The use of rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia* spp.) as wood alternative in red winemaking

by

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Thesis presented in partial fulfilment of the requirements for the degree of
Master of Agricultural Sciences

at
Stellenbosch University
Department of Viticulture and Oenology, Faculty of AgriSciences

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March 2015

Declaration

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Date: 12 December 2014

Summary

Wine knowledge has increased drastically in recent years with the aid of scientific experimentation. The producers of wine have increasingly incorporated new wine knowledge into the marketing and innovation of wine products. In parallel with the above, the consumer market has become more aware of winemaking practices and their influence on consumer health. The use of alternative methods and additives in wine are means by which producers have been able to create innovative wine products.

This study focused on the addition of rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia spp.*) plant material to red wine. Consumer liking, as well as descriptive sensory analysis (DSA) of different red wines made from a range of cultivars with the addition of rooibos and honeybush plant material, was investigated. Another aim of the study was to observe the effect of oxygen addition to red wines treated with rooibos and honeybush plant material.

The consumer studies included a number of events where wines treated with rooibos and honeybush material were compared with other commercial wines. The results could not clearly indicate whether consumers liked or disliked the wine products with added rooibos and honeybush plant material. Further refinement of wines with the addition of rooibos and honeybush can serve as topics for future research. Focus on the potential health benefits which can be added to wine from rooibos and honeybush plant material, as a result of inherent antioxidants within both these species, may serve as a novel research topic. The DSA panels identified aroma descriptors that could be related to the addition of wood and leaf or only rooibos and honeybush wood to wine.

The addition of oxygen to wines treated with rooibos and honeybush plant material indicated that aromas associated with these treatments are relatively stable in wine undergoing oxidation. This could possibly pave the way for future research on the topic of low sulphur dioxide containing wines. As a result of this study, a commercial wine product called *“Rooibos wine*” was developed and became available in retail. Further research on this topic and the impact of the addition of rooibos and honeybush material to wine may be of great benefit for the production of innovative wines with unique aroma profiles.

Opsomming

Kennis oor wyn het oor die afgelope paar jaar vooruitgegaan met behulp van wetenskaplike eksperimentering. Wynprodusente sluit toenemend nuwe wynkennis in die bemarking en innovering van wynprodukte in. Parallel hiermee het die verbruikersmark baie meer bewus geword van wynbereidingspraktyke en die invloed daarvan op verbruikersgesondheid. Die gebruik van alternatiewe metodes en byvoegings in wyn is maniere waarop produsente innoverende wynprodukte kan skep.

Hierdie studie het gefokus op die byvoeging van rooibos (*Aspalathus linearis*) en heuningbos (*Cyclopia* spp.) plantmateriaal by rooiwyn. Verbruikers se voorkeur, sowel as die beskrywende sensoriese analise (*descriptive sensory analysis [DSA]*) van diverse rooi wyn kultivars waarby rooibos en heuningbos plantmateriaal gevoeg is, is geanaliseer. Nóg ’n doelwit van die studie was om die effek van suurstofbyvoeging by rooiwyne wat met rooibos en heuningbos plantmateriaal behandel is waar te neem.

Vir die verbruikerstudies is geleenthede aangebied waar wyne wat met rooibos en heuningbos materiaal behandel is met kommersiële wyne vergelyk is. Die resultate kon nie duidelik aandui of die verbruikers gehou het of nie van wynprodukte wat met rooibos en heuningbos behandel is. Verdere verfyning van wyne met bygevoegde rooibos en heuningbos kan as onderwerpe vir toekomstige navorsing dien. Die verhoogde gesondheidvoordele van wyn wat met rooibos en heuningbos plantmateriaal behandel is as gevolg van die inherente anti-oksidante eie aan die spesies, kan dien as fokus vir ’n nuwe navorsingstema. Die DSA-panele het beskrywende terme vir aromas geïdentifiseer wat verband kan hou met die byvoeging van die hout/blaar of slegs die rooibos- en heuningboshout by die wyn.

Die aromas in wyn wat met rooibos en heuningbos behandel is, is gevind om relatief stabiel te bly tydens oksidasie. Dit kan moontlik die weg baan vir toekomstige navorsing oor lae swaweldioksied-bevattende wyne. As gevolg van hierdie studie is ‘n kommersiële wynproduk, genaamd *“Rooiboswyn*”, ontwikkel en is dit in die handel beskikbaar. Verdere navorsing oor hierdie onderwerp en die impak van die byvoeging van rooibos en heuningbos materiaal by wyn sal moontlik van groot voordeel wees vir die produksie van innoverende wyne met unieke aroma profiele.

This thesis is dedicated to the glory of God and to my parents.
Hierdie tesis is opgedra aan die glorie van God asook aan my ouers.

Biographical sketch

Alet de Wet was born in Bloemfontein on 11 May 1989. She attended La Rochelle Girls' High School and matriculated in 2007. After a year of training as a ballet teacher she enrolled for a BScAgric degree at Stellenbosch University, majoring in Viticulture and Oenology. She received her Bachelor's degree in 2012 and enrolled for a MScAgric degree in Oenology at the same University in 2013.

Acknowledgements

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

- Prof WJ du Toit, Department of Viticulture and Oenology, Stellenbosch University, for acting as my supervisor – many thanks for all the time and input in the study
- Nina Muller, Department of Food Science, for all the guidance and many hours of discussion
- Trevor Strydom and Michael van Niekerk from Audacia wine farm, Cape Winelands, Stellenbosch, South Africa, for your innovation, dedication and perseverance, which have been inspiring
- Cape Natural Tea Products®, Bellville, South Africa, for supplying all the plant material
- All the experimental cellar staff – Marisa Nell, Edmund Lakey and Andy van Wyk, your help was greatly appreciated
- Prof Martin Kidd, for all statistical analyses and interpretations, particularly the details and extra analyses
- Financial support from THRIP, Stellenbosch University and the NRF
- All involved at the sensory lab, Department of Viticulture and Oenology, Stellenbosch University
- My friends at the department: Erna Blancquaert, Anke von Mollendorff, Christo Kotze, José Luis Aleixandre, Cannelle Amandine Vanille Roy and Jennifer Langlois
- My friends who helped me over numerous weekends, in particular Siobhan Swart, as well as Arnold Vlok for his immense emotional support
- My family, Schalk, Mari, Schalk jr, Francois, Liezl and baby Reuben de Wet – your love and trust has acted as a guide and hope

Preface

This thesis is presented as a compilation of five chapters. Each chapter is introduced separately.

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General introduction and project aims

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Introduction

Oak wood plays an important role in the tertiary aroma development of many wines and has thus remained popular in winemaking practices. Despite the popularity of barrels, the use of barrels in winemaking has decreased – mainly because of high costs related to their production and shipping. Alternative oak products (AOP) like powders, chips and staves have been widely used as a more cost-effective wine making practice. Wood used for wine production should impart favourable aromas and flavours to the wine and should not have a negative impact on the wholesomeness of wine. The continued search for unique and innovative wine products has sparked interest in using indigenous or alternative wood in wine (Alañón *et al*., 2013).

South African indigenous wood may serve as an alternative wood product in wine, as South African wine laws permit the usage of such products (SAWIS). Rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia*) are both part of the Cape *fynbos* biome, and specifically the *Fabaceae* family. Both rooibos and honeybush are indigenous to the Western and Eastern Cape provinces of South Africa (Du Toit *et al*., 1998; Small & Catling, 2009) and have been harvested and processed mainly in order to produce herbal teas (Joubert *et al.*, 2008). The development of pharmaceutical products and extracts of rooibos and honeybush has also become popular in recent years (Joubert, 1996). Research concerning the antioxidant capacity of both rooibos and honeybush has been an important focus of the research portfolio of the ARC Infruitec-Nietvoorbij, Stellenbosch (Joubert *et al*., 2008), viz. especially that of “fermented” honeybush and rooibos. Both rooibos and honeybush are mainly sold in the “fermented” form (Joubert & De Beer, 2011), i.e. produced through a high-temperature oxidation process required for the development of the characteristic flavour and colour of these two herbal teas.

Rooibos and honeybush both have distinctive aroma profiles and as such potentially could aid in the development of wines with unique aromatic profiles. The aroma attributes associated with rooibos infusions include a host of favourable aromas and tastes, including woody, honey and caramel aromas and a slight sweet taste (Koch *et al*., 2012). Various honeybush species have also been investigated in terms of the aroma profile that they could contribute to tea, iced drinks and other beverages. Although the respective *Cyclopia* species each have their own distinctive sensory profile, a number of terms have been associated with the general aroma profile of honeybush. These terms include “floral”, “fruity”, “sweet-associated” and “woody”, with “plant-like notes” (Theron *et al.,* 2014).

The health-promoting potential of both these plant species has been investigated in various capacities (Joubert & De Beer, 2011). The interest in extracts of rooibos and honeybush as food

additives has increased. Attention to consumer behaviour within the marketplace has highlighted the trend of consumers choosing healthy food products. Consumers are attentive to the addition of synthetic preservatives to food and many search for naturally preserved products (Hoffman *et al*., 2014). The use of rooibos and honeybush, both containing a number of potent antioxidants (Joubert *et al*., 2008), as wine preservative or antioxidant agent may have future potential.

Curiosity about alternative wood that might be more cost-effective and help create innovative wine products has led to the investigation of alternative, indigenous wood sources. This study researched the use of alternative wood in wine. Research on this topic commenced after Audacia, a private commercial wine cellar in the Cape Winelands, South Africa, contacted the Department of Viticulture and Oenology (DVO) at Stellenbosch University with the suggestion of researching the use of rooibos and honeybush wood in wine. The Audacia wine farm had a vested interest in the commercialisation of a wine product with the addition of rooibos and honeybush wood and so worked alongside the DVO in this study.

Project aims

The study focused on the addition of rooibos and honeybush material to wine, with this material acting as alternative wood product. The main aims of this study were:

I. **to assess consumer acceptability of red wines treated with rooibos and honeybush material**

II. **the sensory characterisation of red wines treated with rooibos and honeybush material**

III. **a preliminary investigation of the stability of aromas associated with rooibos and honeybush treatments when exposed to oxidation**

Rooibos and honeybush tea material is normally sold as a mixture of approximately 90% leaf and 10% stem material (Anonymous, 2002). This mixture was tested in wine, as well as only the stem material, which will be referred to as wood in this study. Wood is normally classified as consisting of mainly the xylem, but for the purpose of this study included the whole stem (Chaffey *et al*., 2002).

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Chapter 2

Literature review

The use of alternative wood and an investigation of oxidative reactions related to wood treatments in wine

Chapter 2: Literature review

The use of alternative wood and an investigation of oxidative reactions related to wood treatments in wine

2.1. GENERAL INTRODUCTION

The usage of alternative wood products in wine has received attention in the last few years as a result of the global pressure to produce distinctive wines at a lower cost (Fernández de Simón *et al*., 2014). Oak wood have traditionally been used to add unique tertiary aromas and compounds to wine for numerous years, but other alternative wood sources, in particular indigenous wood, may also serve as flavour contributor to wine or other alcoholic beverages (Young *et al*., 2010). Similar to the process by which oak introduces new aroma and chemical compounds to wine (Garde-Cerdán & Ancín-Azpilicueta, 2006) the use of other types of wood such as cherry-, acacia- and chestnut wood may also contribute a host of unique aromas and chemical compounds to wine. These compounds, such as phenolics, may impact the anti-oxidant capacity, colour stability and aroma of the wine (Cerezo *et al*. 2008). In South Africa rooibos, *Aspalathus linearis*, and honeybush (*Cyclopia* spp.) have been widely used in some beverage industries, but their suitability serving as alternative wood products for wine production is not known.

This review will focus on alternative oak wood products as well as alternative sources of wood, which will include a discussion on the processing of honeybush and rooibos as well as the chemical and aroma characteristics linked to these South African plants. As alternative wood in wine may also influence the anti- oxidant capacity of a wine, oxidation in wine will also be presented in this review. The usage of novel products in wine production may influence sensory characteristics and therefore consumer studies of such products are important for possible commercialisation of these products and will also be touched on in this review.

2.2. ALTERNATIVE OAK WOOD PRODUCTS AND ALTERNATIVE WOOD

Global economic pressures have favoured the search for cheaper alternatives in winemaking, as well as dual purpose products that can enable cost cutting within wine production. Innovative wine products that have unique aroma and chemical properties (Young *et al*., 2010) may thus be created. One avenue of research of wine additives that has been highlighted in the last few years is that of wood additions in wine (Fernández de Simón *et al*., 2014).

2.2.1. ALTERNATIVE OAK PRODUCTS

Wood and wine, particularly red wine, have been coupled in traditional winemaking techniques since the reign of the Roman emperors (Sanz *et al*., 2012a). The use of wood vessels as a means of storage of wine served the original purpose of pairing these two natural products (Guchu *et al*., 2006). The use of barrels, particularly for high quality wines, has remained common practice due to

the extraction of unique oak aroma compounds from the barrels during wine maturation in oak barrels (Campbell *et al*., 2005). The introduction of alternative storage containers such as stainless steel tanks created the opportunity to decrease the use of oak wood barrels.

With the innovation of storing wine in stainless steel tanks the effect of tertiary aroma development in wine as a result of oak aroma extraction and the influence of oxygen diffusion through the barrel had to be adjusted for. The introduction of alternative oak wood products (AOP) that could be used in a stainless tank was thus investigated and implemented. The use of oak wood has remained an integral part of winemaking with barrels and alternative oak products (AOP). Other benefits of using oak wood barrels include the oxidative reactions that ensue as oxygen diffuses through the natural wood pores in a barrel (Del Àlamo & Nevares, 2014). These oxidative reactions assist in the extraction of aroma-derived components from the wood, as well as promoting the colour stability and natural clarification of the wine (Garde-Cerdán & Ancín-Azpilicueta, 2006).
The species, source and manufacturing process of oak wood plays a primary role in the aroma components extracted from the wood throughout the maturation of wine (Sanz *et al*., 2012a). Not only have various oak species been employed as raw material for barrels, but other types of wood have also been considered as raw material for barrel production and alternative products. In this manner the use of chestnut wood has been approved by the IOV (L'Organisation Internationale de la Vigne et du Vin) as an acceptable wood source for barrel production. The potential of new wood sources has thus become apparent and an entirely new area of research into the effectiveness of various other wood sources and their possible contribution to the aroma of the wine has been developed (Fernández de Simón *et al*., 2014; Young *et al*., 2010).

2.2.2. ALTERNATIVE WOOD, ROOIBOS AND HONEYBUSH PLANT MATERIAL

Not only has the occurrence of alternative oak products become more prevalent in winemaking practices, but the use of alternative wood other than that of oak has been implemented. The use of chestnut wood has been implemented extensively for use in barrel production, specifically in Italy (Fernández de Simón *et al*., 2014), the use of chestnut serves as a cost effective alternative to oak barrels.

Acacia wood has been of interest in the production of vinegar products, as the wood's composition promotes the permeability of air and, in this way, encourages the production of acetic acid in the wine. The profiling of Acacia wood with regard to its phenolic composition was investigated (Sanz *et al*., 2012a), but further research is required to explain the sensorial impact that the use of Acacia would have on the balance and perception of aromas in red wines.

The use of mulberry wood has led to the extraction of a compound called picceatannol. This compound has drawn much attention due to its potential as additive in anti-leukaemia medication, as well as in anti-melanoma treatments. Picceatannol has a structure related to that of a

hydroxystilbene phytoalexin that is effective in the above-mentioned treatments (De Rosso *et al*., 2009). The potential of alternative wood in wine therefore not only presents an opportunity to create wines with unique flavour and aroma profiles, but also can be promoted with regard to the potential health benefits these woods could contribute to a wine product.

The use of indigenous wood and its relevance to winemaking is mentioned in a study by Fernández de Simón *et al*. (2014), raising the question if South African indigenous wood could serve as an alternative wood product. In this manner the costs related to the importation of oak barrels from the USA and Europe can be reduced. The possibility of creating wines with unique aroma profiles may also become a secondary benefit. Table 2.1 provides a summary of the various alternative wood sources, as well as traditional oak wood, and the potential aromas these wood types may contribute to wine production.

Table 2.1: Type of wood used in winemaking practices with the aromas the wood can contribute to wine or vinegar (NA: Not applicable)

Type of wood	Geographic source	Species	Major contributing aromas	Chemical compounds responsible	References
Oak	French	*Quercus robur; Quercus sessilis*	Spice and smoke	Eugenol and guaiacol	Guchu *et al*., 2006
Oak	American	*Quercus alba*	Coconut, vanilla, woody, spice	*Cis* and *trans* oak lactones, eugenol	Arapitsas *et al*., 2004; Guchu *et al*., 2006
Oak	Hungarian	*Quercus petraea; Quercus robur*	Vanilla, toasty	Vanillin, furfural	Guchu *et* al., 2006
Oak	Spanish	*Quercus pyrencia*	Coconut, vanilla, woody	*Cis* and *trans* oak lactones, vanillin	Fernández de Simón *et al*., 2010
Oak	Portuguese	*Quercus pyreniaca*	Vanilla, woody	Vanillin, oak lactones	Caldeira *et al*., 2002
Oak	Russian	*Quercus robur*	Spice and smoke	Eugenol and guaiacol	
Oak	Bulgarian	*Quercus sessilis*	Spice and smoke	Eugenol and guaiacol	Garde-Cerdán & Ancín-Azpilicueta, 2006
Chestnut	Mediterranean/ Portuguese	*Castanea sativa*	Vanilla, caramel (brandy production)	Vanillin, furanic compounds	Caldeira *et al*., 2010; Fernández de Simón *et al*., 2014
Cherry	NA	*Prunus avium*	Red fruit (vinegar products)	Future work	Cerezo *et al*., 2008; Fernández de Simón *et al*., 2014
False acacia	NA	*Robinia pseudoacacia*	Vinegar	Acetification	Sanz *et al*., 2012b; Fernández de Simón *et al*., 2014
Mulberry	NA	*Morus alba; Morus nigra*	Horse stable, medicinal	Ethylphenol	Guchu *et al*., 2006; De Rosso *et al*., 2009; Fernández de Simón *et al*., 2014
Ash	NA	*Fraxinus excelsior; Fraxinus vulgaris*	Future work	Future work	Fernández de Simón *et al*., 2014
Beech	NA	*Fagus sylvatica*	Future work	Future work	Fernández de Simón *et al*., 2014
Alder	NA	*Alnus glutinosa*	Future work	Future work	Fernández de Simón *et al*., 2014

2.2.2.1 ORIGIN OF ROOIBOS (*ASPALATHUS LINEARIS*) AND HONEYBUSH (*CYCLOPIA*) WOOD

Indigenous to South Africa are the wide array of plants comprising the Cape fynbos biome (Joubert et al. 2011). One of the families that occur within the *fynbos* biome is the *Fabaceae* family. Rooibos, *Aspalathus linearis*, and honeybush (*Cyclopia* spp.), make up the two genera in the *Fabaceae* family related to legume-type plants (Dahlgren, 1968). Records of the explorer Carl Thunberg from 1772 suggest the use of rooibos and honeybush by the Khoi people as an infused drink. The Khoi people developed a primitive, but efficient, harvest and processing methodology that is closely related to how rooibos and honeybush are processed in modern-day factories (Joubert *et al.*, 2008).

Since the early explorers recorded their use, both rooibos and honeybush have sparked interest among consumers as remedies for common ailments. Interest in the medicinal qualities of rooibos tea grew rapidly in the early 1900s due to the physician Dr P. le Frais Nortier, who promoted the use of rooibos tea as a hot drink and encouraged the cultivation of rooibos plants in the mountainous areas of the Western Cape, South Africa. Local inhabitants were employed by the physician and his colleagues to search for rooibos seeds in the mountains. During this search, thousands of seeds were retrieved from ant hills, where they had been stored by ants. Black ants collect the seeds after they are shot from seed caps (Morton, 1983).

Rooibos, *Apalathus linearis,* occurs wild in and among the Cederberg mountains, stretching northwards to the town of Nieuwoudtville and encompassing Clanwilliam and Citrusdal. Reports have concluded that the area under rooibos plant cultivation currently, specifically in the Clanwilliam vicinity, is in the order of 36 000 ha (Pretorius, 2007). Rooibos plants occur wild and there are four variants. The variant that serves as raw material in rooibos tea processing is that of “Rooi Tea” or “Red Type”, alternatively known as the Rocklands variant. The Rocklands variant occurs wild, growing sporadically in the mountains. When harvested wild, the Rocklands variant is called the Cederberg type. The demand for rooibos tea started to outweigh the supply of wild harvested Cederberg-type bushes. As a result, a commercial cultivar with finer, thinner leaves was developed and is known as the Nortier type (Morton, 1983; Joubert & De Beer, 2011).

Rooibos has gained popularity in recent years, primarily because it does not contain any caffeine (Morton, 1983). Much research has thus been done on the health properties of rooibos. The first speculation on the health-promoting quality of rooibos can be related to 1968, when Mrs Annetjie Theron was trying to sooth her toddler, who suffered severe milk allergies. The toddler had to drink soybean derivatives as a result of the allergy. In order to heat the soybean formula, Mrs Theron added a part rooibos tea. This mixture immediately calmed the child, soothing indigestion and other stomach cramps (Joubert & Ferreira, 1996). The remedy was remarkable and led to a surge among lobbyists for the further commercialisation of rooibos tea (Morton 1983). Rooibos tea’s marketability as a natural health product was seen as beneficial and profitable.

Honeybush (*Cyclopia*) is a member of the Fabaceae family and can be distinguished from other genera on closer inspection of the flower shape. The meaning of *Cyclopia*, originating from the Greek *cyclops*, refers to a circular shape and highlights the spherical attachment of the honeybush flower calyx to that of the pedicle. This point of morphology, unique to the *Cyclopia* spp., can be used as a distinguishing factor when identifying honeybush plants (Joubert & De Beer, 2011).

Honeybush is a perennial shrub-like plant reaching 3 m in height. The bushes are generally located in lower areas close to the coastline, as well as in higher mountainous plots fringing the Eastern and Western Cape shoreline. The bushes grow vigorously in shaded sites on south-facing mountains. Several species of honeybush have been classified with particular reference to how they have adapted to survive the damaging fires that occur regularly in honeybush habitats. The survival techniques include some species being able to re-sprout after fire damage. The sprouting species can be distinguished morphologically from the non-sprouting species by the noticeable lignotuber that forms part of its botany, from which new shoots sprout after a damaging fire. Non-sprouting species are able to generate new plants from seeds present in the soil as part of the soil seed bank. The hardy honeybush seed coat becomes damaged in fire conditions and thus promotes the germination of the seeds. When cultivated, sulphuric acid is used to damage the seed coats and promote germination (Schutte *et al*., 1995; Joubert & De Beer, 2011).

In this study, the focus was on the species *Cyclopia intermedia* and *Cyclopia subternata*, as the material supplied for use in the experimentation was a mixture of these two species. *C. intermedia* can be classified as a *sprouter* and grows very slowly, thus can be harvested only every two to three years. This lag in consecutive annual harvesting causes *C. intermedia* not to be cultivated. *C. subternata* can be harvested annually and thus is used as a cultivation product (Joubert & De Beer, 2011).

Although the industry is relatively small, the three main commercial species are *Cyclopia genistoides*, *C. subternata* and *C. intermedia*. The ARC Infruitec-Nietvoorbij Stellenbosch is involved in pre-harvest and post-harvest research on honeybush, primarily to determine processing parameters that would result in ideal product quality (Joubert & De Beer, 2011), but also to establish the species-specific sensory and phenolic profile of honeybush tea (Theron *et al*., 2014). Other organisations are also involved in furthering honeybush production. The South African National Botanical Institute (SANBI, Kirstenbosch) has been instrumental in furthering wide plantings of honeybush with a focus on its sustainable development as a profitable cultivation product. In collaboration with SANBI, the Agricultural Research Council (ARC) has also assisted in the development of research on the health benefits of various *Cyclopia* species (Joubert & De Beer 2011).

2.2.2.2 PRODUCT PROCESSING OF ROOIBOS AND HONEYBUSH WOOD

The resilience of both rooibos and honeybush, making them able to grow in seemingly unfavourable environments, including sandy, acidic soil and natural fire hazard areas, highlights the importance of environmental protection plans for the fynbos biome. The potential of rooibos and honeybush for use as material in foodstuffs and other products is very highly regarded due to the fact these species are indigenous to South Africa (Joubert & De Beer, 2011).

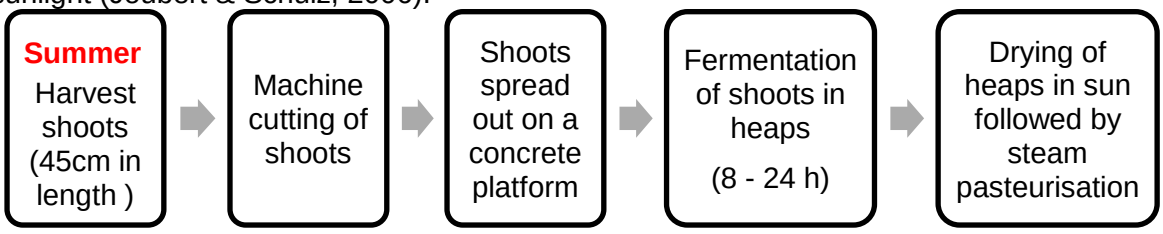
Much of the methodology of current rooibos and honeybush tea processing originates from the original practices of the Khoi inhabitants in the Western Cape, as observed by explorers and scientists from the 18th to 20th century. Refer to Figure 2.1 for a general overview of both rooibos and honeybush product processing. Rooibos plants are widespread in the Cederberg Mountain areas and are harvested wild, but an increased demand for rooibos products in recent years has led to large-scale harvesting of cultivated crops. The part of the production that is harvested wild is mixed with cultivated produce in order to comply with grading standards and consumer preferences (Small & Catling, 2009). The grading of rooibos crops has become highly regulated in order to maintain a fixed standard in the rooibos industry. Professional, expert graders are appointed to award a grade standard between A to D for particular batches of tea. The criteria used by graders are based on the flavour, physical appearance and red colour of fermented rooibos. The criteria for green rooibos are adapted to the required greener product colour. Most commercial rooibos tea batches are graded B to C, whereas rooibos material destined for extraction products, as in ice tea, generally will be graded D, being the lowest quality rooibos product (Joubert & Schulz, 2006).

Rooibos bushes are planted as seedlings and can be used as cultivation crop for up to seven years. After seven years, the extensive lignification causes large amounts of dead wood to become part of the bush. The shoots are harvested in summer before the rooibos bushes are able to flower. The presence of flowers causes the quality of the rooibos product to decrease very rapidly. The shoots cut are up to 45 cm in length, as cutting longer shoots lowers product quality. The yield of utilisable tea product from a fully grown rooibos shrub can be up to 226 grams of dried plant material (Morton, 1983). After transporting shoots from the field to a concrete platform, machines cut the shoots into smaller pieces and these are spread out in heaps. Damaging the shoots by cutting initialises enzymatic activity, resulting in polyphenolic oxidation. During the cutting and shredding of the harvested material, polyphenols are released, resulting in the browning of the plant material and the development of the inherent flavour of rooibos through oxidation.

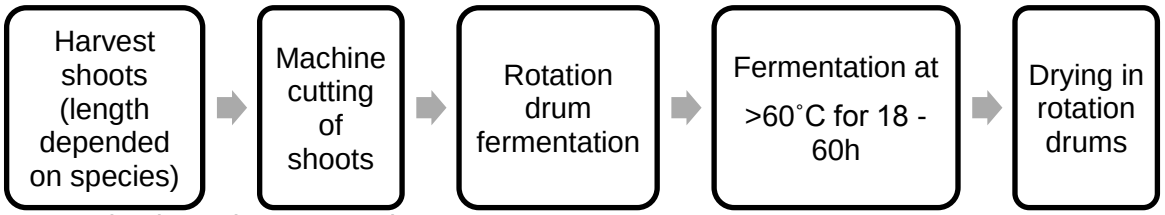
The harvested material consists of leaves and stems, which are later, sieved in order to split the two plant fractions. The teabags contain a third stems together with a majority of leaves. The red-brown polyphenols are readily absorbed onto the stems and cause the colouration of the harvested material. In the rooibos industry, the oxidation of polyphenols by oxidative enzymes is called

"fermentation" (Joubert & De Beer, 2011; Joubert & Schulz, 2006). Throughout this thesis the oxidation period of the rooibos and honeybush heaps will be referred to as fermentation.

In order to assist the enzymatic action, the piles of rooibos shoots are wetted. As reported by Morton (1983), the amount of water added to each pile can be amended according to the following ratio: 10 L of water for every 35 kg of cut shoots. The piles or heaps of rooibos are agitated regularly by a tractor turning the piles over. Each producer will have his or her own means by which to enhance the fermentation of the rooibos, either by covering the piles with plastic or varying the thickness of the shoot heap. The variation in heap thickness can be from 15 to 30 cm, depending on the rapidness of the drying desired. The period of fermentation, from 8 to 24 h, is also regulated according to the producer's needs, as well as prevailing temperatures in the processing area. Fermentation takes place at night and the drying of the rooibos occurs in direct sunlight (Joubert & Schulz, 2006).



A: Rooibos product processing



B: Honeybush product processing

Figure 2.1: Flow diagram of rooibos and honeybush product processing

Other factors that determine how long the shoots are fermented include how long the shrub has been used for cultivation and how extensive the young growth is on the harvested bushes. The temperature in the fermentation heap can reach 42˚C when the oxidation process is at its peak. The end point for the fermentation period is highly dependent on the speed of flavour development in the fermenting piles. The producers seek the sweet, honey-like flavour generally associated with fermented rooibos. When this flavour has developed, the heaps are spread over a larger area in order for rapid sun drying to commence. Steam treatment as a pasteurisation mechanism is also applied to the fermented and sieved shoot pieces to ensure microbial stability (Joubert *et al.*, 2008; Joubert, & De Beer, 2011; Joubert, 2012).

The harvesting of honeybush material depends on the species used. Harvesting prior to the flowering period is highly recommended for *Cyclopia* species. Harvesting in the flowering period causes great pressure on the plant and may lead to lignifications or die-back. Previously, the presence of flowers in the harvested material was believed to enhance the honey, floral, fruity and

sweet-associated aroma of honeybush products. Sensory analysis of honeybush products containing flowers versus a product without flowers was investigated by Du Toit and Joubert in 1999. The results of their study indicated that there were significant differences in the aroma profile of tea infusions when the flowers were present in the final processed product. The presence of flowers in the product led to an overall sweet-associated aroma profile (Du Toit & Joubert, 1999). The increasing demand for honeybush products has led to the extension of the harvesting period into late summer and autumn. Traditionally, the harvesting of honeybush was completed in the flowering period, which occurs in spring from September up until October. As stated, the presence of flowers gives a sweeter aroma and flavour, but jeopardises the well-being and effective growth of the plant. In order to continue the sustainable growth of wild honeybush sources, the prevention of die-back has become essential. *C intermedia* is cut back as close to the base as possible at harvest, as this action promotes new growth for the following harvest as a result of this species being a sprouter (Du Toit *et al*., 1998; Joubert, 2008; Joubert. & De Beer 2011).

When harvested, the honeybush material is transported back to the processing site, where it is quickly cut into smaller pieces of two to three millimetres which rapidly initiate oxidative actions in the fermentation period. Mechanical fodder cutters were employed earlier as a tool to cut the harvested stems, but in later years the use of a tobacco cutter was preferred, as the final cut product would be more uniform (Du Toit *et al*., 1998). The quality parameters of honeybush include the visual appearance of the product to a great extent, as well as the development of aroma and flavour characteristics, including honey-like, sweet, floral and fruity notes. Having good even coloration of the white stems to dark red brown serves as a grading criterion and measure of fermentation progress. To assist the even colouration of stems, the practice of pre-wetting has proven to be adequate. The water added to the cut material acts as medium for polyphenols, inherent to the stems, to come to the fore and become rapidly oxidised by enzymatic action (Joubert *et al*., 2011).

Traditionally the use of curing heaps was employed for honeybush, as with rooibos production. The use of the heap to initiate the fermentation process is cheap and can be completed easily. Variation in the quality, as well as a lack of process control by using fermentation heaps, has encouraged other methods of honeybush fermentation (Du Toit & Joubert, 1999). Most importantly, the use of fermentation heaps offers no control over the presence of extensive microbial growth within the heap, particularly as a result of the duration of curing in a heap, which can last up to five days. The high temperature of the curing heap and the extent of water present in the anaerobic environment within the heap cause large amounts of mould and bacterial growth (Du Toit *et al*., 1998).

The presence of mould growth on fermented honeybush is believed to decrease the product quality by giving the product a faded appearance and negatively influencing the flavour profile of the final product (Du Toit *et al*., 1998). Rotation drums for fermentation, as well as for drying, are employed

in modern-day processing plants and increase product quality by reducing the time required for fermentation. The temperatures of 60˚C and higher in the rotation drums act as an effective anti-microbial treatment (Du Toit *et al*., 1999). *C. intermedia* is currently exposed to fermentation periods of 60 h at 70˚C, while *C. subternata* undergoes fermentation for 18 to 24 hours at 80 to 85˚C (Joubert. & De Beer, 2011). The drying of the material also occurs in rotation drums, with the time of drying dependent on the species and the moisture content of the material.

The processing period for honeybush products is very important, as the steps taken in this time benefit the characteristic colour and aroma development associated with honeybush. The flavour and colour of the fermented honeybush serve as tools to assess the quality of the fermented product.

2.2.2.3 NON-VOLATILE COMPOSITION OF ROOIBOS AND HONEYBUSH

The increased popularity of honeybush and rooibos tea worldwide can be attributed to their increased association with health benefits. Large amounts of polyphenols form part of the chemical composition of both of these fynbos plants. In the case of rooibos, the presence of unique antioxidant compounds like aspalathin has sparked much interest in the potential of rooibos products as health supplements (McKay & Blumberg, 2007; Joubert *et al*., 2008).

When considering rooibos and its chemical composition, the presence of powerful antioxidants, including aspalathin, nothofagin and various flavonoids, indicates the potential of the health-benefiting properties of rooibos. An in-depth discussion of the antioxidants present in rooibos and honeybush plant material continues in the subsequent sections of this literature review.

The compound nothofagin has to date only been identified in red beech trees (*Nothofagus fusca*) and in rooibos (Joubert & Ferreira, 1996). This compound has not been studied very extensively, but does possess antioxidant properties (Joubert *et al*., 2012).

The presence of particular compounds ascribed to honeybush and rooibos can be seen in Tables 2.2 and 2.3 In general, the levels of these compounds can differ to a large extent due to differences from year to year, as well between regions (McKay & Blumberg, 2007). As mentioned in section 2.2.2.2 (Product processing of rooibos and honeybush), the presence of flowers in harvested honeybush causes a stronger sweet aroma in the product, but decreases the overall polyphenolic content (Du Toit & Joubert, 1999). Thus, not only do year-to-year harvests bring about differences within the plant polyphenolic content, but the origin and mixture of leaves, stems and flowers also have an effect on the concentration of the chemical compounds. Studies done by Joubert *et al*. (2012) looked at the phenolic variation over three harvest seasons of rooibos and stipulated that, in order to quantify representative quantities of phenolic compounds within rooibos material, a very large sample size is required.

The three phenolic compounds present in most honeybush species is that of mangiferen, iso-mangiferin and hesperidin. In a study by De Beer & Joubert (2010), a high phase liquid

chromatography method for honeybush was established and refined. The phenolic content and chemical structure of C. *subternata* and *C. intermedia* were studied extensively. Together with the most prominent phenolic compounds present in honeybush, the following compounds were also identified in the above-mentioned species: luteolin, eriocitrin, narirutin and eriodictyol (Joubert & De Beer, 2010).

The mineral content of rooibos varies in different studies. Joubert *et al.,* (2008) reported that earlier analyses of rooibos mineral content were not completely correct and the more recent analysis techniques determine the mineral content accurately. Sodium (Na) and potassium (K) are the most abundant minerals, followed by magnesium (Mg), calcium (Ca) and phosphorous (P). Contradictory to previous reports, the presence of iron in rooibos extracts is much less and is only indicated as trace amounts. Honeybush, in contrast, has been shown to have high levels of calcium (McKay & Blumberg, 2007; Joubert, 2008).

Table 2.2: Phenols that occur in rooibos (*Aspalathus linearis*) material.

Chemical Composition of Rooibos (*Aspalathus linearis*) and Honeybush (*Cyclopia*)		
Chemical compounds occuring in *Aspalathus linearis*		
Compound Type	**Names**	**Reference**
Aspalathin Aspalalinin Nothofagin	dihydrochalcone-*C*-glucoside cyclic dihydrochalcone dihydrochalcone-C-glucoside	**Joubert, 2012**
Flavanones	dihydro-orientin dihydro-isoorientin hemiphlorin	**Joubert, 2008**
Flavones	orientin isoorientin vitexin isovitexin luteolin luteolin-7-*O*-glucoside chrysoeriol	**Preedy, 2014** **Joubert, 1996** **Joubert, 2008**
Flavonols	Aglycone compound - quercitin quercitin-3-O-robonibioside hyperoside isoquercitrin rutin	**Joubert, 1996**
Coumarins	esculetin esculin	**McKay, 2007** **McKay, 2007**
Phenolpropanoid	phenylpyruvic acid-2-O-glucoside (PPAG)	**Joubert E, 2012**
Other	phenolic acids (caffeic, ferulic, vanillic , ρ-hydrobenzoic acid, protocathechuic and ρ-coumaric) lignans flavone diglycosides (+)- catechin	**McKay, 2007** **Joubert, 2008**

Table 2.3: Phenols occurring in honeybush (*Cyclopia* spp.) material.

Chemical compounds occuring in all chemically studied species of *Cyclopia*		
Compound Type	**Names**	**Reference**
Xanthones	Mangiferin Isomangiferin	**McKay, 2007** **De Beer, 2012**
Flavanone	Hesperidin	**De Beer, 2012**
Chemical composition of species specific to this study. C. intermedia (fermented) and C. subternata (unfermented)		
Compound Type		**Reference**
Xanthones	mangiferin isomangiferin	**McKay, 2007**
Flavanones	hesperetin eriodictyol naringenin eriocitrin narirutin naringenin-5-*O*-β-D-glucopyranoside eriodictyol-5-*O*-β-D-glucopyranoside eriodictyol-7-*O*-β-D-glucopyranoside	**de Beer, 2012** **Joubert *et al.*, 2011** **De Beer et al., 2012** **Joubert *et al.*, 2011** **Joubert *et al.*, 2011**
Flavones	luteolin 5-deoxyluteolin scolymoside diosmetin	**De Beer et al., 2010** **De Beer et al., 2012**
Isoflavones	formononetin a formononetin-diglucoside afrormosin calycosin wistin orobol pseudobatigenin fujikinetin isosakuranetin	**Joubert *et al.*, 2011**
Flavonols	kaempferol glucosides	**McKay, 2007**
Coumestants **Do not occur in C. subternata**	mediacagol flemmichapparin sophoracoumestan	**Du Toit, 1998** **McKay, 2007**
Varied compounds	epigallocatechin gallate *p*-coumaric acid tyrosol tyrosol derivatives	**Joubert, 2008**
Tannins	proanthocyanidin	**Joubert *et al.*, 2011**

2.3 AROMA CHARACTERISTICS ASSOCIATED WITH OAK WOOD, ROOIBOS AND HONEYBUSH

When assessing the complex matrix of wine numerous factors play a role in a change of the aroma and aroma development. Not only will the primary cultivar aroma play a role, but secondary aroma development by yeast and tertiary aroma development during aging all play a role in the final aroma of a wine (Preedy, 2014). Each aroma contributor can be isolated and studied in light of its unique aroma contribution. The investigation of wood aroma compounds and the potential extraction in wine thus form an integral part in shedding light on the aromatic profile of a wine.

2.3.1 OAK WOOD AROMA COMPOUNDS

Naturally occurring aroma constituents of oak wood include so-called whisky lactones (cis- and trans-β-methyl-γ-octalactone), which are associated with toasty, coconut and, in high concentrations, vanilla-like aromas (Díaz-Plaza, 2002). The effects of toasting on the formation of essential oak aromas are a key factor in the potential quality of wine-related oak wood products. The degradation of the structural polymers hemicelluloses and lignin serve as extensive reserves of impacting aroma constituents. Toasting and the degree to which it is preformed depend mainly on the cooper's preference and the methodology of the cooperage (Campbell *et al*., 2005).

Oak lactones (cis- and trans-β-methyl-γ-octalactone) have a strong sensorial perception in wine. Oak lactones differ in flavour perception based on the formation of two isomers of this compound. This differentiation serves as a good distinguishing measure between different types of oak wood, specifically when comparing the American and French oak species with one another. American oak (*Q. Alba*) has considerably higher amounts of oak lactones in the wood. High values of oak lactones have been utilised innovatively by winemakers to produce wines that are strongly distinguishable by having prominent vanilla-like aromas (Arapitsas *et al*., 2004; Fernández de Simón *et al*., 2014).

Three stages of toasting are implemented by cooperages. Toasting levels can vary from heavy to light. Heavy toast imparts more char-like and smoky aromas. Medium toast is suited to the maturation of more fruity wines, which should have oak aroma without dominating the varietal wine characteristics. Lastly, light toasting levels focus the aroma profile on extractable constituents that are naturally present in the wood, particularly the oak lactones (Garde-Cerdán & Ancín-Azpilicueta, 2006). Heavier toasting degrades the structure of the wood, causing more contact between the wine and the wood surface, which may lead to the increased extractability of higher ellagitannins (Jordão, 2012).

With every level of toasting, different oak aromas become apparent. Therefore the degree of toasting applied to the inside of a barrel or alternative products will form a unique set of aromas in the processed wood. Thermal heat directly disrupts the composition of the structural polymers. Thus lignin degradation leads to the formation of vanillin in medium toasted and guaiacol in heavily

toasted wood. Guaiacol (chemically described as *o*-methoxyphenol) is related to smoky, char-like aromas. Vanillin contributes favourably to the vanilla aroma present with medium to light toasting (Arapitsas *et al*., 2004).

Similarly, eugenol is also formed with the breakdown of lignin as a result of heat application. This compound is of particular interest, as it is a favoured volatile phenol, adding clove, spice and smoky aroma descriptors to wine (Arapitsas *et al*., 2004). The degradation of hemicelluloses by heat exposure during toasting causes the formation of furfural, which gives an almond, toasty aroma quality to wood-treated wines (Garde-Cerdán & Ancín-Azpilicueta, 2006). Supplementary reactions like the Maillard reaction also add some aroma compounds that can have a pronounced effect on the complexity of wine. Aromas such as toast-like, sweet and caramel have been related to the end products of the Maillard reaction, namely cyclotene, maltol and furaneol (Guchu *et al*., 2006).

When considering the use of alternative oak products in relation to the extractable aroma that will be obtained, the surface-to-volume ratio between the wood powder, chips, cubes or staves and the available wood surface area of a barrel will have a considerable impact on the extractability of aroma compounds. The available wood-to-wine surface area within a barrel amounts to only 40% surface area. When comparing this ratio to the much higher surface area presented by wood alternatives, a higher aroma extraction can be expected (Garde-Cerdán & Ancín-Azpilicueta, 2006).

2.3.2 ROOIBOS AND HONEYBUSH AROMAS

Aromas ascribed to honeybush and rooibos products may differ from year to year, as variations in plant material occur within different areas and harvest seasons (Koch *et al.*, 2012)

Rooibos production in the Ceres and Clanwilliam areas has sought to establish a geographic indication (GI) for rooibos tea. In order to be assigned a GI for a particular product, the distinguishing attributes of that product in terms of composition and aroma and as obtained from its environment of origin have to be established. To unlock the sensory profile of rooibos, Koch *et al.* (2012) studied the aroma profile of rooibos in one production season, whereas Jolley (2014) included samples of a further four production seasons. These two studies indicated that the primary and secondary aroma profiles of rooibos can be described as follows: The primary sensory profile (i.e. all rooibos production batches illustrate these attributes) is predominantly made up of “rooibos-woody”, “fynbos-floral” and “honey” aroma notes, while “fruity-sweet”, “caramel” and “apricot” aroma notes are the predominant sensory attributes of the secondary profile (80% of rooibos production batches illustrate these aroma attributes).

Huba *et al. (*1985) expanded on the latter, and analysed the main volatile compounds in rooibos material by means of GC and GC-MS. These compounds are summarised in Table 2.4.

Table 1.4: Volatile components of brewed rooibos, identified by GC and GC-MS (Huba *et al.*, 1985, McKay & Blumberg, 2007).

Compound
Guaiacol
6-methyl-3,5-heptadien-2-one isomer
Damascenone
Geranylacetone
β-Phenylethyl alcohol
6-Methyl-5-hepten-2-one

The aroma profile of a number of honeybush species have also been subjected to sensory and chemical characterisation. Erasmus (2015) determined the generic and defining aroma of *C. genistoides, C. maculata, C. subternata* and *C. longifolia.* The following aroma attributes were present in all *four Cyclopia* species: “fynbos-floral”, “woody” and “fynbos-sweet”. These attributes were present in 100% of the production samples (> 200). The “characteristic” sensory profile of honeybush can thus be defined as a “fynbos-floral”, “woody”, “fynbos-sweet” aroma with a sweet taste and slight astringent mouthfeel. This generic profile differs slightly from that proposed by Theron *et al.* (2014), possibly because the latter study included only 58 production samples. Erasmus (2015) was also able to distinguish between the four *Cyclopia* species in terms of sensory profiles. This resulted in species-specific aroma and flavour wheels, i.e. quality tools that could be very valuable to the honeybush industry. *Cyclopia maculata* and *C. subternata* were characterised as being reasonably similar in sensory profile. Both can be described as having “caramel” and other sweet-associated notes. *Cyclopia longifolia* and *C. genistoides* were also characterised as being reasonably similar. Cyclopia *genistoides* was defined as being high in “rose geranium” flavour as well as bitterness, while *C. longifolia* had a slightly less prominent “rose geranium” flavour and no strong bitter taste.

Le Roux *et al.* (2012) identified the aroma-active compounds present in a number of *Cyclopia* species. The presence of a large percentage of volatile compounds encourages the distinction of honeybush as a unique aroma profile. With the identification of volatile compounds in the honeybush species, *Cyclopia subternata*, by means of gas chromatography paired with mass spectrometry (GC-MS), the majority of the influential aroma compounds could be identified as terpenoids and terpene derivatives (Le Roux *et al*., 2012). Similarly, the study done by Cronje

(2010) looked at the aromas that will be most influential in contributing to aroma as assessed by a panel using the method gas chromatography-olfactory (GC-O) analyses (Table 2.5).

