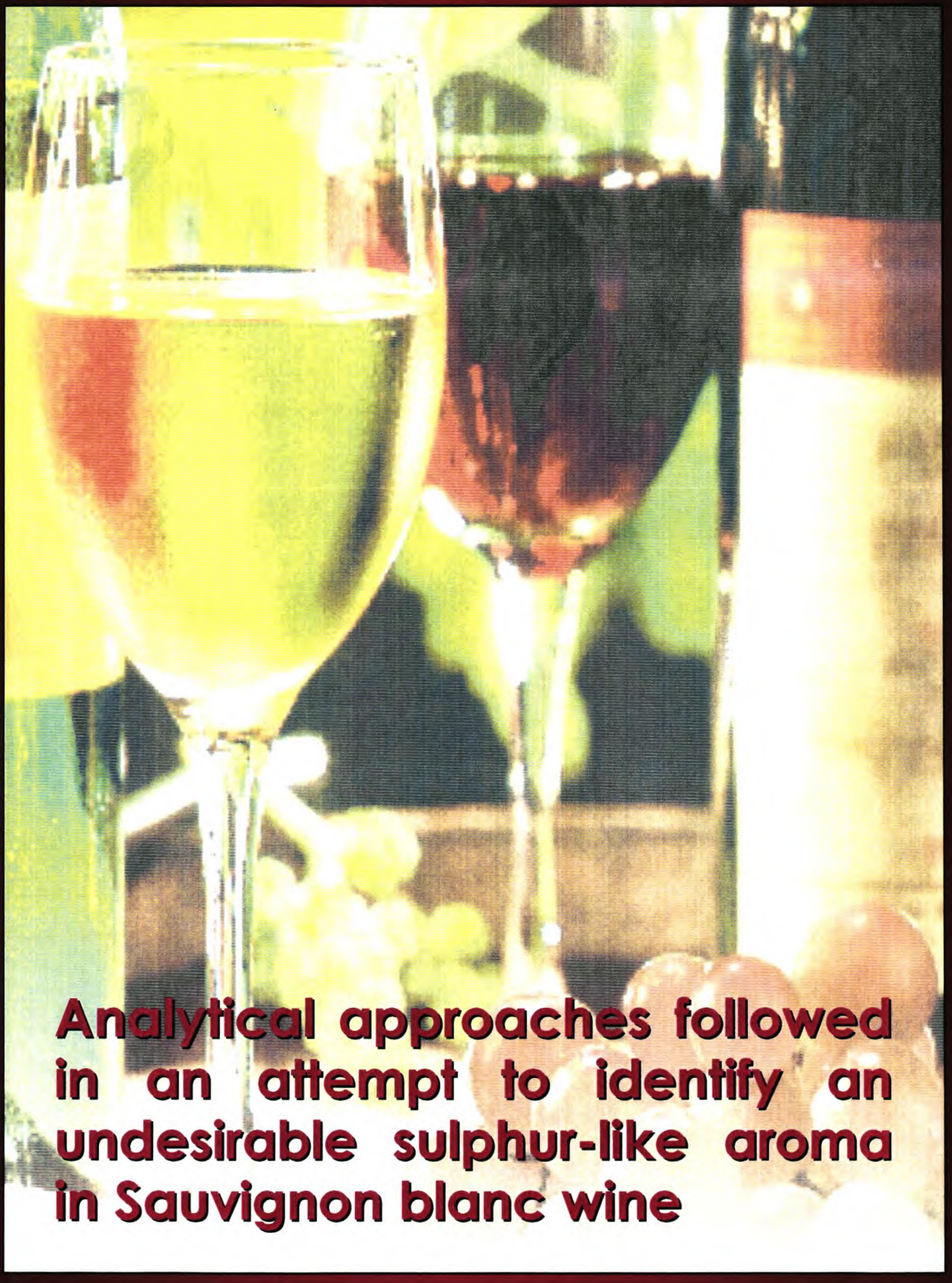
CHAPTER 6



Analytical approaches followed in an attempt to identify an undesirable sulphur-like aroma in Sauvignon blanc wine

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CHAPTER 6

ANALYTICAL APPROACHES FOLLOWED IN AN ATTEMPT TO IDENTIFY AN UNDESIRABLE SULPHUR-LIKE AROMA IN SAUVIGNON BLANC WINE

Summary

Sauvignon blanc wines, produced with the use of a commercially-available ascorbic acid/meta preparate and the French yeast strain, VL3C, presented a strong undesirable sulphur-like aroma. Techniques, namely gas chromatography-mass spectrometry to analyse 4-mercapto-4-methylpentan-2-one, comparison of ascorbic acid/meta preparates and analysis of headspace volatiles by solid phase microextraction together with sniffing, were applied. The compenent(s) responsible for the relevant sulphur-like off-flavours could not be identified. Further investigations in this regard are necessary.

Introduction

Aroma is used as a parameter to recognise and evaluate grapes and wines from a specific cultivar. The reason for a wine being accepted or rejected is fundamentally based on its smell or aroma. These aromas can be classified into primary aromas (varietal or prefermentative aromas derived from grapes), secondary aromas (fermentative aromas which develop during the fermentation process) and tertiary aromas (post fermentative aromas which develop during ageing and storing of wine) (Cordonnier & Bayonove, 1981). A considerable amount of research has been done to characterise the volatile profile of wine by classical methods of flavour volatile analysis, like gas chromatography (GC), mass spectrometry (MS) and the recently developed solid phase microextraction (SPME).

Sauvignon blanc has a characteristic varietal aroma of grassy, green pepper, asparagus, tomato leaf, vegetative or herbaceous nuances. Methoxypyrazines, especially 2-methoxy-3-isobutylpyrazine (ibMP), 2-methoxy-3-isopropylpyrazine (ipMP) and 2-methoxy-3-sec-butylpyrazine (sbMP), are considered to be the most important components responsible for the above mentioned nuances (Augustyn *et al.*, 1982; Allen *et al.*, 1988; Allen *et al.*, 1991; Lacey *et al.*, 1991).

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Aromatic volatile thiols are formed during the alcoholic fermentation of Sauvignon blanc must. Their aromas, reminiscent of and similar to those identified in box tree, broom, black currant, grapefruit, passion fruit and cat urine have also been recognised to be typical of Sauvignon blanc wine aroma. In this respect a few sulphur-containing components identified as 4-mercapto-4-methylpentan-2-ol, 3-mercaptohexan-1-ol, 3-mercaptohexyl acetate, 3-mercapto-3-methylbutan-1-ol and 4-mercapto-4-methylpentan-2-one (MMP), are responsible for these nuances (Darriet *et al.*, 1995; Tominaga *et al.*, 1996; Tominaga *et al.*, 1998a).

Since the threshold value of MMP is as low as 0.1 $ng.l^{-1}$ in water and 3 $ng.l^{-1}$ in wine, this component can often, in too high concentrations, present a cat urine character (Darriet *et al.*, 1995; Marais & Swart, 1999). Wine tasters and consumers regard this sulphur-like aroma as negative and not typical of Sauvignon blanc wine. However, low concentrations of MMP can contribute to the complexity and overall quality of Sauvignon blanc wine.

Often, locally produced Sauvignon blanc wines possess undesirable, sulphur-like aromas. One of the recently identified causes was the use of a commercially available ascorbic acid/meta preparate (Swart *et al.*, 2000a). This preparate, together with the French yeast strain, VL3C, known for the production of cultivar-typical Sauvignon blanc wines in France, were especially responsible for the intense sulphur-like off-flavours. It was accepted that MMP could have been responsible for this aroma.

The purpose of this investigation was to identify the component(s) responsible for the undesirable aroma of Sauvignon blanc wine. To achieve this, different analytical approaches were followed.

Materials and methods

1. Determination of MMP

1.1 Wines

Sauvignon blanc wines produced from grapes from the Robertson and Stellenbosch regions (1998 vintage), were used (Swart *et al.*, 2000a; 2000b).

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1.2 Reference compounds

4-Mercapto-4-methylpentan-2-one was kindly donated by Dr. P. Etiévant from INRA, Dijon, France. 6-Mercapto-1-hexanol (Fluka, code 63762) was used as internal standard.

1.3 Extraction of volatile thiols

The method, decribed by Tominaga *et al.* (1998b) was used with a few minor adjustments. Sodium hydroxide (10 N) (Merck, code 10252) was used to adjust 500 ml of wine, containing 2.5 nmol 6-mercapto-1-hexanol, to pH 7. The wine was successively extracted with two volumes of 120 ml dichloromethane (Merck, code 32222), each subjected to magnetic stirring for 7 minutes. The two organic phases were combined and centrifuged for 5 minutes at 4000 r.p.m. Using a separating funnel, the organic phase was recovered and extracted twice with 20 ml of p-hydroxymercuribenzoate solution (Sigma, code H0642)(1mM in 0.01N sodium hydroxide) for 8 minutes each. The aqueous phases (pH>7) obtained, were then combined, and by the slowly addition of 5% hydrochloric acid (Merck, code 100319) adjusted to pH 7.

A basic anion exchanger column (1.5 x 3 cm) (Dowex-1-chloride, Sigma, code 1x2-100) was activated, using 50 ml of 0.1 M hydrochloric acid and afterwards rinsed with 100 ml deionised water. The adjusted p-hydroxymercuribenzoate complex was loaded onto the column and 25 minutes were allowed for the percolation to finish. The column was washed with 10 ml of potassium phosphate buffer (2 mM, pH 7.2) (Sigma, code P0662) and 50 ml of 0.1 M sodium acetate (pH 6) (Merck, code 10236) to which 0.1 M sodium chloride (Merck, code 582 2320) had been added.

A previously purified (3 X 5 ml dichloromethane) cysteine solution (853 mg/ 80 ml) (pH 7) (Sigma, code C4820) was used to eluate the volatile thiols from the column. The percolation was completed in 40 minutes. The eluate, containing the thiols, was extracted with successive volumes of 4 ml, 3 ml and 3 ml of dichloromethane by means of magnetic stirring for 8 minutes each. The organic phase, recovered from each extraction was collected, combined and dried on anhydrous sodium sulphate (Merck, code 10264). The combined organic phase was transferred and concentrated to 10 µl under nitrogen flow in a waterbath at 10°C. All the glassware were washed with deionised water, dried and rinsed with dichloromethane before use, as a precaution against contamination.

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1.4 Calibration

Chardonnay wine (vintage 1998) was used for the calibration purpose. Increasing quantities MMP were added to wine (500 ml lots), each lot containing 2.5 nmol of the internal standard (6-mercapto-1-hexanol) (**Table 1**). The wines were extracted by the same method, described under section 1.3. All extractions were done in duplicate.

Table 1. MMP concentrations used for setting up a standard curve.

Volume MMP [1.6 ng.ml^{-1}] added to 500 ml wine (µl)	Final concentration of MMP in wine (ng/l)
62.5	0.2
315.5	1.0
1562.5	5
3125.0	10
4687.5	15
6250	20

1.5 Gas chromatography/mass spectrometry (GC/MS)

The conditions for GC/MS, described by Tominaga & Dubourdieu (1997), were used and were as follows:

Column:	PB-20 (SGE, 50 m x 0.22 mm i.d. x 0.25 µm)
Injection temperature:	250°C
Temperature programme:	1 min. at 35°C
	3°C.min^{-1} up to 230°C
	2 min. at 230°C
Carrier gas:	Helium
Column flow rate:	1.2 ml.min^{-1}
Injection mode:	Splitless
Injection volume:	2 µl

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4-Mercapto-4-methylpentan-2-one and 6-mercapto-1-hexanol (internal standard) were detected in SIM mode, according to the following selected ions:

4-mercapto-4-methylpentan-2-one:	*m/z* 75; *m/z* 132
6-mercapto-1-hexanol:	*m/z* 39; *m/z* 55; *m/z* 67; *m/z* 87; *m/* 101

2. Comparison between ascorbic acid/meta preparates

2.1 Wines

The same wine making procedures (Swart *et al.*, 2000a) were followed to produce Sauvignon blanc wines (2000 season). A newly developed commercially available ascorbic acid/meta preparate (donated by AEB Africa Pty Ltd.) was compared to the commercially available preparate used previously (Swart *et al.*, 2000a). The French yeast strain, VL3C, was used for the production of both wines.

2.2 Liquid-liquid extraction of wine

The wines were extracted for 20 hours using Freon 11 (Marais, 1986). 2-Ethyl-1-hexanol (0.5 ml at 80 $\mu g.l^{-1}$) was used as internal standard.

2.3 Gas chromatography/Mass spectrometry

The freon extracts were analysed by gas chromatography (HP 5880) using the following conditions:

Column:	Supelcowax 10 fused silica
	(60 m x 0.32 mm i.d. x 0.25 µm)
Injector temperature:	200°C
FID temperature:	250°C
Temperature programme:	10 min. at 60°C
	$1°C.min^{-1}$ up to 190°C
	30 min. at 190°C
Carrier gas:	Helium
Column flow rate:	$1.5 ml.min^{-1}$
Split ratio:	60:1
Injection volume:	0.8 µl

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Peak identities were confirmed by mass spectrometry (Finnigan Mat GCQ mass spectrometer, coupled to a Finnigan 9610 gas chromatograph), using the same conditions as given above.

3. Solid phase microextraction and sniffing

3.1 Extraction method

A SPME holder for manual sampling (Supelco Co., Sigma-Aldrich, cat no. 57330-U), equipped with a fibre (10 mm length), coated with poly (dimethylsiloxane) (100 µm thickness) was used to adsorp the headspace volatiles of the Sauvignon blanc wines. A volume of 100 ml wine, kept in a closed plastic bottle, was temperated to 30°C in a waterbath. After the addition of 10 g of sodium chloride (Merck, code 582 2320) to the wine, the SPME holder was installed so that the fibre was exposed to the headspace of the wine (Garcia *et al.*, 1996; Hayasaka & Bartowsky, 1999). The collection procedure was performed for 1 hour, while the wine was continuously stirred and kept at 30°C.

