DEVELOPMENT OF A SENSORY LEXICON AND SENSORY WHEEL FOR ROOIBOS (*ASPALATHUS LINEARIS*) AND THE ROLE OF ITS PHENOLIC COMPOSITION ON TASTE AND MOUTHFEEL

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Declaration

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Date: 23 February 2011

Abstract

The sensory characteristics and chemical composition of rooibos infusions were analysed to establish the extent of variation in sensory attributes and compositional parameters, and investigate whether correlations exist between the sensory characteristics and the phenolic composition. To capture as much potential variation as possible, 69 rooibos samples were collected throughout the 2009 harvesting season from different production areas. The samples were also representative of the different quality grades (A, B, C or D) which are based on the appearance of the leaves, and the colour and flavour of the infusion.

Quantitative descriptive analysis was used to develop sensory profiles for each of the rooibos infusions. Considerable variation in the sensory characteristics was observed, highlighting the need for standardised terminology that can be used to describe what is often referred to as “characteristic” rooibos flavour. Aroma, flavour, taste, and mouthfeel descriptors were generated and assembled into a rooibos sensory wheel. The most recurring attributes were selected to compile a rooibos sensory lexicon which provides a definition and a reference standard for clarification of each term. A combination of “honey”, “woody” and “fynbos-floral” notes with a slightly sweet taste and subtle astringency may be regarded as “characteristic” rooibos attributes indicative of good quality tea. Pleasant “caramel” and “fruity-sweet” notes were also observed in a number of infusions, whereas negative descriptors, associated with poor quality rooibos, included “green” and “hay” flavours.

Large variation in the composition of rooibos infusions was revealed through the quantification of soluble solids (SS), total polyphenols (TP), tannins and 14 monomeric phenolic compounds, as well as spectrophotometric colour measurements. High quality rooibos was associated with higher levels of SS, TP, tannins and phenolic compounds than low quality rooibos. Correlations between the compositional parameters and sensory attributes of the infusions indicated that several non-volatile compounds, including enolphenylpyruvic acid-2-glucoside, quercetin-3-glucoside, and iso-orientin, were associated with the characteristic sweet taste of rooibos, while bitterness was related to certain flavonoids such as luteolin, quercetin and aspalathin. The only compound significantly correlated to astringency was rutin, although it is likely that aspalathin and several other monomeric flavonoids also contribute to astringency. The tannin content was not associated with astringency possibly because of the limitations associated with the tannin quantification method.

To determine whether compositional changes resulting from steam pasteurisation of rooibos leaves influence the sensory quality of rooibos infusions, differences in the phenolic composition and sensory attributes of infusions, prepared from unpasteurised and pasteurised rooibos, were analysed. Steam pasteurisation significantly reduced the SS, TP and aspalathin content of rooibos infusions, as well as the absorbance, especially at a wavelength of 450 nm. It also resulted in significant reductions in the intensities of most of the aroma and flavour attributes, especially the “green” notes associated with low quality

rooibos. After steam pasteurisation the prominent “green” flavour of certain samples was frequently replaced by a “hay” flavour. The taste attributes, sweetness and bitterness, remained unchanged, whereas the astringency of rooibos infusions decreased significantly.

Uitreksel

Die sensoriese eienskappe en chemiese samestelling van rooibostee is geanaliseer ten einde die mate van variasie van die sensoriese sowel as die chemiese profiel te bepaal, asook om vas te stel of korrelasies bestaan tussen die sensoriese eienskappe en die fenoliese samestelling van rooibostee. Om soveel as moontlik potensiële variasie in te sluit, is 69 rooibos monsters tydens die 2009 oesseisoen van verskillende produksiegebiede versamel. Die monsters is ook verteenwoordigend van die verskillende kwaliteitgrade (A, B, C en D), wat op grond van die voorkoms van die teeblare, en die kleur en geur van die tee toegeken is.

Kwantitatiewe beskrywende analise is gebruik om ‘n sensoriese profiel vir elke rooibostee op te stel. Aansienlike variasie in die sensoriese eienskappe is waargeneem. Dit het die behoefte aan gestandaardiseerde terminologie onderskryf, wat gebruik kan word om die term “karakteristieke” rooibos geur in meer detail te omskryf. Spesifieke terme vir aroma, geur, smaak en mondgevoel is geformuleer, en gebruik om ‘n sensoriese wiel vir rooibostee saam te stel. Terme wat die meeste voorgekom het, is gekies om in ‘n sensoriese leksikon vervat te word wat ‘n definisie en ‘n verwysingstandaard vir elke term voorstel. Die “karakteristieke” sensoriese eienskappe van goeie kwaliteit rooibostee kan soos volg gedefinieer word: ’n kombinasie van “heuning”, “houtagtige” en “fynbos-blom” geure met ‘n effense soet smaak en ‘n sagte vrankheid. Aangename “karamel” en “vrugtige-soet” geure is ook waargeneem, terwyl terme soos “groen” en “hooi” met swak kwaliteit rooibostee geassosieer is.

Groot variasie in die samestelling van rooibostee is verkry deur kwantifisering van die inhoud van oplosbare vastestowwe (SS), totale polifenole (TP), tanniene, en 14 monomeriese fenoliese verbindings, asook spektrofotometriese kleurmetings. Hoë kwaliteit rooibostee is geassosieer met hoër vlakke van SS, TP, tanniene en fenoliese verbindings in vergelyking met lae kwaliteit rooibos. Korrelasies tussen laasgenoemde parameters en die sensoriese eienskappe het aangedui dat sekere nie-vlugtige verbindings, bv. fenielpirodruiwesuurglukosied, kwersetien-3-glukosied en iso-orientien, geassosieer is met die kenmerkende soet smaak van rooibos, terwyl ‘n bitter smaak geassosieer is met spesifieke flavonoïede soos luteolien, kwersetien and aspalatien. Die enigste verbinding wat betekenisvol gekorreleer het met vrankheid was rutien, maar dit is waarskynlik dat aspalatien en ander monomeriese flavonoïede ook bygedra het tot vrankheid. Die tannieninhoud het nie verband gehou met vrankheid nie, vermoedelik as gevolg van die beperkinge van die chemiese metode vir tannienbepaling.

Om te bepaal of stoompasteurisasie van rooibos die sensoriese kwaliteit en chemiese samestelling van rooibostee beïnvloed, is die verskille in fenoliese samestelling en sensoriese eienskappe van die tee, berei van ongepasteuriseerde en gepasteuriseerde rooibos, ondersoek. Stoompasteurisasie het ‘n betekenisvolle verlaging teweegebring van die SS, TP en aspalatien inhoud, sowel as van die absorbansie van die tee, veral by ‘n golflengte van 450 nm. Dit het ook gelei tot ‘n statisties betekenisvolle vermindering van die intensiteit van die meeste aroma en geur eienskappe, veral die sogenaamde “groen” eienskap wat met swak kwaliteit

rooibos verbind word. As gevolg van stoompasteurisasie is die opmerklike “groen” geur van sekere ongepasteuriseerde monsters herhaaldelik vervang deur ‘n “hooi” geur. Die smaakeienskappe, soet en bitter, was onveranderd, terwyl vrankheid statisties betekenisvol afgeneem het as gevolg van pasteurisasie.

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"And whatever you do, do it heartily, as to the Lord and not to men"

[Colossians 3:23]

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Chapter 1

INTRODUCTION

"Cigarette smoke", "tobacco", "cigar", "horse barn", "earthy", "bland" – these are some of the terms suggested by different internet users attempting to describe the flavour characteristics of rooibos tea in an online discussion forum on teas (www.teachat.com/viewtopic.php?t=2345). It is quite unlikely that tea which exhibits such flavour notes would awaken the interest of tea lovers, and successfully compete with the overwhelming variety of herbal teas, fruit infusions and blends that are populating the international tea market. However, the national and international popularity of rooibos, South Africa's unofficial national beverage, has in fact increased substantially over the last decade as is reflected by the export volumes which have been boosted from about 1800 tons in 1999 to more than 6200 tons in 2009 (S. Snyman, South African Rooibos Council, 2010, personal communication). It is thus safe to assume that the abovementioned flavour attributes do not accurately describe the sensory character of rooibos tea.

A number of internet sources also offer a variety of positive descriptors for rooibos flavour including "nutty", "woody", "fruity", "vanilla" and "caramel". The inconsistencies in the descriptive terms used to characterise rooibos flavour highlight, firstly, the fact that there is considerable variation in the sensory profiles of rooibos available on the market, and secondly, that there is a lack of standardised terminology with which to describe "good quality" rooibos. If these aspects are not addressed, the potential of this unique product may be limited as a result of the detrimental consequences on the market growth of rooibos. Ensuring a consistent supply of high quality tea with unchanging product attributes is crucial for maintaining and enlarging the market share of rooibos.

When examining the quality requirements stipulated by the South African regulations governing the sale of rooibos, it is not surprising that such disparities have evolved in the way that the sensory characteristics of rooibos are described. For rooibos to be deemed fit for sale, it must only "have the clean, characteristic taste and aroma" of rooibos (Anonymous, 2002). No further explanation of the term "characteristic" is given that elaborates on the sensory attributes associated with it. Variation in sensory quality due to differences in production areas, environmental conditions and processing parameters is not taken into account. Consequently, any batch of rooibos can be declared as having a "characteristic" rooibos flavour, which means that negative flavour characteristics do not necessarily create a barrier for the sale of rooibos on the national or international market.

However, rooibos must also comply with the food safety standards specified in the regulations, which focus mainly on the microbial safety of the product. Because of this, all rooibos is steam-pasteurised followed by drying to reduce its moisture content to less than 10% (J. Basson, Rooibos Ltd., Clanwilliam,

South Africa, 2009, personal communication). When this steam treatment was introduced in the 1980s, consumers that had been familiar with the product for years complained that pasteurised rooibos did not taste the same way it did prior to pasteurisation (Food & Beverage Reporter, 2005). Others have said that the changes caused by steam pasteurisation have a positive effect on its sensory quality by "softening" the flavour and "strong medicinal smell" of rooibos (Food & Beverage Reporter, 2005). Although this anecdotal evidence suggests that steam pasteurisation has a perceptible effect on the sensory quality of rooibos infusions, these changes, which may be beneficial or detrimental to the sensory quality, have not yet been accurately described or quantified.

With the continuing increase in the international demand for rooibos, it may awaken an interest in the production of rooibos in other countries, which would pose a major threat to the South African rooibos industry. In order to protect this exclusive South African product the development of a geographic indication (GI) for rooibos has been set in motion (Gerz & Bienabe, 2006). A GI is a label that is reserved for products which acquire their characteristic and defining qualities as a result of their geographical location, e.g. Champagne, Tequila or Parma Ham (Grazioli, 2002). In this way producers can distinguish their product based on its specific origin-related characteristics. However, in order to establish a GI for rooibos a suitable, well-defined description of the "typical" sensory profile of South African rooibos is required. Again, the inadequacy of the term "characteristic rooibos" flavour becomes apparent, and the need for more descriptive terminology is exposed.

One way of describing the sensory attributes of a product while taking into account the variation in sensory characteristics, is by developing a so called sensory lexicon. A sensory lexicon is a set of words that describe the sensory attributes of a product, along with definitions and/or reference standards for clarification (Drake & Civille, 2002). Sensory lexicons can be used as tools to describe, compare and monitor different products in industry- or research-related environments. By providing a standardised set of terms they facilitate the communication of attributes in a way that may be comprehended even by those unfamiliar with the product. Alternatively, descriptive terms can be assembled into a more convenient format that is referred to as a sensory wheel. Such wheels have been developed for a number of products. The red wine flavour wheel, for instance, developed in 1984, was well-received by the wine audience (Noble *et al*., 1987) and is still in use even after more than 20 years. The successful application of this sensory tool in other industries suggests that the development of a rooibos sensory lexicon and wheel would make a valuable contribution to the industry. Not only would these tools improve the communication between various role players of the industry, but they would also provide the means to compare the sensory profiles of rooibos during quality control or product development. They could also be employed to describe the effect of different production areas or processing parameters on the sensory characteristics of rooibos tea. It may then be possible to develop rooibos niche products that can be differentiated on the basis of their sensory profiles.

The development of attribute descriptors may also be useful when investigating the correlation between instrumental or physical data and the sensory quality, since the descriptive terms have direct application to the multitude of compounds found in a product (Drake & Civille, 2002). Taste and aroma characteristics of food and beverage products are derived from non-volatile and volatile compounds, respectively (Jackson, 2009). Therefore, specific descriptive terms for taste and aroma attributes may be closely associated with a number of components that have a high flavour impact, which would depend not only on their concentration in the product, but also on their respective detection thresholds. More than 120 volatile compounds have been identified in rooibos (Habu *et al*., 1985; Kawakami *et al*., 1993), each of which has specific aroma characteristics such as floral, fruity, woody and green notes (Arctander, 1969). Rooibos also contains numerous non-volatile phenolic compounds (Rabe *et al*., 1994), some of which are commonly associated with bitter and astringent characteristics (Bravo, 1998). Rooibos tea is well-known for its naturally slightly sweet taste. It has been speculated whether aspalathin, a phenolic compound which is unique to rooibos, is responsible for this sweetness due to its its dihydrochalcone structure (Rabe *et al*., 1994). However, this was disproved by a recent study in which aspalathin was found to exhibit a bitter taste quality (Reichelt *et al*., 2010). The component(s) that give(s) rise to the sweet rooibos taste remain unidentified. Unlike black tea, which has strongly astringent mouthfeel characteristics that are associated with the tannin content of a black tea infusion, rooibos exhibits a soft mouthfeel with a subtle astringency. The importance of the content of polyphenolic and tannin-like compounds with regard to the mouthfeel characteristics of rooibos infusions has also not yet been established. If key compounds responsible for the sensory characteristics of rooibos can be identified, these may be used as indicators of the quality and sensory properties of rooibos tea. This would facilitate the development of a prediction model with which the sensory quality of rooibos can be estimated by analysing its chemical composition.

Taking all of the above into consideration, the objectives of this study were thus to develop a defined set of descriptive terms in the form of a rooibos sensory lexicon and sensory wheel, to employ these tools to determine whether correlations exist between rooibos sensory attributes and the phenolic composition of a rooibos infusion and, based on the findings obtained from these analyses, to characterise the changes in the sensory attributes of rooibos due to steam pasteurisation in relation to heat-induced changes in the phenolic composition of the infusions.

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Chapter 2

LITERATURE REVIEW

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1. Rooibos (*Aspalathus linearis*)

1.1 Introduction

The leguminous shrub *Aspalathus linearis* grows in the Cedarberg area of the Western Cape province of South Africa. This unique plant is indigenous to the fynbos biome and is found in the Citrusdal, Clanwilliam and Nieuwoudtville regions (Fig. 1). One type of *A. linearis,* the selected and improved Nortier type, is cultivated for commercial purposes (Joubert *et al*., 2008a). Only small amounts of wild-growing tea is also harvested and processed (Malgas *et al*., 2010). Processing of the leaves and stems of *A. linearis* results in the development of the characteristic organoleptic qualities of rooibos. Rooibos is the Afrikaans term for "red bush", a descriptive term referring to the colour of the processed leaves as well as the water infusions prepared from these dried leaves. This popular herbal tea is unique to South Africa and may be considered as the country's unofficial beverage (Wilson, 2005).

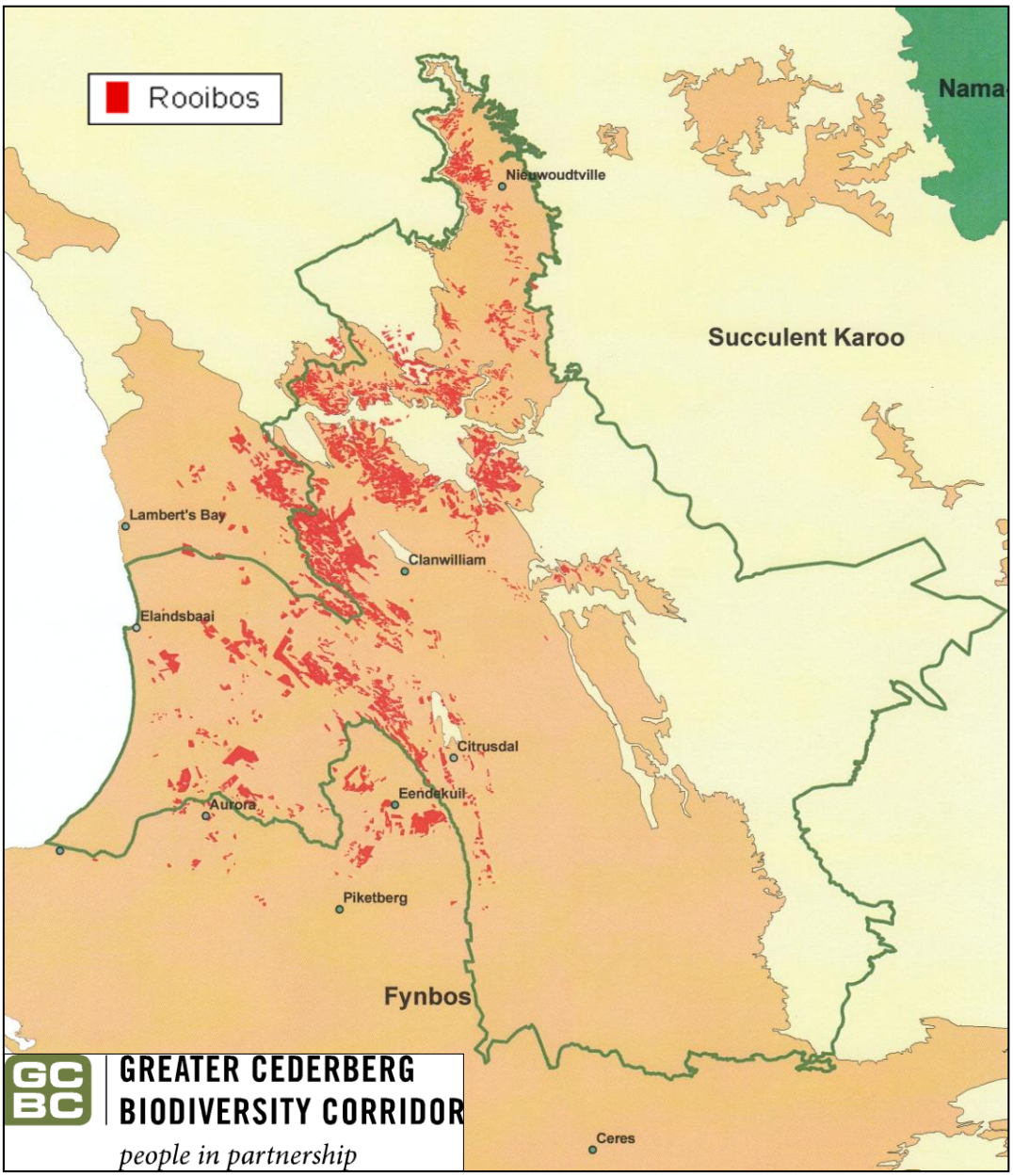


Figure 1 Distribution of *Aspalathus linearis* (Map supplied by the South African Rooibos Council, 2010).

1.2 History and use by the food and beverage industry

The first recorded consumption of rooibos tea can be dated back to 1772 when a travelling botanist reported that the Khoi people were using rooibos as a beverage (Joubert *et al*., 2008a). However, the modern use of rooibos started only much later during the early 1900s. Rooibos leaves and stems were boiled in water resulting in a brew with a unique flavour, aroma and colour. The hot brew was usually served with milk and sugar. Consumers' demand for convenience led to the introduction of the tea bag and nowadays rooibos tea is mostly prepared using tea bags instead of loose-leaf tea. For one cup of rooibos tea, one tea bag (ca. 2 g rooibos) is infused for 2 min to 5 min in freshly boiled water. Sugar and milk are added as desired (Joubert *et al*., 2008a).

Nowadays, a large offering of rooibos tea products is available. Various types of flavoured rooibos (such as vanilla, honey and lemon), tea mixtures (e.g. honeybush, buchu and fennel) and over 30 different brands are available on the South African market (Snyman, 2000). A number of rooibos ice teas with interesting flavours, such as Passion-Currant and Pineapple, have been introduced into the ice tea market (Food and Beverage Reporter, 2006). Ice tea has become a highly popular beverage as reflected by a considerable increase in sales in the ready-to-drink beverage sector.

Numerous innovative ideas have surfaced over the last years that have revamped the image of traditional, old-fashioned rooibos tea to give it a more modern and sophisticated feel. A relatively new commodity on the rooibos market is unfermented rooibos for which demand on the international market has gradually been increasing due to its higher antioxidant activity and alleged health effects (Food & Beverage Reporter, 2004). Unfermented rooibos was first introduced in the mid-1990s for a scientific study on antioxidant activity of rooibos (Von Gadow *et al*., 1996) and it then became necessary to differentiate between the two processed forms of rooibos to avoid confusion, especially in scientific literature (E. Joubert, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, 2010, personal communication). Joubert and co-workers (Schulz *et al.*, 2003) were, therefore, the first to formulate and use the terms green and traditional rooibos to refer to unfermented and fermented rooibos, respectively.

Another novel concept is that of Red Espresso®, a tea espresso that is easily prepared from finely ground rooibos leaves (Food & Beverage Reporter, 2007). Even the successful international company Starbucks has now put rooibos on the international stage by offering a range of vanilla-flavoured products (Food & Beverage Reporter, 2010). The use of rooibos as an ingredient or flavour in other products, locally and internationally, has also been on the rise, driven by its association with health and wellness. Rooibos can now be found in yoghurt, bakery and confectionery products, and fruit bars (Food & Beverage Reporter, 2007).

1.3 A functional beverage: Rooibos and its health benefits

Consumers have always demanded that food and beverages should be high in quality, convenient, nutritious and inexpensive (Wilson, 2005). However, over the years consumers have become increasingly health-conscious and more sensitive to the benefits of food other than its basic nutritive value. Foods that provide additional physiological benefits that may prevent disease or promote health are referred to as functional foods (Hasler, 1996). The demand for functional products has increased significantly and is still on the rise. The global market value for supplements, functional foods and beverages is forecasted to reach $176.7 billion in 2013. Functional beverages are predicted to be the fastest growing segment obtaining the largest share of the functional market and boasting a 10.8% compound annual growth rate (Roberts, 2009). Rooibos, along with several other medicinal teas such as green tea, can be classified as a functional beverage product (Wilson, 2005). Not only is rooibos caffeine-free and low in tannins (Morton, 1983), but anecdotal evidence suggests that it also has anti-allergic effects, reduces nervous tension and alleviates ingestion, heartburn and nausea (Joubert *et al*., 2008a). A wide variety of beneficial physiological effects has been investigated by numerous research groups. These effects include antioxidant properties, antimutagenic properties, hepatoprotective effects, phyto-oestrogenic properties and miscellaneous other effects such as immune system modulation, vasodilatory effects, antihemolytic effects, anti-aging properties, antimicrobial and antiviral effects, dermatological effects and anti-allergic effects (Joubert *et al*., 2008a).

1.4 Marketing and export

Tea, particularly green and black teas (*Camellia sinensis)*, can be regarded as one of the most popular beverages in the world (Cabarello *et al*., 2003) which can be attributed to various factors such as its sensory properties, relatively low retail price and associated health benefits (Cabarello *et al*., 2003). There are more than 2000 different types of tea available on the market (Snyman, 2000). Product differentiation is thus essential in order to be successful in this crowded industry. For this reason it is of essence that rooibos tea is clearly distinguishable from other teas and herbal infusions especially in terms of flavour. Only if it can be clearly defined and described can it be marketed effectively in this competitive market.

Commercial sales of rooibos started in 1904 and demand increased during the Second World War when it was considered a cheap alternative to black tea. However, during the 1950s the rooibos market was running at a loss due to a decrease in demand, overproduction and poor and inconsistent quality. The government, therefore, set up a marketing organisation called the Rooibos Tea Board to ensure that marketing was regulated and that some sort of quality grading system was in place. In 1993 the one-channel marketing system and the Rooibos Tea Board were abolished (Joubert *et al*., 2008a). Presently most producers sell their produce to a central processing yard that processes about 75% of all rooibos that is produced (J. Basson, Rooibos Ltd., Clanwilliam, South Africa, 2009, personal communication). This results in

better standardisation of tea quality since batches of different quality grades can be blended to obtain a final product with acceptable quality.

The estimated production volume for the 2009/2010 season lies between 18 000 and 20 000 tons with the local sales of rooibos being between ca. 4500 tons and 5000 tons per annum (S. Snyman, South African Rooibos Council, 2010, personal communication). International demand for rooibos has risen steadily over the last two decades from about 750 tons in 1993 to more than 7000 tons in 2007. A decline in rooibos exports occured from 2007 to 2009 (Fig. 2) which may be explained by the global economic crisis. Nevertheless, the amount of rooibos exported during these three years is still higher than it was before 2007. The major rooibos-importing country is Germany with a share of about 50% of all exports, followed by the Netherlands, the United Kingdom, Japan and the USA (Table 1).

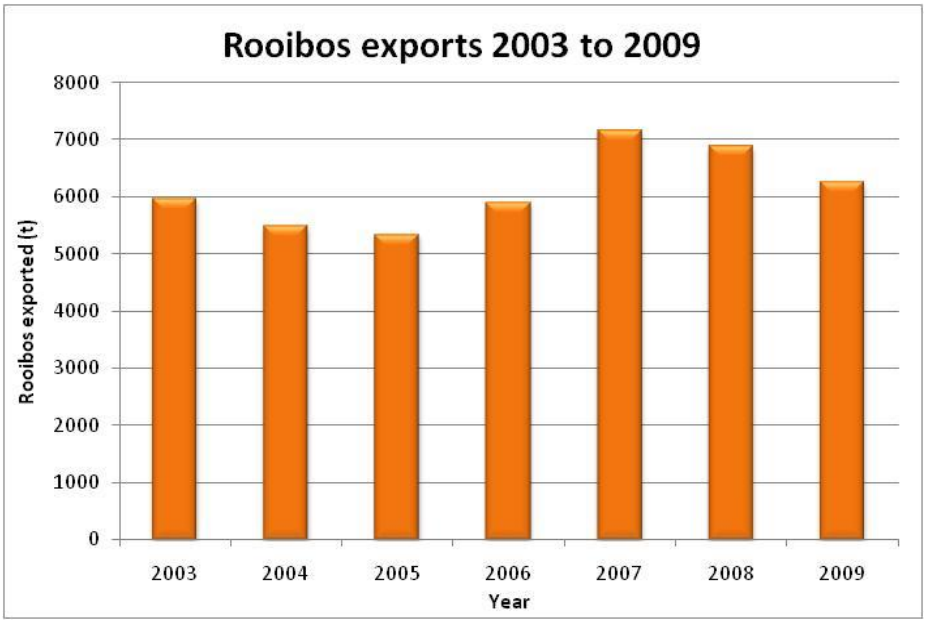


Figure 2 Rooibos exports from 2003 to 2008. Data supplied by the South African Rooibos Council (2010).

Table 1 Rooibos export values for major rooibos importing countries for 2003 to 2009

	Amount exported (t) (percentage of total exports)						
Country	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**
Germany	4525	4063	3383	3354	3935	3495	3019
	(75.9%)	(74.0%)	(63.3%)	(56.9%)	(54.8%)	(50.8%)	(48.2%)
Netherlands	455	520	721	798	781	754	956
	(7.6%)	(9.5%)	(13.5%)	(13.5%)	(10.9%)	(10.9%)	(15.3%)
UK	170	150	307	426	527	783	787
	(2.8%)	(2.7%)	(5.7%)	(7.2%)	(7.3%)	(11.4%)	(12.6%)
Japan	474	286	274	312	413	260	380
	(7.9%)	(5.2%)	(5.1%)	(5.3%)	(5.8%)	(3.8%)	(6.1%)
USA	123	275	218	387	383	341	279
	(2.1%)	(5.0%)	(4.1%)	(6.6%)	(5.3%)	(5.0%)	(4.4%)
Australia	8	13	44	56	86	69	78
	(0.1%)	(0.2%)	(0.8%)	(0.9%)	(1.2%)	(1.0%)	(1.2%)

(data supplied by the South African Rooibos Council, 2010)

1.5 Tea processing

During the hot summer months and early autumn (January to April) rooibos is harvested by topping the plants to a height of ca. 45 cm. The shoots are shredded to a length of 3 to 4 mm. This initiates enzymatic oxidation of the polyphenols present in the leaves which leads to browning (Joubert & Schulz, 2006). Heaps of shredded plant material are then made, water is added and the plant material is bruised to accelerate the oxidative process in the plant material. The term “fermentation” is commonly used in the industry to describe this oxidation process. When water is added polyphenols are extracted from the leaves and absorbed by the coarse light-coloured stems leading to a more uniformly coloured product. Adequate aeration of the fermenting plant material is important so that uniform oxidation can take place throughout the rooibos heaps. Therefore, fermentation heaps are turned over several times during the fermentation process. Because of the importance of aeration for the oxidation reactions, insufficient aeration leads to a low quality product as a result of under-fermentation (Joubert, 1998).

Fermentation takes place overnight and usually takes between 12 and 14 hours at an optimum temperature of between 38°C to 42°C (Joubert & Schulz, 2006). However, fermentation time can vary from 8 to 24 hours depending on a number of factors such as young growth, age of the bush and cultivation area (Joubert, 1994). Changes occur in the plant material during fermentation: Leaves acquire the typical red-brown colour of fermented rooibos, and the aroma changes from resinous, hay-like and grassy to sweet, apple-like or honey-caramel (Joubert, 1994). Colour development occurs rapidly after comminution of the

plant material (Joubert, 1996) while the sweet, honey-like rooibos aroma only develops after about 12 to 15 hours (Joubert & De Villiers, 1997). It has also been observed by farmers that as soon as bees are attracted to the fermentation heaps the tea has reached its optimum aroma (E. Joubert, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, 2010, personal communication). As soon as the characteristic rooibos aroma has developed the heaps are spread out in a thin layer to dry in the sun. Since fermentation continues when the tea is still moist, drying should commence as soon as possible to prevent over-fermentation. In the case of adverse weather conditions drying may take up to 24 hours which results in poor quality tea (Joubert, 1994). Dried, fermented rooibos is then sieved, graded and steam-pasteurised before being packed. During processing numerous factors play a role in determining the final quality of the tea since the tea manufacturing process is not carried out under controlled conditions. Along with other factors, such as the composition of the plant, this results in considerable variation in the quality of the rooibos end product.

1.6 Chemical composition

The range of compounds present in a rooibos infusion is responsible for the flavour, colour and functional properties of the tea. A number of research groups have analysed the chemical composition of the tea as well as the changes in the chemical profile that take place during fermentation.

The most important compounds in fermented rooibos tea that significantly impact the organoleptic properties of the tea are the phenolic components especially the flavonoids and their oxidized polymeric products that are formed during fermentation (Joubert, 1994). Phenolic compounds that have been isolated from *A. linearis* are summarised in Table 2. A high yield of flavonoids and an exceptionally high content of *C*-glycosides are found in rooibos. The most important flavonoid is the C–C linked dihydrochalcone glucoside, aspalathin, which to date has only been found in rooibos (Joubert *et al*., 2008a). The cyclic dihydrochalcone, aspalalinin, has also been isolated only from rooibos (Shimamura *et al*., 2006). Another rare dihydrochalcone *C*-glucoside found in *A. linearis* is nothofagin, the 3-dehydroxy analogue of aspalathin, the presence of which has only been confirmed in two other species, *Nothofagus fusca* (Joubert *et al*., 2008a) and *Schoepfia chinensis* (Huang *et al*., 2008).

Fermentation of rooibos leaves results in a decrease in the average total polyphenol content indicating a loss of soluble polyphenols during oxidation (Schulz *et al*., 2003). It has been shown that aspalathin is converted to dihydro-iso-orientin and dihydro-orientin when exposed to sunlight and oxygen (Koeppen & Roux, 1965) with the simultaneous formation of polymeric substances. This oxidation process was confirmed by Marais *et al*. (2000). Investigations by Krafczyk and Glomb (2008) and Krafczyk *et al*. (2009) shed further light on the chemical changes taking place during fermentation of rooibos leaves. The formation of aspalathin dimers during oxidation was established Krafczyk *et al*. (2009). Aspalathin was also found to be the most important compound taking part in the browning reactions although other compounds contribute to some extent to the colour formation. Krafczyk *et al*. (2009) concluded that the browning reactions which occur during oxidation of *A. linearis* plant material are mainly non-enzymatic. However, the rapid

discolouration of plant material taking place after bruising of rooibos leaves suggests that the mechanism responsible for the change in colour is in actual fact enzyme-mediated oxidation (Joubert & De Villiers, 1997). The role of enzymes in initiating oxidation is supported by the fact that by inactivating the enzymes through steam treatment the green colour of the rooibos leaves is retained (E. Joubert, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, 2010, personal communication).

Other phenolic compounds that have been identified include the flavones orientin, iso-orientin (Koeppen & Roux, 1965), vitexin, iso-vitexin, chrysoeriol (Rabe *et al*., 1994) and luteolin (Snyckers & Salemi, 1974), as well as the flavonols rutin, iso-quercitrin (Koeppen *et al*., 1962), quercetin (Snyckers & Salemi, 1974), hyperoside (Bramati *et al*., 2002), luteolin-7-*O*-glucoside (Kazuno *et al*., 2005) and the flavan-3-ol (+)-catechin (Ferreira *et al*., 1995). Furthermore, the presence of various phenolic acids (e.g. *p*-hydroxybenzoic acid, protocatechuic acid, caffeic acid, vanillic acid, *p*-coumaric acid, ferulic acid), lignans and the coumarin, esculetin, has been established (Rabe *et al*., 1994; Shimamura *et al*., 2006; Krafczyk & Glomb, 2008). Richfield (2008) also isolated tannin-like flavonoid oligomers containing dihydrochalcone monomers, which were present at low concentrations in unfermented rooibos.

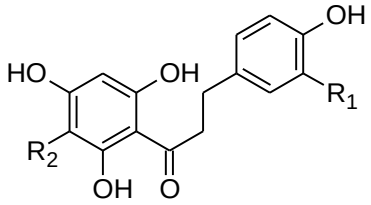
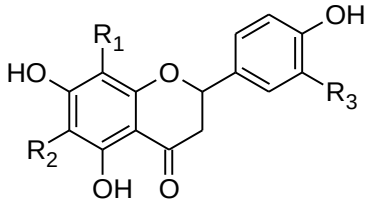
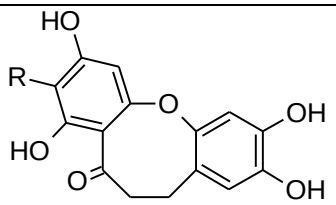
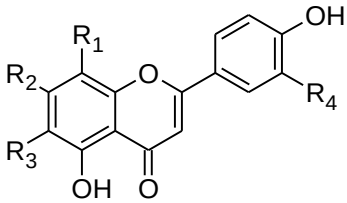
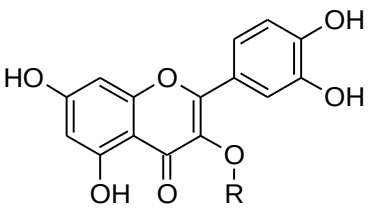
A number of research groups have quantified several of the major compounds isolated from rooibos. Fermented rooibos contains about 34.25% total polyphenols and about 2.96% flavonoids expressed as mass percentage of hot water soluble solids (Joubert *et al*., 2005). Other sources state the estimated polyphenol content of a cup of tea (150 ml) as 82 to 88 mg (Joubert & Schulz, 2006). As for the individual flavonoids, aspalathin, rutin and orientin are the major constituents of aqueous rooibos infusions (Bramati *et al*., 2002), whereas the aglycones, luteolin, quercetin and chrysoeriol are present in small quantities only (Toyoda *et al*., 1997). The content of the major individual flavonoids determined by various research groups is shown in Table 3. Different samples of green rooibos were found to differ significantly in aspalathin and nothofagin levels (Joubert & Schulz, 2006). Not only did samples from different plantations vary in their flavonoid levels, but so did bushes within the same plantation. It was concluded that genetic variation plays an important role in determining the level of aspalathin and nothofagin present in rooibos. A recent study analysed the variation in the composition of rooibos infusions prepared from more than 100 different production batches from the 2009 harvest (Joubert, 2010, unpublished data). Considerable ranges of total polyphenol (19% to 31%) and total flavonoid (1.72% to 5.83%) content, expressed as percentage of the freeze-dried rooibos extract prepared from the infusions, were observed. Aspalathin and nothofagin contents differed by factors of 10 and 6 respectively (Joubert, 2010, unpublished data).

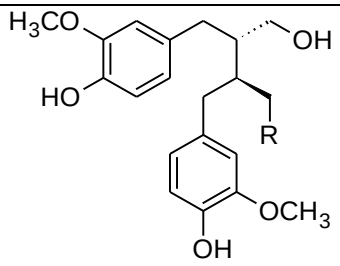
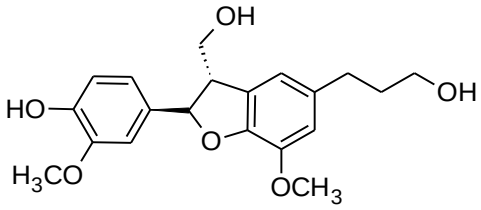
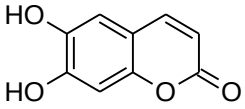
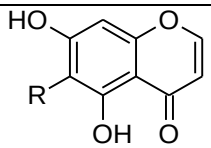
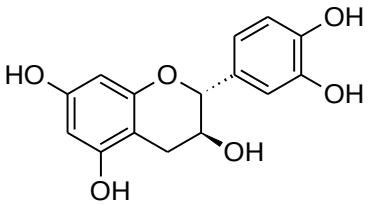
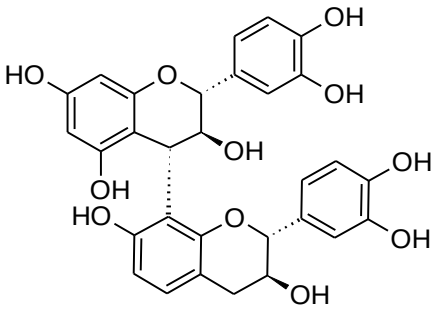
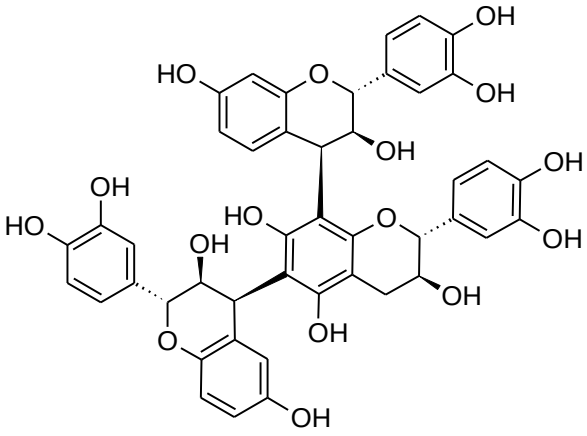
Compared to black tea (*C. sinensis*), the tannin content of rooibos leaves is very low and has been estimated as 3.2% (Reynecke *et al*., 1949) and 4.4% (Blommaert & Steenkamp, 1978). However, other research groups have shown that as much as 50% of a dried water extract of fermented rooibos was composed of complex tannin-like substances (Ferreira *et al*., 1998) while a methanol extract was found to contain 14% tannin (Joubert *el al*., 2008a). The tannins have been described as irregular heteropolymers of the procyanidin type with (+)-catechin and (-)-epicatechin as chain-extending units and (+)-catechin as

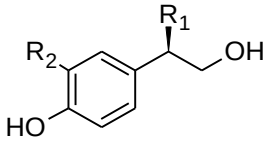
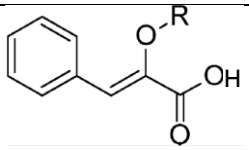
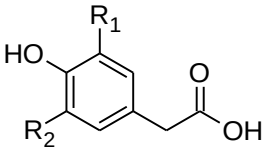
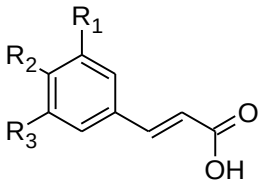
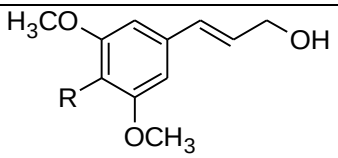
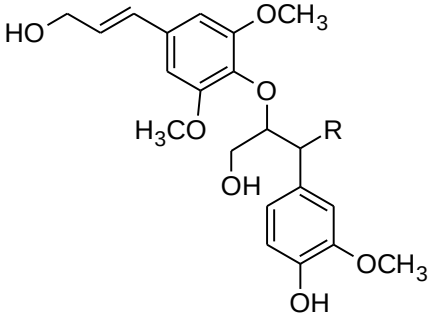
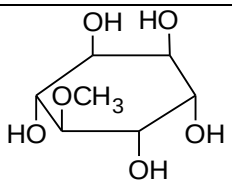
terminal unit (Ferreira *et al*., 1998; Marais *et al.,* 1998). The dimer procyanidin B3 and a profisinidin triflavanoid (Ferreira *et al*., 1995) are also present at very low concentrations.

Unlike black tea, rooibos does not contain caffeine although the alkaloid sparteine has been identified (Van Wyk & Verdoorn, 1989). Various minerals including sodium, potassium, magnesium, calcium, phosphorus and iron, and fluoride are also present in a rooibos infusion (Joubert *et al*., 2008a). Volatile compounds of fermented tea have been characterised by Habu *et al*. (1985) and Kawakami *et al*. (1993). Some of the major volatiles that were identified include guaiacol, damascenone, dihydroactinidiolide, geranylacetone, β-ionone, 5,6-epoxy-β-ionone and benzaldehyde. More information on these aroma compounds is given in Section 6.5.

Table 2 Secondary metabolites identified in *Aspalathus linearis* plant material (Joubert *et al.,* 2008a)

Structure	Compound type, names and substituents
	**Dihydrochalcones** aspalathin[a,b,c]: R_1 = OH, R_2 = *C*-β-D-glucosyl nothofagin[d]: R_1 = H, R_2 = *C*-β-D -glucosyl
	**Flavanones** dihydro-orientin[d]: R_1 = *C*-β-D -glucosyl, R_2 = H, R_3 = OH dihydro-iso-orientin[d]: R_1 = H, R_2 = *C*-β-D-glucosyl, R_3 = OH hemiphlorin[d]: R_1 = *C*-β-D-glucosyl, R_2 =R_3 = H
	**Cyclic dihydrochalcones** aspalalinin[d]: R = *C*-β-D-glucosyl
	**Flavones** orientin[a,b,d,e]: R_1 = *C*-β-D-glucosyl, R_2 = R_4 =OH, R_3 = H iso-orientin[b,e]: R_1 = H, R_2 = R_4 =OH, R_3 = *C*-β-D-glucosyl vitexin[a,b,d]: R_1 = *C*-β-D-glucosyl, R_2 = OH, R_3 = R_4 = H iso-vitexin[b,d]: R_1 = R_4 = H, R_2 = OH, R_3 = *C*-β-D-glucosyl luteolin[b,d]: R_1 = R_3 = H, R_2 = R_4 = OH luteolin-7-*O*-glucoside[d]: R_1 = R_3 = H, R_2 = *O*-β-D-glucosyl, R_4 = OH chrysoeriol[b]: R_1 = R_3 = H, R_2 = OH, R_4 = OCH_3
	**Flavonols** quercetin[b,d]: R = H iso-quercitrin[b,d,e]: R = *O*-β-D-glucosyl hyperoside[d]: R = *O*-β-D-galactosyl rutin[e]: R = *O*-β-D-rutinosyl quercetin-3-*O*-β-D-robinoside[d]: R = *O*-robinosyl

	**Lignans** secoisolariciresinol[d]: R = OH secoisolariciresinol-*O*-glucoside[d]: R = *O*-glucosyl
	vladinol F[d]
	**Coumarins** esculetin[d]
	**Chromones** 5,7-dihydroxy-6-*C*-glucosyl-chromone[c]
	Flavan-3-ols
	(+)-catechin[c]
	procyanidin B3[c]
	bis-fisetinidol-(4β,6:4β,8)-catechin[c]

	**Phenylethanol derivatives** *p*-hydroxyphenylethanol[d]: $R_1 = R_2 = H$ vanylglycol[d]: $R_1 = OH$, $R_2 = OCH_3$
	**Phenylpyruvic acid derivatives** 3-phenyl-2-glucopyranosyloxypropenoic acid[f]: R = *O*-glucosyl
	**Phenolic carboxylic acids** *p*-hydroxybenzoic acid[b,d]: $R_1 = R_2 = H$ protocatechuic acid[b]: $R_1 = OH$, $R_2 = H$ vanillic acid[b]: $R_1 = OCH_3$, $R_2 = H$ syringic acid[c]: $R_1 = R_2 = OCH_3$
	**Hydroxycinnamic acids and derivatives** 3,4,5-trihydroxycinnamic acid[b]: $R_1 = R_2 = OH$ *p*-coumaric acid[b,d]: $R_1 = R_3 = H$, $R_2 = OH$ caffeic acid[b]: $R_1 = R_2 = OH$, $R_3 = H$ ferulic acid[b]: $R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$ sinapic acid[b]: $R_1 = R_3 = OCH_3$, $R_2 = OH$
	**Phenylpropanoid glycoside** syringin[d]: R = O-glucosyl
	**Other** 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxy-1-(*E*)-propenyl)-2,6-dimethoxyphenoxy]propyl-β-D-glucopyranoside[d]: R = *O*-β-D-glucosyl
	**Inositol** (+)-pinitol[c]

[a] Koeppen *et al*., 1965
[b] Rabe *et al*., 1994
[c] Ferreira *et al*., 1995
[d] Shimamura *et al*., 2006
[e] Koeppen *et al*., 1962
[f] Marais *et al*., 1996

Table 3 Content of major flavonoids in fermented rooibos

Compound	% of Dried Extract (m/m)				
Aspalathin	0.152 - 1.552[a]	0.53[b]	0.061[c]	0.350[d]	0.123[e]
Nothofagin	0.029 - 0.178[a]	0.11[b]	0.017[c]	0.110[d]	
Iso-orientin	0.472 - 1.034[a]		0.085[c]	0.052[d]	0.083[e]
Orientin	0.442 - 0.897[a]		0.076[c]	0.161[d]	0.100[e]
Rutin	0.140 - 1.221[a]				0.127[e]
Vitexin	0.050 - 0.080[a]	0.46[b]	0.017[c]	0.236[d]	0.033[e]
Iso-vitexin	0.048 - 0.081[a]	ND[b]	0.011[c]	0.089[d]	0.027[e]
Luteolin-7-*O*-glucoside	0.012 - 0.031[a]				
Hyperoside	0.027 - 0.087[a]				
Luteolin		0.084[b]	trace[c]	trace[d]	0.003[e]
Chrysoeriol			trace[c]	trace[d]	0.002[e]
Quercetin				trace[d]	0.011[e]

[a] Joubert *et al.,* 2010
[b] Richfield, 2008
[c] Joubert *et al.,* 2005
[d] Joubert & Schulz, 2006
[e] Bramati *et al*., 2002

2. Grading and quality of rooibos

2.1 The rooibos grading system: Development and current state

Grading systems are put in place to enable successful standardisation and commercialisation of a product by improving control over its overall quality and thereby increasing consumer satisfaction. When developing such systems, quality parameters need to be identified, defined and measured. In order to be useful and reliable, measurement of these parameters must be quick, simple, scientifically validated and actually correlated to the way that consumers perceive product quality (Feria-Morales, 2002). These principles also apply to the grading of rooibos.