Table 2.5: Aroma compounds as identified by gas chromatography-olfactory (GC-O) for fermented *Cyclopia subternata,* as adapted from Cronje (2010)

Odour active compound	Associated aromas
(*E*)-2-nonenal	Green, aldehydic, cucumber and fatty
(*E,Z*)-2,6-nonadienal	Green vegetable, violet leaf and cucumber
10-*epi*-γ-eudesmol	Sweet, floral and woody
(*E,E*)-2,4-decadienal	Fatty, waxy, fried and orange-like
epi-α-muurolol	Herbaceous and spicy
epi-α-cadinol	Woody and herbaceous
Geraniol	Sweet, citrus-like, rose and floral
Linalool	Floral-woody and refreshing
(*E*)-β-damascenone	Sweet, earthy, fruity green-floral, woody
(*E*)-β-damascone	Minty, tea-like and fruity (citrus and apple)
(*E*)-β-ionone	Raspberry-like, woody, cedar, fruity warm
3,4-dehydro-β-ionone	Slight leather, saffron
(7E)-megastigma-5,7,9-trien-4-one	Dried fruit, tea-like and spicy

2.4 OXIDATIVE MECHANISMS IN RED WINE AND ANTIOXIDANTS

Together with the potential aromatic impact of rooibos and honeybush plant material in wine the addition of unique antioxidant compounds that can curb the effects of oxidation may also carry merit. Both rooibos and honeybush have been noted in having potential health benefits as a result of antioxidant compounds as part of the composition of these materials (Joubert, 2008). The following section will deal with general oxidation in wine as well as anti-oxidants in oak, rooibos and honeybush.

2.4.1 OXIDATIVE SUBSTRATES IN RED WINE

The chemical steps of oxidation in wine relate to redox interactions between electron-rich and electron-poor substrates, which are catalysed by the presence of a metal, such as ferrous iron. Oxidation occurs continuously within many foodstuffs and other natural products, leading to in general to an overall loss of quality. This loss in quality as a result of oxidation, when related to

wine, refers to the development of off-flavours and colour changes within wine (Waterhouse & Laurie, 2005). The exposure of wine to oxygen is not always unwanted, especially in red wine, as limited oxidation reactions in red wine may improve its sensory profile. The oxidation of wine in barrels by means of oxygen transfer through the wood pores or oxidation by other vinification practices, such as pump-overs, encourages colour stabilisation and a reduction in tannic compounds (Waterhouse & Laurie, 2005).

Phenols form the foundation of red wine, as their presence contributes to the colour, as well as other sensory qualities, of the wine (Atanasova *et al*., 2002). Not only can the colour of red wine be attributed to anthocyanins, but the structure and mouthfeel properties of wine can to a large extent be associated with tannins, as both compounds form part of the phenolic group (Gómez-Plaza & Cano-López, 2011).

Red wine can be considered less sensitive to the detrimental effects of increased oxygen exposure than white wine, as there are larger amounts of phenols present in red wine. Phenols make up a large portion of the wine matrix. When considering the capacity of red wine to consume up to 180 mL O_2/L, as explained in a study by Singleton in 1987, the action of phenols as oxidative substrates has to be considered. Phenols thus make up the majority of the oxidative substrate in comparison to the smaller amounts of other antioxidants that also consume oxygen, namely ascorbic acid, sulphur dioxide and ethanol. Singleton (1987) also made reference to wine and how the wine style can influence the amount of oxygen that the wine can handle before the negative products of oxidative reactions become apparent. Light-style white wines can consume an estimated 60 ml/L oxygen in comparison to a full-bodied tannic wine, that can consume up to 600 ml/L of added oxygen (Singleton, 1987).

The numerous hydroxyl groups as part of the structure of phenols act as ready proton donors and take part in a range of reactions within the wine matrix (Gómez-Plaza & Cano-López, 2011). A closer inspection of the structure of phenols relates oxidative reactions to the ortho-hydroxyl groups present on the benzene ring, and various aromatic substitutive reactions have been related to the meta-hydroxyl groups (Fulcrand *et al*., 2006). The oxidative substrates other than phenols in wine are not present at sufficient levels to account for the large amount of potential oxygen that can be consumed by the wine. Thus, as noted by Gómez-Plaza & Cano-López (2011), the ability of red wine to consume oxygen depends on the concentration of polyphenols present in the wine.

Phenolics present in grapes and wine have been categorised into two main groups, namely the flavonoids and non-flavonoids. The flavanoid group includes anthocyanins, which are responsible for the red colour in grape skins and wines. When present in wine as the positive flavylium ion, anthocyanins yield a red colour. The flavylium ion is susceptible to various reactions, specifically oxidative reactions (Fulcrand *et al*., 2006).

Wine tannins are categorised into two different groups according to their origin. Hydrolysable tannins are introduced to wine by oak exposure or as an ingredient of tannin additives. The second group of tannins are condensed tannins. Condensed tannins are inherent in grapes and consist of monomeric subunits of flavan-3-ols (Herderich & Smith, 2005). The ability of tannins to bind with protein gives an idea of the tannin content of red wines. The tannin molecules bind to proteins present in the human mouth and, as such, cause the perception of astringency and bitterness in the wine (Gómez-Plaza & Cano-López, 2011).

2.4.2 WOOD PHENOLS

The majority of wood phenolic molecules consist of non-flavanoid compounds, namely ellagic tannins formed by the monomers gallic acid and ellagic acid. The presence of ellagitannins could have a pronounced effect on the sensory profile of wine. The tannins that occur most prominently in wood are hydrolysable tannins. Two non-flavanoids, namely gallic acid and ellagic acid, serve as the monomeric building blocks of the hydrolysable tannins (Jordão, 2012). Hydrolysable tannins like ellagitannin have been connected to the astringency of wine. The two most prominent ellagitannins occurring in wine are vescalagin and castalagin, as seen in Fig. 2.2 (Puech *et al*., 1999).

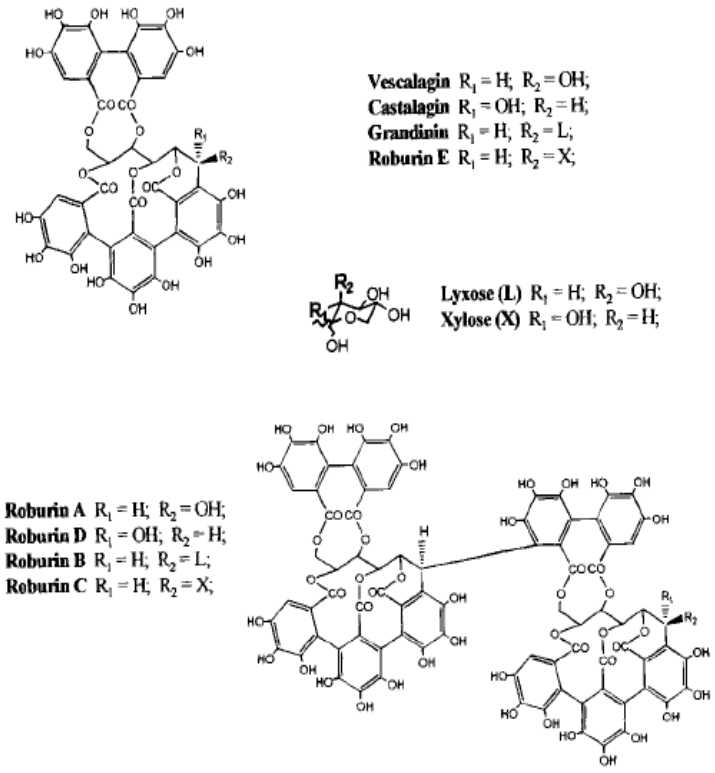


Figure. 2.2: The structure of the eight ellagitannins identified in oak heart wood (from Puech *et al*., 1999).

2.4.3 ANTIOXIDANTS PRESENT IN ROOIBOS AND HONEYBUSH PLANT MATERIAL

Natural antioxidants have grown in popularity as additives in food and cosmetic products. The occurrence of adverse resistance reactions to synthetic antioxidants has increased (Hoffman *et al*., 2014). These reactions have spurred the use of natural antioxidant-rich products (Joubert & Ferreira, 1996). Polyphenolic compounds form an important part of plant cells and the potential of phenols, especially flavonoids, as antioxidant has been noted in recent years. Rooibos and honeybush contain sufficient amounts of phenols that participate in a large number of bioactive reactions (McKay & Blumberg, 2007). Honeybush and rooibos have been investigated in terms of their antioxidant composition. The health-benefitting properties of rooibos have acted as an effective marketing tool in increased consumer consumption (Joubert *et al*., 2012). The development of rooibos tea production has thus continued as preferred beverage for kidney, cardiac and baby patients. Subsequent health benefits include relief of nervous tension, digestive aid, curing dermatological aliments and supporting sound sleep (Von Gadow, 1997; Morton 1983). These proposed health benefits connected to rooibos can be related to the action of antioxidants, which make up the chemical structure of rooibos material (Von Gadow, 1997). The soothing effect of rooibos tea with regard to spasmodic diseases caused by allergies has been connected to quercetin, a flavonol in rooibos (Joubert & Ferreira, 1996).

Aspalathin is one of the major contributing antioxidants present in and unique to rooibos plants. Aspalathin is a dihydrochalcone C-glucoside (β-hydroxy-dihydrochalcone) that does not occur readily in nature (Joubert & De Beer, 2011). Aspalathin plays an important part in the reactions that occur when green rooibos is fermented. Aspalathin has been recognised as contributing to the characteristic red colour of fermented rooibos (Joubert *et al*., 2012). Green rooibos, thus rooibos that has not undergone fermentation and still possesses its green harvested colour, has a much higher level of polyphenolic compounds. The fermentation process reduces the levels of aspalathin in the rooibos product to a large extent. With the fermentation of green rooibos, oxidative reactions cause aspalathin to form two products, namely orientin and iso-orientin, which are both flavones (Joubert *et al*., 2010).

Together with aspalathin, another compound that is unique to rooibos is phenylpyruvic acid glucoside (PPAG), which has been indicated to be important in anti-diabetic actions (Joubert *et al*., 2013). This substance, however, can add to bitterness in rooibos. Some of the other flavonoids present in rooibos have gained attention, as they are effective antioxidants. These compounds structurally include 3’ and 4’-dihydroxy positions and, as such, can readily reduce oxidative agents like superoxides. Included in this group are the flavonoids rutin, luteolin, orientin and orientin derivatives (Joubert & Ferreira, 1996).

Honeybush products have also been reported to have health-giving properties, mainly attributed to the phenolic compounds that form part of *Cyclopia* spp. With honeybush not having any caffeine and a low tannin content, infused beverages have been popular, specifically in the case of

indigestion or cardiac patients. Honeybush has also been noted to contain pinitol, which has anti-diabetic functions (Du Toit *et al*., 1998). As mentioned previously, the three most prominent phenolic compounds present in most honeybush species are mangiferen, iso-mangiferin and hesperidin. In honeybush, a few of the phenols that can be extracted from the plant material, specifically from *C. subternata* (De Beer *&* Joubert, 2010), have received much attention with regard to potential and known health benefits. As can be seen in Table 3, the flavone compound scolymoside, as well as flavonoids eriocitrin and hesperidin, are of particular interest as they possess strong antioxidant activity. In the abovementioned study, the importance of certain plant parts used with regard to antioxidant activity is again highlighted, as the leaves of *C*. *subternata* were proven to have significantly higher numbers of antioxidant compounds than the stems.

2.5 CONSUMER AND SENSORY METHODS

Consumer acceptability of a product relies on many factors, which includes intrinsic quality of the wine together with the market visibility and market trends (Charters & Pettigrew, 2007). Various sensory methodologies can thus be implemented in order to have a clearer understanding as to the perception of a new wine product made from alternative wood sources for instance, which will be briefly discussed in the following section

2.5.1 METHODOLOGIES FOR TESTING SENSORY WINE QUALITY AND CONSUMER LIKING OF WINES

Consumer behaviour forms an integral part of product development and marketing. Interest from consumers concerning product production has become a keen selling point, particularly in the wine industry (Forbes *et al*., 2009). Whether consumers like a product (or not) has to be considered in product development, and this is usually settled through market research (Nicolas *et al*., 2010). Consumers have become especially aware of sustainable practices that are environmentally sound and have human health benefits (Forbes *et al*., 2009). Wine product differentiation has become essential in the wine marketplace. Consumer tests can be used to establish whether a wine product will be successful in a highly competitive product sphere (Charters & Pettigrew, 2007).

The nine-point hedonic scale has been used extensively in product research, i.e. where the primary aim is to determine consumer liking of products when tasted blind. This methodology was first implemented by the US military in order to rate menu options. The bi-polar rating scale is a simple measure of rating with nine word categories, set out from “like extremely” to “dislike extremely”. The use of numbers together with words is avoided, as variations in data sets have been noted between only number and only word nine-point scales when presented to consumers (Nicolas *et al*., 2010). Blind tastings are important in order to receive unbiased data. Information about a product prior to tasting can skew the opinion of a consumer. The consumer may have a

brand preference or pre-set idea about a particular product if label or oral information is shared prior to a tasting, thus affecting the rating given on a hedonic scale (Siegrist & Cousin, 2009).

The method often used to quantify product aroma or other sensory attributes is descriptive sensory analysis (Lawless & Heymann, 2010; Piggot, 2011). In this method, a panel of assessors (eight to 12) have to undergo numerous sessions in which the product of interest is discussed and consensus is reached among the panel members in terms of the sensory attributes describing the sensory profile of the product(s), as well as the intensities of the respective attributes in the sample set. The sensory terms can be aroma qualities or tactile sensations related to the product (Piggot, 2011). These terms are them employed as means by which intensity ratings related to a product can be quantified in test sessions, with the latter being driven by the experimental design of the research project in question (Tomic *et al*., 2013).

2.5.2 DESCRIPTIVE ANALYSIS RELATED TO ALTERNATIVE WOOD IN WINE

The use of alternative wood sources in wine maturation has not been studied or applied extensively. The use of alternative wood, such as chestnut wood, has become more apparent in brandy production. Sensorial data has been obtained for brandy aged in chestnut wood by means of a trained panel (Caldeira *et al*., 2002). The sensory attributes of wine vinegar products aged in various wood sources, including cherry, acacia, oak and chestnut, were compared by means of descriptive sensorial analysis in a study by Cerezo *et al*. (2008). The trained sensory panel evaluated the vinegars based on taste. Vinegars aged in oak had a distinctive vanilla aroma versus cherry-aged vinegar, which had a stronger fruity aroma as rated by the panel. The vinegars aged in cherry and oak wood were preferred by the panel.

2.5.3 CONSUMER LIKING CONCERNING WOOD TREATMENTS IN RED WINE

In order to remain market relevant and increase the demand by consumers for wine products, the use of product innovation and unique consumer preference-driven products have to be introduced (Ortega-Heras *et al.*, 2010; San-Jaun *et al*., 2012). The way to establish the movement of trends as led by consumer demand requires data collection, either in the form of preference tests or liking by means of hedonic scaling (Ortega-Heras *et al.*, 2010). Young *et al*. (2010) looked at the sensory implications of indigenous wood from New Zealand compared to American oak (*Quercus alba*) chips in un-aged Chardonnay wine. The wine was exposed to 12 different species of wood chips, including *Q. alba.* An analysis of the treated wines included a large-scale consumer liking test. The 180 consumers' liking scores on a hedonic scale was recorded for six treated wines, four of which were treated with indigenous wood species. The results of this study indicated that there is potential to use indigenous wood as an additive in wine. The consumers significantly ($p < 0.05$) liked the wines that had the indigenous wood *Totara* added to the un-oaked chardonnay wine.

2.6 CONCLUSIONS

The development and refinement of alternative wood for winemaking practices may become more common in the future. The presence of rooibos and honeybush wood in wine can create unique aroma profiles that distinguish these wine products. So also can the addition of potent antioxidants, like aspalathin, serve as a research topic in relation to wine. The added antioxidants may have a favourable effect on the stability of the wine as a result of antioxidant reactions (Madrera *et al*., 2010). The addition of unique compounds to the wine, like aspalathin, also has the potential to act as chemical marker for wines that have rooibos added during the winemaking process, as aspalathin is unique to rooibos (Fernández de Simón *et al*., 2014).

However, to date the study of rooibos and honeybush material added to wine has not been reported on. Therefore clear descriptions of the potential aroma characteristics obtained in wine as a result of honeybush and rooibos material treatments are still not known. The general dosage requirements to obtain favourable aroma characteristics also need to be established before the commercialisation of such a wine product can be completed effectively. Consumer liking of wines treated with both rooibos and honeybush bears much relevance in order to gauge the commercialisation potential of the treated wine product. Consideration as to the stability of wine aroma and soundness of wines treated with rooibos and honeybush plant material in the oxidative environment experienced during red wine making also merits investigation.

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Chapter 3

Research results

Sensory profile and consumer liking of wine treated with rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia* spp.)

Chapter 3
Sensory profile and consumer liking of wine treated with rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia spp.*)

3.1. INTRODUCTION

The commercialization of a new wine product is reliant on the preference and product acceptability of the consumer market (Young, 2010). Innovation of wine products is continuously relevant to wine producers, especially with the pressure to stand out in the competitive wine market. Economic pressures as a result of the global economic downturn have spurred on the use of new and/or alternative production methods and additives (Fernández de Simón, 2010). The latter includes the usage of cheaper alternative wood products such as chips or staves. The use of cost-effective multi-purpose additives in wine making, i.e. additives that will complete numerous actions in the wine, give the winemaker a valuable means to save on cost. The potential of rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia spp.*) plant material as cost- effective dual purpose additives could be a very attractive alternative to conventional wine making practices. Not only could the rooibos and honeybush additions serve as potential contributors to the development of unique aromas, but there is also the possibility of adding additional antioxidants and this can be regarded as a secondary advantage (see Research Chapter 4).

The particular use of rooibos and honeybush plant material and/or honeybush and rooibos wood is novel and has not been presented to the consumer market in order to gauge the consumer acceptability thereof. When conducting consumer preference tests of products, it is possible to assess whether a product has the potential of being successful. When new products or a new product concept is launched, consumer testing is vital and might ensure that the new product range or product concept is not rejected by potential consumers (Lawless & Heymann, 2010). The use of consumer testing has gained popularity in wine research (King *et al.*, 2010). In order to develop a wine product with market potential, consumer data collection is required (Ortega-Heras *et al.*, 2010).

Consumer acceptance testing is thus an effective means by which to judge how a new product would compete in the current wine market. Interest in consumer acceptance of wine products that were treated with alternative wood sources was reported on by a study done in New Zealand by Young, 2010. Within this study 5 indigenous wood sources as well as *Querces alba* (American oak) were selected and added in chip form to un- oaked Chardonnay wine. These wines were then presented to 180 consumers in order to collect hedonic liking data concerning the wines. With analysis of the data, big variation among liking of the treated wines was noted. Some groups of consumers showed much preference to some of the wines where different groups of consumers showed no preference to the same wines. The species most liked in wine was that of the *Totara*

wood species. The control wine (no wood added) also had significant *liking* results. From this study the potential of an indigenous wood species or alternative wood source was displayed.

The nine-point hedonic scale is usually used when it is important to determine consumer preference and acceptability (Sidel & Stone, 1993). Consumer testing is usually conducted when new raw materials are used, when processing is adapted or when product packaging has changed. Furthermore, consumer testing can be conducted via the internet, or using home tests or central location tests. Central location tests (CLT) are usually conducted at research laboratories, designated facilities at supermarkets or, in the case of wines, at winery tasting rooms (Lawless & Heymann, 2010).

In the light of the above, the aim of this study was to ascertain whether red wines can be produced using wood and leaf material from two indigenous South African herbal teas, viz. rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia* species). Rooibos and honeybush wood was also further tested in wine as an alternative wood product. Consumer acceptance was tested by means of consumer liking tests to determine the potential of rooibos and honeybush as wood alternatives when producing red wines. Together with extensive consumer tests, expert wine judges and a sensory descriptive panel were also used to determine the general wine quality, as well as the sensory quality associated with a group of experimental wines that have been treated with rooibos and honeybush wood.

3.2. MATERIALS AND METHODS

3.2.1. Wine samples used for consumer testing at events 1 to 5

For the *consumer liking tests* an array of commercial wines were sourced and experimental wine samples were produced for the *five* events as indicated in Table 3.1. The treatments that the wines underwent are also indicated in Table 3.1.

The experimental wines used for **events 1 to 3** were all Shiraz, with no added sulphur dioxide. These wines were all produced at Audacia wine cellar in the Cape Winelands, Western Cape, South Africa. Rooibos (*Aspalathus linearis*) or honeybush (*Cyclopia intermedia* and *Cyclopia subternata*) wood/leaf material was added to the Shiraz wines at a dosage level of 5 g/L. It is important to note that the wood/leaf mixture does not comply with South African legislation, which only permits the addition of wood products in wine (SAWIS). However, the aim of this study was, *inter alia*, to ascertain whether a combination of wood and leaf material would influence consumer preference positively or negatively. The wood/leaf mixture was obtained from Cape Natural Tea Products®, Bellville, South Africa. The stem-to-leaf ratio was determined by the producer. The chips were 5 to 7 mm in size, with leaves mixed throughout. Sieves of various sizes are used in the processing of rooibos and honeybush plant material and the finer the sieve, the finer the material in the end product (Joubert *et al.*, 2008).

For event 4, Merlot wine, also from Audacia wine cellar, was treated separately with either rooibos or honeybush wood chips. Only the wood fraction (chips were 5 to 7 mm in size) of the rooibos and honeybush material was used so as to comply with South African legislation (SAWIS). After the chips were removed, the wine treated with honeybush wood chips was blended with wine treated with rooibos wood chips. The blend consisted of 20% rooibos-treated wine and 80% honeybush-treated wine and was made according to the preference of the winemaker of Audacia cellar.

For event 5, a Merlot wine from the 2010 vintage was used. This wine did not have oak additions, only marginal oak extraction as a result of storage in old oak barrels. This Merlot wine also underwent sulphur dioxide addition just after malolactic fermentation (MLF) (40 mg/L). The wine for event 5 was treated with rooibos and honeybush wood chips for four months.

As indicated in Table 3.1, the above-mentioned experimental wines were compared to the commercial wines at the respective consumer testing events. The commercial wines used were chosen by an independent panel that had a vested interest in determining how the experimental wines would compare to the selected commercial wines. The experimental wines were of potential commercial interest and the liking data therefore acted as a means to determine how the general wine consumer viewed the acceptability and potential of rooibos- and honeybush-wooded wine products compared to other commercial wines.

Table 3.1: Commercial and experimental wines used for consumer liking test, number of consumers assessed per event, and plant material treatment of the experimental wines.

Event	Commercial wine samples (Commercial) versus experimental wines (Experimental) used	No. of consumers assessed	Plant material treatment of experimental wines	Aims of the respective experiments
1	Four experimental wines used (RB, HB, W, B) [1]	104	RB: leaves and wood chips HB: leaves and wood chips W: oak chips and wooden barrel	Consumer acceptability was determined by *liking* scores of experimental wines (wine treated with rooibos and honeybush plant material) in comparison to a range of cheap and expensive commercial wines from the same cultivar and a range of vintages.
2	Two experimental wines were used, with four commercial wine samples (RB, HB, Stellar, Obik, Au, Rieb) [1]	108	RB: leaves and wood chips HB: leaves and wood chips	
3	Two experimental wines were used, with four commercial wine samples (RB, HB, Al, ER, RV, EI) [1]	118	RB: leaves and wood chips HB: leaves and chips	
4	One experimental wine was used, with five commercial wine samples (Au, Stellar, EE, RV, LU and WATER) [1]	69	Au: Blend of honeybush and rooibos wood-treated wines, 80% honeybush-treated wine with 20% rooibos-treated wine	Consumer acceptability, by means of consumer *liking* scores, of a rooibos and honeybush wood treated wine was compared to commercial wines from the same vintage and cultivar.
5	Six experimental wines were compared to each other (Control, RB 5 g/L, HB 5 g/L, RB 10 g/L, HB 10 g/L and blend (all the RB- and HB-treated wines)	85	RB: wood chips HB: wood chips	Consumer acceptability of experimental wine, treated with varying dosages of rooibos and honeybush wood were determined by consumer *liking* scores.

*RB = Rooibos material (*Aspalathus linearis*) and HB = Honeybush material (*C. intermedia* and *C. subternata)*; [1] Wines for events 1 to 3 were all Shiraz wine; [2] Wines for event 4 were all Merlot wine; [3] Wines used for event 5 were Merlot wine from the 2010 vintage treated with the indicated rooibos and honeybush wood as seen above.

Label keys*: Events 1 to 3*: W - Oak chip-wooded (4 g/L French oak) Audacia Shiraz; B - Blend of 50% rooibos-treated wine and 50% honeybush-treated wine; HB – Honeybush; RB – Rooibos; Stellar - Stellar Organics 2012; Obik - Obikwa Shiraz 2012; Au - Audacia Shiraz 2012; Rieb - Riebeek Cellars Shiraz 2012; Al - Alto Shiraz 2010; ER - Ernie Els Shiraz 2011; RV - Rust en Vrede Shiraz 2011; EI - Eikendal Shiraz 2011; *Event 4* - Au = Merlot 2013, treated with RB and HB wood; LU - Lushof; EE - Ernie Els Merlot 2013; WATER - Waterkloof Merlot 2013.

3.2.2. Testing of consumer liking at events 1 to 5 and testing of wine quality of wines presented at events 1 to 3

For the consumer liking tests, consumers were recruited randomly as they walked past a tasting area that was set up at a local weekend market in the Stellenbosch area, South Africa (see Addendum B for general pictures of the market set-up). In order to recruit sufficient consumers over two days for each event, three to four postgraduate students from Stellenbosch University were employed to ask consumers if they would like to join the consumer tasting in return for having their names put into a lucky draw competition as an incentive. The consumers were asked to complete a general socio-demographic questionnaire (an example of the questionnaire used can be found in Addendum C. The socio-demographic data included details of gender, age, occupation, frequency of wine consumption and some questions regarding knowledge of the preservation of wine, in particular the addition of sulphur dioxide to wine. The socio-demographic data collected were used to identify possible trends or consumer market segments (Lawless & Heymann, 2010). The consumers were given four to six wines to assess, depending on the event (see Table 3.1). The wines were served in standard ISO wine tasting glasses, with a random three-digit code on each glass to ensure the anonymity of the samples. The wine was poured behind a screen, out of sight of the consumers. Into each glass, 30 mL of wine was poured and covered with a petri dish to concentrate aromas in the headspace area of the glass. The wine samples were chilled in all the consumer tests (15˚C $\pm$1ºC). Each consumer received a set of wines, sorted randomly per consumer. Consumers rated each wine sample by selecting a point on the nine-point hedonic scale, i.e. between “like extremely” to “dislike extremely” (see Addendum C for examples of the tasting sheets used for the consumer testing).

For the expert tastings, the 12 wines presented to the consumers in events 1 to 3 (as seen in Table 3.1), were also presented to a panel of 28 expert wine tasters that included winemakers, wine researchers and wine ambassadors. The panel had to generate descriptors, using free description, and also had to rate the wines for general wine quality on a nine-point quality scale (see Addendum D for an example of the tasting sheet and the free description sheet presented to the expert panel). All twelve wines were given to the expert tasters at the same time. The wines were poured into standard clear ISO wine tasting glasses 30 min prior to tasting. The wine was kept at a temperature of 15˚C $\pm$1ºC prior to tasting. Each glass contained 30 mL of wine and was covered by a petri dish to concentrate aroma in the headspace of the wine glass. Each glass was marked with a random three-digit code and all samples were served in a random order to each assessor. Assessors were seated in a lecture hall environment with spaces in between them to avoid interaction. Water was given as refreshment and a small gift was presented to each panellist as a reward for joining the expert tasting.

3.2.3. Samples used for sensory descriptive analysis

Two descriptive sensory analysis experiments were completed for this study at the Department of Viticulture and Oenology (DVO), Stellenbosch University, South Africa. The first experiment investigated the aroma impact of varying dosages of rooibos and honeybush leaf/wood material when added to an un-oaked commercial red wine (Namaqua, Vredendal, South Africa). The second experiment tested the impact of varying dosages of rooibos and honeybush on the sensory profile of an aged 2010 Merlot (Audacia). In this instance, only wood material was used.

The store-purchased red wine used, packaged in a box, had no previous oak additions (Namaqua). The plant material, honeybush and rooibos, was added once the wine was purchased. The rooibos and honeybush plant material consisted of a mixture of wood chips (5 to 7 mm in size) and leaves. The samples were supplied by Cape Natural Tea Products®, Bellville, South Africa. The leaf and chip material was added directly to the un-oaked red wine and left in contact with the wine for two weeks, after which it was removed and descriptive sensory analysis was conducted. The treatments applied to the red wine can be seen in Table 3.2.

Table 3.2: Wine treatments used for first experiment (commercial red wine, with added rooibos and honeybush wood and leaf material).

Wine sample name	Treatment - addition of RB and HB wood and leaf material
Control	No addition of plant material
RB low	1 g/L RB
RB medium	2 g/L RB
RB high	5 g/L RB
HB low	1 g/L HB
HB medium	2 g/L HB
HB high	5 g/L HB

*RB = rooibos; HB = Honeybush

For the second experiment, an aged 2010 Merlot wine from Audacia was used. The treatments were all completed at Audacia wine cellar. Only rooibos and honeybush wood chips were added directly to the wine, the wine was then transferred from a tank to be stored in old oak barrels and remained in contact with the wine for four months before they were removed. The treatments can be seen in Table 3.3. Wine F was a tank sample of the 2010 Merlot wine. Descriptive sensory analysis of the wines was undertaken, commencing after the four months of extraction was completed.

Table 3.3: 2010 Merlot treated with rooibos and honeybush wood.

Wine	Treatment - addition of RB and HB wood
A	Control Merlot 2010
B	5 g/L RB
C	5 g/L HB
D	10 g/L RB
E	10 g/L HB
F	20 g/L HB (Tank)

* RB = Rooibos; HB = honeybush

3.2.4. Descriptive sensory analysis

Descriptive sensory analysis (DSA) was used in order to generate descriptors for the treated wines and also to rate the intensities of the various sensory attributes. The treated commercial boxed red wine and 2010 Merlot wines, shown in Table 3.2 and Table 3.3 respectively, were subjected to sensory analysis in two separate DSA sessions using the same nine female assessors for the respective sensory analysis sessions. Training sessions were conducted twice weekly for four consecutive weeks. Each training session was 2 h long and a total of eight training sessions were completed, see Addendum E for examples of the training tasting sheets.

The descriptor list for the commercial boxed red wine included the following aroma notes, measured orthonasally: berry, dried fruit, prune, rose, black pepper, vanilla, rooibos, honeybush, green apple, sherry and green vegetative. The retronasal and mouthfeel attributes included astringent mouthfeel, rooibos aftertaste, honeybush aftertaste and sulphur compounds. For most of the attributes, two reference standards were presented to the panel for training purposes, one fresh reference and one soaked in a neutral red wine. These standards included fresh green apple, sherry wine, beta-mercapto-ethanol (sulphur compound), fresh rose water, fresh red berries, dried fruit, dried prunes, black pepper seeds, vanilla pods, honey bush tea and rooibos tea. All the standards were presented as is; therefore the standards were not assessed in wine.

Descriptors generated by the descriptive panel for the 2010 Merlot included similar descriptors to those for the commercial red wine. The descriptors for the Merlot wine included the following aroma notes: berry, rooibos, vanilla, tobacco, prune, jammy, honeybush, floral, black pepper, cherry, raisin and cooked vegetable. Taste and flavour descriptors generated by the panel included the following terms: sweet taste, sour taste and bitter taste, astringent mouthfeel, as well as fruity, tea, spicy/pepper and tobacco flavour; the latter flavour notes were analysed retronasally.

The test sessions were completed in a sensory laboratory with ambient lighting, good ventilation and a constant temperature of 20 ± 2°C. The tasters were presented with the wines in dark ISO

wine tasting glasses at a volume 30 mL per glass. The wine was kept at a temperature of 15˚C prior to tasting. Petri dishes were placed on each glass just after pouring to contain the aroma notes in the headspace area. The glasses were filled 30 min prior to testing. The assessors were seated in individual booths in order to avoid any panel interaction throughout the test session. Intensity scores were marked on 120 mm unstructured line scales. Each line scale was labelled “none” to “intense”. The glasses were marked with unique three-digit codes and all the samples were randomised per assessor within each replication using a Williams Latin Square design. As a means of palate cleansing, purified water and unsalted crackers were provided to the panel.

For the commercial red wine, a total of seven wine samples were evaluated per assessor in triplicate using descriptive analysis (Table 3.2). These wines were tested for aroma and flavour notes. For the 2010 Merlot experiment (Table 3.3), the panel assessed six wines. These wines were also presented in triplicate. A 15 min break was taken after each repetition to avoid sensory fatigue. As indicated above, all samples were again randomised per judge per replicate, with a total of three replicate sessions over three days.

3.2.5. Statistical procedures

For DSA, the panel performance was assessed using PanelCheck® software (Version 1.4.0, Nofima, Ås, Norway). Analysis of variance (ANOVA) was conducted for all the aroma attributes generated by the tasting panel for the commercial red wine experiment, as well as for the 2010 Merlot experiment, using Statistica (version 10 and 12, Statsoft Inc., Tulsa, USA). Two-dimensional principle component analysis (PCA) bi-plots were generated using the sensory data. The PCA plots were drawn up in Statistica and PanelCheck, but only the Statistica plots are shown.

Statistica was also used to determine student t-test at 95% confidence intervals and $p \leq 0.05$ in order to analyse the liking data for the experimental wines, as well as that of the commercial wines. Indications of the significant differences between treatments and commercial samples, based on the liking of the wine samples, could be obtained by the t-test.

The consumer socio-demographic data were analysed with CART (classification and regression trees). CART is used to organise large data sets according to a number of elected classes incorporating in a regression tree that sets up a statistical model for the dependant variable (Romano *et al.,* 2014). The use of CART was employed to detect any trends between liking and consumer segments. The CART analysis was only employed for the data collected over the first three consumer testing events (see Table 3.1). The CART analysis was also completed in Statistica (Statistica, version 12, Statsoft Inc., Tulsa, USA).

3.3 RESULTS AND DISCUSSION

3.3.1 Consumer testing (events 1 to 3)

Fig. 3.1 displays the summation of the consumer liking results for events 1 to 3. From Fig. 3.1 a clear distinction can be made between the experimental RB and HB wines and the commercial wines. Significant differences in liking can be seen between the last block of wines (ER, RV EI and AI) and B, RB and HB. The latter three experimental wines received liking scores close to five, representing “neither like nor dislike”. In contrast, the commercial wines had liking values ranging from six to seven, indicating a significantly higher preference ($p \leq 0.05$) for the commercial wines. Considering that the scale of liking was from one to nine, and having the experimental wines being scored at approximately 5 and the commercial wines at approximately 6 to 7, it can be concluded that the consumers did not reject the experimental wines outright. In view of this, the experimental wines could be regarded as being “just” acceptable and thus reasonably drinkable. The lower scores from the consumers for the experimental wine could simply be due to the consumers not being familiar with the aromas associated with rooibos and honeybush wood products in wine. Another consideration may be that the base wine used for the experimental wine was not of the same quality as a number of the other commercial samples used in this event. This could have influenced the low scores given to the experimental wine by the consumers. These results encourage the further development and refinement of wine treated with honeybush and rooibos plant material in future harvest seasons.

The socio-demographic data, as well as some responses relating to wine consumption by the consumers, are listed in Table 3.4. For events 1 to 3 a total of 330 target consumers (157 male and 173 female consumers) indicated that they were interested in tasting the wines. The majority of the consumers were white South Africans, or visitors from Europe. The consumers tested represented the up-and-coming young wine consumer, i.e. 25 to 34 year olds. Although no criterion was placed on the consumers chosen for the liking assessments, the majority of the consumers indicated that they drink wine more than once per week. This reasonably high frequency of wine consumption (Table 3.4, section 2) is beneficial for this study, as these wine consumers could be regarded as having a reasonably established knowledge of wines and most probably also are quite adventurous when it comes to trying new trends in winemaking practices (Bester, 2011). As seen in Table 3.4, section 2, a large percentage of the consumers knew that wine contains sulphur, while a large number of them also preferred to buy wines with no preservatives or no/low sulphur. Therefore the majority of consumers were aware of the fact that sulphur is used in winemaking. The consumers therefore were mindful of the practices used in winemaking, specifically concerning winemaking practices that could impact on health (Forbes *et al*., 2009). Most of the consumers preferred full-bodied dry red wine (Table 3, section 3), therefore also an indication that they were

not novices when choosing wine. The latter is substantiated by the fact that the majority of the assessed consumers had been drinking wine for more than 10 years.

The results of the CART analysis can be seen in Fig. 3.2. The variables that showed the most significance when related to the dependant variable *liking* were the *treatments* of the wine and the *age* of the consumers. All the socio-demographic and wine consumption questions in Table 3.4 were considered for CART analysis; however, as mentioned, only “age” and “treatments” were significant enough to be included in the CART analysis. The CART analysis thus showed that a segment of consumers over the age of 45 constantly gave the highest liking scores (> 6.5) for a large range of wines when compared to the consumers aged younger than 45 (Fig. 3.4). The latter range of 10 wines included nine commercial wines and only one of the experimental wines, viz. the RB/HB blend. This result indicates that the red wine blend (i.e. a blend of RB- and HB-treated wine) was liked by the consumers older than 45 and younger than 45, i.e. similarly to the other nine commercial wines.

3.3.2 Expert tasting of wines evaluated at events 1 – 3

Wine quality, as determined by wine experts, has been researched in many studies, although the results are quite diverse (Lawless *et al.*, 1997). The main criterion used in judging the quality of wine was that of assessing the so-called “soundness” of the wine. The soundness of a wine refers to the degree that the wine is free from off-odours and other faults that might make the wine unacceptable for a consumer or taster (Ferreira *et al.*, 2009). The twelve wines presented to the 330 consumers in events 1 to 3 were also given to a panel of 28 experts that included winemakers, wine researchers and wine ambassadors (see section 3.2.2 for a he detailed description of the tasting set-up).

In Fig. 3.3, the quality ratings of the wines treated with rooibos plant material (RB) and honeybush plant material (HB) are compared with those of the commercial wines. One commercial wine, Stellar, together with the honeybush- and rooibos-treated wines (B, RB and HB), scored significantly lower ($p \leq 0.05$) for overall wine quality when compared to the rest of the commercial wines. The CA plot in Fig. 3.4 displays the distribution of terms obtained from the free description by the expert panel. According to Fig. 3.4, all the experimental wines are situated on the right-hand side of the CA plot and associated with terms such as “herbal tea”, “floral”, “ripe fruit”, “caramel” and “jammy”. Some of the afore mentioned attributes could be described as being typical of Shiraz (Herderich *et al*., 2007); however, terms such as “floral”, “caramel”, herbal tea” and even “jammy” have also been illustrated in rooibos (Koch *et al.,* 2012) and honeybush tea infusions (Theron *et al.,* 2014). Furthermore, the presence of oxidative descriptive terms for the experimental wines (RB and HB), as can be seen in Fig. 3.4, is not completely out of place, as the experimental wines were suspected to be oxidised. The latter wines had been bottled a few months earlier and the lack of any sulphur dioxide additions may have caused spoilage and oxidation by the oxygen picked up

during bottling. The consumer studies for events 1 to 3 were completed a few months prior to the expert tasting and faults in the experimental wines may not have been relevant or noticeable to the consumers at that stage. This probably also led to the lower quality ratings given to the experimental wines by the expert panel, as seen in Fig. 3.3. Fig. 3.4 enables deductions to be made considering the influence of the treatment of the wine with plant material. The normal Audacia 2012 Shiraz (AU) was associated with mocha, tobacco and chocolate descriptors, whereas the same Shiraz wine treated with rooibos and honeybush plant material was associated with descriptors such as herbal tea, ripe fruit, jam and meaty. As already mentioned, some of these attributes could be a direct effect of the plant material (Koch *et al.*, 2012, Theron *et al*., 2014), or the natural aromas associated with Shiraz could have been influenced by that of the honeybush and rooibos plant material.

3.3.3 Consumer testing (event 4)

Consumer testing (event 4, Table 3.1) was conducted in order to compare a treated wine with commercial samples from the same cultivar and vintage in terms of consumer degree of liking. At the time of event 4, the experimental wine had been refined into a commercial wine called *Rooibos wine*, produced with the addition of wood material, namely *Aspalathus linearis* (rooibos) and *Cyclopia* (honeybush), as alternative wood products. The purpose of event 4 was to gauge consumer acceptability of the new commercial product treated with honeybush and rooibos (AU) and to compare it to other commercial wines. The red wines were all very young, as they were presented roughly six months after they had been produced.

According to Fig. 3.5, the treated commercial wine (AU) did not differ significantly ($p > 0.05$) in liking from only LU, with STELLA, EE, WATER and RV receiving significantly higher liking scores. Interestingly, EE and WATER did not differ significantly ($p > 0.05$) from Stella. Comparing such young red wines may have led to the consumer liking scores not being very high, and one could assume that further ageing may have resulted in higher scores for the full sample set of six Merlot wines. Although AU was scored significantly lower ($p \leq 0.05$) than most of the commercial Merlot wines (Fig. 3.5), the results illustrate that the treated wine (AU) was neither liked nor disliked, again some indication that there were consumers who liked the aroma and flavour of rooibos and honeybush as wood alternatives, thus showing a winemaking concept with marginal potential.

Similar consumer socio-demographic results were shown for this consumer study (event 4) when comparing the results of this experiment to those discussed in section 3.3.1 (events 1 to 3). In event 4 (Table 3.5, section 1), a slightly higher percentage of male consumers (59%) were assessed compared to female consumers (41%). The most represented age group was the 20- to 24-year-old category. Again, the major ethnic group was white consumers. In section 2 (Table 3.5), the highest percentage of consumers drank wine more than once per week, again indicating that most of the consumers who completed the tasting were not wine novices. The most important

extrinsic factor when buying wine was “price”, with choice of cultivar being the second most important factor. There was a 90% “yes” answer on the question if the consumers were aware that wine contains sulphur. Interestingly, the majority of the consumers indicated that they would like to buy a non-sulphur or low-sulphur wine. There definitely is a consumer market willing to buy wine products that are produced with low or zero preservatives, but many consumers perceive these products to be more expensive, as can be seen in a study done by Forbes *et al.* (2009). From a health perspective, consumers might say that they want natural wine products free from potentially harmful additives, but in actual fact this is not always true. When given the choice between a cheaper or more expensive wine product, consumers would rather choose a less expensive product, even when the more expensive wine has potential health benefits (Forbes *et al*., 2009).

3.3.4 Descriptive sensory analysis of treated wines

3.3.4.1 Descriptive analysis of an un-oaked commercial red wine treated with rooibos and honeybush wood and leaf material

The influence of varying plant material levels in un-oaked commercial red wine was investigated (refer to section 3.2.3 for the application of wine treatments). This experiment was conducted to assess the influence of different plant material levels on the sensory characteristics of the experimental wines. In this instance the treated wines were tested for aroma notes, astringency, and whether the rooibos aroma and honeybush aroma (tested orthonasally) resulted in a lingering flavour or aftertaste (tested retronasally).

As can be seen from the PCA bi-plot (Fig. 3.6), the main separation on PC1 was due to the type of plant material used (honeybush versus rooibos), whereas dosage level was responsible for separation on PC2 (High versus Low). According to Fig. 3.6, the control treatment was associated strongly with berry aroma. This association was also significant ($p > 0.05$), as indicated by the ANOVA results (Fig. 3.7). The rooibos and honeybush aromas and respective aftertastes increased linearly with increasing plant material dosage levels, as can be seen in the ANOVA results (Figs 3.8, 3.9, 3.11 and 3.12, respectively). These results could possibly be used for future research, to ultimately build a robust model acting as a guide to winemakers, i.e. to determine the amount of plant material to add to wine in order to end up with a desired wine style.

The honeybush aroma, as seen in Fig. 3.10, displayed higher mean intensity ratings at **honeybush high** (5 g/L) than the same dosage of rooibos material (Fig. 3.8). In contrast, the mean intensities of the respective aftertastes for rooibos and honeybush, seen in Fig. 3.9 and 3.11, were very similar. This indicates that aroma perception (orthonasal) is usually more prominent than aftertaste (retronasal aroma perception) (Aubry, 1999). Interestingly, astringency, the mouthfeel attribute (as seen in Fig. 3.12), also increased with increasing plant material addition. The honeybush treatments affected astringency more prominently than rooibos; however, the rooibos

treatment with 1 g/L plant material was not judged to be significantly higher in astringency than the control wine ($p > 0.05$). It should be borne in mind that this wine was not a very full red wine and this experiment should be repeated with a fuller red wine such as Cabernet Sauvignon or Shiraz to determine the effect on astringency.

3.3.4.2 Descriptive analysis of an aged un-oaked Merlot wine treated with rooibos and honeybush wood

The results of the descriptive sensory analysis of the Merlot wine sample treated with rooibos and honeybush wood material are shown in Fig. 3.13 and 3.14. Fig. 3.13 illustrates treatment by replicate means, whereas Fig. 3.14 illustrates the treatment means. Both PCA plots (Fig. 3.13 and 3.14) show that there is a clear distinction between the wine treatments. The control wine shows a close grouping with berry aroma and berry flavour in both plots, indicating that this aroma note is part of Merlot's basic aroma profile (Pineau, 2009). This is also illustrated in Fig. 3.15 and Fig. 3.16, where the berry note of the Merlot control sample was significantly higher ($p \leq 0.05$) than that of the treated wines. It is also clear that the berry aroma was less affected by honeybush addition than by rooibos addition, i.e. the berry intensity of the Merlot wine was more prominent ($p \leq 0.05$) in the wines treated with honeybush than those treated with rooibos (Figs 3.15 and 3.16). Thus, the aroma descriptor berry-like could be regarded as being related to the wine, irrespective of the treatment (Fig. 3.15). Jammy aroma and prune aroma also seemed to stem from the Merlot wine (Figs 3.17 and 3.18). The addition of rooibos or honeybush significantly decreased the jam-like aroma in the treated wines (Fig. 3.17); conversely, the prune aroma was significantly ($p \leq 0.05$) more prominent in the samples with added rooibos than those with added honeybush (Fig. 3.18), but still significantly lower than in the control. The latter result illustrates that the inherent prune aroma of this Merlot wine is supressed by rooibos, but highly supressed by the addition of honeybush, especially when adding 10 and 20 g of honeybush per L of wine.