3.2 Gas chromatography (GC)/Sniffing

Immediately after the extraction procedure, the SPME fibre was inserted into the injection port of the gas chromatograph (Varian 3800), coupled with an olfactory detector, and removed after 5 minutes. Sniffing of the headspace phase of the samples was performed for the duration of the GC-run. The SPME extract was analysed under the following gas chromatographic conditions:

Column:	Supelcowax 10 fused silica
	(60 m x 0.3 mm i.d. x 0.25 µm)
Injector temperature:	250°C
FID temperature:	250°C
Temperature programme:	5 min. at 60°C
	2°C.min^{-1} up to 190°C
	30 min. at 190°C
	10°C.min^{-1} up to 210°C
	38 min. at 210°C
Carrier gas:	Helium
Column flow rate:	2.5 ml.min^{-1}

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Split ratio: 10:1

Peaks or areas, where possible sulphur-like aromas were detected, were marked.

3.2 Mass spectrometry

The same chromatographic conditions, used for analysing the headspace SPME extract (section 3.2) were used for the MS analyses. A Finnigan Mat GCQ mass spectrometer, coupled to a Finnigan 9610 gas chromatograph, was used.

Results and discussion

1. Determination of MMP

Results obtained from the sensory evaluation of the wines analysed in this study (Swart *et al.*, 2000a), showed high intensities of a sulphur-like aroma in wines treated with a combination of the commercially available ascorbic acid/meta preparate and the French yeast strain, VL3C. These wines were regarded as poor quality wines and not typical for Sauvignon blanc. It was assumed that MMP could be responsible for this sulphur-like aroma, and it was therefore important to identify and quantify MMP in these wines.

Mass spectra of MMP and the internal standard (6-mercapto-1-hexanol) are shown in **Figures 1** and **2**. A calibration curve (**Figure 3**), suitable for analysing MMP, was set up by increasing concentrations of MMP, added to a neutral Chardonnay wine (**Table 1**). No MMP was found in the wines, and it can be accepted that this component was not responsible for the perceived sulphur-like aroma. Consequently it was decided to try out other methods of identification.

2. Comparison between ascorbic acid/meta preparate

Two Sauvignon blanc wines (2000 season) were produced from the same juice, using the original and the newly developed, commercially available ascorbic acid/meta preparate, respectively. The wines, treated with the newly developed preparate, showed no sulphur-like off-flavours, while the wines treated with the original preparate, showed the same, prominent sulphur-like nuances, as found in the wines from the 1998 season. Chromatographic analyses showed prominent differences between the two wines. The chromatogram of the wine with the sulphur-

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like aroma is shown in **Figure 4**. At retention times, 46.28 min. and 69.22 min, two additional peaks were observed in the wine treated with the original ascorbic acid/meta preparate. They were identified as furfural and furfurol, by analysing authentic samples thereof (Fluka, code 48070; Fluka, code 48090, respetively), under the same chromatographic conditions (**Figures 5** and **6**). Furfural and furfurol are degradation products of the hydrolysis of pentose sugar or ascorbic acid, and are associated with almond-like, baked and toasted aromas (Rodriques *et al.*, 1991; Jackson, 1994; Bartowsky & Henschke, 1995; Solomon *et al.*, 1995). Although they can be considered as negative for wine quality, they were probably not involved in the development and the perception of the relevant sulphur-like off-flavours. The actual component(s), responsible for the off-flavour, was/were probably too low in concentration to be detected on the chromatogram, generated by this extraction method.

3. Solid phase microextraction and sniffing

Since the sulphur-like aroma was so potent and distinctive, it was decided to investigate the headspace aroma of the wines. During the SPME analyses, sniffing of the volatile extract was simultaneously performed. A number of possible sulphur-like nuances were observed during the GC-run. These peaks or areas are indicated on the chromatogram **Figure 7**, and the corresponding mass spectra in **Addendum 1** (**Figures 1** to **8**). Identification of these peaks was hindered by specific problems, namely the incompleteness of the MS component library, contaminating fragments, probably as a result of column bleed (**Figures 2, 4** and **6**) and the general complexity of this identification. Nevertheless, with the available knowledge, it appears that the mass spectra of the relevant components were not typical of sulphur-containing components. The search for the identities of the sulphur-like aromas should be continued and sulphur-specific SPME fibres and GC-detectors should be applied.

Conclusions

It was not possible to identify the component(s) responsible for the perceived sulphur-like off-flavour of Sauvignon blanc wines, by applying the specific methods in this study. Despite the fact that it appears that the newly developed, commercially available ascorbic acid/meta preparate does not produce any sulphur-like off-flavours, similar undesirable off-flavours still often occur in locally produced

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Sauvignon blanc wines. Further research is needed to identify the factors determining the development of these sulphur-like aromas and to identify the responsible components.

Acknowledgements

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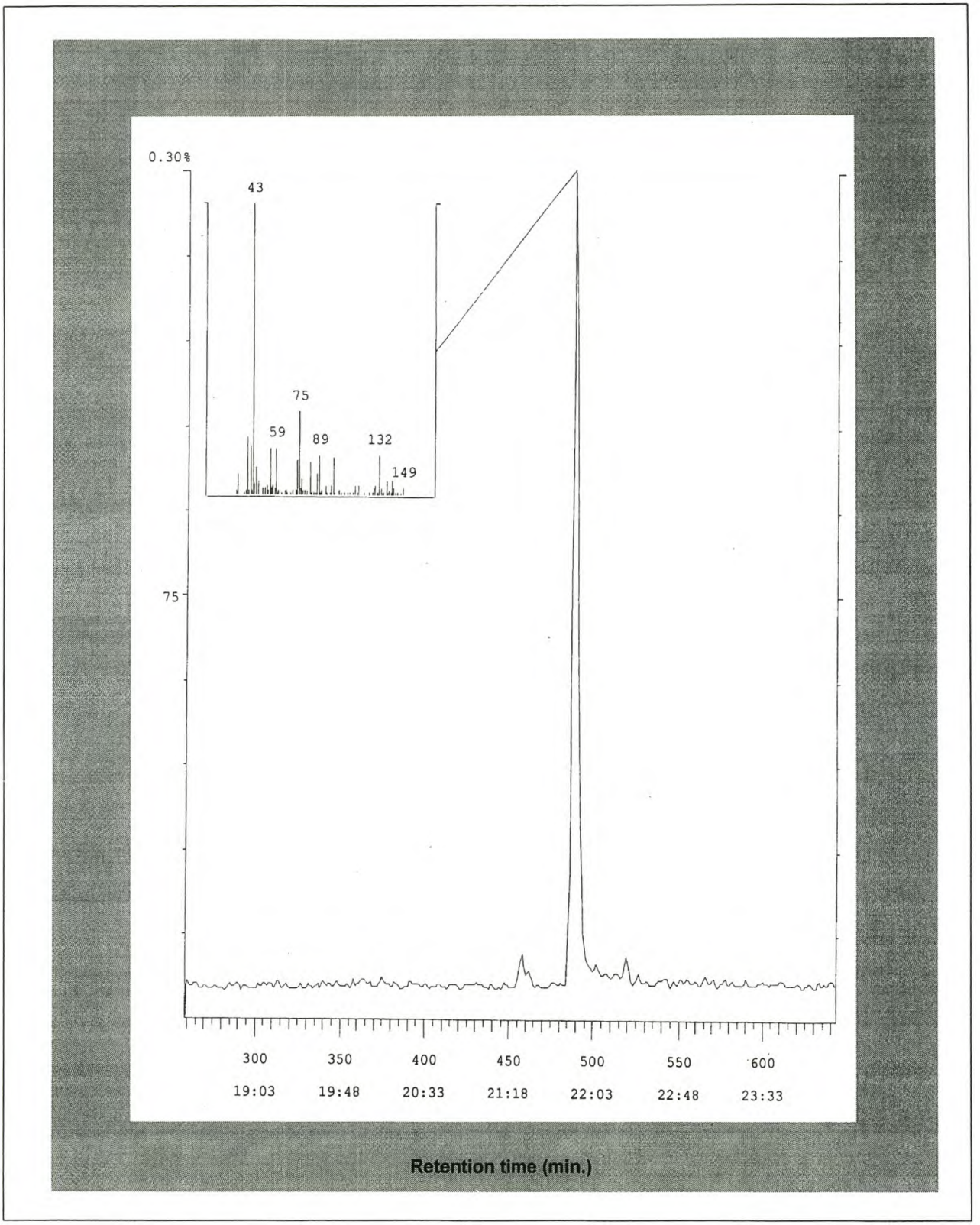

Figure 1. Mass spectrum of 4-mercapto-4-methylpentan-2-one (Darriet *et al.*, 1995).

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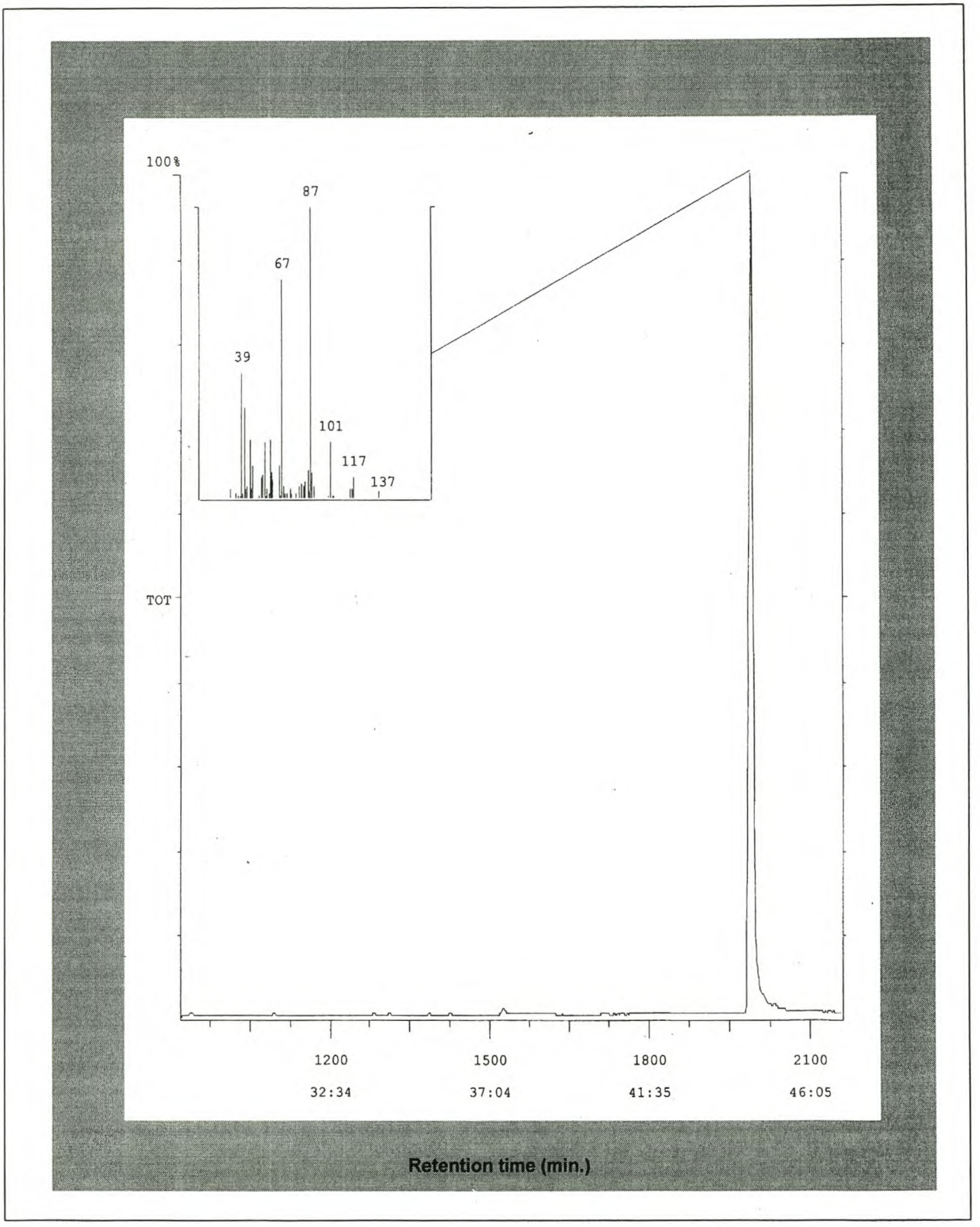

Figure 2. Mass spectrum of 6-mercapto-1-hexanol (internal standard).