Before the establishment of the Rooibos Tea Board no grading or quality control system was in place. Standardisation of the product was initiated by the Rooibos Tea Board and subsequently a need for a quality grading system arose (Joubert, 1994). At first, sieved tea received from farmers was graded into six different grades (A1, A2, A3, B1, B2 and Undergrade) by four members of a special committee. Grading was purely subjective, non-mechanical and based on cut, colour and aroma of the **dried** rooibos leaves and stems. In 1965 a new grading system was put in place and a mechanical sieving system was introduced which separated rooibos into classes according to cut length. Tea classification was, however, still based on cut, colour and aroma of the **leaves and stems**. In 1985, evaluation of the taste, colour and aroma of a tea **infusion** was then added to the grading system giving rise to different quality grades termed Super, Choice

and Standard, with Super representing tea of highest quality. Several minor adjustments were made to the system over the years and in 1992 another grade (Selected grade) was introduced (Joubert, 1994). Finally, rooibos tea was grouped into three categories (A, B and C) reflecting strong, medium or poor characteristic aroma and taste attributes. Also, fine and coarse tea was separated according to cut size for use in tea bags or loose tea packaging.

Currently, each tea processing company uses their own methods of grading rooibos. The most structured evaluation system used in the industry is employed by Rooibos Ltd. It is based on the grading system described above and entails the following: Each batch of rooibos received from the producers is mechanically sieved to obtain the yield, which refers to that size fraction which will be graded, processed and marketed. Grading is then performed by an experienced taster. Firstly, the appearance of the dry and wet tea leaves is evaluated followed by the appearance (colour and brightness) and flavour (aroma, taste and mouthfeel) of an infusion prepared from the tea leaves according to a standard protocol (J. Basson, Rooibos Ltd., Clanwilliam, South Africa, 2009, personal communication). A list of terms is available that outlines the ideal and undesirable attributes of the dry leaves, the wet leaves and the infusion. The **appearance of the dry and wet leaves** acts as an indicator of poor quality and processing practices since over-fermentation results in dull-brown leaves and produces a watery infusion with a woody aroma. Shiny, brick-red leaves of uniform colour are regarded as high quality (Joubert, 1994). The **colour of the infusion** should be clear, brick-red-brown with an orange-yellow tint at the rim of the cup. Under-fermentation results in an undesirable, prominent orange-yellow tint; brown or turbid extracts are also detrimental to the visual quality of the infusion and are usually associated with over-fermentation (Joubert, 1994). Joubert (1995) investigated the use of tristimulus colour measurements of rooibos infusions as potential objective quality indicator. The red colour (a* values) of rooibos infusions was found to play an important role during visual evaluation of the rooibos quality.

In order to analyse **tea flavour** tea tasting is carried out by sucking rather than sipping in order for the liquid to be drawn to the back of the mouth and up to the olfactory nerve in the nose (J. Basson, Rooibos Ltd., Clanwilliam, South Africa, 2009, personal communication). The flavour of the tea can be regarded as the most significant determinant in terms of tea quality since it ultimately affects the degree of liking of the product by the consumer. The **aroma** of a rooibos infusion, a critically important characteristic for any tea, must be free from foreign notes (musty, woody, old honey or straw), and no green notes should be present. The strength of the 'characteristic', sweet, honey-like rooibos aroma determines the grade awarded to the aroma of the sample. High quality rooibos tea is expected to have a strong, full-bodied, sweet, 'characteristic' **taste** and must be free from sour, bitter, salty, musty or foreign tastes. A slightly grassy taste is permitted only for certain grades.

According to these guidelines the tea taster assigns a mark out of 10 for each of the grading parameters to every batch of rooibos. Each of these parameters is multiplied by a certain factor reflecting the impact that this parameter has on the tea quality. The weights assigned to the grading parameters differ

according to the cut of the leaves which determines whether the tea will be used in tea bags or as loose tea. The taste and aroma of the tea infusion have the largest impact on the grade that is awarded to the tea. After the primary grading of the batch has been completed a panel of seven judges re-evaluates the tea and, if necessary, makes adjustments that may affect the final grade achieved by the batch. According to the final mark awarded to the batch the tea is assigned one of seven grades (AA, A, B, C, D, E or F) of which tea of grades E and F is not distributed for consumption as tea. Of the total production volumes received over the last three years about 86% to 93% of all batches were awarded grades B and C (J. Basson, Rooibos Ltd., Clanwilliam, South Africa, 2009, personal communication).

Even though an improved, structured grading system is now in place the system has limitations and the grade awarded to a certain batch of rooibos still only gives an overall impression of tea quality. Objective indicators of rooibos quality have not yet been found and very little research has been conducted to do so.

2.2 Problems and limitations of the current grading system

Considering the criteria of a good grading system that were mentioned above (quick, simple, scientifically validated, correlated to consumer perception) it becomes evident that developing a reliable, pertinent grading system for a product as complex as tea is indeed a challenging endeavour. Although the sensory evaluation by expert tasters is inexpensive, quick and simple this grading procedure has some limitations since the quality standards depend solely on the subjective opinion of the tasters and the results cannot be scientifically validated. The tasters act as the instrument that measures and quantifies certain quality parameters. An expert taster can be defined as “a person with considerable experience and proven ability in sensory assessment of a given product under specified conditions” (Land & Shepherd, 1984). Due to long exposure to a single product they develop an acute sensitivity to its characteristics and are, therefore, able to make rapid judgments in terms of its quality. However, the day-to-day perceptiveness of an expert taster may differ; his judgement may also be influenced by external factors and may thus not be free from bias (Feria-Morales, 2002). One might also wonder how tasters would define and quantify terms such as “strong” or “poor” taste and aroma, and whether different tasters have a different understanding of terms such as “strong” or “characteristic rooibos”. Furthermore, developing the special sensitivity and abilities of such experts takes time and experience which makes the replacement of these individuals a critical matter for any company.

Grading determines the market value that will be awarded to each batch of tea (Joubert, 1995); therefore, the results of tea grading have financial implications for both the producer and the processor. Since these parties have conflicting interests with regard to financial compensation, problems may arise. Producers may not be satisfied with the grade awarded to their produce and may question the capabilities and bias of the tasters.

The abovementioned limitations have also been identified by black tea (*C. sinensis*) producers and processors. However, although considerable effort has been made to correlate certain black tea parameters

with results from sensory evaluation, a single, reliable and objective scientific method to determine tea quality has not yet been found. Section 6.6 will expand on this aspect. Also, since instrumental methods of analysis are mostly slow and work-intensive, tea tasting remains the most widely used method to determine the quality of tea (Cabarello *et al*., 2003).

2.3 Quality control and regulations

In order to achieve long-term growth in the market, products must be of consistent quality, and efficient quality control procedures must be in place, governed by regulations stipulated by the appropriate governing bodies. The South African regulations relating to quality standards for rooibos specify that all rooibos should "have the clean, characteristic taste and aroma and clear, distinctive colour of rooibos" and "may contain not more than 10% white sticks" (Anonymous, 2002). However, no definitions or reference standards are provided for the terms "characteristic" and "distinctive". This means that manufacturers have the freedom to set their own quality standards in terms of colour, flavour and mouthfeel of rooibos infusions. All other specifications are concerned with food safety standards. Regulatory control is limited to moisture content, pesticide residues and microbial contamination. The Perishable Products Export Control Board (PPECB) is in charge of monitoring the quality standards of rooibos intended for the export market. However, the PPECB does not evaluate the flavour and colour of the product and only deals with food safety issues (Snyman, 2000). Furthermore, there are no specifications regarding the composition, polyphenol content or antioxidant activity of rooibos despite the fact that these parameters are becoming increasingly important for health-conscious consumers. In the case of green rooibos, a high level of aspalathin, for example, would be desirable for the cosmetic and functional food markets, and some extract manufacturers are already using total polyphenol content and total antioxidant activity as part of their product specifications (Joubert & Schulz, 2006). Should minimum levels of such parameters be required, another level of quality control of rooibos would be created. In an attempt to provide a more comprehensive rooibos quality control program to a South African sustainability initiative, Rutgers University evaluates a variety of parameters including colour, taste, aroma, moisture content, total phenol content and antioxidant capability of tea samples (Erickson, 2003). The data is recorded on a product specification sheet which is made available to farmers and prospective buyers (Erickson, 2003). This quality control program is, however, only used for a small percentage of the total amount of rooibos that is produced and marketed.

A closer look at the regulations dealing with rooibos quality standards raises several questions: Do producers and processors agree on what they define as "characteristic" rooibos? How would one describe the sensory attributes of "characteristic" rooibos flavour? What do consumers regard as a good quality cup of rooibos, and is there consensus among them? In order to produce a product that is of consistent quality in terms of flavour the meaning of terms such "characteristic" and "strong" must be clear. The flavour of different quality grades must be defined, distinct and distinguishable to be able to successfully market and differentiate between products according to consumer needs. Also, the concept of "characteristic" rooibos

flavour is likely to be completely foreign to overseas markets and consumers; therefore, it would be difficult to promote rooibos tea among these markets without being able to accurately describe the unique flavour characteristics of the tea. Rooibos is, to a large extent, still used in blends or as a flavoured product so that consumers are not familiar with its natural, characteristic taste. The export potential of rooibos may consequently be limited by its poor differentiation on the crowded herbal tea market simply because of a lack of proper terminology.

2.4 Variation in and effect of processing conditions on rooibos quality

Studies on other plants have shown that a number of factors may result in considerable variation in the composition of the plant tissue. These include plant distribution, the genetic make-up of the seedling, seasonal effects, light intensity, drought and climate. All of these may contribute to a certain extent to the differences in the composition and quality of the plant product (Aherne & O'Brien, 2002). It has been shown, for example, that the accumulation of plant flavonoids increases in response to certain stress factors such as increased light exposure (Aherne & O'Brien, 2002). Research has been done to analyse the factors contributing to the variation in the composition of black tea. Astill *et al*. (2001) investigated the effects of product and preparation variables on the chemical composition of *C. sinensis* extracts and found that the variety, growing environment, manufacturing conditions and particle size of the tea leaves had a significant impact on the composition of the tea leaves and the final infusions. Lin *et al*. (2003) also found significant differences in the phenolic composition of *C. sinensis* infusions prepared from old and young leaves indicating that the age of the tea leaves may influence the composition of the tea infusion. Seasonal variations in the phenolic composition of Australian-grown fresh tea shoots were studied by Yao *et al.* (2005). Tea harvested during warmer months had significantly higher levels of epigallocatechin gallate, epicatechin gallate and total catechin gallates compared to tea harvested in cooler months. The opposite was true for levels of catechin and epigallocatechin. Different environmental factors were proposed to be the cause of these seasonal variations including the differences in day length, sunlight and temperature across the seasons. Comparable studies have not yet been conducted for *A. linearis* but it can be expected that such factors would have similar effects on the composition of rooibos leaves which in turn would result in variation in the sensory quality of tea infusions.

Environmental factors are not the only source of variation in rooibos quality. The processing method of rooibos is still carried out according to the traditional method with limited control being exercised over the processing parameters. The guidelines for rooibos processing variables such as fermentation and drying times and temperatures are rather general and based on the experience of the producer. Fermentation periods, for example, can range from 8 to 24 hours and farmers commonly determine when to stop the rooibos fermentation by simply evaluating the change in aroma taking place during the process. Adverse weather conditions, low night temperatures or inexperience of the farmer may result in over- or under-

fermentation which would affect the quality of the final product and lead to tea of inconsistent quality being produced (Joubert, 1994).

Rooibos has been processed under controlled conditions (Joubert, 1994; Joubert & De Villiers, 1997; Joubert, 1998). Special equipment, including a rotary fermentation unit and a deep-bed drying unit (Joubert & Müller, 1997; Joubert *et al.*, 1998), was used for bruising, fermenting and drying of rooibos plant material. In this way certain processing parameters, such as fermentation time and temperatures, aeration during fermentation, and drying temperatures, were controlled and their effect on rooibos quality was determined. Aeration improved the quality of rooibos taste (Joubert, 1998) while a fermentation period of 10 to 14 hours at temperatures of 38°C to 42°C delivered the best results in terms of sensory quality (Joubert & De Villiers, 1997). Different mechanisms were proposed to be responsible for these differences in sensory quality based on the findings by Goldstein and Swain (1963) that the degree of oxidation and polymerisation of polyphenols determines their effect on taste and astringency. Joubert and De Villiers (1997) postulated that insufficient oxidation and polymerisation of rooibos polyphenols would affect the sensory properties of the infusion. Also, temperature affects the reaction rate of the enzymatic and chemical oxidation of polyphenols (Hillis & Inoue, 1967), and this may be true for the oxidation of aspalathin and nothofagin to various polymeric brown products (Marais, 1996). Furthermore, temperature influences the formation of degradation products of β-carotene and other flavour precursors which could impact rooibos aroma. It was also found that drying at 40°C resulted in a better aroma than when drying was carried out at 70°C. Finally it was stated that more research is needed to determine the effect of processing conditions on tea volatiles as well as their impact on overall tea flavour (Joubert & De Villiers, 1997).

Although there was some benefit in controlling certain parameters during rooibos processing, implementation of such procedures would be difficult due to practical and financial implications. Therefore, rooibos is still being produced using uncontrolled, traditional processing methods and the variation in quality of samples received for grading is quite considerable. Not one batch will be identical to the next in terms of taste and aroma which makes grading, product differentiation and the definition the term “characteristic” even more challenging.

It has been shown that variation in certain processing parameters affect the flavour and mouthfeel of rooibos infusions; however, the term “quality” was not properly defined or quantified. “Quality” in literature was evaluated by an expert panel of the Rooibos Tea Board (Joubert & De Villiers, 1997; Joubert, 1998) and not by a trained sensory panel using descriptive sensory analysis. The capabilities of such expert tasters is not questioned here; however, it would be useful to describe the quality of an infusion in more precise and defined terms which other researchers, marketers and even consumers can understand and interpret. Despite the importance of the taste, aroma and mouthfeel of rooibos tea no research has been done to accurately describe the sensory attributes of rooibos infusions.

2.5 Pasteurisation of rooibos

Tea in general is considered to be a well-preserved product since it has a low moisture content of 7% to 12% and is, therefore, thought to be microbiologically safe (Lund *et al*., 2000). For this reason the rooibos regulations specify that the moisture content of rooibos may not be more than 10% (Anonymous, 2002). Although microbial counts on rooibos increase during pre-fermentation handling stages and open-air processing (Du Plessis & Roos, 1986), spoilage of the final product is rare due to the low water activity of the tea leaves and stems. Furthermore, boiling water used to prepare the tea infusion would greatly reduce microbial risks. However, should the water temperature be too low, some pathogens and bacterial spores might survive causing illness in susceptible individuals.

During 1984 the rooibos trade took a major blow when an outbreak of *salmonella* disrupted the industry. Exports were stopped, contaminated product was taken off the shelves and a major drop in sales occurred (Snyman, 2000). Consequently, a study was carried out in which high numbers of coliform bacteria including *Escherichia coli* type I and *Salmonella* spp. were recovered from processed tea (Du Plessis & Roos, 1986). Because different batches of tea were blended together to standardise the quality, it led to large-scale contamination and wide-spread occurrence of microbes in the packaged product. Steam pasteurisation of rooibos leaves was subsequently introduced to reduce the microbial load and the number of potential pathogens on fermented rooibos. In order to establish the optimum rooibos moisture content and pasteurisation time a study was done to evaluate different combinations of processing parameters and their effect on microbial numbers and sensory quality. A three-member test panel of the Rooibos Tea Board found that the organoleptic quality of dry rooibos pasteurised at 99.5°C for 2 minutes at moisture level of 10% was acceptable (Du Plessis & Roos, 1986). Currently the major South African rooibos processor steam-pasteurises rooibos at 96°C for 60 seconds. Since this treatment results in a slight increase in the moisture content of the dried rooibos the leaves are dried to a moisture content of less than 10% (J. Basson, Rooibos Ltd., Clanwilliam, South Africa, 2009, personal communication). Microbiological quality is thoroughly monitored through laboratory testing to ensure that the required regulatory standards are met.

Standley *et al*. (2001) and Joubert *et al*. (2005) found that the soluble solids content and total polyphenol content of unpasteurised and pasteurised samples did not differ significantly from each other. However, since the individual samples originated from different plantations and were likely to differ considerably in their composition, the results are not conclusive. Recently, the effect of steam pasteurisation on the colour and the composition of rooibos was re-investigated (Joubert *et al.*, 2010). A 2-minute steam pasteurisation treatment at more than 96°C significantly decreased the soluble solid and the total polyphenol contents as well as the absorbance colour measurement of the rooibos infusions. The levels of most individual flavonoids were also reduced by pasteurisation (Joubert *et al.*, 2010).

The impact of steam pasteurisation on the volatile compounds of rooibos has not yet been analysed. However, Kawakami *et al*. (1993) showed that the composition of the volatile fraction of rooibos depends on the extraction method. The extract of volatiles from rooibos that was brewed for 10 minutes contained only

50 components compared to 123 compounds in the extract prepared using a steam distillation and extraction (SDE) method. Also, the aroma of the brewed extract resembled that of rooibos tea more closely than the aroma of the SDE extract. However, some lactone compounds were decomposed and lost during SDE extraction. These results show that some volatile compounds present in rooibos can be driven off by certain processes involving water and high temperatures, and that the loss of certain volatiles impacts the aroma of a tea infusion.

A number of factors play a role in terms of the release of volatile compounds during steam pasteurisation. These include their specific boiling points and polarities (Ingham *et al*., 1995), and physicochemical interactions between the volatiles and the food or beverage matrix (Kinsella, 1989). The boiling point temperatures and molecular weights of a selection of rooibos volatiles are shown in Table 4. The compounds exhibiting "green" aroma characteristics generally have lower boiling points and molecular weights compared to compounds associated with fruity or floral aromas. "Green" volatiles would, therefore, be driven off quicker during pasteurisation which would influence the aroma, and thereby also the sensory quality of rooibos infusions.

Table 4 Boiling points and molecular weights of selected "green" and "non-green" volatiles in rooibos

Volatiles <u>not</u> associated with green aroma			**Volatiles associated with green aroma**		
Compound	**[1]Boiling Point (°C)**	**[1]Molecular weight**	**Compound**	**[1]Boiling Point (°C)**	**[1]Molecular weight**
Dihydroactinidiolide	296	180.24	β-Cyclocitral	212	152.23
5,6-Epoxy- β-ionone	276	208.30	6-Methyl-5-hepten-2-one	174	126.20
β-Damascenone	275	190.28	*Trans*-2-hexenol	159	100.16
β-Ionone	268	192.30	*Cis*-3-hexenol	157	100.16
Geranyl acetone	247	194.31	3-Heptanone	147	114.19
2-Phenylethyl alcohol	220	122.16	*Trans*-2-hexenal	142	98.14
Guaiacol	206	124.14	Hexanal	131	100.16
Methyl heptadienone	190	124.18	*Trans*-2-pentenal	124	84.12
Benzaldehyde	178	106.12	1-Penten-3-ol	115	86.13
Average	240	160.23	Average	151	106.83

[1]The Good Scent Company (2010)

The effect that steam pasteurisation has on the sensory quality of a rooibos infusion remains unknown, even though anecdotal evidence suggests that it leads to a less prominent flavour. The changes in taste, aroma and mouthfeel that may result from this heat treatment have not yet been accurately described or quantified. It is not known whether the introduction of this process resulted in a beneficial or detrimental change in sensory attributes of the tea, and whether pasteurisation may be a necessary step in producing tea with the typical sensory characteristics of rooibos as perceived nowadays by the consumer.

In contrast to this, the critical importance of firing (high temperature drying) for the development of black tea (*C. sinensis*) flavour is well-recognised (Sanderson *et al*., 1976). Firing is the final stage of black tea processing whereby tea at a moisture content of 45% to 50% is dried to about 3% moisture by blowing hot air at about 90°C through the fermented leaves for about 20 minutes (Wickremasinghe, 1978). Several changes occur during this process including the arrest of fermentation reactions, breakdown of chlorophyll and the formation of polyphenol-protein complexes resulting in a reduction of astringency. Firing also causes a significant loss of extractable solids as well as a decrease in flavan-3-ols (Sanderson *et al*., 1976). These changes all contribute to the change in flavour from green, harsh and strongly astringent (fermented, un-fired tea) to the flowery, slightly green, pleasantly astringent and mild black tea flavour of the fired tea (Sanderson *et al*., 1976). Firing is also said to be essential for the development of black tea aroma because certain volatile compounds are lost during the heat treatment (Wickremasinghe, 1978). Ravichandran and Parthiban (1998) examined the changes in volatile flavour compounds as a result of hot-air drying of black tea leaves which resulted in a decrease in the total amount of volatiles. Also, a major decline of compounds imparting an undesirable grassy odour to the tea was observed, while other compounds, associated with a sweet, floral aroma, increased. Consequently, the aroma quality improved during the drying process as compounds associated with a green flavour were volatilised (Ravichandran & Parthiban, 1998).

Wang *et al*. (2000) compared the quality of green tea infusions prepared from steamed (temperatures of 95-100°C) and roasted (temperatures of 160-230°C) tea leaves, as well as the changes in flavan-3-ol composition and sensory quality of these infusions. The sensory characteristics of a high quality tea were described as a balanced taste of bitterness, astringency and a persistent sweet aftertaste. It was found that the taste of the steamed tea was more bitter, more astringent and less sweet than that of the roasted sample. The levels of phenolic compounds and flavan-3-ols in the steamed tea extract were higher compared to the roasted tea which explains the decrease in bitterness and astringency of the roasted tea infusion. It was concluded that high-temperature treatment results in a greater loss of flavan-3-ols and a concomitant change in sensory qualities of the tea.

Although heat treatment of black and green teas is somewhat different to steam pasteurisation of rooibos, and the chemical composition of *C. sinensis* is quite different from that of *Aspalathus linearis*, comparisons can be made since both processes involve heating of tea leaves at relatively high temperatures. A number of questions can thus be asked: Do similar changes occur in fermented rooibos during pasteurisation comparable to those seen in fired black tea; i.e. is there a loss of volatiles or a decrease in astringency? In which way does the change in the polyphenol and flavonoid levels of a rooibos infusion impact its sensory characteristics (aroma, taste and mouthfeel)? May pasteurisation be essential for the development of "characteristic" rooibos flavour as perceived by the modern consumer? These questions can only be answered by an investigation focused specifically on the differences in the sensory attributes and chemical composition of rooibos infusions prepared from unpasteurised and pasteurised tea leaves.

3. Chemical analysis of rooibos

A number of research groups have analysed rooibos using certain chemical and/or instrumental methods. A summary of selected studies is given in Table 5. Some studies have focused on the characterisation of compounds in rooibos while others have examined the quantification of certain compounds.

Table 5 Selected studies on the composition and chemical parameters of rooibos

	Analysis	Author(s)
Green / unfermented rooibos	• Identification and quantification of the major **flavonoids** by HPLC-DAD and LC-MS/UV-Vis analysis	Schulz *et al*., 2003
	• Development of an NIR spectroscopic method to predict **aspalathin** content	Schulz *et al*., 2003
	• Quantitative characterisation of **major flavonoids** using by HPLC-DAD	Bramati *et al.*, 2003
	• In-situ identification of **aspalathin** and quantification of the **dihydrochalcones** in rooibos plant material by FT-Raman spectroscopy	Baranska *et al*., 2006
	• Identification of **carotenoids** and **lignins** by FT-Raman spectroscopy	Baranska *et al*., 2006
	• Quantification of **aspalathin, nothofagin and soluble solid content** by means of NIRS	Manley *et al.*, 2006
	• UV spectrophotometry as a rapid method for determining the **dihydrochalcone** content and **total polyphenol** content of rooibos water extracts	Joubert *et al*., 2008b
Fermented rooibos	• Gas chromatographic (GC) determination of **volatile components**	Habu *et al*., 1985 Kawakami *et al*.,1993
	• Qualitative characterisation of **phenolic metabolites** by ^{1}H NMR spectroscopy	Rabe *et al*., 1994
	• Transmission **colour** measurement of rooibos extracts using Hunter Lab or CIELAB scales	Joubert, 1995
	• Quantitative determination of **aspalathin** and **nothofagin** by HPLC	Joubert, 1996
	• Objective **colour** measurement of leaf colour in terms of the CIELAB scale	Joubert, 1996
	• Quantitative characterisation of **flavonoid compounds** by HPLC-DAD	Bramati *et al*., 2002
	• Characterisation of **phenolic compounds** using multilayer countercurrent chromatography (MLCCC) and preparative HPLC	Krafczyk & Glomb, 2008

The sensory and visual quality of a rooibos infusion is determined largely by a number of factors including the total polyphenol content, the levels of individual flavonoids, the tannin content and the soluble solids content. Standard methods of analysis for each of these parameters have already been set up and will be discussed briefly.

3.1 Total Polyphenol Content (TP)

A number of analytical techniques have been applied to measure the polyphenol content in plant extracts. Some of these methods make use of the characteristic absorption peaks of phenolic compounds in the ultraviolet range while others exploit the reaction of polyphenols with metal ions or oxidising agents and the subsequent formation of colour complexes. Their radical scavenging activity is another useful characteristic that lends itself to the quantification of these compounds (Beecher *et al*., 1999).

A reliable method that has been used most often for determining the content of all phenolic compounds in rooibos infusions is the Folin-Ciocalteau method of Singleton and Rossi (1965). This method, combined with the method of Peri and Pompei (1971) for tannin determination in wine, was first used by Blommaert and Steenkamp (1978) to determine the tannin content of rooibos tea. Since then the Folin-Ciocalteau method for polyphenol determination has been used extensively for estimating the polyphenol content of rooibos tea. The method relies on a colour-forming reaction that is produced by monohydric phenols, polyphenols, flavonoids, tannins and other readily oxidised substances (Singleton & Rossi, 1965). It, therefore, gives an indication of the level of all phenolic compounds present in a sample. Although the method is non-specific it is nevertheless useful for estimating the polyphenol content of tea (Beecher *at al*., 1999). The method involves spectrophotometric measurements of the colour intensity of the relatively stable blue complex that is formed. Since gallic acid is used as the standard for setting up a standard curve results are expressed in gallic acid equivalents (GAE). Recently the method has been adapted for use in a multiplate reader to quantify the the polyphenol content of rooibos (Joubert *et al*., 2009). The use of 96-well microplates greatly reduces the time of analysis when a large number of samples must be evaluated.

3.2 Flavonoids

The measurement of the individual flavonoid content of food and beverage products is usually done using high-performance liquid chromatography (HPLC) although modern separation systems, such as capillary zone electrophoresis, have also been applied to a limited extent (Merken & Beecher, 2000). A variety of chromatographic techniques can be used to separate, characterise and quantify the individual polyphenols found in tea. It has been said that for routine analysis of black tea, HPLC, using a reversed phase column and ultraviolet-visible detector, has “become the ‘work horse’ instrumental method” (Beecher *et al*., 1999).

Similarly, HPLC has become the method of choice to quantify the level of individual flavonoids present in rooibos infusions and extracts. Joubert (1996) developed the first HPLC method for quantification of rooibos phenolic acids and the major flavonoids including the dihydrochalcones, aspalathin and nothofagin. Separation was achieved on a 250 mm C18 column with a run time of 120 min. Several years later Bramati *et al*. set out to quantify the levels of 10 individual flavonoids (aspalathin, rutin, orientin, iso-orientin, vitexin, iso-vitexin, quercetin, luteolin, chrysoeriol, iso-quercitrin + hyperoside) in fermented (Bramati *et al*., 2002), as well as unfermented rooibos extracts (Bramati *et al*., 2003). In the same year Schulz *et al*. (2003) were

able to improve the sensitivity of the method developed by Joubert (1996), as well as reduce the analysis time to less than half that of the original method. Alongside aspalathin and nothofagin, iso-orientin, orientin, rutin and iso-quercitrin could be resolved by this method. A slightly different approach of characterising rooibos polyphenols was used by Krafczyk and Glomb (2008). They first used multilayer countercurrent chromatography (MLCCC) to separate the phenolic compounds in rooibos extracts, followed by preparative high-performance liquid chromatography (HPLC) to obtain pure flavonoids. This two-step method, however, does not lend itself to the routine analysis of rooibos phenolic compounds. A slightly modified version of the method of Joubert (1996) using a shorter C18 coumn has been applied by several research groups including Joubert *et al.* (2004), Joubert *et al*. (2005) and Pengilly *et al*. (2008) for the quantification of the major rooibos flavonoids. However, this method is not ideal because co-elution of rutin and iso-quercitrin, and aspalathin + phenylpyruvic acid glucoside (PPAG) occurs. To achieve their separation another method with a runtime of 30 min (Joubert *et al.,*2009) must be used resulting in a total runtime of 90 min for the quantification of major flavonoids and PPAG. For this reason, an alternative HPLC method is currently being developed at the ARC Infruitec-Nietvoorbij by which the major monomeric phenolic compounds can be separated and quantified in less than half of the time (T. Beelders, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, 2010, personal communication).

3.3 Tannins

Since tannins play an important role in a number of food and beverage products, including wine and tea, the capacity to analyse and quantify tannins in such products has become an issue of interest not only amongst researchers of various fields such as chemistry, food science, plant physiology and agriculture, but also for the industry. What makes reliable tannin quantification a rather difficult task is their large chemical diversity, as well as the structural similarity of tannins and other phenolic substances resulting in non-tannin components interfering with the tannin measurement. Finding a selective, simple and robust method of tannin quantification has, therefore, been an ongoing pursuit that started as far back as the 1950s (Owades *et al*., 1958). Makkar (1989), Schofield *et al*. (2001) and Herderich and Smith (2005) have reviewed existing methods which can be grouped according to their basis of quantification namely colorimetric, gravimetric, chromatographic and polymeric precipitation analyses (Sarneckis *et al*., 2006).

However, many of these are laborious, costly or not suitable for large sample sets, and disadvantages and limitations have surfaced for each method. Many colorimetric tannin detection methods lack selectivity for tannins (Porter *et al*., 1986; McMurrough & McDowell, 1978; Sun *et al*., 1998) and interference by phenolic and other compounds is commonly encountered. Gravimetric methods, which rely on the precipitation of tannins by metal ions or other compounds, are appealing since no standards are required for calibration, but a lack of sensitivity and low repeatability in samples with low tannin levels are some of the drawbacks of this analytical technique (Reed *et al.*, 1985; Makkar *et al.*, 1995). Chromatographic analyses, primarily HPLC-based, have often been used for tannin analysis (Prieur *et al.*, 1994; Kennedy *et al.*, 2001;

Peng *et al*., 2001; Downey *et al*., 2003); however, these methods necessitate the use of expensive equipment and are thus difficult to implement in an industry setting (Sarneckis *et al*., 2006).

One of the most defining properties of tannins is their ability to precipitate proteins. They readily interact with other biological molecules such as polysaccharides and alkaloids as well as with metal ions (Schofield *et al*., 2001). This characteristic has been successfully exploited for tannin quantification by the selective precipitation of tannins using various polymers such as bovine serum albumin (BSA) (Hagerman & Butler, 1978). Protein precipitation methods have been described as a fast and precise tool for tannin measurement in grapes and wines (Harbertson *et al*., 2003). They are frequently used in settings with limited laboratory facilities since they allow for a large number of samples to be analysed (Hagerman, 1987). Furthermore, compared to other methods tannins quantified by protein precipitation correlated particularly well with the phenomenon of astringency (Kennedy *et al*., 2006). It has been found, however, that many factors can have an impact on the protein precipitation of tannins, e.g. pH, isoelectric point, ionic strength, protein and tannin conformation and temperature (De Freitas & Mateus, 2001). Also, Jensen *et al*. (2008) found that the calculated tannin content of both very diluted and concentrated wine samples were underestimated, and they consequently defined a valid range of the tannin response. Another shortcoming of the method is the interference with absorbance measurements by the added protein precipitant (Sarneckis *et al*., 2006).

Because of these drawbacks Sarneckis *et al*. (2006) developed the methyl cellulose precipitable (MCP) tannin assay, a tannin quantification method using the polymer methyl cellulose to precipitate tannins. This simple and robust assay can tolerate a broad range of pH values and ethanol concentrations and can consequently be adapted for tannin quantification in a large number of plant-derived products including coffee and black and green teas (Mercurio *et al*., 2007). The assay involves centrifugation of the polymer-tannin precipitate and absorbance measurements at 280 nm (A_{280}) of the supernatant solution from control and treatment samples. The difference in A_{280} values of control and treatment samples is converted to monomer equivalents using a suitable standard curve. The MCP tannin assay has been adapted to allow for more efficient, high throughput measurements that use smaller volumes and smaller centrifuges without reducing the reproducibility of the measurement. The absorbance of samples is measured by a multiplate spectrophotometer in a 96-well microplate resulting in a much more time-efficient analysis (Mercurio *et al*., 2007).

Kennedy *et al*. (2006) proposed that because of the complex, heterogeneous nature of wine tannins, tannin concentration values can vary considerably depending on the method of quantification that is used. Mercurio and Smith (2008) set out to compare two precipitation methods for the quantification of grape and wine tannins. Strong correlations were found between the MCP tannin assay and the Adams-Harbertson (A-H) protein precipitation tannin assay. However, they reported significant differences (about three-fold) in the tannin content quantified by the two analytical techniques with the MCP tannin assay precipitating more tannin material than the A-H assay. Harbertson and Downey (2009) also found differences in the

measured tannin content of grape skin determined by MCP and protein (BSA) precipitation techniques. Significantly lower tannin concentrations were calculated using methyl cellulose as a precipitant suggesting that there are differences in the nature of the tannins precipitated by methyl cellulose and protein (BSA). It was concluded that the extraction solvent, the analytical methodology and the complex nature of tannins affected the measurement and that, unless an analytical tannin polymer standard is made available and all methods have been rigorously evaluated, such conflicting and erratic results will continue to hamper reliable tannin quantification.

Tannin quantification in rooibos has not received much attention to date. The tannin content of rooibos plant material is estimated as 3.2% (Reynecke *et al*., 1949) and 4.4% (Blommaert & Steenkamp, 1978) while up to 50% of the dry mass of a rooibos water extract is composed of complex tannin-like substances (Ferreira *et al*., 1998). Joubert (1984) even reported that as much as 80% of the rooibos tea flavonoid content are tannins. Tannin content was determined by precipitating tannins using cinchonine sulphate (Peri & Pompei, 1971) and calculating the difference between total phenolic compounds and non-tannins (Joubert, 1984). Richfield (2008) proposed that the conflicting values obtained for the rooibos tannin content are partly due to differences in the plant material used since tannin levels of plants generally increase with the age of the tissue as well as under physiologically stressful conditions. In a flow-through extraction system the extraction conditions, such as temperature and flow rate of the solvent, were found to affect the measured tannin concentration (Joubert, 1988). Tannins of high molecular weight would have a lower diffusion rate through the plant material and this rate would increase in proportion with temperature. Taking all of the abovementioned limitations of tannin assays into consideration it should be noted that the quantification of tannin in rooibos infusions can only provide an estimate of the actual tannin content of the sample.

3.4 Soluble solids (SS) content

The soluble solids content of a tea sample gives an indication of the strength of the infusion that was prepared from it, and it, therefore, has an impact on the sensory quality of the tea infusion. Joubert (1984) found that the levels of total phenolic compounds, tannins and flavonoids were higher in rooibos leaves than in “stems” (waste material with high stem content) resulting in the SS content of rooibos leaves also being higher compared to that of the stems. Furthermore, it was shown that fermentation of rooibos plant material is accompanied by a decrease in soluble solids as the polymerisation of polyphenolic compounds reduced their solubility (Joubert, 1984). A low level of soluble solids can, therefore, be an indication of a large percentage of tea stems mixed with tea leaves or poor solubility due to over-fermentation.

According to Joubert (1988), who analysed the effect of extraction temperature, extract:tea mass ratio and the flow rate of water on the yield of soluble solids from rooibos in a flow-through batch system, SS content of extracts increased linearly with increasing temperature. The highest temperature that was investigated was 90°C. Extraction of soluble solids was more efficient at lower flow rates due to longer

contact times, as well as higher mass ratios which favoured mass transfer. Joubert and Hansmann (1990) found that the soluble solid content of rooibos extracts obtained by fixed-bed batch extraction first increased and decreased rapidly after which a gradual decline in the extract concentration was seen. After a 5-minute extraction period about 50% of the total amount of soluble solids extracted in 24 min was recovered. Joubert (1990) also examined the effect of extraction time on the extraction of rooibos polyphenolic compounds and found a similar pattern of extraction: The phenolic content of the extract increased at first reaching a maximum after about 3 min at a flow rate of 0.09 m^3/h followed by a sharp decline and a more gradual decrease towards the end of the extraction time. The increase in polyphenol content of soluble solids with time flattened off after about 8 and 5 min for flow rates of 0.09 m^3/h and 0.18 m^3/h respectively. After completion of extraction a higher yield of polyphenols was obtained at a flow rate of 0.09 m^3/h (Joubert, 1990). Furthermore, Jaganyi and Wheeler (2003) showed that the aspalathin concentration of a rooibos infusion initially increased rapidly after which the concentration approached equilibrium after 60 to 90 min. The first order rate constant of aspalathin was determined ($k = 23.4 \times 10^{-4}\ s^{-1}$) and it was found to be five times smaller compared to that of green tea flavanols.

All of these factors affect the level of soluble solids extracted from rooibos leaves and stems when preparing a rooibos infusion and can, therefore, influence the sensory attributes of the tea. Since polymeric substances, such as the tannin-like compounds that have been found in rooibos extracts (Joubert, 1984), have slower diffusion rates than smaller, simpler compounds such as quercetin and luteolin, it may be expected that the ratio of polymeric to monomeric polyphenolic compounds may increase with extraction time (Joubert, 1990). This may influence the taste and astringency of a rooibos infusion.

The AOAC has developed a standard method to determine the water-soluble solid content of tea leaves (AOAC, 1975). This gravimetric method of SS determination has been modified and applied by Joubert (1988), and has since been used and adapted as a routine analysis in rooibos research (e.g. Joubert, 1995; Joubert, 1996; Von Gadow *et al*., 1997; Joubert, 1998; Joubert *et al*., 2005; and Pengilly *et al*., 2008).

3.5 Colour

The colour of a tea infusion is an important parameter that has a significant impact on the perception of its quality. In the literature the colour of both black and rooibos tea infusions has commonly been measured using tristimulus colour parameters (Joubert, 1996; Joubert & De Villiers, 1997; Joubert, 1998; Wang *et al*., 2000; Liang *et al*., 2003; Wang *et al*., 2004; Liang *et al*., 2007; Wang & Ruan, 2009). Joubert (1995) has investigated the relationship between soluble solid content of rooibos extracts and colour parameters as well as the effect of turbidity on colour parameters in order to examine whether objective colour parameters could be used to predict the quality of rooibos tea extracts. Tristimulus colour parameters were measured using a Colorgard 2000 systems with a TM-M transmission attachment. Dilution of rooibos extracts causes a shift from a red-brown to a yellow hue. Also, extracts with a high a* value, corresponding

to a red colour, obtained higher colour ratings by an expert panel. This reflects the importance of a red tint for the visual quality of rooibos infusions.

Absorbance measurements with a spectrophotometer have also been used to quantify the colour of wine and tea. Spectrophotometric measurements at different wavelengths have been used to determine the tint and colour density of red port wines and a scan over the total visible spectrum was said to provide more comprehensive information about the colour of the wines (Bakker & Arnold, 1993). Joubert (1984) quantified the "total colour" (absorbance at 460 nm, A_{460}) as well as the "tint" of rooibos extracts (ratio of A_{450}/A_{550}). It was found that the A_{460} value of rooibos leaves was higher than that of rooibos stems. It was proposed that this was as a result of the higher tannin content of rooibos leaves. Rooibos stem material had a higher A_{450}/A_{550} ratio compared to leaf material indicating that there are differences between rooibos leaves and stems in terms of their phenolic composition. Heating of rooibos extracts resulted in a decrease of both the A_{460} value and the A_{450}/A_{550} ratio, while an increase in the fermentation time caused an increase in the A_{460} value and a decrease in the A_{450}/A_{550} ratio. The extent of polymerisation of the phenolic compounds was most likely responsible for these changes (Joubert, 1984).

In another study absorbance measurements (A_{420}) were used to quantify the effect of heating on the colour (i.e. brown colour formation) of rooibos ice tea samples (Joubert *et al.,* 2009). Heat treatment of ice teas resulted in browning as reflected by a significant increase in absorbance at 420 nm. The increase in absorbance was ascribed to changes in the phenolic composition of rooibos ice teas such as oxidative changes and formation of brown polymers. It may be concluded that spectrophotometric measurements can provide useful information about the colour of rooibos infusions while also reflecting changes in their phenolic composition.

4. Sensory analysis

Sensory analysis can be defined as a scientific discipline used to evoke, measure, analyse and interpret reactions to characteristics of foods and beverages as they are perceived by the senses (Stone & Sidel, 1993). It is based on training a panel of judges on how to analyse a specific product in order to improve their sensitivity, reliability and consistency. The conditions of analysing the product do not usually relate to the normal way it is consumed since the aim is to determine the variation in the product (Stone & Sidel, 1993).

4.1 Descriptive analysis and quantitative descriptive analysis

Descriptive analysis is one of the most useful tools for sensory analysis. It involves the discrimination and description of qualitative (attributes) and quantitative (intensity) sensory characteristics of a product by a trained panel of judges to generate a complete sensory description of a product (Meilgaard *et al*., 1999). The analysis can incorporate all kinds of product attributes such as its flavour (aroma + taste), mouthfeel or texture, or it can focus only on certain individual aspects (Stone, 1992).

Many methods of descriptive analysis have been developed over the years. One of these methods is quantitative descriptive analysis (QDA). This method relies on the ability of a number of assessors to describe perceptions of a product in a reliable and repeatable manner (Stone, 1992). The aim is to train a panel of assessors so that each individual can identify certain product attributes, rate their intensities and establish the order of detection (Stone *et al*., 1974). The basic procedure of QDA can be summarised as follows (Stone, 1992):

- Selection, screening and training of panellists
- Development of flavour and mouthfeel vocabulary by the trained panellists
- Compiling a list of sensory attributes, usually in order of dectection
- Quantifying attribute intensity on an anchored, unstructured line scale
- Data analysis using various statistical techniques

It is critical that panellists use the same frame of reference or comparison when rating product attributes and their intensities. For this reason, extensive panel training must be conducted to ensure that the panel is calibrated by developing definitions and/or providing concrete reference standards for each perceived attribute. Reference standards may be food and beverage products, chemicals or other substances which communicate the concept of the product attributes and ensure that panellists have the same understanding of the language used to describe the attributes (Drake & Civille, 2002). Although reference standards may not be identical to the perceived product attribute they are useful for calibrating the sense of smell or taste of the assessors since the reference standards remain unchanged throughout the training.