When the wines were treated with varying dosages of rooibos (Table 3.3), the treatments RB 5 g/L and RB 10 g/L grouped closely with the aroma attributes rooibos, vanilla, tobacco and prune, as seen in Fig. 3.13 and Fig. 3.14. The analysis of variance (ANOVA) results also showed that the aroma attribute intensities for rooibos (Fig. 3.19) and vanilla (Fig. 3.20) were significantly higher ($p \leq 0.05$) in the samples treated with rooibos. Similar high intensities were obtained for tobacco aroma (Fig. 3.21) and tobacco flavour (Fig. 3.22) in the wine treated with rooibos. Interestingly, only the wine sample treated with 20 g /L HB illustrated a prominent tobacco aroma and flavour, whereas the other two HB treatments (5 g/L HB and 10 g/L HB) showed virtually no perceptible intensity of these two sensory attributes (Figs 3.21 and 3.22, respectively). According to Koch *et al.* (2012), rooibos tea has been noted to contribute a tobacco aroma. This, however, is not always the case with all honeybush species. According to Theron *et al.* (2014), only *Cyclopia maculata* has a tobacco-like aroma, primarily as a results of the chemical compound 2,3-dehydro-α-ionone. The

Cyclopia species used in this study were a mix of *C. subternata* and *C. intermedia.* Thus, the occurrence of 2,3-dehydro-α-ionone seems unlikely, although interaction between the wine aroma molecules and wood treatments could have resulted in the development of a tobacco-like note. This tobacco-like note was especially prevalent when a high concentration of honeybush wood was added to the wine (20 g/L).

The wines treated with honeybush wood (viz. HB 10 g/L and HB 20 g/L; Table 3.3) grouped more closely to the aroma notes floral, cherry and honeybush, as seen in Fig. 3.13 and Fig. 3.14. This result is also illustrated in the ANOVA results (Figs 3.23, 3.24 and 3.25). The three honeybush wood dosages (HB 5 g/L, HB 10 g/L and HB 20 g/L) resulted in perceptible low to moderate intensities, especially floral aroma and honeybush aroma, which both illustrated intensities of > 40 for HB 10 g/L and HB 20 g/L, respectively. These two aroma notes are well established in honeybush tea infusions and it seems that this is also the case in a young, unwooded Merlot wine treated with honeybush wood material. As indicated in Fig. 3.25, a cherry note was just perceptible when using a dosage of HB 5 g/L, while the intensity of the cherry aroma was significantly higher at the two higher honeybush dosages, with an intensity score of > 25 for the latter two HB dosages.

Floral flavour (Fig. 3.26), measured retronasally, illustrated similar low to moderate intensities, as shown in Fig. 3.23, with HB wood being the main contributor in this instance. As expected, both honeybush and rooibos wood contributed to the flavour attribute, *tea flavour,* especially RB 10 g/L and HB 20 g/L (Fig. 3.27). Also, both honeybush and rooibos wood resulted in a barely perceptible spicy/pepper flavour, with HB 20 g/L illustrating a mean intensity of 5. Both herbal teas are known to result in a spicy note (Koch *et al.,* 2012; Theron *et al.,* 2014). A recent study (Erasmus, 2015) on the sensory profile of honeybush tea indicated that *C. subternata* can result in a spicy/cassia-like aroma and flavour.

The basic taste modalities (sour taste, sweet taste and bitter taste), as depicted in Figs 3.29, 3.30 and 3.31 respectively, were mostly not affected by treatment, except perhaps for bitter taste, where HB 20 g/L resulted in a significantly higher bitter taste perception ($p \leq 0.05$). The reason for this is not clear. Only *Cyclopia genistoides* is known for its bitter taste in tea infusions (Theron *at al.,* 2014), but this species was not used in these experiments. There also was no clear trend when testing the astringency levels (Fig. 3.32). All the samples showed low levels for astringency ($p > 0.05$), except for the HB 20 g/L sample, in which the perceptible astringency level was significantly higher than that of the control sample. It is well known that bitterness and astringency are often seen as “twin sensations”, as almost all phenolic compounds that cause astringency are also bitter (Bajec & Pickering, 2008). It thus can be assumed that the phenolic content of the HB 20 g/L sample most probably contributed to the bitter taste and astringency of the treated Merlot wine (Figs 3.31 and 3.32 respectively).

To summarise, the sensory characteristics, namely berry and jammy aroma, were both lowered by the rooibos and honeybush treatments, while the vanilla, rooibos and tobacco notes were increased by rooibos. Cherry, floral and honeybush aromas were all increased by the HB treatments. The sour and sweet taste did not differ between the treatments, while bitter taste and astringency were both increased significantly by the HB 20 g/L treatment.

3.3.5 Consumer liking of Merlot with different dosage levels of honeybush and rooibos wood chips (event 5)

The wines that had undergone treatment with rooibos and honeybush wood chips at various dosage levels were finally tested for consumer degree of liking, using central location tests at a wine market (see treatments in Table 3.3). The base wine used was aged Merlot from the 2010 vintage (see sample details in section 3.2.3). As seen in Fig. 3.33, the consumers showed the highest liking score for the HB 5 g/L wine, and this wine was liked significantly more than any of the other honeybush- and rooibos-treated wines ($p \leq 0.05$). This shows that wine samples with higher dosages of honeybush and rooibos wood were less liked by consumers. However, all the wines showed liking scores of > 5 on the nine-point hedonic scale, which could indicate that consumers did not perceive the treated wines as being totally unacceptable.

Some general consumer information can be seen in Table 3.6 (sections 1 to 2). The consumers were mainly in the 20- to 24-year-old category, with an equal number of male and female consumers. The majority of consumers were of the white ethnic group. This group of consumers had a reasonably high frequency of wine consumption, as most of the consumers indicated that they drank wine two to three times a week. Regarding the extrinsic factors that drive the purchase of wine, this group of consumers indicated that “cultivar” plays a significant role when purchasing wine. These consumers will also purchase a wine when recommend by a friend or colleague. Tasting notes on the back label of a bottle of wine was the least important factor as ranked by the consumers. Similar results on the role of extrinsic factors were indicated by Bester (2010) when researching the generation Y consumer.

3.4 CONCLUSIONS

Consumer liking is a valuable tool in wine research when it is important to get an indication of how the general wine consumer will accept a new, innovative wine product. The consumers indicated that they neither liked nor disliked the wines produced with honeybush (HB) or rooibos (RB), therefore no real aversion or high degree of liking was indicated. Although only marginally, the wines treated with low levels of honeybush and rooibos were, however, generally preferred more by the consumers. In most cases, the mean liking scores of the treated wines were significantly lower than those of the control wines, the liking scores for the treated wines were usually just

above *five* on the nine-point hedonic scale. This indicates that the common consensus among the 399 consumers was that they did not outright reject the wines treated with honeybush and rooibos products.

The usage of a consumer study in wine research can be very helpful. Not only is the acceptability of a new product illustrated, but the supplementary socio-demographic questionnaires can also shed light on how consumers behave when purchasing wine and which extrinsic factors drive their wine choices. Knowledge of the consumer market is very important, especially in a highly competitive wine market (Lattey *et al.*, 2009). It will be important to market this new concept of wine production. The general, older consumer is not always open to new ideas, however, young consumers are often more open to new innovations (Bester, 2011) and it might be a good idea to market this new innovation to the up-and-coming Y-consumer.

The descriptive sensory analysis indicated how the typical wine aroma, taste and mouthfeel could be adapted by adding honeybush and/or rooibos wood. Aroma notes associated with the rooibos treatments were vanilla, tobacco and rooibos, whereas the honeybush treated wines were described as having floral, cherry and honeybush notes. The taste and mouthfeel of the wines were influenced to a limited extent by the rooibos and honeybush treatments. These results should however be further substantiated with applications on other red wine cultivars.

This research has indicated that it is possible to create an innovative wine product with unique aromas and flavours with the addition of honeybush and rooibos material, especially the wood fraction. However, further research is necessary on the exact dosage of the respective treatments, as well as a blend of the two treatments.

3.5 LITERATURE CITED

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ADDENDUM A

Results figures and tables

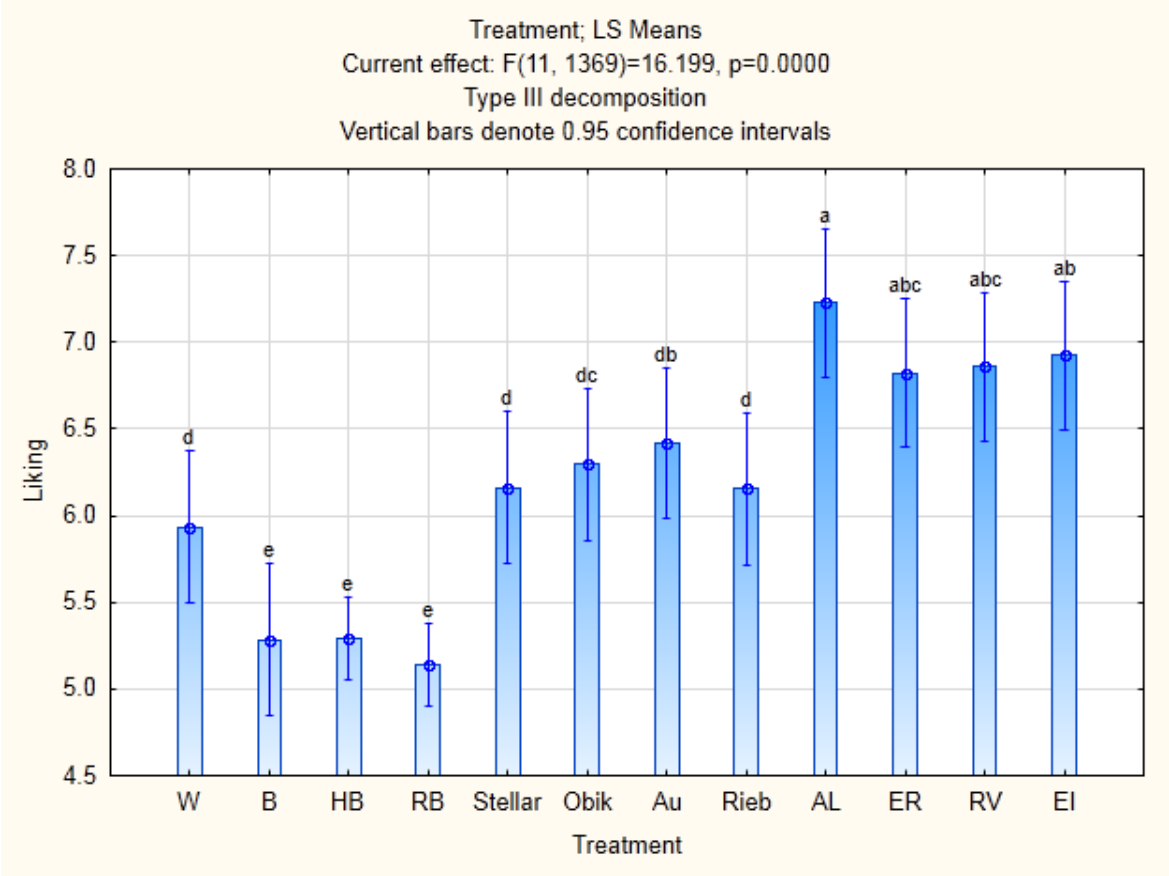


Figure 3.1: Mean consumer liking scores for wines tasted at events 1 to 3. Letters indicate significant differences between sample means. Vertical bars denote 0.95 confidence intervals.

Label keys: W - Wooded Audacia Shiraz; B - Blend 50% rooibos and 50% honeybush; HB – Honeybush; RB – Rooibos; Stellar - Stellar Organics; Obik - Obikwa Shiraz; Au - Audacia Shiraz 2012; Rieb - Riebeek Cellars Shiraz; Al - Alto Shiraz 2010; ER - Ernie Els Shiraz 2011; RV - Rust en Vrede Shiraz 2011; EI - Eikendal Shiraz 2011.

Table 3.4: Sections 1-3: Socio-demographic data of consumers tested at events 1 to 3 (see Table 3.1).

Section 1 of Table 3.4

Socio- demographic Data				
Questions	**Number of Observations (n= 330) and percentage**			
Gender	**Male**		**Female**	
	157	48%	173	52%
Age	**18-20**		10	3%
	20- 24		87	27%
	25-34		97	30%
	35-44		44	13%
	45-54		42	13%
	55-64		34	10%
	65+		13	4%
Nationality	**African**		7	2%
	Asian		1	0%
	Coloured		8	2%
	Indian		5	2%
	White		309	94%
Do you live in RSA?	**Yes**		**No**	
	312	95%	17	5%
What is your occupation?	**Student**		65	20%
	Working		249	76%
	Stay at home parent		15	5%

Section 2 of Table 3.4: : Questions related to wine consumption

Questions relating to consumer wine consumption				
Questions	**Number of Observations (n= 330) and percentage**			
How often do you drink wine?	**Every day**	78	24%	
	More than once per week	148	45%	
	Once per week	48	15%	
	More than once per month but less than once per week	35	11%	
	Once per month	6	2%	
	Only during parties or festive events	15	5%	
What would be the most important factor when buying wine?	**Tasting Notes on the back of the label**		12%	
	Awards Won		13%	
	Cultivar		34%	
	Price		34%	
	The name of the wine estate		21%	
	Other		17%	
Do you know that wine contains sulphur?	**Yes**	283	86%	**No** 46 14
Would you prefer to buy preservative free wines above wines with preservatives?	**Yes**	225	69%	**No** 99 31%
Would you prefer a low sulphur/no sulphur wine above a wine with sulphur?	**Yes**	238	75%	**No** 79 25%
Have you ever been enrolled for a wine tasting course?	**Yes**	84	25%	**No** 246 75%

Section 3 of Table 3.4: Questions related to wine ci=consumption

Questions	Number of Observations (n= 330) and percentage		
How would you rate your own wine knowledge?	I don't know much, but enjoy wine - complete novice	76	23%
	I know quite a bit	74	22%
	I work in the wine industry (not a winemaker)	19	6%
	I know a little	147	45%
	I am a wine expert	2	1%
	I work in the wine industry, I am a winemaker/ assitant winemaker/viticulturist	11	3%
Which type of wine do you prefer?	Red	227	69%
	White	76	23%
	Rosé	24	7%
Which kind of wine do you prefer regarding red wine?	Light and Fruity	Full bodied	
	120 36%	210	64%
Regarding sweetness, which level of sweetness do you prefer?	Dry	230	70%
	Semi- sweet	78	24%
	Sweet	20	6%
Which Cultivars do you like in a wine to drink?	Pinotage		34%
	Merlot		35%
	Shiraz		42%
	Other		9%
	Blends		38%
	Cabernet Sauvignon		43%
How long have you been drinking wine?	0 to 2	19	6%
	2 to 5	71	22%
	5 to 10	80	24%
	10 +	160	48%
Regarding the packaging of a bottle of wine, which kind of design do you prefer?	a "funky" design with a colourful label	83	26%
	a classic label	236	74%

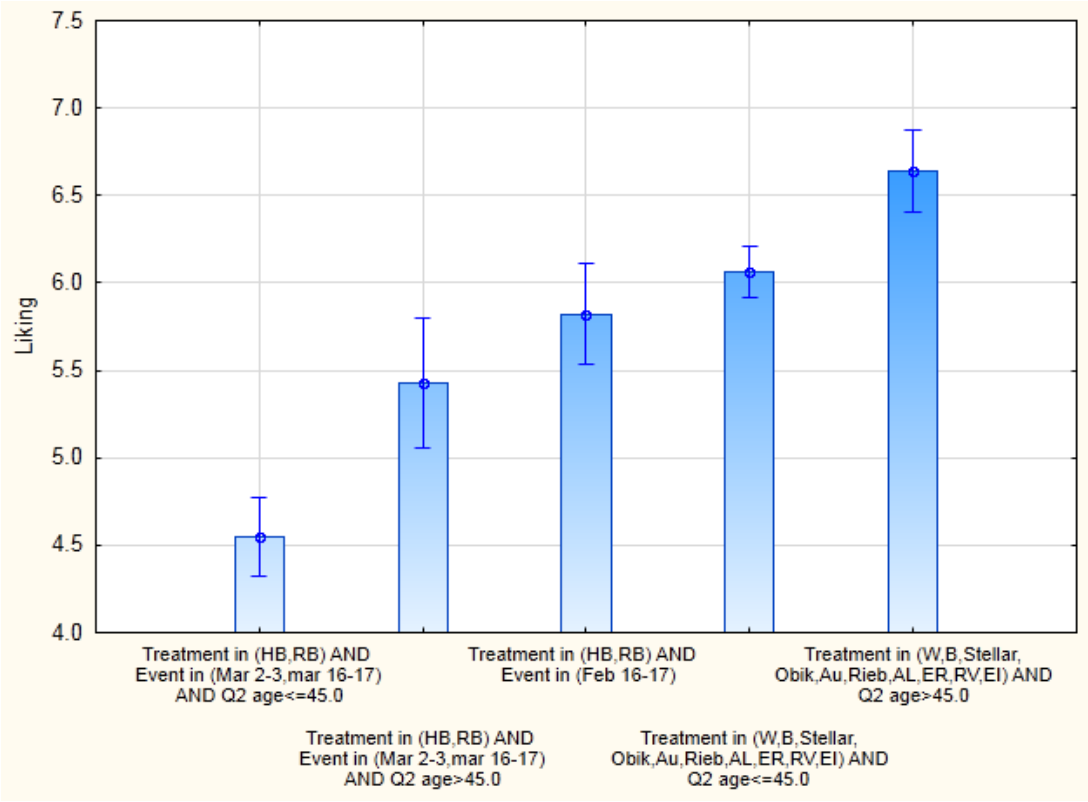


Figure 3.2: CART analysis of socio-demographic data, fixed variables (treatments and events), differing variables (age and liking score), Event 1 = Feb 16-17, Event 2 = Mar 2-3 and Event 3 = Mar 16-17.

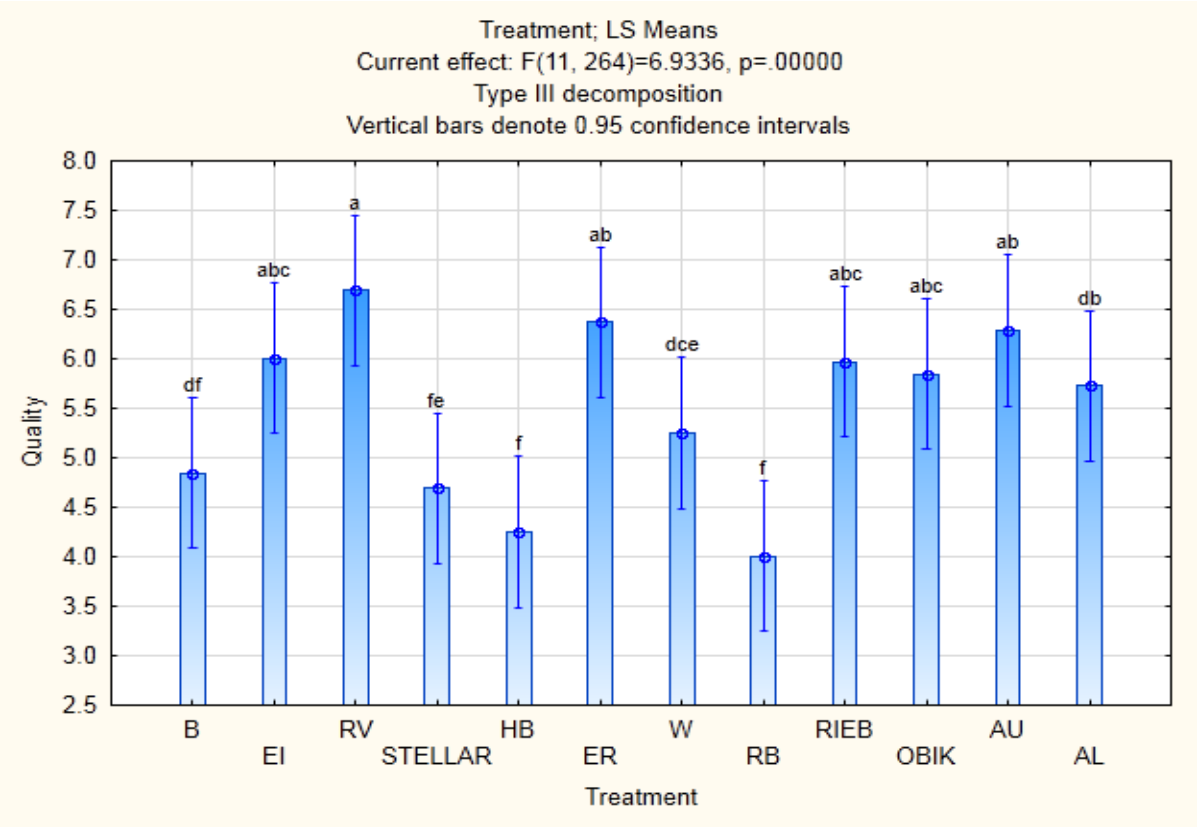


Figure 3.3: Expert panel quality ratings of wines evaluated at events 1 to 3. Letters indicate significant differences between sample means. Vertical bars denote 0.95 confidence intervals.

Wine keys: W - Wooded Audacia Shiraz; B - Blend 50% rooibos & 50% honeybush; HB – Honeybush; RB – Rooibos; Stellar - Stellar Organics; Obik - Obikwa Shiraz; Au - Audacia Shiraz 2012; Rieb - Riebeek Cellars Shiraz; Al - Alto Shiraz 2010; ER- Ernie Els Shiraz 2011; RV - Rust en Vrede Shiraz 2011; EI - Eikendal Shiraz 2011.

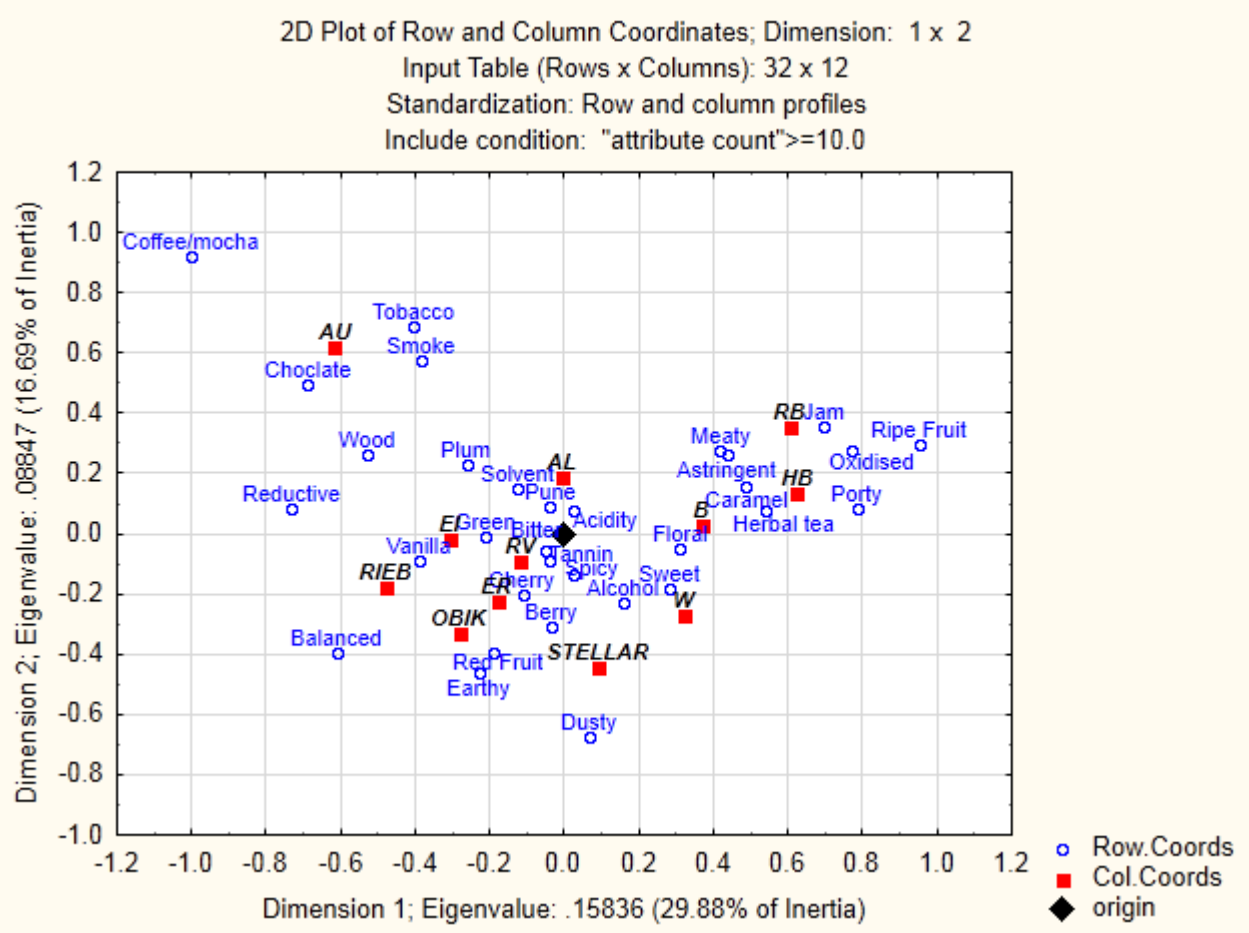


Figure 3.4: CA plot showing the grouping of wines according to free description by an expert panel. This sample set was also analysed for consumer liking during events 1 to 3.

Wine keys: W - Wooded Audacia Shiraz; B - Blend 50% rooibos and 50% honeybush; HB - Honeybush; RB - Rooibos; Stellar - Stellar Organics; Obik - Obikwa Shiraz; Au - Audacia Shiraz 2012; Rieb - Riebeek Cellars Shiraz; Al - Alto Shiraz 2010; ER - Ernie Els Shiraz 2011; RV - Rust en Vrede Shiraz 2011; EI - Eikendal Shiraz 2011. Letters indicate significant differences between sample means. Vertical bars denote 0.95 confidence intervals.

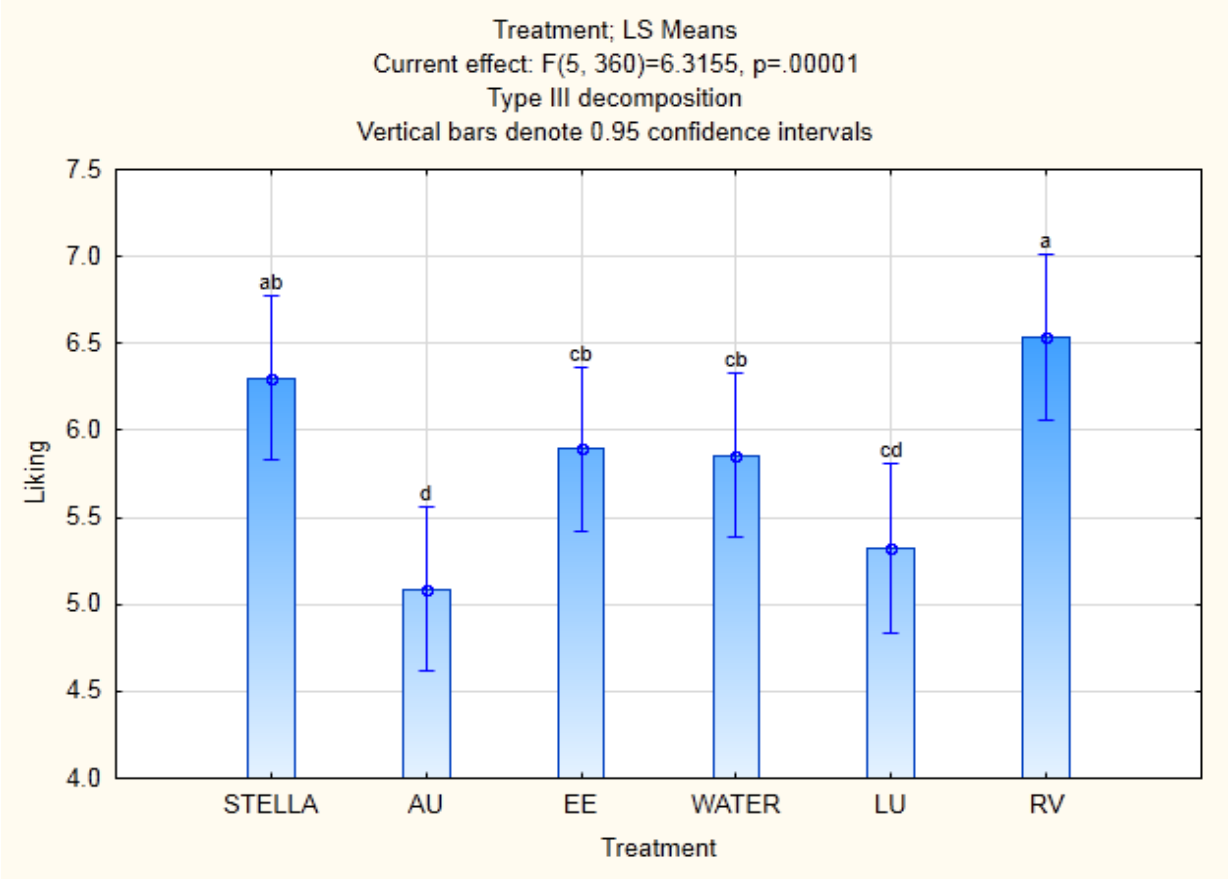


Figure 3.5: Consumer liking of commercial Merlot wine blend treated with rooibos and honeybush wood (AU) and five other commercial wine samples from the same cultivar and vintage (Stella, EE, Water, LU and RV).

Table 3.5 Section 1 to 3: Consumer socio-demographic data and questions related to wine consumption

Section 1 of Table 3.5

Socio- demographic Data				
Questions	**Number of Observations (n= 69) and percentage**			
Gender	**Male**		**Female**	
	41	59%	28	41%
Age	**18-20**		2	3%
	20- 24		23	33%
	25-34		16	23%
	35-44		11	16%
	45-54		11	16%
	55-64		2	3%
	65+		4	6%
Nationality	**African**		3	4%
	Asian		1	1%
	Coloured		2	3%
	White		63	91%
Do you live in RSA?	**Yes**		**No**	
	62	90%	7	10%
What is your occupation?	**Student**		23	33%
	Working		42	61%
	Stay at home parent		4	6%

Section 2 of Table 3.5: Questions related to wine consumption

Questions relating to consumer wine consumption				
Questions	**Number of Observations (n= 69) and percentage**			
How often do you drink wine?	**Every day**	13	19%	
	More than once per week	20	29%	
	Once per week	13	19%	
	More than once per month but less than once per week	16	23%	
	Once per month	3	4%	
	Only during parties or festive events	3	4%	
What would be the most important factor when buying wine?	**Tasting Notes on the back of the label**		25%	
	Awards Won		14%	
	Cultivar		35%	
	Price		41%	
	The name of the wine estate		23%	
	Other		14%	
Do you know that wine contains sulphur?	**Yes**	62	90%	**No** 7 10%
Would you prefer to buy preservative free wines above wines with preservatives?	**Yes**	46	67%	**No** 23 33%
Would you prefer a low sulphur/no sulphur wine above a wine with sulphur?	**Yes**	47	69%	**No** 21 31%
Have you ever been enrolled for a wine tasting course?	**Yes**	17	25%	**No** 52 75%

Section 3 of Table 3.5: Questions related to wine consumption

Questions	Number of Observations (n= 69) and percentage		
How would you rate your own wine knowledge?	I don't know much, but enjoy wine - complete novice	12	17%
	I know quite a bit	14	20%
	I work in the wine industry (not a winemaker)	1	1%
	I know a little	38	55%
	I am a wine expert	2	3%
	I work in the wine industry, I am a winemaker/ assitant winemaker/viticulturist	2	3%
Which type of wine do you prefer?	Red	53	77%
	White	13	19%
	Rosé	3	4%
Which kind of wine do you prefer regarding red wine?	Light and Fruity	Full bodied	
	27 40%	41	60%
Regarding sweetness, which level of sweetness do you prefer?	Dry	46	67%
	Semi- sweet	19	28%
	Sweet	4	6%
Which Cultivars do you like in a wine to drink?	Pinotage		36%
	Merlot		46%
	Shiraz		48%
	Other		14%
	Blends		38%
	Cabernet Sauvignon		58%
How long have you been drinking wine?	0 to 2	11	16%
	2 to 5	16	23%
	5 to 10	14	20%
	10 +	28	41%
Regarding the packaging of a bottle of wine, which kind of design do you prefer?	a "funky" design with a colourful label	21	30%
	a classic label	48	70%

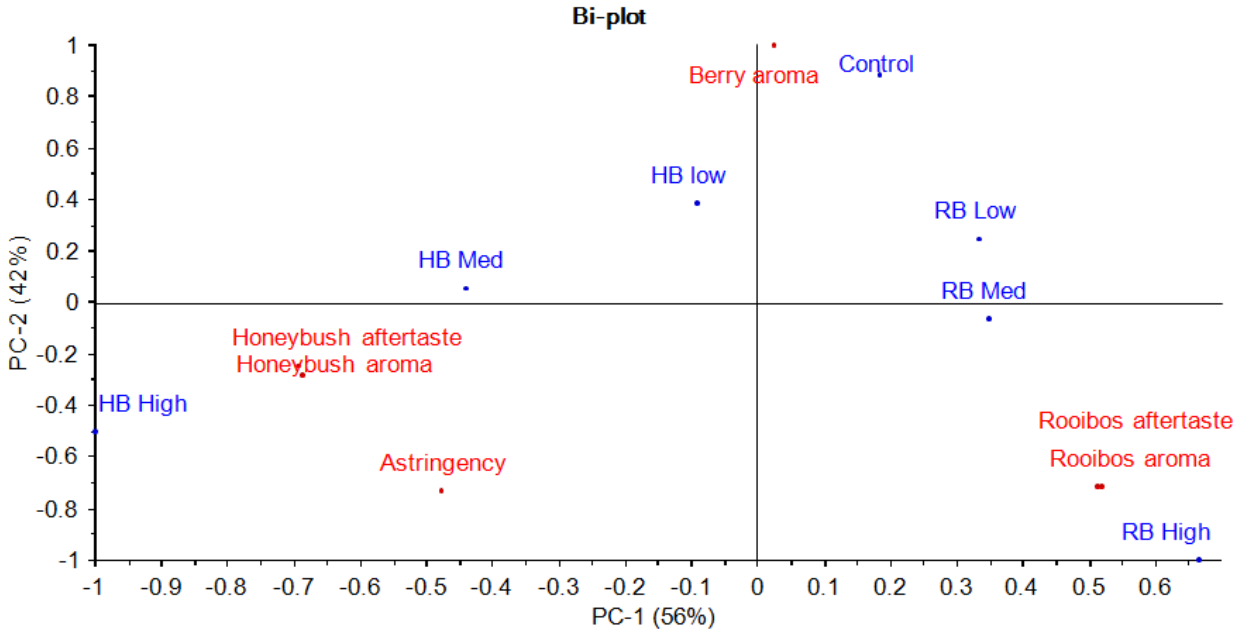


Figure 3:6: PCA bi-plot of the sensory characteristics of un-oaked commercial red wines. Treatments are indicated in blue and sensory attributes in red., RB= rooibos, HB= honeybush RB/HB low= 1 g/L, RB/HB medium= 2 g/L, RB/HB high= 5 g/L

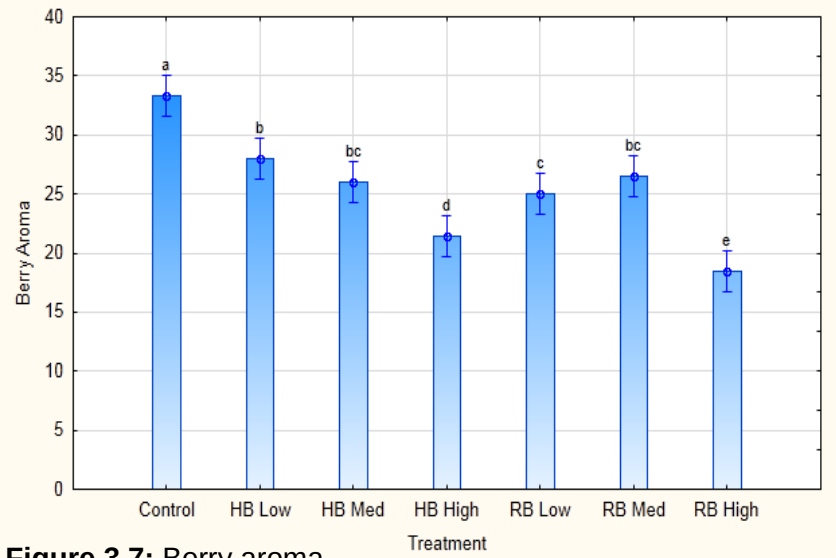


Figure 3.7: Berry aroma

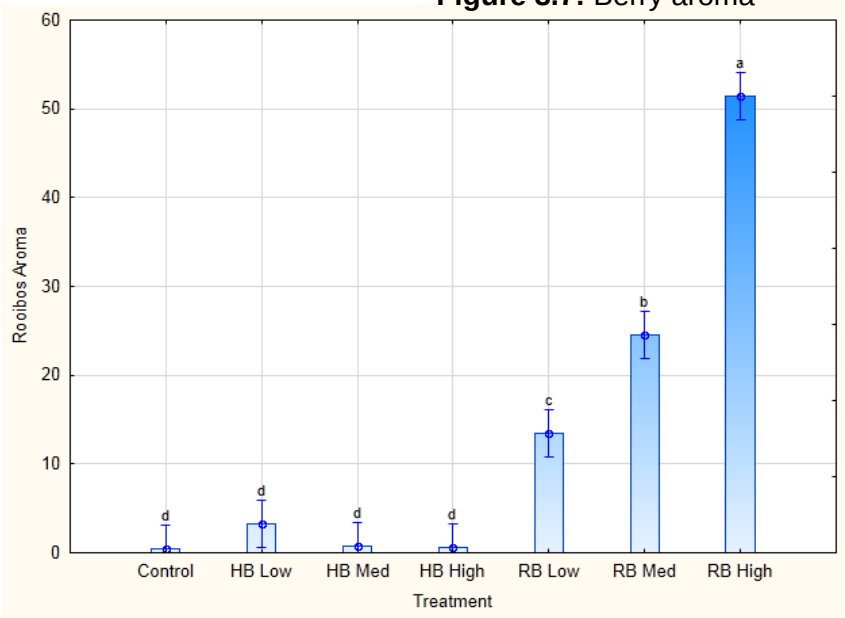


Figure 3.8: Rooibos aroma

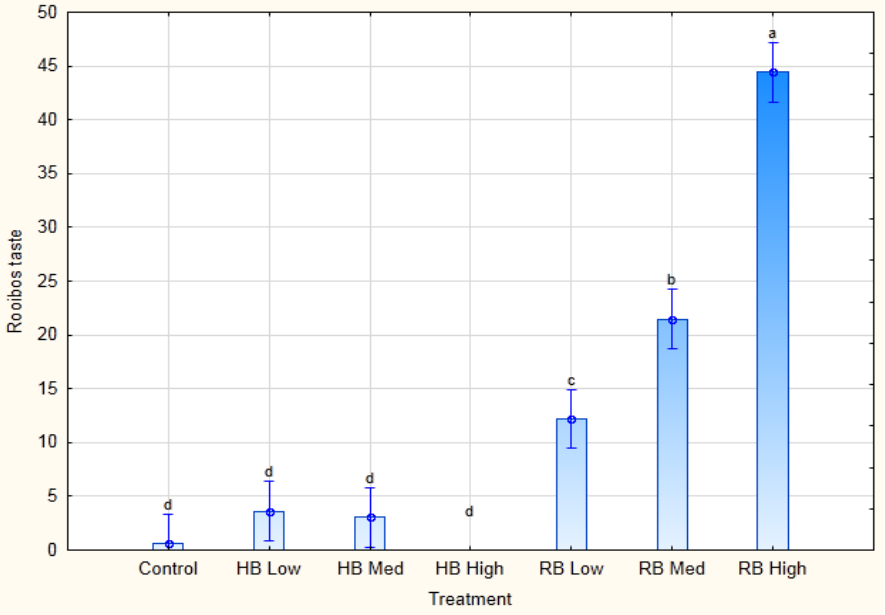


Figure 3.9: Rooibos flavour

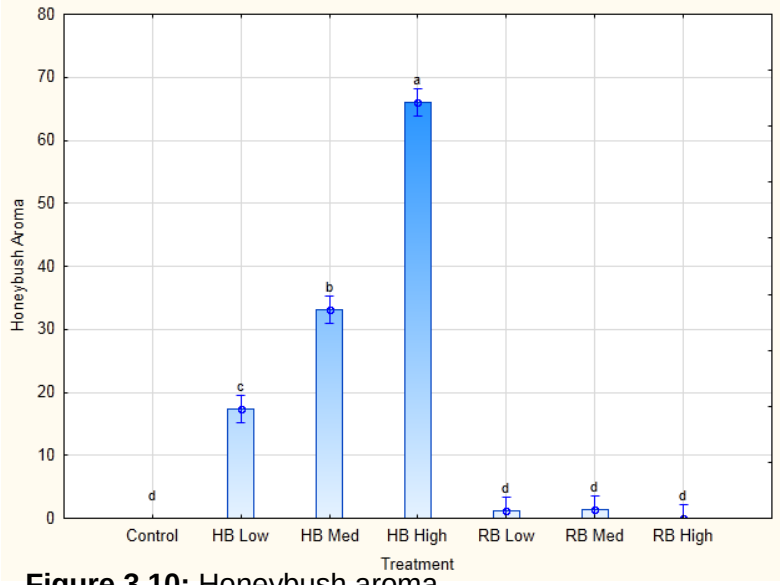


Figure 3.10: Honeybush aroma

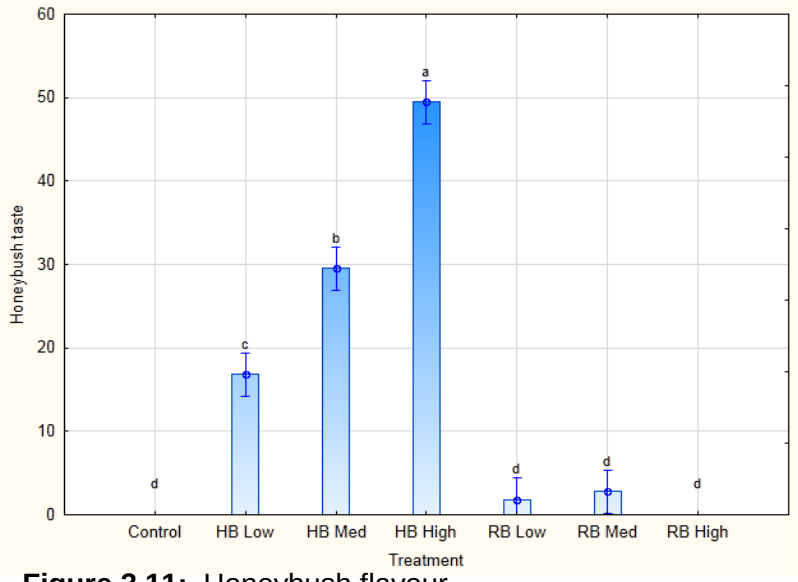


Figure 3.11: Honeybush flavour

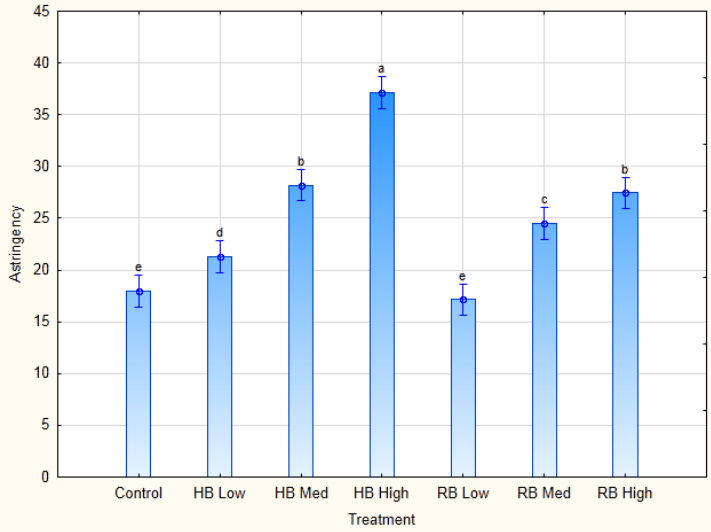


Figure 3.12: Astringency

Figures 3.7 to 3.12: ANOVA results of the main sensory characteristics investigated in an un-oaked commercial red wine treated with rooibos and honeybush wood and leaf material.

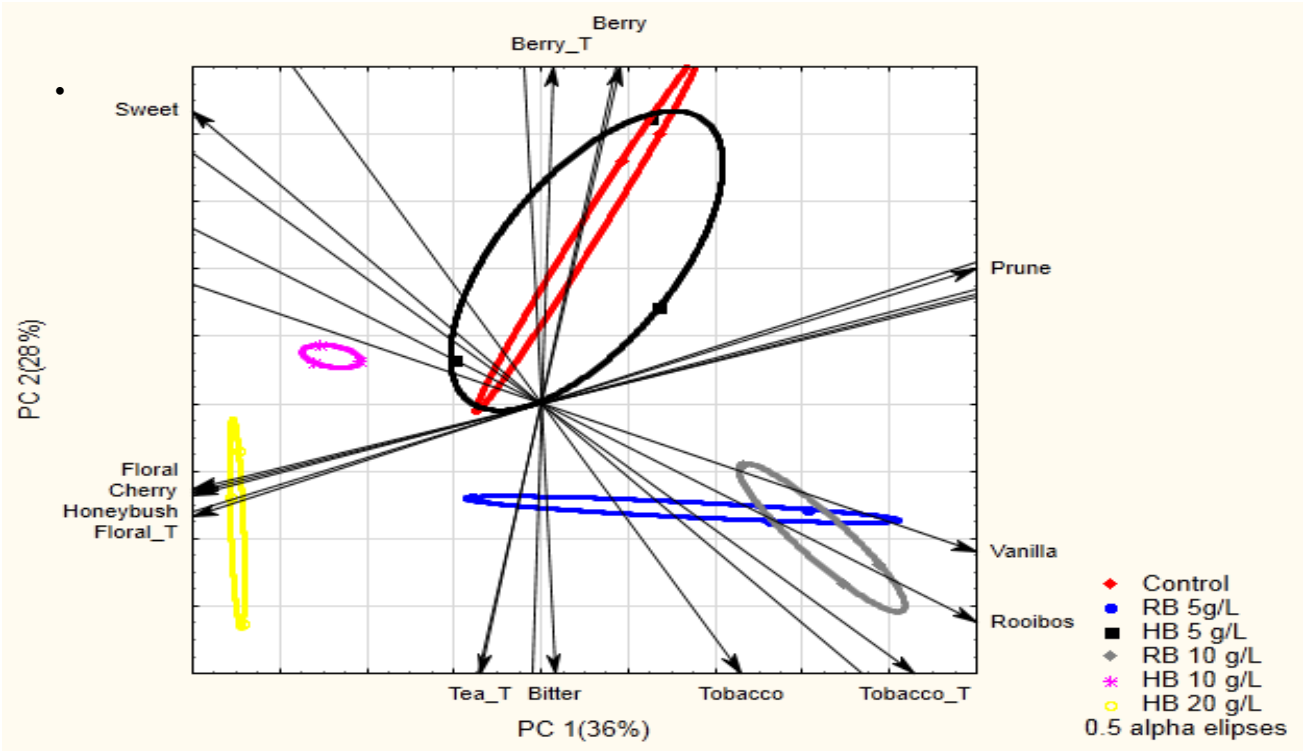


Figure 3.13: PCA bi-plot displaying the association of a selection of aroma and flavour descriptors and the respective treatment replicates of a Merlot wine treated with different levels of honeybush and rooibos wood chips.