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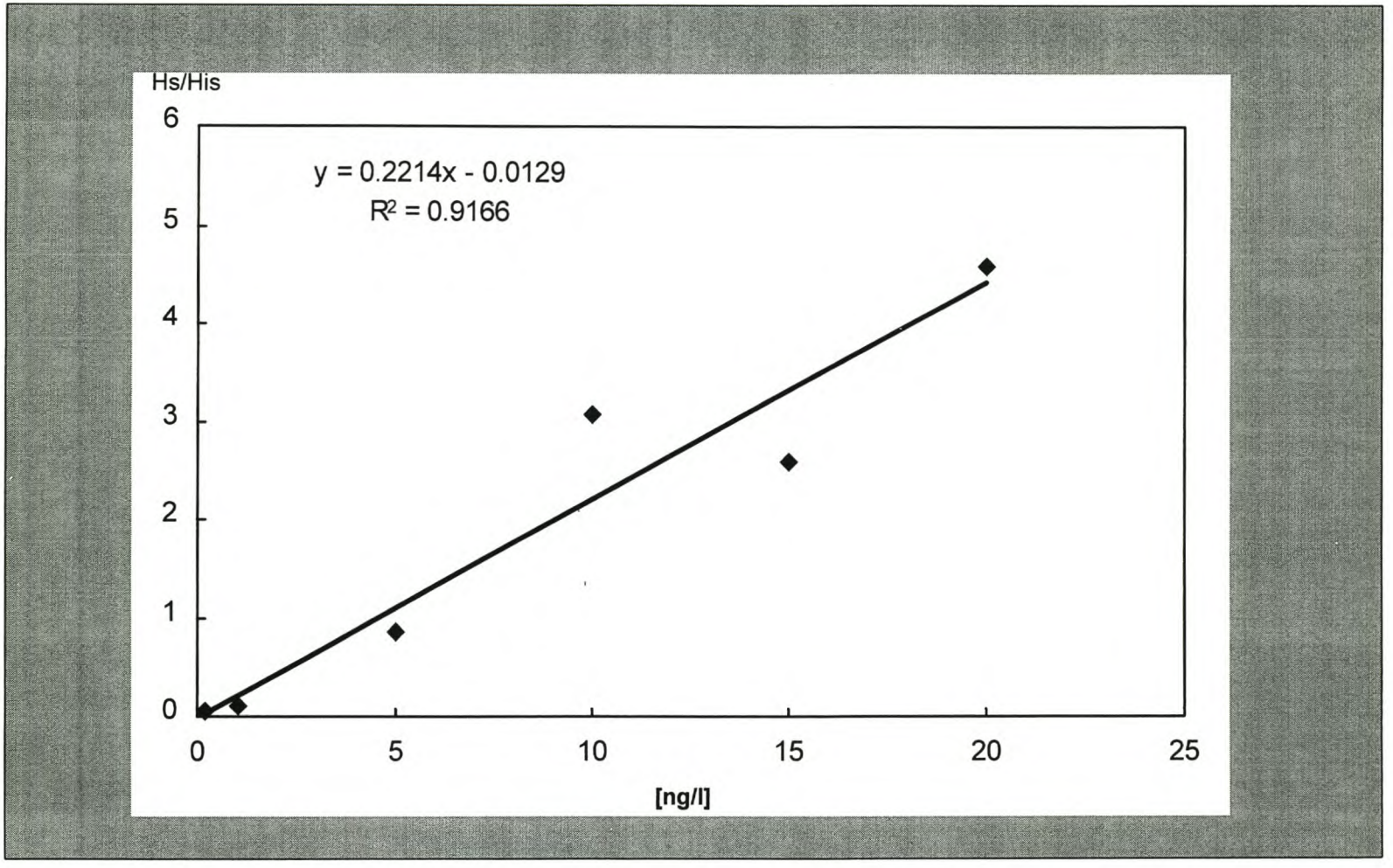

Figure 3. Calibration curve for the analysis of 4-mercapto-4-methylpentan-2-one. Hs/His: Ratio of the area of MMP to that of the internal standard (6-mercapto-1-hexanol).

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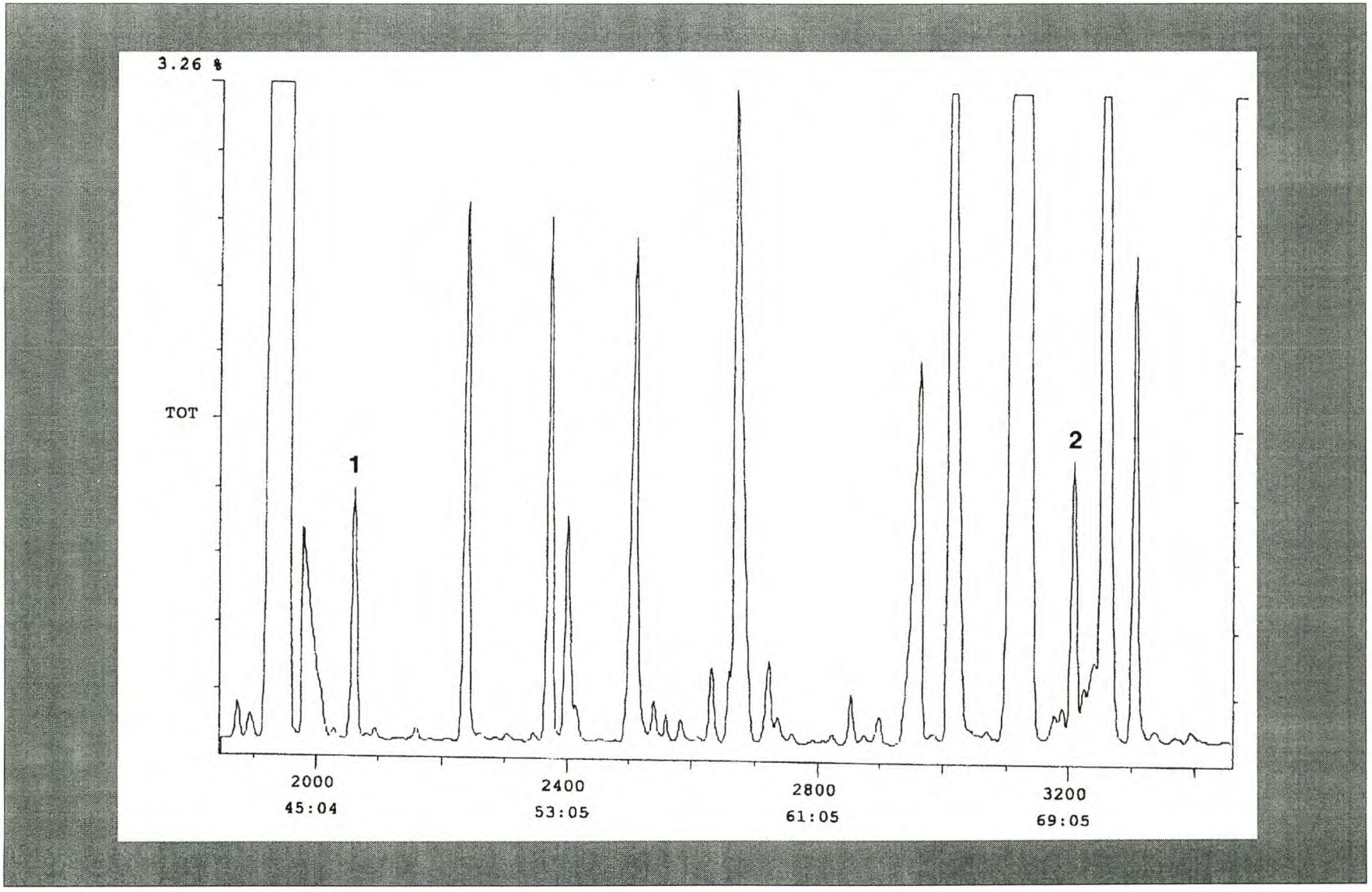

Figure 4. Part of chromatogram of Sauvignon blanc wine treated, with original, commercially-available ascorbic acid/meta preparate. Peak 1 = furfural; peak 2 = furfurol.

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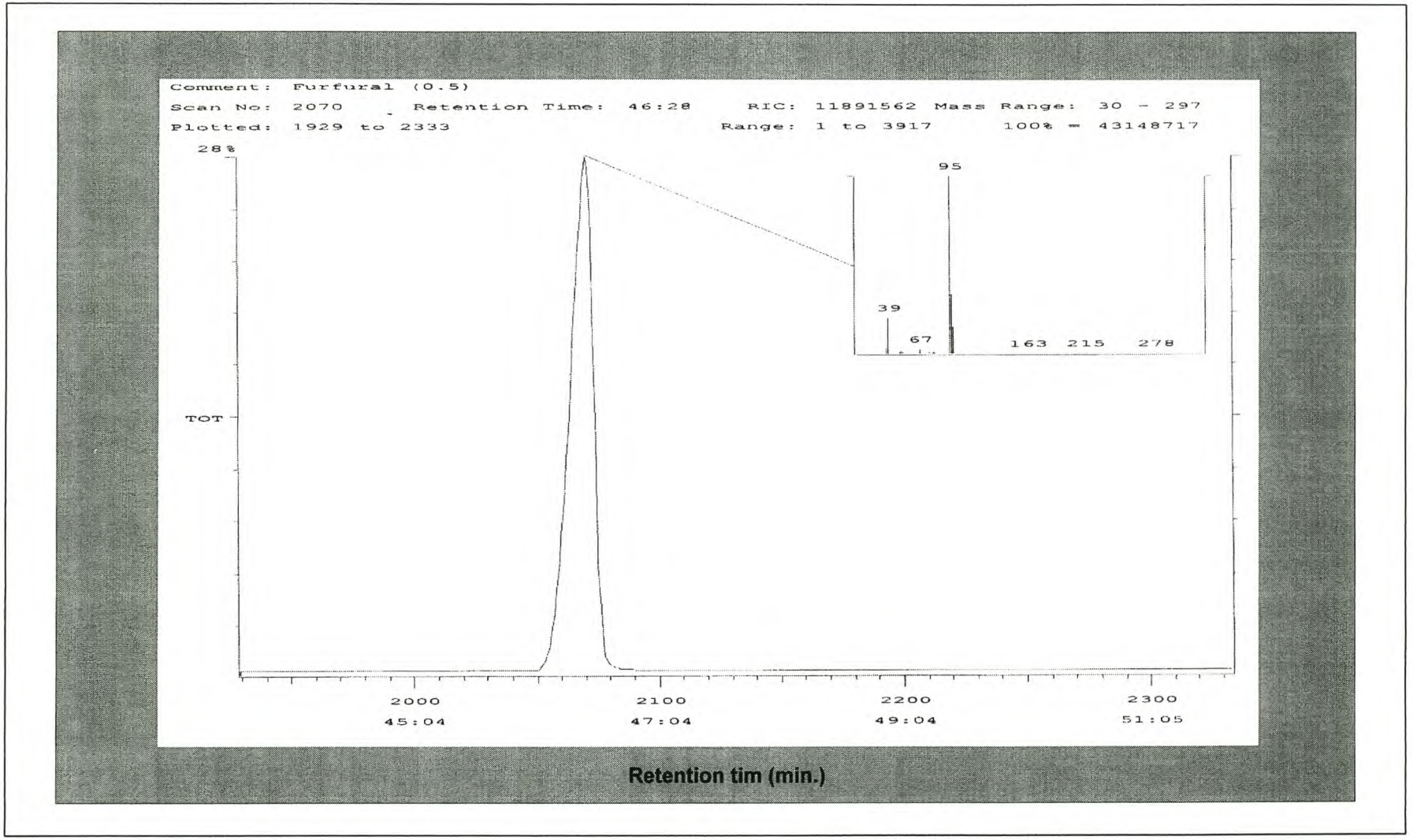

Figure 5. Mass spectrum of furfural.

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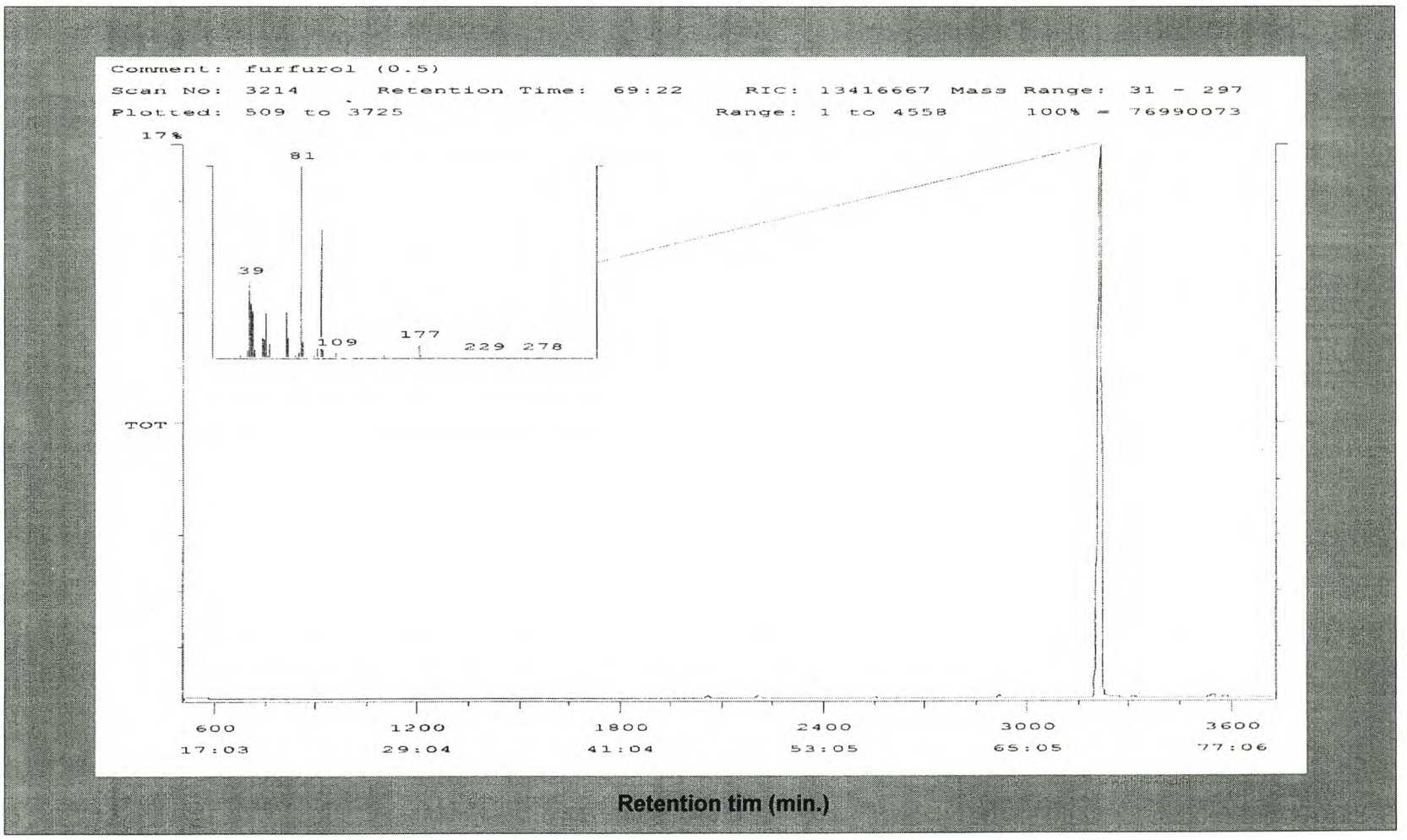

Figure 6. Mass spectrum of furfurol.