References can be qualitative, quantitative or both. Qualitative reference standards, the most important training component, demonstrate the nature of an attribute, while quantitative reference standards, which are not always used during QDA training, represent the upper intensity limit for a specific attribute. This maximum intensity becomes the reference point that panellists can refer to when rating the intensity of a specific product attribute. By using reference standards panel variability is reduced because it prevents panellists from using their own criteria to judge attributes and intensities (Munoz & Civille, 1998).

Since panel performance is a deciding factor that affects the reliability of the results it should be carefully evaluated and monitored throughout the analysis. Several practices are recommended to improve panel performance including careful screening and selection of panellists, a comprehensive training program and scaling standardisation (Drake & Civille, 2002). Precision of the panel reflects the variability of the scores allocated to replicates of one sample and is an important aspect regarding panel performance (Carbonell *et al*., 2007). Homogeneity among panellists in their scoring paths is another feature of panel performance that should be monitored. This also includes the level of use of the scales: some panellists use the full length while others use only a part of the scale (Carbonell *et al*., 2007). Statistical techniques and software tools (e.g. PanelCheck, Nofima Mat, Norway) can be used to evaluate the parameters relating to panel performance and in this way the effectiveness and reliability of the panel can be verified. Judge reproducibility and reliability can be analysed by examining the internal consistency (Judge*Treatment interaction) and temporal stability (Judge*Replication interaction) associated with the panel members

(Prichett-Mangan, 1992; Carbonell *et al*., 2007). In this way, judges who are not able to rate samples reliably can be identified and removed.

There are numerous applications of QDA including the identification, quantification and documentation of sensory characteristics for research guidance or product maintenance, the correlation of chemical or instrumental measurements with sensory attributes, defining specifications for controlling product quality and consistency, and examination of changes in sensory attributes during the production process (Stone, 1992; Meilgaard *et al*., 1999). Often there are no alternative analytical methods which would provide the information that is generated by descriptive analysis (Hootman, 1992).

4.2 Sensory lexicons

A sensory lexicon can be defined as a set of terms used to describe the sensory attributes of a product along with reference standards and definitions for clarification (Drake & Civille, 2002). QDA is used to generate and quantify the terms of which the sensory lexicon will be composed. The major steps for setting up a sensory lexicon include collecting a product frame of reference, generating appropriate descriptive terms, reviewing reference standards and examples, and developing the final list of descriptors. When selecting the samples used for descriptor development it is essential to choose samples that cover the widest range of possible characteristics in that product category so that all potential product variability is captured in the lexicon (Drake & Civille, 2002). Once a list of terms has been generated and attributes have been compared to references for clarification, the list of terms is reduced by merging similar terms, eliminating redundant terms and listing the attributes in order of appearance (Drake & Civille, 2002). An excerpt from a sensory lexicon set up for green tea is shown in Fig. 3 (Lee & Chambers, 2007).

In order to generate a reliable and pertinent lexicon several aspects must be taken into consideration: attribute intensities must be anchored consistently; terms must be precise, clear and appropriately defined and/or reference standards provided. Also, terms must be discriminating (i.e. able to differentiate between products), descriptive, relevant and non-redundant (Drake & Civille, 2002; Kreutzmann *et al*., 2007).

Many examples of the sensory characterisation of various products can be found and sensory profiles have already been set up for a wide variety of products by using QDA. Several examples of sensory lexicons or sensory profiles for specific products are shown in Table 6. Sensory profiling of tea is limited to green tea and so far no sensory lexicon has been set up for rooibos tea. The only sensory-related research that has been done on rooibos has been conducted by Dos *et al.* (2005) who analysed the effect of a number of different factors on the sensory attributes and preference of rooibos tea. The effects of gender and age of the assessor, serving size of the tea sample, tea type (natural vs. vanilla-flavoured rooibos), tea-making technique and water quality were examined. Age was a significant factor that influenced the the degree of liking of the tea taste and aroma. Also, the use of spring water for tea-making was recommended since tea prepared with spring water was preferred over tea prepared with tap water.

TABLE 1.
DEFINITIONS OF ATTRIBUTES FOR GREEN TEA EVALUATION

Attributes	Definition	Reference
Green	Sharp, slightly pungent aromatics associated with green plant/vegetable matter, such as asparagus, Brussels sprouts, celery, green beans, parsley, spinach, etc.	Fresh parsley water = 9.0 (flavor) 25 g of fresh parsley, rinse, chop, and add 300 mL of water. Let it sit for 15 min. Filter and serve liquid part
Floral/perfumy	The somewhat sweet aromatics generally associated with fruit and flowers	Geraniol Pure = 8.0 (aroma) Put 1 drop geraniol in 200 mL of distilled water in a large-size snifter. Cover
Fruity	A sweet, floral, aromatic blend, reminiscent of variety of ripe fruits such as apricots, peaches	Blackberry WONF 3RA654 (McCormick & Wild Inc., Hunt Valley, MA) (character reference) Place one drop of chemical on a cotton ball in a medium size snifter. Cover
Astringent	The drying, puckering sensation on the tongue and other mouth surfaces	0.03% "Alum solution" Alum (McCormick & Co., Inc.) = 1.5 0.050% "Alum solution" Alum = 2.5 0.10% "Alum solution" Alum = 5.0 0.15% "Alum solution" Alum = 7.5

Figure 3 Excerpt from a green tea sensory lexicon (Lee & Chambers, 2007).

Table 6 Examples of sensory profiles of various food and beverage products

Product	Reference
Beer	Mecredy *et al*., 1974
Chicken flavour	Lyon, 1987
Wine	Noble *et al*., 1984
	Mirarefi *et al*., 2004
Fish	Prell & Sawyer, 1988
	Chambers & Robel, 1993
Cheese	Piggot & Mowat, 1991
	Drake *et al.,* 2001
	Retiveau *et* al*.,* 2005
Champagne wines	Vannier *et al*., 1999
Chocolate ice cream	Prindiville *et al*., 1999, 2000
Soymilk	Day N'Kouka *et al.,* 2004
	Chambers *et al.,* 2006
Honey	Galan-Soldevilla *et al*., 2005
Mandarin juices	Carbonell *et al*., 2007
Carrots	Kreutzmann *et al*., 2007
Green tea	Lee & Chambers, 2007
Grapes	Le Moigne *et al*., 2008

4.3 Applications of sensory lexicons

The importance of sensory lexicons is often underestimated and there are a number of useful applications of such profiles for the industry, for processors, researchers and consumers (Lee & Chambers, 2007). Applications include the correlation of sensory data with instrumental, chemical or physical measurements, especially when key flavour attributes are represented by a group of substances present in small amounts. Chemical analyses would, therefore, be ineffective in providing a clear picture of the effect on the sensory characteristics of the product (Drake & Civille, 2002). Furthermore, sensory lexicons provide a means of describing and discriminating between products which makes them a useful tool for the industry when comparing and monitoring products and product consistency for quality control purposes. They can also be used for profiling new and competitive products and are commonly used in the food and beverage industry

throughout the product development process for benchmarking flavour characteristics during research and development, developing flavours in prototypes, determining drivers of liking when creating new product formulations, and for monitoring product attributes during production (Drake & Civille, 2002).

Sensory characterisation can also be used for various research purposes. Examples of such applications include the following: Examining the maturation and aging of Cheddar cheese (Piggot & Mowat, 1991; Muir & Hunter, 1992), determining the effect of starter culture on Cheddar cheese flavour (Drake *et al*., 1996), developing a meat-like process flavouring made from vegetable protein (Wu *et al*., 2000), differentiating fluid milks and analysing the effects of fat content, storage and other processing conditions on the sensory attributes of milk (Lawless & Claassen, 1993; Phillips *et al*., 1995; Watson & McEwan 1995; Bom Frost *et al*., 2001; Chapman *et al*., 2001), and discriminating between champagne wine samples according to maturity and production origin (Vannier *et al*., 1999). These examples demonstrate the value of sensory profiles to researchers as well as to the industry.

4.4 Sensory wheels

Sensory attributes can be arranged in a wheel format to form a sensory wheel which is a simple and more convenient representation of the product characteristics. In a flavour wheel there are usually two levels of attributes. The terms near the centre of the circle are the more general, basic characteristics while the more specific descriptive attributes are found on the outer ring of the circle (Lawless & Heymann, 1998). Sensory wheels can be set up for flavour, aroma or even mouthfeel attributes or these attributes can be combined in one sensory wheel. An examples of a flavour wheel for wine is shown in Fig. 4 (Noble, 1984).

Sensory wheels were developed for quality control and product development purposes to communicate clear terminology. The development of the beer flavour wheel 30 years ago was an important milestone for the beer industry. It provided a clear, accepted set of terms used for sensory analysis of beer and is still in use today (Schmelzle, 2009). A wine aroma wheel was generated in 1984 (Noble *et al.*, 1984) to facilitate communication among winemakers, marketing personnel, wine researchers and wine writers, as well as consumers, and it was received rather positively by members of the wine industry (Noble *et al*., 1987). Furthermore, Gawel *et al.* (2000) have assembled a mouthfeel wheel which summarises terminology that can be used to describe the mouthfeel sensations elicited by red wines. A flavour wheel has also been developed for black tea as shown in Fig. 5 (Bhuyan & Borah, 2001).

To be of use to members of the rooibos industry the attributes described in its sensory lexicon should be arranged in the form of a sensory wheel since it is more convenient and can be used more easily by rooibos processors, graders, extract producers and flavour companies.

Figure 4 Flavour wheel for wine with three levels of sensory attributes (Noble, 1984).

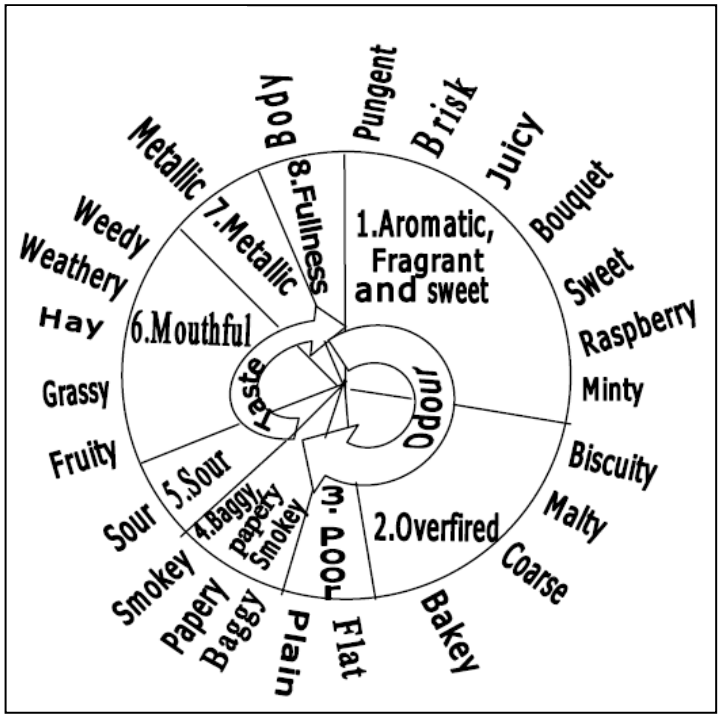


Figure 5 Black tea flavour wheel illustrating the international flavour terminology for tea as accepted by the Indian Tea Research Association (Bhuyan & Borah, 2001).

5. Statistical techniques

Sensory studies mostly involve more than one response variable since multiple variables are measured for each sample. Sensory panel data are always presented in the format of a three-way table with assessors, samples and attributes representing the three dimensions. The objective of a study is then to determine how the different measurements relate to each other, i.e. to examine the differences and similarities between samples and assessors, as well as to analyse the correlation structure among the attributes. Analysing sensory data is often problematic because of the individual differences between the assessors. The perception of a sample, the understanding of the attributes and the use of the scales will, to a certain extent, be different for the different panellists. A powerful model describing sensory data must then be able to handle such differences, and to distinguish between assessor-specific variation and sample-specific variation (Bro *et al.,* 2008).

There is a range of multivariate statistical analyses that have become important tools for evaluating sensory data. A number of different approaches have been taken to generate useful results (Meilgaard *et al*., 1999). Multivariate analysis of variance (MANOVA) can be used for testing the effect of samples and/or assessors for all attributes simultaneously. More commonly, however, Generalised Procrustes Analysis (GPA) is applied to sensory data where each assessor slice is treated as a matrix, followed by a Principal Component Analysis (PCA) of the average or consensus matrix. Regular PCA involves the analysis of all individual sensory profiles and subsequent ANOVA of the most significant components (Luciano & Næs, 2009). Using PCA the positioning of the samples with respect to each other and their characterising attributes can be displayed (Mirarefi *et al*., 2004). Also, when setting up a sensory lexicon, PCA can be used to determine whether individual sensory attributes can be combined into a smaller set of components so that redundant terms are eliminated (Lee & Chambers, 2007).

Sensory data are frequently averaged over the assessors to reduce the three dimensions of the data by one dimension. Although this results in a more simplified data structure for further analyses, potentially significant information and insight about the individual differences among the assessors are lost (Dahl *et al*., 2008). Because of these limitations three-way factor analyses have been developed that are able to analyse all three dimensions simultaneously. An example of this approach is Parallel Factor analysis (PARAFAC) (Luciano & Næs, 2009). Although PCA and PARAFAC produce similar interpretations of sensory data, PCA assumes that there are no significant individual differences between assessors, while PARAFAC takes into consideration the fact that the assessors are not all equal. Therefore, PARAFAC is better able to deal with variation found in sensory data and consequently generates a more meaningful picture of the patterns within the data set (Bro *et al*., 2008).

Correlation analysis, PCA and cluster analysis are used to study data in which all variables are equal in status while regression analysis, principal component regression, partial least squares (PLS) and discriminant analysis (DA) apply when variables in a data set are classified as independent or dependent, with the aim of predicting the value of the dependent using the independent variables (Meilgaard *et al*., 1999). These

techniques can be used to find relationships between attributes as well as to visualise the relationships between the different samples. It is generally recommended that more than one of these statistical methods should be used to analyse multivariate data since each one generates a slightly different picture of certain correlations and relationships that are hidden in the data sets (Palmer, 1974).

6. Tea taste, tea aroma and their link to tea quality

6.1 Polyphenolic compounds

Polyphenols are secondary metabolites that are widely distributed in the plant kingdom. These compounds can be grouped into several classes including phenolic acids, flavonoids and lignans (El Gharra, 2009). Proanthocyanidins, often referred to as condensed tannins, can be defined as complex, soluble, phenolic substances (Cabarello *et al*., 2003) that readily precipitate proteins. They are, therefore, often responsible for the astringent character of certain fruit and beverage products (e.g. grapes, apples, wine, beer and tea) (El Gharra, 2009). Flavonoids are polyphenols that are found often in vascular plants, fruits, vegetables, grains and tea. There are thousands of flavonoids which differ in their chemical structure and characteristics. They include anthocyanidins, catechins, flavanones, flavonols and flavones (Merken & Beecher, 2000). A rooibos infusion is characterised by a wide variety of such phenolic compounds, ranging from simple phenolic acids to complex oligomeric flavanols and lignans as summarised in Table 2.

6.2 Taste and mouthfeel propterties of polyphenols

It is well known that phenolic compounds generally contribute to the taste of numerous food products of plant and animal origin. Polyphenols often contribute to a sweet or bitter taste or astringent mouthfeel depending on their solubility and structure (Cabarello *et al*., 2003). This also applies to the polyphenolic compounds found in black tea, green tea and rooibos tea infusions. The combination of flavonoids and other phenolic compounds in rooibos is believed to be largely responsible for the unique taste of a rooibos infusion. However, to date, no research has investigated this aspect except for a study done by Reichelt *et al.* (2010a). They developed a novel method called LC Taste® by which the separation of compounds by High Temperature Liquid Chromatography is combined with sensory analysis based on a similar concept as gas chromatography olfactometry (GC-O). An extract of unfermented rooibos tea was fractionated followed by sensory analysis of the fractions by trained assessors. Several fractions had “bitter, tea-like and herbal notes” while others exhibited a “sweetish, honey-like taste” (Table 7).

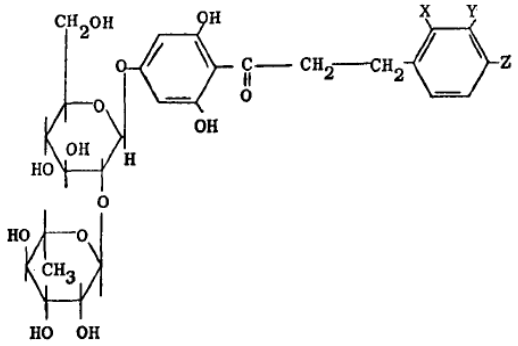
Table 7 Sensory evaluation of rooibos tea fractions obtained by LC Taste®

Fraction	Taste quality	Compound
1	Sweet (weak)	
2	Herbal, tea (weak)	
3	Bitter, plastic	
4	Bitter, herbal, woody, musty	
5	Fruity, herbal, tea-like	
6	Herbal, musty, bitter, sweetish (weak)	Aspalathin
7	More bitter	
8	Bitter	
9	Bitter	
10	Bitter	
11	Bitter, tea-like	Nothofagin
12	Bitter, sweeter, tea-like	
13	Bitter, earthy, woody	
14	Fruity, sweet, bitter, honey, raspberry	
15	Sweetish, bitter, honey, tea-like	
16	Green, bitter, hay-like	

Reichelt *et al*., 2010a

Rooibos is generally described as having a naturally sweet taste. It has been suggested that the dihydrochalcone, aspalathin, which has only been isolated from rooibos (Joubert & Schulz, 2006), may be largely responsible for this sweetness (Rabe *et al*., 1994) since it is well known that some dihydrochalcones have sweet taste characteristics and are even used as sweeteners (Shahidi & Naczk, 2004). The onset of sweetness of dihydrochalcone sweeteners is said to be quite slow but a lingering sweetness persists in the mouth (DuBois *et al*., 1981). Furthermore, it has been found that the threshold values for the dihydrochalcone neohesperidin dihydrochalcone is very low (0.0006-0.00076% w/v) and that its sweetness rating is about 1000 times that of sucrose (Inglett *et al*., 1969). Dihydrochalcones can thus be intensely sweet and powerful taste compounds even at very low concentrations. However, not all dihydrochalcones necessarily have a sweet taste (Fig. 6). Sweetness results from a combination of certain structural features such as the number and position of hydroxyl groups as well as the presence of other groups such as OCH_3 (Inglett *et al*., 1969). Before the recent study by Reichelt *et al.* (2010a) the sensory character of the dihydrochalcones aspalathin and nothofagin was unknown. The study revealed that the fraction of green rooibos known to contain aspalathin only had a “weak, sweetish taste” and “herbal, musty and bitter notes”, while a “bitter, tea-like” taste quality was associated with the fraction containing nothofagin. Sensory analysis of a pure aspalathin solution with a concentration of 100 ppm confirmed that this compound is not associated with a sweet taste. The compound(s) responsible for the natural sweetness of rooibos, therefore, remain to be discovered.

Some compounds that do not exhibit strong intrinsic taste characteristics may, however, have taste modulating effects (Reichelt *et al*., 2010b). Certain compounds, for example, in Yerba Santa (*Eriodictyon californicum*) extracts are well known for their bitter masking properties (Reichelt *et al*., 2010b). Such compounds would thus be able to significantly alter the taste characteristics and thereby the sensory quality of certain products. Reichelt *et al.* (2010a) investigated whether certain rooibos fractions showed taste modulating effects, i.e. sweetness-enhancing or bitter-masking properties, but none of the fractions were found to contain taste modulating compounds. It should be noted that fractions of green rooibos extracts were analysed. Since fermentation of rooibos plant material involves complex oxidation reactions and the formation of complex polymeric compounds (Koeppen & Roux, 1965; Marais *et al*, 2000; Krafczyk & Glomb, 2008) it may be expected that the fractions obtained from fermented rooibos may have different sensory characteristics compared to those characterised for unfermented rooibos fractions.



X	Y	Z	Sweetness
H	H	H	0[1]
OH	H	H	0
H	OH	H	110
H	H	OH	110
H	OH	OH	0[1]
H	OCH_3	OH	0[1]
H	OH	OCH_3	950
H	OH	OCH_2CH_3	1,100
H	OH	$OCH_2CH_2CH_3$	2,000

Figure 6 Sweetener specificity of selected dihydrochalcones (Inglett *et al*., 1969).

When examining the effect that certain polyphenols have on the taste of foods or beverages it is also important to consider that different compounds have different taste thresholds. This means that the concentration of a compound does not necessarily determine the impact it has on the sensory properties of the product. For example, the taste thresholds for individual phenolic acids found in food can range from 30 ppm (protochatechuic acid) to 240 ppm (syringic acid) (Shahidi & Naczk, 1995). It has also been found that a combination of phenolic acids may produce a synergistic effect which considerably lowers the detection thresholds of the individual acids: The bitter and astringent taste of *p*-coumaric acid and the sour taste of ferulic acid were perceived at 48 ppm and 90 ppm respectively, while the combination of the two acids resulted in a sour and bitter taste at 20 ppm (Shahidi & Naczk, 1995). The interplay of different polyphenolic compounds must, therefore, be considered when evaluating the sensory characteristics of a combination of compounds in a food or beverage product.

Polyphenolic compounds, especially tannins with molecular weights between 500 and 3000 (Bajec & Pickering, 2008), are well known to cause astringency, a complex phenomenon which has been defined as "the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins" (ASTM, 1995). The mechanism of astringency has been extensively reviewed; however, there still exist widely differing opinions regarding this matter. It is proposed that precipitation of salivary proteins, or more specifically proline-rich proteins, and/or their binding to polyphenolic compounds reduces oral lubrication resulting in an increase in the perceived friction in the mouth cavity (Bennick, 2002). It has also been found that procyanidins bind directly to oral epithelial cells and that binding is affected by concentration, pH and temperature (Payne *et al*., 2009). This means that even phenolic compounds of relatively low molecular weight, such as flavan-3-ol monomers, dimers and trimers and hydroxybenzoic acids, may elicit an astringent mouthfeel response (Lesscchaeve & Noble, 2005).

While simple phenols may bind weakly to proteins, it is generally accepted that astringency increases with the degree of galloylation and polymerisation of the phenolic compounds (Bajec & Pickering, 2008). However, should the molecular weight become too high the polymeric compound becomes insoluble, losing its ability to bind to proteins, and thereby also to impart astringency. Furthermore, it has been established that even small differences in the configuration of flavonoids can lead to significant differences in their sensory properties: Chirality, bond location and the identity of monomeric units have all been found to have a significant effect on bitterness and astringency (Lesschaeve & Noble, 2005). Epicatechin, for example, is more bitter and astringent than its chiral isomer, catechin (Lesschaeve & Noble, 2005). The presence of other compounds including polysaccharides as well as sucrose and aspartame can also influence astringency (Lesschaeve & Noble, 2005; Troszyńska *et al.,* 2010).

Compared to black tea, rooibos has a considerably lower tannin level and consequently also a substantially less astringent mouthfeel. Nevertheless, astringency can still be perceived indicating that certain components in a rooibos infusion are capable of inducing an astringent mouthfeel. It is likely that the tannins in rooibos, such as procyanidin B3, are largely responsible for the effect. Some of the simpler polyphenolic compounds may also contribute to the astringency of an infusion such as (+)-catechin. This flavan-3-ol is present in rooibos at low levels (Ferreira *et al*., 1995; Krafczyk & Glomb, 2008) and has been found to impart an astringent oral sensation at a threshold concentration of 410 µmol/ml water (Scharbert *et al*., 2004).

6.3 A comparison: Black tea taste

A large number of studies have been done to investigate which non-volatile compounds are responsible for typical black tea taste. Millin *et al*. (1969) suggested two approaches to this task. Firstly, the preparation of pure samples containing all components present in tea at their normal concentrations and describing the taste of these samples was proposed. This is a complex endeavour since there are at least 80 non-volatile components and the authors suggested disregarding those substances present at concentrations below their

threshold values. The second approach involves the fractionation of the components of an infusion, evaluating the taste of each of these fractions alone or in combination, and subfractionating those fractions with a significant taste impact. Some of the conclusions from these approaches are as follows: Flavanol and flavonol components produced a slightly astringent and metallic taste; simple monomeric phenolic compounds did not significantly affect the tea taste; theaflavin and other oxidation products were said to be largely responsible for astringency; the polysaccharide fraction had a malty taste; numerous non-volatile substances played no direct role in the tea taste; and the molecular weight distribution of the oxidation products and their association with proteins impacted their effect on sensory quality. Finally, it was stated that "for a tea of good quality a correct balance of these substances is necessary" (Millin *et al*., 1969).

Using a similar approach, Sanderson *et al.* (1976) analysed a black tea infusion chemically and sensorially to reveal the chemistry underlying the taste of black tea. An infusion was fractionated and each fraction was analysed in order to identify the compounds responsible for each component of black tea taste. It was found that polyphenols are essential elements of the taste of black tea infusions while caffeine was responsible for the bitter taste. Polyphenols were also found to be responsible for causing astringency, a characteristic attribute of black tea (Sanderson, 1976).

Several other studies have since tried to elucidate the correlation between the taste of and the compounds found in a black tea infusion. The results of all of these studies have been quite contradictory. Some researchers found the theaflavins and thearubigins to be important (Collier *et al.*, 1973; Robertson & Derek, 1983) while others have revealed that several flavan-3-ols are the most significant taste compounds (Scharbert *et al*., 2004; Scharbert & Hofmann, 2005). Scharbert and Hofmann (2005) have investigated the impact of 51 black tea taste compounds by calculating their dose-over-threshold (Dot) factors (the ratio of the concentration and the taste recognition threshold of a compound), preparing a solution of the 51 compounds and evaluating this solution using sensory analysis. They were able to group all components in the black tea infusion into six groups according to their effect on taste and mouthfeel, e.g. astringent, bitter, sour, sweet and umami taste compounds. After it was found that the artificial reconstituted cocktail did not differ significantly from an authentic tea infusion, taste omission experiments were performed. These results led to a reduced, simplified solution containing only the 12 most significant components (caffeine, flavonol-3-glycosides and catechins). Once again it was determined that the taste profile of this solution did not differ significantly from the complete solution containing 51 compounds. These results are significant since it was clearly demonstrated that only a small number of the many non-volatile compounds found in a tea solution actually contribute to black tea taste.

Regarding the progress made and the extent of research done on black tea taste it becomes clear that no easy solution may be anticipated when investigating the taste of rooibos tea on a molecular level. No similar fractionation studies or taste reconstitution and omission experiments have as yet been conducted for rooibos infusions. It is not known what impact the different phenolic compounds and other components of the infusion have on the sensory properties of the tea. Furthermore, Dot values have not yet been

calculated for any of the compounds found in rooibos. Evidently, considerable gaps exist in the understanding of rooibos tea taste.

6.4 Amino acids, proteins and polysaccharides

A significant part of the total soluble solids of a black tea infusion is composed of amino acids and peptides (14%), as well as simple carbohydrates (11%) (Millin *et al*., 1969). It is well known that amino acids are often associated with bitter or sweet-tasting properties (Kawai & Hayakawa, 2005). Theanine, a unique amino acid found only in *C. sinensis*, makes up half of the weight of amino acids (Millin *et al*., 1969). It has been boldly stated that it is the main component responsible for the umami-like taste of tea (Sakato, 1957; Shu *et al.,* 2006) and that it exhibits sweet, sweetish-brothy or mellow taste qualities (Jujena *et al*., 1999; Wang *et al.,* 2010). However, there appears to be some discrepancy in these claims since Millin *et al*. (1969) found that theanine was present at levels close to its detection threshold since only 25% of a trained sensory panel could detect it. Scharbert and Hofmann (2005) also stated that "theanine does not exhibit any sweet or umami-like taste as reported in literature" after it had been established that the amino acid was far below its taste threshold concentration. Millin *et al*. (1969) proposed that the amino acid might serve as a flavour potentiator or interact with other components, and that protein or peptide material found in black tea may associate with certain phenolic compounds changing the taste characteristics of an infusion. The association of tea protein with phenolic compounds was confirmed by Wu & Bird (2010). It is also known that black tea polysaccharides are composed of a polysaccharide part and a protein part consisting of different amino acids (mainly valine, alanine, glycine and glutamic acid) (Wang *et al*., 2008a). In addition, amino acids are likely to act as important precursors for volatile compounds of black tea aroma which would have an impact on the aroma characteristics of an infusion (Millin *et al*., 1969).

Not only are there discrepancies in the findings relating to black tea amino acids, but results for polysaccharides in black tea infusions are also inconsistent to a large extent. As mentioned before, the low molecular weight fraction of polysaccharides was found to impart a certain "maltiness" to the taste (Millin *et al*., 1969), while Scharbert and Hofmann (2005) found that soluble carbohydrates did not have any impact on flavour.

Currently, very little is known about the protein and carbohydrate content of rooibos infusions. It is not know whether *A. linearis* may contain unique or rare amino acids, such as theanine, and whether these have a significant impact on the taste of the infusion. The composition of rooibos polysaccharides has been examined by Nakano *et al.* (1997) and Pengilly *et al.* (2008). The majority of sugar units of the polysaccharides in the fermented plant material were glucose and xylose (Pengilly *et al.,* 2008) while Nakano *et al.* (1997) also found a significant amount of mannose (Table 8). It should be noted that the tea analysed by Pengilly *et al*. (2008) was subjected to enzyme hydrolysis so that the values obtained also reflect the composition of insoluble polysaccharides. Quantitative data for rooibos polysaccharides and information on their sensory character have not yet been encountered in literature.

Table 8 Sugar components of rooibos polysaccharides

Monosaccharide	Percentage of total neutral sugars	
Glucose	66.8 [a]	63.8 [b]
Xylose	20.6 [a]	10.6 [b]
Arabinose	4.2 [a]	
Galactose	3.5 [a]	10.2 [b]
Mannose	2.6 [a]	16.3 [b]
Rhamnose	2.0 [a]	
Fucose	0.3 [a]	

[a] Pengilly *et al.*, 2008

[b] Nakano *et al.*, 1997

6.5 Volatile compounds and aroma

The importance of volatile compounds and olfaction in relation to flavour perception has been emphasised and elucidated by numerous authors (Sanderson *et al*., 1976; Dutta *et al*., 2003a). It has even been stated that it is the number and the ratio of volatile components that determine the quality of tea (Dutta *et al*., 2003a).

The aroma characteristics of green rooibos are often described as grassy, hay-like or resinous, while fermented rooibos tea aroma is described as sweet, honey-like and caramel. It follows that a considerable change in the type and level of volatile compounds must occur during rooibos fermentation. More than 120 volatile substances have been identified in rooibos tea extracts (Joubert & Schulz, 2006). However, olfactory studies aimed at identifying those volatiles responsible for the aroma attributes of rooibos tea infusions have not been carried out. Some volatile compounds found in rooibos, such as ionones, are known to have a floral to woody aroma (Arctander, 1969), while a grassy aroma is associated with *cis*-3-hexenal and *trans*-3-hexenal. Phenylacetaldehyde has a sweet or clover aroma (Nursten & Woolfe, 1972) and damascenone is related to a rotting grass/compost aroma (Ames & MacLeod, 1990). Other descriptions include sweet and honey-like (Kumazawa & Masuda, 2001), apple, rose and hay (Table 9).

Each volatile compound has its own detection threshold value and will only contribute to the aroma of a product if its concentration exceeds this treshold concentration. The odour impact of any volatile compound thus depends on the ratio of its concentration to its threshold concentration. Table 9 summarises the aromas associated with the major components of a rooibos tea vacuum steam distillate oil (Habu *et al.*, 1985) and the threshold values of the individual compounds. All of these odour notes can contribute to the aroma of a rooibos infusion depending on the concentration and detection threshold levels of the volatile compounds.

Table 9 Aroma attributes and threshold values of the major volatile compounds in a rooibos distillate oil

Name	Odour [a,b]	Odour threshold [c] (ppb)
Benzaldehyde	almond, burnt sugar, sweet, bitter almonds, marzipan	350 – 3500
β-Damascenone	apple, rose, honey, fruity, floral, rotting grass	0.002
Dihydroactinidiolide	black tea, hay, peach, gardenia sweet, floral, tea-like, fruity, tobacco	Not available in literature
Geranyl acetone	magnolia, green, fresh-floral, sweet-rosy	60
Guaiacol	smoke-like, sweet, medicinal, antiseptic	3 – 21
5,6-epoxy- β -ionone	fruity, sweet, woody, raspberry, floral, hay-like	Not available in literature
β-Ionone	violet, floral, raspberry, powerful sweet, woody	0.007
Methyl heptadienone	spicy, cinnamon, coconut, slightly fruity, sweet	380
6-Methyl-5-hepten-2-one	green-fruity, green-banana, unripe berry, oily green, pungent-herbaceous	50
2-Phenylethyl alcohol	honey, rose, sweet, spice, lilac, hyacinth-like	750 – 1100

[a] Arctander, 1969
[b] Acree & Arn, 2004
[c] Leffingwell & Associates, 2008

Hongsoongnern and Chambers (2008) have analysed the aroma characteristics of a number of compounds that have been associated with “green” aroma notes. Several of these volatiles have also been found in the steam distillate of rooibos (Habu *et al.*, 1985), and their odour characteristics are given in Table 10. It was found that the aroma character of some of these compounds changed at different concentrations indicating that not only the threshold value but also the concentration of a volatile compound must be considered when evaluating the aroma of a product (Hongsoongnern & Chambers, 2008).

Table 10 Volatile compounds in rooibos associated with a "green" aroma

Volatile Compound	Odour characteristics
Hexanal[a]	Musty, earthy, green-grassy/leafy
***Trans*-2-hexenal**[a]	green-grassy/leafy, sweet, almond
***Cis*-3-hexenol**[a]	green-grassy/leafy , green-viney, musty/earthy
***Trans*-2-hexenol**[a]	green-peapod, green-viney
1-penten-3-ol[a]	green-grassy/leafy, sweet
***Trans*-2-pentenal**[a]	Green-grassy/leafy, sweet
3-heptanone[a]	Minty, artificial banana
β-cyclocitral[b]	Green / cut grass

[a] Hongsoongnern & Chambers, 2008
[b] Dravnieks, 1985

Volatile compounds do not only provide valuable information about the flavour of tea; they may also give insight into how the tea was processed. This was demonstrated by Ravichandran and Parthiban (1998) who examined the changes in two groups of volatiles during black tea manufacturing. Group 1 comprised volatiles formed by fatty acid degradation which impart an undesirable grassy aroma, while Group 2 compounds, associated with a sweet floral aroma, were derived from the degradation of carotenoids, terpenoid glycosides or amino acids. The total of these compounds as well as the ratio of the two groups was significantly different between green leaf material and fermented tea. Togari *et al*. (1995a) as well as Wang *et al*. (2008b) also showed that it is possible to distinguish between unfermented and fermented teas (i.e. green, Oolong and black teas) on the basis of their volatile compounds. Togari *et al*. (1995a) stated that changes in certain volatiles (e.g. *trans*-2-hexenal) could be used as an indicator for the degree of fermentation, and consequently as an indicator for the quality control of tea. Wang *et al*. (2008b) compared the total concentrations of five volatile flavour compounds (*trans*-2-hexenal, benzaldehyde, methyl-5-hepten-2-one, methyl salicylate and indole) and showed that the sum of these volatiles was significantly higher in fully fermented tea (i.e. black tea). It was also found that fermentation may cause the loss of grassy and green flavours whereas the fruity and floral character increases.

The lack of literature on similar studies for rooibos suggest that a lack of understanding exists of the link between tea quality and rooibos volatiles. The change of volatile compounds during the different rooibos processing stages has not yet been analysed. Furthermore, so far no compounds have been identified that may reflect the degree of fermentation and consequently the flavour quality of rooibos.

6.6 Predicting tea quality using chemical and instrumental data

There is a vast number of studies that have examined the use of objective quality parameters to predict the sensory quality of black and green teas (Table 11).

The difficulty of correlating sensory attributes and chemical compounds has been widely recognised. This is largely due to the fact that the relative amount of a compound in a food product does not necessarily reflect its impact on sensory characteristics because of differences in threshold values and effects of the food matrix (Drake & Civille, 2002). Also, not all volatile components in food or beverage products are odour-active (Friedrich & Acree, 1998). A technique that is often used to investigate the sensory characteristics of specific fractions of various products is gas-chromatography olfactometry. This involves sniffing and describing the aroma characteristics of individual compounds of the GC effluent by a trained panellist, and is often the first step of relating chemical compounds to their impact on sensory quality (Drake & Civille, 2002). This technique, however, does not provide information on the effect of the compounds when chewed or swallowed and ignores the effects of saliva, pH, temperature and interactions between the volatiles. For this reason descriptive analysis is often done simultaneously with instrumental or chemical analysis and the results are evaluated using univariate and multivariate statistical techniques (Drake & Civille, 2002).

Table 11 Selected studies on the quality evaluation and estimation of black and green teas

Article Title	Author(s)
Spectrophotometric measurements of theaflavins and thearubigins in black tea liquors in assessment of quality in teas	Roberts & Smith, 1961
Estimation of the market value of central African tea by theaflavin analysis	Hilton & Ellis, 1972
The effect of fermentation temperature on the quality parameters and price evaluation of Central African black teas	Cloughley, 1980
Phenolic composition of black tea liquors as a means of predicting price and country of origin	McDowell *et al*., 1991
Model for predicting black tea quality from the carotenoid and chlorophyll composition of fresh green tea leaf	Taylor *et al*., 1992
Prediction of quality and origin of black tea and pine resin samples from chromatographic and sensory information. evaluation of neural networks	Tomlins & Gay, 1994
Relating sensory properties of tea aroma to gas chromatographic data by chemometric calibration methods	Togari *et al*., 1995b
Flavanol composition and caffeine content of green leaf as quality potential indicators of Kenyan black teas	Obanda *et al*., 1997
Application of capillary electrophoresis to tea quality estimation	Horie & Kohata, 1998
Analysis of caffeine and flavan-3-ol composition in the fresh leaf of *Camellia sinensis* for predicting the quality of the black tea produced in Central and Southern Africa	Wright *et al*., 2000
Tea quality prediction using a tin oxide-based electronic nose: An artificial intelligence approach	Dutta *et al.*, 2003b
Evaluation of the composition and sensory properties of tea using near infrared spectroscopy and principal component analysis	Yan, 2005
Estimation of tea quality by infusion colour difference analysis	Liang *et al*., 2005
Application of chemical composition and infusion colour difference analysis to quality estimation of jasmine-scented tea	Liang *et al*., 2007
Prediction of Japanese green tea ranking by gas chromatography mass spectrometry-based hydrophilic metabolite fingerprinting	Pongsuwan *et al*., 2007
Identification of the green tea grade level using electronic tongue and pattern recognition	Chen *et al*., 2008
Chemical and instrumental assessment of green tea sensory preference	Liang *et al*., 2008
Evaluation of Chinese tea by the electronic tongue: Correlation with sensory properties and classification according to geographical origin and grade level	He *et al*., 2009
Analysis of chemical components in green tea in relation with perceived quality, a case study with Longjing teas	Wang & Jianyun, 2009
A novel iTongue for Indian black tea discrimination	Bhondekar *et al.,* 2010

The following are a few selected examples of the many attempts to predict the sensory quality of tea from instrumental data. To find a reliable way of predicting quality, sensory attributes of tea infusions have been correlated with various chemical and instrumental parameters. Nakagawa *et al*. (1972, 1981) related non-volatile compounds in green tea to their taste using univariate and multiple linear regression analysis and found that the interaction of components was important for the prediction of taste. Davies (1983) identified certain substances in black tea that correlated with the sensory ranking of black tea tastes. Artificial neural networks have also been applied to predict the sensory quality of black tea based on HPLC profiles of phenolic compounds (Tomlins & Gay, 1993). Togari *et al*. (1995b) correlated certain sensory properties of black, Oolong and green tea samples with the GC profiles of their volatile flavour components by using multivariate calibration models. Obanda *et al*. (1997) analysed the correlation between the score awarded to black tea by two professional tasters and the chemical components of the green leaf, the theaflavin and thearubigin content of the black tea liquor, as well as the total colour and brightness of the liquor. Significant positive correlations between the tasters' scores for the black tea infusions and the levels of epicatechin gallate, epigallocatechin gallate and caffeine in the green leaf were established. In a similar study the chemical composition and colour differences of black tea infusions, and their relationship with sensory quality were analysed (Liang *et al*., 2003). Sensory quality was a measure of tea taste (35%), aroma (30%), infusion colour (15%) and the appearance of the dry (10%) and infused (10%) leaves. The content of caffeine, nitrogen, amino acids, polyphenols, theaflavins, total catechins, a number of individual catechins and infusion colour indicators significantly correlated with the sensory quality. Another group attempted to develop a fast and reliable model to establish the quality or grade of green tea samples by means of metabolomics or "chemical fingerprints" (Pongsuwan *et al*., 2007). GC data and multivariate statistical techniques were employed to evaluate green tea quality. This approach provided some useful information on the quality of green tea and may be used as a consistent, quick and informative screening technique (Pongsuwan *et al*., 2007). Wang & Ruan (2009) correlated properties of leaf and infusion colours, chemical components and volatile compounds of Longjing teas with the scores of a tasting panel and formulated a prediction model that "may be interesting for potentially estimating the quality and designing quality assessment equipment of Longjing tea" (Wang & Ruan, 2009)

Because the flavour of any type of tea largely depends on the combination, the levels and threshold values of the volatile compounds that are found in the tea infusion (Dutta *et al*., 2003b) there have been several attempts to use various ratios of GC peak areas as measures of tea quality (Wickremasinghe *et al*., 1973; Yamanishi *et al*., 1978; Owuor *et al*., 1986; Baruah *et al*., 1986; Mahanta *et al*., 1988; Yamanishi *et al*., 1989; Owuor, 1992). In several of these studies on black tea volatiles, the compounds are divided into two groups: Group 1 is composed of compounds imparting an undesirable grassy odour to the tea infusion including isomers of hexanal, hexanol, hexenal and hexenol. Group 2 comprises compounds such as linalool, geraniol, benzaldehyde and ionones which are associated with the sweet, floral aroma of black tea (Ravichandran & Parthiban, 1998). The flavour quality of a black tea infusion was found to be linked to the

so-called flavour index, i.e. the ratio of the sum of Group 2 volatiles to that of Group 1 (Owuor, 1992; Ravichandran & Parthiban, 1998). These ratios may, therefore, be useful indicators of the sensory quality of tea.

Furthermore, the use of capillary electrophoresis, the electronic nose and the electronic tongue for tea classification and quality estimation have been examined (Legin *et al.*, 1997; Horie & Kohata, 1998; Ivarsson *et al.*, 2001; Dutta *et al*., 2003b; Chen *et al*., 2008; He *et al*., 2009). An electronic nose can identify and estimate the concentration of an odourant sample by means of various gas sensors, with different sensitivities, together with a signal processing system (Dutta *et al*., 2003b). Using this technology, Dutta *et al.* (2003b) were able to discriminate between teas that had been produced under different processing conditions, e.g. over-fermentation and under-fermentation. Yu and Wang (2007) and Tudu *et al.* (2009) have also applied the electronic nose to tea quality estimation and discrimination. More recently the electronic tongue has awakened a keen interest among researchers. It has even been said that it might partially replace the expert tasters' routine work since it is able to produce objective measurements in a time-efficient, consistent and cost-effective way (Scampicchio *et al.*, 2006). The instrument is based on an array of non-specific chemical sensors that show partial specificity to different components in a solution. Pattern recognition tools, such as principal component analysis or artificial neural networks, are used to generate qualitative, as well as quantitative information relating to the composition of the solution (Legin *et al.*, 1997). Chen *et al*. (2008), He *et al*. (2009) and Bhondekar *et al*. (2010) all obtained satisfactory results when using the electronic tongue for black tea discrimination and classification. The appeal of such electronic instruments, especially for use in quality control and for grading purposes, is that they deliver a fast, reliable, repeatable, real-time measurement eliminating problems such as subjectivity and fatigue often associated with expert tasters.

In light of these studies it once again it becomes clear that extensive research, similar to that available for green and black teas, is still needed for rooibos tea to investigate whether a feasable, reliable and efficient way to correlate the sensory quality of rooibos with instrumental parameters may be found. Nevertheless, it should be said that although the studies on black and green tea have delivered useful and interesting results, the methods used for quality estimation or prediction are not widely applied in commercial tea production and marketing because of time and cost implications. Sensory evaluation of tea by expert tasters remains the most common practice used to evaluate the sensory quality and sensory characteristics of tea.