Keys: Berry_T = Berry flavour, Tea_T = Tea flavour, Floral_T = Floral flavour and Tobacco_T = Tobacco flavour.

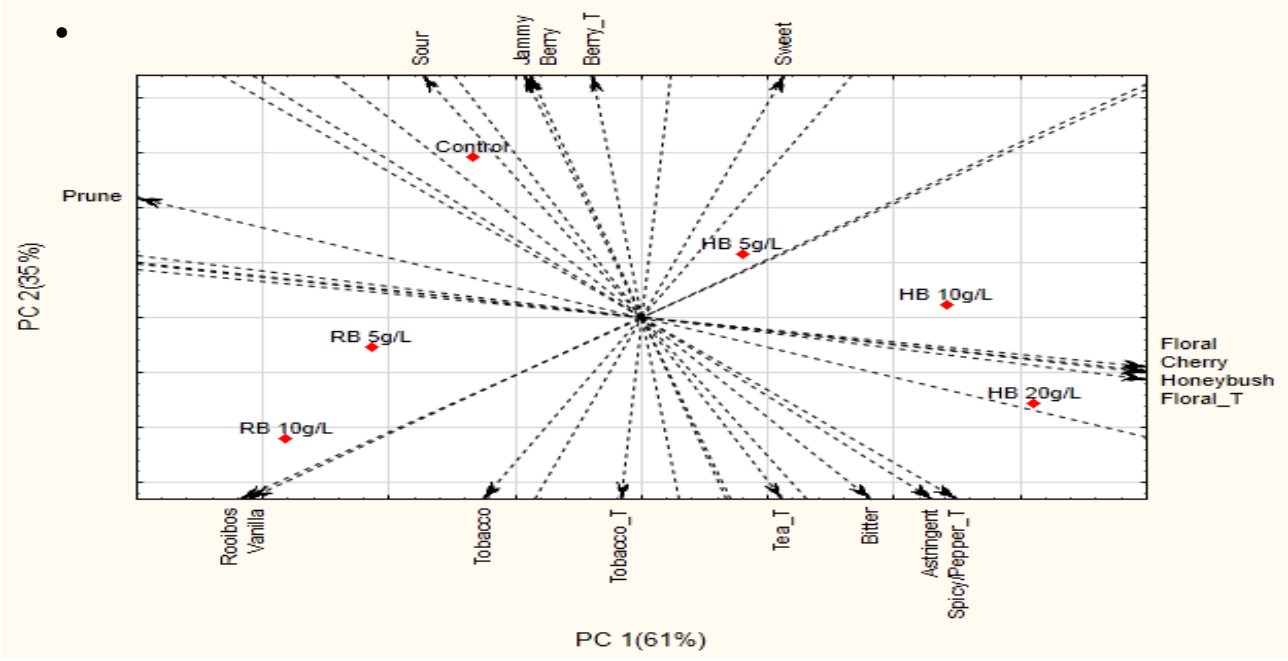


Figure 3.14: PCA bi-plot of Merlot wines that were treated with various dosages of rooibos and honeybush wood chips. Treatment means were used in PCA.

Keys: Berry_T = Berry flavour, Tea_T = Tea flavour, Floral_T = Floral flavour and Tobacco_T = Tobacco flavour.

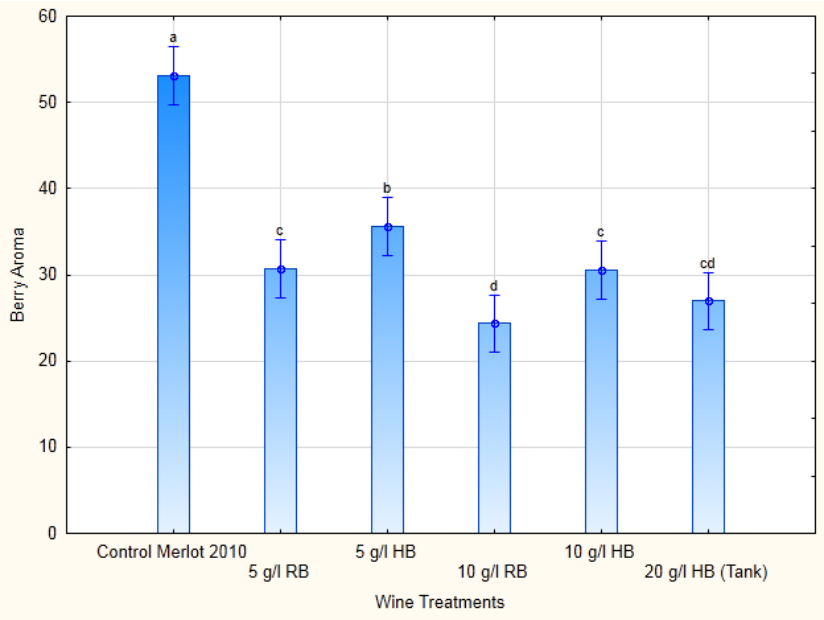


Figure 3.15: Berry aroma

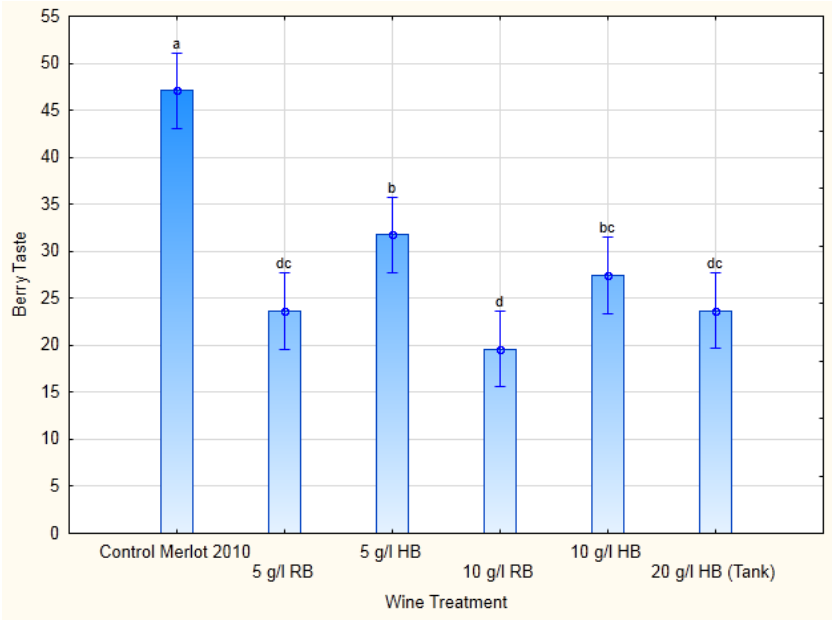


Figure 3.16: Berry flavour

Figures 3.15 - 3.16: ANOVA results of the main sensory characteristics of a Merlot wine treated with different levels of honeybush and rooibos wood chips.

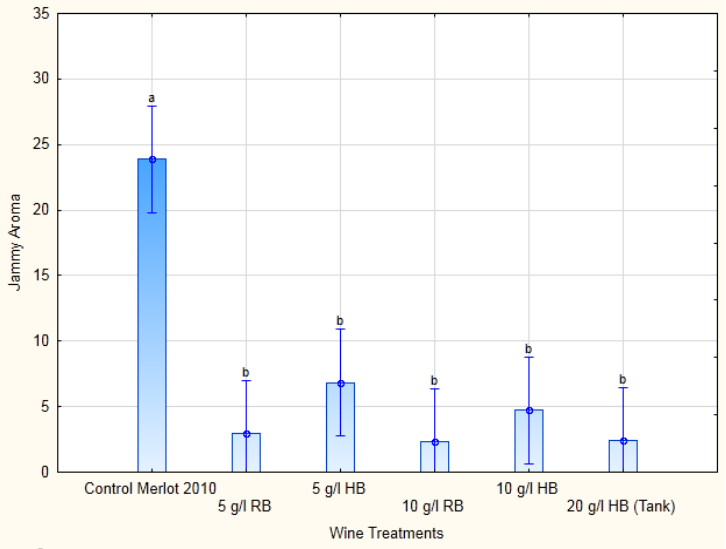


Figure 3.17: Jammy aroma

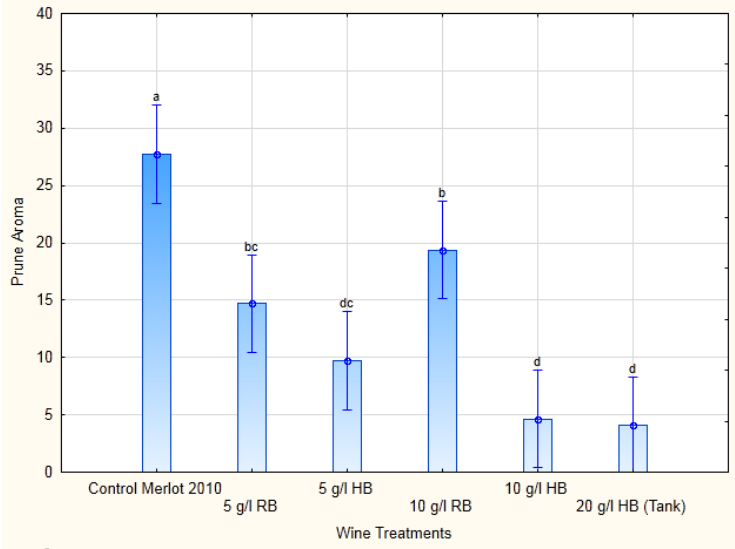


Figure 3.18: Prune aroma

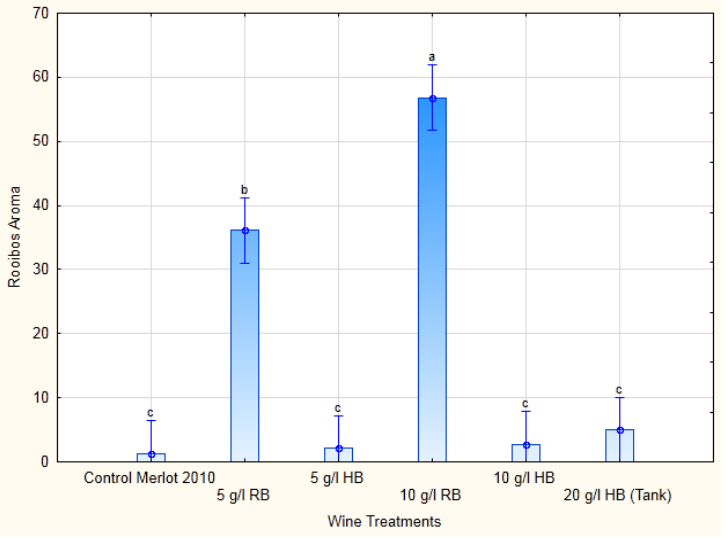


Figure 3.19: Rooibos aroma

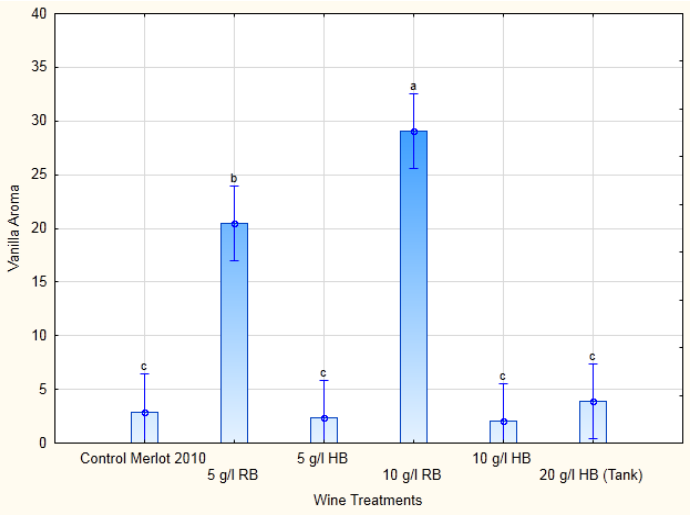


Figure 3.20: Vanilla aroma

Figures 3.17 - 3.20: ANOVA results of the main sensory characteristics of a Merlot wine treated with different levels of honeybush and rooibos wood chips.

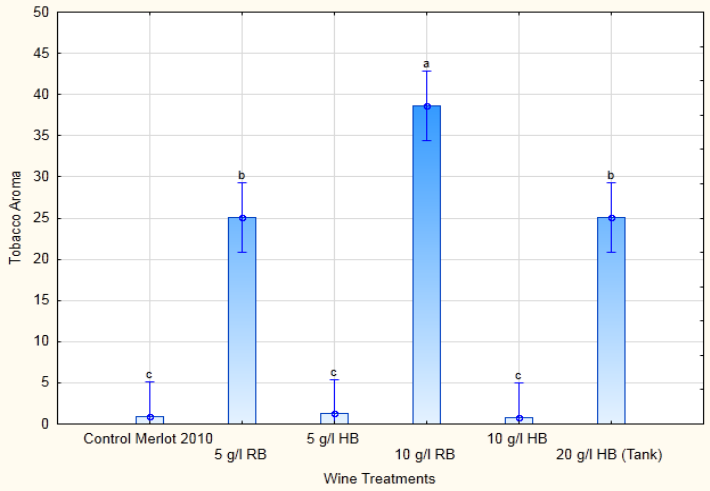


Figure 3.21: Tobacco aroma

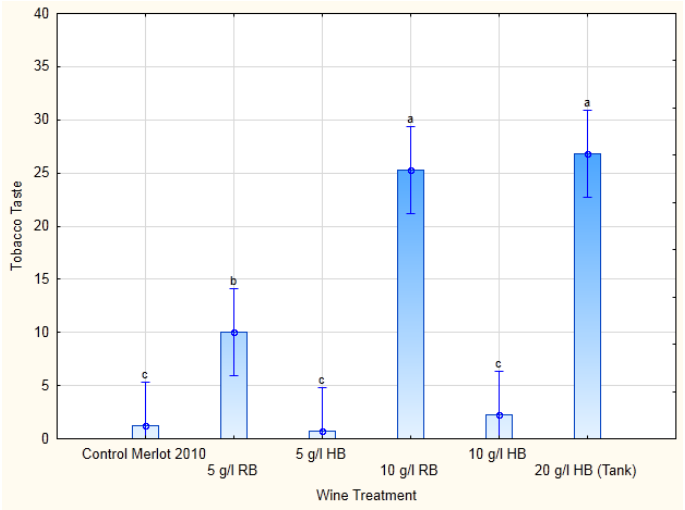
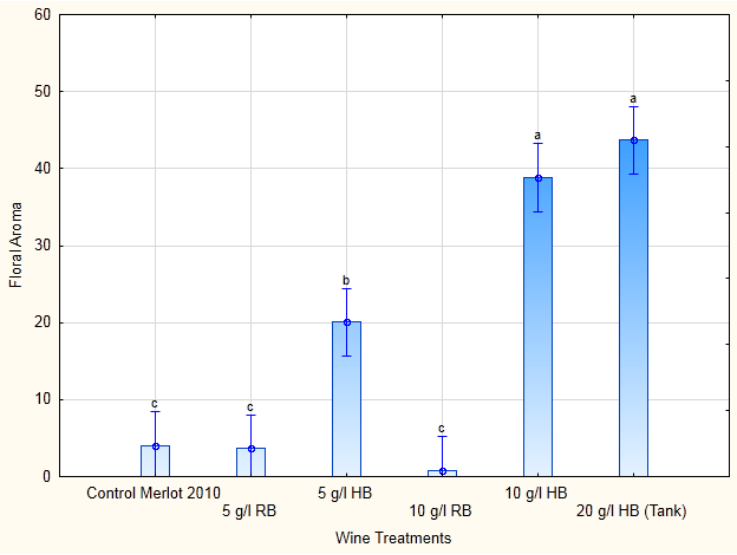


Figure 3.22: Tobacco flavour



Figur 3.23 Floral aroma

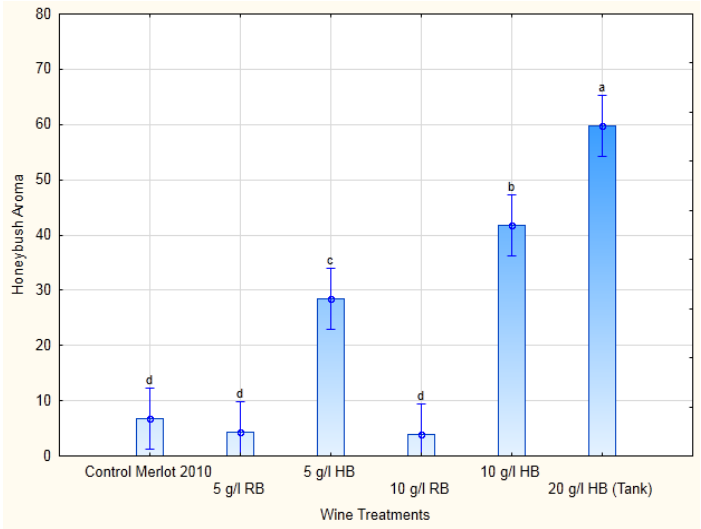


Figure 3.24 Honeybush aroma

Figures 3.25 - 3.28: ANOVA results of the main sensory characteristics of a Merlot wine treated with different levels of honeybush and rooibos wood chips.

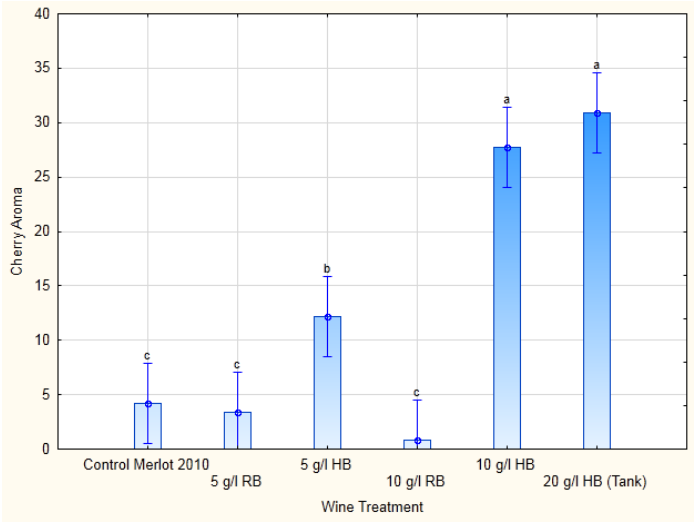


Figure 3.25: Cherry aroma

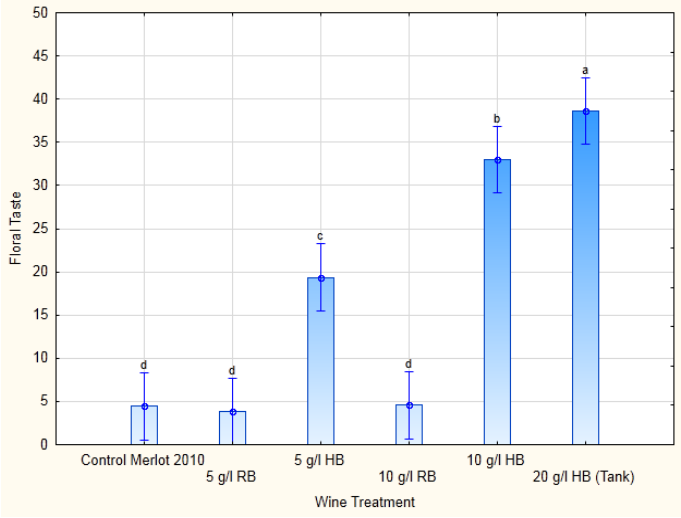


Figure 3.26: Floral flavour

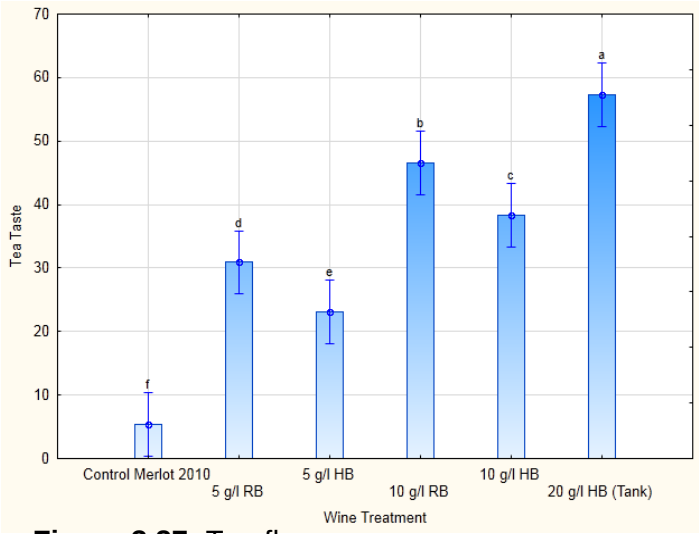


Figure 3.27: Tea flavour

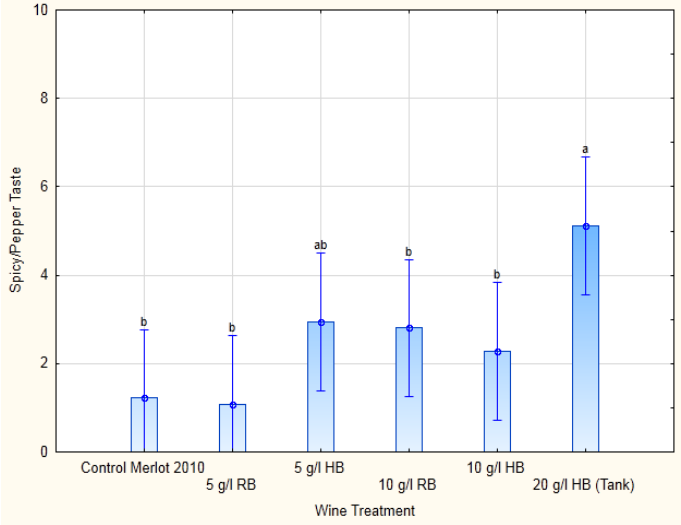


Figure 3.28 Spicy/pepper flavour

Figures 3.25 - 3.28:ANOVA results of the main sensory characteristics of a Merlot wine treated with different levels of honeybush and rooibos wood chips. .

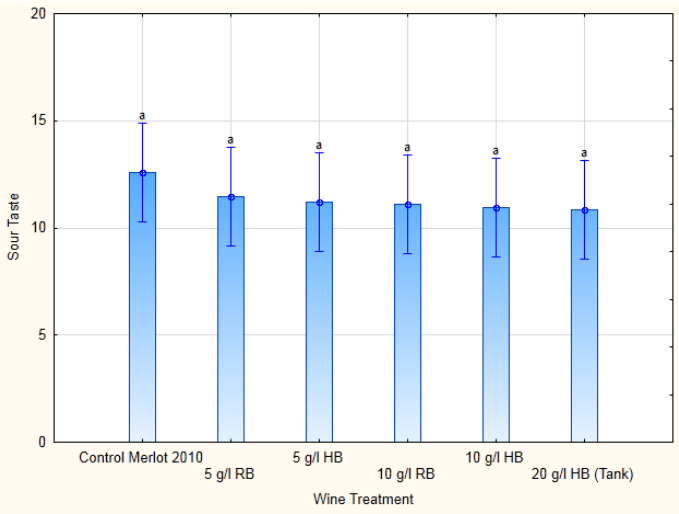


Figure 3.29: Sour taste

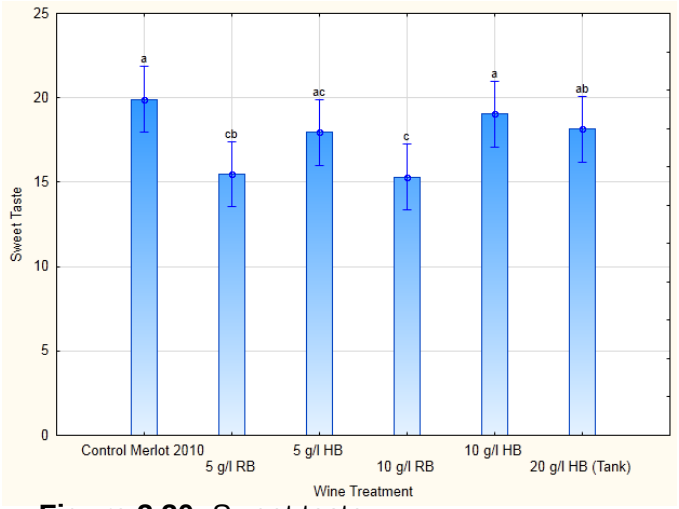


Figure 3.30: Sweet taste

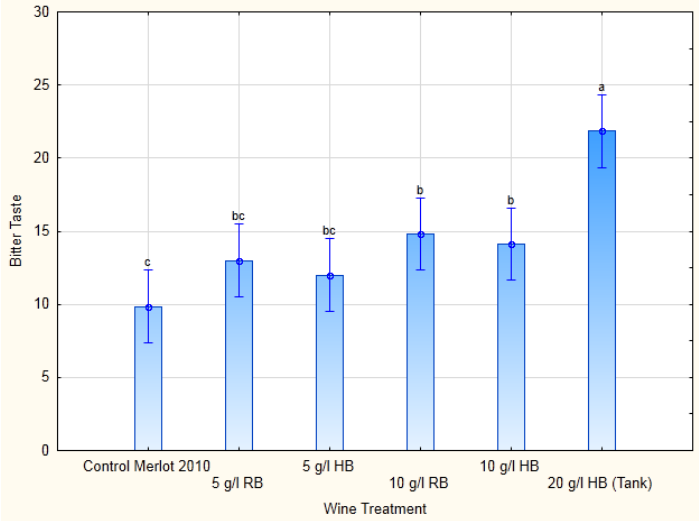


Figure 3.31: Bitter taste

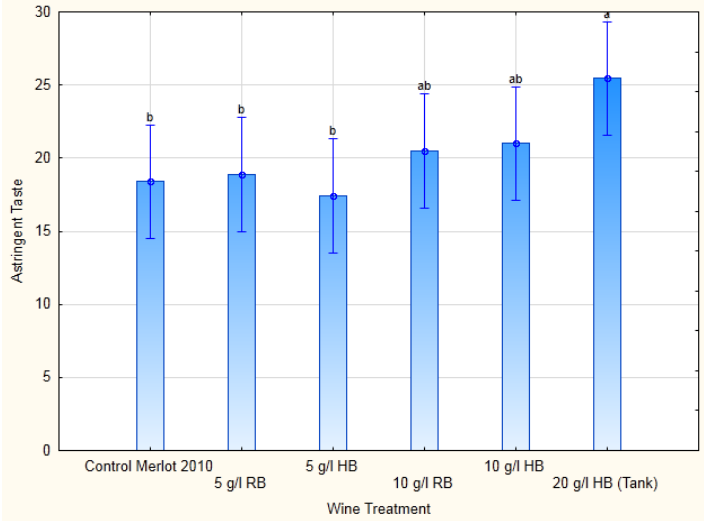


Figure 3.32: Astringency

Figures 3.29 - 3.32:ANOVA results of the main sensory characteristics of a Merlot wine treated with different levels of honeybush and rooibos wood chips.

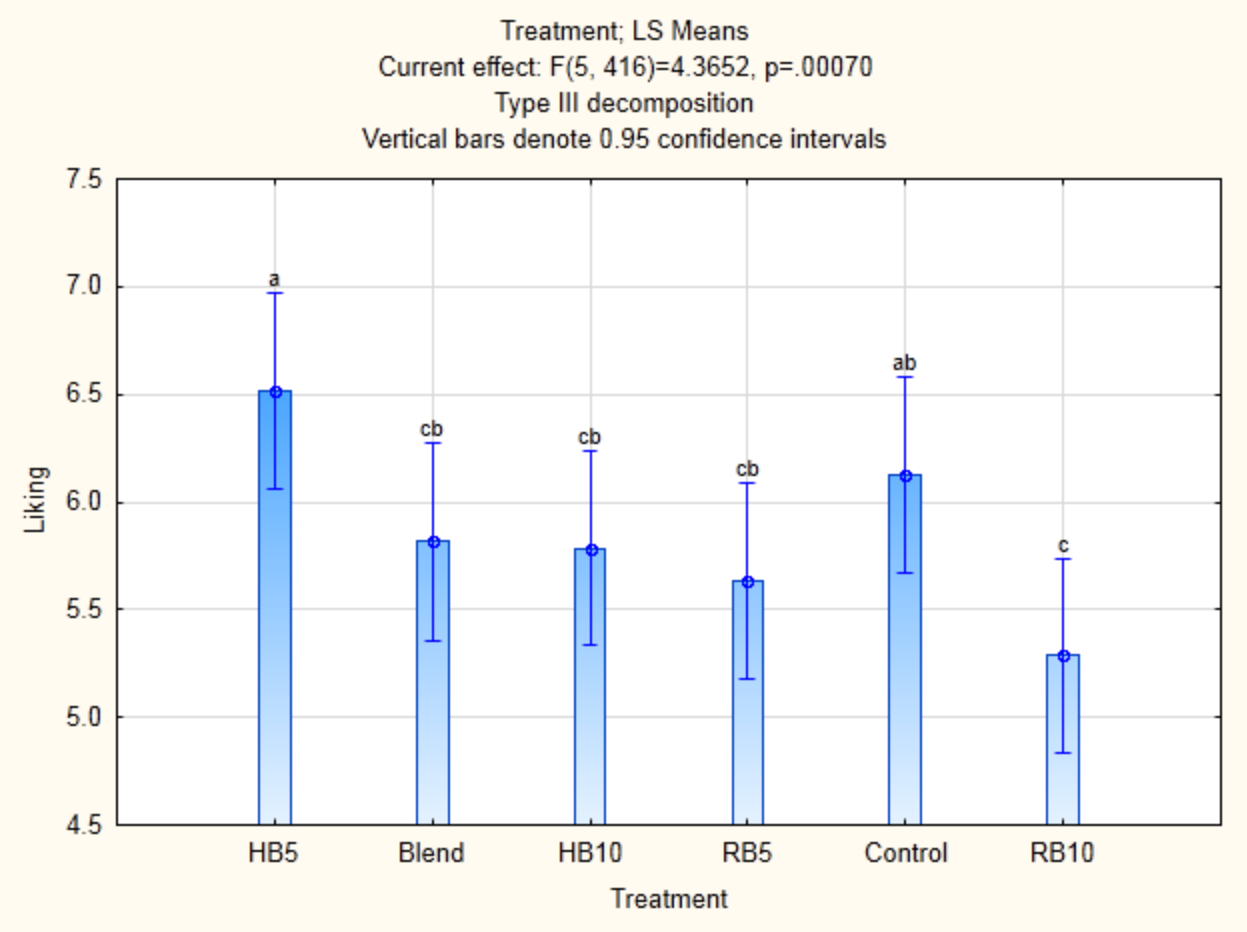


Figure 3.33: Consumer liking of 2010 Merlot wine treated with RB (rooibos) and HB (honeybush) wood chips at varying dosages.

Table 3.6 Section 1 to 2: Consumer socio-demographic data and questions related to wine consumption

Section 1 of Table 3.6: Socio-demographic data

Socio- demographic Data				
Questions	**Number of Observations (n= 85) and percentage**			
Gender	**Male**		**Female**	
	43	51%	42	49%
Age	**18-20**		7	8%
	20- 24		31	36%
	25-34		26	31%
	35-44		6	7%
	45-54		4	5%
	55-64		7	8%
	65+		4	5%
Nationality	**African**		4	5%
	Asian		1	1%
	Coloured		1	1%
	White		78	93%
Do you live in RSA?	**Yes**		**No**	
	81	95%	4	5%
What is your occupation?	**Student**		34	40%
	Working		45	53%
	Stay at home parent		2	2%
	Retired		4	5%
How often do you drink wine?	**Every day**		18	21%
	2-3 times per week		36	42%
	Once per week		20	24%
	2-3 times per month		2	2%
	Once per month		3	4%
	Only during parties or festive		6	7%
What would be the most important factor when buying wine?		**Rank from 1-9 (scale of importance)**	**Obersvations for each ranking**	
	Tasting Notes on the back of the label	1	14	
	Stickers on the bottle	6	16	
	Awards Won	6	16	
	Cultivar	8	19	
	Price	6 and 7	18	
	The name of the wine estate	7	15	
	Word of mouth	8	25	
Do you know that wine contains sulphur?	**Yes**	**No**	**Unsure**	
	42 50%	9 11%	33 39%	
Have you ever been enrolled for a wine tasting course?	**Yes**	**No**		
	11 13%	74 87%		

Section 2 of Table 3.6: Questions related to wine consumption

Questions	Number of Observations (n= 85) and percentage		
How would you rate your own wine knowledge?	I work in the wine industry (not a winemaker)	3	4%
	I know a little	73	86%
	I am a wine expert	7	
	I work in the wine industry, I am a winemaker/ assistant winemaker/viticulturist	2	2%
Which type of wine do you prefer?	Red	59	69%
	White	21	25%
	Rosé	5	6%
Which kind of wine do you prefer regarding red wine?	Light and Fruity	35	42%
	Full bodied	49	58%
Regarding sweetness, which level of sweetness do you prefer?	Dry	58	68%
	Semi- sweet	23	27%
	Sweet	4	5%
Which Cultivars do you like in a wine to drink?	Pinotage		38%
	Merlot		49%
	Shiraz		32%
	Other		20%
	Blends		39%
	Cabernet Sauvignon		66%
How long have you been drinking wine? (Years)	0 to 2	7	8%
	2 to 5	25	29%
	5 to 10	24	28%
	10 +	29	34%
Regarding the packaging of a bottle of wine, which kind of design do you prefer?	a "funky" design with a colourful label	13	15%
	a classic label	72	85%

ADDENDUM B

General market set-up and tasting area

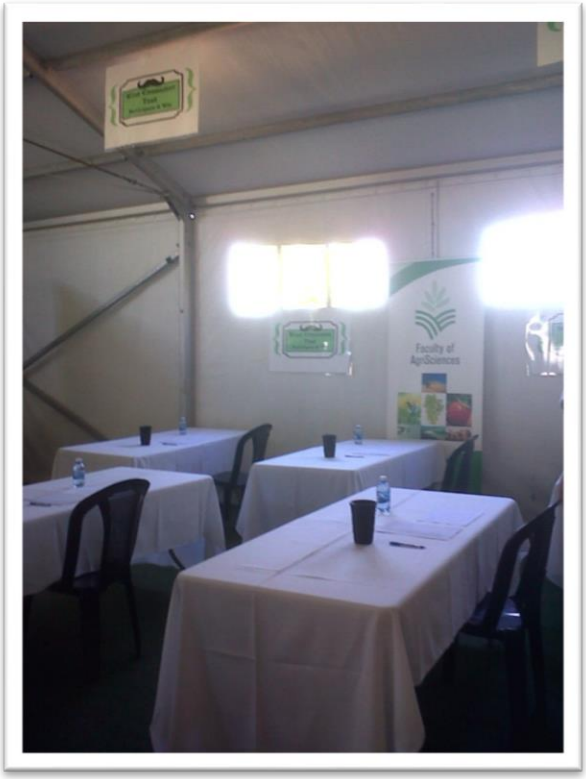


Figure. 3.34: Consumers were placed at separate tables in order to avoid any interaction. Water and plain crackers were provided for each consumer, as well as a spittoon.

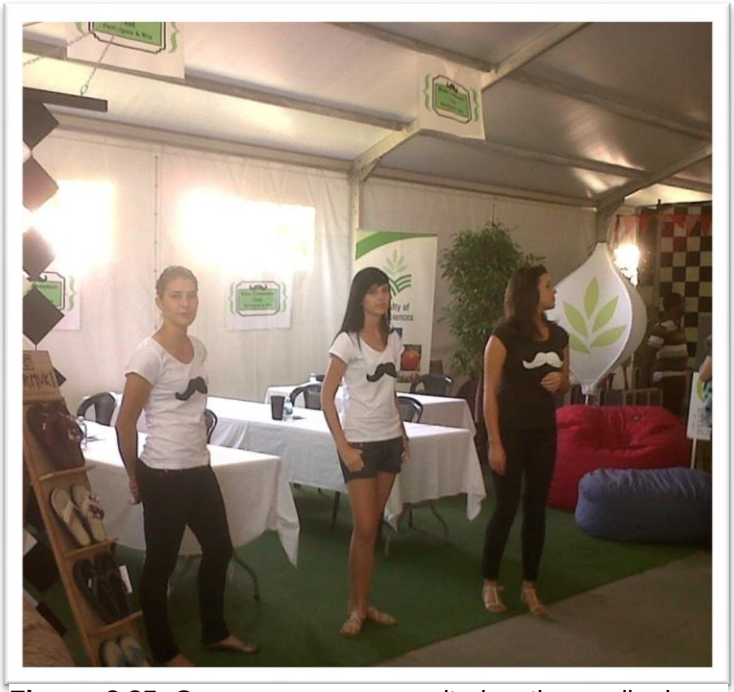


Figure. 3.35: Consumers were recruited as they walked past the tasting area by a group of girls who were employed to recruit consumers.

Figure 3.36: Tasting samples and nine-point hedonic tasting sheet.

ADDENDUM C

Two examples of the consumer socio-demographic questionnaire and liking page used within consumer testing

Red Wine Liking Questionnaire

Judge nr …… Date ……...

The data will be treated confidentially. Your name will only be used for the lucky draw and will not be captured with the rest of the data.
Please tick the appropriate box

1. Are you male or female?
   □ Female □ Male

2. How old are you?
   □ 18 - 20 □ 20 – 24 □ 25 – 34 □ 35 – 44 □ 45 – 54 □ 55 – 64 □ 65 +

3. Which ethnic group do you most closely identify with?
   □ African □ Asian □ Indian □ Coloured □ White

4. What is you nationality? _____

5. Do you live in South Africa?
   □ Yes □ No

6. Which of the following apply to you?
   □ I do not own a house or a flat, I rent a place
   □ I live in the house that I own
   □ I own a house that I rent out
   □ I own multiple properties that I rent out
   □ I own a car

7. What is your occupation?
   □ Student
   □ Stay at home parent
   □ Working
   If working, please specify your occupation _____

8. How often do you drink wine?
   □ Every day
   □ More than once per week
   □ Once per week
   □ More than once per month but less than once per week
   □ Once per month
   □ Only during parties or festive events

9. What would be the most important factor when buying wine?
   □ Price
   □ Tasting notes on the back label
   □ Awards won (award stickers on the bottle)
   □ The name of the wine estate
   □ Cultivar
   □ Other
   If other please specify _____

10. Do you know that wine contains sulphur?
    □ Yes □ No

11. Would you prefer to buy preservative free wines above wines with preservatives?
    □ Yes □ No

12. Would you prefer a low sulphur/no sulphur wine above a wine with sulphur?
    □ Yes □ No

13. Have you ever been enrolled for a wine tasting course?

□ Yes □ No

If Yes, which one? _____

14. How would you rate your own wine knowledge?

□ I don't know much, but enjoy wine – complete novice
□ I know a little
□ I know quite a bit
□ I am a wine expert
□ I work in the wine industry (not a winemaker)
□ I work in the wine industry, I am a winemaker/assistant winemaker/viticulturist

15. Which type of wine do you prefer?

□ Red □ White □ Rose

16. Which kind of wine do you prefer regarding red wine?

□ Light and fruity □ Full bodied

17. Regarding sweetness, which level of sweetness do you prefer?

□ Dry □ Semi sweet □ Sweet

18. Which cultivar(s) do you like in a wine to drink? (you can pick more than one)

□ Pinotage □ Merlot □ Shiraz
□ Other □ Blends □ Cabernet Sauvignon

19. How long have you been drinking wine?

□ 0 to 2 years □ 2 to 5 years □ 5 to 10 years □ More than 10 years

20. Regarding the packaging of a bottle of wine, which kind of design do you prefer?

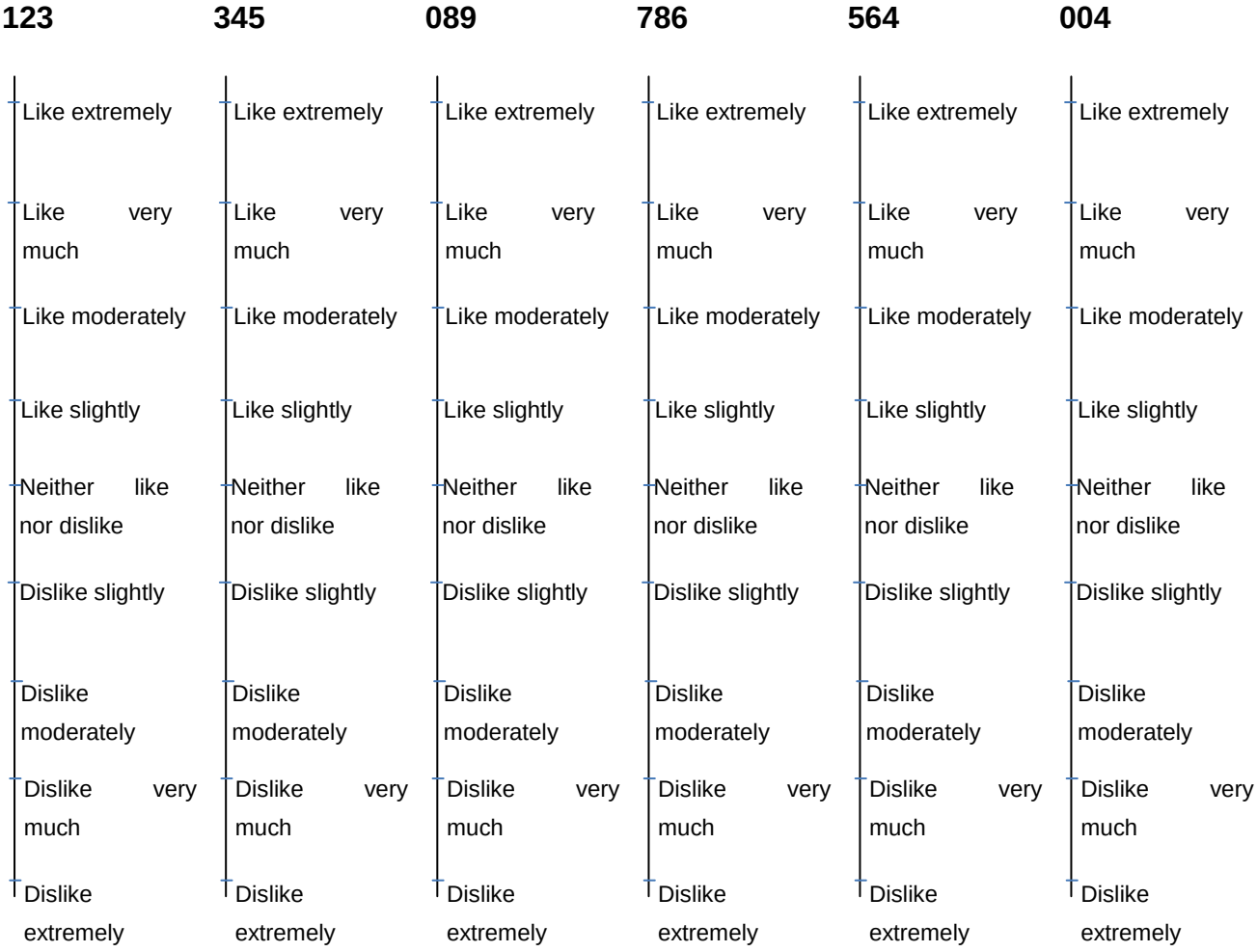
□ A "funky" design with a colourful label □ A classic label

Thank you for your time, we hope to see you at the market again.

9-point hedonic scale tasting sheet presented to consumers

Judge nr …….. Date……

Please taste the wines from left to right.
Please indicate on the 9-point scale how much you like the wine



Example of questionnaire and tasting sheet presented to consumers

Consumer Test - Red Wines

Judge nr …….. Date…….

Mobile ……..

The data will be treated confidentially. Your name will only be used for the lucky draw and will not be captured with the rest of the data.

Please tick the <u>appropriate</u> box

1. Are you male or female?
   - ☐ Female ☐ Male

2. How old are you?
   - ☐ 18 - 20 ☐ 21 – 24 ☐ 25 – 34 ☐ 35 – 44 ☐ 45 – 54 ☐ 55 – 64 ☐ 65 +

3. With which ethnic group do you most closely identify with?
   - ☐ African ☐ Asian ☐ Indian ☐ Coloured ☐ White

4. What is you nationality? _____

5. Do you live in South Africa?
   - ☐ Yes ☐ No

6. Which of the following apply to you?
   - ☐ I do not own a house or a flat, I rent a place
   - ☐ I live in the house that I own
   - ☐ I own a house that I rent out
   - ☐ I own multiple properties that I rent out
   - ☐ I own a car

7. What is your occupation?
   - ☐ Student
   - ☐ Stay at home parent
   - ☐ Working
   - ☐ Retired

If currently working, please specify your occupation _____

8. How often do you drink wine?
   - ☐ Every day
   - ☐ 2-3 times per week
   - ☐ Once per week
   - ☐ 2-3 times per month
   - ☐ Once per month
   - ☐ Only at parties or festive events

Please turn to next page for tasting of 6 red wines

Tasting sheet for 6 red wines

Please taste the wines from left to right.
Please indicate on the 9-point scale how much you like the wine

Code	Code	Code	Code	Code	Code
Like extremely	Like extremely	Like extremely	Like extremely	Like extremely	Like extremely
Like very much	Like very much	Like very much	Like very much	Like very much	Like very much
Like moderately	Like moderately	Like moderately	Like moderately	Like moderately	Like moderately
Like slightly	Like slightly	Like slightly	Like slightly	Like slightly	Like slightly
Neither like nor dislike	Neither like nor dislike	Neither like nor dislike	Neither like nor dislike	Neither like nor dislike	Neither like nor dislike
Dislike slightly	Dislike slightly	Dislike slightly	Dislike slightly	Dislike slightly	Dislike slightly
Dislike moderately	Dislike moderately	Dislike moderately	Dislike moderately	Dislike moderately	Dislike moderately
Dislike very much	Dislike very much	Dislike very much	Dislike very much	Dislike very much	Dislike very much
Dislike extremely	Dislike extremely	Dislike extremely	Dislike extremely	Dislike extremely	Dislike extremely

Please turn to next page for general questions

General Questions

1. How important are these aspects when purchasing wine?

IN EACH CASE **CIRCLE** THE NUMBER THAT BEST FITS YOUR ANSWER

	1 2 3 4 **5** 6 7 8 9 Unimportant — Not sure — Like Extremely
Price	Unimportant 1 2 3 4 5 6 7 8 9 Extremely Important
Tasting Notes-Back Label	Unimportant 1 2 3 4 5 6 7 8 9 Extremely Important
Awards Won (Stickers on the bottle)	Unimportant 1 2 3 4 5 6 7 8 9 Extremely Important
The name of the wine estate	Unimportant 1 2 3 4 5 6 7 8 9 Extremely Important
Cultivar	Unimportant 1 2 3 4 5 6 7 8 9 Extremely Important
Word of Mouth	Unimportant 1 2 3 4 5 6 7 8 9 Extremely Important

2. Would you prefer a low sulphur/no sulphur wine above a wine with sulphur?
□ Yes □ No □ Not Sure

3. Have you ever been enrolled for a wine tasting course?
□ Yes □ No

If Yes, which one? _____

4. How would you rate your own wine knowledge?
□ I know a little about wine, but I am not involved in the wine industry
□ I am a wine expert, but I am not involved in the wine industry
□ I work in the wine industry (not a winemaker)
□ I work in the wine industry, I am a winemaker/assistant winemaker/viticulturist

5. Which type of wine do you prefer?
□ Red □ White □ Rosé

6. Which kind of wine do you prefer regarding red wine?
□ Light and fruity □ Full bodied

7. Regarding sweetness, which level of sweetness do you prefer?
□ Dry □ Semi-sweet □ Sweet

8. Which cultivar(s) do you like in a wine to drink? (You can pick more than one)
□ Pinotage □ Merlot □ Shiraz
□ Cabernet Sauvignon □ Blends □ Other …….....