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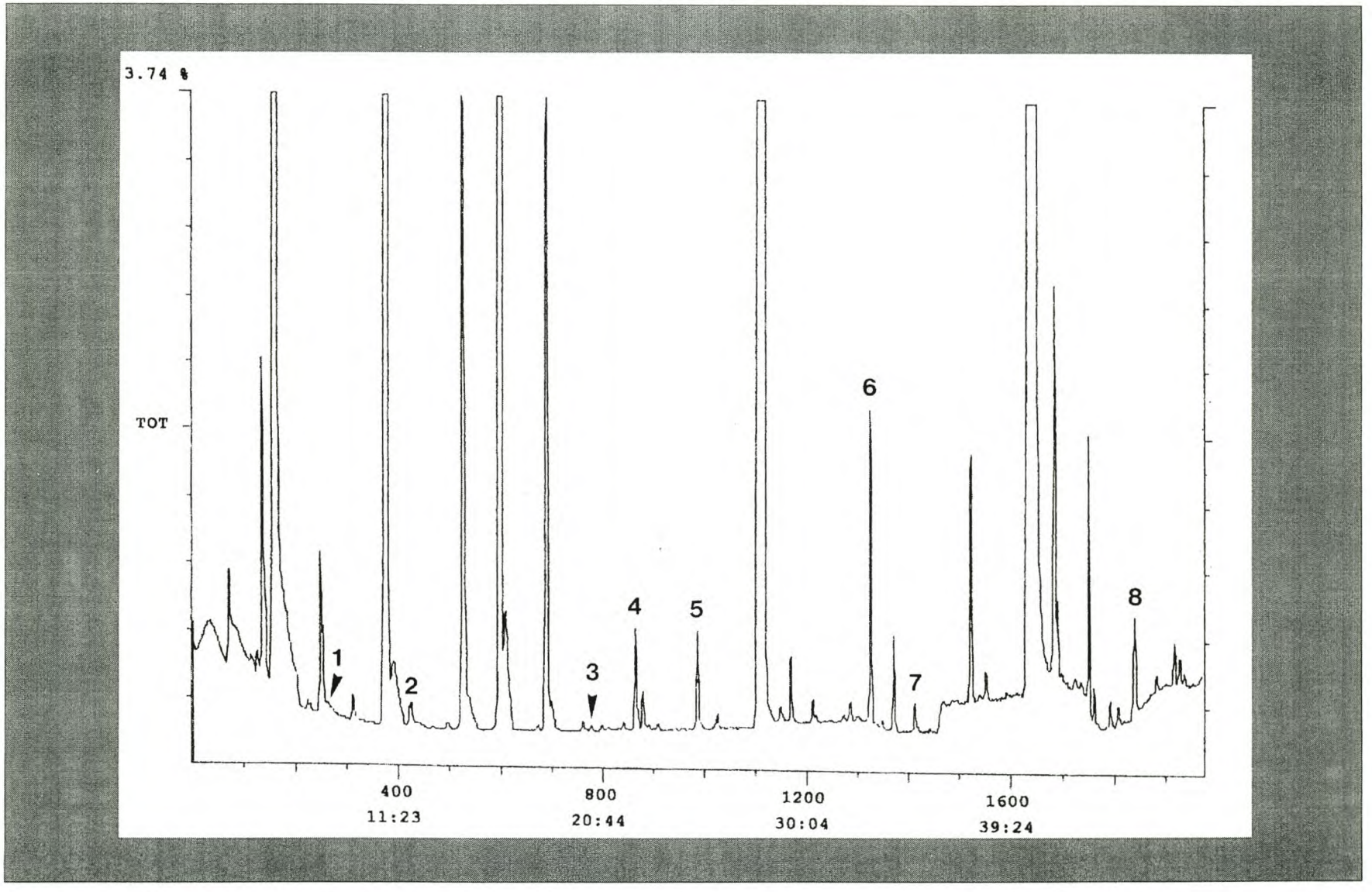

Figure 7. Chromatogram of SPME analysis of Sauvignon blanc wine headspace. Peaks 1 to 8 indicate components, possibly responsible for sulphur-like off-flavours.

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Addendum

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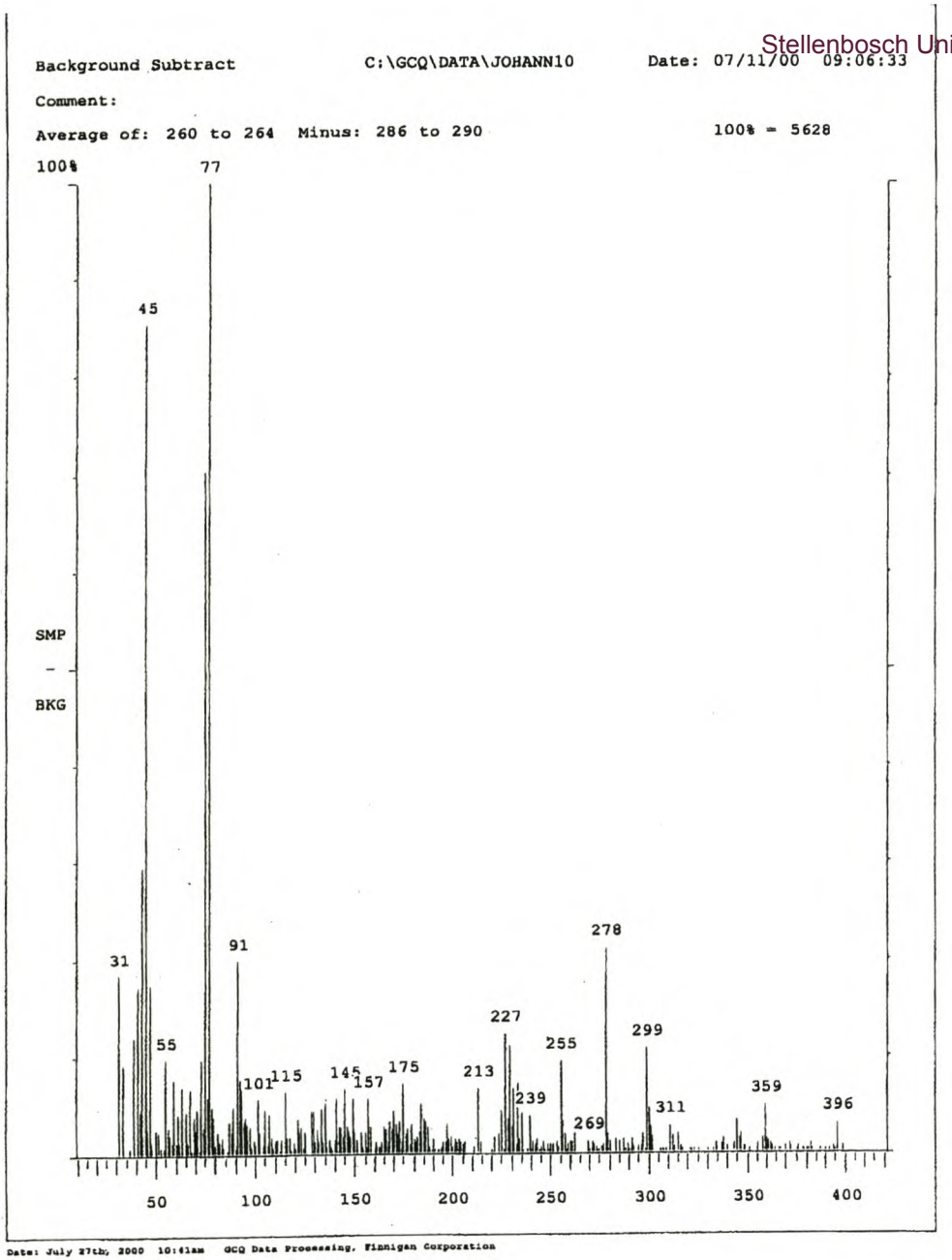

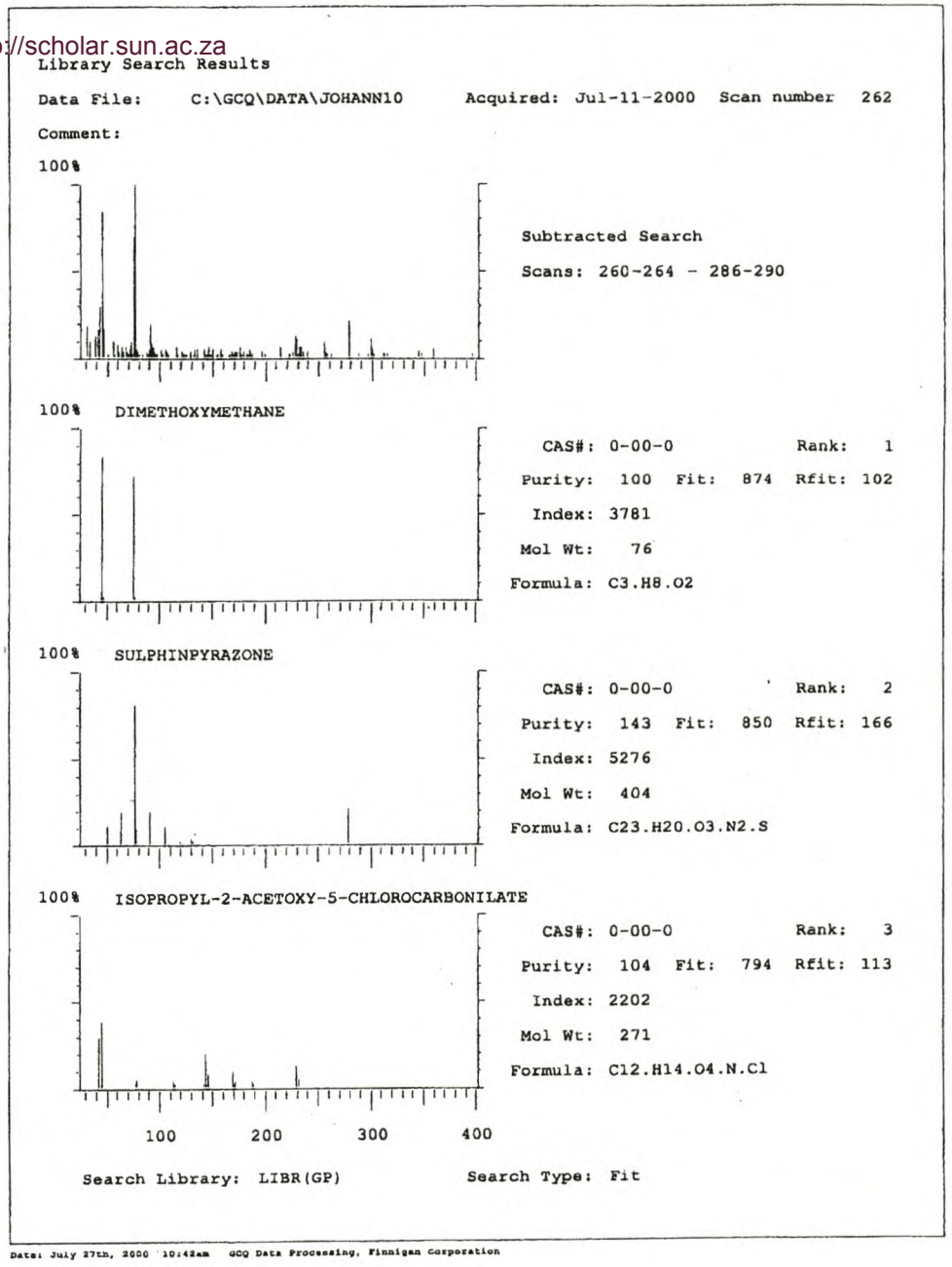

Figure 1. Mass spectrum and library search results for peak 1. Peak number corresponds to that in Figure 7.

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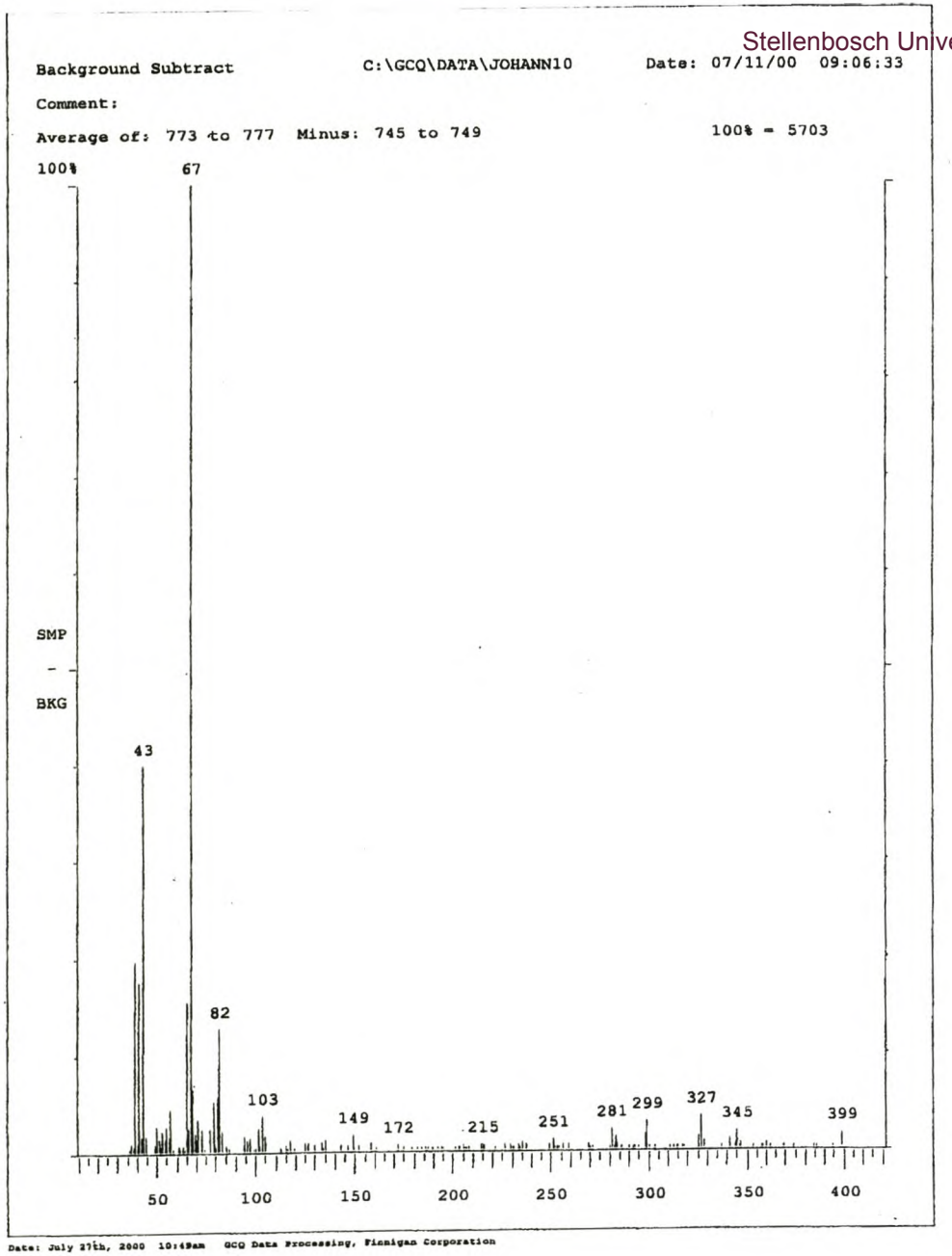