6.7 Interactions between volatile and non-volatile compounds

The abovementioned studies highlight the complexity revolving around the analysis of tea flavour and tea quality which are largely determined by the array of non-volatile and volatile compounds. What makes this matter even more complicated is the fact that analysing the effect of these two groups of compounds separately may not be able to paint a clear picture of their final impact on flavour because of various

interactions that may take place between volatiles and non-volatiles. Taste can increase the apparent intensity of aromas whereas the perceived taste intensity can be amplified by certain aromas (Noble, 1996). Stevenson *et al*. (1999), for instance, found that certain aromas enhanced the tasted sweetness while others suppressed it: A sweet smelling caramel aroma suppressed the sourness of citric acid and enhanced the sweetness of sucrose, whereas aroma notes that were less sweet suppressed the perceived sweet taste of sucrose. Buettner and Beauchamp (2010) showed the opposite to be true as well: Fruity aroma was rated higher when evaluated in a sweet medium compared to a non-tasting medium, whereas fruitiness was drastically reduced in the presence of a salty stimulus. Also, it has been found that taste and aroma stimuli interact most strongly when the associations are similar; e.g. peanut butter aroma does not enhance sweetness while strawberry aroma does (Frank & Byram, 1988).

Aroma-taste relationships have also been analysed in black tea: Scharbert and Hofmann (2005) examined the effect of nose clamps on the intensity ratings of certain black tea taste qualities (e.g. astringent, bitter, and sweet) for recombinate tea solutions. In absence of nose clamps a slight increase in the perception of astringency, bitterness and sweetness was observed. Since the taste profile of the volatile distillate was described as tasteless in presence of nose clamps, but as slightly bitter when clamps were omitted, the results demonstrate that olfactory responses to tea volatiles can modify the perception of non-volatile components (Scharbert & Hofmann, 2005). Evidently, the discrimination between the perception of a taste and a retronasally perceived aroma with an unplugged nose is difficult, if not impossible (Noble, 1996).

Furthermore, it has been established that physical or chemical interactions (e.g. hydrogen bonding and hydrophobic interactions) may take place between certain volatiles and non-volatiles (Thanh *et al*., 1992; Charles-Bernard *et al*., 2005). The magnitude of these interactions depends on the physico-chemical properties of the compounds involved, e.g. the structure, volatility, polarity, molecular mass and solubility of the volatile compounds (Le Thanh *et al*., 1992). Such interactions between rooibos volatiles and non-volatiles would modify the sensory character of the individual components and could, therefore, influence the sensory quality of the tea.

7. Summary

Rooibos tea is a popular South African beverage that is low in tannin and caffeine-free. The range of polyphenols found in the tea is assumed to be responsible for the taste and mouthfeel characteristics of a rooibos infusion. The flavonoid composition of a rooibos infusion is unique, largely because of the high levels of the rare dihydrochalcones, aspalathin and nothofagin.

Despite the importance of this product on local and export markets, there exists a lack of standardised terminology to describe the flavour and mouthfeel of infused rooibos. The current quality standards laid down in the regulations relating to rooibos are vague, and no defined chemical or sensory specifications have been established. It is simply stated that rooibos “shall have the clean, characteristic taste and aroma

and clear, distinctive colour of rooibos" (Anonymous, 2002). However, it is not specified what precisely is meant by "characteristic" rooibos taste and aroma and "distinctive" colour. Also the considerable variation in quality found between different batches of rooibos due to differences in production areas, environmental conditions and processing parameters is not taken into account. The quality assigned to the tea is not determined according to a standardised, uniform grading system, but by in-house evaluation procedures. Quality, therefore, relies on the subjective opinion of expert tea tasters that use their own set of terminology to describe tea flavour and aroma.

A rooibos flavour lexicon and flavour wheel would be a helpful tool that could aid the improvement of rooibos grading and marketing. Flavour lexicons have found application in a number of industries and are used for describing and discriminating between products, comparing and monitoring products and product consistency, as well as profiling new and competitive products. Flavour wheels are a graphical representation of a collection of product characteristics and provide terminology that can be used to describe these. Due to their simple and convenient nature they may prove to be useful to rooibos processors, graders, extract manufacturers and flavour companies.

A multitude of studies have examined black tea quality in relation to its chemical composition and colour. Instrumental techniques employed for quality estimation and discrimination of black tea samples include GC and capillary electrophoresis, as well as electronic noses and tongues. Quality of black tea has been correlated with the levels of non-volatiles such as caffeine, amino acids, polyphenols and catechins. However, none of these techniques have been widely applied in commercial tea manufacture because of their limitations and cost and time implications. Similar research has not yet been conducted on rooibos tea.

One of the critical control points during processing of rooibos includes steam pasteurisation in order to reduce the microbial load on the product. This heat treatment has been shown to affect the chemical composition of rooibos infusions. However, the effect that steam pasteurisation has on the flavour and aroma of rooibos tea has not yet been characterised. Studies conducted for green and black teas indicated that heat treatment of the tea leaves resulted in important changes in the sensory quality of the tea infusions. It would be interesting to determine the extent of changes in flavour and aroma of rooibos due to pasteurisation.

8. References

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Chapter 3

SENSORY PROFILING OF ROOIBOS TEA AND THE DEVELOPMENT OF A ROOIBOS SENSORY WHEEL AND LEXICON

Table of Contents

1. Abstract

Rooibos samples were collected throughout the 2009 harvesting season from different geographic areas and different producers to capture as much potential variation in rooibos sensory characteristics as possible. The quality (i.e. the appearance of the leaves, and the colour and flavour of the infusion) of 69 samples was evaluated by expert graders and samples were grouped into four quality grades. Using quantitative descriptive analysis (QDA) the sensory profiles of the rooibos samples were developed and 123 aroma, flavour, taste, and mouthfeel descriptors were generated. A rooibos sensory wheel was created by selecting 27 flavour, taste and mouthfeel attributes and grouping these terms together to form a logical, convenient and user-friendly overview of the sensory descriptors associated with rooibos. The most frequently occuring descriptors were selected to compile a rooibos sensory lexicon of 14 flavour, taste and mouthfeel attributes along with a definition and reference standard for each term. It was found that the term "characteristic" rooibos flavour may be described as a combination of honey, woody and fynbos-floral notes with a slightly sweet taste and subtle astringency. Also, differences in the sensory characteristics between and within different quality grades were established with low-quality tea often being associated with green, hay-like and musty flavours and a bitter or sour taste. High-quality tea was generally associated with pleasant rooibos attributes including honey, floral and caramel notes and a sweet taste. No apparent distinction between samples was found based on the geographic location of the production areas of the teas. Variation in rooibos sensory attributes may, therefore, be more significantly impacted by processing than by the location of the plantation.

2. Introduction

The unique sensory characteristics of rooibos tea – its brick-red colour, pleasant aroma and naturally sweet taste – have boosted the popularity of this South African herbal tea, which has even been referred to as the country's unofficial beverage (Wilson, 2005). The demand for rooibos has grown considerably over the last few decades with the total demand being amplified from 4,700 tons in 1994 (Watson, 2008) to about 12,500 tons in 2009 (Genis, 2009). A large percentage of this amount flows to international markets, and exports have grown steadily from only 1,800 tons in 1999 to about 6,300 tons in 2009, with Germany, the Netherlands and the UK being the major importers of the South African product (S. Snyman, South African Rooibos Council, 2010, personal communication). This rise in the demand for and consumption of rooibos can be attributed partly to the health benefits that are associated with this hot beverage. Health and wellness is becoming increasingly important to consumers and this is reflected in their interest in food and beverage products that offer certain health benefits such as high levels of antioxidants (Datamonitor, 2010). The functional drink market, of which neutraceutical drinks hold a share of 17.4%, has been growing steadily, reaching a value of $8.4 billion in Europe in 2008 (Datamonitor, 2010).

As a result, the international tea market is becoming more and more crowded as numerous new herbal teas, fruit infusions, blends and flavours are introduced, e.g. Aloe Vera Tea, Cactus Blossom Tea, Carob Tea, Eucalyptus Tea and Goji Berry Tea (Anonymous, 2010). With such an overwhelming variety of teas to choose from, product differentiation becomes a challenging but essential endeavour. For this reason it is crucial that the attributes of a product can be properly defined and described. Also, consumers expect that these product attributes remain unchanged every time they purchase the product. This is achieved by means of quality control and sensory assessment of the final product. Often, companies make use of sensory lexicons or sensory wheels that offer a collection of descriptive terms with which to describe the attributes of a certain product (Drake & Civille, 2002). Such tools have proven to be useful since they facilitate and improve communication between different role players in the industry by standardising the terminology that is used when discussing certain product characteristics.

No such product attribute descriptors have yet been established for rooibos tea; instead the term "characteristic" rooibos flavour is often encountered. Even in the official South African regulations outlining the quality standards of rooibos it is simply stated that "all rooibos shall have the clean, characteristic taste and aroma of rooibos" (Anonymous, 2002). The statement is rather vague, and although South African consumers that are familiar with the product might have an understanding of the concept of "characteristic" rooibos flavour, it would be difficult to communicate this to foreign markets. Furthermore, the variation in the sensory attributes of different batches of rooibos has not yet been established. Many years ago a grading system was put in place when rooibos was still marketed through a one-channel system. A modified system is currently employed by the major rooibos marketing company to distinguish between high quality and low quality tea for commercial purposes. However, if the sensory quality of a certain batch of rooibos is rated according to the strength of the "characteristic" rooibos flavour then how would one determine if the perceived flavour of that specific batch is indeed "characteristic" if an accepted definition for this term does not exist? Also, the final grade awarded to a batch of rooibos depends not only on the flavour characteristics of the tea infusions, but also on the appearance of the dry and wet leaves as well as the appearance of the rooibos infusion (J. Basson, Rooibos Ltd., Clanwilliam, South Africa, 2009, personal communication). The specific sensory attributes of a batch of rooibos may, therefore, not always be accurately predicted by the overall quality that is assigned to the batch by expert graders.

In view of the above-mentioned shortcomings, this study was conducted to characterise and quantify the sensory attributes associated with rooibos flavour and mouthfeel in order to paint a more comprehensive picture of what is frequently referred to as "typical" or "characteristic" rooibos flavour. By analysing the sensory characteristics of a broad range of rooibos samples the variation in sensory quality between different quality grades of rooibos was established. The sensory profiles of these rooibos tea samples were summarised in a rooibos sensory lexicon containing descriptive terms, definitions and reference standards for each attribute. Rooibos producers, processors, marketers – both local and overseas – and researchers can benefit from this lexicon which can assist in differentiating and characterising the

sensory attributes of rooibos tea. Also, a selection of sensory attributes was assembled in a simple and convenient format by developing a rooibos sensory wheel that reflects the variation in sensory profiles of samples harvested on different rooibos plantations throughout one harvesting season.

3. Materials and methods

3.1 Rooibos samples

Over a three-month period (March to June 2009) the quality control personnel of Rooibos Ltd. (Clanwilliam, South Africa) randomly collected one sample each from 69 different batches of rooibos obtained from 64 different rooibos plantations. The yield fraction of each sample was graded according to their in-house grading system by which a quality grade is awarded to each batch based on the appearance of the dry and wet leaves, the appearance of the infusion, as well as the flavour of the infusion. The samples comprised nine Grade A samples and 20 samples each of Grades B, C and D. The complete list of samples can be found in Addendum 1. Each sample was given a code that reflects its quality grade and a sample number that was assigned to each sample in the order that the different batches of rooibos were received; e.g. Sample B13 was the 13th Grade B sample that was received from Rooibos Ltd. A rooibos reference sample was made up by blending six batches of B-grade rooibos samples of the 2008 season provided by Rooibos Ltd to the Agricultural Research Council (ARC-Infruitech Nietvoorbij, Stellenbosch, South Africa).

3.2 Sample preparation

Tea preparation. Freshly boiled, distilled water (900 g) was poured onto 17.4 g dry tea leaves. After stirring for about 5 sec the tea leaves were infused for 5 min. The tea infusion was then poured through a fine-mesh tea strainer into a 1 l stainless steel thermos flask. Approximately 100 ml of tea was served in a white porcelain mug covered with a plastic lid to prevent evaporation and loss of volatiles.

Temperature maintenance. A number of measures were taken to keep the temperature of tea as constant as possible: The thermos flasks were filled with boiling water to heat the inner surface of the flask, and emptied just before adding the rooibos infusion. Tea mugs were preheated in an industrial thermofan oven (Hobart CSD 1012) set to 70°C. Throughout the sensory analysis sessions the mugs were kept in scientific waterbaths (SMC, Cape Town) with the temperature regulator set to 65°C.

3.3 Quantitative descriptive analysis (QDA)

The panel*.* Nine female judges participated in the study. They were selected based on availability and interest. Most of them had extensive experience with descriptive analysis of a wide range of products. None of them, however, had previous experience with the sensory analysis of rooibos tea.

Panel training. The training of the panel was conducted according to the consensus method described by Lawless and Heymann (1998). The panellists were informed about the background and the objectives of the study. The panel then received instructions on the sample analysis procedure. They were asked to first remove the plastic cap that had been placed on the cup and to swirl the cup several times before evaluating the aroma of the tea. Thereafter, they were instructed to evaluate the flavour, taste and mouthfeel by sucking, rather than sipping, a mouthful of the tea infusion off a round tablespoon. The panel was also requested to cleanse their palates at regular intervals using water and unsalted water biscuits (Woolworths, South Africa).

During the first part of the training, panellists were exposed to a number of rooibos samples to become familiar with the product and the analysis protocol. During 22 one-hour sessions 6 to 8 of the 69 samples were analysed and compared to one another, and the panel generated flavour, taste, aroma, and mouthfeel terminology. *Aroma* was defined as the fragrance or odour perceived through orthonasal analysis while *flavour* referred to the retronasal perception in the mouth. The term '*taste'* was used to describe the basic sweet, sour, salty and bitter taste modalities. *Mouthfeel* was described as the tactile sensations that occurred in the oral cavity after sipping the tea infusion. In an open discussion led by the panel leader descriptive terms were suggested and deliberated by the panel members, and each new term was recorded. Relationships and redundancies among the terms were discussed, and definitions for the most recurring sensory descriptors were developed. The suitability of a number of reference standards was tested to identify those that best described the specific sensory attributes. In each session the panel was also given the reference rooibos sample which was consistently prepared from the same batch of tea throughout the sensory analysis period.

During the training sessions 85 aroma and 38 flavour, taste and mouthfeel descriptors were generated. Twenty-seven of these terms were selected for inclusion in the sensory wheel based on whether they were relevant, unambiguous, non-redundant and non-hedonic. For efficient sensory profiling the number of attributes was further reduced to a set of 17 mutually exclusive terms (8 aroma descriptors, 5 flavour descriptors, 3 taste descriptors and 1 mouthfeel descriptor) based on their frequency of quotation and their discriminative power. A score sheet was then developed which showed each of the 17 descriptors together with a 10 cm unstructured line scale anchored on both sides with two word descriptors – "none" and "prominent" (Addendum 2). During the final training sessions the panel practised intensity rating of the individual attributes on the unstructured line scales. Maximum and minimum intensity values for the 17 attributes were discussed and frequently compared to the attribute intensity values that had been established for the reference rooibos sample. The intensity of attributes that were not perceived in the reference sample was rated in relation to the sample with the highest intensity for that attribute.

Intensity rating. After training had been completed, the panel was requested to use the score cards to rate the intensities of the 17 attributes for each of the 69 samples during 40 sessions spread out over 8 weeks. On 3 days per week 2 sessions were conducted per day during which 6 and 8 samples were analysed,

respectively. Panellists were requested to take a 10 minute break between the sessions to avoid panel fatigue. Each rooibos sample was analysed in duplicate, on two non-consecutive days in order to test for panel reproducibility and reliability. Samples were labelled with three-digit codes and presented to each panellist in a randomised order. The reference rooibos sample, however, was labelled as such so that it could be identified by the panellists and used for comparison to the other samples.

3.4 Statistical analysis

The data for each sensory attribute rated for each tea sample by all panellists were collected and analysed using various statistical techniques. Panel performance was monitored using *PanelCheck* Software (Version 1.3.2, Nofima Mat, Norway). The data were also subjected to test-retest analysis of variance (ANOVA) using SAS® software (Version 9.2; SAS Institute Inc, Cary, USA) to test for panel reliability. Judge*Replication interaction and Judge*Sample interaction were used as measures of the panel precision and homogeneity, respectively. The Shapiro-Wilk test was used to test for non-normality of the residuals (Shapiro & Wilk, 1965). In the event of significant non-normality ($p < 0.05$) outliers were identified and removed until the data were normally distributed. Principal component analysis (PCA) was conducted using XLStat (Version 7.5.2, Addinsoft, New York, USA) to visualise and elucidate the relationships between the samples and their attributes.

4. Results and discussion

4.1 General

When developing a sensory lexicon it is important that samples with a wide range of sensory profiles are included in the development process so that as many potential sensory attributes as possible are captured in the lexicon. For this reason samples should be specifically selected to cover different sources of variation, such as seasonal effects and production areas (Drake & Civille, 2002). Rooibos samples were, therefore, selected throughout the rooibos harvesting period, as well as from different production areas and harvested by different producers.

The rooibos reference sample that was presented to panellists during each training and testing session served as a fixed point which all other samples could be compared to, thereby allowing panellists to calibrate their sensory perception at the start of each session. The reference sample was composed of a blend of six Grade B rooibos samples and its sensory attributes were considered to be well-representative of the sensory profile typically associated with rooibos.

In order to ensure that no sample differences arise from variations in the preparation of the tea infusions it is necessary to ensure that a standardised tea preparation protocol is followed. The extraction of rooibos soluble solids is determined by various factors such as the tea-to-water ratio, the length of the infusion, the water temperature, and the type of water used (Joubert, 1988; Joubert, 1990; Joubert &

Hansmann, 1990; Dos *et al.*, 2005). These factors were thus kept as constant as possible without reducing the practicality of the protocol. The tea-to-water ratio used in this study is specified in the official rooibos regulations (Anonymous, 2002) and is also used by Rooibos Ltd. for grading purposes. The water used to prepare tea infusions is also a critical factor that may affect tea quality. Its pH, ion concentration and hardness are some of the factors that have been found to influence the quality of Oolong tea extracts (Chen *et al*., 1997). Dos *et al.* (2005) found that the type of water used to prepare a rooibos infusion can affect the clarity and acceptability of the tea. Therefore, instead of tap water, distilled water was used for preparing the rooibos infusions.

Furthermore, temperature maintenance of the tea samples during sensory analysis is critical because it was found during preliminary experiments that the sensory characteristics of the tea infusions changed as the temperature of the tea decreased. The temperature-dependence of green tea sensory attributes was also noted by Lee *et al.* (2008) who outlined several adaptations to the tea preparation protocol, with the aim of keeping temperature fluctuations between the samples as small as possible. Some of these adaptations were adopted for the preparation of the rooibos infusions, e.g. the use of pre-heated thermos flasks and waterbaths.

4.2 Panel performance

It is important to monitor panel performance throughout the sensory analysis period because a number of problems relating to the training, stability and maintenance of the quality of the panel are frequently encountered (Tomic *et al.*, 2010). A number of techniques have been developed to improve panel performance by detecting a lack of precision, homogeneity and discrimination ability. These include both univariate and multivariate statistical methods (Prichett-Mangan, 1992; Dijksterhuis, 1995; Latreille *et al.*, 2006; Tomic *et al.*, 2010).

Often, the first step of analysing sensory data, even before monitoring panel performance, is the use of a 3-way ANOVA to assess the importance of the sensory attributes in detecting significant differences between the samples. This involves modelling samples, assessors, replicates and their interactions and testing for the sample effect using *F* tests (Tomic *et al.*, 2010). The attributes found to have a significant sample effect should be considered for further analysis. ANOVA is also useful for analysing panel performance measures, reflecting the reliability of a sensory panel: Precision is a measure of panel reproducibility that relates to the variability of the scores given to the replicates of one sample (Judge*Replicate interaction) (Prichett-Mangan, 1992). Homogeneity reflects inconsistencies in the scoring patterns of the assessors and is estimated from the Judge*Sample interaction (Carbonell *et al*., 2007).

Table 1 shows the ANOVA table for the 17 sensory attributes evaluated during QDA. Judges were a significant source of variation in all 17 attributes ($p<0.001$). The tea samples were a significant source of variation for all attributes ($p<0.05$) indicating that all attributes could significantly discriminate between the samples. However, significant Judge*Replication interaction ($p<0.05$) was found for 10 attributes reflecting a

low level of precision and, therefore, a lack of panel reproducibility from one replication to the next. Also, significant Judge*Sample interaction effects ($p<0.05$) were observed for 12 attributes indicating that the panel did not agree on the order of intensity across the tea samples for these attributes. Furthermore, the significant Sample*Replication interactions for the attributes "dusty/musty" aroma ($p<0.001$) and "floral" flavour ($p<0.001$) and aroma ($p<0.05$) suggest that, in terms of these attributes, the tea samples were not constant over the two replicates.

Further analysis using PanelCheck software (data not shown) indicated that panellists experienced some difficulties in evaluating the attribute intensities of samples. It should be noted that the sample set was considerably large (n = 69) and many of the samples were similar to each other in their sensory quality making it difficult to distinguish between them. Also, accurately quantifying the intensity of certain attributes was found to be challenging because of the low intensity of specific attributes in some of the samples. Certain samples were found to have one or two highly prominent aroma notes or flavour characteristics that could be easily identified while other samples could only be described as having low levels of a number of attributes. Rating the intensity of a combination of attributes at low levels proved to be a challenge for the panel which influenced the results of the sensory analysis.

Although panel performance is essential for reliable sensory analysis there are certain aspects that should be considered. Wolters and Allchurch (1994) stated that it is not always appropriate to remove attributes that are difficult to perceive and quantify. Such attributes may be very weak or the assessors may not have a comprehensive understanding of the descriptor; therefore, it is likely that panellists will give a different rating for such attributes on repeated presentation of the samples. When removing such "difficult" attributes, reliability of the data may be increased. Consequently, however, information on certain aspects of the sensory characteristics of the product is lost, which is unfavourable when the objective of sensory analysis is the development of a sensory profile.

Panel training and sample analysis were spread out over a period of more than five months including a six-week period during which no sessions were held. Over time the panel became increasingly familiar with the attributes and the sample analysis, and more confident in rating the attributes. It may, therefore, be expected that the panel improved in their ability to evaluate the samples. Also, throughout the continuous exposure to the samples their understanding of certain attributes may have improved. This may have resulted in a modification of their perception of an attribute and in differences in the intensity rating between the replications, despite the use of a reference sample. PCA scores and loadings plots can be used to analyse panel repeatability over more than one replication (e.g. Vannier *et al.,* 1999; Kreutzmann *et al.*, 2007; Bro *et al.*, 2008). This approach was used to visualise the differences between the two replications of the 69 samples. The percentages of explained variance of the first two Principal Components (PCs) for replication 1 and 2 were 37.56% and 39.31% respectively (data not shown) reflecting a slight increase in the explained variance from Rep 1 to Rep 2. The scores and loadings plots of the averages for each grade and each replication are shown in Fig. 1. A movement of all 4 score vectors representing Rep 1 from the bottom

left to the top right quadrant towards their corresponding Rep 2 vector can be seen. Projected onto the loadings plot this movement indicates that the association between the scores of Rep 2 and the attributes is stronger than for Rep 1, which suggests that the panel performance and their ability to rate attribute intensities increased over time. However, the translation of the vectors is almost parallel to one another so in essence the results that were obtained are the same.

Table 1 F-values for 17 attributes as obtained by ANOVA

Attribute	Judge (J)	Tea (T)	Rep (R)	J x T	J x R	T x R
AFloral	105.76***	1.81***	11.06***	1.23**	6.01***	1.33*
AHoney	32.36***	1.65**	0.07	1.07	2.97**	0.82
AWoody	37.64***	2.83***	14.69***	1.47***	11.81***	1.06
AGreen	26.65***	3.04***	0.62	1.72***	1.85	1.09
AHay	12.22***	1.65**	3.79	1.20*	1.77	0.85
ACaramel	20.99***	5.40***	0.00	1.23**	1.92	0.98
ASweet (Fruity)	31.38***	1.53**	19.55***	1.34***	2.10*	0.97
ADusty (Musty)	4.91***	2.23***	0.15	1.28**	0.62	1.93***
FFloral	123.31***	1.42*	57.97***	1.36***	6.59***	1.72***
FWoody	54.69***	3.30***	93.37***	1.28**	6.97***	1.24
FGreen	25.19***	2.67***	0.06	1.45***	1.21	0.86
FHay	56.93***	2.08***	9.42**	1.29**	4.73***	1.05
FCaramel	13.60***	3.43***	0.03	0.99	2.31*	1.27
TSweet	137.55***	1.64**	4.96*	1.00	9.01***	0.76
TSour	10.72***	1.41*	2.63	1.11	1.11	1.10
TBitter	15.35***	1.33*	4.48*	1.16*	4.52***	0.98
Astringent	109.90***	2.74***	48.54***	1.07	6.55***	1.20

*, **, *** indicate significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively

T x J = Tea by Judge interaction; J x R = Judge by Replication interaction; T x R = Tea by Replication interaction

Except for astringency, the letters “A”, “F” and “T”, and in front of an attribute name refer to aroma, flavour and taste attributes, respectively.

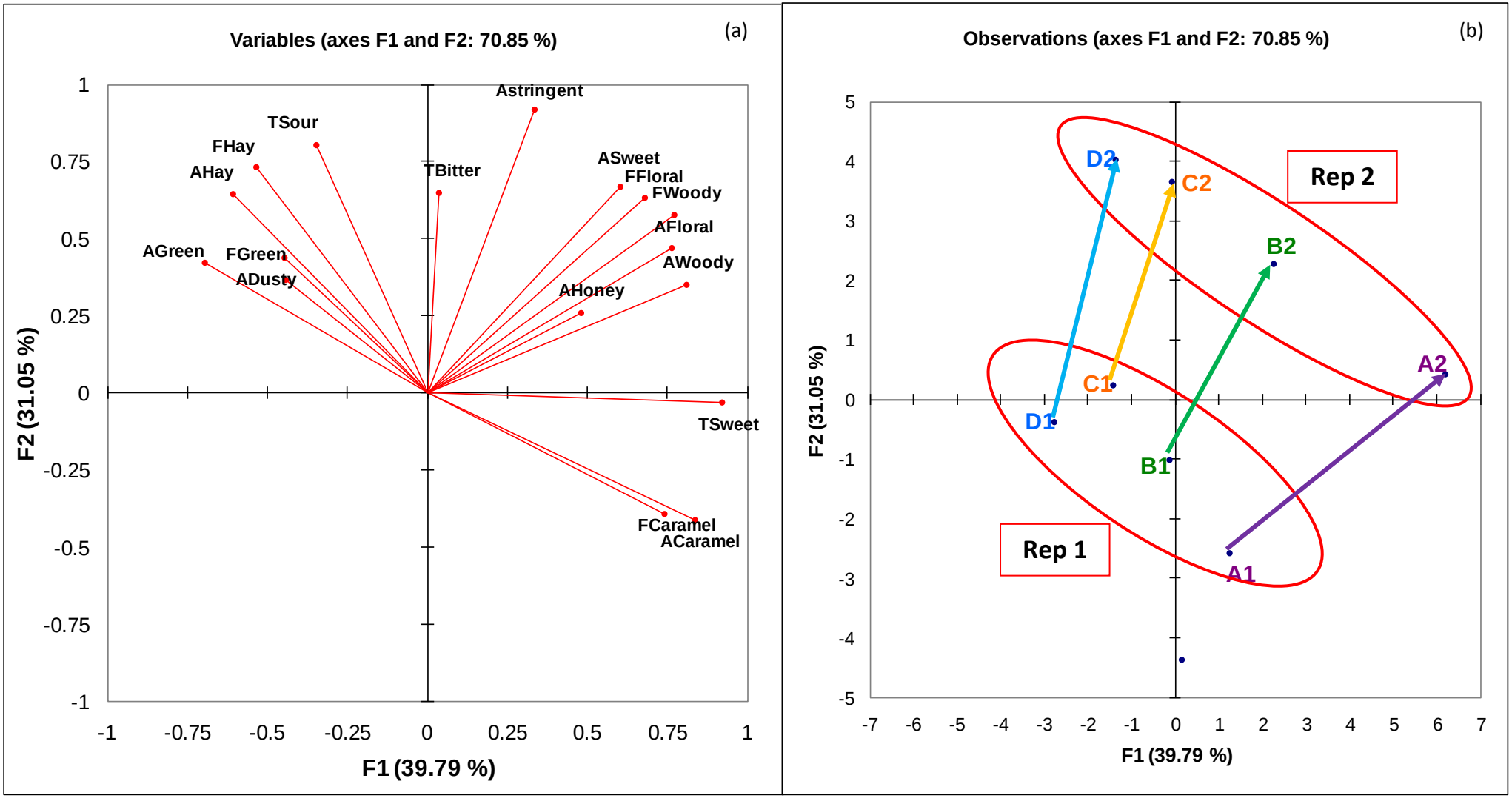

Figure 1 PCA loadings (a) and scores (b) plots reflecting the differences between sample replications. On the loadings plot the letters "A", "F" and "T" in front of the attribute name, except for astringency, refer to aroma, flavour and taste attributes, respectively. The letters A, B, C and D in the scores plot reflect the quality grade of the sample while "Rep" refers to replication. The numbers "1" and "2" associated with the letters A, B, C and D refer to replications 1 and 2, respectively.

4.3 Sensory attributes

The complete list of 123 descriptive terms generated during the training sessions can be found in Addendum 3. However, efficient sensory profiling requires the reduction of the number of terms to about 10 to 20 (Vannier *et al.*, 1999). Therefore, this list was greatly reduced by grouping together similar terms, eliminating redundancies and disregarding those attributes that were perceived in only a small number of samples. The reduced list of 17 attributes is shown in Table 2.

Table 2 Final aroma, flavour (F), taste (T) and mouthfeel (MF) attributes used for QDA

Aroma Attributes	**Flavour, Taste and Mouthfeel Attributes**
Floral	Floral (F)
Woody	Woody (F)
Plant-like / Green	Plant-like / Green (F)
Hay / Dried grass	Hay / Dried grass (F)
Caramel	Caramel (F)
Sweet (Fruity)	Sweet (T)
Honey	Sour (T)
Dusty / Musty	Bitter (T)
	Astringent (MF)

Reichelt *et al*. (2010) analysed the taste quality of a number of rooibos fractions obtained by High Temperature Liquid Chromatography and formulated a number of descriptors that were identical to those obtained in this study including “sweet”, “bitter”, “woody”, “green”, “hay-like”, ”fruity”, ”honey” and ”musty”. These results confirm the significance of the major attributes that were described in this study (Table 2).

In order to paint a better picture of the relative importance of the 17 major attributes, i.e. their intensity perceived in an infusion as well as their prevalence amongst the samples, graphs were drawn up that show the average intensity range for each individual attribute and the percentage of samples that exhibit each attribute plotted against its average intensity (Fig. 2). The attribute “honey” aroma obtained, on average, the highest score followed by “woody” flavour, “floral” aroma, “woody” aroma, “sweet” taste and “astringency”. These six attributes were perceived in all of the 69 rooibos samples. It may be concluded that the “characteristic” or “typical” rooibos sensory profile may be described as a combination of honey, woody and floral flavours with a sweet taste and a slightly astringent mouthfeel. The “floral” note does not refer to a typical, perfume-floral aroma, but rather to a natural, fynbos-floral fragrance, while the “honey” aroma of rooibos samples can be likened to the scent associated with fynbos or wild-flower honey or Alyssum flowers.

It was found that the intensities of the fynbos-floral and honey-like notes of the tea infusion increased as the tea cooled down.

"Caramel" aroma is also an important attribute since it occurred in more than 90% of the samples. From Fig. 2 it can be seen that this attribute obtained the largest range of average intensity scores from about 5 to more than 40 out of 100. "Sweet aroma", "floral" flavour, "hay" flavour and "green" aroma also occurred frequently in the rooibos samples but their average intensity scores were fairly low with maximum scores of less than 25 out of 100. "Bitter" and "sour" taste, "caramel" flavour, and "dusty/musty" aroma were also rated low in intensity (less than a maximum of 15 out of 100) and were perceived in less than 20% of the samples.

A simple and convenient way of visualising differences in sensory profiles between samples is by means of a spider plot. The attribute intensities for two rooibos samples with distinctly different sensory characteristics are shown in a spider plot in Fig. 3. One of the samples (C18) has a strong green flavour with woody notes and a slightly sour taste, whereas the other sample (A5) may be described as having a "characteristic" rooibos sensory profile with woody, honey and floral notes, a sweet taste and a slightly astringent mouthfeel.

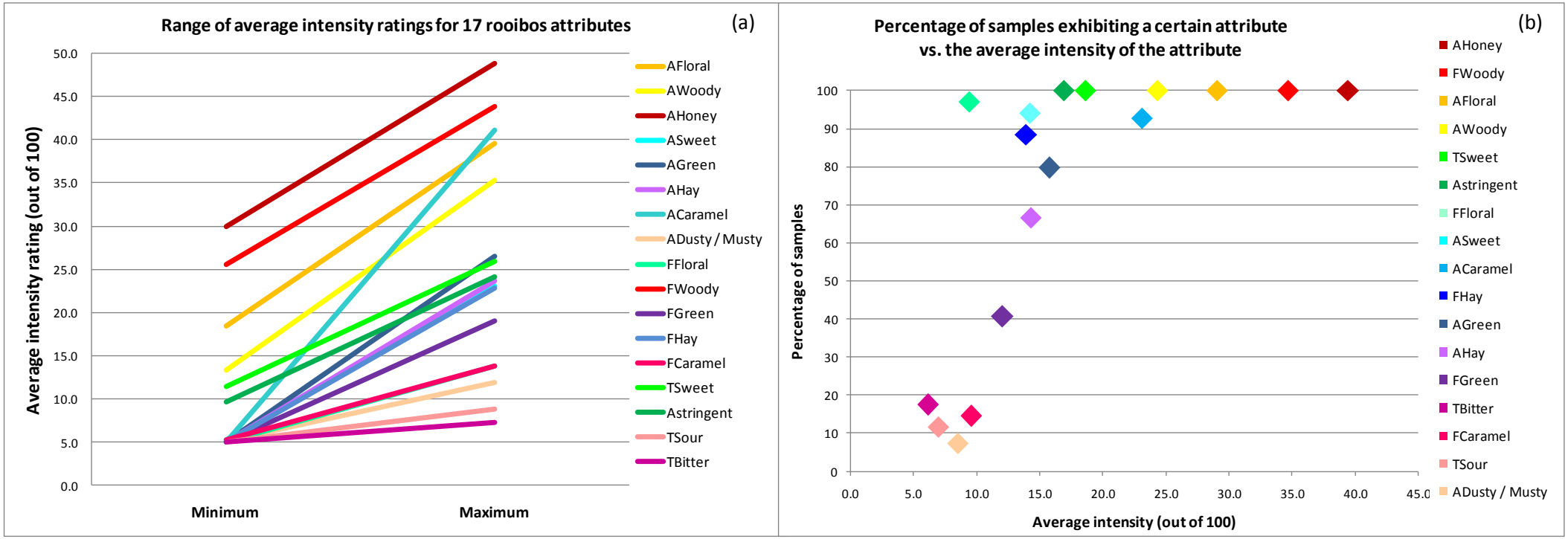

Figure 2 Minimum and maximum average intensity ratings (averages over 9 judges, 2 replications and 69 samples) for each attribute (a). Scatter plot showing the percentage of samples exhibiting a certain attribute vs. the average intensity of the specific attribute (b). Except for astringency, the letters "A", "F" and "T" in front of an attribute name refer to aroma, flavour and taste attributes, respectively. For Fig. 2(b) only those samples were considered that had an average intensity rating of ≥ 5%.

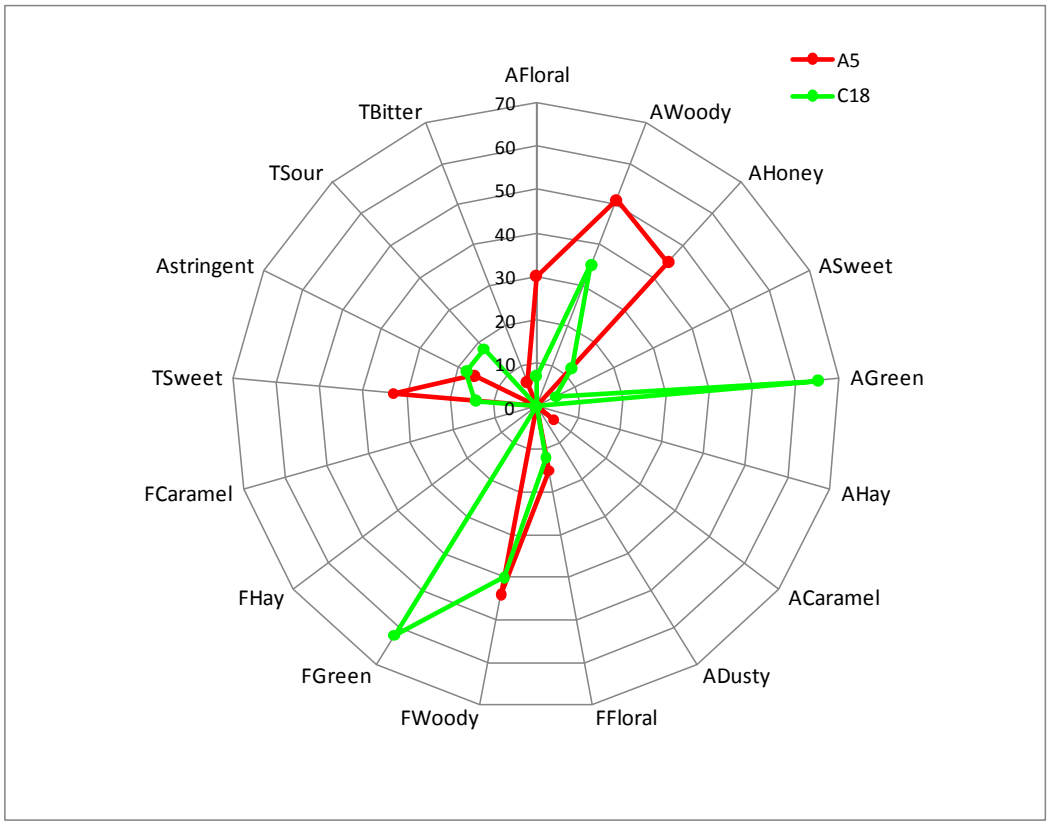

Figure 3 Spider plot for two rooibos samples with strong green flavour (**C18**), and strong woody, honey and floral notes (**A5**). Except for astringency, the letters "A", "F" and "T" in front of an attribute name refer to aroma, flavour and taste attributes, respectively.

4.4 Relationships between sensory attributes

PCA plots are commonly used in sensory analysis to display the relationships between attributes as well as between individual samples. The PCA loadings plot (Fig. 4) displays the positioning of and association between the rooibos attributes. This plot shows the separation between positive sensory characteristics ("floral", "woody", "honey", "caramel" and "sweet") and negative attributes ("green", "hay", "dusty/musty", "bitter", "sour") along the x-axis. The positioning of the 69 rooibos samples relative to each other is reflected by the corresponding scores plot (Fig. 4).

PCA plots can be used to indicate whether certain attributes are redundant and may be reduced to a simplified set of terms to prevent different attributes from being used to describe the identical sensory characteristic. They can also demonstrate whether correlations exist between an aroma and flavour attribute that has been analysed by nose (orthonasal, ON) and by mouth (retronasal, RN), respectively. Figure 4 shows that most of the ON and RN attributes ("green", "hay", "woody", "floral" and "caramel") were closely associated with one another, indicating that these notes were perceived similarly on the nose, as well as the tongue. However, "sweet" taste and "sweet" aroma lie further apart, which may be explained by the fact that the latter referred to a fruity-sweet fragrance, whereas sweet taste was exhibited by almost all rooibos samples, including those that did not have a fruity-sweet aroma.

Attributes that seem to be highly correlated on a PCA loadings plot can, however, sometimes refer to very different sensory aspects, and it is possible that attribute grouping may arise from a general tendency of certain attributes to change in a similar way over a large group of samples (Wolters & Allchurch, 1994; Talavera-Bianchi *et al.*, 2010). For this reason, it is often more useful to examine the relationships between sensory attributes by analysing their correlation coefficients (Table 3). Significant ($p<0.05$) and strong positive correlations existed between the aroma and flavour attributes for "green" ($r = 0.894$), "caramel" ($r = 0.838$), "hay" ($r = 0.779$) and "woody" ($r = 0.727$). The correlation coefficients for "floral" and "sweet" aromas and flavours were much weaker ($r = 0.366$ and $r = 0.302$, respectively), but they were nevertheless significant ($p<0.05$).

The mechanism of perception of aromas and flavours is different: While aromas are perceived through the nostrils, flavours are detected "in mouth" by transportation of the stimulus from the back of the throat up to the olfactory receptors in the nasal cavity (Ross, 2009). Because of this the sensory perception of a certain attributes perceived by RN and ON analysis may differ. This was shown by Aubry *et al.* (1999) who examined the differences between the sensory profiles of wines by ON and RN evaluation. The results for ON and RN analysis were correlated for most of the descriptors, including "wood" ($r = 0.84$), "cut grass" ($r = 0.73$) and "caramel" ($r = 0.68$), indicating that these attributes were understood and used in the same way during ON and RN analysis (Aubry *et al.,* 1999). However, other descriptors, notably fruity notes, seemed to be more effectively evaluated by nose. This was also noted by Sauvageot and Vivier (1997), and it has also been found to be true for the "fruity-sweet" and "honey" aromas of rooibos samples which were not perceived very strongly by in-mouth analysis.

4.5 The relationship between sensory attributes and quality

It has already been mentioned that the positive and negative sensory attributes, respectively, lie to the right and to the left of the loadings plot (Fig. 4). When analysing the corresponding scores plot it is evident that no distinct clusters are formed based on the quality grade of the samples. Nevertheless, the nine Grade A samples (except for A8) are separated from most Grade D samples (except for 4 samples; D4, D6, D9, D16) along the first Principal Component (F1) which indicates that high quality Grade A samples associate with the positive attributes in the right quadrants, while most Grade D samples correspond to the negative attributes in the left quadrants. Grade B and C samples, however, were distributed evenly all over the four quadrants. This indicates that the sensory quality of a sample is not necessarily in accordance with the quality grade assigned to it, and that there is considerable variation in sensory characteristics within each quality grade. For instance, some of the Grade C samples (e.g. C11 and C5) were found to have strong "woody", "honey" and "floral" notes, whereas others (e.g. C18 and C19) had a prominent "green" or "hay" flavour.

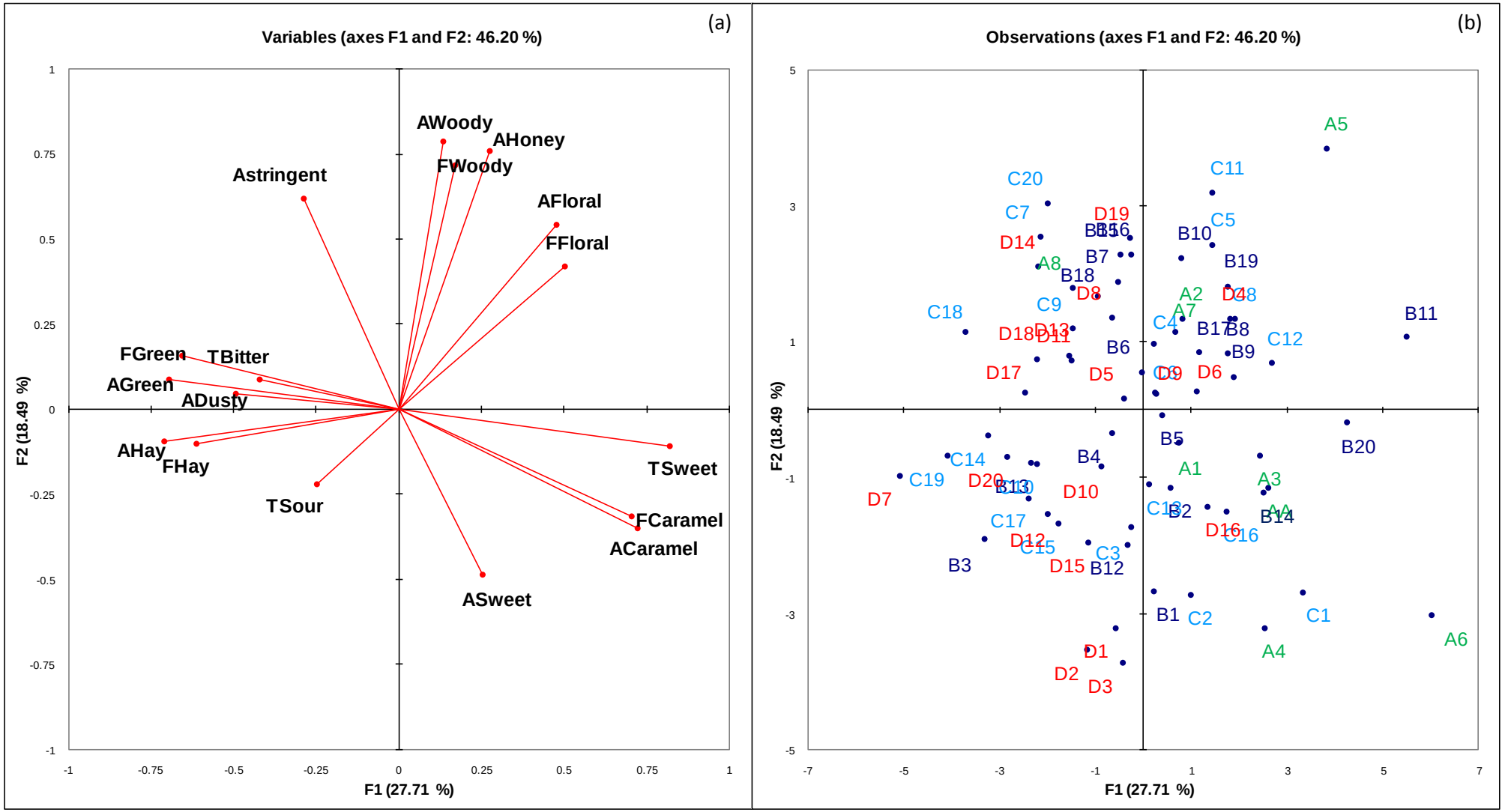

Figure 4 PCA loadings (a) and scores (b) plots showing the positioning of the 17 sensory attributes and of the 69 rooibos samples, respectively. In the loadings plot the letters "A", "F" and "T" in front of an attribute, except for astringency, refer to aroma, flavour and taste attributes, respectively. The letters A, B, C and D in the scores plot reflect the quality grade of the sample.