9. How long have you been drinking wine?
□ 0 to 2 years □ 2 to 5 years □ 5 to 10 years □ More than 10 years

10. Regarding the packaging of a bottle of wine, which kind of design do you prefer?
□ A “funky” design with a colourful label □ A classic label

THANK YOU FOR YOUR PARTICIPATION
SEE YOU AGAIN SOON

ADDENDUM D

Expert tasting sheet and free description sheet

Example of tasting sheet used in expert tasting in order to rate experimental wines

Judge name ……..
Date……

Please **smell** and **taste** the wines from left to right. Do not re-taste the wines. Remember to **write down the code** of the wine.
Please indicate the **quality** of the wine on the 9-point scale

Code:	**Code:**	**Code:**	**Code:**
Excellent ☐	Excellent ☐	Excellent ☐	Excellent ☐
Very Good ☐	Very Good ☐	Very Good ☐	Very Good ☐
Good ☐	Good ☐	Good ☐	Good ☐
Fairly Good ☐	Fairly Good ☐	Fairly Good ☐	Fairly Good ☐
Neither Good nor Bad ☐	Neither Good nor Bad ☐	Neither Good nor Bad ☐	Neither Good nor Bad ☐
Fairly Poor ☐	Fairly Poor ☐	Fairly Poor ☐	Fairly Poor ☐
Poor ☐	Poor ☐	Poor ☐	Poor ☐
Very Poor ☐	Very Poor ☐	Very Poor ☐	Very Poor ☐
Extremely Poor ☐	Extremely Poor ☐	Extremely Poor ☐	Extremely Poor ☐

Example of free description sheet used for expert tasting

New Wine Innovation Project
Expert Tasting: Free Description

Judge nr: …… **Date:……..**

Please smell and taste all twelve wines and write down at least **3 descriptors:**

Code:……..
Code:……..
Code:……..
Code:……..
Code:……
Code:……..
Code:……
Code:……..
Code:……..
Code:……..
Code:……..
Code:……..

ADDENDUM E

Examples of the descriptive panel training session tasting sheet

General set-up of descriptive analysis tasting (training session).

Judge Nr…… **Session 4** **18 November 2013**

Aroma

1. Smell all of the standards nr. 1-15. Try to identify the odour. Write your answer in column A
2. Mark the box in column C to tell us how good the standard is.

	A	B	C			
Nr of Aroma Std	What do you think is the aroma?	What is the aroma according to the instructor?	How close is the aroma of the reference standard (that you perceived) to the aroma of the real product (according to your memory)?			
			Quite close	Good enough	Slightly	Not at all
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						

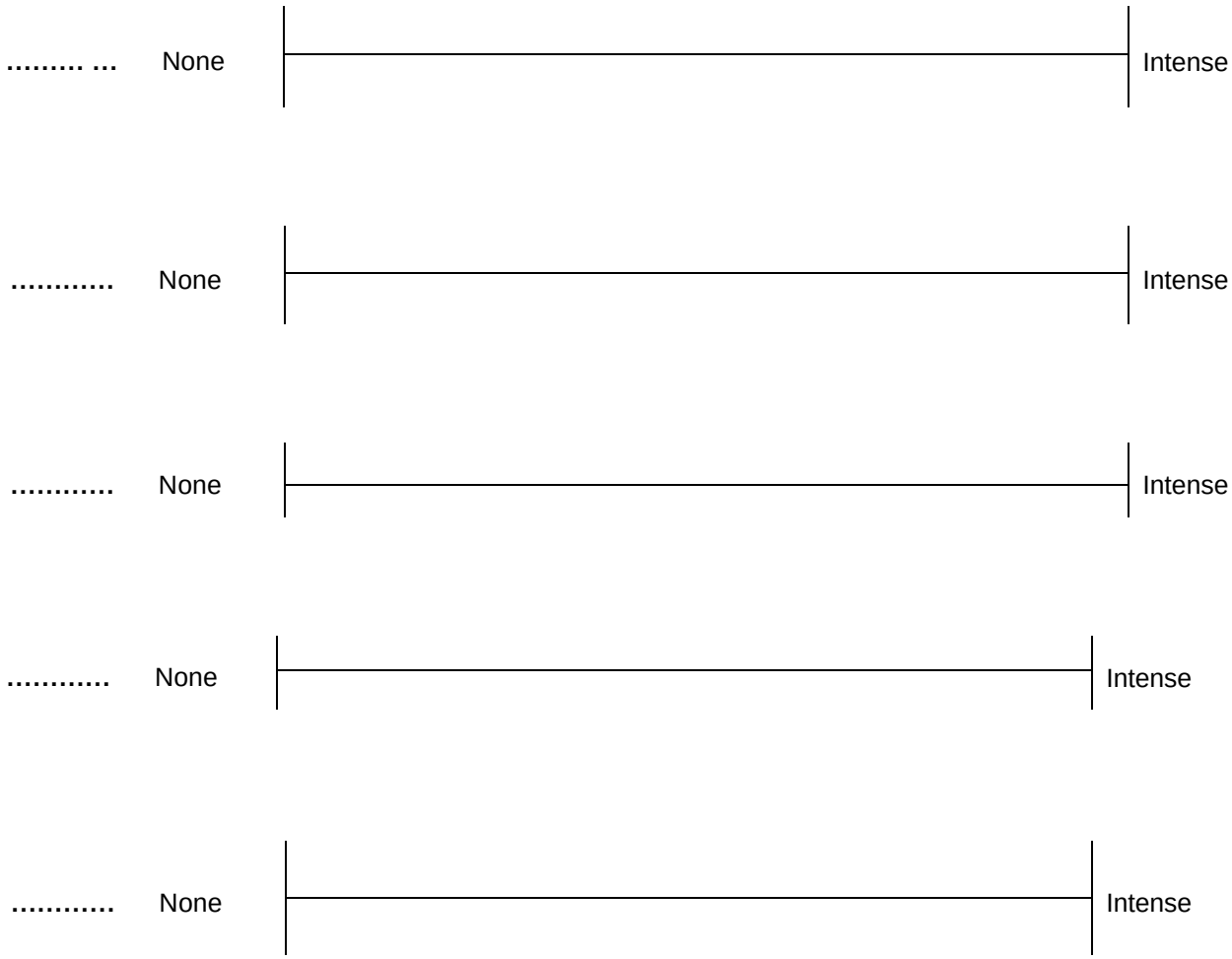
Wines

3. Please write down the most prominent attributes that you will use to describe the wines.

Sample Nr.	Aroma Descriptors/Attributes	Taste Descriptors/Attributes
A		
B		
C		
D		
E		
F		

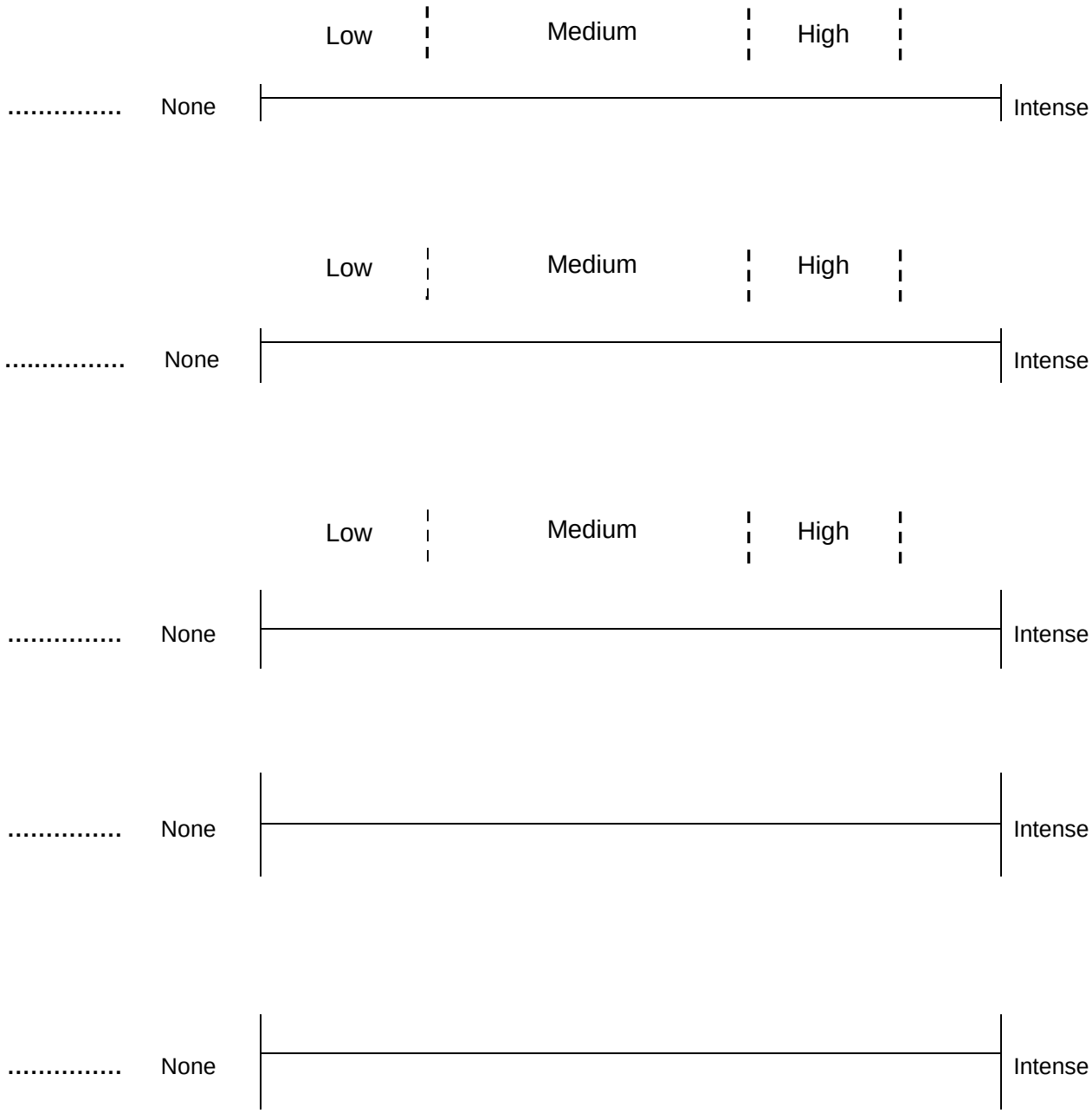
Wines - AROMA

4. Please evaluate the aroma of the wines and scale the attributes



Wines - TASTE

1. Please evaluate the aroma of the wines and scale the attributes



Chapter 4

Research results

The effect of oxidation on red wine treated with rooibos (*Aspalathus linearus*) and honeybush (*Cyclopia*) plant material

Chapter 4

The effect of oxidation on red wine treated with rooibos (*Aspalathus linearus*) and honeybush (*Cyclopia*) plant material

4.1. INTRODUCTION

The exposure of wine to oxygen (O_2) is often inevitable due to winemaking practices like pump overs, grape crushing and wine transferral methods, including barrel filling. Over- and under-exposure to oxygen can cause off-flavours to develop in wine. Exposure to oxygen thus has to be managed either by reductively handling the wine, introducing O_2 with micro-oxygenation, adding antioxidants such as sulphur dioxide (SO_2) or ascorbic acid, or using inert gases like nitrogen to displace O_2 (Caillé, 2010).

Too little O_2 exposure of a wine may lead to the formation of unpleasant reductive aromas such as vegetative, rotten and sulphur-like off-notes (Ugliano *et al*., 2012). The introduction of controlled amounts of O_2 into red wine could be beneficial due to an increase in colour stability and a decrease in astringency as a result of oxidative reactions (Waterhouse & Laurie, 2006). However, too much O_2 exposure may lead to unwanted oxidation of the wine (Du Toit *et al.*, 2006a), leading to the formation of high levels of sotolon and certain aldehydes.

Natural antioxidants, like glutathione and ascorbic acid, serve a significant purpose in wine by scavenging oxidative agents. By removing oxidative agents rapidly, the development of off-flavours and microbial spoilage can be managed. SO_2 can serve as an antioxidant, as well as an anti-microbial agent in wine in common wine-making practices, and still is a popular wine preservative. The occurrence of allergic reactions and the frequency of asthmatic cases as a result of SO_2 have highlighted the need to decrease SO_2 usage in wine (Fracassetti *et al*., 2013).

Honeybush and rooibos tea have both been reported to have health-benefiting attributes (Joubert *et al*., 2008). These health benefits include a strong antioxidant activity, which is inherent to these fynbos species. This antioxidant capacity has recently been investigated as a means of reducing oxidation in foods. Hoffman *et al.* (2014) investigated the use of rooibos in meat products to limit the oxidation of fats and thus the development of rancidity. However, it is unknown how stable the aromas derived from these species are in wine containing low levels of SO_2, and if these treatments could contribute to producing wines with lower SO_2 levels. The main aims of this chapter were therefore to investigate the effect of timing of rooibos and honeybush additions to wine as well as the stability of aroma characteristics of wine treated with rooibos and honeybush when exposed to oxidation.

4.2. MATERIALS AND METHODS

NOTE: The experimental work was divided into two phases for this study. The two phases will be referred to as *phase 1* and *phase 2. Phase 1* aimed at investigating the stability of aroma characteristics associated with rooibos and honeybush treatments in red wines when exposed to oxygen, as well as different times of addition. *Phase 2* aimed at elucidating the stability of aroma characteristics associated in rooibos and honeybush treated red wines exposed to oxidation. In phase 1, different trials were performed using wood and leaf material from both rooibos and honeybush. In phase 2, only wood materials from rooibos and honeybush were used. Phase 1 was regarded as a pilot, preliminary study in which biological repeats were not always included.

Rooibos (*Aspalathus linearis*) or honeybush (*Cyclopia intermedia* and *Cyclopia subternata*) wood/leaf material was added to the wines in phase one. The wood/leaf mixture did not comply with the South African legislation, which only permits the addition of wood products to wine (SAWIS). The wood/leaf mixture was obtained from Cape Natural Tea Products®, Bellville, South Africa. The fraction of stem/wood to leaf, as well as the mixture of *C. intermedia* to *C. subternata*, was determined by the producer. The wood chips were 5 to 7 mm in size, with leaves mixed throughout. Sieves of various sizes are used in the processing of rooibos and honeybush plant material, and the finer the sieve, the finer the material in the end product. The sieves are selected to fit the size of plant material (leaf and wood) that is desired for the end product (herbal tea or extract material) (Joubert *et al.*, 2008). For the phase 2 experimentation only the wood or chip fraction was used, with the chips being 5 to 7 mm in size. The mixture of *C. intermedia* to *C. subternata* was determined by the producer. The chips were added directly to the wine in the glass bottles. Note that “fermented” honeybush and rooibos plant material was used for this study. Both rooibos and honeybush are sold commercially in the so-called “fermented” form (Joubert *et al*., 2011), i.e. produced through a high-temperature oxidation process that is required for the development of the characteristic sweet-associated flavour and dark colour of both herbal teas.

4.2.1. Phase 1: Wines treated with rooibos and honeybush wood/leaf material

4.2.1.1. Winemaking techniques and treatments

The experimental work concerning oxidation and ageing reactions in the treated wines was divided into the following experiments as indicated in Table 4.1:

Table 4.1: Phase 1 – Experiments and general objectives.

Experiment titles		Experimental objectives
Experiment A	Enhanced oxidation in red wine as a result of H_2O_2 addition	Investigate the stability of aroma compounds derived from honeybush and rooibos material in wine exposed to H_2O_2
Experiment B	Oxygen addition to red wines with or without the addition of plant material (rooibos and honeybush) and the sensory impact thereof	Investigate the stability of aroma compounds derived from rooibos and honeybush plant material in wine exposed to oxygen
Experiment C	Sensory analysis of the aromas in commercial wine after nine months barrel ageing in contact with plant material (rooibos and honeybush)	Determining the timing of plant material addition in wine (during fermentation or after MLF) and quantifying aroma development in treated wines

4.2.1.2. Experiment A: Enhanced oxidation in red wine as a result of H_2O_2 addition

Shiraz grapes were sourced from Audacia wine farm, Cape Winelands, South Africa. The harvested grapes, with a weight of 250 kg, were mixed to ensure homogeneity. Batches of 20 kg of grapes were made into separate wines. The grapes were crushed and de-stemmed in separated 20 kg quantities. All the winemaking steps were completed in the experimental cellar located at the Department of Viticulture and Oenology (DVO), Stellenbosch University, South Africa. The following nine treatments were applied:

Table 4.2: Experiment A, treatments and descriptions.

Treatment number	Treatment	Treatment description
1	+SO_2	80 mg/L SO_2 added to wine just before treatment with H_2O_2
2	No SO_2	No sulphur addition, as well as no plant material used
3	Control	SO_2 addition at 30 mg/L prior to fermentation, followed by 50 mg/L SO_2 added to wine prior to bottling
4	RB 5 g/L	5 g/L rooibos leaves and stems added to the must prior to fermentation, no addition of SO_2
5	RB 10 g/L	10 g/L rooibos leaves and stems added to the must prior to fermentation, no addition of SO_2
6	RB 20 g/L	10 g/L rooibos leaves and stems added to the must prior to fermentation, no addition of SO_2
7	HB 5 g/L	5 g/L honeybush leaves and stems added to the must prior to fermentation, no addition of SO_2
8	HB 10 g/L	10 g/L honeybush leaves and stems added to the must prior to fermentation, no addition of SO_2
9	HB 20 g/L	10 g/L honeybush leaves and stems added to the must prior to fermentation, no addition of SO_2

*RB = Rooibos; HB = Honeybush

The wine was inoculated with *Saccharomyces cerevisiae* (WE 372, Anchor Biotechnologies) and placed within a temperature- controlled room at 25˚C and fermented until fermentation was completed. Di-ammonium phosphate (DAP) was added to the fermenting must at 0.5 g/L on the second day of fermentation. Twice daily punch downs were done in order to mix the skins, juice and honeybush or rooibos plant material. The punch downs enabled increased tannin and colour extraction. With alcoholic fermentation completed (residual sugar < 4 g/L, using a Grapescan™ FT 120 instrument (Foss Electric, Denmark) (Nieuwoudt *et al.*, 2004)), the skins were pressed and the rooibos and honeybush material was removed. After the completion of malolactic fermentation (MLF) (malic acid < 0.2 g/L using a Grapescan™ FT 120 instrument, Foss Electric, Denmark) (Nieuwoudt *et al.*, 2004)), the wines were bottled in 750 mL glass bottles (Consol, Stellenbosch, South Africa).

Hydrogen peroxide was used as oxidation agent in order to induce enhanced oxidation in all the treatments as listed in Table 4.2. Hydrogen peroxide (30%, Pinnacle Pharmaceuticals Ltd) was added at the equivalent of 500 mg/L SO_2 removal. This high dosage was used to induce a high degree of oxidation in the wine. The higher H_2O_2 addition was chosen in order to evaluate how well rooibos and honeybush could act in potentially decreasing oxidation, but also the resultant effect on the aroma profile. The wines were kept at 20˚C for one week before being tasted. All treatments of experiment A (N=9) were analysed in triplicate for the respective sensory analyses.

4.2.1.3. Experiment B: Oxygen addition to red wines with or without the addition of plant material (rooibos and honeybush) and the sensory impact thereof

Shiraz and Merlot wines were collected in 50 L canisters from Audacia winery after the completion of MLF and divided into 4 L bottles, as portrayed in Fig. 4 1. The SO_2 concentrations in the wines were below 5 mg/L, as analysed by an external laboratory (Vinlab, Stellenbosch, South Africa).

Each 50 L wine canister was split up into 10 x 4.5 L glass bottles.

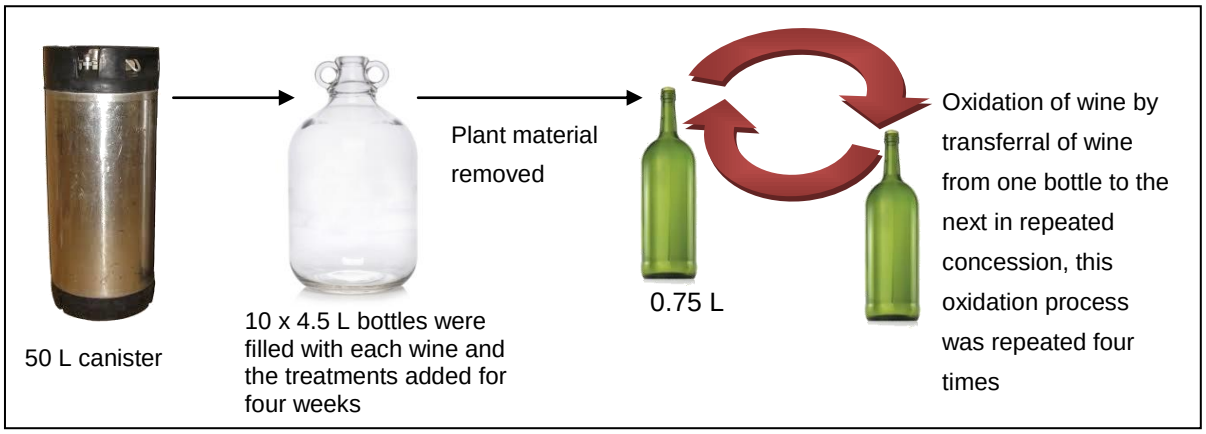


Figure 4.1: Summary of experiment B process (Photographs: Wilko, Consol).

Fermented rooibos and honeybush (10 g/L) was added to each 4.5 L glass bottle. Each treatment was represented by two 4.5 L glass bottles for both Merlot and Shiraz. To prevent oxidation, the bottles were filled to the rim, allowing no headspace.

The wine was kept at a constant temperature of 15˚C for four weeks. The plant material was then removed and the wine was oxidised by pouring the wine from one bucket to another until dissolved oxygen levels of 7 to 8 mg/L were reached. The treated wine was then bottled in 0.75 L glass bottles and stored at 15˚C for three weeks to ensure the consumption of the added oxygen. The O_2 consumed by the treated wines was measured by oxygen sensors glued inside the glass bottles (Pst3; PreSens, Regensburg, Germany). The spot sensors reflect a fluorescent red light when a blue light-emitting diode (LED) provides an excitation pulse when held on the outside of a glass bottle parallel to the spot on the inside of the glass. The fluorescent red light reflects the amount of oxygen present in the wine. This particular method of analysis is referred to as oxoluminescence (Coetzee, 2014). The spot sensors enabled the measurement of dissolved oxygen levels without having to open the bottles, which would have risked the introduction of additional oxygen. After the dissolved oxygen levels had decreased to below 0.5 mg/L, an additional 7 to 8 g/L of dissolved oxygen was added to the wines as described above. This process was repeated four times, after which the wines were collected for tasting.

All treatments for experiment B (N=8) were analysed in triplicate for the chemical and sensory analyses.

4.2.1.4. Experiment C

This experiment was performed using commercial Shiraz wine and performed at the Audacia winery. The wine was prepared using standardised commercial winemaking practices and treated as specified in Table 4.3. In treatment 1 and 2, the leaf and wood material was only in contact with the wine for the duration of alcoholic fermentation (eight days), while in those treatments where it was added after MLF this period was nine months (treatments 3 and 4), after which sensory analyses were performed. All these treatments were stored in old fourth-fill oak barrels after the completion of MLF. All treatments of experiment C (N = 6) were analysed in triplicate for the chemical and sensory analyses.

Table 4.3: Experiment C, treatments and descriptions.

Treatment number	Treatment	Treatment description
1	RB + No SO_2	No SO_2 added. Rooibos added at 5 g/L at the onset of fermentation.
2	HB + No SO_2	No SO_2 added. Honeybush added at 5 g/L at the onset of fermentation.
3	RB + SO_2	SO_2 added at 20 mg/L prior to fermentation. Rooibos added at 5 g/L after MLF.
4	HB + SO_2	SO_2 added at 20 mg/L prior to fermentation. Honeybush added at 5 g/L after MLF.
5	Control	No SO_2 added pre-fermentation or post-MLF. Aged in old wooden barrels.
6	SO_2 + Oak	SO_2 added prior to fermentation (20 mg/L) post-MLF (30 mg/L). Ageing occurred in old barrels with the addition of 3.5 g/L French oak staves.

*RB = Rooibos; HB = Honeybush

4.2.1.5. Sensory analysis

For the tasting of the wines of phase 1, a trained sensory panel consisting of nine assessors took part in the sensory analysis of the treated wines from experiments A to C. Descriptive sensory analysis was used as the test technique in order to generate aroma descriptors for the treated wines, and also to rate intensities of the various aromas (Lawless & Heymann, 2010). Training was done twice a week in four training sessions that lasted two hours each. Panellists thus each completed eight hours of intensive training per experiment (A, B and C). The panel generated descriptors for the experimental wines after panel consensus was reached. The wines were only evaluated for aroma attributes, and these included berry, dried fruit, prune, rose, black pepper, vanilla, rooibos, honeybush, green apple, sherry and green vegetative. Astringent mouthfeel, rooibos aftertaste and honeybush aftertaste were also tested. For most of the attributes, two reference standards were presented to the panel for training purposes – one fresh reference and one soaked in a neutral red wine. These reference standards included fresh green apple, sherry wine, beta-mercapto-ethanol (sulphur compound), fresh rosewater, fresh red berries, dried fruit, dried prunes, black pepper seeds, vanilla pods, honeybush material and rooibos material.

Once the training phase was completed, sensory testing was conducted within a formal sensory laboratory with ambient lighting, good ventilation and a constant temperature of 20 ± 2°C. The tasters were presented with the wines in dark ISO wine tasting glasses and the sample size was 30 mL. The wine was kept at a constant temperature of 15˚C prior to tasting. The tasters were

seated in individual booths in order to avoid interaction throughout the tasting session. Lids (Petri dishes) were placed on each glass just after pouring to concentrate the aroma in the headspace. The glasses were filled 30 min prior to testing. Intensity ratings were marked on 120 mm unstructured line scales, one line scale per attribute. Each line scale was labelled “none” to “intense”. The glasses were marked with unique three-digit codes and all the samples were randomised within each replication. Palate cleansers included purified water and unsalted crackers.

Using descriptive sensory analysis, a total of nine wine samples were evaluated by the trained panel for experiment A, while this number was eight and six for experiments B and C respectively. Each sample was analysed in triplicate. Each test session lasted two hours, during which the respective replicates were completed. After each replicate the assessors had a 15 min break to avoid sensory fatigue. A complete randomised block design was used, ensuring that all samples were tested by all judges (Lawless & Heymann, 2010).

During the training of the sensory panel, panel performance was assessed using PanelCheck® software (Version 1.4.0, Nofima, Ås, Norway). Analysis of variance (ANOVA) was performed to determine attribute means using Statistica (Statistica, version 10, Statsoft Inc., Tulsa, USA). Principle component analysis (PCA) was conducted using attribute means after data weighing, and centring was applied (Unscrambler X, version 10.1, CAMO Inc., Oslo, Norway).

4.2.1.6. Chemical analyses

The colour density and total phenolics, as well as the total red pigment content of the treated wine, were determined by an Analytic Jena Specord 50 UV/VIS spectrophotometer (Analytic Jena, Germany) according to Du Toit *et al*. (2006b). The acetaldehyde concentrations were determined by means of the enzymatic method using the Arena 20XT enzyme robot (Thermo Electron Oy, Finland).

4.2.2. Preliminary extraction trail of rooibos and honeybush wood material in model wine

Model wine was made using distilled water with an alcohol percentage of 12% v/v. The pH of the model wine solution was adjusted to 3.5 using 1 N sodium hydroxide (1 N, Wynland Laboratories, Wellington). Both rooibos and honeybush stems were added at 5, 10, 20 and 40 g/L dosages to the model wine to a final volume of 100 mL each. The rate of extraction was monitored over a period of two weeks with spectrophotometer measurements at 280 nm to determine total phenolic content (Somers & Evans, 1977). The treatments were kept at room temperature.

4.2.3 Phase 2: Oxidation of wine treated with rooibos and honeybush wood

4.2.3.1. Wines used and treatments

The Merlot wine used in phase 2 of this study was obtained from the Audacia cellar just after the completion of MLF and stored in a 200 L stainless steel tank at 4˚C until treatments were applied. The wine was analysed by Vinlab, an external accredited laboratory in Stellenbosch, South Africa, and the free and total sulphur dioxide concentrations were found to be 0 and 3 mg/L respectively.

The wine was divided into 4.5 L glass canisters and treated as indicated in Table 4.4. All transferrals of wine were done while carbon dioxide gas was blown over the wine and into the 4.5 L glass containers prior to being filled with wine. The honeybush and rooibos wood material was supplied by Cape Natural Tea Products, Bellville, South Africa. The wood was processed by the supplier, thus the stems were cut into 5 to 7 mm chips. The chips were added directly to the wine in the glass bottles.

The wine was exposed to the rooibos and honeybush wood treatments for two weeks before the wood was removed and the 4.5 L canisters were combined into PVC food-grade bins according to each treatment. Wine samples were taken for acetaldehyde analysis and spectrophotometric analysis at this stage. The wine was bottled in 0.75 L bottles for each of the respective treatments. All the treatments (in triplicate) were exposed to six consecutive oxygen (O_2) exposures (time stages 0 to 5). The oxygen was added to the wine by pouring the wine from the bottle into a bucket; the wine was then transferred from one bucket to another for five turns, after which it was returned to the bottled and sealed. At every oxidation stage a batch of wine was removed just prior to oxidation and kept in the -4˚C refrigerator in order to represent that particular stage for tasting at the end of the oxidation trial. Each wine treatment, as seen in Table 4.4, was made up of three replications. Each of these replications consisted of two 750 mL bottles. Acetaldehyde and spectrophotometric samples were removed at the concessive oxidation stages.

Table 4.4: Treatments of Merlot wine prior to wine oxidation.

Number	Treatment	Treatment description
1	O_2	Control wine with no addition
2	SO_2	SO_2 (100 mg/L) added
3	RB 10	Rooibos wood addition at 10 g/L
4	HB 10	Honeybush wood addition at 10 g/L
5	RB 40	Rooibos wood addition at 40 g/L
6	HB 40	Honeybush wood addition at 40 g/L

*RB = Rooibos; HB = Honeybush

The O_2 consumption in the treated wines was measured by oxygen sensors glued inside the glass bottles (Pst3; PreSens, Regensburg, Germany). As mentioned in section 4.2.1.3, the spot sensors enabled the measurement of oxygen consumption without having to open the bottles and risk the potential introduction of additional oxygen. Oxygen was again added at each stage after the oxygen levels decreased to lower than 0.5 mg/L. The samples were stored at a constant temperature of 20˚C. All the bottles containing the Pst3 sensors were kept in closed boxes in order to prevent light exposure.

4.2.3.2. Acetaldehyde analysis

The analysis of total acetaldehyde was completed by the Central Analytical Facility of Stellenbosch University using an Arena 20XT enzyme robot (Thermo Electron Oy, Finland) with an enzymatic method. All samples were analysed in triplicate and on the same day as sampling.

4.2.3.3. Spectrophotometric analysis

The treated wines were analysed after every oxidation stage. For each analysis, wine samples were poured into 1 mm quarts cuvettes for colour density measurements. To determine the red, brown and purple/violet colour in the treated wines, absorbencies were measured at 520 nm, 420 nm and 620 nm respectively (Glories, 1984). The spectrophotometer used was an Analytic Jena Specord 50 UV/VIS spectrophotometer (Analytic Jena, Germany). The total phenol and total red pigment content were also determined by diluting wine samples with 1 M HCl, coupled with a waiting period of 3 h. The total phenol and total red pigment content was then calculated by measuring absorbencies at 280 nm and 520 respectively with a 10 mm quartz cuvette (Ribereau-Gayon *et al*., 2006; Somers & Evans, 1977).

4.2.3.4. Sensory analysis

For the sensory analysis of the treatments (indicated in Table 4.4), a trained panel consisting of 12 panel members was used. The full sample set consisted of 18 wines, i.e. six treatments and three replications tested at three oxidation stages (T0, T3 and T5). Descriptive sensory analysis of the treated wines by the panel resulted in a list of aroma descriptors and enabled the panel to rate the intensities of the respective aroma attributes. Seven two-hour training sessions were conducted within two weeks. The panel generated descriptors for the experimental wines after panel consensus was reached. The wines were only tested for aroma and the descriptors included black fruit, red fruit, yellow fruit, prunes, rose, apple skin, sherry, medicinal, sulphur, fresh herbaceous, dry herbaceous, vanilla, honey, black pepper, white pepper, rooibos tea, black tea, biltong, seaweed, artificial candy, cloves/nutmeg and hydrogen sulphide. The attributes were illustrated by reference standards during training and the standards were all fresh, prepared every second or third day depending on the viability of the aroma, see Addendum G, Table 4.7.

The test sessions were conducted in a sensory laboratory with ambient lighting, good ventilation and a constant temperature of 20 ± 2°C. The tasters were presented with 30 mL of wine served in dark ISO wine tasting glasses. Lids (Petri dishes) were placed on each wine glass just after pouring to prevent any loss of aroma, thus concentrating the aroma in the headspace. The wine was kept at a constant temperature of 15˚C, prior to pouring. The glasses were filled 30 min prior to the testing. Intensity ratings were marked on 120 mm unstructured line scales, one for each attribute. Each line scale was labelled “none” to “intense”. The glasses were marked with unique three-digit codes and all the samples were randomised within each replication. For palate cleansers, purified water and unsalted crackers were provided to the assessors. The tasters were seated in individual booths to avoid interaction among the tasters during the tasting session. Three test sessions were completed by the panel over three days. The 18 wines used for training were presented to the panel in the three testing sessions. Six of the 18 wines were given to the tasters over three flights, with a break between each flight. Session 1 represented the wines from oxidation stage 0, session 2 the wines from oxidation stage 3 and session 3 the wines from oxidation stage 5. All the wines were presented in triplicate and each flight was randomised for each assessor.

4.2.3.5. Statistical procedures

Panel performance was verified during training using PanelCheck® software (Tomic *et al.*, 2010). Statistical analysis was conducted with the software program Statistica 12 (Statsoft Inc., Tulsa, USA). Analysis of variance (ANOVA) and student t-test at the 95% confidence intervals were conducted to analyse the significant differences ($p \leq 0.05$) between treatments for each of the sensory and chemical attributes. Thereafter, principal component analysis (PCA) was conducted for the sensory results. The PCA plots were drawn up in both PanelCheck® and Statistica, but only the Statistica graphs are shown.

4.3 RESULTS AND DISCUSSION

4.3.1. Phase 1: Sensory and chemical profile of wines treated with rooibos and honeybush wood/leaf material

4.3.1.1. Experiment A

Acetaldehyde is a product of oxidation in wine and may lead to the occurrence of pungent aromas. Changes in total acetaldehyde levels may give an indication of the progression of oxidation, as the majority of acetaldehyde formation in wine can be linked to the auto-oxidation of ethanol and various phenolic substrates (Wilderandt & Singleton, 1974). Acetaldehyde results are displayed in

Fig. 4.2. As can be seen in Fig. 4.2, the lowest levels of acetaldehyde were seen in the ***Control*** sample, which was treated with 30 mg/L sulphur dioxide (SO_2) early in vinification and 50 mg/L prior to bottling. Acetaldehyde binds preferentially to SO_2 in wine when sulphur dioxide is in its free bisulphite form. Therefore, the addition of 50 mg/L SO_2 prior to bottling served as an effective method to prevent the formation of acetaldehyde as a result of enhanced oxidation by H_2O_2 addition (Waterhouse & Laurie, 2006).

The highest levels of acetaldehyde were seen in the treatment ***No SO_2***,, as well as in the treatments ***RB 5 g/L*** and ***RB 10 g/L***. Conversely, the addition of honeybush material caused significantly lower levels of acetaldehyde compared to that of rooibos material at 5 and 10 g/L. Honeybush material at 20 g/L had significantly lower acetaldehyde levels when compared to the other treatments besides for the ***Control*** treatment. Acetaldehyde in red wine can participate in polymerisation reactions by forming ethyl bridges between anthocyanins and other phenolic moieties, thereby leading to changes in the aldehyde levels over time (Du Toit *et al*., 2006a). Trends observed between these treatments could be due to differences in the phenolic composition of the honeybush and rooibos material (Erasmus, 2015; Jolley, 2014).

The spectral results shown in Table 4.5 represent the brown, red and purple/violet colour components as measured post-H_2O_2. Brown tints measured at 420 nm were lowest within the treatment ***+ SO_2***, as seen in Table 4.5. The late addition of SO_2 at 80 ppm prior to bottling served as an effective antioxidant and thus prevented the browning action as a result of enhanced oxidation (Singleton, 1987). As mentioned by Waterhouse and Laurie (2006), the most prevalent action that SO_2 has in wine is to reduce any H_2O_2 present in the wine matrix, while it can also bleach brown quinones. The red colour measured at 520 nm in treatment ***+ SO_2*** had the lowest value when compared to the other treatments, which can be due to the bleaching effect that the SO_2 could have on the red flavylium ion. As seen in Table 4.5, the biggest differences in measurement occurred at 520 nm in all treatments. The ***Control*** treatment displayed the highest red colour absorbance, measured at 8.28 AU. With the honeybush treatments the red colour decreased steadily as the dosage increased. Changes in the red colour with the rooibos and honeybush wood/leaf treatments may be due to the addition of colour pigments extracted from the two wood/leaf products. Part of the compositional fraction of rooibos has been called “uncharacterized brown material” (Joubert *et al.*, 2008). This brown matter extracted from the rooibos additions therefore can have an impact on the spectral colour of wine. The decrease in red colour measured at 520 nm for the rooibos treatments when compared to the ***Control*** treatment (see Table 4.5) could be explained by the addition of brown matter from the rooibos material. However, the brown colour of the wine measured at 420 nm did not differ immensely from the ***Control*** treatment, suggesting a possible polymerisation or precipitation of the wine colour components. Honeybush also adds colour pigments when extracted into a water or alcohol medium. The colour extracted has been described as “dark reddish brown” (Joubert *et al.*, 2011)

The highest colour density was observed in the ***Control*** treatment, possibly as a result of the increased red colour measured at 520 nm. Colour density is determined by the sum of readings at 420 nm, 520 nm and 620 nm. High colour density readings were also recorded in the honeybush treatments, i.e. at 5 and 10 g/L. Total red pigments, which are an indication of the total pool of red colour, can be seen in Table 4.6 and range normally from 10 to 30 AU in red wine (Boulton, 2001). The total red pigment content in red wines normally decreases steadily during wine ageing due to polymerisation and precipitation (Du Toit, 2006a). The ***+ SO_2*** and ***Control*** treatments had the highest total red pigment levels. By inhibiting oxidation to a large extent, the SO_2, probably led to less precipitation, as well as bleaching of the flavylium ions, thereby leading to a lower percentage of colour in the red form (Table 4.6).

The addition of higher plant material dosages in treatments ***RB 20 g/L*** and ***HB 20 g/L*** led to lower total red pigment colour values, as seen in Table 4.6. The addition of both rooibos and honeybush could have led to increased polymerisation and, as such, lowered the total red pigment content due to precipitation. The polymerisation of phenolic compounds can result in the precipitation of colour pigments or other phenolic compounds like tannins, causing a smoother mouthfeel and colour stability in the wine (Garde-Cerdán & Ancín-Azpilicueta, 2006). These trends were also observed in the total phenol levels, being higher in the SO_2 treatments and lower in the ***No SO_2*** treatment, and also where higher levels of honeybush and rooibos were added.

The results of the sensory analysis of experiment A can be seen in Fig. 4.3. From the principle component analysis (PCA) bi-plot (Fig. 4.3), deductions can be made regarding the sensory impact of the rooibos and honeybush leaf/wood material. According to principal component one (PC1), the samples separated according to species. Treatments 4 (***RB 5 g/L***) and 5 (***RB 10 g/L***) grouped closely together and were associated with the aroma attributes prune and vanilla on the right-hand side of PC1. Similarly, treatment 6 (***RB 20 g/L***) was closely related to dried fruit and a distinctive rooibos aroma, also on the right side of PC1. The higher dosage of rooibos material thus was associated with an increase in perceived intensity of rooibos aroma. On the opposite side of PC1 the aromas were more closely associated with the honeybush treatments. The aromas that were associated with honeybush treatments 8 (***HB 10 g/L***) and 9 (***HB 20 g/L***) were rose, black pepper and honeybush.

In PC2, the rooibos and most of the honeybush treatments (20 g/L and 10 g/L) lie in the bottom right and left quadrants respectively, and thus separate from most of the treatments that do not contain any form honeybush or rooibos (Fig. 4.3). Where sulphur dioxide was added to the wine – treatments 1 (***+ SO_2***) and 3 (***Control***) – it is clear that these treatments were associated with a sulphur-like aroma. These two treatments, together with treatment 2, containing ***No SO_2***, are all situated in the top two quadrants of PC2. The latter indicates that treatments 1, 2 and 3 (***+ SO_2, No SO_2, Control***) are not associated with the rooibos- and honeybush-associated aroma descriptors,

but with aromas that have been connected to oxidative degradation in wine as a result of increased acetaldehyde formation. The acetaldehyde levels in the rooibos treatments (***RB 5g/L*** and ***RB 10 g/L***) were not significantly ($p > 0.05$) different to that of the ***No SO_2*** treatment (Fig. 4.2), but were not associated with oxidative aromas (Fig. 4.3), indicating a possible masking effect of the rooibos treatments on oxidative aromas.

Acetaldehyde in wine has been connected with aromas such as green apple and sherry, as seen in Figs 4.4 and 4.5 (Liu, 2000). It is interesting to note that sample 7 (Fig. 4.3), with ***HB 5 g/L***, was associated more with the samples not treated with HB and RB (upper quadrants of PC2), than with those treated with HB and RB plant material (lower quadrants of PC2). This could be as a result of the natural oxidation taking place and/or the fact that the percentage of HB used was not high enough to combat oxidation.

The ANOVA results of experiment A, i.e. the respective aroma attributes are presented in bar graphs (Figs 4.4 to 4.13). The ***No SO_2*** treatment had significantly higher oxidative aromas (sherry and green apple), as seen in the PCA bi-plot (Fig. 4.3). Sherry aroma was also perceived in the ***HB 5 g/L*** treatment (> 15 intensity value). The ***Control*** and + ***SO_2*** treatments showed negative reductive sulphur aromas (Figs 4.4 and 4.5).

As seen in Fig. 4.6, berry aroma was perceived in all the wines, but significantly ($p > 0.05$) so in the ***Control*** and ***HB 5 g/L*** treatment. Both dried fruit aroma (**RB 20 g/**L and ***RB 10 g/L***; Fig. 4.7) and prune aroma (***RB 5 g/L*** and ***10 g/L***; Fig. 4.8) were significantly higher in most of the rooibos treatments ($p \leq 0.05$). The treatments ***RB 5 g/L*** and ***RB 10 g/L*** led to significantly higher vanilla attributes ($p \leq 0.05$), but this aroma attribute decreased at ***RB 20 g/L*** (Fig. 4.9), to even less than some of the HB treatments. The rooibos aroma was highest in the ***RB 20 g/L*** treatment. The lower dosages of rooibos resulted in significantly lower rooibos aroma intensities, although the latter two RB treatments still resulted in perceptible rooibos aromas (Fig. 4.10).

Aromas that were associated with honeybush treatments were rose, black pepper and honeybush. The honeybush aromas were prominent in ***HB 20 g/L*** (> 70), and significantly less so in ***HB 10 g/L*** (> 25) and ***HB 5 g/L*** (> 10) (Fig. 4.11). The rose and black pepper aromas followed a similar trend (see Figs 4.12 and 4.13 respectively). Both aroma attributes increased significantly in intensity with increasing dosages of honeybush material, especially the rose aroma intensity, which was perceived at a high intensity (> 60) in the ***HB 20 g/L*** treatment. It is well known that tea infusions produced from some of the *Cyclopia* species associate strongly with a rose-like/rose perfume aroma, especially *C. genistoides* and *C. maculata* (Erasmus, 2015). These two *Cyclopia* species were not used as plant material in this experiment, but the strong inherent floral aroma of the honeybush species used in this experiment, especially in treatment ***HB 20 g/L***, could have induced the “development” of a rose-like aroma.

Aromas associated with rooibos leaf and wood additions can be summarised as rooibos, dried fruit and vanilla, while honeybush treatments may lead to honeybush, rose and black pepper aromas. Vanillic acid is present in the composition of rooibos and, as such, could lead to the formation of vanillin. Vanillin, if present in sufficient amounts, can increase vanilla aromas. This possibility should be researched, however, in order to confirm the relation between potential volatile compounds in rooibos fractions and the sensory impact that they may have (Joubert & Schultz, 2012).

Rose and black pepper aromas are generally pleasant aromas, specifically the black pepper aroma, which has been associated with Shiraz wine. The compound rotundone has been identified as a key component of black pepper aroma and can also be present in red wines (Huang *et al*., 2014). The effect of honeybush additions to the Shiraz wine could either have enhanced the potential rotundone already present in the wine, or could have added rotundone or other volatile compounds associated with a spicy aroma. The development of a sensory wheel encompassing a variety of honeybush species indicated that the spicy notes associated with this herbal tea were those of cinnamon/cassia, particularly in the case of *Cyclopia maculata* (Theron *et al.*, 2014). *C. maculata* was not used in this study, but *Cyclopia subternata* was used in a mixture with *Cyclopia intermedia* for all the experimental work in this study. The sensory attributes ascribed to *C. subternata* in the study of Theron *et al.* (2014) indicate that this honeybush species has strong rose, geranium and perfume characteristics. *C. intermedia* was described as having a significant sweet taste and an aroma profile closely related to that of *C. sessiliflora* and *C. genistoides,* both of which display apricot jam and green aroma characteristics (Theron *et al*., 2014).