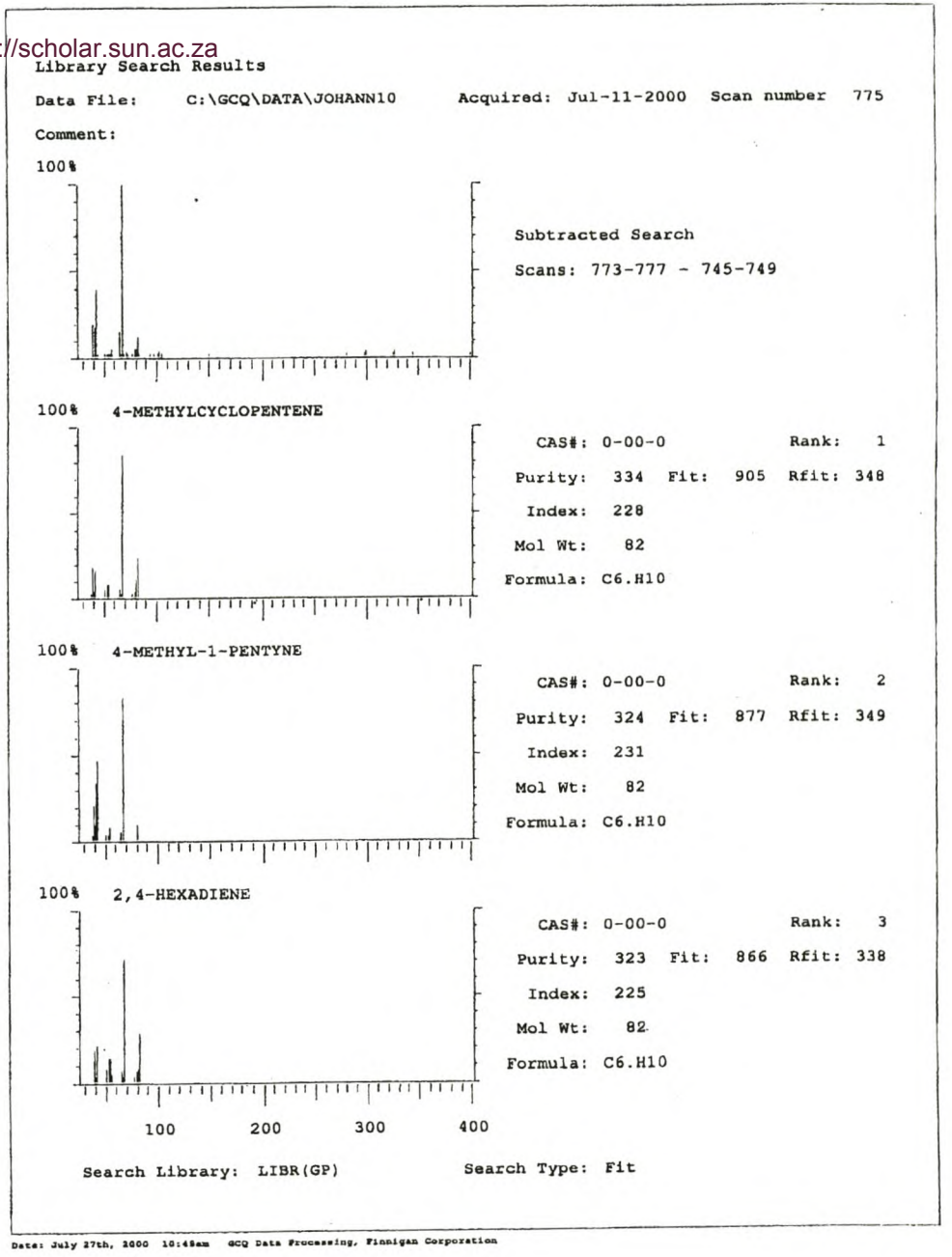

Figure 2. Mass spectrum and library search results for peak 2. Peak number corresponds to that in Figure 7.

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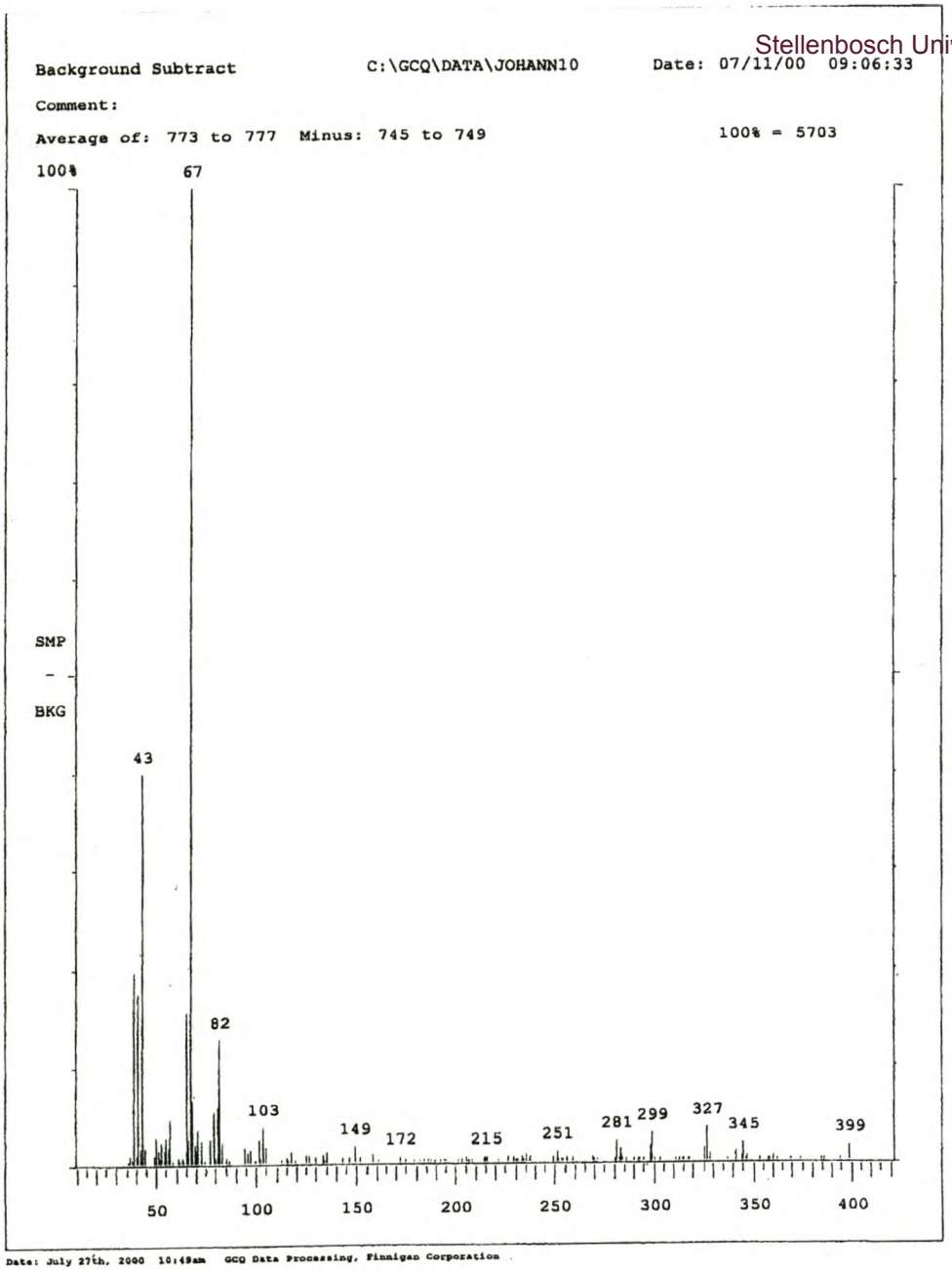

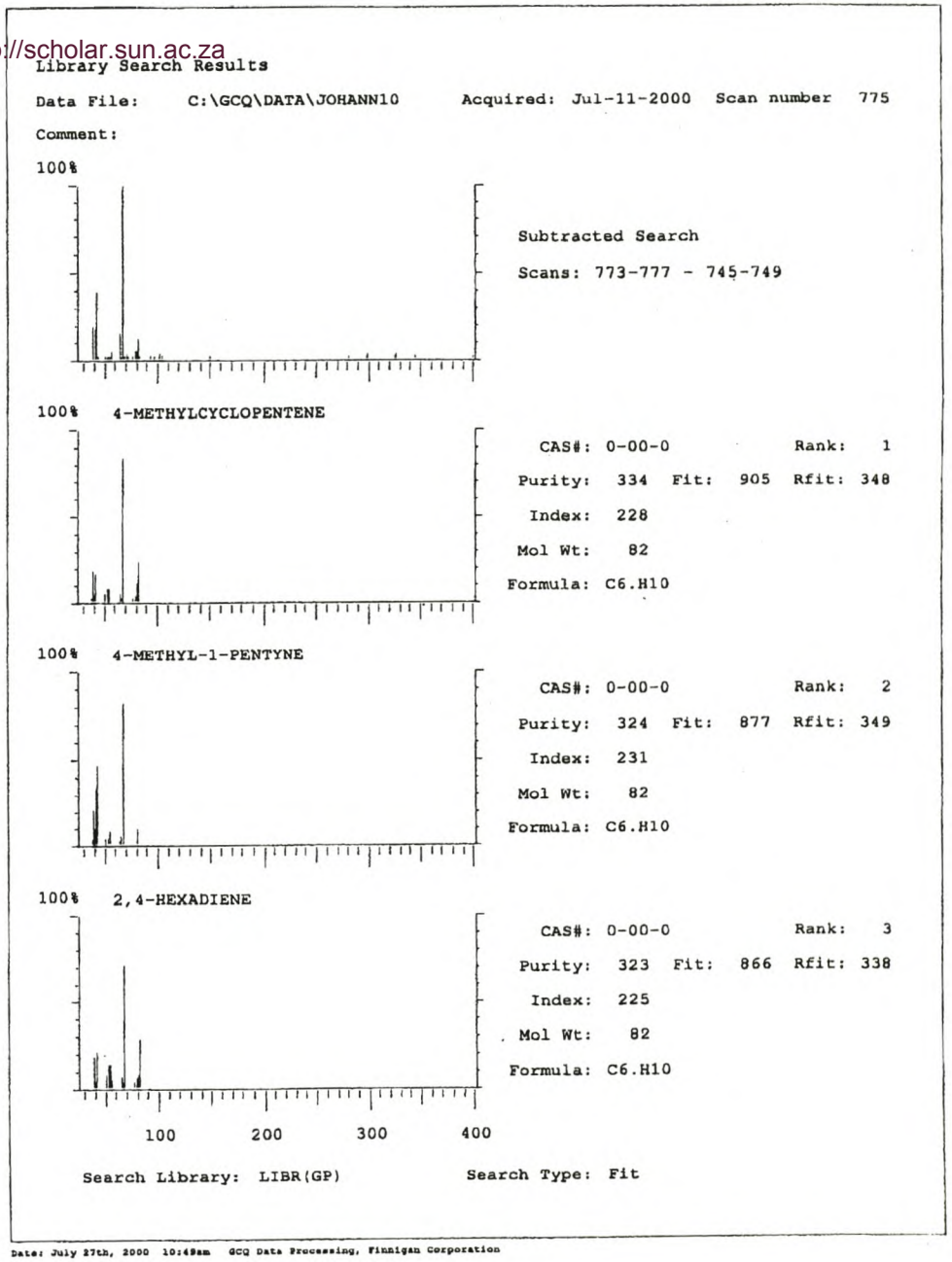

Figure 3. Mass spectrum and library search results for peak 3. Peak number corresponds to that in Figure 7.