Table 3 Correlation matrix showing Pearson's correlation coefficients (r) for all sensory attributes

Variables	AFloral	AWoody	AHoney	ASweet	AGreen	AHay	ACaramel	ADusty	FFloral	FWoody	FGreen	FHay	FCaramel	TSweet	Astring.	TSour	TBitter
AWoody	**0.292**	1															
AHoney	**0.610**	**0.505**	1														
ASweet	-0.154	**-0.306**	**-0.420**	1													
AGreen	-0.220	-0.136	-0.225	-0.121	1												
AHay	**-0.532**	0.058	**-0.272**	-0.194	0.230	1											
ACaramel	0.028	-0.014	-0.200	**0.318**	**-0.534**	**-0.359**	1										
ADusty	**-0.328**	0.151	-0.114	**-0.275**	0.056	**0.607**	**-0.333**	1									
FFloral	**0.366**	**0.361**	**0.291**	0.070	-0.145	**-0.400**	**0.241**	**-0.243**	1								
FWoody	**0.244**	**0.727**	**0.421**	-0.115	-0.105	-0.067	0.038	0.093	**0.328**	1							
FGreen	-0.162	-0.065	-0.178	-0.116	**0.894**	0.189	**-0.486**	0.023	-0.068	-0.049	1						
FHay	**-0.485**	0.056	**-0.248**	-0.063	0.126	**0.779**	**-0.246**	**0.493**	**-0.397**	0.032	0.116	1					
FCaramel	0.076	-0.011	-0.123	**0.302**	**-0.394**	**-0.329**	**0.838**	**-0.262**	**0.249**	0.000	**-0.387**	**-0.346**	1				
TSweet	0.159	0.130	0.082	**0.302**	**-0.593**	**-0.473**	**0.588**	**-0.278**	**0.369**	0.202	**-0.553**	**-0.365**	**0.597**	1			
Astringent	0.088	**0.535**	0.209	-0.042	**0.343**	0.148	**-0.246**	-0.054	0.209	**0.518**	**0.435**	0.190	**-0.249**	**-0.273**	1		
TSour	-0.157	-0.133	-0.232	**0.254**	**0.259**	0.056	-0.033	-0.125	-0.172	-0.203	**0.334**	0.138	-0.031	-0.175	**0.238**	1	
TBitter	-0.142	-0.086	-0.083	-0.094	**0.252**	0.136	-0.231	0.235	-0.144	0.023	**0.262**	0.016	**-0.322**	**-0.450**	0.153	-0.016	1

= Significantly different from 0 ($p < 0.05$)

= $r > 0.5$

= $r < -0.5$

All values in bold are significantly different from 0 with a 5% significance level. Except for astringency, the letters "A", "F" and "T" before the attribute descriptors refer to aroma, flavour and taste attributes, respectively.

The positioning of the averages for the four quality grades and their association with the sensory attributes is shown in Fig. 5. The quality grades are distributed from highest to lowest quality (i.e. from A to D) from the right to the left of the plot. This indicates that the expert graders at the rooibos processing facility are able to distinguish, to a certain extent, between batches of rooibos with positive and negative sensory attributes based on the overall flavour, as well as the appearance of the leaves and of the infusion (see Chapter 2, Section 2.1, for a description of rooibos grading). A PCA loadings plot (Fig. 6) displays how the sensory attributes quantified by QDA associate with the "Flavour" rating awarded to the samples by expert graders at the rooibos processing facility. This plot confirms that the overall "Flavour" score is associated with the positive rooibos attributes found on the right-hand side of the plot.

Furthermore, the differences in average attribute intensities between the quality grades are displayed in Fig. 7. From the 17 sensory attributes used for QDA only 8 differed significantly between the grades (data not shown) and these 8 attributes are shown in Fig. 7. On average, low quality rooibos is associated with higher intensities for "green" and "hay", and with lower intensities for "caramel", woodiness and sweet taste. These results may have been anticipated since the flavour score awarded to a batch of rooibos by the expert graders is based on the absence of foreign flavours – including musty, straw-like or green notes and a bitter or sour taste – as well as the strength of the "characteristic", sweet, honey-like rooibos aroma (Joubert, 1994; J. Basson, Rooibos Ltd., Clanwilliam, South Africa, 2009, personal communication). High quality rooibos may thus be described as a sweet and woody tea without green or haylike flavours.

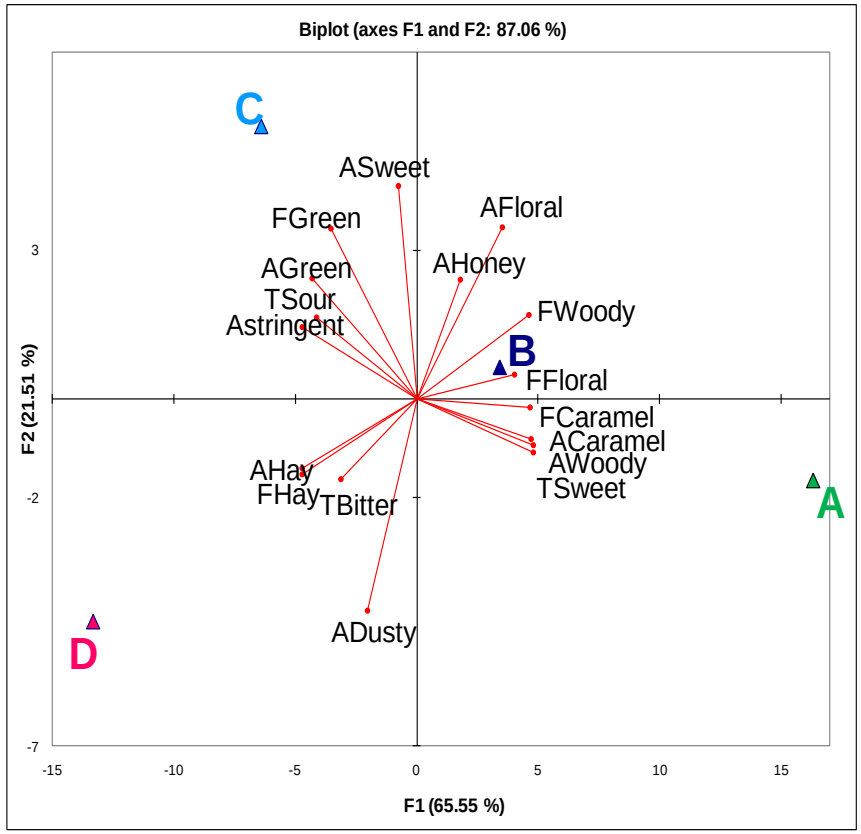

Figure 5 PCA biplot showing the means for each quality grade (A, B, C and D) as well as the descriptive attributes. Except for astringency, the letters "A", "F" and "T" in front of the attribute name refer to aroma, flavour and taste attributes, respectively.

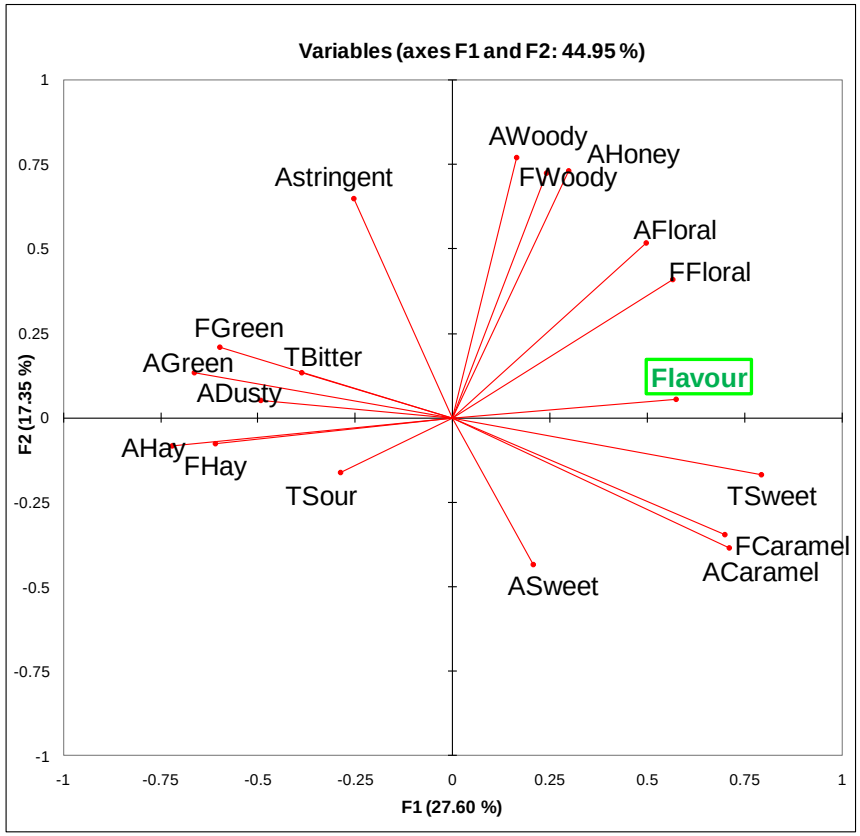

Figure 6 PCA loadings plot showing sensory attributes and the "Flavour" grading parameter used by export graders from a rooibos processing facility. Except for astringency, the letters "A", "F" and "T" in front of the attribute name refer to aroma, flavour and taste attributes, respectively.

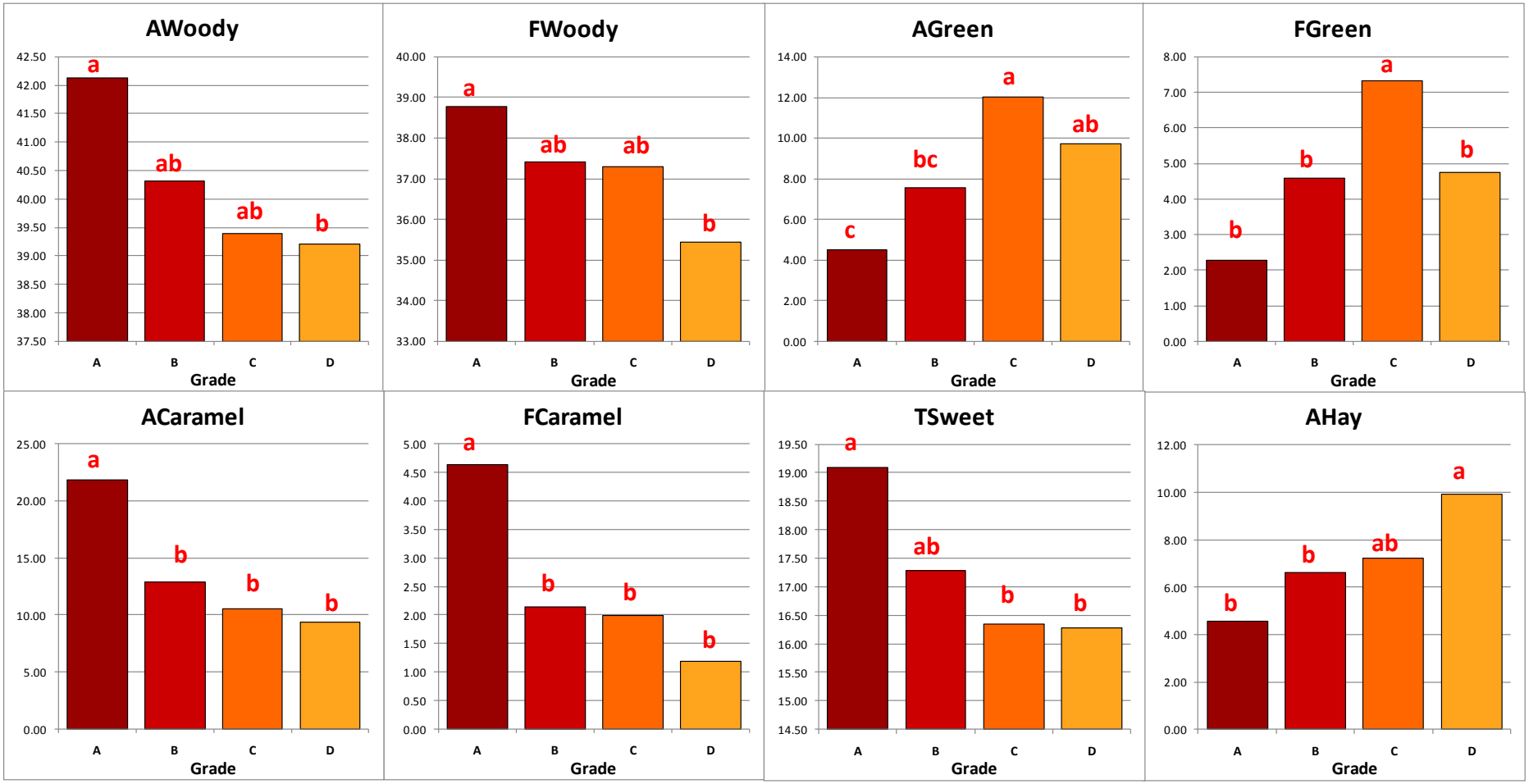

Figure 7 Average attribute intensities for each quality grade. Only those attributes are shown which differed significantly between grades. Bars with different alphabetical letters are significantly different from each other ($p<0.05$). Except for astringency, the letters "A", "F" and "T" in front of the attribute name refer to aroma, flavour and taste attributes, respectively.

4.6 Astringency

One attribute that deserves special mentioning is the mouthfeel attribute, astringency. In Figures 4 and 5 astringency lies on the left-hand side of the plots and, therefore, associates to some extent with the negative attributes such as “green” and “bitter”. However, astringency is not necessarily a negative characteristic; on the contrary, it is one of the most essential and defining attributes of black tea (*Camellia sinensis*), and also plays an important role in rooibos tea flavour.

It has been found that sensory panels have struggled to accurately describe astringency, and that this sensory phenomenon is the cause of much confusion among panellists. Lea and Arnold (1978), for instance, stated that astringency and bitterness in ciders may often be confused by untrained panellists. Also, a number of studies have broken down astringency into more than one mouthfeel characteristic. Sanderson *et al.* (1976) divided the astringent sensation in black tea into two components: “tangy astringency”, described as sharp and puckery with little after-taste effect, and “non-tangy astringency”, a mouth-drying, mouth-coating phenomenon with a lingering after-taste effect, typical of unripe bananas. The mouthfeel characteristics of various astringents were described by Lawless *et al.* (1994) as “drying”, “roughing” and “puckery“, while “astringency“ was considered to be a combination of these three attributes. Recognising the confusion that exists around the term “astringency”, Gawel *et al.* (2000) assembled terminology describing the mouthfeel sensations elicited by red wines into a two-tiered mouthfeel wheel. Astringency was broken down into seven terms including “drying”, “complex”, “harsh”, “surface smoothness” and “particulate”, each of which in turn grouped together a number of more specific terms. Also, variations in astringent sensations of red wines exist, and a lexicon providing definitions for 16 astringent sub-qualities has been developed (Gawel *et al.*, 2000). Astringency may, therefore, be considered as a multifaceted sensation that may not have one straight-forward definition. These differences in mouthfeel characteristics may arise from differences in the structure of the astringent compounds. Differences in chain length, degree of galloylation and epigallocatechin content have been found to influence the intensity of the individual astringency attributes such as “drying”, ”chalkiness”, “pucker” and “coarseness” (Vidal *et al.*, 2003).

Taking all of this into consideration it seems likely that the astringent sensation exhibited by rooibos infusions may have been perceived as pleasant in some of the samples, while in others it may have been perceived negatively. Some of the original taste and mouthfeel descriptors generated by the panel (Addendum 3) included “soft/smooth”, “round” and “subtle”. These terms may refer to astringent sub-qualities that were not defined in the sensory lexicon. A closer investigation into rooibos tea astringency could, therefore, lead to the development of a number of mouthfeel sub-qualities with different effects on the perceived sensory quality of rooibos samples.

4.7 Distribution of samples according to geographic area

In order to find out whether the sensory attributes of the rooibos samples are linked to the geographic area of the plantation where they were harvested, the samples were divided into five groups according to their production area as shown in Table 4. The distribution of the individual samples across the rooibos production area in the Western Cape province of South Africa, is shown in Fig. 8.

Table 4 Number of samples of each quality grade for each production area

Area	No. of samples per grade				Total
	A	B	C	D	
Nardouw/Pakhuys	5	7	6	1	19
Nieuwoudtville	2	2	0	2	6
Olifantsrivier Bergreeks	2	8	4	4	18
Sandveld	0	1	5	9	15
Vanrhynsdorp	0	2	5	4	11

Information provided by J. Brand, Rooibos Ltd., Clanwilliam

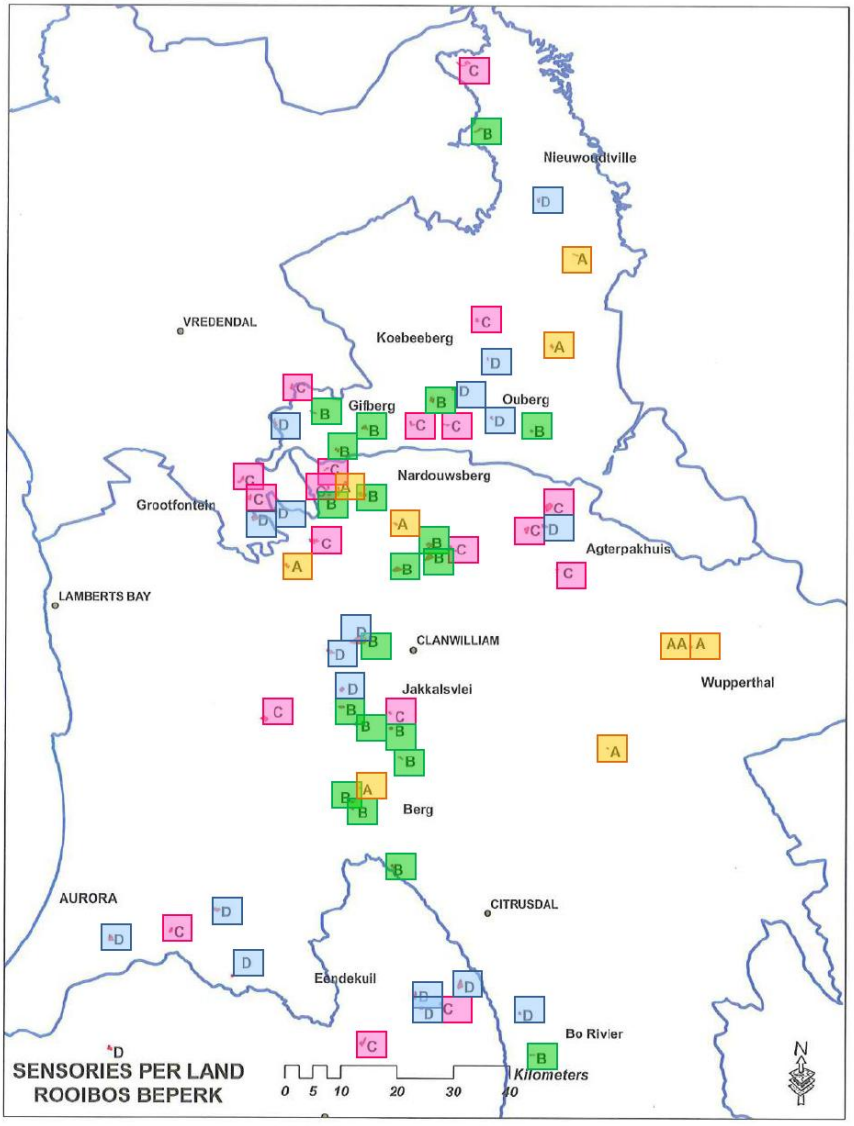

Figure 8 Locations of the plantations where individual rooibos samples were produced and harvested (Map supplied by J. Brand, Rooibos Ltd., Clanwilliam).

A PCA scores plot with the production areas as data labels (Fig. 9) shows that there was no distinct grouping or clustering according to the production area, although certain trends could be observed. As shown in Table 4 most high-quality Grade A samples (5 out of 9) originated from the Nardouw/Pakhuys area. The PCA scores plot (Fig. 9) shows that many of the samples from this area lay far towards the right of the plot reflecting a strong association with the positive rooibos attributes such as “floral” and “caramel” flavour and sweet taste. All tea samples originating from the Nieuwoudtville area, except for one, were distributed over the bottom two quadrants (Fig. 9). Since these samples lay opposite the attributes astringency and “woody” flavour it is possible that rooibos tea from this area has low intensities of astringency and woodiness. Tea samples from Vanrhynsdorp were generally low in quality (9 out of 11 samples were Grade C or D) and most of them associate with negative attributes, e.g. “plant-like” or “hay-like”, on the left hand side of the y-axis. Although most low-quality Grade D samples originated from the Sandveld area (9 out of a total of 20 Grade D samples), no noticeable clustering of the Sandveld samples was observed. Instead these low-quality samples (14 out of 15 were Grade C and D) are spread throughout the four quadrants. The same is true for the Olifantsrivier Bergreeks samples, although a large proportion of these samples (10 out of 18) are of higher quality (Grade A and B).

It is necessary to view these results in light of the limitations of the study. Firstly, it should be noted that the aim of this study was not to distinguish between samples based on the geographic location of their production areas. Consequently, samples were not selected according to a well-designed and suitably randomised field plan, and it is likely, therefore, that a significant sampling error contributes to the variation in the data. Also, differences in rooibos processing may impact the quality of rooibos infusions more significantly than the geographical location of the rooibos plantation (J. Basson, Rooibos Ltd., Clanwilliam, South Africa, 2010, personal communication). Although the production area determines whether Grade A tea can be produced, the differences in quality between Grade B and Grade C teas are linked to the processing practices of the producer (J. Brand, Rooibos Ltd., Clanwilliam, South Africa, 2009, personal communication). This means that the rooibos producer and his skill and expertise of processing rooibos also contributes toward the variation in sensory attributes between samples.

These factors would explain the wide distribution of samples originating from the same production area across the PCA scores plot (Fig. 9). It follows then that, in order to investigate the effect of the geographical area of the plantation on the sensory characteristics of rooibos tea, a more targeted and controlled approach would be required to deliver reliable results that can be interpreted without the above-mentioned limitations. Studies linking sensory attributes to geographical production areas have already been carried out for wines (Noble *et al.*, 1984b; Guinard & Cliff, 1987; Heymann & Noble, 1987; Vilanova & Soto, 2005). Attribute ratings of samples provided sufficient discriminatory power to facilitate the differentiation between samples from different geographical locations. Fischer *et al.* (1999), on the other hand, observed “tremendous” variation among wines from the same vineyard, whereas the geographic location of the vineyard had only a minor impact on the sensory properties of the wines in comparison with the vintage and

the wine estate. Such findings indicate that similar studies on rooibos may deliver interesting results which might be valuable to rooibos producers and marketers.

The impact of the rooibos production areas on the sensory quality of rooibos tea may also be of interest if this unique South African beverage obtains the status as a geographical indicator (GI). A geographical indication is a label that is reserved for products which acquire their characteristic and defining qualities as a result of their geographical location (e.g. Champagne, Florida Oranges and Parma Ham) (Grazioli, 2002). Since it allows consumers to identify the product while conveying a message about its unique features, it may be a very attractive marketing tool. Registration of rooibos as GI is underway (S. Snyman, South African Rooibos Council, 2010, personal communication), and it may, therefore, be useful to determine in which way the geographic location of the rooibos plantations may influence the sensory quality of "typical" South African rooibos.

4.8 The sensory wheel

Based on the frequency of quotation, 20 flavour attributes and 7 taste and mouthfeel attributes (Table 5) were selected from the list of descriptive terms generated by the panel during the training sessions. Seventeen of these descriptors were subjected to QDA while the remaining 10 terms did not occur frequently enough in the rooibos samples to be considered for inclusion in the QDA process. The 27 terms were assembled to form a two-tiered wheel (Fig. 10). The primary descriptors forming the outer tier are generic terms that group together a certain class of adjectives, while the more specific attributes are found in the inner tier. The terms were also grouped together based on a positive (13 descriptors) or a negative (14 descriptors) association with the sensory quality of the tea.

Aroma wheels, flavour wheels and even mouthfeel wheels have been developed for products such as beer (Meilgaard *et al*., 1979; Schmelzle, 2009), wine (Noble *et al*., 1984a; Gawel *et al.*, 2000; Mirarefi *et al*., 2004), cheese (Berodier *et al*., 1997), black tea (Bhuyan & Borah, 2001), brandy (Jolly & Hattingh, 2001) pawpaws (Duffrin & Pomper, 2006) and a range of Australian natural products (Smyth, 2010). A comparison of the attributes found in these sensory wheels showed that many of the terms are frequently used as descriptors for a variety of products. A number of common first- and second-tier descriptors that are found on other aroma and flavour wheels include the following:

"floral" (e.g. rose, lily, geranium, perfumey, violets, jasmin, orange blossom)
"fruity" (e.g. berry, citrus, cooked/processed, apricot)
"sweet" (e.g. caramel, honey, butterscotch, toffee)
"spicy" (e.g. cinnamon)
"vegetative" (e.g. fresh / cut grass, hay / straw, stemmy)
"earthy" (e.g. musty, dusty, paperbark, papery, wet cardboard, sweaty, mouldy)
"woody" (e.g. stemmy, smokey, burnt)

The basic taste modalities (sweet, sour, bitter), as well as astringency form part of almost all sensory wheels. All of these descriptors were also included in the rooibos sensory wheel.

It should be noted that this study was only the first attempt of developing a rooibos sensory wheel. The descriptors used for this wheel are limited to the 69 samples that were analysed spanning only one season (2009) of the rooibos harvest. It can be expected that certain modifications may have to be made over time as the wheel is presented to and used by interested parties. After the wine aroma wheel was developed by Noble *et al.* in 1984 (Noble *et al*., 1984a) it was positively received by the wine industry, wine writers and consumers. However, after having collected constructive suggestions from the industry, modifications to the original wine aroma wheel were proposed by the authors in 1987 (Noble *et al.*, 1987). Certain attributes were removed from the wheel while others were added; the order of the attributes was reorganised and reference standards were provided for each of the terms. Such changes, which lead to an improvement and refinement of the terminology, may also be anticipated for the rooibos sensory wheel. Finding accurate definitions and suitable reference standards for each descriptor of the sensory wheel may be an important step towards improving the usefulness and value of the wheel. Also, by analysing a number of samples from a second season of rooibos harvests the range of the flavour wheel could be further expanded.

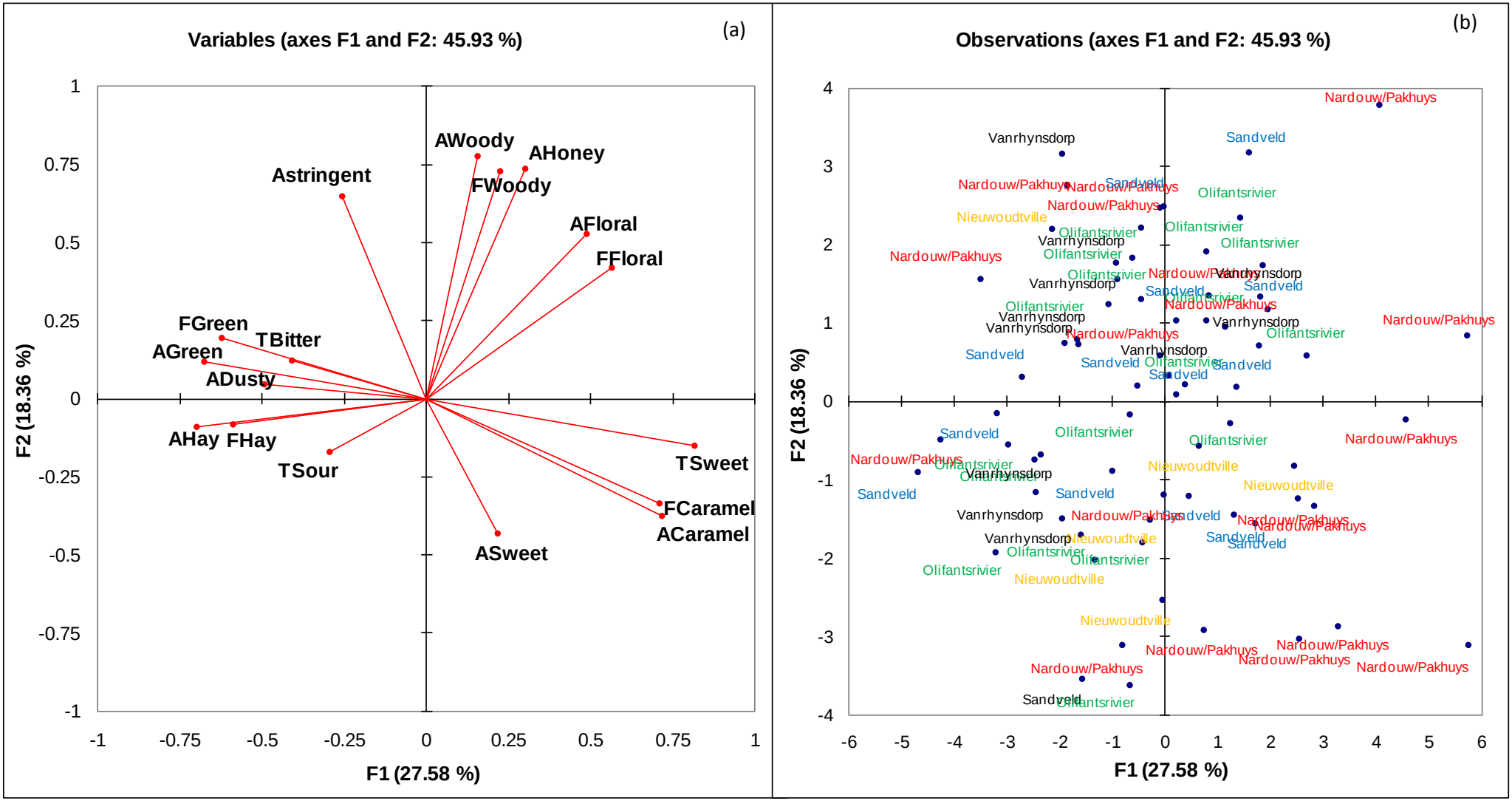

Figure 9 PCA loadings plot (a) and scores plot (b) with production areas as data labels. In the loadings plot the letters "A", "F" and "T" in front of an attribute, except for astringency, refer to aroma, flavour and taste attributes, respectively.

Table 5 Rooibos flavour and mouthfeel terminology grouped into two tiers

Type of attribute	1st tier term	2nd tier term
Flavour	**Sweet**	Caramel*
		Honey*
	Fruity*	Citrus
		Berry
		Hot apricot jam
	Woody*	Bushy / stemmy
		Smokey / burnt
	Floral	Fynbos*
		Perfume
	Spicy	Cinnamon
	Vegetative	Green grass*
		Hay / Dried grass
		Seaweed
		Rotting plant water
	Chemical	Medicinal
		Rubber
	Micro	Musty* / mouldy
		Sweaty
	Earthy	Dusty*
		Wet hessian
Taste and Mouthfeel	**Basic**	Sweet*
		Bitter*
		Sour*
	Mouthfeel	Soft / Smooth
		Astringent*
		Flat / Bland
		Harsh Astringency

Attributes with an asterix (*) indicate those attributes that were analysed during QDA.

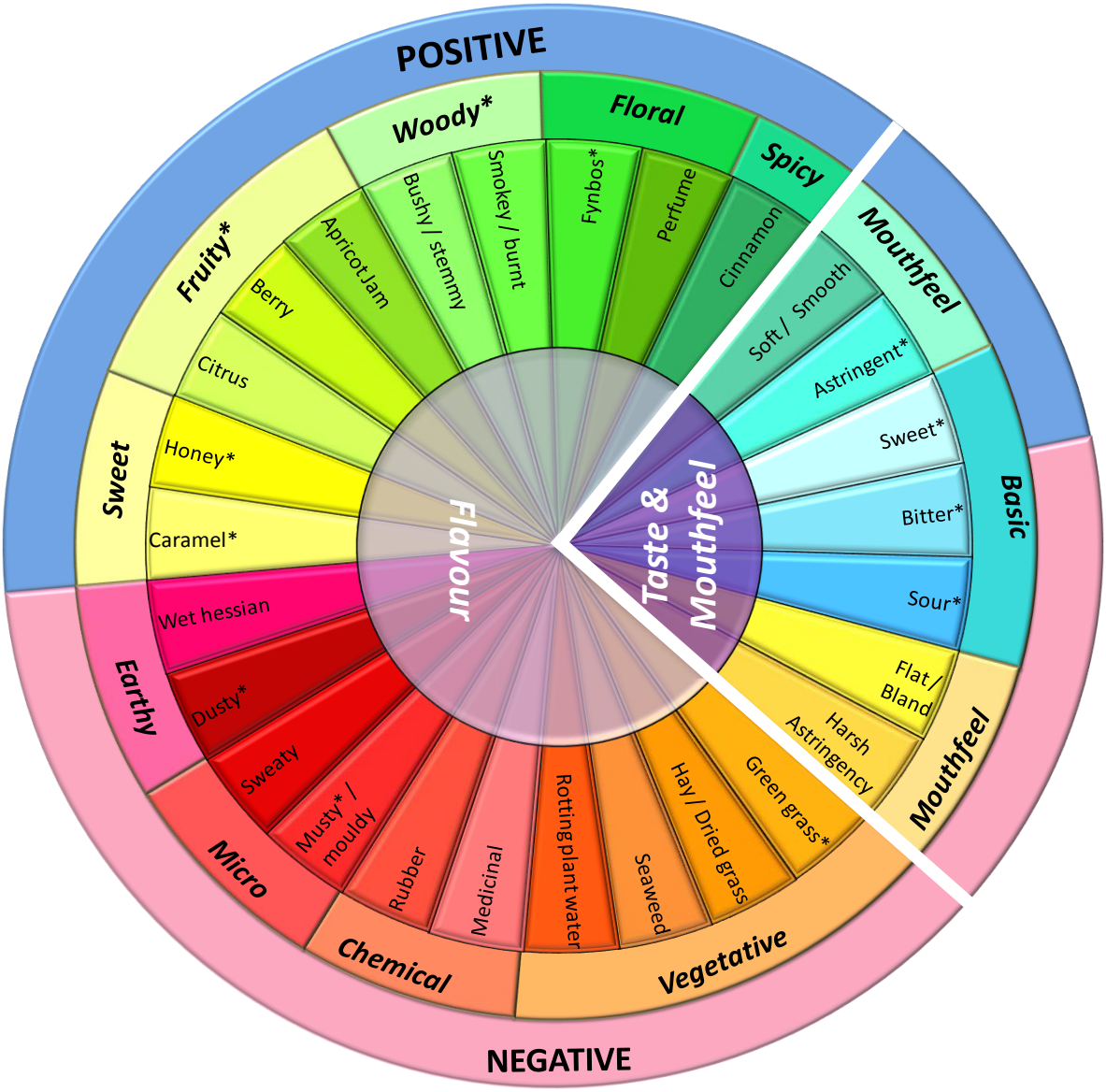

Figure 10 Rooibos sensory wheel comprising 27 terms that describe the sensory attributes of 69 rooibos infusions. Attributes marked with an asterix (*) indicate those attributes that were analysed during QDA.

4.9 The sensory lexicon

The rooibos sensory lexicon developed in this study is shown in Table 6. It comprises a descriptive term together with a definition and reference standard for each term. As mentioned earlier, five pairs of attributes determined orthonasally and retronasally ("floral", "woody", "green", "hay" and "caramel") were grouped together under five "flavour" terms reducing the number of terms from 17 to 12. Furthermore, on the score card used for QDA (Addendum 2) the attribute "green grass" shown in the lexicon had been grouped together with the descriptor "plant-like/green". This is because some samples had a strong plant-like flavour, whereas the green note of other samples was described more accurately as freshly cut green grass. Since these two descriptors refer to "green" flavours the panel was instructed to rate both flavour notes as "plant-like/green"; should the sample have a strong "green grass" character the panel was asked to note this under the heading "Other". For the purpose of the sensory lexicon it was decided to separate these "green" descriptors, since it was possible to distinguish between these aroma notes. Also, the terms "dusty" and "musty" were grouped together as one attribute during sensory analysis of the samples. Dusty and musty aroma notes did not occur very frequently in the rooibos samples (Fig. 2). However, if such an aroma note was perceived it was often a combination of a musty and a dusty odour. Consequently, it was decided to group these terms together despite the fact that their definitions are not the same. In their review on flavour lexicons Drake and Civille (2002) mention that descriptors may be linked together in this way to assist with conceptualising a particular attribute.

A literature study of other sensory lexicons revealed that many of the terms in the rooibos lexicon have also been used by other researchers. "Floral", "fruity", "sweet", "musty", "straw-like" / "dried straw", "cut grass", "bitter", "sweet", "sour" and "astringent" have all been used as descriptors for green tea (Lee & Chambers, 2007; Lee *et al*., 2008). Many of these descriptors have also been used for other food and beverage products including soy milk (Chambers *et al*., 2006), honey (Galan-Soldevilla *et al*., 2005), vegetables (Talavera-Bianchi *et al*., 2010) and various other products (Hongsoongnern & Chambers, 2008).

However, most of the rooibos attributes have a specific meaning within the context of rooibos tea. For this reason it is essential to assign specific definitions and reference standards to each descriptor when developing a sensory lexicon. The importance of reference standards has been emphasised and discussed by a number of authors (Bainey, 1986; Munoz & Civille, 1998; Drake & Civille, 2002). Providing panellists with a food, a chemical or other substances that communicate the concept of a specific attribute increases their understanding of the attribute and improves the clarity of the attribute terminology (Drake & Civille, 2002). References can be qualitative or quantitative (Munoz & Civille, 1998). Qualitative references are essential when developing sensory lexicons since they allow for clarification of the terminology for future use or comparison. Quantitative references, however, are not used as frequently during sensory analyses (Munoz & Civille, 1998). In this study the reference standards that were used were all qualitative, since they were not used to indicate the perceived intensities of attributes. Attribute intensities were rated in relation to the reference rooibos samples, or to the samples exhibiting the most prominent or the weakest intensities of a

specific attribute. This type of intensity scaling is referred to as product specific scaling which is based on the concept that attribute intensities are rated within the boundaries of the product category being studied (Munoz & Civille, 1998). Obtaining suitable qualitative reference standards that accurately exemplify specific attributes is a challenging task because of the unique sensory characteristics of each food and beverage product. Civille and Lyon (1996) compiled a book of standardised definitions and reference standards for a wide range of flavour descriptors. However, the proposed definitions and references are not always well-suited for the product being evaluated. The search for appropriate references, therefore, cannot be limited simply to a literature study but requires a time-consuming examination of a wide range of products and chemicals until the most suitable substance has been identified.

An attribute that requires special attention is “plant-like/green”. This attribute is often used for a wide range of products to describe a variety of flavour notes. Lee and Chambers (2007) found that the term “green” was too general to describe the different aroma notes in green tea and, therefore, broke down this general descriptor into a number of more specific terms with vegetable references for each note including “spinach”, “parsley”, “celery”, “green beans” and “green herb-like”. Hongsoongnern and Chambers (2008) evaluated about 30 products including fresh and processed vegetables, fruits, herbs, and green tea, and found that the sensory characteristic “green” is not a single attribute but can be characterised as unripe, peapod, grassy/leafy, viney, fruity or combinations of these. Examining the definitions developed in the present study it can be said that the descriptor most closely related to the “plant-like/green” attribute of rooibos would be “green-grassy/leafy”, which is defined as “a green aromatic associated with newly cut grass and leafy plants; characterised by sweet and pungent character” (Hongsoongnern & Chambers, 2008).

Also, Hongsoongnern and Chambers (2008) found that other attributes, e.g. “musty/earthy”, “astringent”, “bitter”, “overall sweet” and “floral”, were intrinsically associated with the green character of many products. In the present study, the correlation coefficients between green flavour and aroma and other attributes (Table 3) show that this attribute significantly correlates with “astringency”, and “sour” and “bitter” taste. The PCA loadings plot (Fig. 4) also indicates that “green” flavour is associated with bitterness, as well as “dusty/musty” aroma, although the correlation between “green” and the latter term is not significant (Table 3). “Green” notes associated with low quality rooibos may thus be coupled to other sensory attributes, especially to sour and bitter taste properties as well as to astringency.

Table 6 Sensory lexicon describing flavour and mouthfeel characteristics of rooibos infusions analysed by QDA

Attributes	Definitions	Reference standards
Floral	The unique, somewhat sweet aromatics associated with flowers of the fynbos vegetation	α-ionone[3] (1.5 µl in 100 ml water)
Woody	Aromatics associated with dry bushes, stems and twigs of the fynbos vegetation	2% (v/v) FTNF Rooibos Extract [Rooibos Ltd., Clanwilliam, South Africa]
Honey	Aromatics associated with the sweet fragrance of fynbos honey	Wild Flower Honey [Hillcrest, South Africa]
Caramel	Sweet aromatics characteristic of molten sugar or caramel pudding	0.4% (v/v) Wild® Natural Flavour Type Caramel, [Comhan Products, South Africa]
Sweet, "Fruity sweet"	An aromatic associated with the sweet smell of non-specific fruit especially berries and apricot jam	1 teaspoon each of Superfine Apricot Jam [All Gold] and Strawberry Jam [All Gold] dissolved in 100 ml hot water
Plant-like / Green [1]	Slightly sour aromatics characteristic of freshly cut green leaves or plant material	Fresh parsley water (25 g of fresh parsley, rinse, chop, add 300 ml water, allow to stand for 10 min, filter and serve liquid part)
Grassy [1]	Aromatics associated with freshly cut grass	*Cis*-3-hexenol[3] (5 µl in 100 ml water); or finely chopped fresh green grass (*Pennisetum clandestinum*)
Hay / Dried grass	Slightly sweet aromatics associated with dried grass or hay	Dried grass (*Pennisetum clandestinum*)
Dusty [2] **Musty** [2]	Earthy aromatics associated with wet hessian or wet cardboard Mouldy aromatics associated with mildew or damp cellars	Place a piece of old, dry tree bark (*Jacaranda mimosifolia*) in 100 ml hot water, allow to stand for 10 min, filter and serve liquid part
Sweet	Fundamental taste sensation of which sucrose is typical	0.1% Sucrose[3]
Sour	Fundamental taste sensation of which citric acid is typical	0.035% Citric acid[3]
Bitter	Fundamental taste sensation of which caffeine is typical	0.03% Caffeine solution[3]
Astringent	The drying, puckering sensation on the tongue and other mouth surfaces	0.7% Alum solution[3]

[1] "Plant-like / green" and "grassy" were grouped together under one attribute during QDA

[2] "Dusty" and "musty" were grouped together under one attribute during QDA

[3] Suppliers of these chemicals are given in Addendum 4

5. Conclusions

The so-called “characteristic” sensory quality of rooibos infusions may be best described as a mixture of honey, woody and fynbos-floral flavours with a slightly sweet taste and a subtle astringent mouthfeel. Caramel flavour and a fruity-sweet flavour were also present in many of the rooibos infusions, while low-quality samples often had off-taints such as grassy, hay-like and musty/dusty notes and a slightly bitter or sour taste. A rooibos sensory wheel was assembled from 27 rooibos attributes. The wheel is a simple and convenient tool that summarises and displays a wide range of product attributes which may be useful to various parties of the industry, as well as to flavour houses and marketers. Also, a rooibos sensory lexicon was developed that is composed of 14 sensory attributes along with definitions and reference standards for each term. The sensory lexicon may find application in future research involving the sensory quality of rooibos, in training sensory panels and quality control personnel of the industry and in communicating the concept of “characteristic” rooibos flavour to international markets since it provides a clear, defined set of terminology. The sensory wheel and lexicon may also facilitate the distinction between high and low quality tea based on its sensory attributes. Variation in sensory characteristics between different rooibos quality grades, as well within each quality grade, has been established, indicating that tea of lower quality does not necessarily have unpleasant or negative sensory attributes. This suggests that rooibos grading, based solely on the appearance of the leaves, the colour of the infusion and the overall flavour rating, may not always accurately predict the sensory characteristics of the tea infusion. Nevertheless, most low-quality samples (Grade C and D) were associated with negative sensory attributes such as green, hay-like, and a bitter and sour taste, while higher-grade samples (Grade A and B) were generally associated with the “characteristic” rooibos attributes, i.e. honey, woody and floral flavours and a sweet taste. No apparent clustering of samples was observed based on the geographical location of the production areas of the teas indicating that variation in rooibos sensory attributes may be affected more significantly by processing than by the location of the plantation. However, since the main aim of this study was not to differentiate between samples based on their geographic origin and representative samples were not selected accordingly, it is possible that the effect of the production area was unapparent as a result of significant sampling errors that contributed to the variation in the data.