The higher acetaldehyde concentration in certain rooibos and honeybush treatments (Fig. 4.2) did not lead to aromas associated with acetaldehyde in the oxidised wine. The honeybush- and rooibos-treated wines had significantly lower intensity ratings of green apple as well as sherry aroma, and a potential masking effect of these off-flavours could have occurred as a result of the honeybush and rooibos treatments.

4.3.1.2. Experiment B

A preliminary tasting of the different stages by three experienced wine experts found oxidation-derived aromas only at stage 4 of experiment B. Stages 1 to 3 did not show any significant oxidation effect and were not evaluated further, thus only stage 4 was tested for aroma attributes using descriptive sensory analysis. According to the PCA bi-plot (Fig. 4.14), the honeybush and rooibos leaf/wood treatments grouped together. The impact of grape cultivar variation therefore did not play a large role and the additions of rooibos and honeybush material, at the concentrations used in this study, dominated the sensory differences between the Merlot and Shiraz cultivars indicated in PC1. The Merlot wine without any addition was rated to be high in asparagus and

green vegetable characters (Figs 4.15 and 4.16), while the untreated Shiraz scored higher in dusty, acetone and berry aromas (Figs 4. 17 to 4.19).

The rooibos treatments added to the Merlot and Shiraz wines led to rooibos/tobacco and vanilla aromas (Figs 4.20 and 4.21). Similarly, the honeybush wood/leaf treatments were associated with aromas of honeybush/rose and black pepper (Figs 4.22 and 4.23). In experiment A, the treatments of honeybush and rooibos induced similar aromas in the wines treated with honeybush and rooibos (Figs 4.11, 4.12 and 4.13). The blackcurrant aroma also increased with the addition of honeybush to both Merlot and Shiraz (Fig. 4.24).

Fig. 4.25 shows the development of an off-flavour in the Shiraz wine treated only with SO_2. It is possible that reductive odours occurred in the wine, causing an intense mouldy/musty/sour dairy smell. Merlot treated with SO_2 showed a very intense apple/sherry aroma (Fig. 4.26). This is surprising, as SO_2 additions bind acetaldehyde, and the latter compound is synonymous with green apple and sherry aromas in oxidised wines (Liu, 2000). The free SO_2 in the wine was analysed and confirmed to be 40 mg/L, which is sufficient to bind acetaldehyde. However, other compounds, such as methional and phenylacetaldehyde, have also been associated with oxidation-derived aromas in wine (Du Toit *et al.,* 2006a).

The potential of rooibos and honeybush treatments to mask oxidation-derived aromas in wine was again observed. The green vegetal aromas, caused by methoxypyrazines and other compounds and associated with cultivars like Merlot, Cabernet Sauvignon and Cabernet franc, could possibly also be masked by the addition of honeybush and rooibos material to wine.

4.3.1.3. Experiment C

Experiment C was performed to assess whether sensory observations made on the experimental scale could also be found on the commercial scale, as well as the potential effect of timing of wood/leaf addition. The two addition times were at the beginning of fermentation and after MLF.

The PCA bi-plot (Fig. 4.27) shows that three sample groupings formed across PC1. The influence of rooibos and honeybush treatments thus drove the separation in PC1. The rooibos additions in treatment 1 and 3 were associated with the aromas rooibos, vanilla, tobacco and prune/dried fruit. The association of rooibos treatments with the above-mentioned aromas was confirmed in the ANOVA results (Figs 4.28 to 4.31). The intensity of rooibos and vanilla in Figs 4.28 and 4.29 was significantly higher in treatment 3, ***RB + SO_2***, indicating that the loss of these particular aromas was higher as a result of the longer exposure to the wood material, with chips added after MLF and remaining in contact with the wine for nine months. The aromas tobacco and prune/dried fruit were perceived at the highest intensities in treatment 3 (***RB + SO_2***) and were significantly less in treatment 1 (***RB + No SO_2***), (Figs 4.30 and 4.31). As the ageing of the treated wines was

undertaken in old oak barrels, aroma loss as a result of slow oxidation through the bung and pores of the barrel is quite possible, and the addition of SO_2 effectively prevented a loss of aroma as a result of oxidation (Del Álamo *et al*., 2010). However, another reason could be that the rooibos and honeybush material was in contact with the wine for a much longer time in treatments 3 (***RB + SO_2***) and 4 (***HB + SO_2***) than in treatment 1 (***RB + No SO_2***) and treatment 2 (***HB + No SO_2***), hence possibly leading to a higher extraction of these aromas from the leaf/wood material.

Treatments 2 and 4 (***HB + No SO_2*** and ***HB + SO_2***) had more honeybush, black pepper and rose aroma characteristics, as can be seen in Figs 4.32 to 4.34. Blackcurrant aroma was again associated with the honeybush treatments, as observed in Fig. 4.35, and significantly so in the ***HB + SO_2*** treatment, indicating the benefit of honeybush material addition after MLF.

Treatment 5 (***No SO_2***) had a prominent acetone aroma, as indicated in Fig. 4.36. The formation of acetone can be connected to spoilage by bacteria that creates volatile acidity or ethyl acetate. The occurrence of bacterial spoilage, such as acetic acid bacterial growth (Du Toit & Pretorius, 2002), is highly possible in this treatment, as no SO_2 was added to the wine. Treatment 6 (***SO_2 + Oak***) had oak-associated aromas such as oak and mocha (Figs 4.37 and 4.38 respectively), probably due to the addition of alternative oak products (Guchu, 2005). The addition of rooibos at 5 g/L and no SO_2 (***RB + No SO_2***) resulted in a significant berry aroma (Fig. 4.39).

In summary, experiment C resulted in similar aromas associated with rooibos and honeybush addition as seen in experiment A and B. The potential of these wood alternative additives on commercial scale was shown in this experiment (Experiment C). As already explained, the addition of rooibos and honeybush wood/leaf material lead to higher intensities of the aroma attributes associated with these alternative wood/leaf material.

4.3.2. Preliminary extraction trial of treated model wine

The use of rooibos and honeybush extracts in cosmetics and food products has been widely commercialised. Extracts are made from the waste material, mostly the harder stem material, in harvested rooibos and honeybush (Joubert *et al*., 2008). The time required to extract the majority of rooibos phenolic compounds and soluble solids is dependent on the time the rooibos material is exposed to the extraction solution, as well as on the temperature of the solution (Von Gadow *et al.*, 1997). To assess the time frame required to expose rooibos and honeybush material to wine, thereby extracting the maximum phenolic content possible, a wine-like model solution was treated with honeybush and rooibos wood material.

Figure 4.40 represents the absorbance (AU) at 280 nm over a period of 18 days. The phenolic content in all the treated samples increased rapidly in the first eight days; thereafter the rate of phenolic extraction slowed and reached a plateau. The treatment that displayed the most rapid and

abundant phenolic extraction was that of ***HB 40 g/L***. In comparison, the ***RB 40 g/L*** treatment was also rapid at the beginning, but it slowed down and reached a plateau at a lower phenolic content. All the treatments peaked in terms of extraction rate after six days, thus displaying a similar trend in terms of the time frame required to extract phenolic compounds from varying dosages of rooibos and honeybush material in model wine solutions. Lower levels of the material also led to lower AU measurements at 280 nm over time. Further research should be done with regard to how a model wine solution treated with honeybush and rooibos would compare to a real wine matrix in terms of the time frame required to extract a maximum or preferred phenolic content.

4.3.3. Phase 2: Oxidation of wine treated with rooibos and honeybush wood

4.3.3.1 Oxidation experiment: Chemical profile of wine treated with rooibos and honeybush wood

The decay rate of oxygen in the Merlot wine treated with rooibos (RB) and honeybush (HB) wood and oxidised over six stages is displayed in Fig. 4.41. In session 1, all the treatments displayed a high decay rate as represented by the decay rate parameters on the y-axis of Fig. 4.41. When a wine is still young there are a large number of phenols present in the wine that can act as oxidative substrates. In this way, phenols to react with oxygen in the wine matrix are highly abundant, consuming the available O_2 at a quick rate (Ugliano, 2013).

The rate of oxygen decay in the wine treated with rooibos at 40 g/L (***RB 40 g/L***) was often significantly higher than the ***Control*** and honeybush treatments. The ***Control*** treatment and ***RB 10 g/L*** showed a similar rate of decay in the first session, emphasising that the initial oxygen consumption cannot be related solely to the antioxidants present in the treated wines. The phenols present in the Merlot wine thus played a significant role in consuming oxygen, as the ***Control*** wine displayed a higher rate of oxygen decay than the ***HB 40 g/L*** treatment. Looking at the total phenol content measured at 280 nm, Figure 4.49, a significantly higher ($p > 0.05$) total phenol content was measured for the ***HB 40 g/L*** treatment. More research will have to be done in order to ascertain how the respective phenol compounds added by the honeybush plant material interact with wine phenols and oxygen in the wine.

A noteworthy result from the first oxidative session shown in Figure 4.42 is the slow rate of oxygen decay attributed to the sulphur dioxide-treated wine. Overall performance of the rate of decay of oxygen by the ***SO_2***-treated wine was significantly lower than that of the other treatments, except in session four, where the ***SO_2*** treatment, together with that of ***HB 40 g/L*** and ***RB 40 g/L***, showed a highly significant rate of oxygen decay. When inspecting the acetaldehyde analysis in Figure 4.48 a significant increase ($p > 0.05$) in acetaldehyde can be seen for the ***SO_2*** treatment at stage three, this can serve as explanation for the peak in oxygen consumption for the ***SO_2*** treatment in stage four, Figure 4.41. The release of free SO_2 from acetaldehyde, colour pigments or other phenolic

compounds at this stage that bind to SO_2 could have occurred, enabling the action of SO_2 to decay oxygen. However, the effect of SO_2 on oxygen consumption in white wines has been investigated (Fracassetti *et al*., 2013), but limited studies has been done on red wines and this needs more attention. In order to quantify the action of SO_2 more effectively, the quantification of free, total and bound SO_2 in the wine for all the oxidative sessions would have been ideal, although these measurements would have had to be made without risking oxygen exposure to the samples.

One of the potential reactions for decreasing oxygen in wine by rooibos additions can be that of the oxidation of the unique antioxidant, i.e. aspalathin, or some other phenolic moiety found only in rooibos (Joubert & Schultz, 2012). The mechanism by which aspalathin is oxidised in an oxidative environment is represented in Fig. 4.53. The oxidation of aspalathin causes the flavones orientin and iso-orientin to form. Fig. 4.41 shows the often significantly higher decay rate of oxygen by the rooibos treatments, in particular the high dosage of rooibos, and raises interesting future possibilities for the ability of rooibos additions in wine to scavenge and decrease oxidative agents. The honeybush treatments did not show the highest decay rate, particularly in the first three sessions (sessions 0 and 3). The high dosage of honeybush at 40 g/L (***HB 40 g/L***) showed a significant decay of oxygen in session 4.

As seen in *Phase 1,* Experiment A, the 520 nm measurements (Table 4.5) showed a similar trend to that of the spectral results in Figure 4.51. The spectral results for 420 nm and 620 nm, Figure 4.50 and 4.52, did not show notable differences between the various treatments, but a high measurement was noted for the ***RB 40 g/L*** at stage 5. At such an advance stage of oxidation the stability of the wine was questionable and further trials will be required to explain the peak in colour for ***RB 40 g/L*** at stage 5. Measurements at 520 nm (Figure 4.51) also showed a higher colour measurement at stage 5 for the ***RB 40 g/L*** treatment similar to the 420 and 620 nm measurements.

4.3.3.2 Oxidation experiment: Sensory profile of wine treated with rooibos and honeybush wood

Treated Merlot wine samples were tested by the sensory panel for an array of aroma attributes using descriptive sensory analysis. The six treatments, as indicated in Table 4.4, were as follows: 1) ***Control*** sample with only O_2 added (***Control***); 2) SO_2 added (***SO_2***); 3) RB wood addition at 10 g/L wine (***RB 10 g/L***); 4) HB wood addition at 10 g/L wine (***HB 10 g/L***); 5) RB wood addition at 40 g/L wine (***RB 40 g/L***); and 6) HB wood addition at 40 g/L wine (***HB 40 g/L***). Each of the six treatments also included three oxidation stages, i.e. time 0 (T0), time 3 (T3) and time 5 (T5), as indicated in section 4.2.3.4.

According to the PCA bi-plot (Fig. 4.54), the samples separated according to treatments along PC1. On the left-hand side of PC1, all the rooibos-treated samples are found, and on the right-

hand side all the honeybush-treated samples are found. The rooibos-treated samples were associated with the aroma attributes rooibos tea, dry herbaceous, honey, vanilla and seaweed, and the honeybush-treated samples were associated with the floral note rose, as well as black tea, artificial candy and biltong aroma. The samples along PC2 split according to no wood treatment (top part of PC2) and the rooibos and honeybush wood treatments (bottom left and right quadrants of PC2 respectively). The ***Control*** sample and the wines treated with SO_2 were associated with the aroma attributes black berries, red berries, fresh herbaceous, black pepper and medicinal. The first three aroma attributes are typical of Merlot wine (Pineau *et al*., 2009).

The aroma attribute, black berries, was quite prominent in all the Merlot wine samples, irrespective of treatment (Fig. 4.55), whereas red berry aroma was perceived at varying intensities in all the treated wines (Fig. 4.56). Figure 4.55 shows the intensity of black berry aroma as being marginally higher in the ***Control*** treatment compared to the rooibos and honeybush treatments over all three oxidative stages investigated. However, this difference was not significant ($p > 0.05$). This indicates a possible masking effect of the natural black berry aroma in the wine by the wood material used. In contrast, the red berry aroma was not strong in all the treatments. Rooibos additions significantly ($p \leq 0.05$) decreased the intensity of red berry aroma compared to the other treatments over all the oxidative stages (Fig. 4.56). With oxidative stage T0, the intensity of red berry was very low in the SO_2 wine sample, but this changed at oxidative stages T3 and T5, when the red berry aroma intensity was evaluated as being significantly higher compared to that of stage T0. It has been shown that SO_2 can bind beta-damascenone, a compound that contributes to the fruity, berry aroma of red wines, which could have been the case in this experiment (Daniel *et al*., 2006).

For black pepper aroma there is a clear distinction between the intensities of the rooibos- and honeybush-treated wines and the ***Control*** and SO_2-treated wines (Fig. 4.57). The rooibos and honeybush wood treatments had low intensities of black pepper aroma compared to the ***Control*** and SO_2 wine samples, which resulted in significantly higher black pepper aromas ($p \leq 0.05$). In contrast, white pepper aroma was significantly ($p \leq 0.05$) higher in the rooibos- and specifically in the honeybush-treated wines compared to that of the ***Control*** and SO_2 treatments (Fig. 4.58). The panel also evaluated the ***Control*** and SO_2 samples as having significantly higher fresh herbaceous notes (Fig. 4.59), whereas only the rooibos treatments at T0, T3 and T5 rated high for the aroma attribute dry herbaceous (Fig. 4.60).

Both wood treatments resulted in reasonably high white pepper intensities, as seen in Fig. 4.58. The compound responsible for the pepper aroma in white and black pepper is rotundone. The difference between white and black pepper is that, for white pepper, the pericarp of ripe peppercorns is removed. Black pepper, on the other hand, is produced by fungal infection of ripe peppercorns. The rotundone content of white pepper is usually double that of black pepper (Wood *et al*., 2008b). Rotundone has also been identified in Shiraz grapes as an impact aroma

compound, in this case being described as black pepper (Wood *et al*., 2008a). Interestingly, the descriptive panel associated black pepper with the SO_2 and ***Control*** treatments, as seen in Fig. 4.57. The wine used in the current experiment, however, was Merlot. Wood *et al.* (2008a) analysed various grape cultivars, as well as Shiraz wine, using sensory analysis. Merlot was not subjected to sensory analysis as an individual wine in the study by Wood *et al*. (2008a), but it did from part of a blended wine (Cabernet Sauvignon/Merlot/Cabernet franc blend). In this case, low levels of roduntone were present, although the panel was able to pick up a slight peppery aroma.

Aromas that were rated significantly higher within the honeybush treatments were black tea, biltong, rose and artificial candy (Figs 4.61 to 4.64). The perceived aromas were often also significantly higher with an increase in wood dosage. As described previously, the two honeybush species used in this study were *C. subternata* and *C. intermedia.* These species have been reported to have floral or rose-like aromas, as well as sweet-associated notes. *C. subternata* in particular has also been associated with fruity-sweet aromas (Theron *et al.*, 2014). In line with the latter tendencies, the aroma descriptors rose and artificial candy-like were indeed present in the wines treated with these two *Cyclopia* species. Interestingly, the aroma descriptor “honeybush” was not generated by the panel during the training phase of this oxidation trial. This can be due to the limited exposure of a number of panel members to fermented honeybush, an indigenous South African herbal tea. Many of the panel members were French, Spanish and Hungarian and thus possibly unfamiliar with the typical aroma of honeybush.

The intensity of the black tea aroma, as seen in Fig. 4.61, was rated at low intensity levels in the honeybush treatments, with a tendency to decrease ($p > 0.05$) with an increase in oxidation. Black tea (*Camellia sinensis*) has no floral notes, but can be described as having a woody aroma. In contrast, fermented honeybush is known to impart a strong floral note (Theron *at al*., 2014). The development of a slight black tea aroma in the honeybush-treated samples could possibility be as a result of the development of a woody note, or because the floral note, typical of all honeybush species, was masked by other compounds in the plant material or wine (Theron *at al*., 2014). It is not clear why the honeybush-treated samples illustrated a definite biltong aroma. The possibility exists that there could have been an interaction between the aromatic compounds (Ferreira, 2010) in the Merlot wine and the honeybush plant material that could have served as a synergistic effect creating a slight savoury biltong aroma.

The aroma attributes that were specifically associated with the rooibos treatments were honey, rooibos tea, vanilla and seaweed (Figs 4.65, 4.66, 4.67 and 4.68). In this study, rooibos and honey aroma have already been reported as among the impact aroma attributes in the respective rooibos treatments (Figs 4.65 and 4.66). Rooibos and honey are also two of the major sensory attributes associated with rooibos tea; on a 100-point scale the intensity is usually > 60 and > 40 respectively (Koch *et al.*, 2012). Vanilla aroma was also indicated at low intensities in the rooibos-treated

samples (Fig. 4.67). Koch *et al.* (2012) indicated that some batches of fermented rooibos tea did indeed illustrate a slight vanilla-like aroma. A seaweed aroma (Fig. 4.68) was also identified in the rooibos-treated wine. In the commercial rooibos industry, this aroma attribute is regarded as a taint as a result of under- or over-fermentation of rooibos plant material (Koch *et al*., 2012). According to Fig. 4.68, the intensity of seaweed aroma remained fairly constant over all the oxidation stages, indicating that it was originally present in the rooibos wood. A medicinal aroma (Fig. 4.69) was detected in all six treated wines, with the ***Control*** and SO_2 samples illustrating the highest intensities. Albeit barely detectable, both the honeybush and especially the rooibos wood resulted in a slight medicinal aroma. Although fermented rooibos, when over-fermented, can easily result in a strong medicinal aroma (Koch *et al*., 2012), it is not clear why the rooibos- and honeybush-treated wines illustrated slight medicinal notes.

4.4 CONCLUSIONS

Aromas typical of fermented honeybush and rooibos plant material, i.e. typically found in rooibos and honeybush tea, were also noted in the rooibos- and honeybush-treated wines. Wines treated with rooibos wood resulted in the following perceptible aroma attributes: rooibos tea, honey, vanilla and dry herbaceous. Aromas that were perceived in the wines treated with honeybush wood included rose, artificial candy, black tea and the savoury-like aroma attribute, biltong. These attributes might seem foreign to the red wine-drinking consumer, but it could definitely add to the complexity of a red wine. However, the aromas associated with these treatments seem to be relatively stable when red wines are exposed to oxygen and could assist in producing wines with reduced levels of SO_2. It will be interesting to ascertain the effect of rooibos and honeybush wood in combination with oak. Future studies thus could include oak-treated wines as well as those containing varying levels of SO_2.

In this study, notice had to be taken of the extreme additions of O_2 throughout the oxidation trials. Extreme additions of O_2 would not be common practice in a commercial wine cellar, but did shed light on the pattern of oxygen decay by the various antioxidants tested. By tracking the oxygen decay within wine using various antioxidant additives, the action over time of these additives could be mapped out, thus creating an opportunity to see at which point of oxygen saturation in wine these antioxidants act at their prime, and if this antioxidant action is relevant when compared to a wine that has no antioxidant additives. The relevance of SO_2 addition to wine became apparent in this study, as the rooibos and honeybush treatments had a faster rate of oxygen decay, as explained in section 4.3.3.

Future advances in sterile winemaking environments and sterile winemaking practices could decrease the use of SO_2 in wine, although maybe not entirely, but the management of SO_2 additions will become a well-defined practice. The management of SO_2 additions throughout the

winemaking process, and especially during ageing, could be considered in the future with the advancement of oxygen monitoring throughout every step of the winemaking process. Allergic reactions to SO_2 and consumer preference for wines with lower SO_2 additions could be catered for. It is clear that more research should be done on the microbial impact of rooibos and honeybush wood additions on wine in the absence of SO_2. The inclusion of rooibos and honeybush can be considered beneficial, as shown by the oxidation decay rate in this study. Creating a timeline of the most beneficial points regarding oxygen consumption by rooibos and honeybush wood material in winemaking practices could lead to the development of low-sulphur or no-sulphur wines with unique aroma constituents contributed by the wood additives.

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Wood, C., Siebert, T.E., Parker, M., Capone, D.L., Elsey, G.M., Pollnitz, A.P., Eggers, M., Meier, M., Vössing, T., Widder, S., Krammer, G., Sefton, M.A. & Herderich, M.J., 2008a. From wine to pepper: Rotundone, an obscure sesquiterpene, is a potent spicy aroma compound. J. Agric. Food Chem. 56(10), 3738-3744.

Wood, C.; Siebert, T.; Parker, M.; Capone, D.; Elsey, G.; Pollnitz, A.; Eggers, M.; Meier, M.; Vössing, T.; Widder, S., 2008b. Spice up your life–The Rotundone story. Expression of Multideisciplinary flavour science, Proceedings of the 12th Weurman Symposium

Addendum F
Results tables and figures

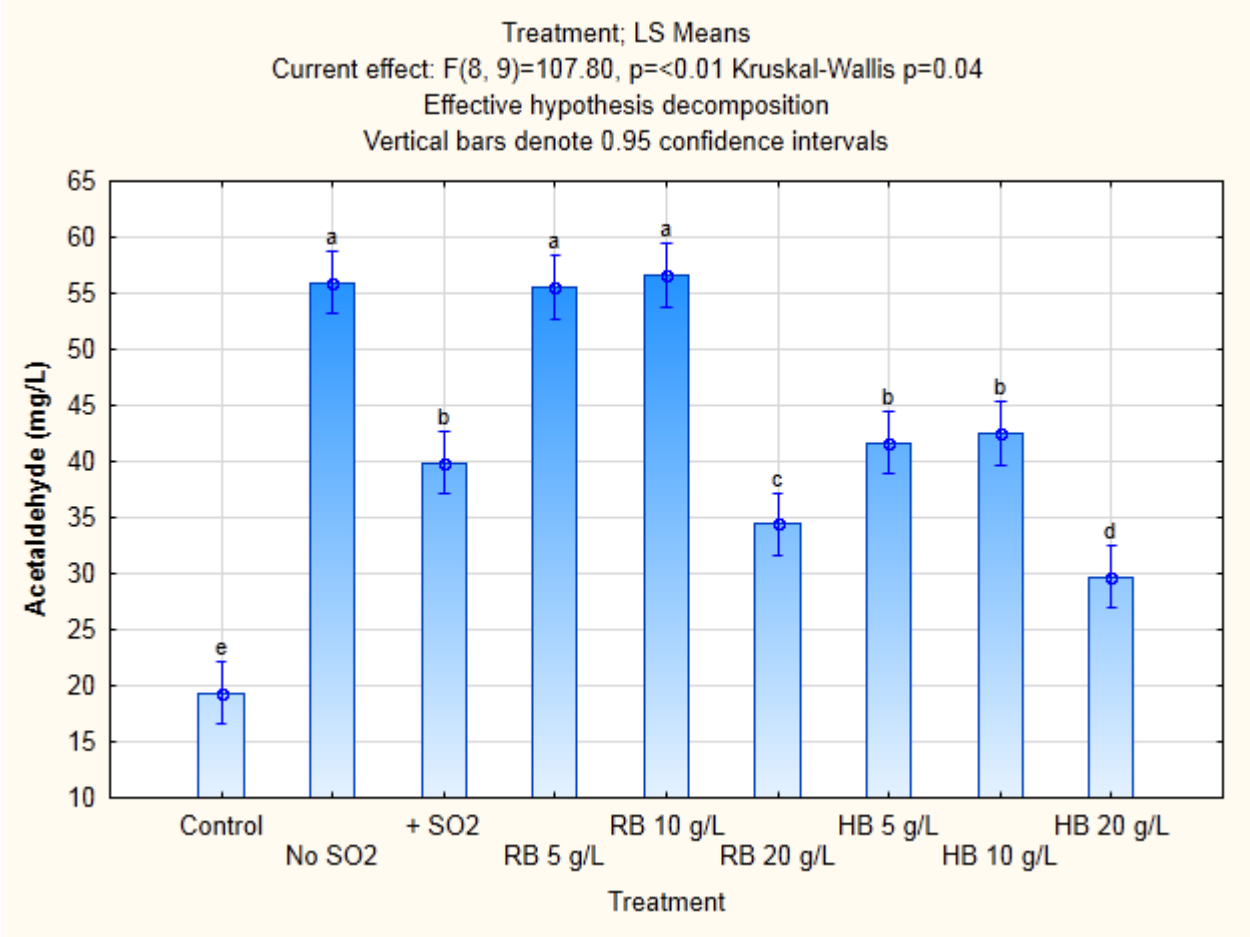

Figure 4.2: Experiment A: Acetaldehyde analysis of Shiraz wine after treatments and induced enhanced oxidation by H_2O_2 additions. ($p<0.01$)

Table 4.5 Experiment A: Absorbency units (AU), measured at varying wavelengths of red wines following treatments, indicate the presence of brown (420 nm), red (520 nm) and purple (620 nm) colour pigments in the wines.

Code	Treatment	420 nm (AU)	520 nm (AU)	620 nm (AU)	*Colour density (AU)
1	+SO_2	3.20	5.12	1.12	9.44
2	No SO_2	4.37	6.79	1.66	12.82
3	Control	4.51	8.28	1.61	14.41
4	RB 5 g/L	4.35	6.69	1.60	12.64
5	RB 10 g/L	4.54	6.74	1.65	12.93
6	RB 20 g/L	4.24	5.37	1.37	10.97
7	HB 5 g/L	4.73	7.31	1.77	13.81
8	HB 10 g/L	4.62	6.86	1.71	13.19
9	HB 20 g/L	4.01	5.29	1.26	10.55

* Colour density refers to the sum of the following absorbency measurements: 420, 520 and 620; RB = Rooibos; HB = Honeybush.

Table 4.6 Experiment A: Total phenol, total red pigment and % of colour in the red form of the treated wines, after the treatments.

Code	Treatment	Total phenols (AU)	Total red pigments (AU)	% of colour in the red form
1	+SO_2	54.93	27.59	34
2	No SO_2	47.51	17.77	72
3	Control	53.41	25.53	56
4	RB 5 g/L	49.86	17.67	72
5	RB 10 g/L	49.00	18.19	71
6	RB 20 g/L	50.58	15.21	72
7	HB 5 g/L	48.17	19.3	72
8	HB 10 g/L	50.11	19.36	68
9	HB 20 g/L	47.79	15.32	69

Absorbency units (AU); RB = Rooibos; HB = Honeybush

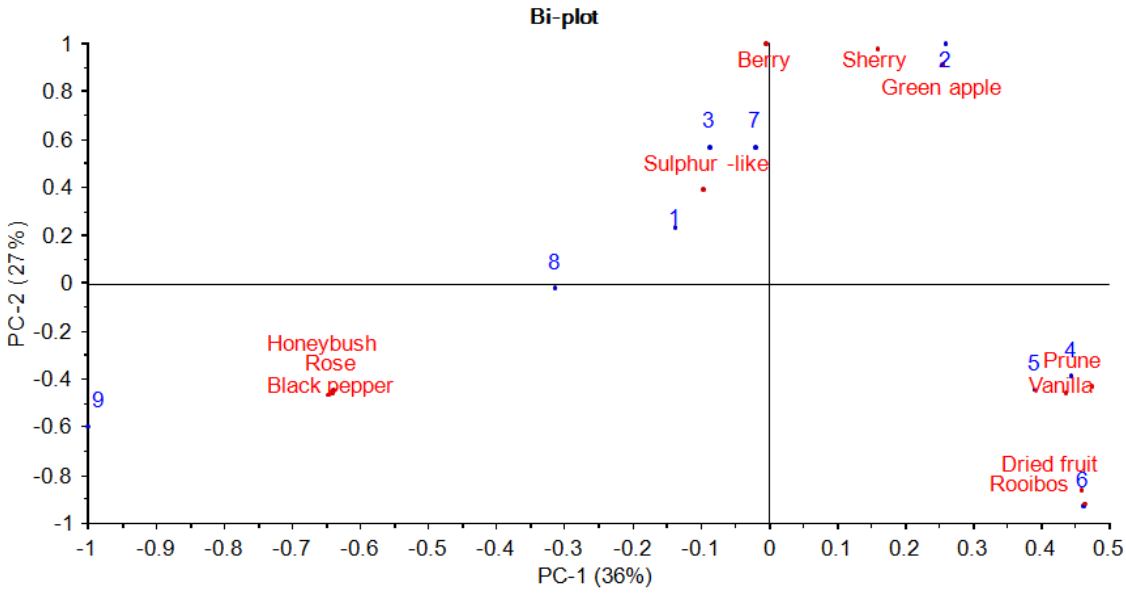

Figure 4.3 : PCA bi-plot of the sensory characteristics investigated in experiment A. Treatment codes are indicated in blue; 1: + SO_2 (80 mg/L SO_2 before H_2O_2), 2: No SO_2, 3: Control (30 mg/L SO_2 and 50 mg/L SO_2), 4: RB 5g/L, 5: RB 10 g/L, 6: RB 20 g/L, 7: HB 5 g/L, 8: HB 10 g/L, 9: HB 20 g/L. RB = Rooibos and HB = Honeybush.

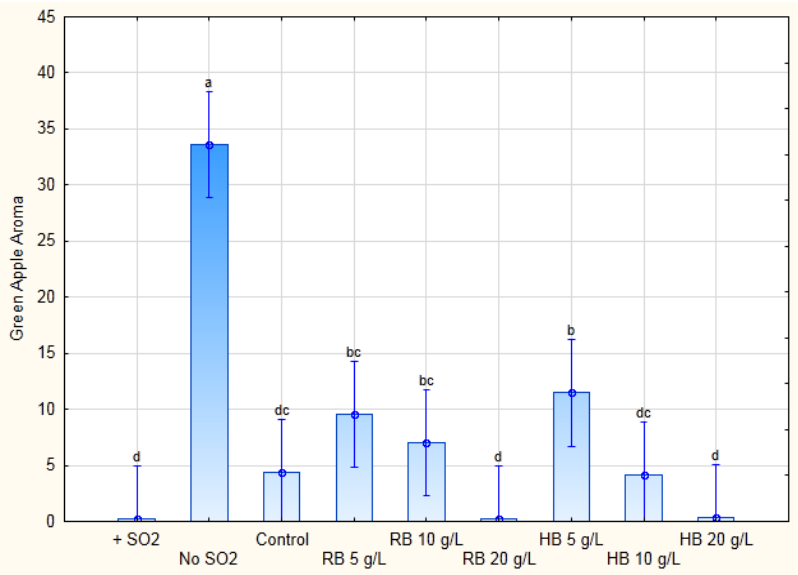

Figure 4.4: Green apple aroma

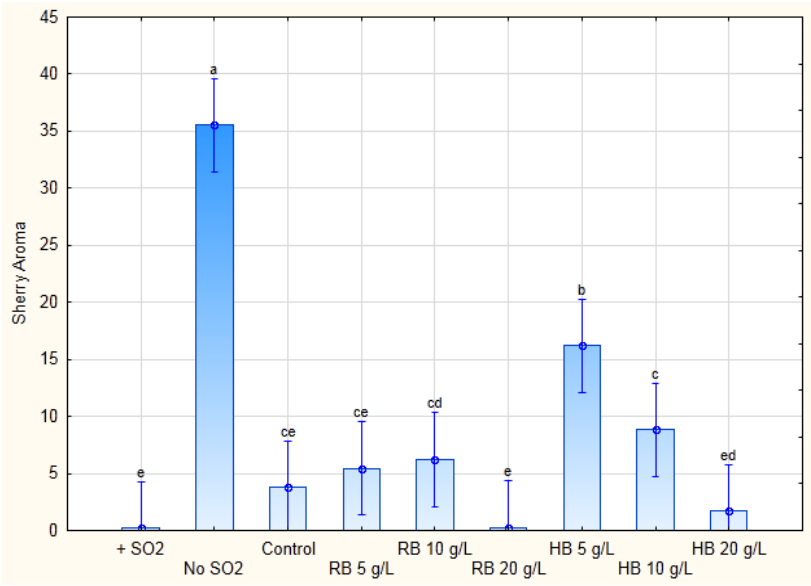

Figure 4.5: Sherry aroma

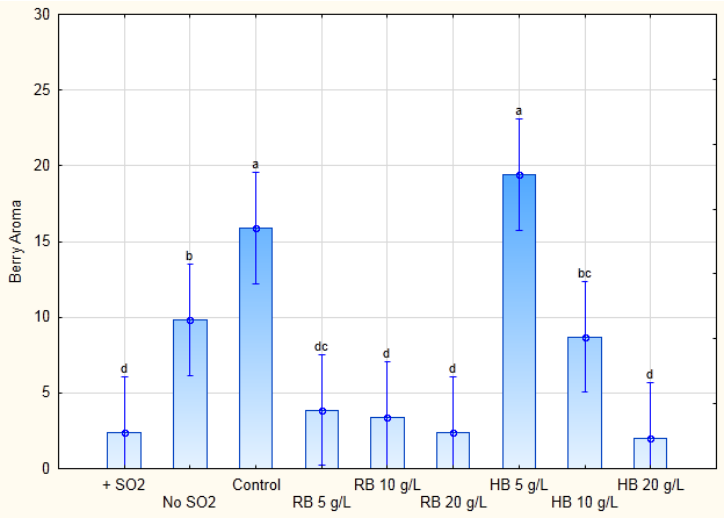

Figure 4.6: Berry aroma

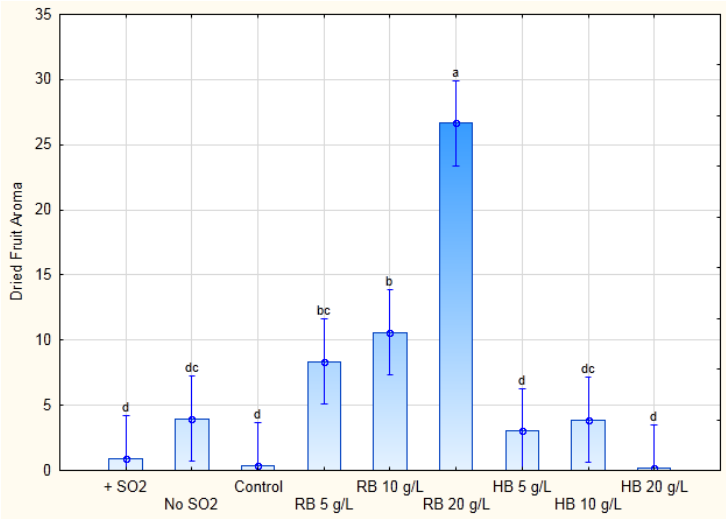

Figure 4.7: Dried fruit aroma

Figures 4.4 to 4.7: Experiment A - ANOVA results for green apple aroma, sherry aroma, berry aroma and dried fruit aroma.

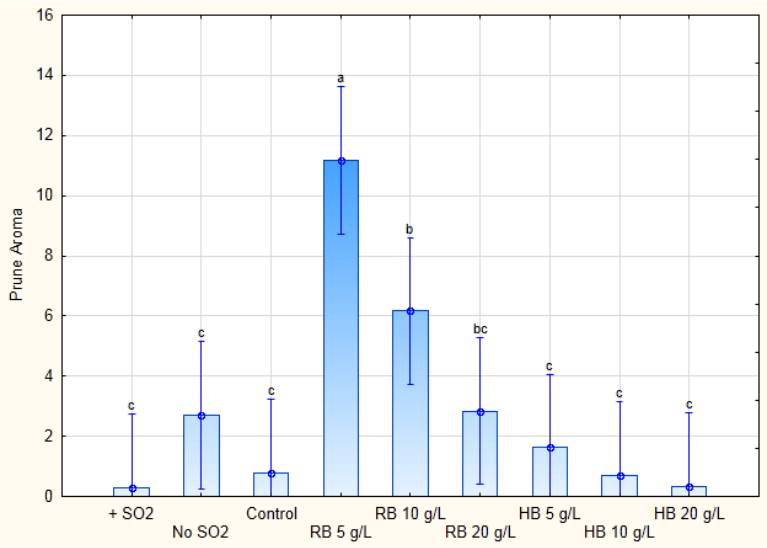

Figure 4.8: Prune aroma

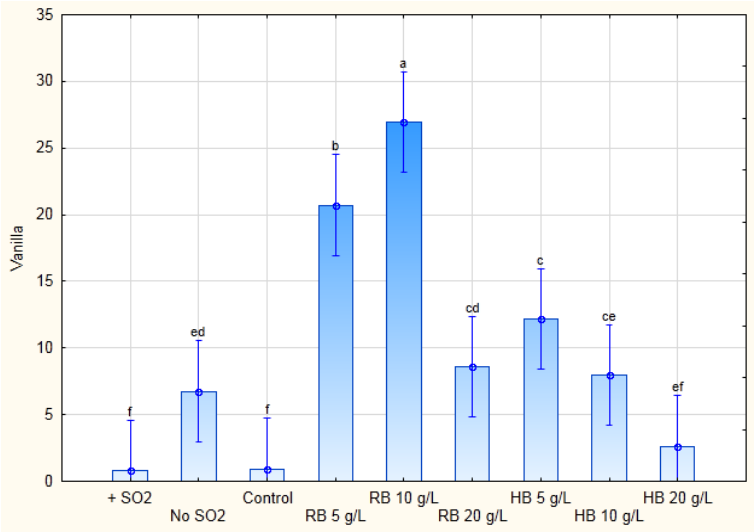

Figure 4.9: Vanilla aroma

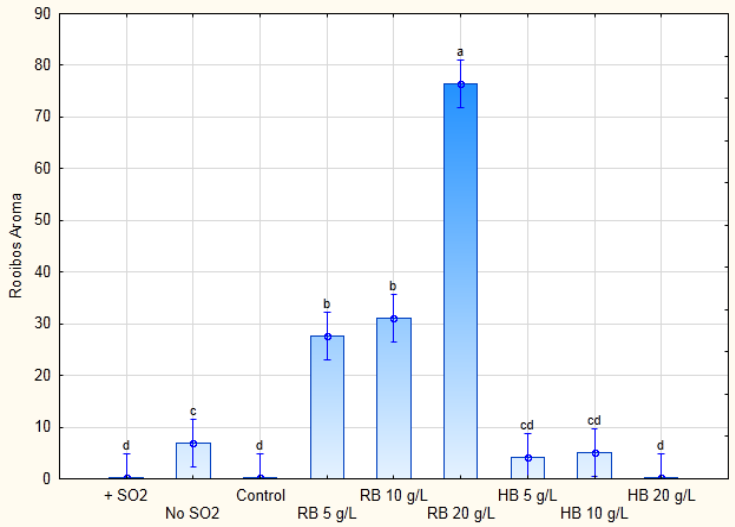

Figure 4.10: Rooibos aroma

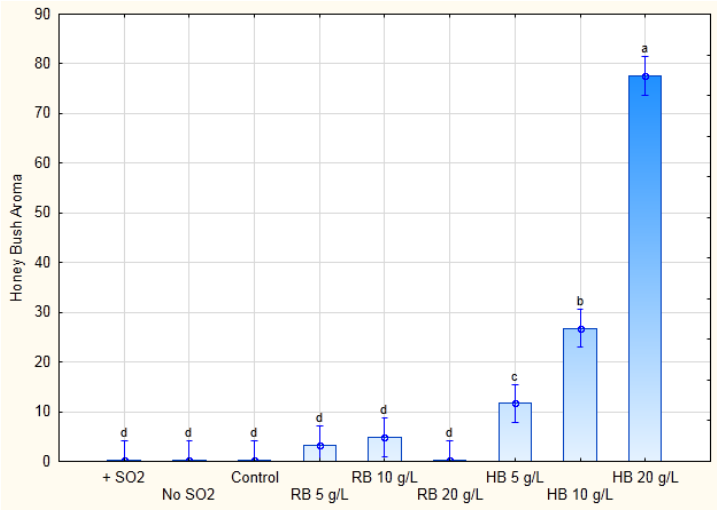

Figure 4.11: Honeybush aroma

Figures 4.8 to 4.11: Experiment A - ANOVA results for prune aroma, vanilla aroma, rooibos aroma and honeybush aroma.

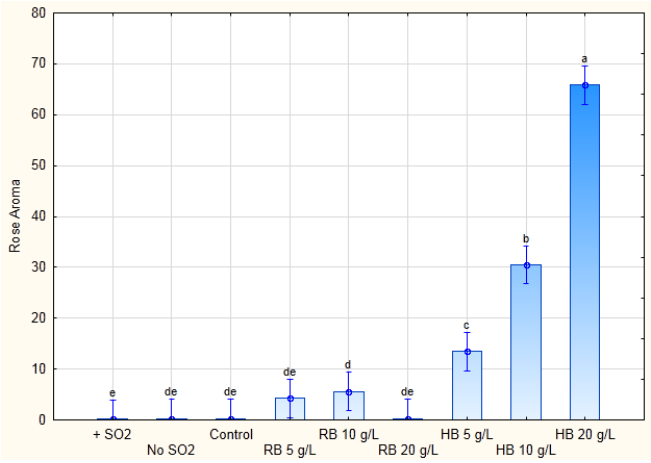

Figure 4.12: Rose aroma

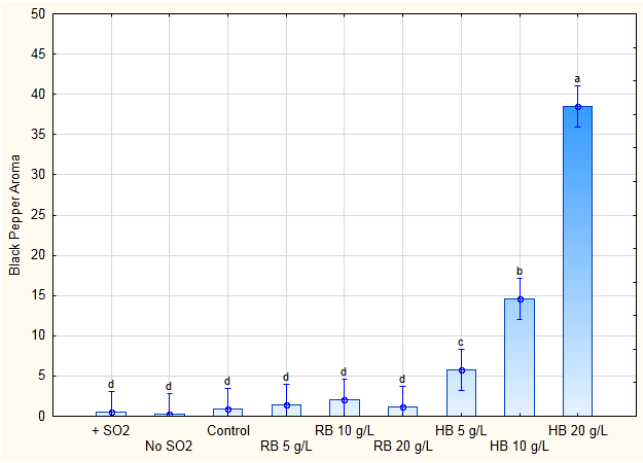

Figure 4.13: Black pepper aroma

Figures 4.12 to 4.13: Experiment A - ANOVA results for rose aroma and black pepper aroma.

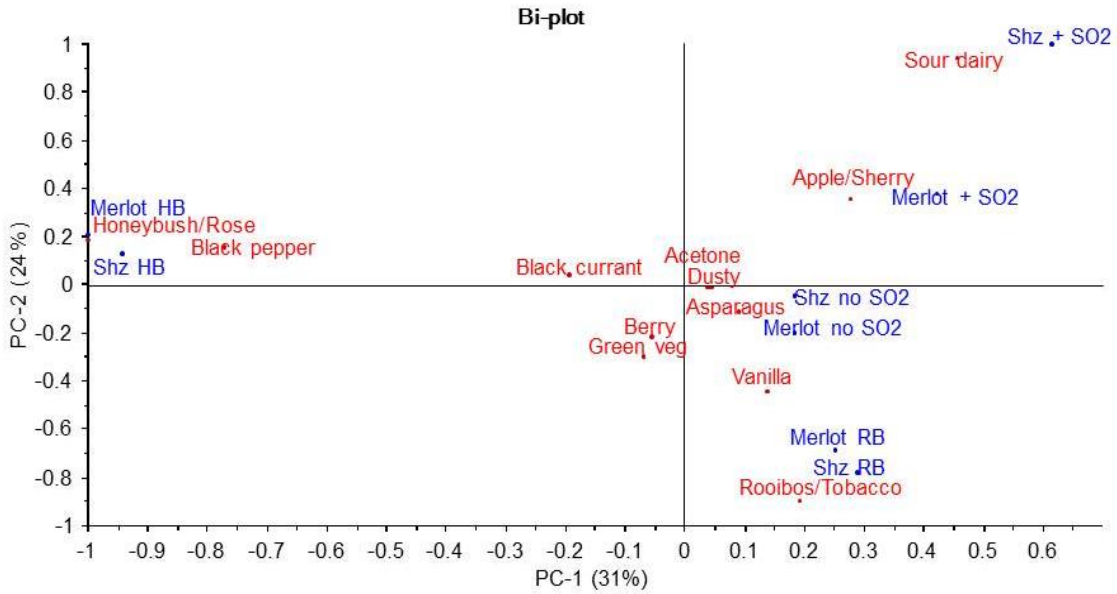

Figure 4.14: PCA bi-plot of the sensory characteristics investigated in experiment B. Treatments are indicated in blue.