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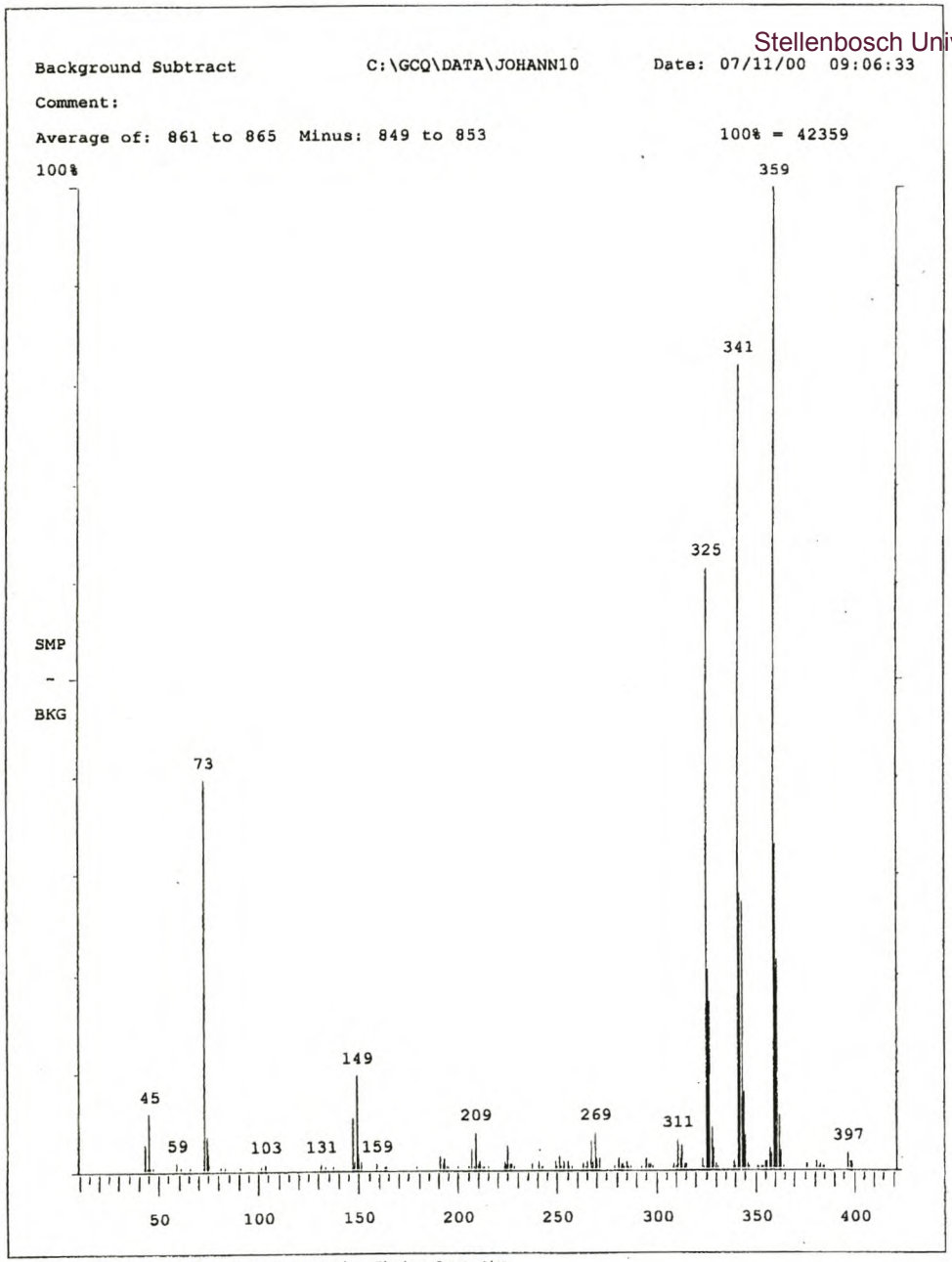

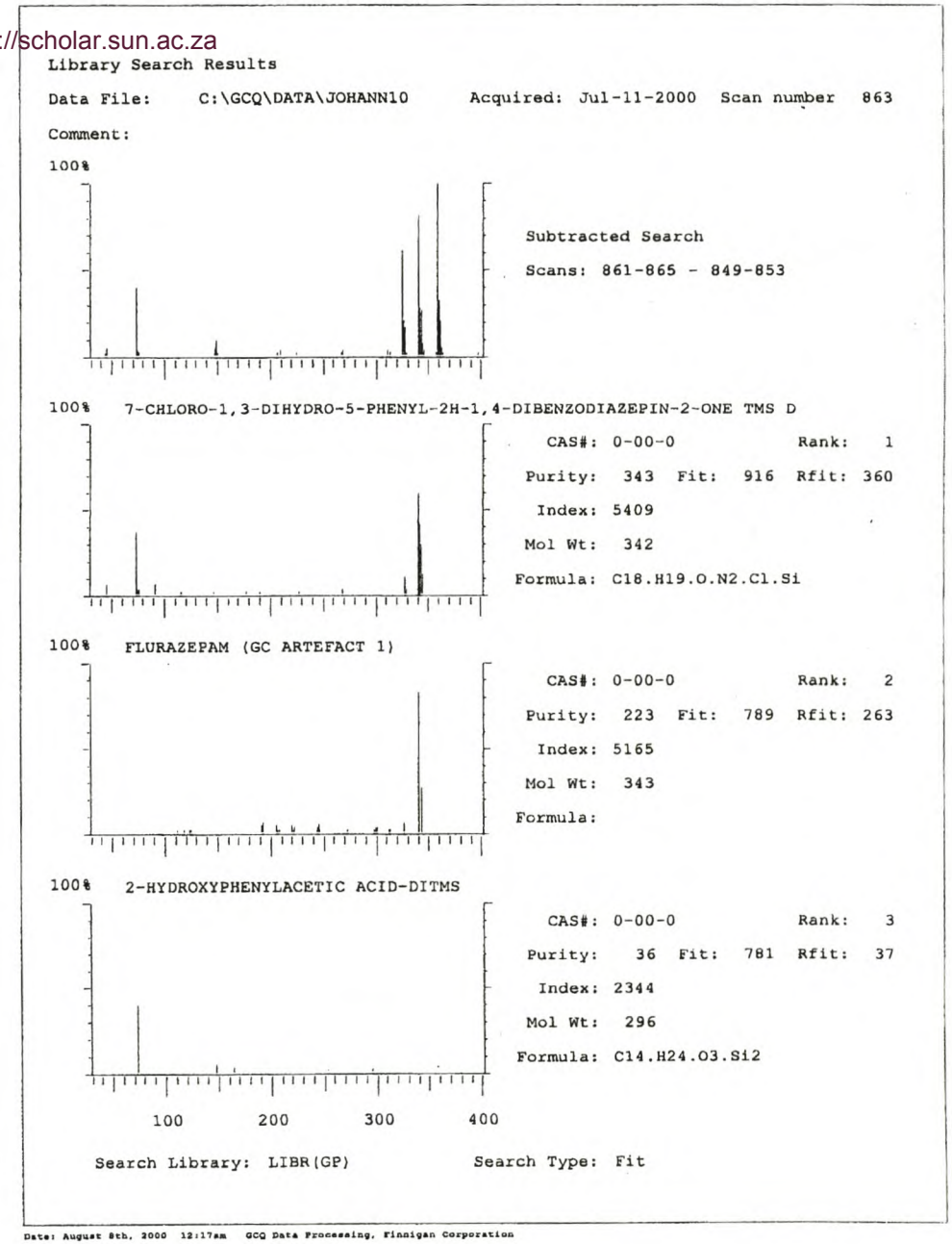

Figure 4. Mass spectrum and library search results for peak 4. Peak number corresponds to that in Figure 7.

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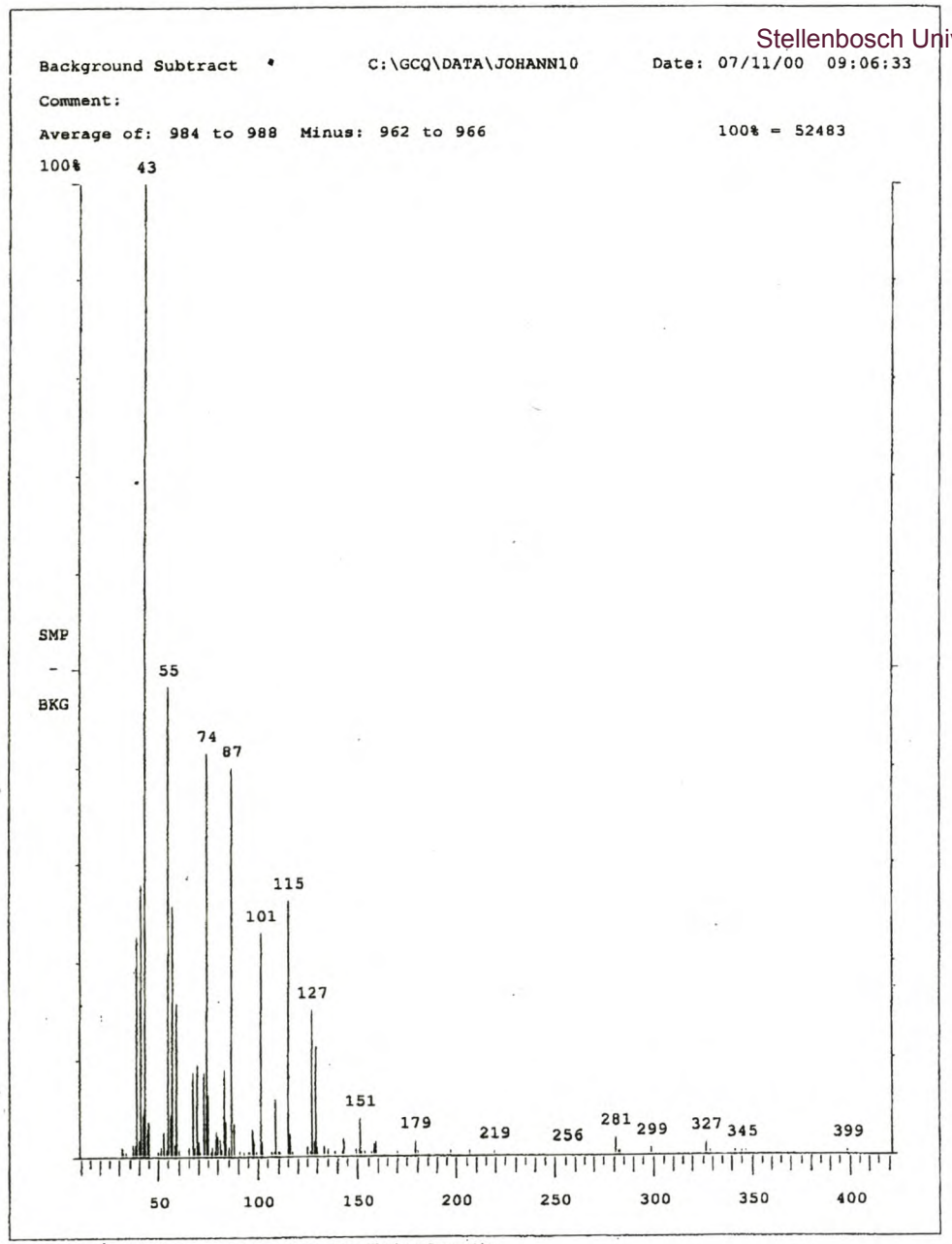

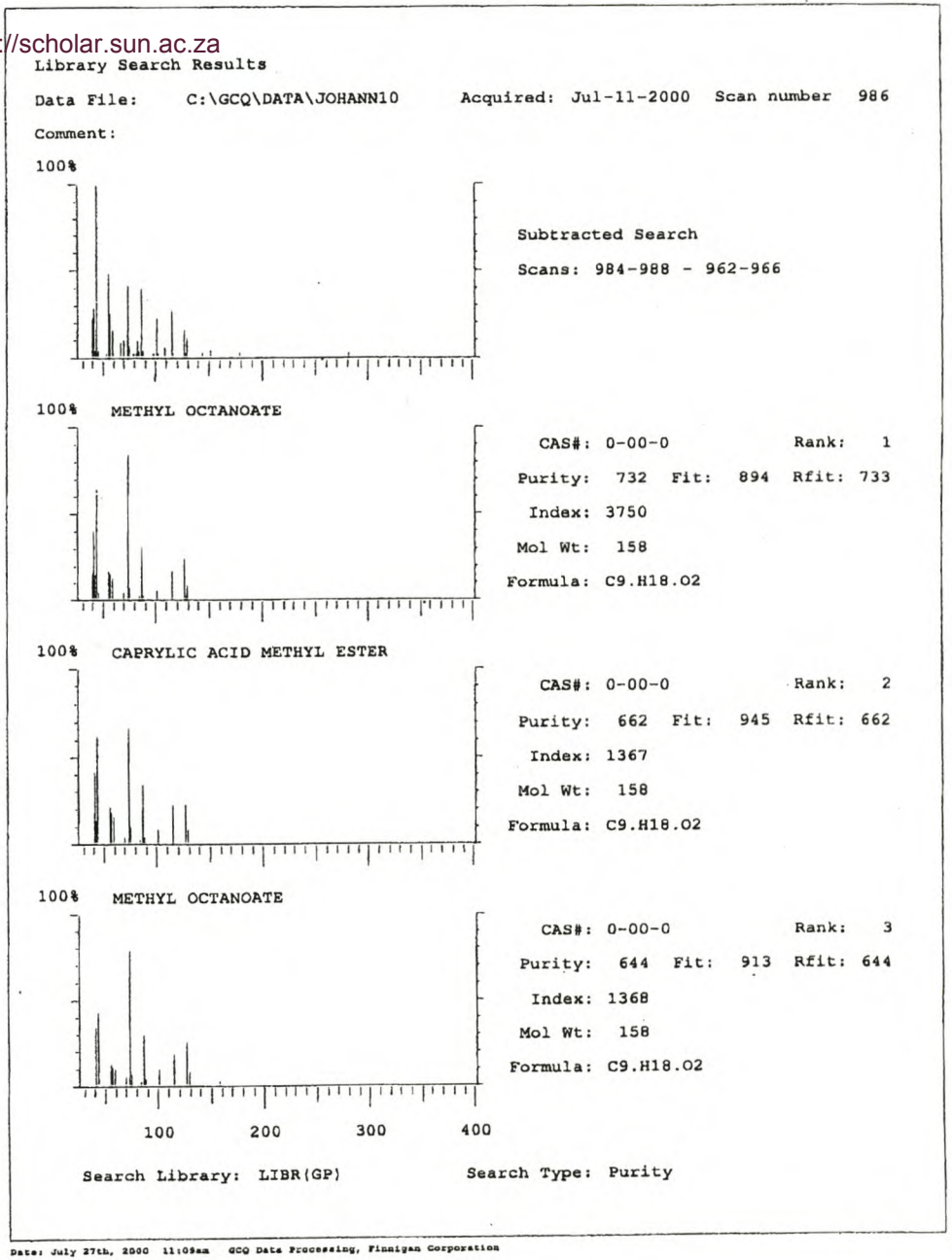

Figure 5. Mass spectrum and library search results for peak 5. Peak number corresponds to that in Figure 7.