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Chapter 4

THE CHEMICAL COMPOSITION OF ROOIBOS INFUSIONS AND ITS RELATION TO THEIR SENSORY QUALITY

Table of Contents

1. Abstract

Quantification of the levels of soluble solids (SS), total polyphenols (TP), tannins and 14 monomeric phenolic compounds, as well as spectrophotometric colour measurements of 69 rooibos infusions, previously subjected to quantitative descriptive analysis, revealed that a large variation exists in the composition of the different rooibos samples. The quality of these batches of rooibos (i.e. the appearance of the rooibos leaves, and the colour and flavour of a rooibos infusion) was rated by expert graders from the industry. Correlations between compositional parameters, quality parameters and sensory attributes revealed that high quality rooibos is associated with higher levels of SS, TP, tannins and monomeric phenolic compounds. Quercetin was most strongly correlated to the infusion colour, while quercetin-3-glucoside, vitexin and luteolin-7-glucoside were associated most strongly with the flavour rating of the tea. Correlation coefficients between flavour/taste attributes and compositional parameters revealed that the negative sensory attributes, "green" and "hay", were negatively correlated with most phenolic compounds. A number of non-volatile compounds, including enolphenylpyruvic acid-2-glucoside, quercetin-3-glucoside, and iso-orientin, were associated with the characteristic sweet taste of rooibos, while bitterness was related to several flavonoids including luteolin, quercetin and aspalathin. The tannin content was not associated with astringency. This may be as a result of methodology which may not have delivered an accurate measurement of the components responsible for the subtle astringency of rooibos infusions. Although astringency was only significantly correlated with rutin, it is likely that aspalathin and several other flavonoids also contributed to astringency. The impact of phenolic oxidation products, amino acids, polysaccharides and volatile compounds on the sensory quality of rooibos tea was not taken into account and should be analysed in future research in order to obtain a more comprehensive overview of their impact on rooibos flavour.

2. Introduction

Food and beverage products are composed of mixtures of different non-volatile and volatile molecules which differ in size, shape and polar properties, and consequently also in their flavour properties (Dutta *et al*., 2003). The human sense of taste, or gustation, is associated with the detection of non-volatile compounds by the tongue, whereas the sense of smell, or olfaction, is related to the detection of volatile compounds by the nose (Dutta *et al*., 2003). The perceived flavour of a product is then the sum of the taste and aroma characteristics of the product. It depends not only on the type, the number and the ratio of the various components, but also on their individual detection thresholds, as well as on certain interactions taking place between the compounds (Drake & Civille, 2002). Taking all of these factors into consideration, it becomes evident why the comprehensive analysis of the flavour of food and beverage products is indeed a complex and challenging task.

A vast amount of research has been conducted on the flavour of black tea (*Camellia sinensis*). It has been well established that the interplay of various non-volatile and volatile compounds in a tea infusion are

responsible for the unique flavour characteristics of this beverage (Hara *et al*., 1995; Scharbert & Hofmann, 2005). Numerous studies have attempted to explain the characteristics of a tea infusion, and to link certain components or parameters to the quality of tea (e.g. Millin *et al.,*1969; McDowell *et al*., 1991; Taylor *et al*., 1992; Wright *et al*., 2002; Liang *et al*., 2003; Scharbert & Hofmann, 2005). Although some of these studies have delivered interesting and promising results, the data reported on the major flavour compounds of tea infusions are quite contradictory, highlighting the difficulties revolving around flavour analysis.

Compared to the extensive amount of research done on black tea flavour, no studies have yet been done to investigate which compounds contribute to the unique flavour and sensory quality of a traditional rooibos infusion. Only one study has analysed the taste quality of different fractions of green (unfermented) rooibos (Reichelt *et al*., 2010). However, except for two dihydrochalcones, aspalathin and nothofagin, no other individual phenolic components of the infusion were directly related to specific taste characteristics. Also, unfermented rooibos was used in the study, and since there is a distinct contrast between the flavour of fermented and unfermented rooibos, different sensory attributes would have been relevant to describe the taste qualities of the rooibos fractions.

The identification of compounds that are essential for rooibos tea flavour and quality can be useful for a number of reasons. Once a group of key components of rooibos flavour has been identified the quantification of these compounds could serve as an indication of the quality and flavour characteristics of the tea. This would make possible the distinction between high and low quality tea based on the levels of certain components, which in turn would enable tea marketers to improve product differentiation of rooibos based on its quality. Also, if certain flavour impact compounds could be linked to specific sensory attributes such as sweetness or astringency, the sensory characteristics of an infusion might be predicted by the levels of these compounds. This could lead to the development of rooibos tea products aimed at niche markets, such as rooibos tea with a particularly sweet taste.

This study was conducted to establish whether significant correlations exist between certain chemical/ instrumental parameters and the sensory characteristics of a rooibos infusion, i.e. its flavour and colour. The focus of the study was on non-volatile compounds and their link to taste and astringency. The variation in the levels of soluble solids, total polyphenols, tannins and monomeric phenolic compounds, as well as in spectrophotometric colour measurements, was related to the sensory profiles of a number of rooibos infusions to reveal patterns or relationships between the composition and sensory quality of rooibos tea.

3. Materials and Methods

3.1 Rooibos samples

The rooibos samples used for this study (n = 69) were the same as those described in Chapter 3.

3.2 Quality rating by expert tasters

Expert graders, associated with the rooibos processing facility that provided the samples, prepared an infusion from each of the 69 rooibos batches. The appearance of the dry rooibos leaves and the infused leaves, and the overall flavour and colour of each infusion were then evaluated, and a score out of 10 was given for each of these four parameters. Each score value was multiplied by a certain predetermined factor according to the weight assigned to each quality parameter. The highest weight was allocated to the flavour of the infusion. The quality grade assigned to each sample (Grade A, B, C and D) was based on the totals of the final scores.

3.3 Sample preparation

The infusions were prepared as described in Chapter 3. A 200 ml aliquot of each infusion prepared for quantitative descriptive analysis was filtered through Whatman No. 4 filter paper and allowed to cool. The soluble solids content of the filtered infusion was determined (Section 3.6), and the remaining part of the infusion transferred into several 2 ml microfuge tubes. These were stored in a freezer at -18°C until required for further analyses. The layout of the sample preparation and analysis protocol is shown in Fig. 1.

3.4 Quantitative descriptive analysis (QDA)

Descriptive sensory analysis was conducted as described in Chapter 3.

3.5 Chemicals

Chemicals required for HPLC analysis were 99.8% acetic acid (Fluka, Sigma-Aldrich, Steinheim, Germany), acetonitrile (LiChrosolv, gradient grade for liquid chromatography, Merck, Darmstadt, Germany) and ascorbic acid (Sigma-Aldrich). Aspalathin (Asp) was supplied by the PROMEC Unit of the Medical Research Council (Parow, South Africa). Iso-orientin (IsoO), iso-vitexin (IsoV), luteolin (Lut), chrysoeriol (Chrys) and hyperoside (Hyp) were obtained from Extrasythese (Genay, France). Sigma-Aldrich provided quercetin (Quer) and rutin (Rut), while Roth (Karlsruhe, Germany) supplied orientin (Ori), vitexin (Vit), quercetin-3-glucoside (Q3g) and luteolin-7-glucoside (Lut7g).

A Modulab water purifier (Continental Water Systems Corporation, San Antonio) was used for the preparation of laboratory grade deionised water. Further purification using a Milli-Q 185 Academic Plus water purifier (Millipore, Bedford, MA, USA) generated HPLC grade water for preparation of the mobile phase. The reagents required for the quantification of the total polyphenol content and tannin content were Folin-Ciocalteu's phenol reagent (Merck), anhydrous sodium carbonate (Saarchem, Gauteng, South Africa), gallic acid (Sigma Aldrich), methyl cellulose (Sigma-Aldrich), ammonium sulphate (Saarchem) and (+)-catechin hydrate (Sigma Aldrich).

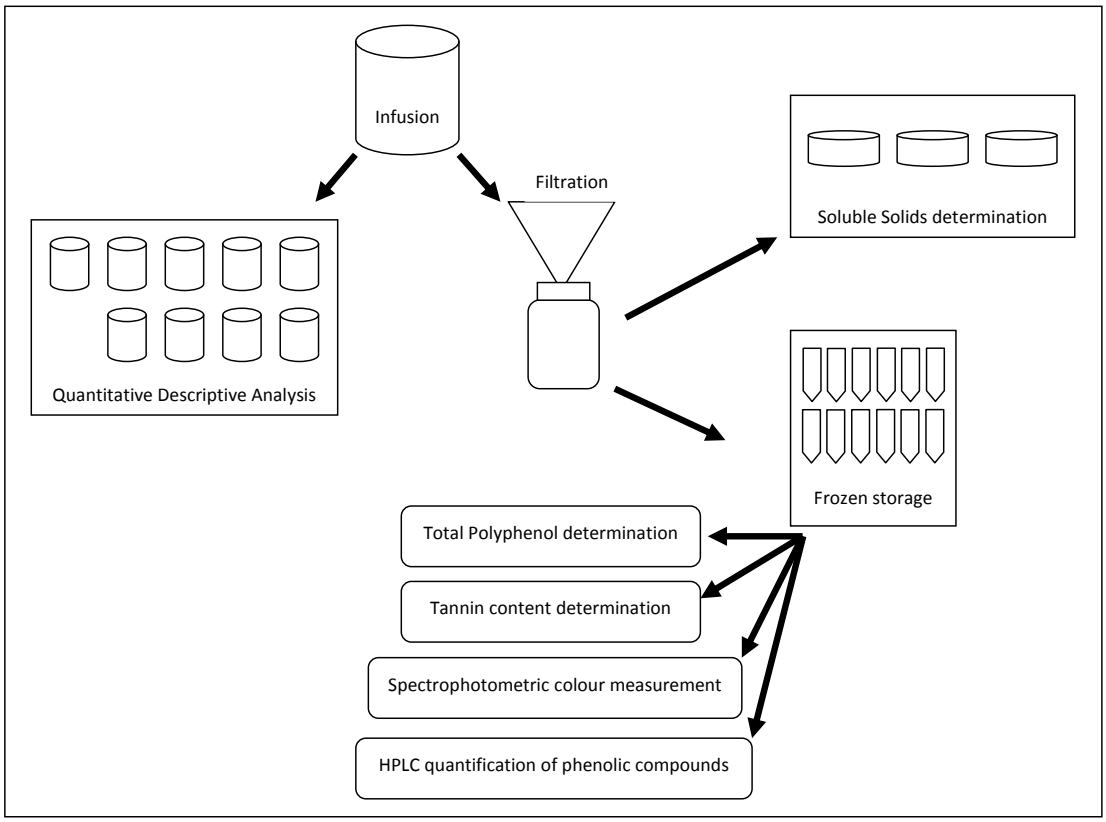


Figure 1 Layout of sample preparation and analyses.

3.6 Determination of soluble solids (SS) content

The SS content of the infusions was determined gravimetrically by evaporating 20 ml aliquots of the rooibos filtrate to dryness on a steam bath (Merck), in triplicate, in pre-weighed nickel moisture dishes, followed by oven drying at 100°C for 1.5 h. The moisture dishes were allowed to cool in a desiccator before re-weighing. Results were expressed in mg/l infusion.

3.7 Determination of total polyphenol (TP) content

The TP content of the rooibos infusions was determined according to the method developed by Singleton and Rossi (1965), scaled-down to 96-well microplate format. After defrosting at room temperature, the filtrate samples were diluted (90 µl sample diluted to 1000 µl) to obtain absorbance values within the range of the calibration curve. Twenty µl of each of the standards, samples and the assay control (deionised water) were transferred in triplicate into a 96-well polystyrene flat bottom plate. Folin-Ciocalteu's reagent (10 x diluted; 100 µl) and sodium carbonate solution (7.5% w/v; 80 µl) were added, followed by mixing of the well contents using an Eppendorf MixMate (Merck, Capte Town, South Africa). Plates were incubated at 30°C for 2 h whereafter absorbance was measured at 765 nm using a Biotek Synergy HT multiplate reader (BioTek Instruments, Winooski, USA). Gallic acid was used to prepare a calibration curve ranging from concentrations of 1 mg/l to 10 mg/l. TP content was thus expressed as mg gallic acid equivalents (GAE)/l infusion.

3.8 Determination of tannin content

An adapted version of the methyl cellulose precipitable (MCP) tannin assay developed by Sarneckis *et al.* (2006) was used for the quantification of the tannin content of the rooibos infusions. In order to ensure that absorbance measurements would fall within the range of the standard curve, samples were diluted to a SS concentration of about 0.8 mg/ml. The first part of the assay was performed in 2 ml deepwell plates. For each sample, a "treatment" and a "control" mixture were prepared on the sample plate. The "treatment" mixture was prepared by transferring, in triplicate, 400 µl of diluted sample and 240 µl methyl cellulose solution (0.04% w/v, preparation according to Sarneckis *et al*., 2006) to the plate. For preparation of the "control" mixture methyl cellulose solution was replaced by 240 µl distilled water. For the standards, only "control" wells without methyl cellulose were prepared. Saturated ammonium sulphate solution (160 µl) was added to all the wells. The plates were then covered with silicone mats, and the contents of the wells were mixed using an Eppendorf MixMate, followed by a 20 min incubation period at room temperature. The plates were then centrifuged at 4000 rpm for 10 min (RCF = 2218) using a Hettich Universal 320 R centrifuge (Labotec, Cape Town, South Africa). After centrifugation, 100 µl of the supernatant from each well were transferred into corresponding wells of a 96-well, flat-bottom UV plate using a multichannel pipette. A Biotek Synergy HT multiplate reader was used to take absorbance readings at a wavelength of 280 nm. The average absorbance values for the triplicate treatment samples were subtracted from the average values of the control samples. This difference was then used to calculate the tannin concentration from the calibration curve. (+)-Catechin was used as the standard for preparing the calibration curve which had a concentration range of 25 to 250 mg/l (Fig. 2). Tannin content was thus expressed as mg catechin equivalents (CE)/l infusion.

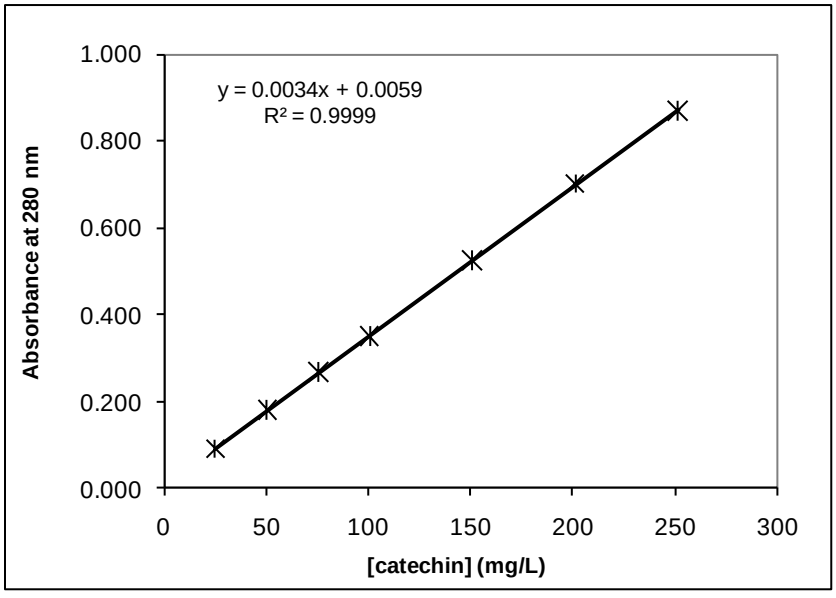


Figure 2 Catechin calibration curve with a correlation coefficient of 0.9999.

3.9 Quantification of monomeric phenolic compounds with HPLC

Quantification of 14 monomeric phenolic compounds was achieved by HPLC-DAD using an Agilent 1200 system (Agilent, Santa Clara, CA, USA) comprising a quaternary pump, autosampler, in-line degasser, column thermostat and diode-array detector (DAD). A Zorbax SB-C18 column (Rapid Resolution HT, 4.6 x 100 mm, 1.8 µm particle size, Agilent) protected by an Acquity UPLC® BEH C18 pre-column (Van Guard, 2.1 x 5 mm, 1.7 µm particle size, Waters, Milford, USA) with an in-line filter frit (2.1 mm, 0.2 µm pore size, Waters) was used for separation of the compounds at 36°C with acetonitrile and 2% acetic acid as the solvents. The flow rate was set to 1 ml/min. The gradient profile of the separation is shown in Table 1. For each of the 12 standards shown in Table 2 a stock solution was prepared using dimethyl sulfoxide. Aliquots of each stock solution were kept frozen and were only defrosted at room temperature about 20 minutes before preparing the standard mixture for injection. In order to set up a standard concentration curve for each compound, 6 different injection volumes (1, 5, 10, 20, 30 and 40 µl) of the standard mixture (Table 2) were injected. The correlation coefficients for each of the calibration curves are given in Table 2.

Table 1 Gradient profile for HPLC analysis

Time (min)	Acetonitrile (%)	2% Acetic acid (%)
0	10.0	90.0
2	10.0	90.0
14	14.8	85.2
38	50.0	50.0
40	50.0	50.0
41	10.0	90.0
50	10.0	90.0

Table 2 Composition of standard mixture

Compound	Concentration in standard mix (µg/ml)	Correlation coefficient of calibration curve
Aspalathin	21.5	1.00000
Iso-orientin	22.0	0.99999
Orientin	20.3	0.99999
Iso-vitexin	11.1	0.99999
Quercetin-3-glc*	10.4	0.99999
Rutin	10.4	0.99999
Vitexin	10.2	0.99996
Hyperoside	5.4	0.99998
Luteolin-7-glc*	5.0	1.00000
Quercetin	2.5	1.00000
Chrysoeriol	2.5	0.99997
Luteolin	1.2	0.99996

* “glc” refers to glucoside

Upon defrosting of the samples, ascorbic acid solution (1% m/v, 100 µl) was added to 1000 µl of each sample. The mixture was filtered through a 0.45 µm Millex-HV hydrophilic PVDF syringe filter (25 mm diameter) (Millipore, Bedford, USA) into an HPLC autosampler vial. A volume of 30 µl of each sample was injected in duplicate. Retention times and spectral characteristics were used for peak identification, and peak area integrations were performed using Chemstation software. The peak areas of aspalathin, enolphenylpyruvic acid-2-glucoside (PPAG) and nothofagin were determined at 288 nm, while all other compounds were quantified at 350 nm. Quantification of all compounds, except for PPAG and nothofagin, was carried out using the appropriate standards curves. PPAG and nothofagin were quantified by means of an absorbance ratio in terms of aspalathin (D. De Beer, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, 2010, personal communication).

3.10 Colour measurements

Spectrophotometric measurements of each sample were carried out using a Biotek Synergy HT multiplate reader. After defrosting of the filtrate samples 100 µl were transferred in triplicate into wells of a 96-well polystyrene flat-bottom microplate, followed by addition of 100 µl distilled water and thorough mixing of the well contents for 30 sec using an Eppendorf MixMate. Absorbance of the 1:1 diluted mixture was measured at 10 nm intervals ranging from 380 nm to 520 nm. Using Gen5 Secure software, values for the integral of the absorbance spectrum were obtained, i.e. the Area Under the Curve (AUC), reflecting the "total colour" of the diluted sample across the wavelength range.

3.11 Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SAS® version 9.2 (SAS Institute, Cary, NC, USA). The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Student's t least significant difference (LSD) was calculated at the 5% level to compare the means of the different quality grades. A probability level of 5% was considered significant for all significance tests. Principal Component Analysis (PCA) was conducted using XLStat software (Version 7.5.2, Addinsoft, New York, USA).

4. Results and Discussion

4.1 Variation in the composition of rooibos infusions

The mean, standard deviation, maximum, minimum and range values for soluble solids, tannins, total polyphenols, monomeric phenolic compounds and AUC absorbance of the hot water infusions, prepared from the different batches of rooibos tea, are summarised in Table 3. The soluble solids content differed considerably between the samples ranging from 1665 to 2945 mg/l infusion. The average TP content was 624.98 mg GAE/l with a range of 390.06 mg GAE/l. The range of the tannin content was even larger ranging

from 236.20 to 798.76 mg CE/l with an average value of 462.82 mg CE/l. Substantial variation was also observed for the phenolic compounds, e.g. the minimum and maximum values for aspalathin and rutin differed by factors of more than 10 and 20, respectively. The major phenolic compounds, based on the average concentrations, were iso-orietin (27.28 mg/l), orientin (20.62 mg/l), aspalathin (16.66 mg/l), vitexin (12.49 mg/l) and PPAG (11.15 mg/l), whereas quercetin, luteolin and chrysoeriol were present at very low concentrations (≤ 1.0 mg/l).

As the water temperature, the ratio of tea leaves to water, and the preparation procedure were kept constant throughout the preparation of the infusions, the differences in composition are attributed to sample variation. Several factors can influence the composition of plant material including plant distribution, the genetic make-up of the seedling, seasonal effects, light intensity, drought and climate (Aherne & O'Brien, 2002; Yao *et al*., 2005). Furthermore, the age of the tea leaves, their particle size as well as tea processing can have an impact on the composition of tea leaves and infusions, as was the case for *Camellia sinensis* tea (Astill *et al*., 2001; Lin *et al*., 2003). Such factors are likely to also affect the composition of rooibos plant material. Limited control is exercised over the rooibos processing parameters. Fermentation periods, for instance, can range from 8 to 24 hours (Joubert, 1994). Consequently, over- or under-fermentation of rooibos may occur due to the inexperience of rooibos producers which would affect the composition of the various batches of rooibos. Aspalathin, for example, has been shown to be very susceptible to oxidative changes during fermentation (Joubert, 1996). The extent of the aspalathin loss would thus be related to the length of the fermentation period.

4.2 The relation between the composition and quality of rooibos infusions

The association between the chemical/instrumental parameters as well as between the individual samples is displayed on PCA loadings and scores plots (Fig. 3). All parameters are distributed on the right-hand side of the loadings plot. Luteolin, chrysoeriol and quercetin lie furthest away from the other parameters. Since they were present at very low levels PCA loadings and scores plots were generated excluding these three compounds. However, these plots, which can be found in Addendum 5, did not provide any additional insight into the distribution of the parameters or of the samples. When examining the spread of the samples across the scores plot (Fig. 3), it is apparent that samples did not form distinct clusters based on their quality grades. This means that, in terms of the chemical/instrumental parameters that were measured, the four quality grades were not distinctly different from one another. Nevertheless, all Grade A samples lie to the right of the plot whereas most of the Grade C and Grade D samples are positioned on the left-hand side. The high quality samples, therefore, associate more strongly with all of the chemical/instrumental parameters whereas low-quality samples are negatively correlated with these parameters. It can be concluded that high quality rooibos is related to higher concentrations of SS, TP, tannins and monomeric phenolic compounds, and higher AUC values than lower grade tea. This trend can also be seen when comparing the average parameter values for the different quality grades (Tables 4 and 5). Except for quercetin, luteolin and

chrysoeriol the mean values for most of the parameters were significantly lower for Grade D than for Grade A samples. Significant differences within the four quality grades followed a less consistent trend.

Instead of analysing the association between parameters displayed on a loadings plot it is often more useful to examine specific and significant correlation coefficients (r). The correlation coefficients for the chemical/instrumental parameters are summarised in Table 6, while those for the chemical/instrumental parameters and the expert grading parameters are shown in Table 7. Several strong correlations are seen in Table 6 whereas poor to very weak correlations are found in Table 7.

Table 6 shows that both the SS content and TP content were significantly correlated to all other chemical/instrumental parameters. Tannin content and "total colour" (AUC) significantly correlated with all other parameters except AUC, quercetin, chrysoeriol and luteolin in the case of tannin, and aspalathin, PPAG and nothofagin in the case of "total colour" (Table 6). From Table 7 it can be seen that the content of SS, TP, vitexin, rutin and quercetin-3-glucoside, as well as the AUC value, significantly correlated with all of the grading parameters indicating that higher levels of these parameters are associated with a higher quality score. Except for nothofagin in the case of flavour, and quercetin, luteolin and chrysoeriol in the case of colour, it would seem that the flavonoids had little impact on the grading parameters.

Having established that high quality rooibos is associated with higher levels of the measured chemical/instrumental parameters, and with the information in Tables 6 and 7, the relationship between rooibos composition and quality were examined more closely.

Dry and wet appearance of rooibos leaves

The appearance of the rooibos stems and leaves is one of the factors that affects the quality grade assigned to a batch of rooibos by expert graders, although this parameter is of lesser importance than the colour and flavour of the infusion (J. Basson, Rooibos Ltd., Clanwilliam, South Africa, 2009, personal communication). Shiny, brick-red leaves of uniform colour are an indication of high quality tea whereas dull-brown leaves are often associated with over-fermentation (Joubert, 1994).

The dry appearance of rooibos was significantly correlated to the TP content (r = 0.511) with vitexin (r = 0.562), rutin (r = 0.538) and luteolin-7-glucoside (r = 0.513) displaying the strongest correlation coefficients (Table 7). The correlation coefficients for these compounds were lower for the wet appearance of the leaves. The significant correlations indicate that polyphenolic compounds may contribute to the visual quality of the fermented rooibos plant material. However, the impact of polymeric fermentation products on the colour of rooibos leaves is probably more imperative than that of individual flavonoids, as is evident when comparing the appearance of unfermented rooibos leaves, which have a greenish colour, with that of fermented rooibos leaves exhibiting a brick-red/brown colour.

Not only is the colour of the tea leaves important when evaluating the dry and wet appearance of rooibos, but it is also stipulated in the regulations that rooibos may contain no more than 10% white sticks, which are defined as "fine sticks of rooibos plant origin, that did not take on the distinctive colour of rooibos during processing, and which detrimentally affect the appearance of rooibos" (Anonymous, 2002). Low

quality rooibos, therefore, often has a higher percentage of white sticks which represent the stem fraction of rooibos. Joubert (1984) found that the levels of SS, TP, tannins and flavonoids were lower in rooibos waste, consisting mostly of stems, compared to rooibos leaves. Consequently, the quality rating of the dry and wet appearance of rooibos containing a large percentage of stems will be lower, and its levels of SS and phenolic compounds will also be lower compared to rooibos with a smaller proportion of stems.

Colour of rooibos infusions

The "total colour" (AUC) of the rooibos infusions correlated significantly with the SS content (r = 0.551) and even more strongly with the TP content (r = 0.649) (Table 6). As for the phenolic compounds, it correlated most strongly with quercetin (r = 0.581). Furthermore, the spectrophotometric colour measurement significantly correlated with the the colour rating assigned to rooibos infusions by expert graders (r = 0.621, Table 7). Consequently, the expert colour grading was also associated with the levels of SS (r = 0.342), TP (r = 0.454) and quercetin (r = 0.381) although the coefficients were even weaker (Table 7). The subjective and objective colour rating, therefore, both reflect the significant impact of polyphenolic compounds, and quercetin in particular, on the brick-red/brown colour of high quality rooibos infusions.

The importance of quercetin was also noted by Wang *et al*. (2004) who found that this flavonoid (at concentrations between 10.0 and 33.0 mg/l) was the most important phenolic compound contributing to the colour of green tea infusions, which was quantified using tristimulus colour measurements. A quercetin solution has a bright yellow colour (Meena & Patni, 2008), whereas solutions of the dihydrochalcones aspalathin and nothofagin only have a very light yellow colour (E. Joubert, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, 2010, personal communication). This could explain why the correlation coefficient for quercetin is higher than those of the dihydrochalcones.

A comparison of the infusion colour of unfermented and fermented rooibos reveals that the former has a lighter amber/yellow colour (Anonymous, 2010), whereas fermented rooibos is well known for its brick-red, brown colour. This shows that the characteristic red-brown colour of traditional rooibos infusions is not related primarily to individual polyphenolic compounds but rather to the polymeric oxidation products that form during the enzyme-catalysed fermentation reactions. No studies have yet been conducted to analyse the effect of these fermentation products on the colour quality of rooibos infusions.

Rooibos that has not been sufficiently fermented results in an extract with a prominent orange-yellow tint, while brown or turbid extracts are typically associated with over-fermented rooibos (Joubert, 1994). The visual colour rating for such tea samples would be low as over- and under-fermented tea is regarded as low quality tea. Since the colour rating was positively correlated with the spectrophotometric AUC measurement, and high quality tea samples had significantly higher AUC values (Table 5), it seems as though high quality tea with a bright brick-red colour will always be associated with higher AUC values. However, the significant differences in the total absorbance values (AUC, Table 5) between Grade A, Grade B and Grades C and D resulted from differences in the SS content of the infusions. When normalising the AUC values in terms of the SS content (average over grade), the differences between the quality grades become

very small (Fig. 4). Differences in absorbance values between grades, therefore, mainly reflect differences in the SS content and not in the hue of the colour.

Furthermore, when evaluating the colour of a rooibos infusion it should be considered that a rooibos extract exhibits dichroism, i.e. dilution of rooibos extracts results in a change of the hue from red-brown to yellow, as is indicated by the yellowish ring that can be observed at the rim of a cup of tea (Joubert, 1995). During spectrophotometric colour measurements the absorbance of a very small volume of a 1:1 diluted infusion sample was measured. Therefore, compounds that would exhibit a brick-red brown colour in a large container used for visual colour evaluation during quality grading would appear yellow during absorbance measurements. The quantification of differences in the colour shades between high and low quality tea could only be achieved by means of tristimulus colour measurements, as conducted by Joubert (1995) who demonstrated that the red colour (a* value) of rooibos infusions plays an important role during visual evaluation of the rooibos quality. Similarly, high quality black tea infusions were also associated with deeper red and yellow colours than low quality infusions suggesting that high quality tea has a higher concentration of red and yellow tea pigments (Liang *et al*., 2003).

The tannin content was neither correlated significantly with "total colour" (r = 0.208, Table 6) nor with the colour rating of the rooibos infusions (r = 0.095, Table 7). This is surprising because it may have been expected that the tannin measurement would have captured the coloured high molecular mass phenolic compounds which form during the fermentation process. The low correlations between tannin content and colour suggest that the MCP tannin assay does not quantify all the components of a rooibos infusion that are responsible for its colour. Kennedy *et al*. (2006) stated that tannin concentration in wines can differ considerably depending on the method of quantification that is used because of the complex nature of tannins and their large chemical diversity. Also, other phenolic non-tannin components with similar structural characteristics can interfere with the tannin measurement (Schofield *et al*., 2001). These factors make reliable tannin quantification a rather difficult task. The MCP tannin assay used in this study is based on the ability of the polysaccharide polymer, methyl cellulose, to precipitate certain compounds present in the rooibos infusion (Sarneckis *et al*., 2006). However, Harbertson and Downey (2009) observed differences in the nature of the tannins precipitated by methyl cellulose and protein (BSA). This suggests that by using another method of tannin quantification it may be possible to precipitate and quantify those compounds that are associated with the colour of a rooibos infusion.

Flavour of rooibos infusions

The flavour rating awarded by expert tasters correlated significantly with the **SS content** as well as the **TP content**. A highly significant correlation was also found between the SS and TP contents (r = 0.911, Table 4) indicating that the SS content is an indirect measure of the soluble polyphenolic compounds present in a rooibos infusion. Also, the SS and TP contents of the high quality samples (Grades A and B) were significantly higher compared to the low quality samples (Grades C and D) (Table 4). This highlights the significant contribution of high levels of phenolic compounds to rooibos quality.

The SS content is a gravimetric measure of the water soluble compounds that are present in a rooibos infusion. On average, the soluble solids consist of almost 30% polyphenolic compounds and 20% tannins while the monomeric phenolic compounds make up about 5%. The oxidation reactions that take place during fermentation of rooibos result in a decrease in SS because the solubility of complex polymers that are formed is reduced (Joubert, 1984). The lower levels of SS and TP of low quality rooibos may, therefore, be related to a larger percentage of tea stems in the rooibos sample (as previously discussed), and/or to a lower solubility of phenolic compounds as a result of over-fermentation. Lower levels of SS and TP are related to lower levels of phenolic compounds as is revealed by the correlation between these parameters, most notably with iso-orientin, orientin, hyperoside, iso-vitexin and vitexin ($r > 0.7$, Table 6).

The contribution of the monomeric **phenolic compounds** to rooibos flavour is poor as is reflected by their correlation coefficients (Table 7). The most important phenolic components contributing towards a high flavour score were quercetin-3-glucoside ($r = 0.537$), vitexin ($r = 0.534$), luteolin-7-glucoside ($r = 0.525$) and rutin ($r = 0.499$). This shows that it is not necessarily the concentration of a compound that determines its impact on flavour – the levels of iso-orientin, orientin and aspalathin were much higher than those of luteolin-7-glucoside, quercetin-3-glucoside and rutin (Table 4) – but rather its detection threshold. The threshold concentrations for rutin, quercetin-3-glucoside, luteolin-7-glucoside and vitexin have been established (Scharbert *et al*. 2004a; Stark *et al*., 2006) (Table 8). In order to investigate the taste impact of these flavonoids their dose-over-threshold (Dot) factors must be calculated as was done for black tea components (Scharbert *et al*., 2004a; Scharbert *et al.*, 2004b; and Scharbert & Hofman, 2005). The Dot factor represents the ratio between the concentration of a compound and its threshold value, and is, therefore, a useful indicator of the impact that each compound has on tea flavour. The Dot factors for vitexin, luteolin-7-glucoside, quercetin-3-glucoside and rutin, based on their average values for the different grades, are shown in Table 8. Except for luteolin-7-glucoside (Grade C and D samples), the Dot values for these flavonoids were all larger than 1, which shows that these compounds are present in concentrations exceeding its threshold value, and thus contribute significantly towards rooibos flavour. Because of the extremely low taste threshold of rutin its Dot factor is very large, highlighting the significant impact of rutin on the sensory quality of a rooibos infusion through its astringency.

Polymeric phenolic compounds with a molecular weight ranging from 500 to 3000 that are able to precipitate proteins are referred to as **tannins** (El Gharras, 2009), and it is these compounds that are usually associated with an astringent mouthfeel. Compared to black tea, rooibos tea has low levels of tannin-like compounds, and very little is known about these polyphenolic substances which have been described as irregular heteropolymers of the procyanidin type with (+)-catechin and (-)-epicatechin as chain-extending units and (+)-catechin as terminal unit (Ferreira *et al*., 1998; Marais *et al*., 1998). Compounds such as the dimer, procyanindin B3, and the trimer, bis-fisetinidol-(4β,6:4β,8)-catechin, have been identified (Ferreira *et al*., 1995), but no quantification has been done to date. The results obtained in this study indicate that high quality (Grade A) rooibos has a significantly higher tannin content than lower quality rooibos (Grades C and D) (Table 5). Furthermore, a significant correlation between the tannin content and the overall flavour rating

(r = 0.381, Table 7) was observed. However, even though the physiological mechanisms of perception of astringency and flavour are fundamentally different (Bajec & Pickering, 2008) the industry expert panel does not evaluate astringency as a separate parameter. Instead it is captured by the flavour rating for the rooibos infusion, despite the fact that an astringent mouthfeel is an important sensory characteristic of rooibos tea. Without noticeable astringency the tea would be perceived as “weak”, whereas excessive astringency would also have a negative impact on its sensory quality. As previously discussed, it is probable that the tannin assay used in the present study does not accurately represent the actual concentration of tannin-like polymeric compounds. Certain tannin-like components in rooibos, such as procyanidin B3, may not have been precipitated using methyl cellulose. This is supported by the findings of Sarneckis *et al*. (2006) who showed that its stereo-isomer, procyanidin B2 (epicatechin-epicatechin), was not precipitated by methyl cellulose. This would result in an underestimation of the tannin content of rooibos infusions.

Table 3 Average values (± standard deviation) as well as minimum and maximum values for the concentrations of soluble solids (SS), tannins, total polyphenols (TP), total colour (AUC) and monomeric phenolic compounds of hot water infusions of rooibos (n = 69)

	SS (mg/l)	TP (mg GAE /l)	Tannin (mg CE/l)	AUC[2]	IsoO (mg/l)	Ori (mg/l)	Asp (mg/l)	Vit (mg/l)	PPAG (mg/l)	Rut (mg/l)	IsoV (mg/l)	Hyp (mg/l)	Lut7g (mg/l)	Q3g (mg/l)	Not (mg/l)	Quer (mg/l)	Lut (mg/l)	Chrys (mg/l)
Mean	2240	624.98	462.82	134.34	27.281	20.618	16.660	12.487	11.151	7.466	7.375	5.013	2.449	1.808	1.245	1.000	0.431	0.321
±SD[1]	*±262*	*±88.77*	*±119.73*	*±16.16*	*±4.955*	*±3.437*	*±7.419*	*±5.692*	*±2.759*	*±3.875*	*±1.353*	*±0.913*	*±2.108*	*±1.568*	*±0.603*	*±0.411*	*±0.114*	*±0.099*
Minimum	1665	439.26	236.20	106.04	13.825	11.046	4.009	1.762	4.710	0.788	3.957	2.634	0.359	0.194	0.202	0.384	0.245	0.152
Maximum	2945	829.32	798.76	175.76	36.902	27.893	41.378	23.822	17.551	16.896	10.507	7.396	9.545	6.727	2.921	2.301	0.787	0.594
Range	1280	390.06	562.56	69.72	23.077	16.847	37.369	22.061	12.841	16.108	6.550	4.762	9.186	6.533	2.719	1.918	0.542	0.443

[1]SD = Standard deviation [2]Area Under Curve

IsoO = Iso-orientin; Ori = Orientin; Asp = Aspalathin; Vit = Vitexin; PPAG = enolphenylpyruvic acid-2-glucoside ; Rut = Rutin; IsoV = Iso-vitexin; Hyp = Hyperoside; Lut7g = Luteolin-7-glucoside; Q3g = Quercetin-3-glucoside; Not = Nothofagin; Quer = Quercetin; Lut = Luteolin; Chrys = Chrysoeriol.

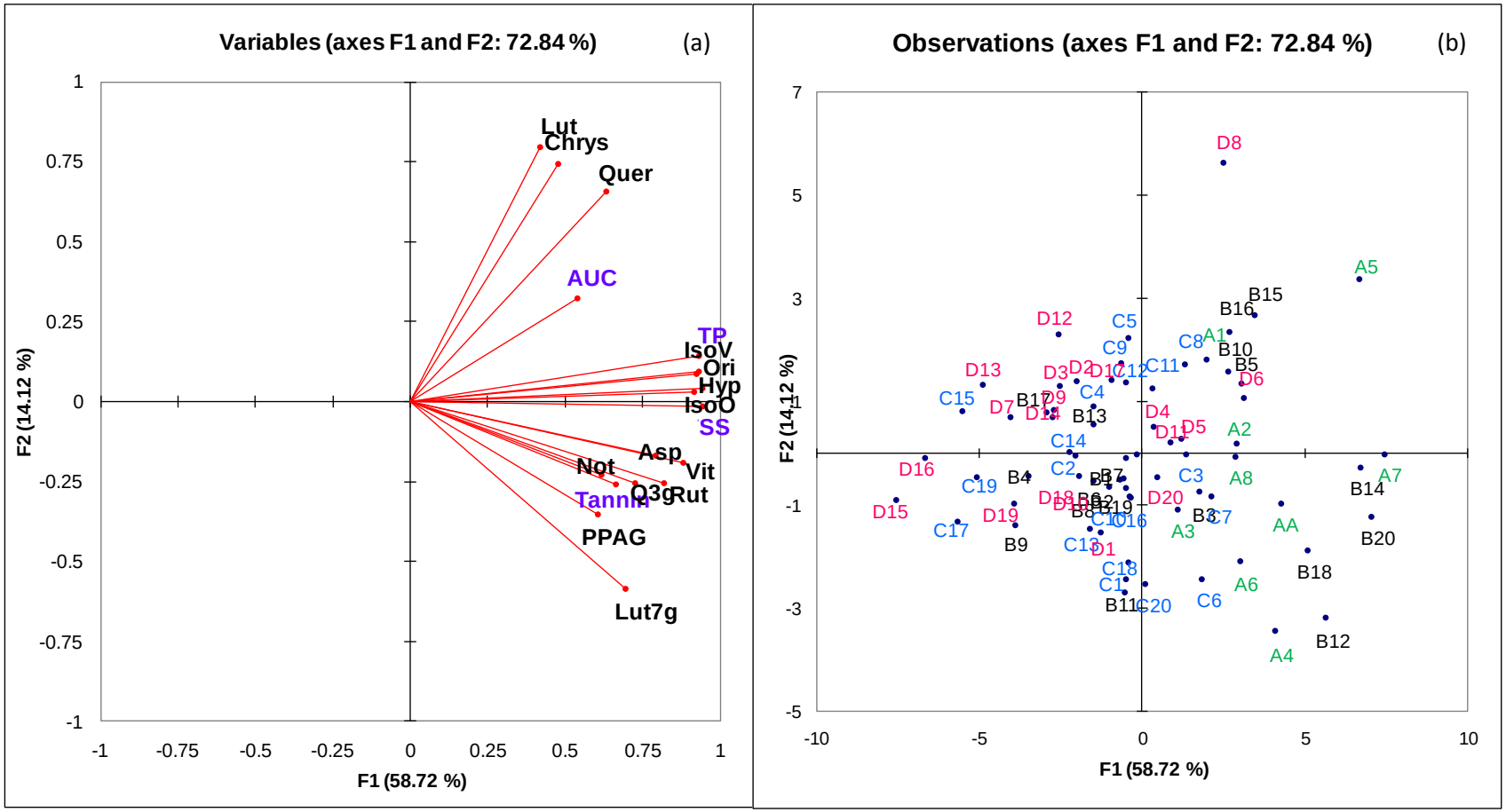


Figure 3 PCA loadings plot (a) and scores plot (b) showing the positioning of chemical/instrumental parameters and samples with respect to each other.

Table 4 Mean values for concentrations for monomeric phenolic compounds for each quality grade (in mg/l)

Grade	IsoO	Ori	Asp	Vit	PPAG	Rut	IsoV	Hyp	Lut7g	Q3g	Not	Quer	Lut	Chrys
A	33.266 a	24.669 a	20.464 a	18.468 a	12.911 a	10.991 a	8.745 a	5.942 a	4.536 a	3.985 a	1.481 a	1.210 a	0.449 a	0.368 a
B	28.442 b	21.395 b	18.914 a	14.019 b	12.260 ab	8.678 ab	7.725 b	5.189 b	3.103 b	2.219 b	1.507 a	1.046 a	0.426 a	0.329 a
C	25.462 bc	19.329 b	16.353 ab	12.552 b	10.563 bc	7.582 b	6.903 b	4.771 b	2.289 bc	1.178 c	1.101 ab	0.931 a	0.406 a	0.301 a
D	25.244 c	19.306 b	13.001 b	8.200 c	9.839 c	4.552 c	6.881 b	4.661 b	1.017 c	1.049 c	1.023 b	0.928 a	0.453 a	0.312 a

Data marked with different letters in the same column were significantly different at $\rho < 0.05$.

Table 5 Mean values for soluble solids, tannin and total polyphenol content and average AUC for each quality grade

Grade	SS (mg/l)	TP (mg GAE /l)	Tannin (mg CE/l)	AUC
A	2521 a	724.95 a	566.12 a	153.63 a
B	2323 b	656.63 b	491.03 ab	140.11 b
C	2156 c	596.80 c	440.30 bc	124.37 c
D	2115 c	576.52 c	410.64 c	129.85 c

Data marked with different letters in the same column were significantly different at $\rho < 0.05$.