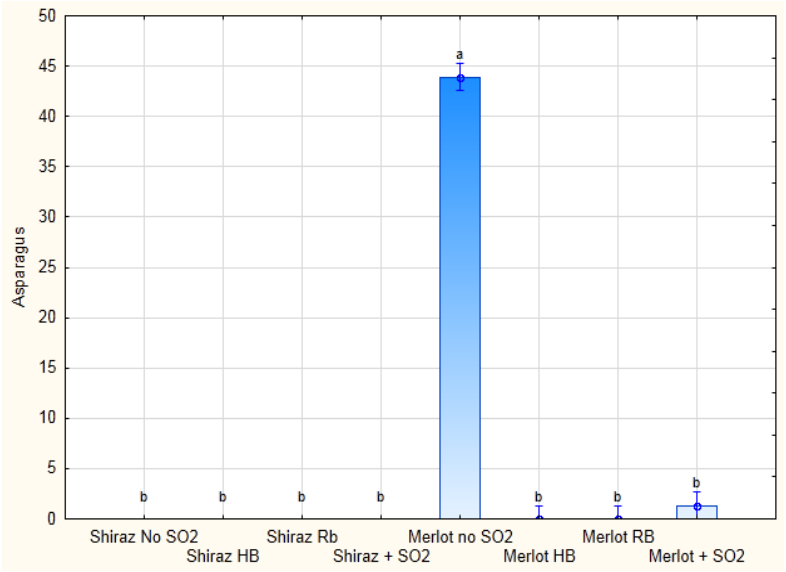

Figure 4.15: Asparagus aroma

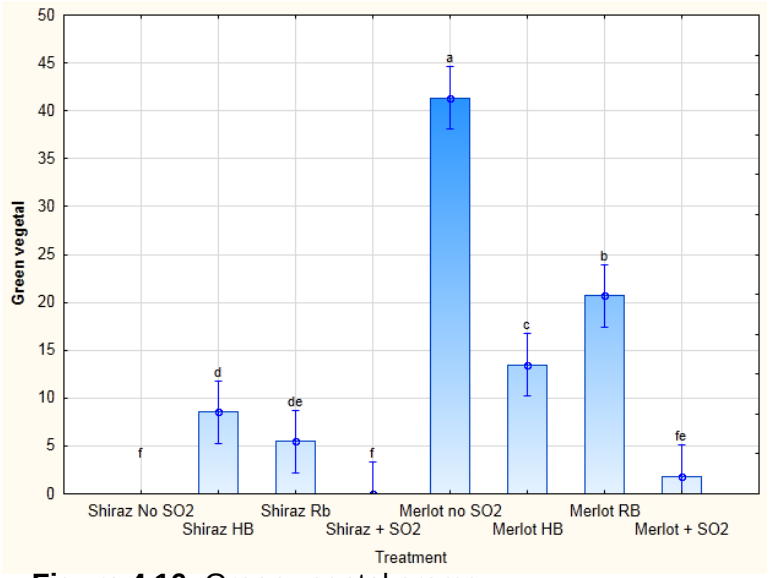

Figure 4.16: Green vegetal aroma

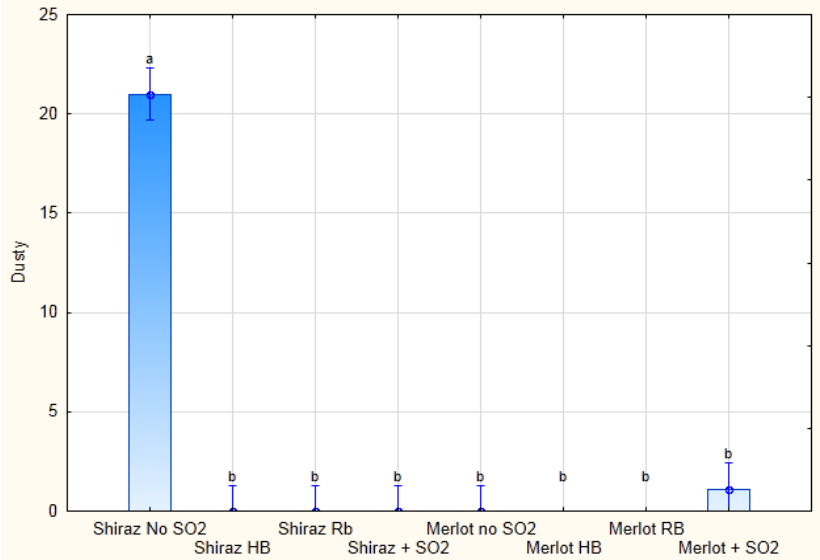

Figure 4.17: Dusty aroma

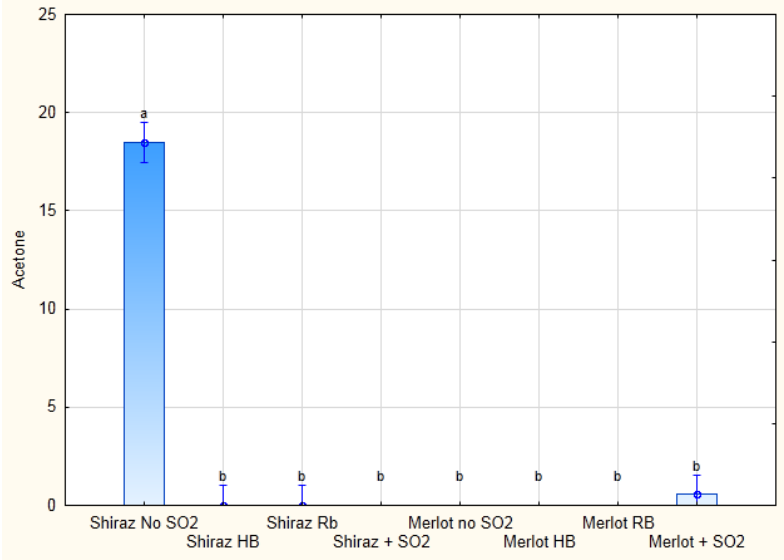

Figure 4.18: Acetone aroma

Figures 4.15 to 4.18: Experiment B - ANOVA results for asparagus aroma, green vegetal aroma, dusty aroma and acetone aroma.

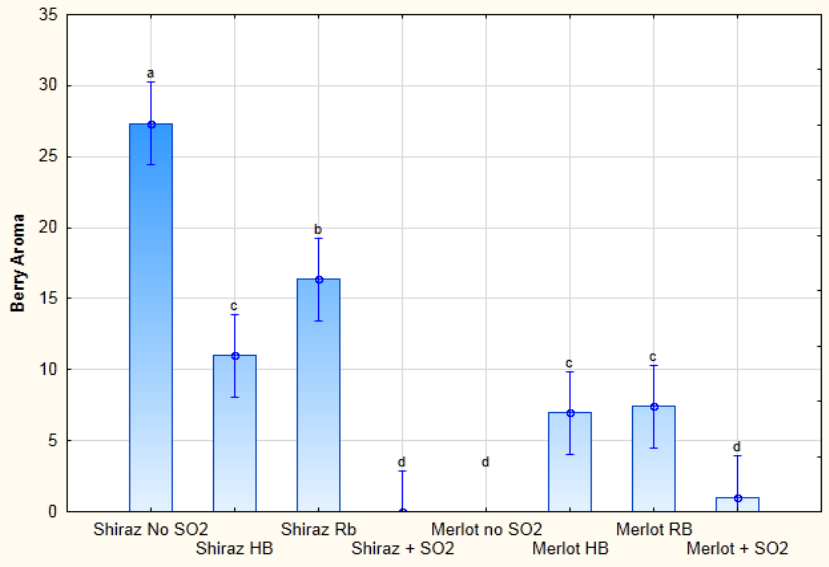

Figure 4.19: Berry aroma

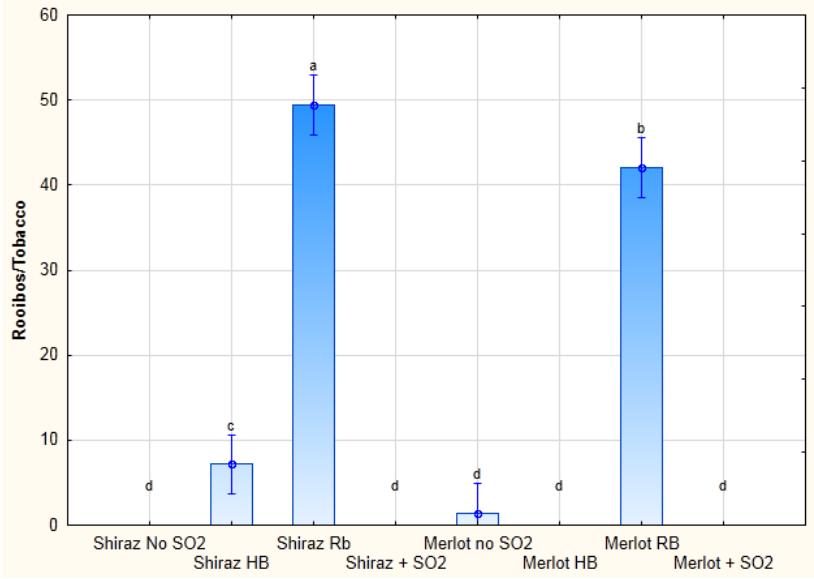

Figure 4.20: Rooibos/tobacco aroma

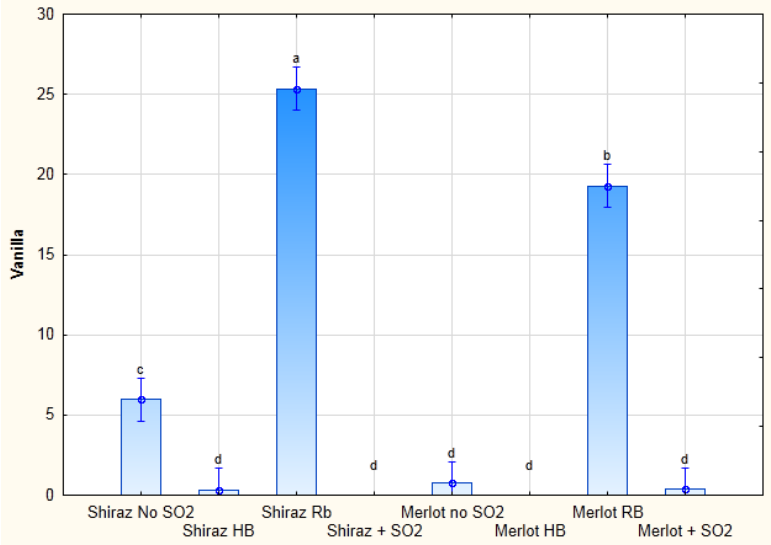

Figure 4.21: Vanilla

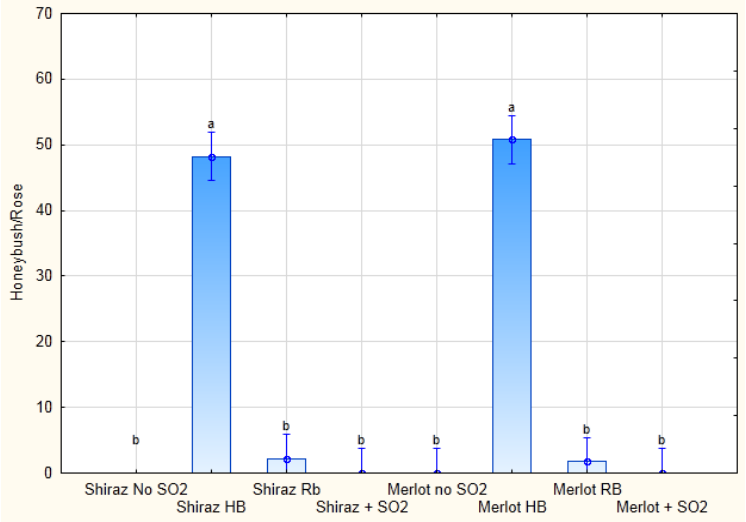

Figure 4.22: Honeybush/rose aroma

Figures 4.19 to 4.22: Experiment B - ANOVA results for berry aroma, rooibos/tobacco aroma, vanilla aroma, honeybush/rose aroma.

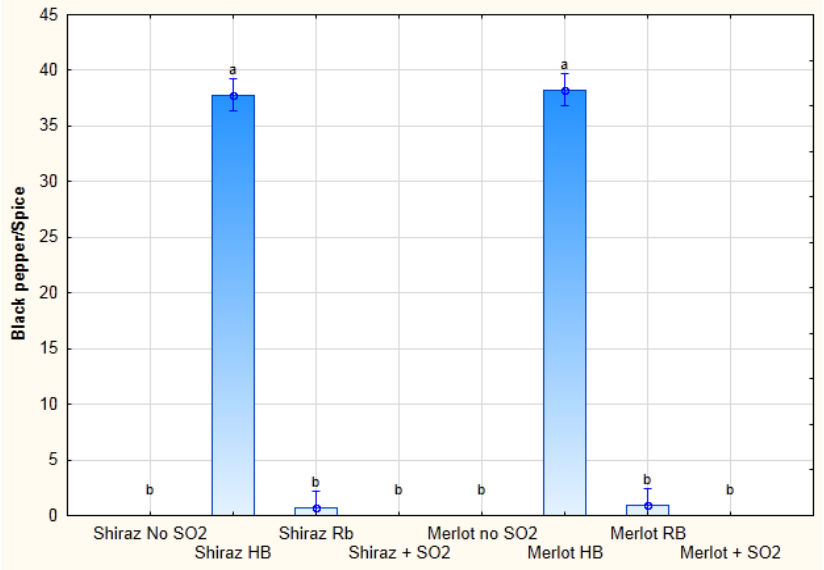

Figure 4.23: Black pepper/spice aroma

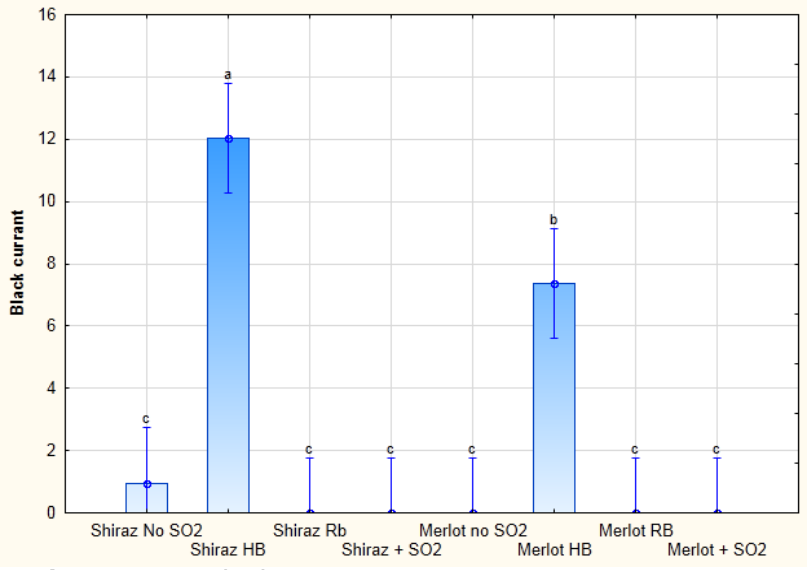

Figure 4.24: Blackcurrant aroma

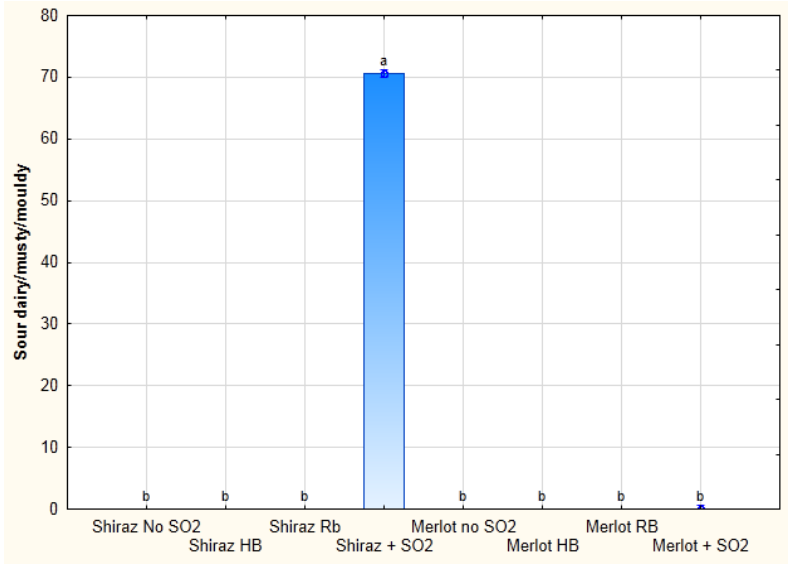

Figure 4.25: Sour dairy/musty/mouldy aroma

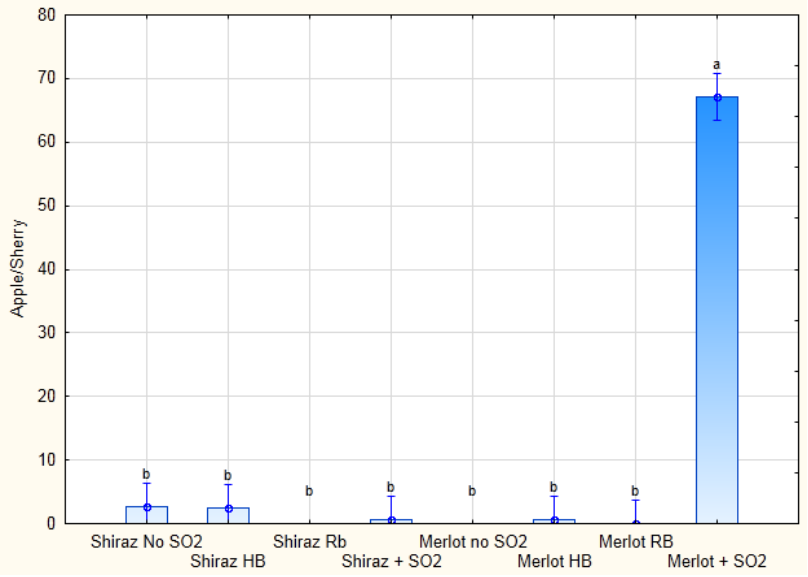

Figure 4.26: Apple/sherry aroma

Figures 4.23 to 4.26: Experiment B - ANOVA results for black pepper/spice aroma, blackcurrant aroma, sour dairy/musty/mouldy aroma, apple/sherry aroma.

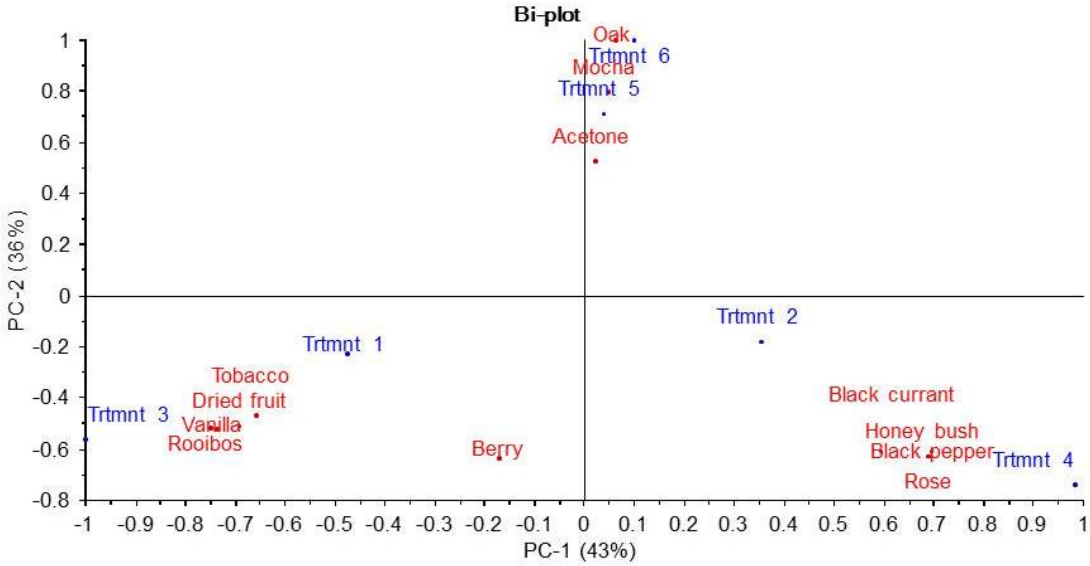

Figure 4.27: PCA bi-plot of the sensory characteristics investigated in experiment C. Treatments are indicated in blue. Treatment 1 = RB 5 g/L + No SO_2; Treatment 2 = HB 5 g/L + No SO_2; Treatment 3 = RB 5 g/L + SO_2; Treatment 4 = HB 5 g/L + SO_2; Treatment 5 = Control; Treatment 6 = SO_2 + Oak; RB = Rooibos; HB = Honeybush.

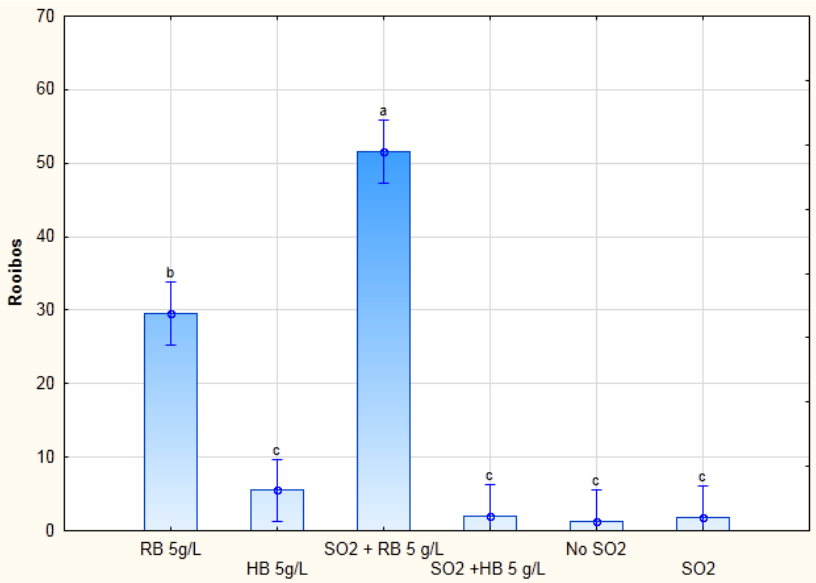

Figure 4.28: Rooibos

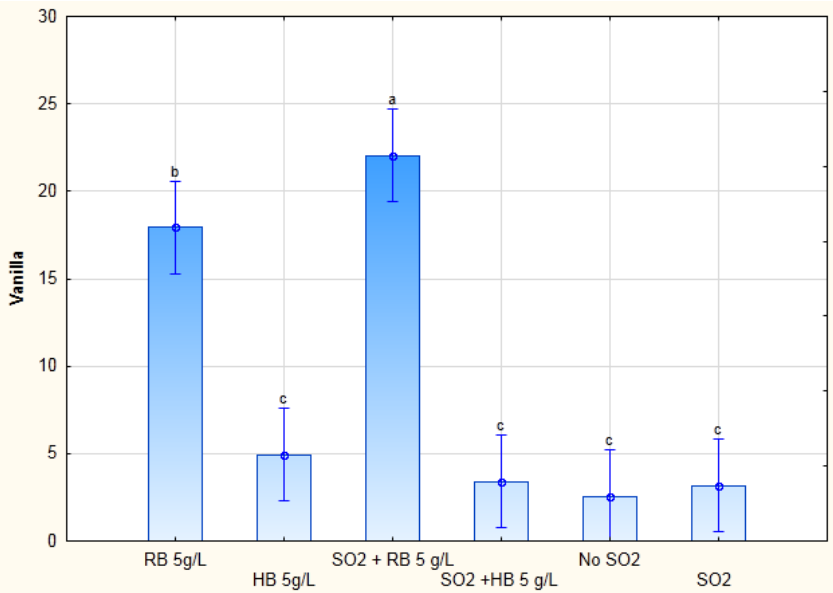

Figure 4.29: Vanilla aroma

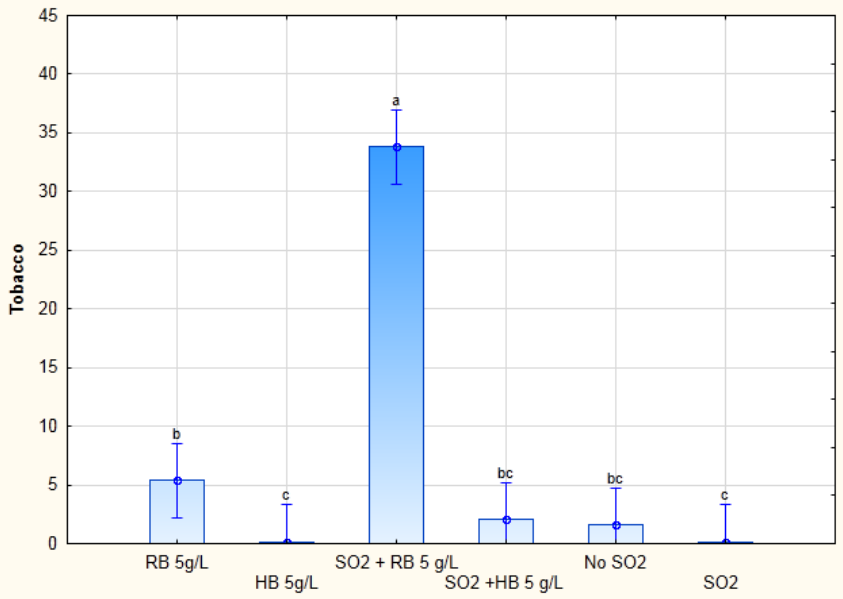

Figure 4.30: Tobacco aroma

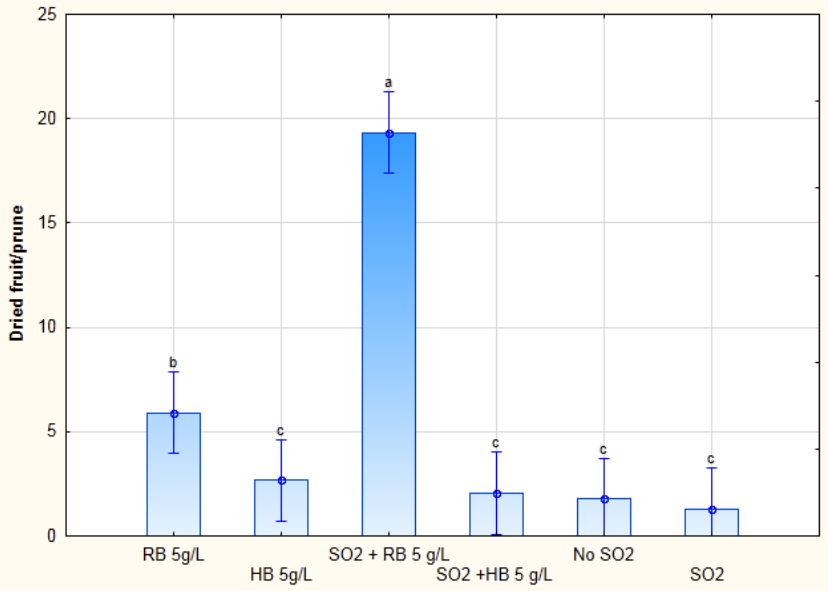

Figure 4.31: Dried fruit/prune aroma

Figures 4.28 to 4.31: Experiment C - ANOVA results for rooibos aroma, vanilla aroma, tobacco aroma and dried fruit/prune aroma.

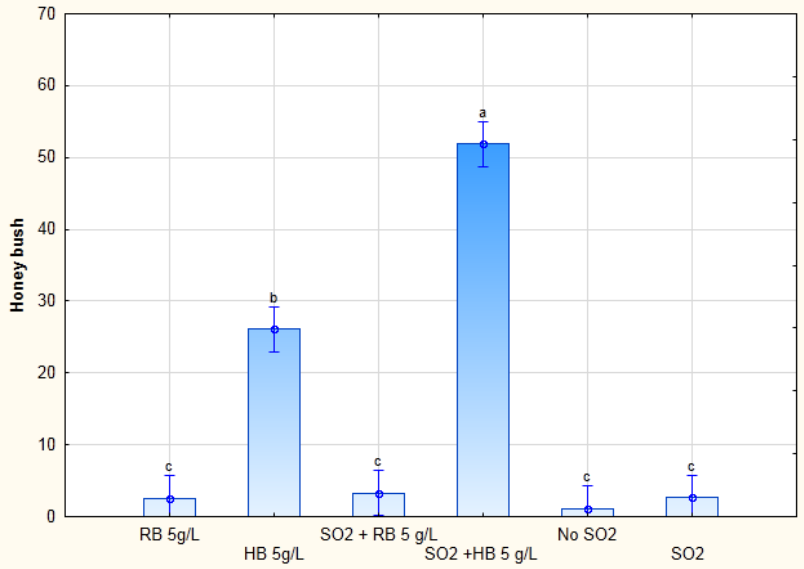

Figure 4.32: Honeybush aroma

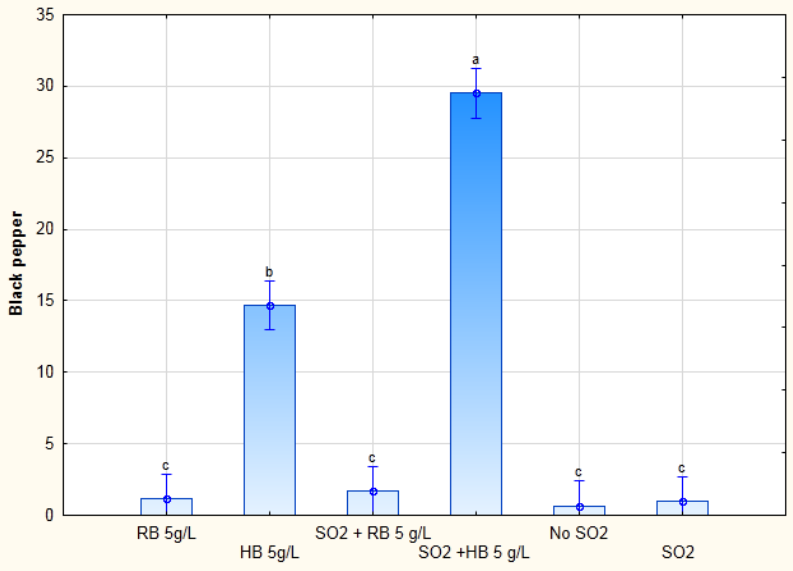

Figure 4.33: Black pepper aroma

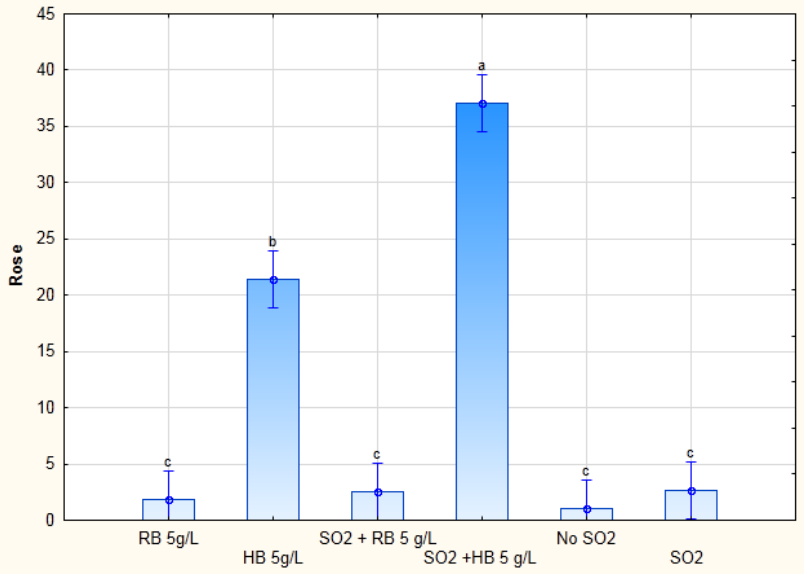

Figure 4.34: Rose aroma

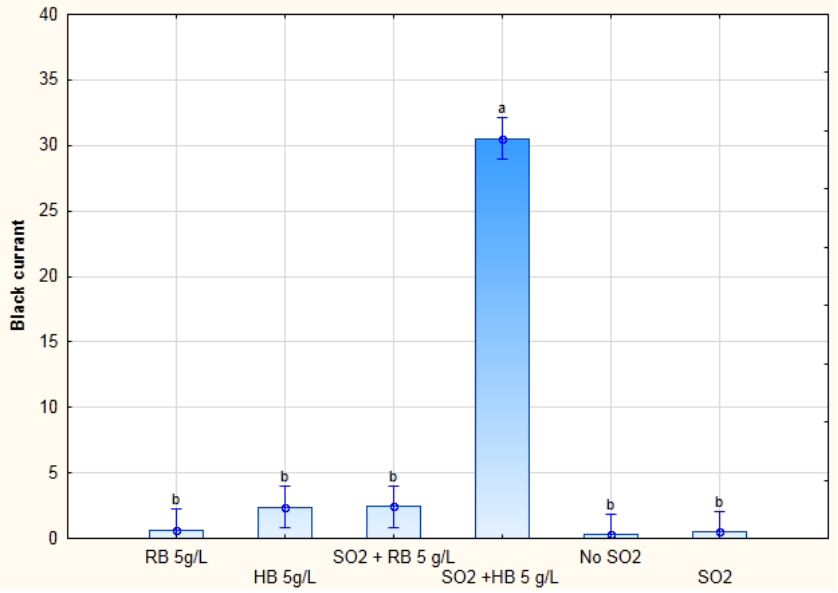

Figure 4.35: Blackcurrant aroma

Figure 4.32 to 4.35: Experiment C - ANOVA results for honeybush aroma, black pepper aroma, rose aroma and blackcurrant aroma.

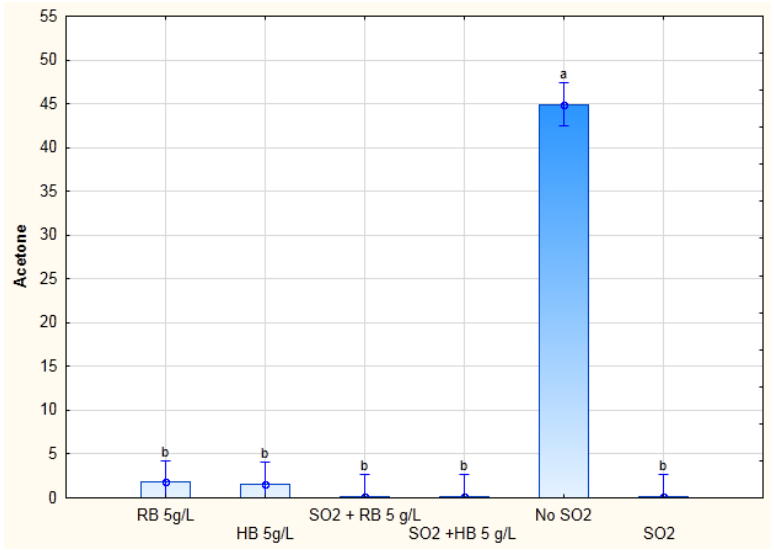

Figure 4.36: Acetone aroma

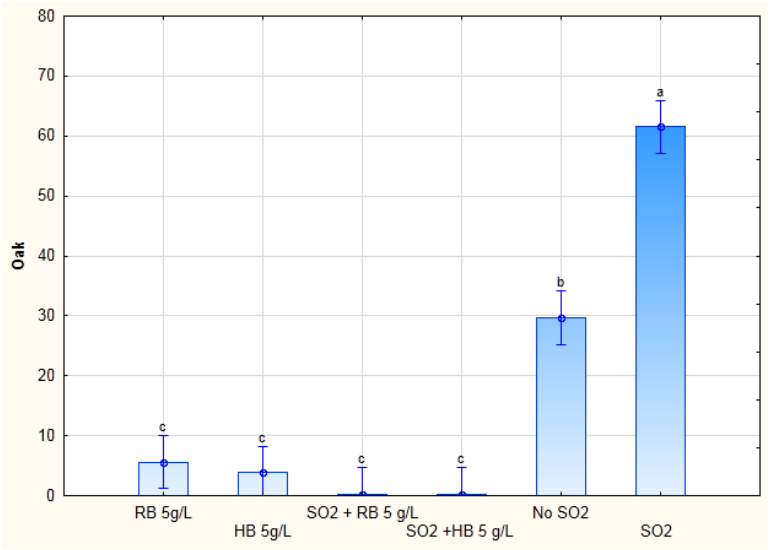

Figure 4.37: Oak aroma

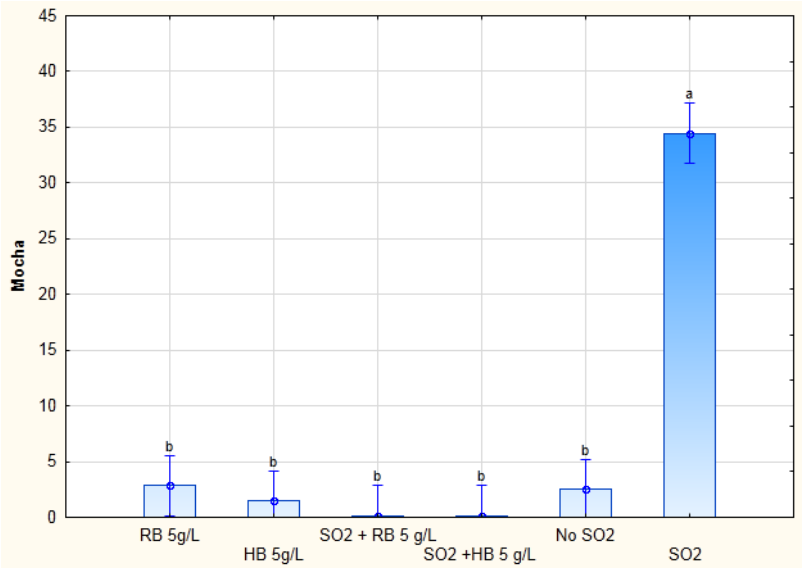

Figure 4.38: Mocha aroma

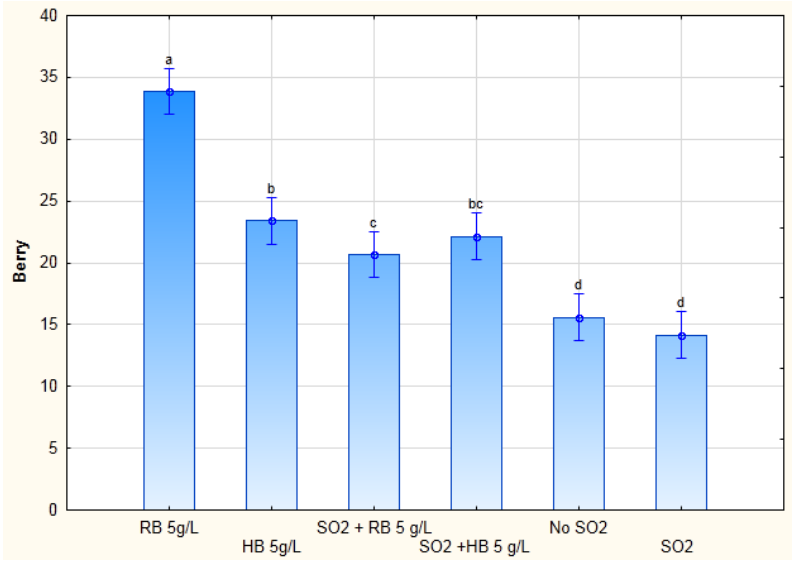

Figure 4.39: Berry aroma

Figure 4.36 to 4.39: Experiment C - ANOVA results for acetone aroma, oak aroma, mocha aroma and berry aroma.

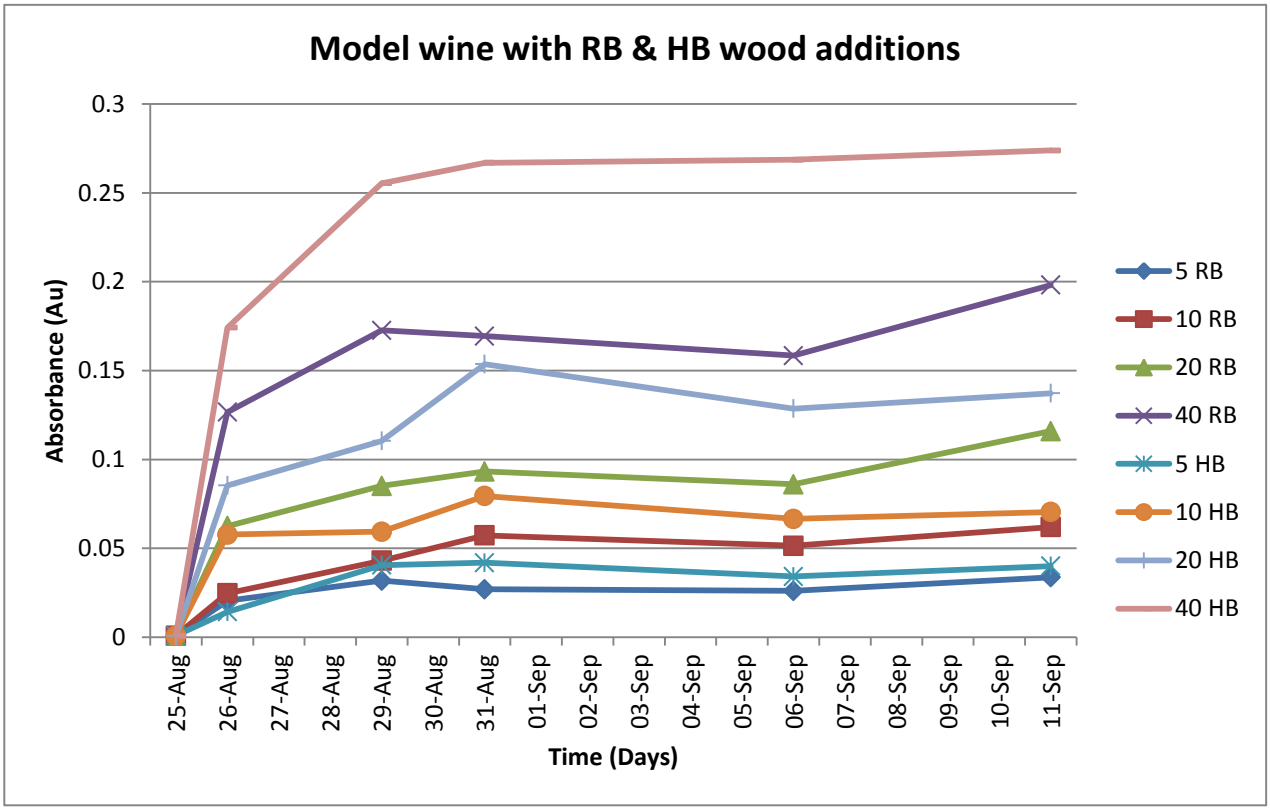

Figure 4.40: Rate of extraction determined over time for increasing rooibos and honeybush wood treatments in model wine; RB = rooibos and HB = honeybush.

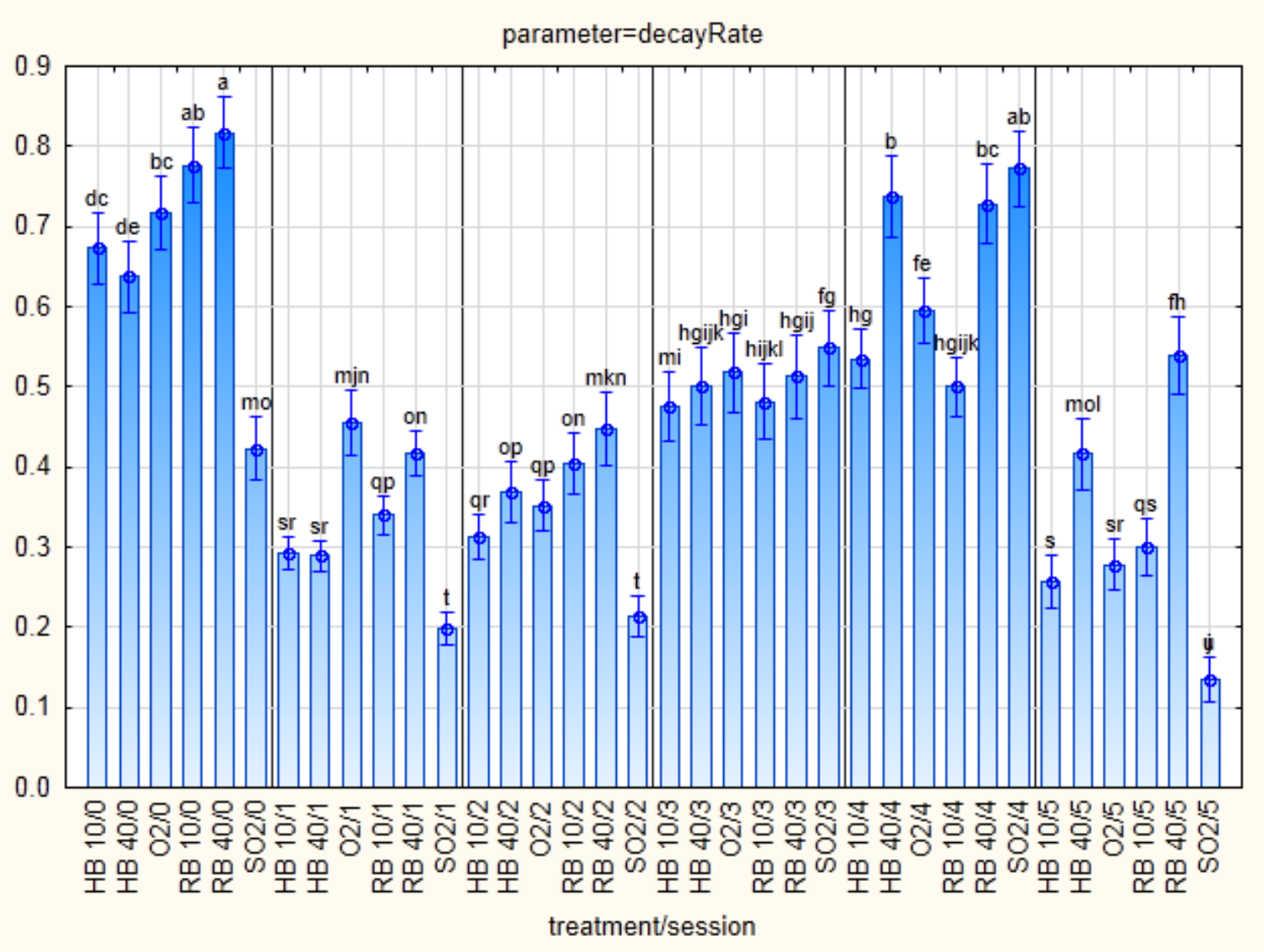

Figure 4.41: Oxygen consumption over six stages (stages 0 to 5), for six treatments (HB 10/3 for instance: indicates that honeybush wood was added at 10 g/L in the wine and the measurement was taken at stage 3).