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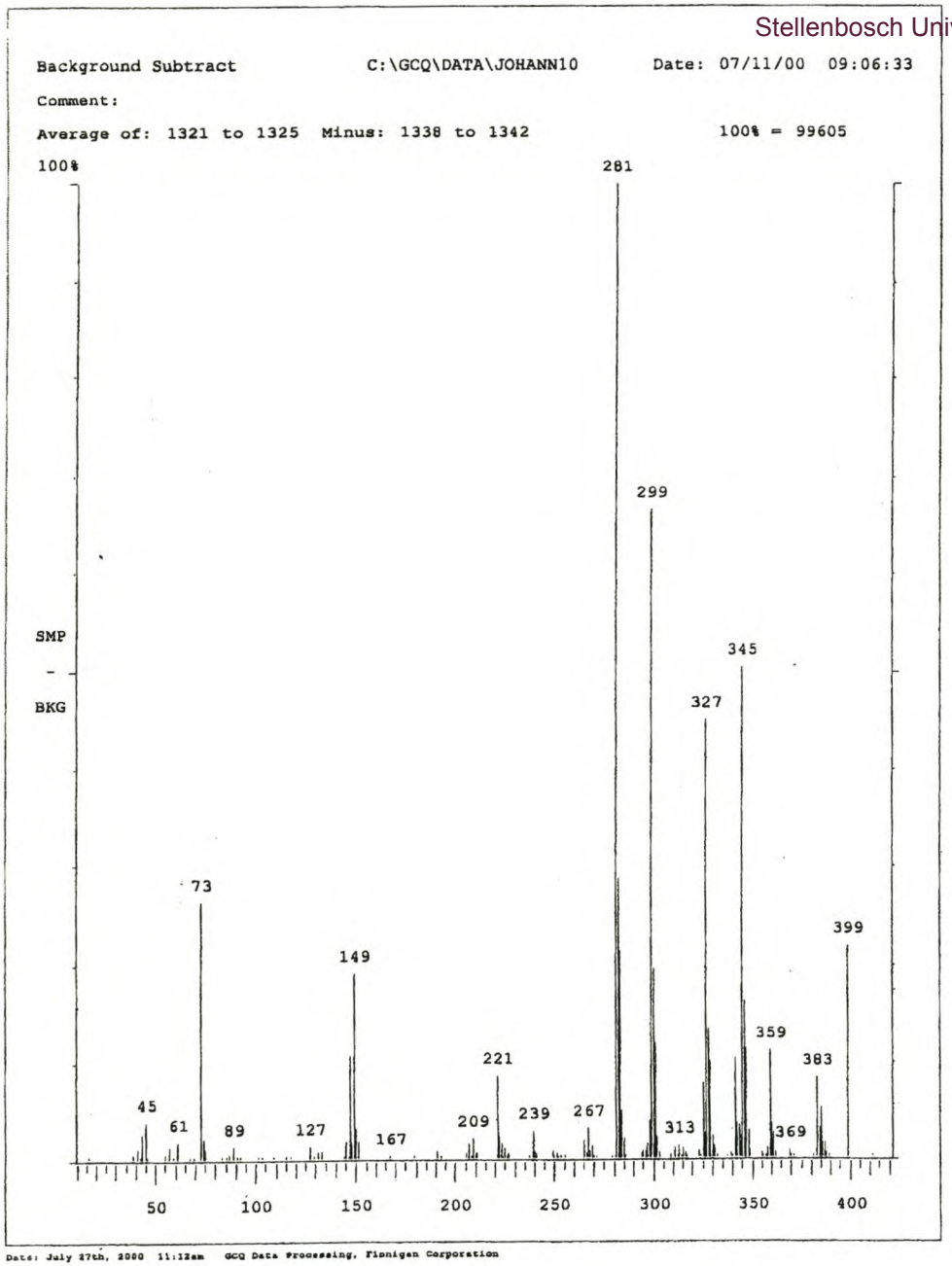

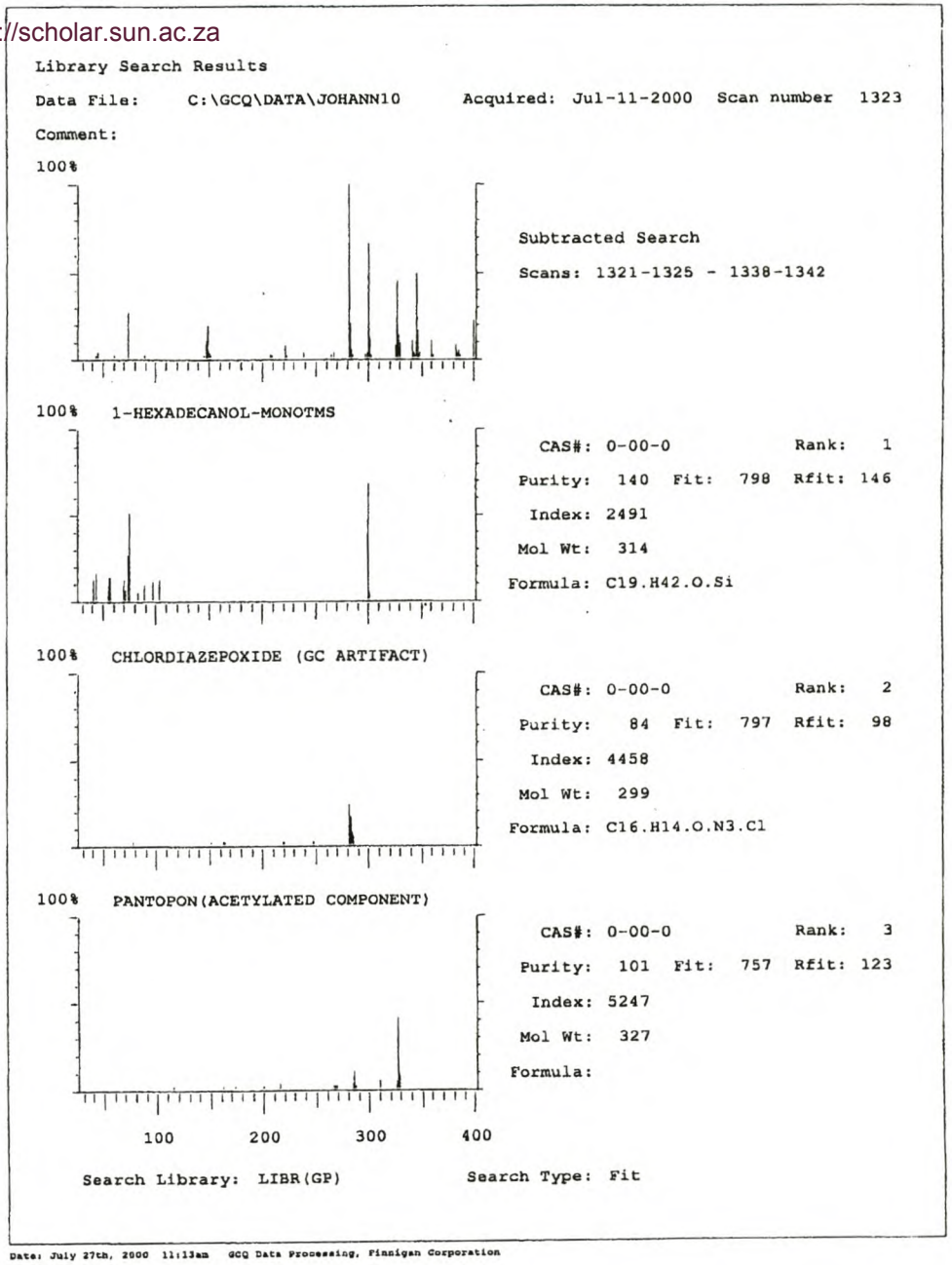

Figure 6. Mass spectrum and library search results for peak 6. Peak number corresponds to that in Figure 7.

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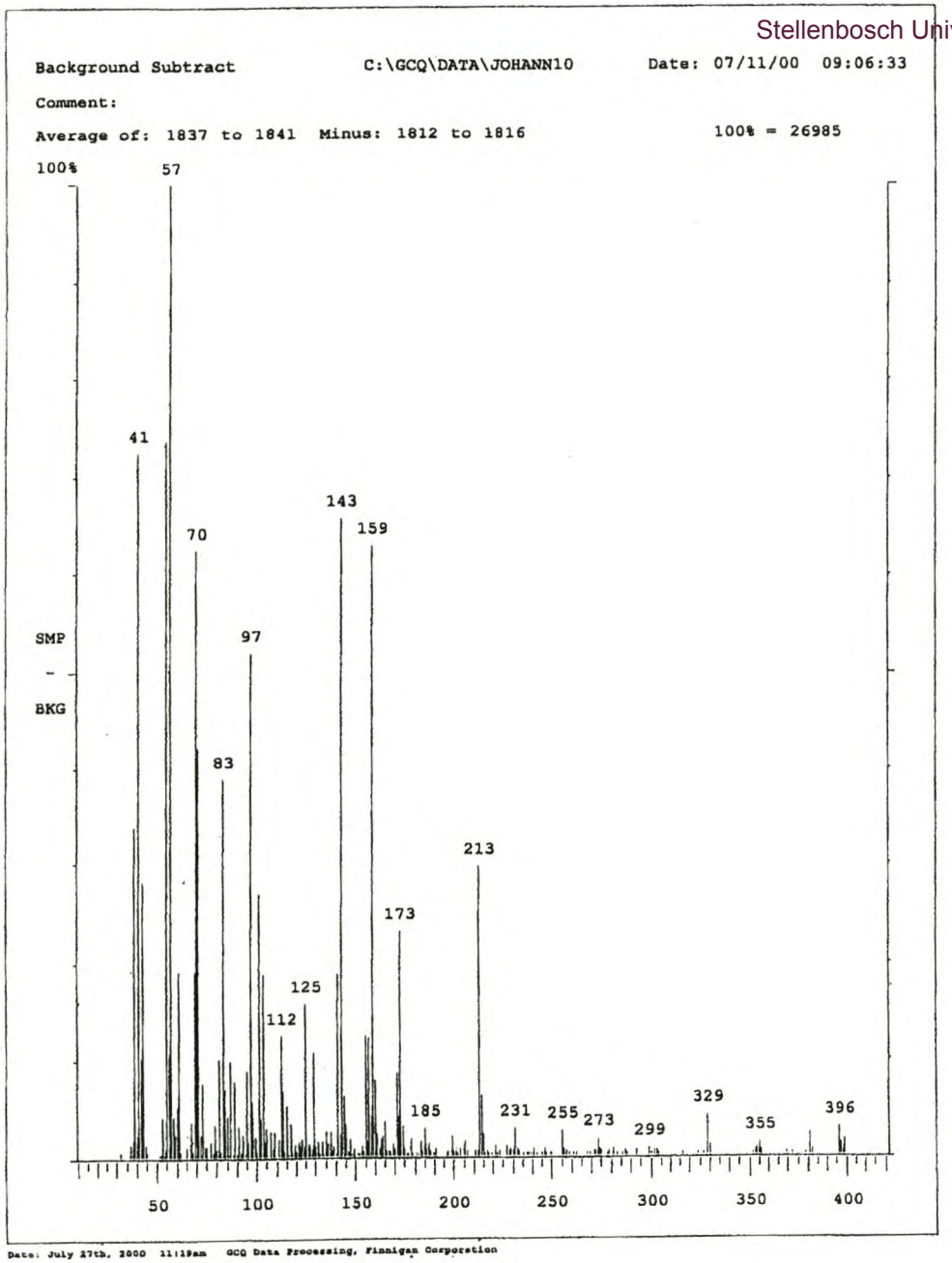

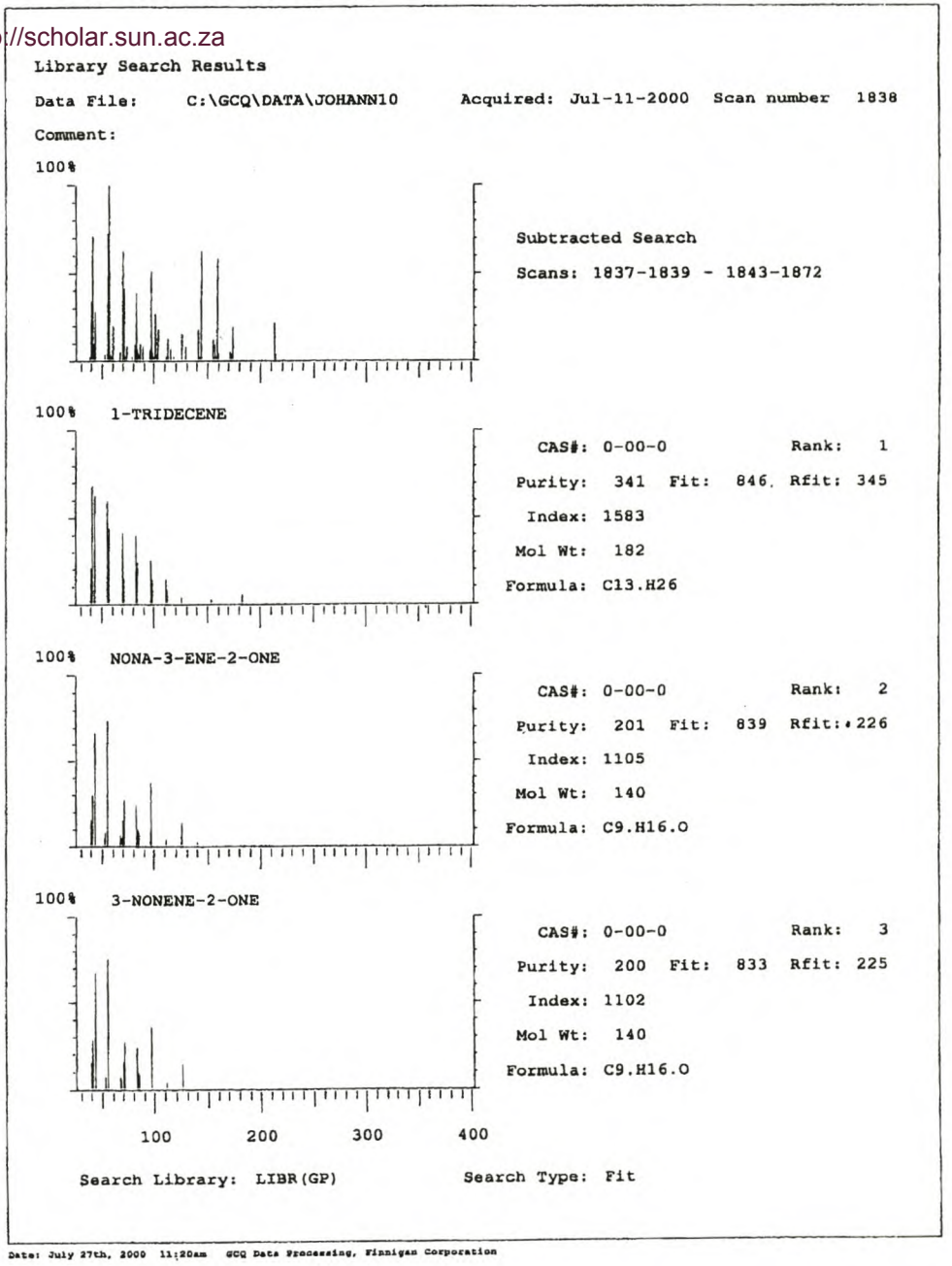

Figure 7. Mass spectrum and library search results for peak 7. Peak number corresponds to that in Figure 7.