Table 6 Correlation coefficients (r) for chemical/instrumental parameters

Parameter	SS	TP	Tannin	AUC
TP	0.911*			
Tannin	0.620*	0.613*		
AUC	0.551*	0.649*	0.208	
IsoO	0.872*	0.829*	0.628*	0.499*
Ori	0.862*	0.810*	0.605*	0.488*
Hyp	0.852*	0.784*	0.586*	0.446*
IsoV	0.845*	0.805*	0.571*	0.506*
Vit	0.809*	0.791*	0.558*	0.365*
Rut	0.768*	0.756*	0.564*	0.348*
Asp	0.710*	0.762*	0.611*	0.158
Lut7g	0.677*	0.579*	0.510*	0.237*
Q3g	0.675*	0.578*	0.382*	0.393*
PPAG	0.602*	0.466*	0.458*	0.216
Quer	0.585*	0.706*	0.224	0.581*
Not	0.529*	0.584*	0.424*	0.141
Chrys	0.419*	0.534*	0.110	0.485*
Lut	0.359*	0.468*	0.087	0.455*

*Values marked with an asterix are significantly different from 0 with a significance level p <0.05

Table 7 Correlation coefficients (r) between chemical/instrumental parameters and quality grading parameters

Parameter	Dry App[1]	Wet App[1]	Colour	Flavour
SS	0.451*	0.283*	0.342*	0.466*
TP	0.511*	0.340*	0.454*	0.467*
Tannin	0.351*	0.237*	0.095	0.381*
AUC	0.390*	0.318*	0.621*	0.317*
IsoO	0.428*	0.316*	0.218	0.496*
Ori	0.400*	0.289*	0.226	0.476*
Hyp	0.380*	0.248*	0.182	0.457*
IsoV	0.372*	0.261*	0.195	0.442*
Vit	0.562*	0.421*	0.303*	0.534*
Rut	0.538*	0.374*	0.292*	0.499*
Asp	0.318*	0.213	0.078	0.321*
Lut7g	0.513*	0.407*	0.167	0.525*
Q3g	0.485*	0.445*	0.261*	0.537*
PPAG	0.389*	0.373*	0.148	0.410*
Quer	0.204	0.075	0.381*	0.162
Not	0.228	0.204	0.025	0.309*
Chrys	0.179	0.135	0.366*	0.100
Lut	-0.086	-0.131	0.244*	-0.082

*Values marked with an asterix are significantly different from 0 with a significance level p <0.05

[1]App = appearance of rooibos stems and leaves

Table 8 Concentrations, taste thresholds and dose-over-threshold (Dot) factors for selected rooibos flavonoids for each quality grade

	Concentration (µmol/l)	Threshold (µmol/l)	Dot factor
Q3g*			
Grade A	8.58	[1]0.65	13.20
Grade B	4.78	[1]0.65	7.35
Grade C	2.54	[1]0.65	3.90
Grade D	2.26	[1]0.65	3.48
Average	**4.54**		**6.98**
Lut7g*			
Grade A	10.12	[2]5.2	1.95
Grade B	6.92	[2]5.2	1.33
Grade C	5.11	[2]5.2	0.98
Grade D	2.27	[2]5.2	0.44
Average	**6.10**		**1.17**
Rut*			
Grade A	18.00	[1]0.001	18002.39
Grade B	14.21	[1]0.001	14213.88
Grade C	12.42	[1]0.001	12418.72
Grade D	7.46	[1]0.001	7455.82
Average	**13.02**		**13022.70**
Vit*			
Grade A	42.71	[2]8.7	4.91
Grade B	32.42	[2]8.7	3.73
Grade C	29.03	[2]8.7	3.34
Grade D	18.96	[2]8.7	2.18
Average	**30.78**		**3.54**

[1]Scharbert *et al.*, 2004a
[2]Stark *et al.*, 2006
*The taste quality of these compounds has been described as astringent in literature.

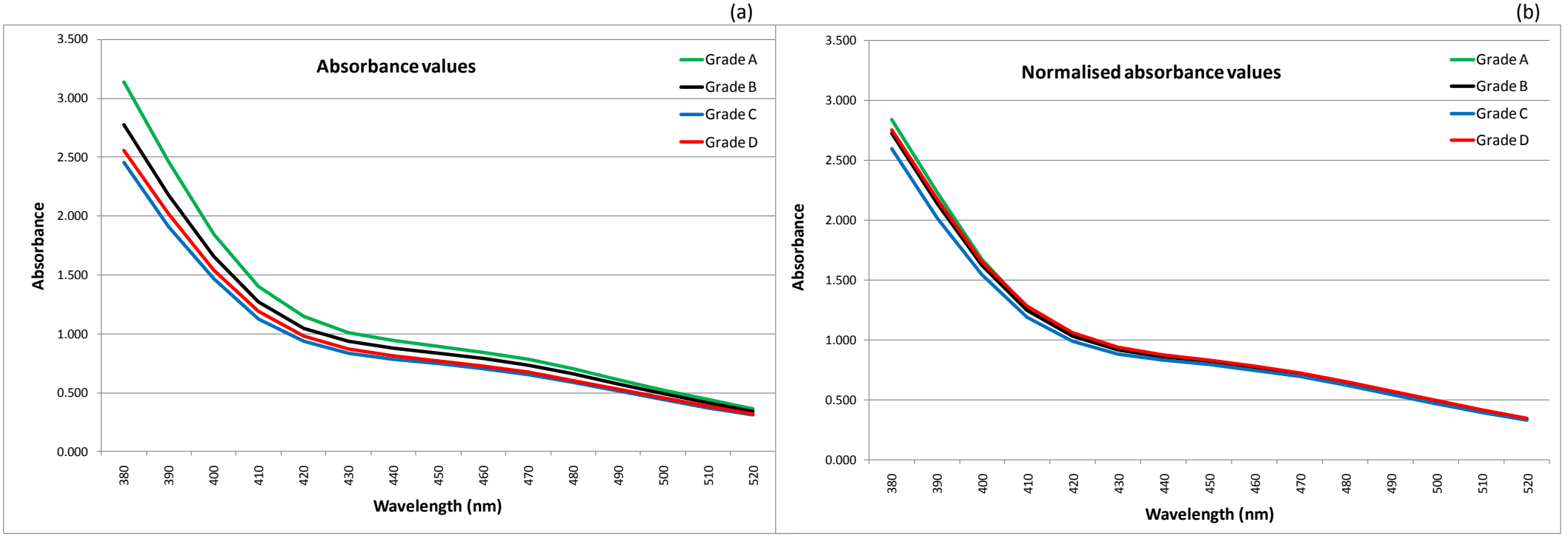


Figure 4 Absorbance values for rooibos quality grades A, B, C and D: Average values "as is" (a) and normalised according to total polyphenol content (b).

4.3 The association between chemical composition and sensory attributes

The positioning of the sensory attributes as determined by QDA together with the chemical/instrumental parameters is shown in the loadings plot in Fig. 5, whereas the corresponding scores plot displays the distribution of individual samples with respect to each other. Most of the loadings vectors were found in the right half of the plot. Only six sensory attributes associated with a negative sensory quality (sour taste, "hay" and "green" flavours and aromas, and "dusty" aroma) were positioned on the left-hand side of the plot. Again, a poor separation of the samples based on their quality grade is seen. However, the same trend is also evident in this scores plot as was seen when only taking into account the chemical/instrumental parameters (Fig. 3): High quality Grade A and Grade B samples are found in the right half of the plot, whereas most of the low quality Grade C and Grade D samples are situated on the opposite side. Luteolin-7-glucoside, quercetin-3-glucoside and PPAG associate more strongly with sweet taste, while quercetin and luteolin are positioned more closely to bitterness and astringency.

Even though PCA plots may provide valuable information relating to the spread of and association between variables and between samples, a better understanding of their relationship is often gained by analysing the correlation coefficients of the data. Because aroma and flavour attributes of the same descriptor were highly correlated (data not shown), and since non-volatile phenolic compounds are linked to the taste and not necessarily to the aroma of a product, only the correlation coefficients between the compositional parameters and the **taste, flavour and mouthfeel** attributes are shown (Table 9). All significant correlations were very weak as reflected by their low correlation coefficients ($r < 0.5$).

"Floral", "woody","caramel", "green" and "hay" flavour

The SS content correlated significantly with the three positive attributes, "floral", "woody", and "caramel", whereas the TP content was associated with "floral" ($r = 0.283$) and "woody" flavour ($r = 0.405$). A number of phenolic compounds also correlate significantly with these attributes. The highest correlation coefficients were obtained for "caramel" flavour and quercetin-3-glucoside ($r = 0.445$), "woody" flavour and quercetin ($r = 0.421$), and "floral" flavour and iso-vitexin ($r = 0.385$). All of the compositional parameters were negatively associated with "green" and "hay" flavour (Fig. 5) which was also reflected by their negative or small correlation coefficients (Table 9).

Taste buds on the tongue can only detect five basic taste modalities – sweet, sour, bitter, salty and umami – which are associated with non-volatile compounds such as sugars, polysaccharides, alcohols, acids, phenolics and nucleic acids (Jackson, 2009). The perception of flavour is then the result of the combination of these basic taste qualities and more specific flavour characteristics that arise from volatile compounds entering the nasal passages through the nose or the back of the mouth (Jackson, 2009). Since the abovementioned rooibos flavour attributes are not part of the basic taste modalities they must be related to volatile compounds which were not quantified in this study. The significant correlations between rooibos

flavours and certain non-volatile compounds, as shown in Table 9, may have occurred by chance, or because of certain interactions between non-volatile and volatile components described in Chapter 2 (Section 6.7). Furthermore, it is possible that samples with high levels of SS and phenolic compounds are intrinsically associated with higher concentrations of volatile compounds responsible for flavour characteristics, resulting in seemingly significant relationships between non-volatiles and flavour characteristics. Since volatiles were not quantified in this study the focus of this discussion is on the basic taste modalities, sweetness and bitterness, as well as the mouthfeel characteristic, astringency.

"Sweet" and "bitter" taste

The TP content was weakly, but significantly correlated to sweetness (r = 0.246) and bitterness (r = 0.256). Several phenolic compounds were also associated with these sensory attributes, e.g. PPAG, quercetin-3-glucoside and iso-orientin correlated with sweet taste, and luteolin, quercetin and aspalathin with bitterness (Table 9). Since more than one phenolic compound correlated significantly with sweetness and with bitterness, it is likely that these taste characteristics are the result of the sum of sweet- or bitter-tasting components rather than one specific sweet or bitter compound. This agrees with the results obtained by Reichelt *et al*. (2010) who found that out of a total of 16 fractions of an unfermented rooibos extract, 4 and 13 fractions had sweet or bitter taste properties, respectively.

From Table 9 it can be seen that all phenolic compounds that were significantly correlated with sweetness did not correlate significantly with bitterness, and vice versa. This may be a reflection of the basic taste character of these compounds, i.e. either bitter or sweet, but never both. Slight changes in the structure of many sweet- and bitter-tasting compounds can cause a change in their taste quality from sweet to bitter (Jackson, 2009). Quercetin, for instance, is associated with bitterness (r = 0.289), while quercetin-3-glucoside significantly correlates with sweetness (r = 0.344, Table 9). The bitter taste quality of quercetin has also been described in literature (Kawakami *et al*., 1995; Drewnowski & Gomez-Carneros, 2000), whereas its glucoside, (quercetin-3-glucoside) was not perceived as bitter (Stark *et al*., 2005). Similarly, changes in the taste quality of catechins due to glycosylation were observed by Stark *et al*. (2007), who demonstrated that the aglycones (-)-epicatechin and (-)-catechin had a bitter taste, while their respective glucosides did not exhibit any bitterness. This shows that such structural modifications of certain non-volatile compounds have an impact on their basic taste characteristics.

Rabe *et al*. (1994) stated that the natural sweet taste of rooibos tea may result partly from the presence of the dihydrochalcone, aspalathin, following demonstration of the non-nutritive sweet characteristics of certain dihydrochalcones (Inglett *et al*., 1969). However, Reichelt *et al.* (2010) evaluated the taste quality of an aspalathin solution at a concentration of 0.1 mg/l and could not identify any sweet taste characteristics. This shows that aspalathin is not responsible for the sweetness associated with rooibos tea, which is also reflected in this study by the low and insignificant correlation coefficient between aspalathin and sweet taste (r = 0.131). However, Reichelt *et al*. (2010) found that the fractions containing

aspalathin and nothofagin were both associated with bitter taste characteristics, which agrees with the significant correlations that were found between these two dihydrochalcones and bitterness (Table 9).

"Sour" taste

Sourness in food or beverage products is caused by small, soluble inorganic cations and not by polyphenolic compounds (Jackson *et al*., 2009), although it has been reported that certain simple phenolic acids, e.g. *p*-hydrobenzoic acid and ferulic acid, have acidic or sour taste characteristics (Huang & Zayas, 1991; Peleg & Noble, 1995). Although a rooibos infusion has an acidic pH (Jaganyi & Wheeler, 2003) it is not generally associated with a sour taste. Sourness was only perceived at a very low intensity in 8 of the 69 samples. Also, no significant correlations were observed between the chemical/instrumental parameters and sour taste. However, sourness was significantly correlated with "green" flavour and "green" aroma (Chapter 3, Table 3). This indicates that sourness may be related either to specific phenolic acids in rooibos, or to the compounds responsible for the green flavour that is often associated with under-fermented rooibos tea. The impact that certain volatiles may have on the basic taste modalities has been previously outlined (Chapter 2, Section 6.7) and in this way, a green aroma may amplify the perceived intensity of a sour taste quality.

Astringency

Only rutin was significantly correlated to the subtle astringency of rooibos infusions (r = 0.238). The correlation coefficients for quercetin (r = 0.236) and aspalathin (r =0.231) were only slightly lower but not significant at a 5% significance level. Scharbert *et al*. (2004a) found that rutin, also known as quercetin-3-*O*-rutinoside, induces a "silky, mouth-drying, and mouth-coating sensation", and that the oral threshold for this compound is extraordinarily low (Table 8). This may explain why rutin was the only flavonoid that was significantly associated with the subtle astringency perceived in rooibos infusions. Whether aspalathin is associated with astringency has not yet been established, whereas the slightly astringent character of quercetin has been mentioned in literature (Stark *et al*., 2005; Ley, 2008). Studies have also shown that astringency is also associated with luteolin (Stark *et al*., 2005), while others revealed that vitexin (apigenin-8-*C*-glucoside), quercetin-3-glucoside and luteolin-7-glucoside have astringent mouthfeel characteristics at different threshold concentrations (Table 8, Scharbert *et al*., 2004a; Stark *et al*., 2006). Even though no significant correlations were seen between these flavonoids and astringency, it is likely that they also contribute to some extent to the mouthfeel sensation of rooibos tea.

Whether compounds elicit an astringent sensation depends on their structural characteristics. For instance, the astringency threshold concentration of catechin glucosides is far below that of their aglycones (Stark *et al*., 2007), suggesting that the flavonoid glucosides in a rooibos infusion may be important contributors to astringency. It has also been proposed that in order for a phenolic compound to exhibit astringency it must have two adjacent hydroxyl groups (McManus *et al*., 1981). Table 10 shows that the correlation coefficient between astringency and aspalathin, which has two hydroxyl groups, was larger than

that of nothofagin, which does not have adjacent hydroxyl groups. The same is true for luteolin and chrysoeriol (Table 10). These findings suggest that aspalathin may exhibit astringent mouthfeel characteristics. Both rutin and quercetin, which displayed the strongest correlation coefficients for astringency, also have two adjacent hydroxyl groups. On the other hand, some of the compounds with two hydroxyl groups, e.g. orientin, iso-orientin, hyperoside and quercetin-3-glucoside, had correlation coefficients that were close to 0 ($r < 0.02$) indicating that other factors influence the capacity for astringency of a compound. These may include chirality, bond location, the structure of the aglycone and the sugar moiety (Scharbert *et al*., 2004a; Lesschaeve & Noble, 2005).

The well-established relationship between polyphenolic tannin-like substances and astringency has already been discussed (Section 4.2). Because the astringency detection threshold of tannins decreases as their molecular weight (M_r) increases (Lea & Arnold, 1978), it was anticipated that polymeric rooibos oxidation products with a high M_r would exhibit astringent mouthfeel characteristics. Unexpectedly, however, the tannin content was not significantly associated with astringency ($r = 0.030$). One of the tannin-like compounds found in rooibos, procyanidin B3, as well as other trimeric and tetrameric procyanidins, has been described in literature as being both astringent and bitter (Delcour *et al*., 1984). Delcour *et al*. (1984) have determined that the taste threshold of procyanidin B3 is 17.3 mg/l. Another study, however, showed that the astringent and bitter taste thresholds for procyanidin B3 isolated from red wine were 116 and 289 mg/l, respectively (Hufnagel & Hofmann, 2008). Because procyanidin B3 is only present in rooibos at low concentrations, and since its detection threshold value is much higher compared to that of the individual flavonoids (Table 8), it is not likely that this polymeric component contributes significantly to astringency of rooibos infusions. In a study analysing the compounds responsible for black tea astringency it was revealed that it was not high M_r polyphenolic compounds, but a series of flavonol glycosides that were the main contributors to astringency (Scharbert *et al*., 2004a). Considering the low correlation between tannin content and astringency that was found in this study it seems likely that this is also true for rooibos infusions. Alternatively, the poor correlation that was obtained may indicate that the tannin measurement did not accurately represent those tannin-like compounds that are associated with astringency as was previously mentioned (Section 4.2).

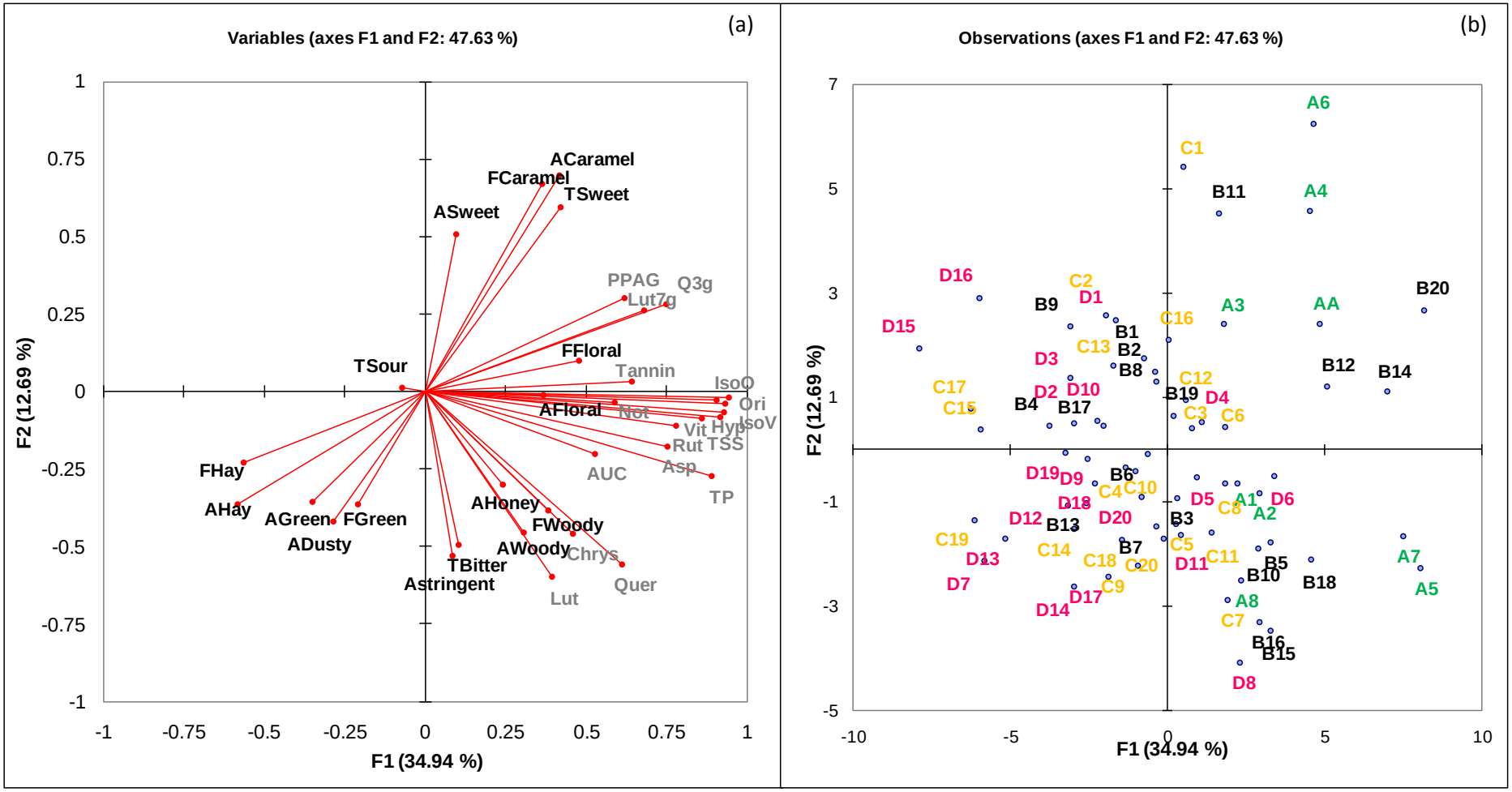


Figure 5 PCA loadings and scores plot showing the distribution of sensory attributes, compositional parameters and flavour and colour ratings (a), and the positioning of individual samples (b). Except for astringency, the letters "A", "F" and "T" in front of an attribute refer to aroma, flavour and taste attributes, respectively.

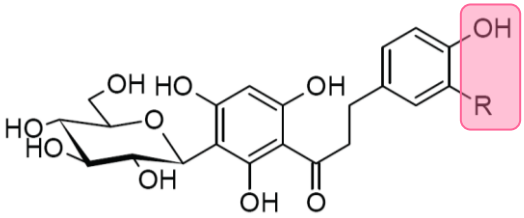
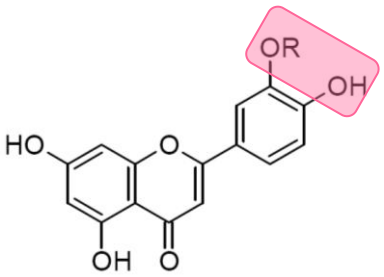
Table 9 Correlation coefficients for flavour (F), taste (T)and mouthfeel attributes, and compositional parameters

Variables	FFloral	FWoody	FCaramel	FGreen	FHay	TSweet	TBitter	TSour	Astringent
SS	**0.366***	**0.309***	**0.247***	-0.064	**-0.437***	**0.318***	0.199	0.012	0.101
TP	**0.283***	**0.405***	0.131	-0.075	**-0.390***	**0.246***	**0.256***	-0.057	0.159
Tannin	0.230	0.086	0.173	0.049	**-0.379***	0.176	0.078	0.037	0.030
AUC	0.088	**0.264***	0.151	**-0.308***	-0.124	**0.244***	0.023	-0.186	-0.027
IsoO	**0.348***	**0.251***	**0.309***	-0.226	**-0.483***	**0.334***	0.116	-0.016	0.017
Ori	**0.364***	**0.277***	**0.307***	**-0.262***	**-0.493***	**0.325***	0.128	-0.058	0.018
Hyp	**0.314***	0.225	**0.278***	-0.178	**-0.520***	**0.268***	0.144	-0.015	-0.003
IsoV	**0.385***	**0.273***	**0.310***	-0.208	**-0.478***	**0.286***	0.114	-0.005	0.045
Vit	**0.324***	**0.367***	0.200	0.075	**-0.377***	0.201	0.176	0.050	0.208
Rut	**0.283***	**0.369***	0.101	0.173	**-0.317***	0.195	0.185	0.082	**0.238***
Asp	**0.335***	0.234	0.035	0.140	**-0.414***	0.131	**0.283***	0.079	0.231
Lut7g	**0.312***	0.210	**0.344***	0.108	-0.232	**0.294***	-0.002	0.150	0.139
Q3g	**0.280***	0.180	**0.445***	**-0.283***	**-0.313***	**0.344***	0.042	0.054	-0.070
PPAG	**0.258***	0.071	**0.374***	-0.069	**-0.459***	**0.356***	-0.023	-0.032	-0.118
Quer	**0.257***	**0.421***	-0.045	-0.115	**-0.292***	0.058	**0.289***	-0.110	0.236
Not	**0.296***	0.077	0.045	0.018	**-0.312***	0.098	**0.240***	0.032	0.036
Chrys	0.051	**0.252***	-0.044	**-0.290***	**-0.271***	0.002	**0.238***	-0.188	-0.014
Lut	0.152	**0.323***	-0.106	-0.175	-0.133	-0.099	**0.300***	-0.195	0.223

*Values in bold and marked with an asterix are significantly different from 0 with a significance level $p < 0.05$

Except for astringency, the letters "A", "F" and "T" in front of an attribute refer to aroma, flavour and taste attributes, respectively.

Table 10 Relationship between structure and astringency of selected phenolic compounds reflecting the importance of adjacent hydroxyl (OH) groups for astringency

	Compound	Correlation coefficient (r) for astringency	Structure
Dihydrochalcones	Aspalathin	0.231	
	Nothofagin	0.036	Aspalathin: R = OH Nothofagin: R = H
Flavone aglycons	Luteolin	0.223	
	Chrysoeriol	-0.014	Chrysoeriol: R = CH_3 Luteolin: R = H

4.4 Other compounds and their effect on tea flavour and quality

The sensory attributes of a rooibos infusion are the result of an interplay of various components each of which may affect the perceived flavour of a tea infusion. The low correlation coefficients that were obtained in this study indicate that other components, that were not quantified in this study, may contribute significantly to the aroma, flavour, taste and mouthfeel characteristics of the rooibos infusions. Examples of such components include other non-volatile phenolic compounds (e.g. catechin, *p*-coumaric acid and ferulic acid), polymeric oxidation products that are formed during fermentation of rooibos, amino acids, polysaccharides, and volatile compounds. Also, interactions between non-volatile and volatile compounds make the elucidation of rooibos flavour an even more complex task. Certain volatiles, for example, associated with floral, caramel, honey and fruity aroma notes, may increase the perceived sweet taste that is related to certain non-volatile compounds, whereas a green aroma may reduce the intensity of the sweet taste and increase that of sour taste. Such phenomena must be kept in mind when analysing tea flavour in a comprehensive way.

5. Conclusions

Chemical/instrumental analysis of a number of rooibos infusions revealed large variations in the levels of soluble solids (SS), total polyphenols (TP), tannins, monomeric phenolic compounds as well as "total colour" measurements. Significant correlations between the concentration of the SS content and TP content, as well as between soluble solids and the levels of monomeric phenolic compounds, suggest that the SS content may be a useful indicator of the phenolic content of a tea infusion. High quality tea was associated with higher levels of SS, TP, tannins and phenolic compounds. The SS content can, therefore, act as good indicator of the quality of the tea since over-fermented rooibos, or rooibos with a high stem content, would generally contain lower levels of SS (Joubert, 1984). Quercetin contributed most significantly to the infusion colour rating by expert graders, as well as to "total colour" (AUC) suggesting that this compound is a key component of the colour of rooibos infusions, despite its low concentration. The flavonoids associated most strongly with the flavour of the infusion include quercetin-3-glucoside, vitexin and luteolin-7-glucoside, which is surprising considering the very low concentrations of quercetin-3-glucoside and luteolin-7-glucoside. However, these two flavonoids have low detection thresholds which greatly increases their flavour impact. The tannin content did not seem to play a critical role in terms of rooibos quality. However, the large coefficient of variation of the tannin quantification suggests that the measured tannin content was not a reliable representation of the actual concentration of tannin-like components in the rooibos infusions.

Correlation coefficients between taste/flavour attributes and compositional parameters were weak ($r < 0.500$) but some of them were nevertheless significant. Although positive correlations were observed between several phenolic compounds and certain flavour attributes it is likely that these sensory attributes are associated with volatile compounds which were not quantified in this study. Sweetness and bitterness were found to be associated with a number of non-volatile compounds including PPAG, quercetin-3-glucoside, and iso-orientin (sweet), and luteolin, quercetin and aspalathin (bitter). Only rutin, which has been previously described as having mouth-drying characteristics at a very low threshold concentration (Scharbert *et al*., 2004a), was significantly correlated to astringency. Results suggested that aspalathin may also have astringent mouthfeel properties. The tannin content did not contribute to astringency, which may be due to the limitations of the tannin quantification, or because other components are in fact responsible for the subtle astringency of rooibos infusions. It should be noted that the sensory impact of only a small fraction of non-volatile rooibos components was analysed in this study. The potential impact of other non-volatile and volatile flavour and aroma compounds, as well as interactions between these components, must be examined in order to paint a more accurate and comprehensive picture of rooibos flavour and how it is related to rooibos quality.

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Chapter 5

THE EFFECT OF STEAM PASTEURISATION ON THE SENSORY PROFILE AND PHENOLIC COMPOSITION OF ROOIBOS INFUSIONS

Table of Contents

1. Abstract

The effect of steam pasteurisation of rooibos leaves on the composition and the sensory characteristics of a tea infusion was analysed to determine whether heat-induced compositional changes influence the sensory quality of rooibos tea. This was achieved by examining the changes in the concentrations of soluble solids (SS), total polyphenols (TP), tannins and 14 individual monomeric phenolic compounds, as well as in 17 aroma, flavour, taste and mouthfeel attributes of the infusions. Changes in the colour of infusions, quantified by absorbance measurements at different wavelengths, were analysed to gain further insight into the changes in the phenolic components. Steam pasteurisation significantly reduced the SS content, TP content and aspalathin content of rooibos infusions, as well as the absorbance, especially at a wavelength of 450 nm. The intensities of the taste attributes, sweetness and bitterness, did not change significantly, since the levels of individual phenolic compounds, which are likely to be associated with these taste qualities, did not decrease significantly. A small but significant decrease in the astringency of rooibos infusions was observed. This may be either due to the thermal degradation of polymeric phenolic compounds, or as a result of the polymerisation and subsequent decrease in solubility of certain monomeric phenolic compounds associated with astringency, most notably aspalathin. Changes in the character of astringency were also observed: Whereas astringency of unpasteurised (UPAS) samples could be described as “green” astringency it was associated more strongly with a woody and hay-like character in pasteurised (PAS) samples. The intensities of most of the aroma and flavour attributes significantly decreased as a result of steam pasteurisation. The extent of these attribute changes was different for the different quality grades of rooibos. “Green” and “caramel” notes exhibited the largest reductions in attribute intensity. The prominent “green” flavour of UPAS samples was frequently replaced by a hay-like flavour after steam pasteurisation.

2. Introduction

Tea and herbal infusions are generally considered as being microbiologically safe, and rarely cause food-borne diseases or food poisoning. Because of their low moisture content (7% to 12%) and water activity (0.4 to 0.6) microbes are generally not able to grow and reproduce on tea leaves (Lund *et al*., 2000). However, the increase in the consumption of herbal teas and infusions necessitates the assurance of their microbiological safety, since it has been shown that these products may in fact carry considerable microbial loads. A number of reports have surfaced which revealed the contamination of medicinal herbs and herbal teas with bacteria and fungi, some of which are potentially toxigenic (Czech *et al*., 2001; Rizzo *et al*., 2004; Tournas & Katsoudas, 2008). These studies highlighted the fact that the microbiological safety of herbal teas should not be presupposed.

The severe consequences associated with poor microbiological quality were suffered by the rooibos industry, when an outbreak of *Salmonella* occurred in 1984 (Snyman, 2000). High numbers of coliform bacteria (up to 3.7×10^7 CFU/g after a 23 h fermentation period) were found on the product (Du Plessis &

Roos,1986). In response to this, steam pasteurisation of rooibos was introduced, after it had been proven to effectively reduce the microbial load of rooibos (Du Plessis & Roos, 1986). This heat treatment was so effective that it has become the industry standard (Food & Beverage Reporter, 2005). Nowadays, rooibos is steam-pasteurised at 96°C for 60 s, after which the leaves are dried to reduce their moisture content below 10% to ensure microbial stability (J. Basson, Rooibos Ltd., Clanwilliam, South Africa, 2009, personal communication). The microbiological quality of rooibos exports is monitored by the Perishable Products Export Control Board (PPECB) (Snyman, 2000).

Even though it is accepted practice to employ heat treatment of food and beverage products for controlling microbiological risks, this often causes considerable changes in their sensory properties which may not always be desirable. Pasteurisation is considered to be a mild heat treatment by which potential microbial hazards are reduced "with minimal chemical, physical and organoleptic changes in the product" (Lewis, 2006). Steam pasteurisation, however, involves the rapid heating of the surface of a product to temperatures higher than 70°C (Castell-Perez & Moreira, 2004). Because of the small particle size of rooibos stems and leaves, the heat transferred from the steam can quickly penetrate the plant material which may cause changes in its composition, and ultimately also in its sensory quality. It has been said that steam pasteurisation of rooibos results in a softening of the flavour and "strong medicinal smell" of rooibos, which may increase the acceptance of the product by consumers (Food & Beverage Reporter, 2005). On the other hand, other consumers, that have been familiar with the product for years, complained about the change in the sensory characteristics caused by pasteurisation (Food & Beverage Reporter, 2005). The anecdotal evidence of noticeable changes in the sensory quality of rooibos infusions due to steam pasteurisation has not yet been scientifically substantiated, nor have such changes been accurately described or quantified. It is thus unclear whether steam pasteurisation is beneficial for or detrimental to the sensory quality of rooibos. The objective of this study was thus to determine the effect of steam pasteurisation of rooibos on the sensory characteristics and the phenolic composition of rooibos infusions, and to establish, thereby, whether compositional changes in these non-volatile compounds could be associated with changes in aroma, flavour, taste and mouthfeel attributes.

3. Materials and Methods

3.1 Rooibos samples

The rooibos samples used for this study (n = 69) were the same as those used in Chapter 3. The complete list of samples can be found in Addendum 1.

3.2 Steam pasteurisation of rooibos samples

In order to determine the effect of steam pasteurisation, one half of each rooibos sample was steam-pasteurised. Rooibos leaves and stems (50 g) were spread out in a thin layer on stainless steel, 30-mesh trays

which were placed in a steam cabinet at 96°C for 60 s. The steam pressure, generated with a THE 400 NM Electropac electrode boiler (John Thompson Boilers, Cape Town), was maintained at 2.76 N/m^2 at the inlet of the cabinet. In order to reduce the moisture content of the steam-pasteurised rooibos below 10% the trays were placed in a dehydrator (Sigge *et al.,* 1998) set to 40°C for 10 min. The dried rooibos was then transferred to air-tight, re-sealable plastic bags.

3.3 Preparation of rooibos infusions

The infusions of unpasteurised (UPAS) and pasteurised (PAS) samples were prepared as described in Chapter 3. A 200 ml aliquot of each infusion, prepared for quantitative descriptive analysis, was filtered through Whatman No. 4 filter paper, allowed to cool and the SS content determined. Several aliquots of the remaining filtrate were transferred into 2 ml microfuge tubes which were stored in a freezer at -18°C until required for further analyses.

3.4 Quantitative descriptive analysis (QDA)

QDA was conducted as described in Chapter 3. No additional training sessions were required since the sensory attributes of the samples were identical to those generated during the panel training described in Chapter 3, and the panel was already familiar with the terminology and sample evaluation procedure. The UPAS sample and its PAS counterpart were presented together with the reference standard so that the panellists could not only compare the two samples directly, but also with the reference standard. The panel was informed that they were to receive a number of sample pairs with each pair consisting of an UPAS sample with its corresponding PAS sample. Samples were labelled with three-digit codes, while the reference sample was labelled as such so that it could be identified by the panellists. The presentation order of the different sample pairs, as well as the order of the PAS and the UPAS sample within each pair was randomised. Nine judges rated the intensity of 17 rooibos aroma, flavour, taste and mouthfeel attributes (Chapter 3, Table 2) for each of the 138 (n = 69 x 2) samples.

3.5 Chemicals

The chemicals and reagents required for this study were identical to those listed in Chapter 4.

3.6 Chemical/Instrumental analyses

All chemical and instrumental analyses were carried out as previously described in Chapter 4.

3.7 Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SAS® software (Version 9.2, SAS Institute, Cary, NC, USA). The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Student's-t least significant difference (LSD) was calculated at the 5% level to compare the UPAS and PAS samples. Principal Component Analysis (PCA) was conducted using XLStat software (Version 7.5.2, Addinsoft, New York, USA).

4. Results

4.1 Changes in sensory attributes

The effect of steam pasteurisation on the sensory characteristics of rooibos infusions can be displayed by means of PCA loadings and scores plots (Fig. 1) which reflect, respectively, the positioning of the sensory attributes with respect to each other, and the translation of the scores vectors across the quadrants. The loadings plot shows that the positive attributes are separated from the negative attributes along the x-axis from right to left. This corresponds to the separation of the score vectors from high quality Grade A rooibos on the right-hand side of the score plot to low quality Grade D tea on the left (Fig. 1). Most of the attributes – except "hay", "dusty" and "caramel" aroma, and "sweet" taste – are distributed across the upper two quadrants of the loadings plot. On the scores plot all four Grade averages of the UPAS samples are positioned in the upper quadrants while the Grade averages for the PAS samples are positioned in the bottom quadrants. This movement from the UPAS sample averages to its PAS counterparts indicates that the score vectors move away from the attributes, revealing a decrease in the attribute intensity as a result of steam pasteurisation.

The sample averages for the attribute intensities of UPAS and PAS samples are displayed in Fig. 2. Steam pasteurisation had a significant effect on the intensities of most of the 17 sensory attributes. The average intensities of the PAS samples were significantly lower for all aroma attributes except for "hay" and "dusty" aroma (Fig. 2). The average intensities of "woody", "floral" and "green" flavours, "sour" taste, and astringency were also slightly, but significantly lower in the PAS samples (Fig. 2).

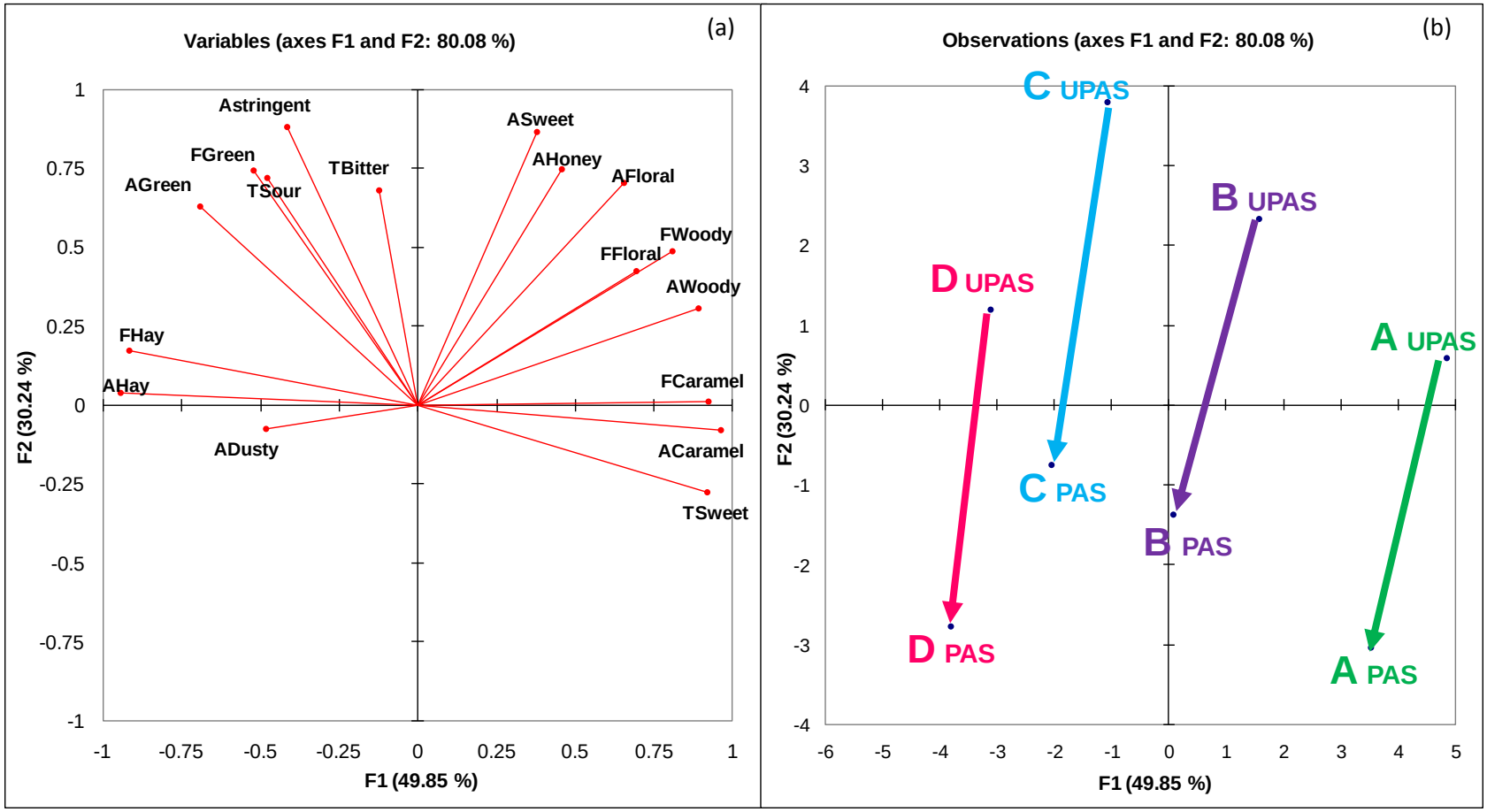

Figure 1 PCA loadings and scores plots showing (a) the positioning of rooibos attributes and (b) GradeXTreatment averages and the changes in sensory characteristics due to steam pasteurisation. Except for astringency, the letters "A", "F" and "T" in front of an attribute refer to aroma, flavour and taste attributes, respectively. The terms UPAS and PAS refer to unpasteurised and pasteurised samples, respectively.

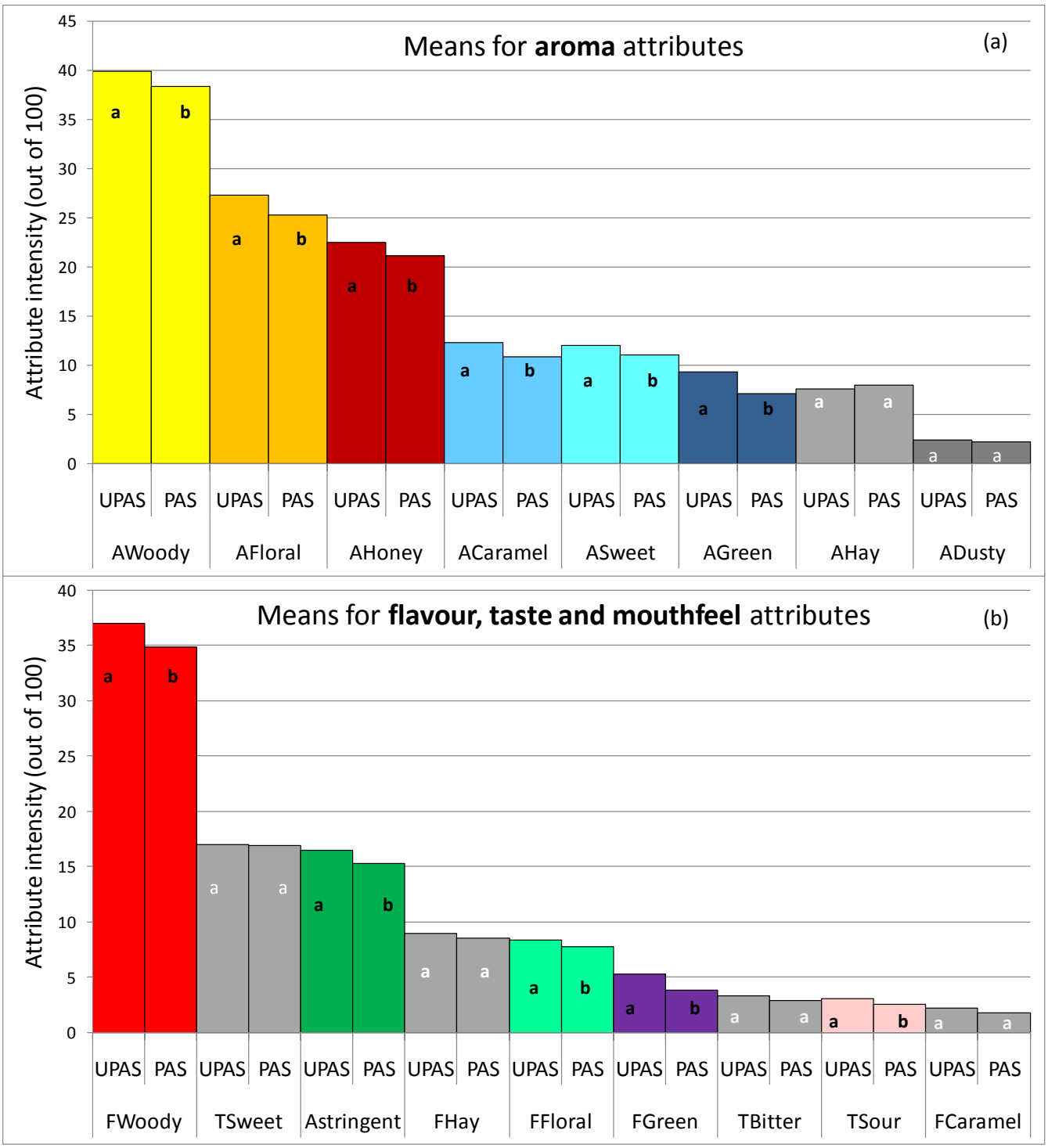

Figure 2 Average intensity values for rooibos aroma attributes (a) and flavour, taste and mouthfeel attributes (b) of unpasteurised (UPAS) and pasteurised (PAS) samples. Bars for the same attribute (same colour) but different letters differ significantly from each other ($p < 0.05$). Except for astringency, the letters "A", "F" and "T" in front of an attribute refer to aroma, flavour and taste attributes, respectively.