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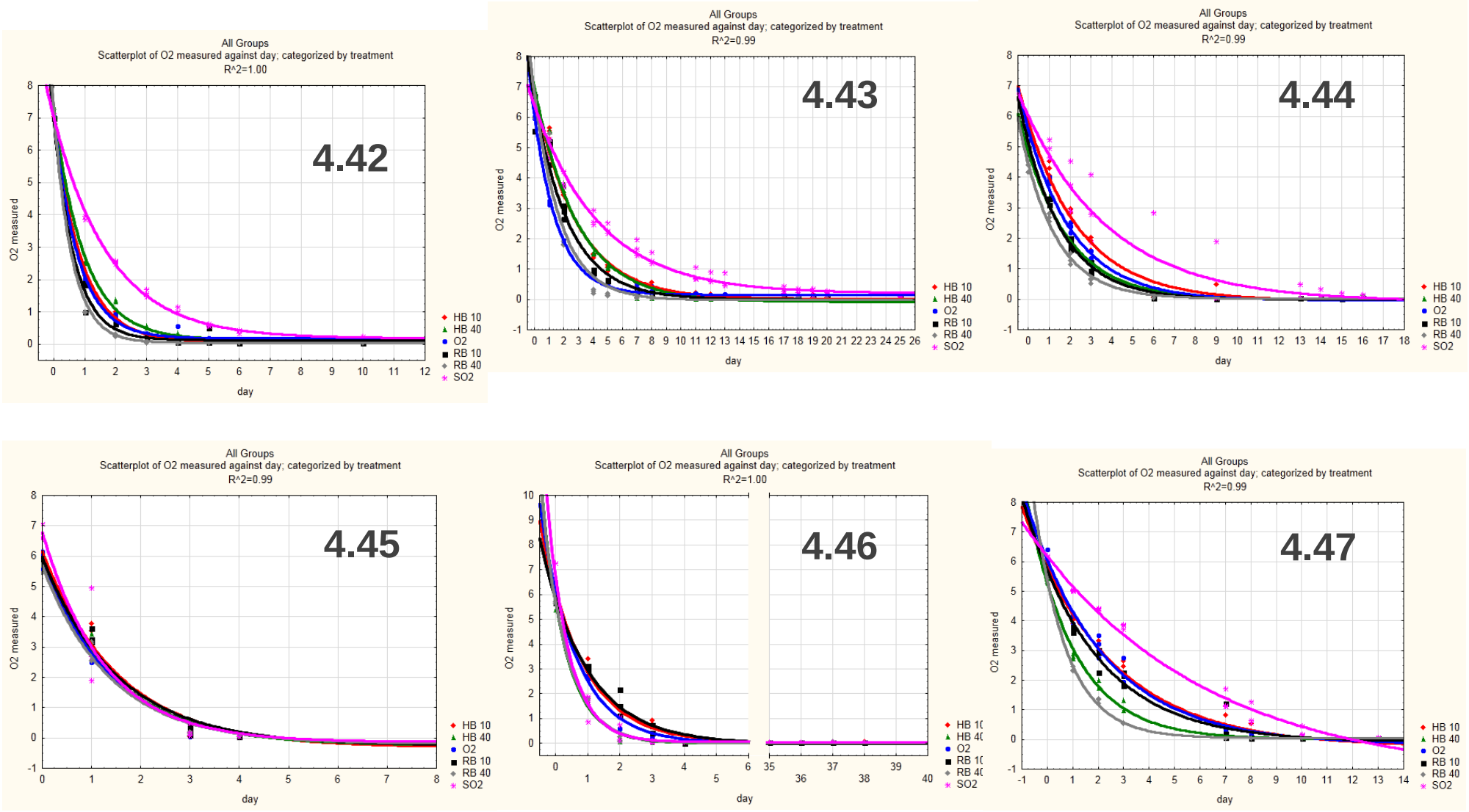


Figures 4.42 to 4.47: Oxygen decay rate of six treatments over six stages (stages 0 to 5)

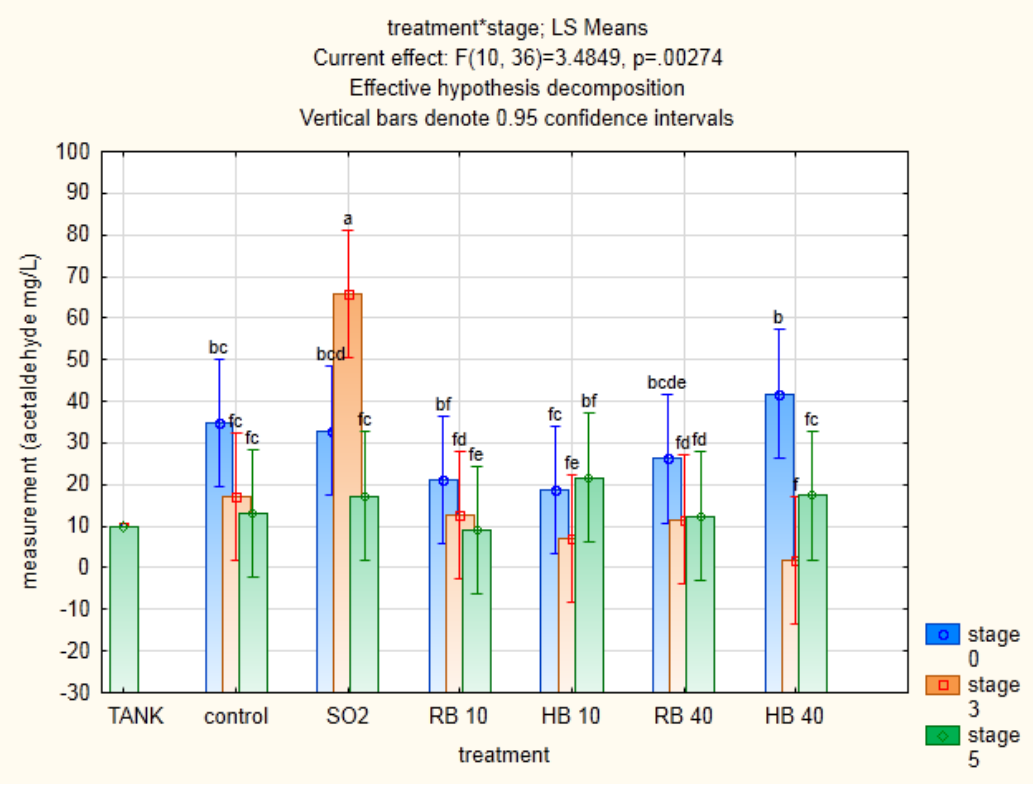


Figure 4.48: ANOVA results for acetaldehyde measured in treated wines at three oxidative stages.

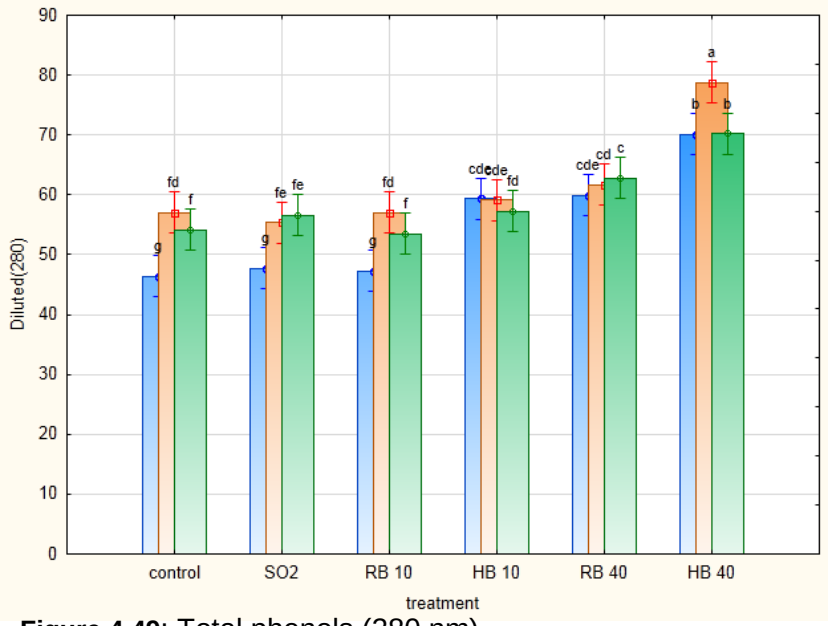


Figure 4.49: Total phenols (280 nm)

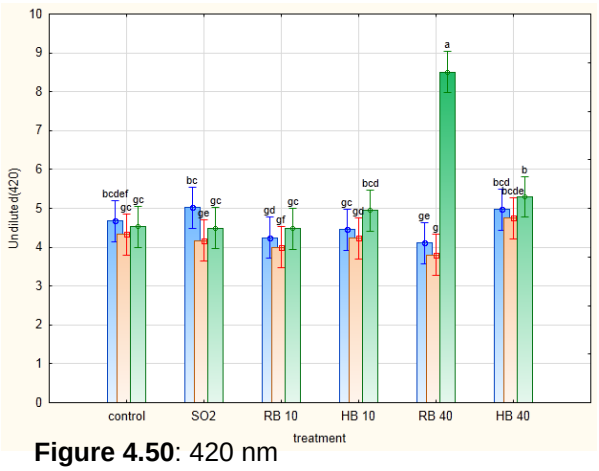
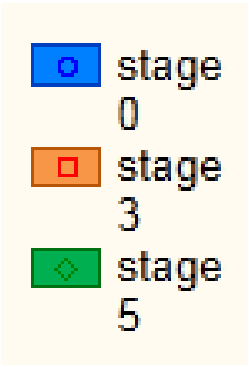


Figure 4.50: 420 nm



Key for chemical ANOVA graphs

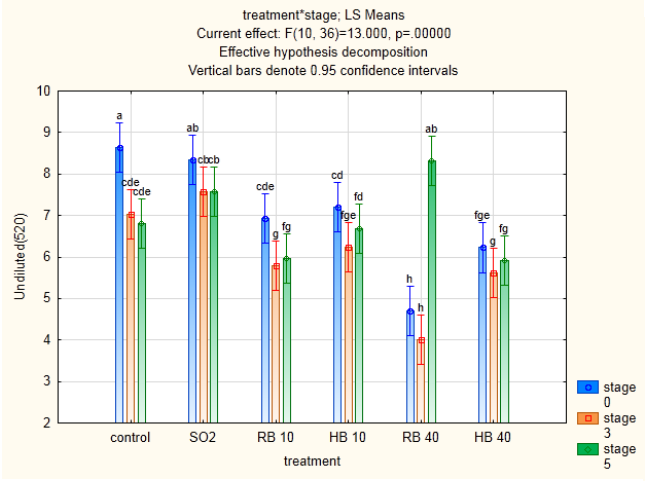


Figure 4.51: 520 nm

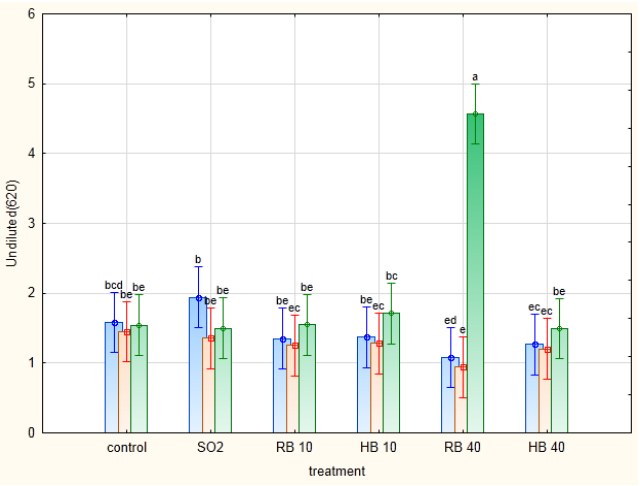


Figure 4.52: 620 nm

Figures 4.49 to 4.52: ANOVA results portraying significant differences between treatments based on spectral measurements 280 nm, 420 nm, 520 nm and 620 nm, at three oxidative stages.

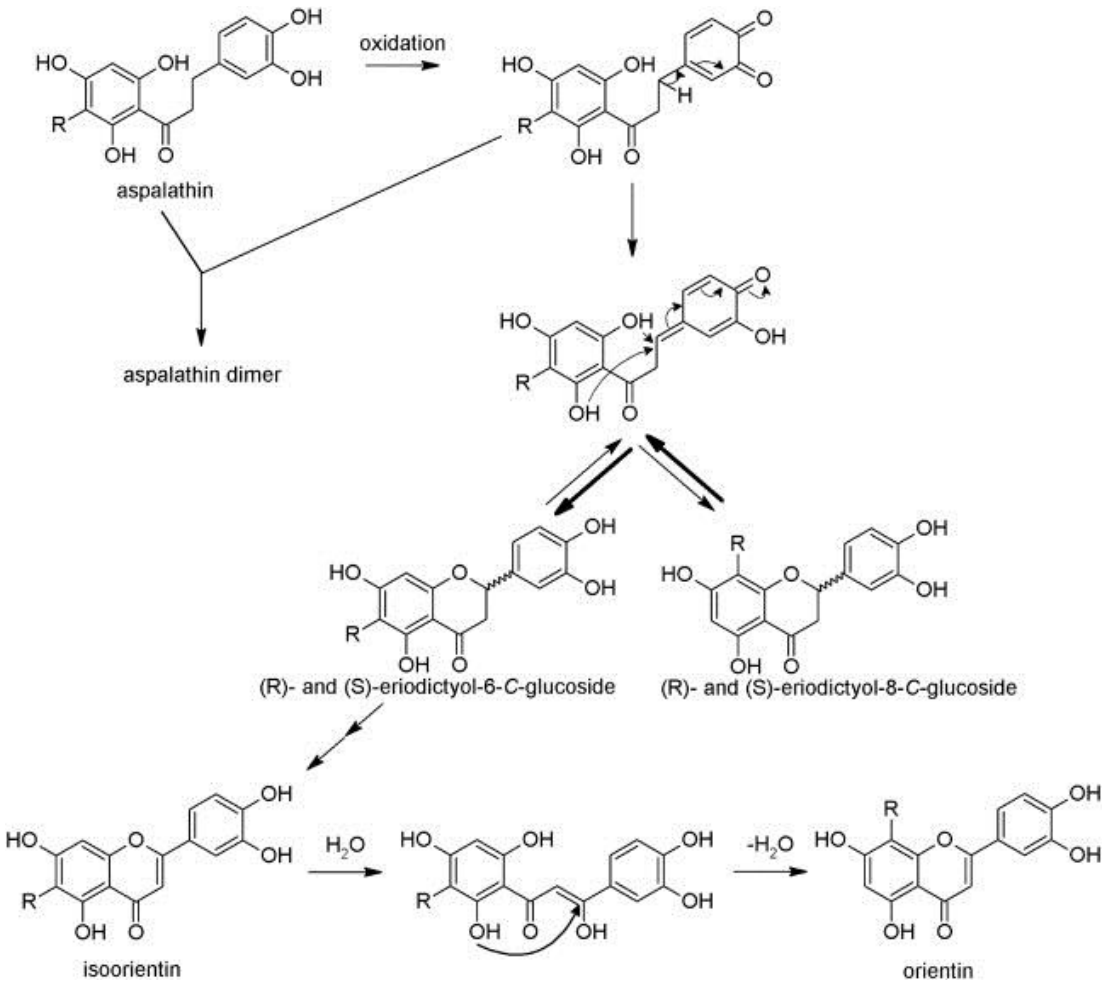


Figure 4.53: Oxidation of aspalathin in rooibos (Joubert & De Beer, 2011).

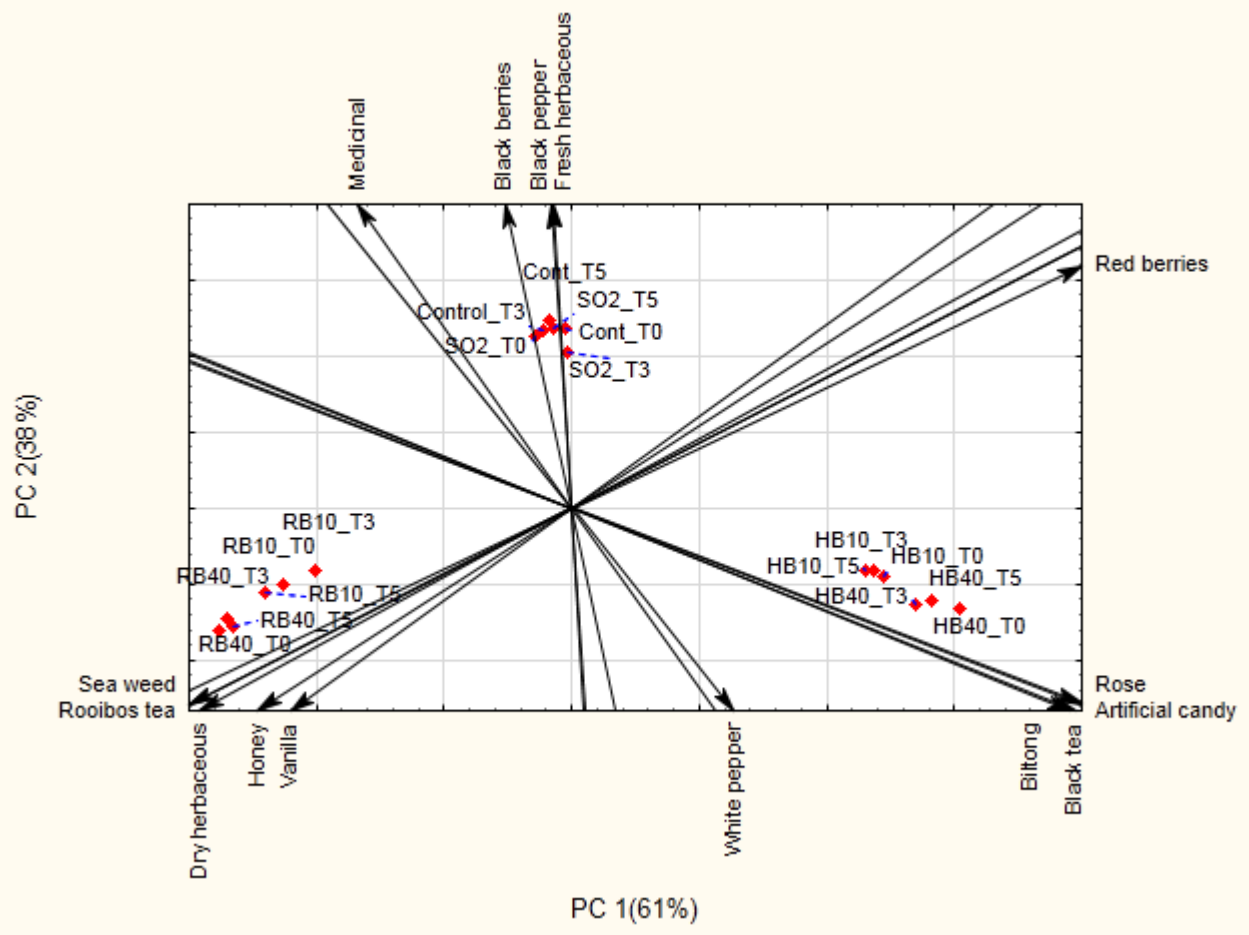


Figure 4.54: PCA bi-plot of aroma descriptors related to 18 wines at three different stages (T0, T3 and T5).

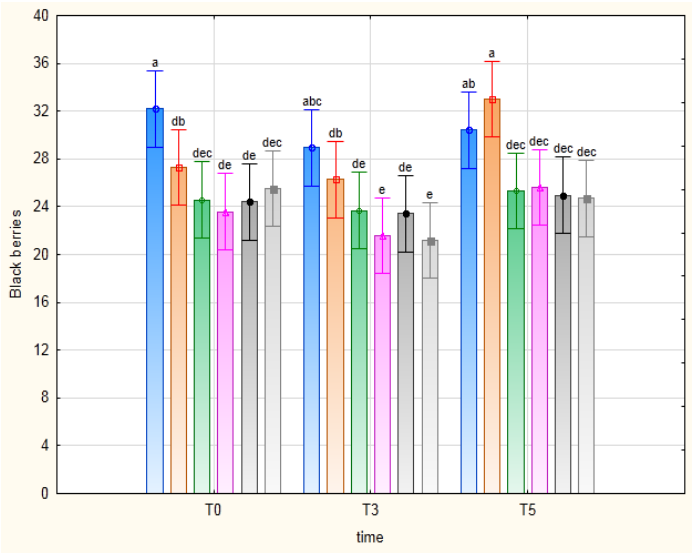


Figure 4.55: Black berry aroma

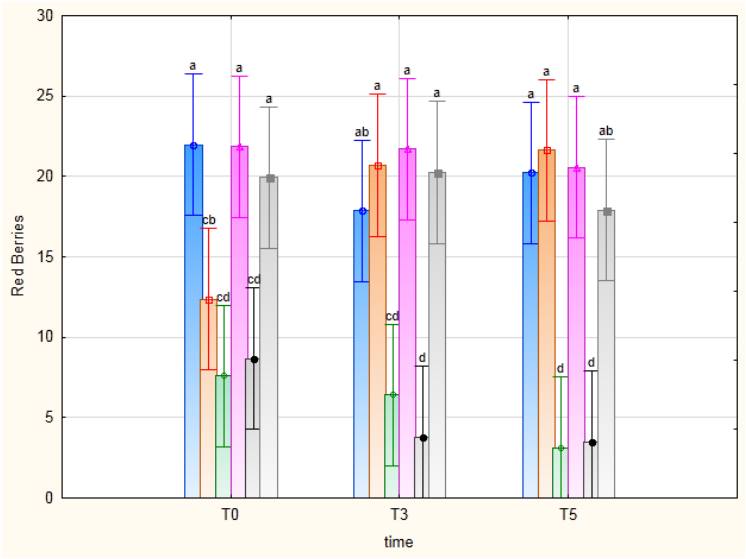


Figure 4.56: Red berry aroma

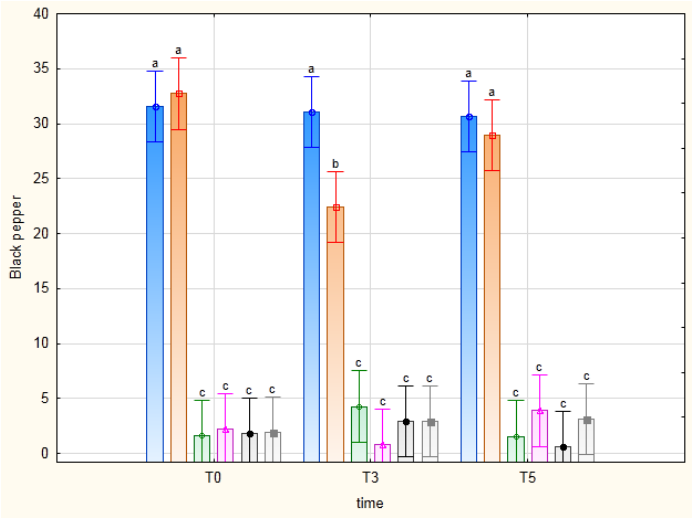


Figure 4.57: Black pepper aroma

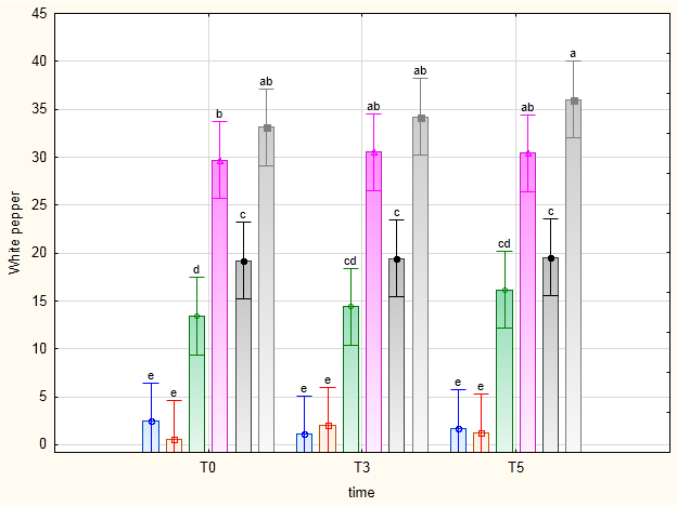
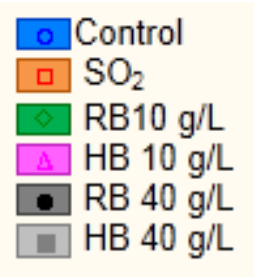


Figure 4.58: White pepper aroma



Figures 4.55 to 4.58: ANOVA results for black berry aroma, red berry aroma, black pepper aroma and white pepper aroma, measured at three oxidative stages (T0, T3 and T5).

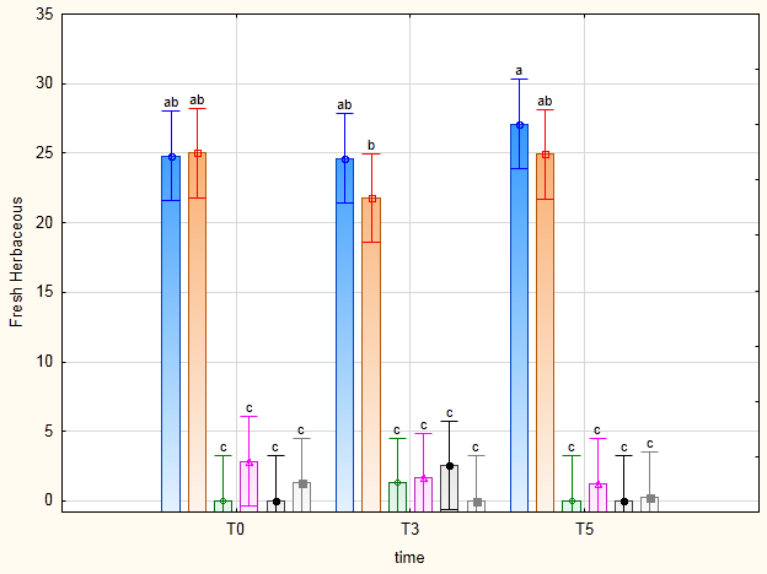


Figure 4.59: Fresh herbaceous aroma

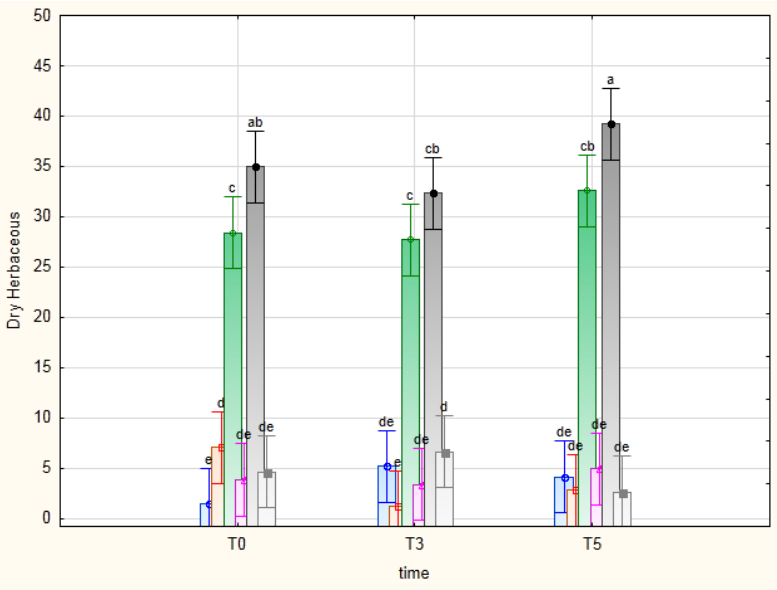
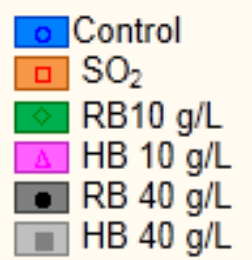


Figure 4.60: Dry herbaceous aroma



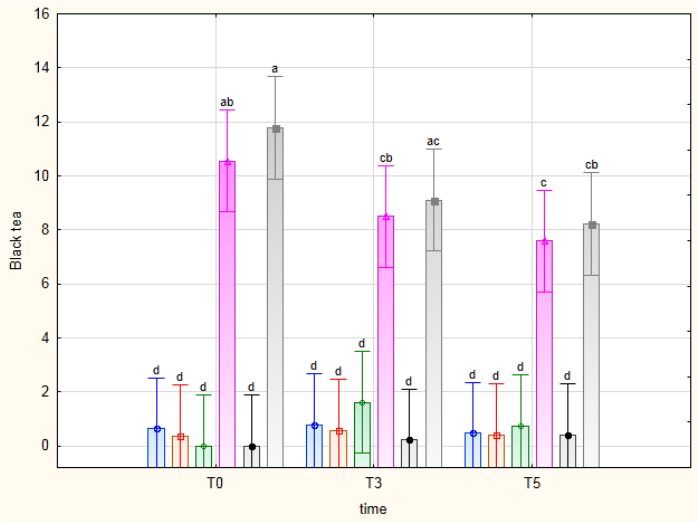


Figure 4.61: Black tea aroma

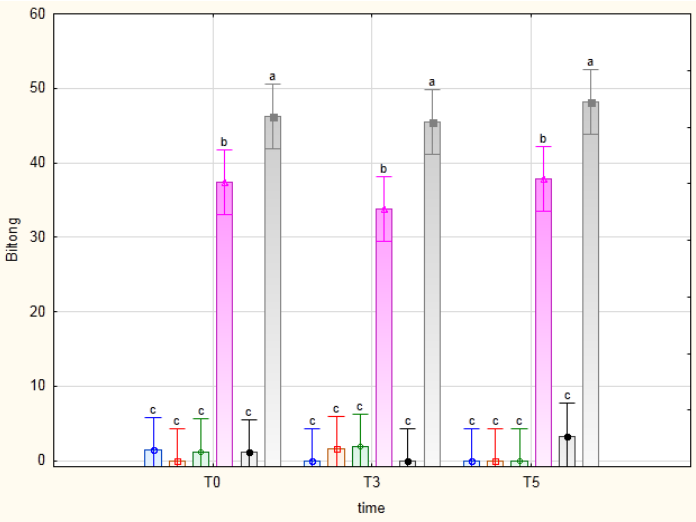


Figure 4.62: Biltong aroma

Figures 4.59 to 4.62: ANOVA results for fresh herbaceous aroma, dry herbaceous aroma, black tea aroma and biltong aroma, measured at three oxidative stages (T0, T3 and T5).

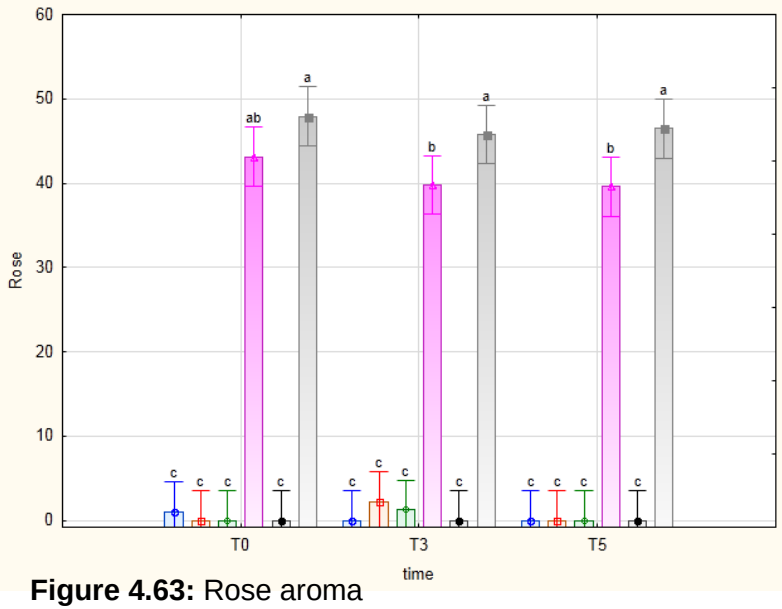


Figure 4.63: Rose aroma

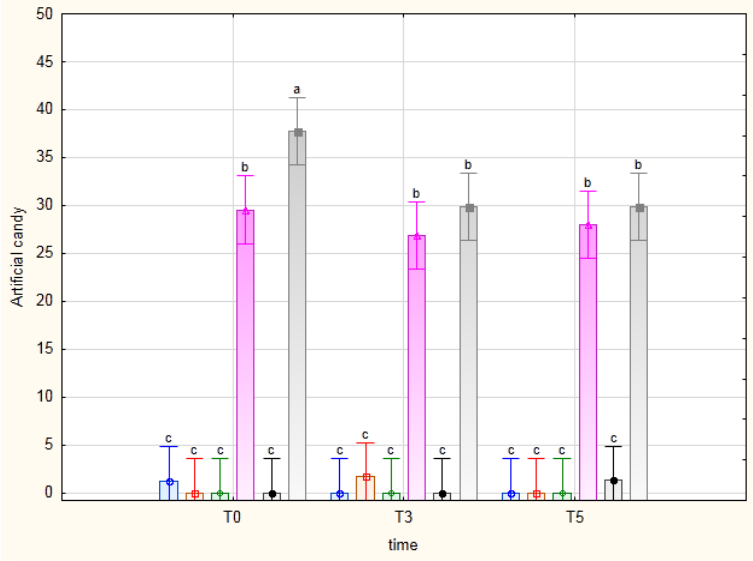
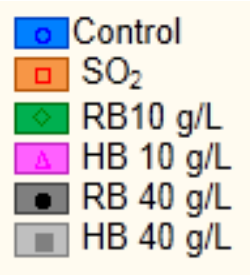


Figure 4.64: Artificial candy aroma



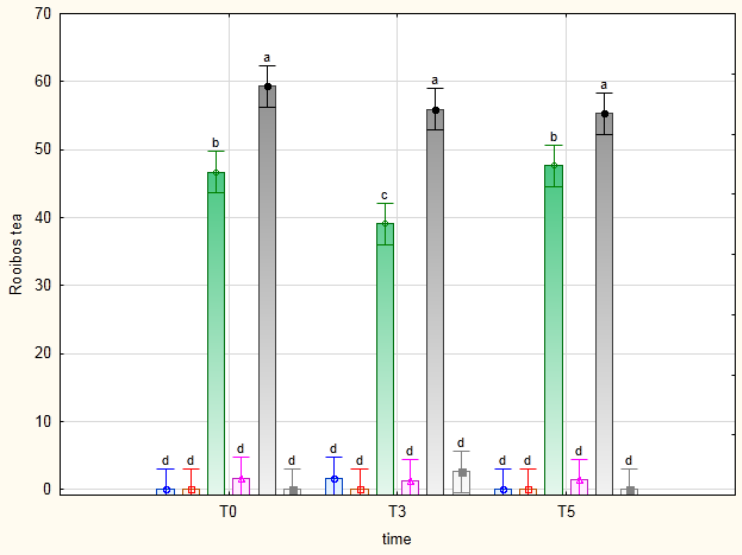


Figure 4.65: Rooibos tea aroma

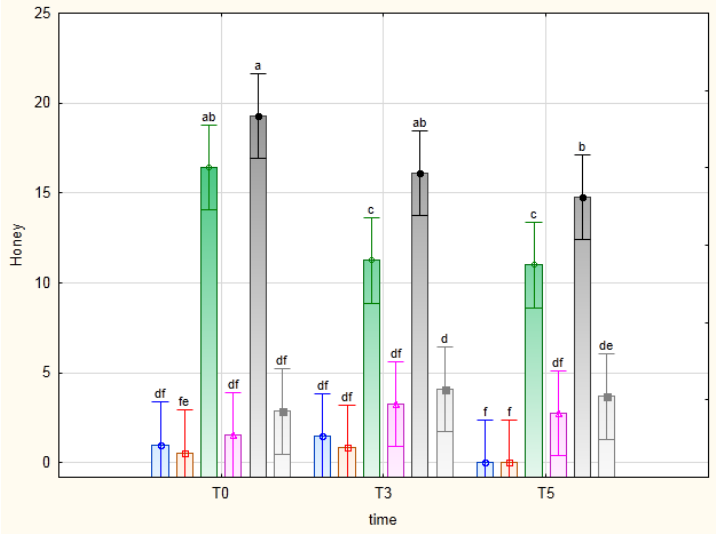


Figure 4.66: Honey aroma

Figures 4.63 to 4.66: ANOVA results for rose aroma, artificial candy aroma, rooibos tea aroma and honey aroma, measured at three oxidative stages (T0, T3 and T5).

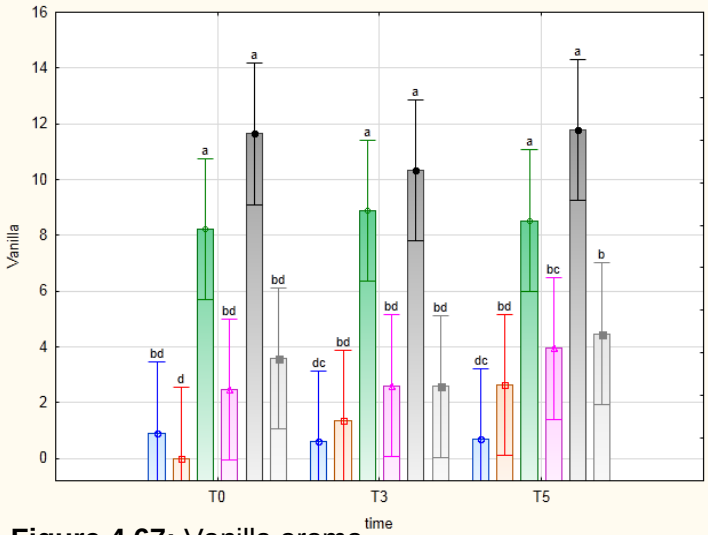


Figure 4.67: Vanilla aroma

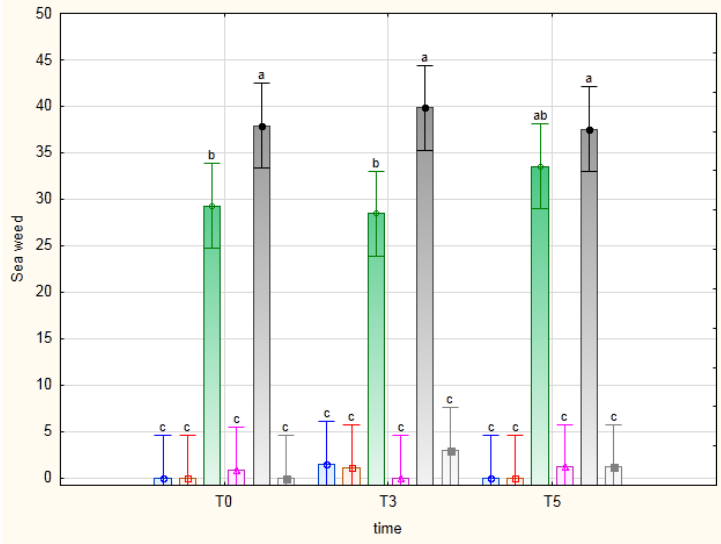


Figure 4.68: Seaweed aroma

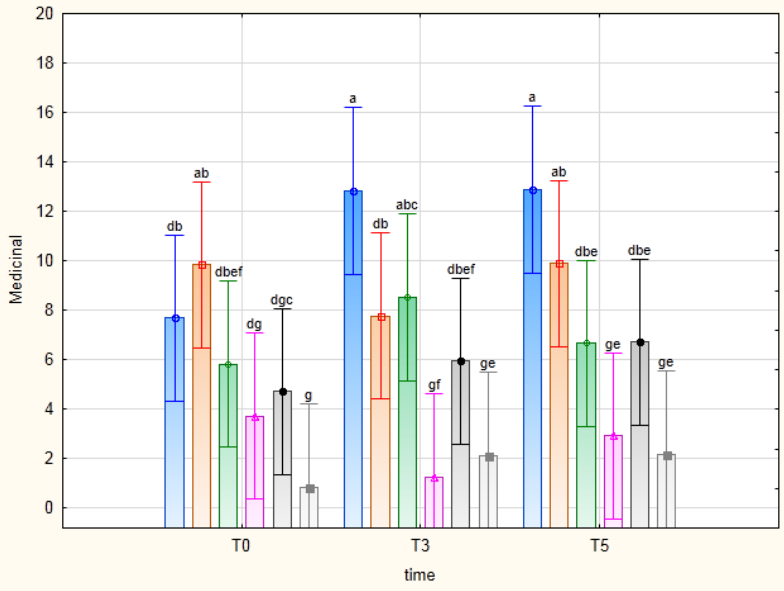
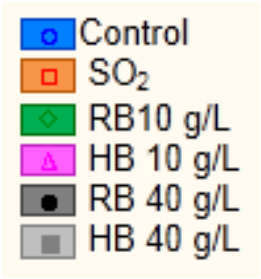


Figure 4.69: Medicinal aroma



Figures 4.67 to 4.69: ANOVA results for vanilla aroma, seaweed aroma and medicinal aroma, measured at three oxidative stages (T0, T3 and T5).

Addendum G

Standards for descriptive sensory analysis and questionnaire presented to the DSA panel

Table 4.7: List of sensory standards used for DSA in *phase 2* of this study.

	Aroma	Standard
1	Black fruit	Mixed black berries
2	Red fruit	Mixed red berries
3	Yellow fruit	Chopped yellow stone fruit
4	Prunes	Dried prunes chopped
5	Rose	Rose water
6	Apple skin	Apple skin left to stand overnight
7	Sherry	Monis Sherry
8	Medicinal	Oral comment was provided to the panellist and agreed on by all
9	Sulphur	2.5% liquid sulphur
10	Fresh herbaceous	Cut green grass
11	Dry herbaceous	Hay
12	Vanilla	Vanilla essence
13	Honey	Fresh honey, heated up
14	Black pepper	Ground black peppercorns
15	White pepper	Ground white pepper
16	Black tea	*Camellia sinensis,* leaves from teabag
17	Biltong	Cut dried biltong
18	Seaweed	Sushi dry seaweed wrap, cut into pieces
19	Artificial candy	Mixed candy
20	Cloves/nutmeg	Mixed spices of cloves and nutmeg
21	Hydrogen sulphide	Oral comment was provided to the panellist and agreed on by all

Judge nr……..

1. Smell all of the standards no. 1-12. Try to identify the odour. Write your answer in column A
2. Mark the box in column C to tell us how good the standard is.

Aroma

	A	B	C			
No of aroma standard	What do you think is the aroma?	What is the aroma according to the instructor?	How close is the aroma of the reference standard (that you perceived) to the aroma of the real product (according to your memory)?			
			Quite close	Good enough	Slightly	Not at all
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						

Chapter 5

General conclusions and future prospects

Chapter 5

General conclusions and future prospects

5.1 GENERAL CONCLUSIONS

The use of alternative wood products in wine production may have relevant place in future winemaking operations. Creating an innovative wine product in the current competitive and crowded wine market is a means by which to attract the attention of new consumers (Alañón *et al*., 2013). The use of alternative wood sources could supplement the range of wines that a winery can offer within the expanding consumer market.

Not only is the innovation of wine products in the competitive wine market an important factor, but sustainable choices in the daily operations of a winery have become a requirement for particular wine certifications in South Africa (Integrated Production of Wine - IPW) (Forbes *et al*., 2009). The use of indigenous wood sources, in particular the use of indigenous wood material, could aid in increasing the sustainable practices of wineries. By using rooibos and honeybush wood, some of which is often wasted in the production of these herbal teas (Joubert *et al.*, 2008), the wood is utilised instead of being discarded.

Interest in the use of natural antioxidants that are present in products has increased in a number of food industries (Joubert & Ferreira, 1996). Consumers are becoming more aware of the fact that various consumable products can either be beneficial or detrimental to their health and, as a result, industry is pushing for the addition of natural preservatives that have inherent health-promoting properties (Hoffman *et al*., 2014). Decreasing the addition of synthetic preservatives such as SO_2 to wine and supplementing it by using products such as rooibos and honeybush could potentially increase the health properties of wine.

The aims of this study thus entailed a number of preliminary investigations into the addition of rooibos and honeybush material to wine. The aroma impact of these alternative wood sources was of interest, as the research reported here was used by Audacia wine cellar in the Cape Winelands to produce a commercial wine product for the consumer market. The results of expert, trained and consumer tastings were all considered in the production of the commercial product, ***Rooibos Wine*****.** This wine is currently available at a big retail chain, while the addition of rooibos and honeybush material to wine has been patented internationally (Anon, 2014).

The results of our study indicate that the consumers did not reject the treated wines outright or described it as undrinkable, however, it does seem as though the new aromas added to the wine might be foreign to the consumer palate. The results clearly indicated that the foreign flavour influenced the liking scores to a certain extent. This could possibly be changed when consumers

have more knowledge of the benefits that these products add to the winemaking process. The experience through which a consumer goes while tasting a wine, as shaped by information about the wine product, can influence the final liking score given to the product by the consumer (Siegrist & Cousin, 2009).

Furthermore, the use of the wood alternatives in the wine imparted unique aroma compounds that seem to impart relative stability to oxidation. Rooibos and honeybush wood could be added to wine at lower concentrations until consumers have more knowledge about the product and the aromas that it imparts to wine. The potential of rooibos and honeybush additions as anti-oxidants in wine during processing was displayed in the oxygen consumption experiments. More research should also be conducted in the management of SO_2 additions together with rooibos and honeybush wood material. In this way both products could simultaneously curb the effects of oxidation. In light of the above, it might be that lower SO_2 wines could be a reality in future.

5.2 FUTURE RECOMMENDATIONS

The general education of the consumer market on the flavour and use of rooibos and honeybush wood in wine may lead to an increase in sales. The consumers assessed in this study did not have any knowledge about the addition of rooibos and honeybush to the wines presented to them. Future studies can include a perception-based consumer study in which a large number of consumers rate the rooibos- and honeybush-treated wines after being briefed about how the wine was made and about the potential health benefits of these wines. These liking results can then be compared to those of consumers that were ignorant of the use of these alternative wood products in the wine prior to tasting.

Continued refinement of the rooibos and honeybush fractions that could be added as wine additives can be implemented. The toasting of these wood fractions can also be considered. By toasting the wood, various aroma profiles may be produced that could have a positive effect when added to wine.

The use of these products for producing a low SO_2 wine also needs attention. Some supplementary chemical analyses that could be included in future studies include methional and phenylacetaldehyde. Both these chemical compounds can indicate the oxidation of wine and contribute to ageing aromas in the wine (Du Toit *et al.,* 2006). By quantifying the increase or decrease of these compounds in the wine, wine aroma stability and ageing potential after treatment with rooibos and honeybush wood sources could be determined.

Further research can also be conducted in order to understand why the rooibos- and honeybush-treated wines in this study displayed stable aroma attributes throughout the oxidation treatments. The question of aroma stability under oxidative conditions can be of benefit to winemakers, as can the masking of green or oxidative aromas by these alternative wood sources.

Research concerning the amount of antioxidants that can be extracted from the alternative wood (rooibos and honeybush) into wine should be continued. Particular tests, like FRAP (ferric-reducing antioxidant potential) and ORAC (oxygen radical absorbance capacity) (Joubert *et al.*, 2012), can be applied to wine products that have undergone treatment with the alternative wood to ascertain the amount of available antioxidants in the wine. The use of rooibos and honeybush, especially the wood fraction, in future winemaking practices thus seems to have great potential.

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