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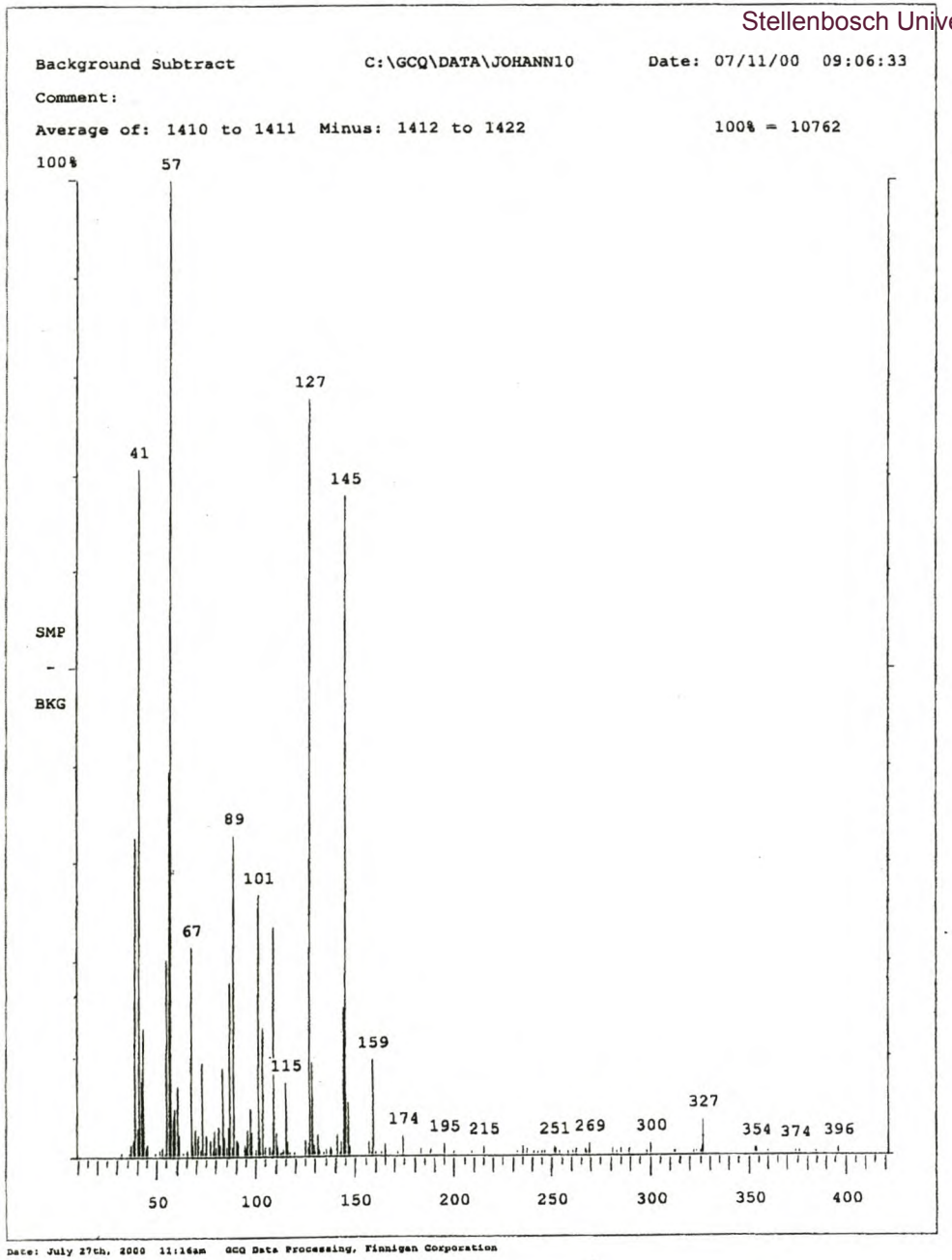


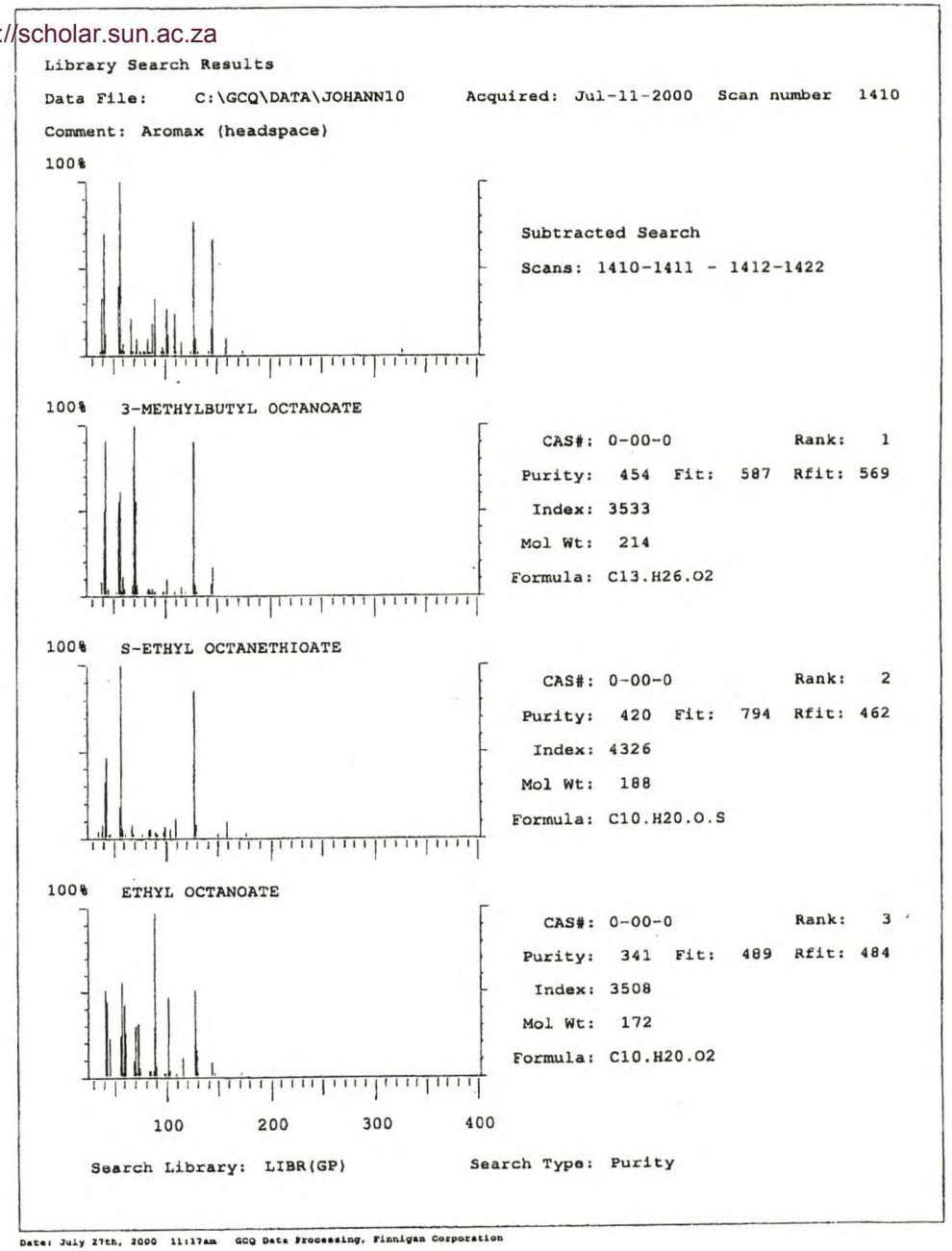
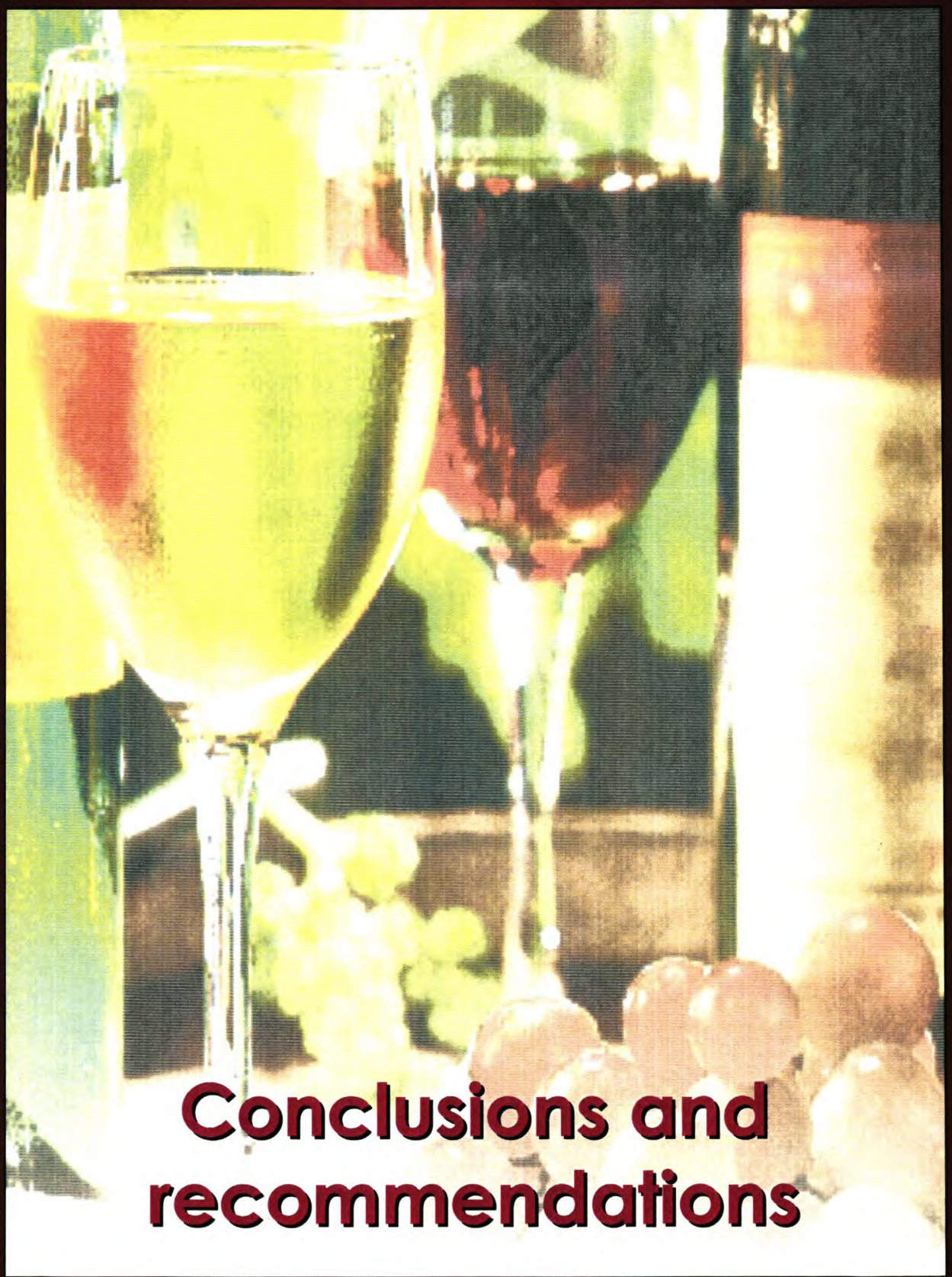


Figure 8. Mass spectrum and library search results for peak 8. Peak number corresponds to that in Figure 7.

CHAPTER 7



Conclusions and recommendations

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CHAPTER 7
CONCLUSIONS AND RECOMMENDATIONS

Vitis vinifera L. cv. Sauvignon blanc wine is highly valued for its distinctive aroma, quality and style. Although typical Sauvignon blanc wines are characterised by the grassy, gooseberry, asparagus and green pepper-like aromas, most of the locally produced wines often possess a neutral character. Also of concern is the occurrence of sulphur-like off-flavours in the wines of this cultivar. Specific impact components, namely ibMP and MMP affect Sauvignon blanc aroma. Depending on the nature and composition of the wine, as well as the concentration levels of ibMP and MMP, different nuances are perceived, which may be ascribed to the combined synergistic action of these two and other components. Apart from these impact components, fermentation-derived esters and higher alcohols also make an important contribution to wine complexity and quality, as long as they do not overpower the cultivar-typical aroma.

Various factors may affect Sauvignon blanc wine composition and quality. For example, region, combinations of ascorbic acid/SO_2 treatments [ascorbic acid/meta preparate, SO_2 (control), pure ascorbic acid/SO_2] and yeast strain (Vin 13, VL3C, NT 116) affect wine composition, wine aroma intensities and overall quality, significantly. High quality Sauvignon blanc wines can be produced by the use of pure ascorbic acid/SO_2 treatment in combination with yeast strain Vin 13 or NT 116 and these treatments are therefore recommended. However, low quality Sauvignon blanc wines with a prominent undesirable, sulphur-like aroma were produced from grapes treated with a commercially available ascorbic acid/meta preparate in combination with the French yeast strain, VL3C. Although a newly developed commercially available ascorbic acid/meta preparate does not produce these off-flavours, undesirable sulphur-like aromas are still often detected in locally produced Sauvignon blanc wines. These aromas, affecting Sauvignon blanc wine aroma and quality negatively, need to be further investigated in order to obtain knowledge to understand and prevent the development of these sulphur-containing components. Furthermore, since the antioxidative, as well as, the oxidative properties of ascorbic

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acid are known, it is recommended that judicious use thereof in combination with SO_2, is essential during wine making.

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