The percentage change in the average attribute intensity between the UPAS and PAS samples was calculated to determine which attributes decreased the most in intensity (Fig. 3). The attributes exhibiting by far the greatest changes in intensity were “green” flavour and aroma which decreased by 32% and 26%, respectively. The other attributes did not decrease as substantially as a result of steam pasteurisation.

Because it was previously shown that the intensities of certain aroma attributes differed significantly between the four quality grades (Chapter 3, Fig. 7), their relative changes in intensities were examined for each quality grade separately (Fig. 4). The “green” aroma of Grades B, C and D decreased considerably, especially that of Grade C samples, whereas the largest reduction in “caramel” aroma was seen for Grade A samples. The “woody” aroma decreased more or less to the same extent for all grades, whereas “hay” aroma showed an increase for Grades B, C and D, but a decrease for Grade A samples.

Figure 5 shows the comparison between the PCA loadings plots for UPAS and PAS samples. Other than a slight anti-clockwise rotation, the relative attribute positioning with respect to each other remained similar. However, while the “green” and “hay” attributes are separate and distinct on the UPAS sample plot, these attributes are closely associated for the PAS samples. Furthermore, “hay” aroma was the only attribute that, on average, increased slightly after steam pasteurisation, even though this increase was not significant (Fig. 3). The change in the “green” and “hay” character of rooibos infusions was also revealed when comparing the sensory profiles of two individual UPAS and PAS sample pairs with strong “green” notes (Fig. 6). The spider plot of sample C7 showed a reduction in the intensity of “green” aroma and flavour as a result of steam pasteurisation. No hay-like notes were perceived after steam pasteurisation. In the case of sample C20, however, the “green” flavour was completely lost during steam pasteurisation and replaced by a hay-like flavour.

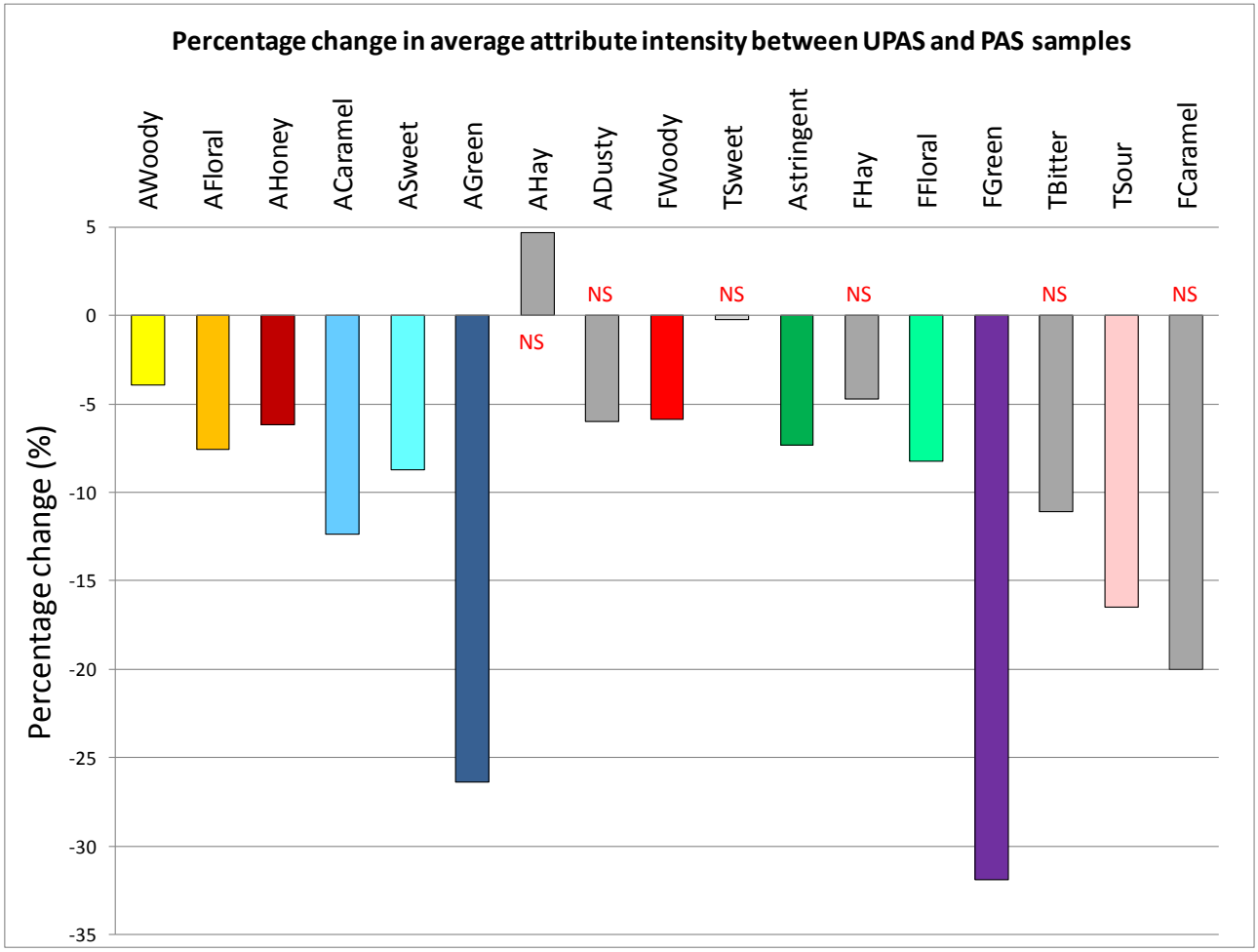

Figure 3 The percentage change in attribute intensity as a result of steam pasteurisation. Except for astringency, the letters "A", "F" and "T" in front of an attribute refer to aroma, flavour and taste attributes, respectively. The terms UPAS and PAS refer to unpasteurised and pasteurised samples, respectively, while NS indicates attributes that did not change significantly.

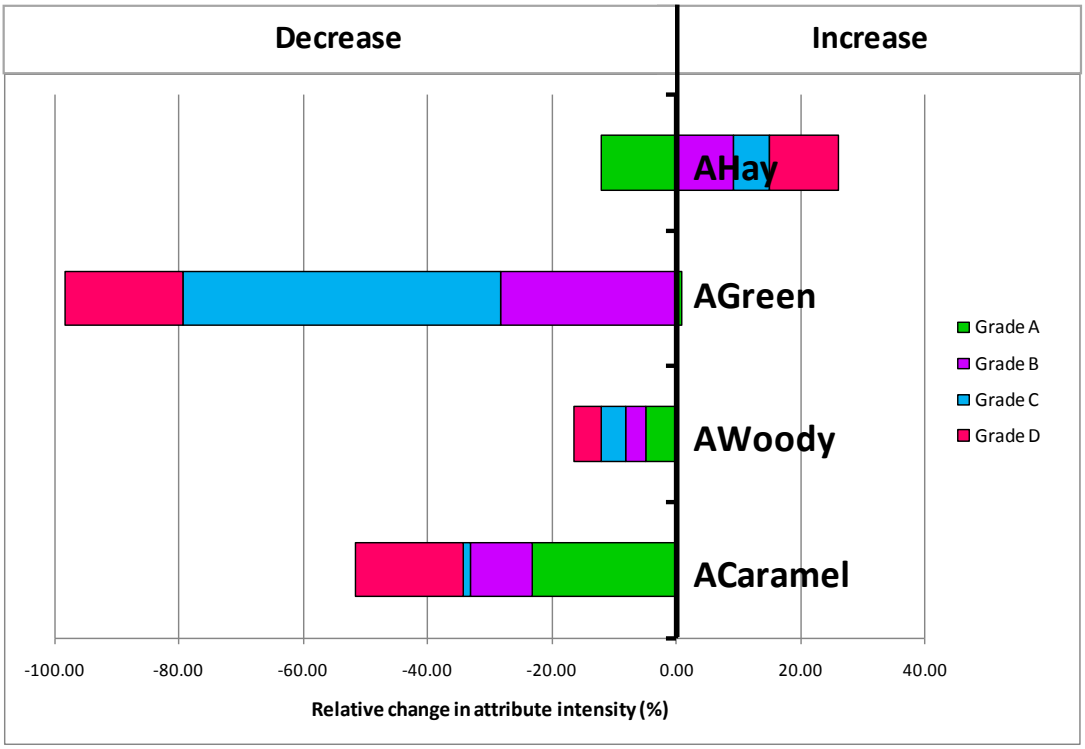

Figure 4 Relative decrease or increase in the intensity of selected aroma attributes caused by steam pasteurisation. The letter "A" in front of an attribute refers to aroma attribute.

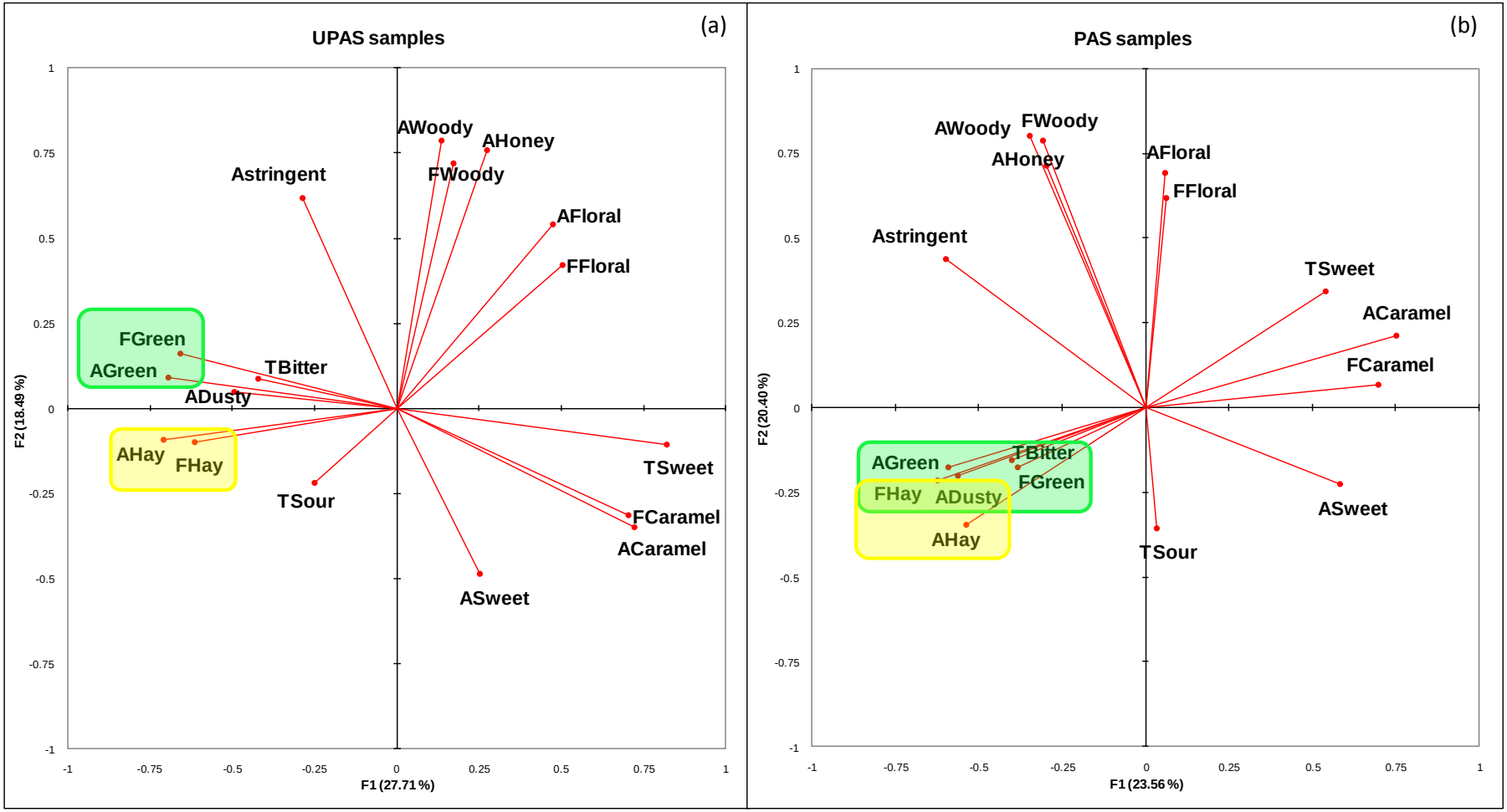

Figure 5 Change in attribute distribution for unpasteurised (UPAS) (a) and pasteurised (PAS) (b) samples indicating a change in the "green" attribute. Except for astringency, the letters "A", "F" and "T" in front of an attribute refer to aroma, flavour and taste attributes, respectively.

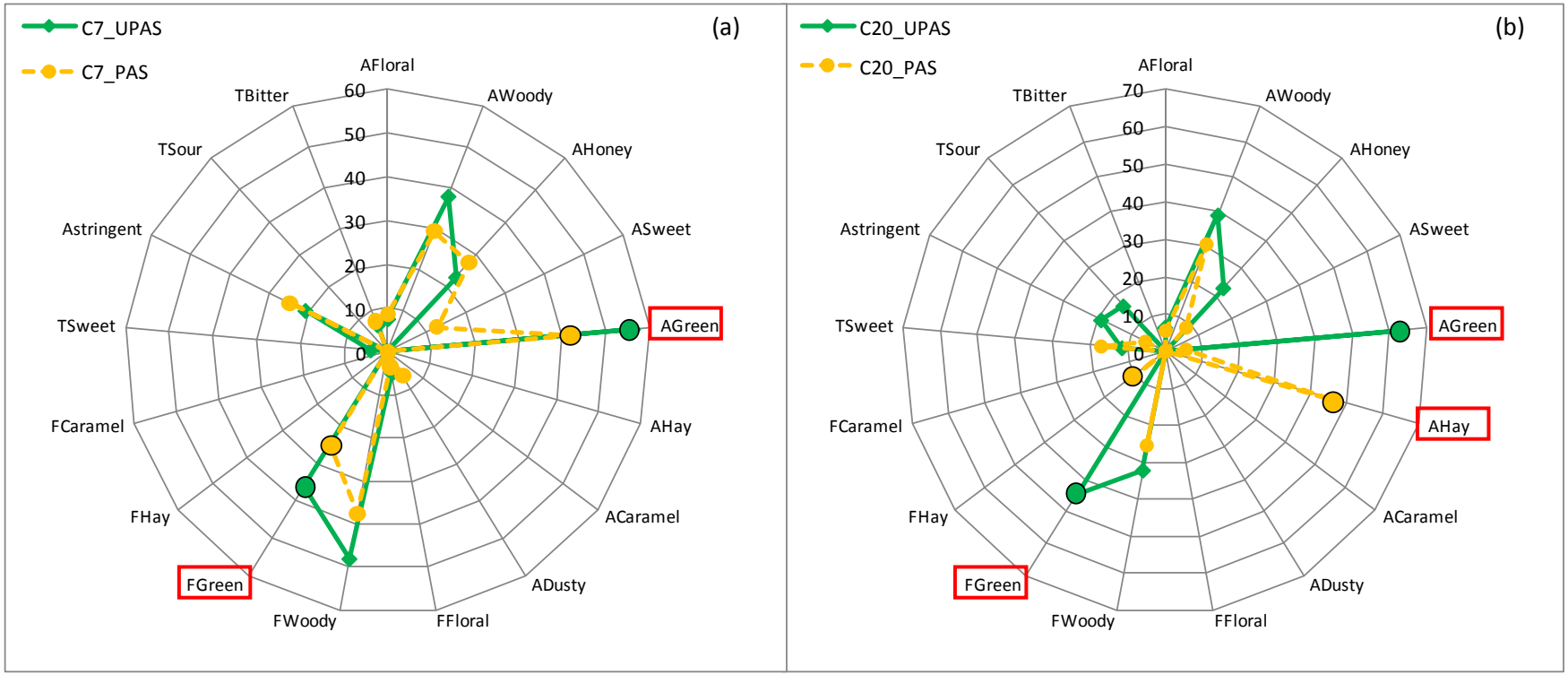

Figure 6 Spider plots reflecting the differences in the sensory profile between unpasteurised (UPAS) and pasteurised (PAS) samples with a strong "green" flavour. The intensity of the "green" flavour of sample C7 was reduced by steam pasteurisation (a), whereas the "green" note of sample C20 was replaced by a hay-like flavour (b). Assessors who were not reliable and consistent in rating "green" flavour and aroma were removed before generating the spider plots. Except for astringency, the letters "A", "F" and "T" in front of an attribute refer to aroma, flavour and taste attributes, respectively.

4.2 Changes in the composition of rooibos infusions

The effect of steam pasteurisation on the chemical/instrumental parameters is summarised in Table 1. The mean values for the contents of SS, TP, tannins and monomeric phenolic compounds, and for the "total colour" (AUC) measurements for UPAS and PAS samples are given. Even though a spectrum of absorbance values was obtained at 15 different wavelengths, all of these values were highly correlated, and, therefore, only the integral of the absorbance spectrum (AUC) was chosen to represent the "total colour". The SS, TP, and aspalathin contents, as well as the "total colour" were significantly higher in UPAS samples than in PAS samples (Table 1). The tannin content and all other individual phenolic compounds did not change significantly as a result of steam pasteurisation, although all the means, except for quercetin, luteolin and chrysoeriol, were slightly lower for the PAS samples.

Relatively weak, but significant correlations existed between the total colour and the TP content ($r = 0.628$), as well as the SS content ($r = 0.551$). To determine whether differences in absorbance measurements resulted from compositional changes of the infusion or from differences in the SS or TP concentration, the absorbance values (A_{380} to A_{520}) were normalised in terms of the TP content and the SS content. The spectra of the actual absorbance values and the values normalised in terms of TP content for UPAS and PAS samples are shown in Fig. 7. The mean absorbance values of PAS samples were consistently lower than those of the UPAS samples, even when normalised. The same results were obtained when normalising in terms of SS content (data not shown). The largest differences in normalised absorbance values of UPAS and PAS samples were observed between A_{440} and A_{460} (Fig. 7).

In order to analyse whether heat-induced changes in the phenolic composition alter the mouthfeel characteristics of astringency, the correlation coefficients between astringency and chemical/instrumental parameters, as well as certain sensory attributes for UPAS and PAS samples were compared (Table 2). The correlations for the SS content and TP content, as well as for certain individual flavonoids (quercetin, aspalathin and vitexin), were significant for PAS samples but not for UPAS samples (Table 2). Also, astringency was significantly correlated with the "green" flavour of UPAS samples, but not of PAS samples, and vice versa for "hay" flavour (Table 2), while a "woody" flavour was significantly correlated with astringency of both UPAS ($r = 0.517$) and PAS ($r = 0.679$) samples.

Table 1 Mean values of chemical/instrumental data for unpasteurised (UPAS) and pasteurised samples (PAS)

Parameter	UPAS	PAS
SS (mg/l)	**2240.0 a**	**2197.3 b**
TP (mg GAE/l)	**624.98 a**	**613.11 b**
Tannin (mg CE/l)	462.82 a	450.99 a
AUC	**134.34 a**	**123.61 b**
Aspalathin*	**16.66 a**	**15.83 b**
Iso-orientin*	27.28 a	27.06 a
Orientin*	20.62 a	20.59 a
Vitexin*	12.49 a	12.37 a
Hyperoside*	5.01 a	4.99 a
Rutin*	7.47 a	7.39 a
Quercetin-3-glc*	1.81 a	1.78 a
Iso-vitexin*	7.38 a	7.30 a
Luteolin-7-glc*	2.45 a	2.43 a
Quercetin*	1.00 a	1.00 a
Luteolin*	0.43 a	0.43 a
Chrysoeriol*	0.32 a	0.32 a
PPAG*	11.15 a	11.12 a
Nothofagin*	1.25 a	1.22 a

* Parameters given in mg/l

Values in the same row with different letters are significantly different. PPAG = enolphenylpyruvic acid-2-glucoside. glc = glucoside.

Table 2 Correlation coefficients (r) between astringency and compositional parameters, and astringency and selected flavour attributes

Parameter	UPAS	PAS
SS	0.101	**0.279***
TP	0.159	**0.327***
Tannin	0.030	0.123
AUC	-0.027	0.158
Rutin	**0.238***	**0.242***
Quercetin	0.236	**0.334***
Aspalathin	0.231	**0.286***
Vitexin	0.208	**0.248***
Luteolin	0.223	0.205
Luteolin-7-glc	0.139	0.114
Iso-vitexin	0.045	0.119
Nothofagin	0.036	0.109
Orientin	0.018	0.140
Iso-orientin	0.017	0.143
Hyperoside	-0.003	0.085
Chrysoeriol	-0.014	0.078
Quercetin-3-glc	-0.070	-0.031
PPAG	-0.118	0.023
FWoody	**0.517***	**0.679***
FGreen	**0.435***	0.152
FHay	0.175	**0.329***

*Values marked with an asterix are significantly different from 0 ($p < 0.05$). The letter "F" refers to flavour attribute.

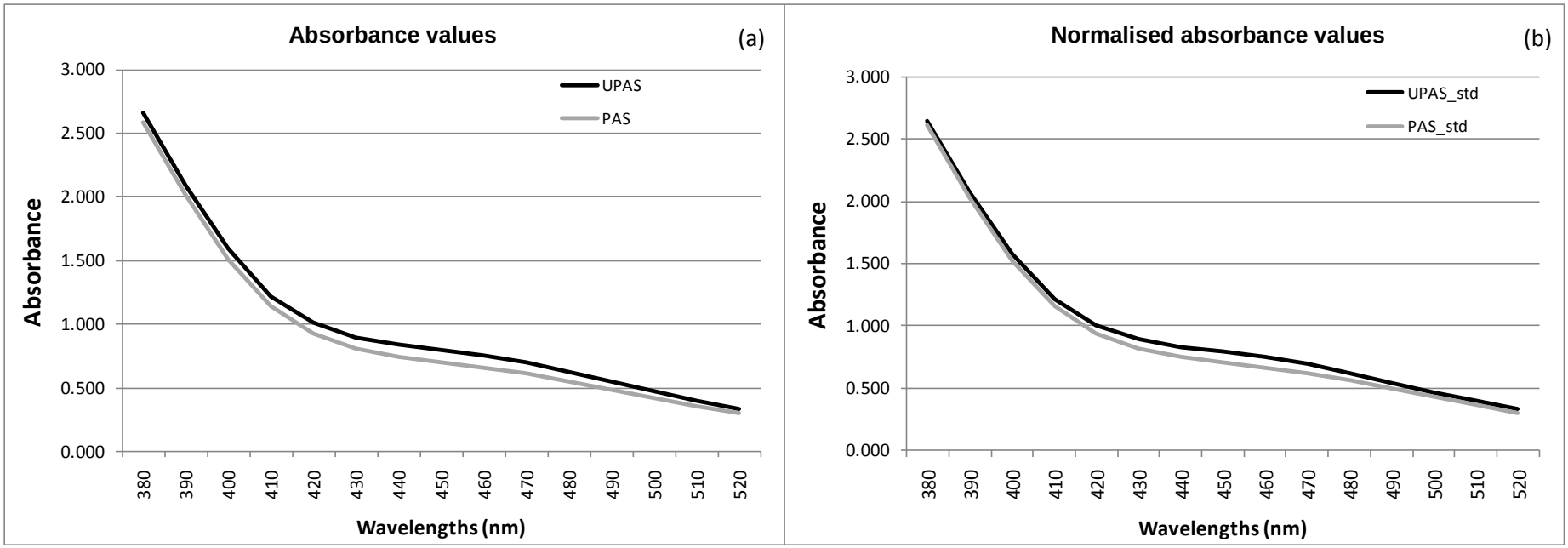

Figure 7 Differences between the absorbance spectra of unpasteurised (UPAS) and pasteurised (PAS) samples. Actual absorbance values (a) and absorbance values that have been normalised according to the average total polyphenol (TP) content (b) are shown.

5. Discussion

The flavour and mouthfeel of a tea infusion is the product of a complex mixture of non-volatile and volatile components, each with its own specific detection threshold. Phenolic compounds, whether monomeric or polymeric, are normally responsible for astringency, and sometimes also for sweet and bitter taste characteristics. Therefore, the focus of this study fell on a selection of both major and minor monomeric phenolic compounds of rooibos. The aroma and flavour notes “woody”, “floral”, “caramel”, “green” and “hay”, on the other hand, are derived from volatile compounds that influence the orthonasal and retronasal sensory perception. The volatile compounds of rooibos infusions were not determined in this study. However, the aroma constituents present in the volatile fraction of rooibos have been identified and quantified in two previous studies (Habu *et al.*, 1985; Kawakami *et al*., 1993), and together with information on their aroma characteristics and physical properties (e.g. boiling points), potential flavour impact constituents may be identified. Changes in the flavour and aroma attributes as related to changes in volatile compounds can thus be deliberated.

5.1 Changes in the SS, TP and aspalathin content

The SS content and TP content are strongly correlated which indicates that the decrease in SS as a result of steam pasteurisation was largely due to the decrease in the level of soluble polyphenols. However, except for aspalathin, none of the individual phenolic components that were quantified in this study were significantly lower after steam pasteurisation. These findings do not correspond to those obtained by Joubert *et al*. (2010a), who not only found a significant decrease in the levels of SS, TP and aspalathin after steam pasteurisation, but also in the levels of iso-orientin, orientin, vitexin, iso-vitexin, hyperoside and nothofagin. In that study, however, rooibos samples were steam-pasteurised for 2 min followed by drying for 20 min, whereas the samples used in the present study were steam-pasteurised and dried for 1 and 10 min, respectively. This suggests that longer steam pasteurisation and drying periods result in greater decreases in the levels of phenolic components.

A decrease in the content of polyphenolic compounds as a result of heat treatments is a well-established phenomenon that has been demonstrated in a number of studies. For instance, a variety of high temperature treatments of *Camellia sinensis* tea leaves (Sanderson *et al*., 1976; Wang *et al*., 2000), red grape pomace (Larrauri *et al*., 1997), mulberry leaves (Katsube *et al*., 2009) and apples (Devic *et al*., 2010) all resulted in a reduction in the phenolic content. Changes in the phenolic composition of *Cyclopia subternata* leaves due to steam treatment were also observed by Joubert *et al.* (2010b) who postulated that eriodictyol-7-*O*-rutinoside was oxidised to luteolin-7-*O*-rutinoside as a result of the steaming process. Furthermore, sterilisation of rooibos iced teas (at 121°C and 135°C) significantly decreased the levels of aspalathin, iso-orientin and orientin, whereas pasteurisation (at 93°C for 30 min) did not (Joubert *et al*., 2009). Heat-induced losses in aspalathin were larger than those of the other flavonoids (Joubert *et al*., 2009), which is

consistent with the results obtained in this study (Table 1). It has been shown that aspalathin is partially converted to the flavones, iso-orientin and orientin, as a result of oxidation reactions (Krafczyk & Glomb, 2008). The aspalathin level was significantly lower in PAS samples, whereas the levels of iso-orientin and orientin were not affected by pasteurisation (Table 1). It is possible that heat-induced degradation of the two flavones was off-set by the concomitant conversion of aspalathin to iso-orientin and orientin. Steam-pasteurisation may also lead to the dimerisation of aspalathin and the formation of other higher molecular weight browning products (Krafczyk *et al*., 2009).

It has been established that heat treatments result in a more substantial reduction of total polyphenols compared to condensed tannins suggesting that tannins are more resistant to thermal degradation due to their more complex chemical structure (Larrauri *et al*., 1997). This may explain why the tannin content of the rooibos infusions was not significantly reduced by steam pasteurisation (Table 1).

The abovementioned studies have shown that high temperature treatments impact phenolic substances, and that the extent of this impact depends on the nature of the specific molecule. The Folin-Ciocalteu assay used in this study for quantification of polyphenols is based on the ability of phenolics to react with oxidising agents. This measurement is affected by different hydroxylation patterns and the degree of polymerisation of polyphenolics (Wang *et al*., 2000). Therefore, even though steam pasteurisation of rooibos was only 60 s long, oxidation, polymerisation or degradation of phenolic components may have occurred, resulting in lower levels of polyphenols being captured in the measurement. It is also possible that handling of rooibos leaves during the steam pasteurisation process (i.e. spreading rooibos leaves on 30-mesh frames, and transferring steamed and dried leaves into plastic bags) may have resulted in a loss of fine rooibos particles and "dust" (<40 mesh) which may have led to lower levels of extractable polyphenolic compounds in an infusion.

5.2 Decreases in absorbance measurements and total colour (AUC)

Absorbance measurements were analysed in order to obtain further insight into the changes taking place in the phenolic composition of rooibos infusions as a result of steam pasteurisation. The differences between the normalised absorbance values of UPAS and PAS samples showed that the heat treatment of rooibos did in fact have an impact on the composition of the infusions (Fig. 7). In Chapter 4 it was established that the strongest correlation coefficient between the individual flavonoids and "total colour" (AUC) was that of quercetin ($r = 0.541$), a compound that exhibits a bright yellow colour (Meena & Patni, 2008). However, since steam pasteurisation did not significantly decrease the quercetin content (Table 1) the differences in absorbance can not be associated with a reduction in the level of quercetin.

The fact that the largest difference in absorbance was observed at about 450 nm suggests that changes must have occurred in compounds with absorbance maxima at approximately 450 nm. Krafczyk *et al.* (2009) demonstrated that oxidation of aspalathin resulted in the formation of higher molecular weight browning products which was accompanied by an **increase** in A_{450}. In the current study, however, a decrease

in aspalathin without a concomitant increase in A_{450} was observed, which could be the result of one or more scenarios, e.g. the brown oxidation products were not formed during pasteurisation, their solubility decreased during steam pasteurisation, or thermal degradation of these compounds resulted in a reduction in absorbance.

5.3 Compositional changes in relation to changes in sensory attributes

Changes in the phenolic composition in relation to changes in taste and mouthfeel attributes

In Chapter 4 the relationship between the basic taste and mouthfeel attributes and the contents of SS, TP and individual flavonoids was elucidated. Based on these findings, the relationship between changes in sensory attributes and changes in chemical/instrumental parameters due to steam pasteurisation of rooibos can be examined.

With regard to the basic taste attributes, **bitterness and sweetness**, no significant changes were observed between UPAS and PAS samples (Fig. 2). Previously it was established that these two taste characteristics correlated significantly with a number of individual flavonoids (Chapter 4, Table 9). Seeing that none of these flavonoids were significantly reduced by pasteurisation (Table 1), it is reasonable that sweet and bitter taste characteristics did not decrease significantly as a result of steam pasteurisation.

Sourness, however, was significantly lower in PAS samples (Fig. 2 and Fig. 3). It was stated in Chapter 4 that phenolic compounds are not usually associated with sour taste characteristics, except for certain phenolic acids (e.g. *p*-hydrobenzoic acid and ferulic acid) which have fairly high detection threshold concentrations (Huang & Zayas, 1991; Peleg & Noble, 1995). Furthermore, sourness significantly correlated with “green” flavour and aroma (Chapter 3, Table 3), and may thus be related to certain volatile compounds responsible for the “green” notes. The fact that “green” flavour and aroma decreased most significantly as a result of steam pasteurisation (Fig. 3) suggests that a reduction in the sour taste quality of certain rooibos infusions is mainly due to the loss of “green” volatiles rather than the loss of sour-tasting phenolic acids.

Astringency was found to be significantly lower in PAS samples (Fig. 2). Reductions in the astringency of black and green tea infusions as a result of heat treatments of *C. sinensis* leaves were observed in other studies (Sanderson *et al*., 1976; Wang *et al*., 2000). Heat-induced changes in the phenolic components, i.e. the decrease in flavan-3-ols and the formation of polyphenol-protein complexes, were proposed as the underlying cause of the change in astringency. Only rutin was significantly correlated to the astringency of UPAS samples (Chapter 4, Table 9), but for the PAS samples quercetin, aspalathin and vitexin also correlated significantly with astringency (Table 2). This indicates that these compounds contribute to the astringency of rooibos infusions as well. Rutin, vitexin and quercetin have been described as astringent in literature (Scharbert *et al*., 2004; Stark *et al*., 2005; Stark *et al*., 2006). However, since the contents of these three compounds did not differ significantly between UPAS and PAS samples (Table 1) the decrease in astringency cannot be attributed to changes in these flavonoids alone. With aspalathin being the only flavonoid that was significantly reduced by pasteurisation, it is possible that the reduction in astringency is associated to a large

extent with the decrease in aspalathin. Furthermore, although the levels of rutin and vitexin were only slightly but not significantly lower after pasteurisation, it is also possible that the cumulative decrease in components exhibiting astringent characteristics may have brought about the slight reduction in the perceived astringency.

The differences in the strength of the correlations between astringency and "green", "hay" and "woody" attributes for UPAS and PAS samples (Table 2) suggest that there was not only a change in the intensity but also in the character of astringency as a result of steam pasteurisation. While astringency was associated with "green" flavour in UPAS samples, it was more strongly correlated to "hay" flavour and "woody" flavour in PAS samples. It is possible that the significant reduction in "green" flavour caused by steam pasteurisation (Fig. 3) enabled panellists to rate the astringent character of the infusion more accurately, which may have lead to an increase in the correlation coefficients for certain compositional parameters (Table 2). Heat-induced changes in the character of astringency were also observed by Sanderson *et al.* (1976) who described the astringency of unfired black tea (i.e. which has not been dried at high temperatures) as "strong non-tangy", while fired tea was described as having "pleasant tangy astringency". Furthermore, terms and definitions for a variety of astringent sensations have been developed, e.g. "resinous" (astringency elicited as if chewing on a piece of raw wood), "sappy" (astringency with high acid and slightly bitter; reminiscent of the astringency elicited by chewing on a green grape stalk) and "unripe" (a negative hedonic grouping consisting of an astringent feel associated with excessive acidity and associated green flavour notes) (Gawel *et al*., 2001). With regard to these definitions it is evident that different types of astringent characteristics may occur, each of which is associated with other sensory properties. Similar descriptions and definitions for astringency may be relevant in the context of the current study. The astringent character of UPAS samples, for example, could be described as "green" astringency which becomes more subtle and "woody" as a result of steam pasteurisation. Also, it has been found that structural differences between tannin-like compounds can influence the intensity of the individual astringency attributes, such as "drying", "chalkiness" and "coarseness" (Vidal *et al.*, 2003). This indicates that changes in the astringency of rooibos infusions may not only depend on the concentration of astringent components, but also on alterations in their structural properties as a result of steam pasteurisation.

Changes in flavour and aroma attributes associated with changes in volatiles

The significant decrease in the intensities of rooibos aroma and flavour attributes could only be associated with the volatile compounds in the rooibos infusions. Even though the volatile fraction of the rooibos infusions was not characterised or quantified in this study, it may be expected that steam pasteurisation resulted in a change in the levels of certain volatiles. The loss of certain volatile compounds during high temperature drying of black tea has been identified as an essential part of black tea aroma development (Wickremasinghe, 1978; Ravichandran & Parthiban, 1998). High temperature drying of black tea leaves, for instance, greatly reduced the levels of volatiles exhibiting an undesirable grassy aroma (e.g. *trans*-2-hexenal), while the levels of other compounds, associated with pleasant sweet and floral notes, increased

(Ravichandran & Parthiban, 1998). Consequently, the aroma quality of black tea extracts was improved. This shows that processing stages which influence the concentration or the ratio of volatiles can result in changes in the sensory quality of an infusion.

This would also be true for steam pasteurisation of rooibos which may give rise to the volatilisation of certain aroma compounds, whereas the level of other volatiles may increase. Depending on the aroma characteristics of these volatiles (e.g. pleasantly sweet, floral, fruity notes vs. green, hay-like or musty notes), such changes in the ratio of volatiles would have a beneficial or a detrimental impact on the overall flavour quality of rooibos tea. The intensities of "green" flavour and aroma changed most drastically due to steam pasteurisation of rooibos (Fig. 3). A rooibos extract contains a number of volatiles that are associated with "green" aroma notes (Chapter 2, Table 8,) so the loss of such compounds during steam pasteurisation is likely to be responsible for the reduction in "green" flavour.

The extent to which volatile compounds are driven off during steam pasteurisation is governed by a number of factors, most notably their specific boiling points and polarities (Ingham *et al*., 1995), as well as physicochemical interactions between the aroma compounds and certain components in the food or beverage matrix (Kinsella, 1989). In a product such as black tea that is subjected to high temperature drying it has been shown that most of its "character impact compounds" have fairly high boiling points (Bondarovich *et al*., 1967). This highlights the importance of the boiling point of a compound in terms of its aroma impact. Volatiles that do not exhibit "green" aroma notes have substantially higher boiling points and molecular weights than the compounds with a "green" odour (Chapter 2, Table 4). During steam pasteurisation, compounds such as *cis*-3-hexenol or hexanal with boiling points less than ca. 160°C would be released quicker than, for example, β-ionone or β-damascenone with boiling points higher than ca. 260°C. The resulting change in the ratio of volatiles would then effect a greater reduction in "green" flavour and aroma compared to "floral" or "caramel" notes which might increase the acceptability of certain batches of rooibos, especially under-fermented rooibos, to the consumer.

The changes in the aroma attributes brought about by steam pasteurisation of rooibos differed for the four quality grades (Fig. 4). This suggests that the original sensory profile of a sample, before steam pasteurisation, determines in which way the heat treatment will affect its sensory characteristics. Unpasteurised Grade C samples, that were most strongly associated with "green" flavour and aroma (Chapter 3, Fig. 5,) thus showed the largest decrease in green aroma (Fig. 4). Similarly, "caramel" flavour and aroma were most closely associated with Grade A samples (Chapter 3, Fig. 5) and, accordingly, these samples decreased to the largest extent in "caramel" aroma (Fig. 4). Furthermore, samples of Grades B, C and D, which decreased in "green" aroma, also increased in hay-like character as a result of steam pasteurisation, whereas Grade A samples, which were not associated with "green" flavour, did not exhibit an increase in hay-like character (Fig. 4).

"Hay" aroma is the only attribute that was not reduced by steam pasteurisation (Fig. 3). The fact that hay-like notes often replaced the strong "green" notes of a number of samples (Fig. 6) shows that the

changes in volatile compounds due to steam pasteurisation did not only result in a reduction in the intensities of certain rooibos aromas and flavours, but also in changes in the sensory character of an infusion. The characteristics of certain volatiles associated with "green" aromas can be concentration-dependent (Hongsoongnern & Chambers, 2008). Therefore, a change in the concentrations of volatile compounds can bring about a decrease in attribute ***intensity***, as well as a change in the ***character*** of the attribute. The slight increase in hay-like character due to pasteurisation of rooibos may thus be explained by a concentration-dependent change in the aroma character of certain "green" volatiles. Furthermore, combinations of certain volatiles have been shown to produce new odour characteristics that are not associated with the individual chemicals (Bott & Chambers, 2006). This means that as the combination of volatiles in the rooibos infusion is modified by steam pasteurisation, they may give rise to new odour characteristics. It is also possible that it was simply the reduction in the strong "green" flavour which allowed for the hay-like notes to be perceived more strongly by the assessors.

6. Conclusions

Steam pasteurisation of rooibos resulted in significant changes in the SS, TP and aspalathin contents and the absorbance and "total colour" (AUC) values of the infusions. These changes may be attributed to thermal degradation of complex polymeric oxidation products or to the oxidation/polymerisation of aspalathin. The anecdotal evidence that steam pasteurisation of rooibos leads to subtle but significant changes in the sensory characteristics of rooibos infusions was confirmed. A significant decrease in the intensity of astringency was observed as a result of pasteurisation and could be associated with the changes in the phenolic composition. Bitter and sweet taste qualities, which may be associated with a number of monomeric phenolic compounds, were not affected by the heat treatment since steam pasteurisation did not significantly alter the levels of these compounds. Most of the aroma and flavour attributes were, however, significantly lower in PAS samples, with "green" flavour and aroma exhibiting the largest decrease in intensity. These changes could be explained by the volatilisation of aroma compounds during steam pasteurisation, bringing about the most significant changes in the sensory quality of rooibos infusions.

Furthermore, the changes in the sensory quality caused by pasteurisation are governed, to some extent, by the original sensory profile prior to pasteurisation. Although the heat treatment reduced the intensities of certain positive attributes, including the "characteristic" rooibos attributes "floral" and "honey" (as established in Chapter 3), it may be able to lead to an improvement in the sensory quality of certain low-quality batches of rooibos. By reducing the levels of volatiles with strong "green" notes, the astringency, as well as the relatively strong "woody" flavour of rooibos, steam pasteurisation may make rooibos, especially under-fermented rooibos, more acceptable to consumers.

7. References

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Chapter 6

GENERAL DISCUSSION AND CONCLUSIONS

The success of any food and beverage product relies greatly on its quality and consistency. Quality control and sensory assessment are used for evaluating product quality and to ensure that consumers can expect a standardised product with unchanging sensory characteristics. Even though the demand for rooibos, both on local and international markets, has been rising steadily over the past 15 years, it remains essential to ensure that the quality of this unique South African product, especially in terms of its sensory quality, is carefully monitored. As the international herbal tea market is becoming more and more crowded with new herbal teas, fruit infusions and tea blends, it becomes increasingly important not only to maintain consistent product quality, but also to successfully differentiate rooibos from its competitors. Effective product differentiation, however, relies to a large extent on the accurate definition and description of the product attributes. The sensory attributes of good quality rooibos tea have, however, not yet been described, and the term "characteristic" rooibos flavour, which is used in the official rooibos regulations, is not able to convey a comprehensive and useful picture of what may be regarded as high quality rooibos.

The objectives of this study were, therefore, to develop a suitable set of terms that can be used for describing the sensory characteristics of rooibos infusions, and to determine whether some of these descriptive attributes are associated with specific non-volatile phenolic compounds, with the aim of identifying some of the major sensory impact compounds of rooibos tea. Based on the findings obtained in these studies, the changes in the sensory characteristics and phenolic composition of rooibos infusions resulting from steam pasteurisation were examined to analyse in what way heat-induced changes in the phenolic rooibos components affect the perceived taste and mouthfeel characteristics of rooibos tea.

The sensory properties of 69 cultivated rooibos samples from the 2009 harvest were evaluated by characterising the aroma, flavour, taste and mouthfeel attributes of their infusions. In order to capture as much variation in sensory characteristics as possible, samples were obtained throughout the harvesting season from different plantations and different production areas. Sensory profiles for all samples were established using quantitative descriptive analysis, and by comparing these profiles the variation in their sensory attributes was established. A selection of attributes was assembled into a rooibos sensory wheel, a simple and convenient representation of a wide range of rooibos attributes that may assist rooibos producers, processors and marketers in characterising and differentiating between the sensory profiles of different batches of rooibos. The most frequently occurring descriptors were summarised in a rooibos sensory lexicon, which provides a definition, as well as a reference standard for each term. This set of well-defined terms lends itself to the training of sensory panels and quality control personnel of the industry, and

may be useful for improving the communication of product characteristics between local and overseas marketers.

Analysis of the sensory profiles of the rooibos samples revealed that the “characteristic” sensory properties of rooibos can be best described as woody, honey and fynbos-floral flavours, with a slightly sweet taste and a subtle astringent mouthfeel. In order to determine how the sensory attributes of rooibos relate to quality, the quality grade, awarded to each sample by expert graders of the industry, was taken into account. This quality rating is based on the appearance of the leaves, the infusion colour and the overall flavour. It was found that high quality rooibos was associated with the abovementioned “characteristic” attributes, as well as with caramel and fruity-sweet flavours, while low quality rooibos was related to plant-like, hay-like and musty flavours, as well as sour and bitter taste characteristics.

Although samples originating from different geographical locations were used in this study, analysis of the effect of production area on the sensory characteristics of rooibos was not an objective of this study. Consequently, no conclusive results can be be obtained regarding the variation in the sensory characteristics of rooibos arising from its production area. In order to determine whether production area may have a significant effect, a more targeted approach and a more controlled sampling plan are recommended. The rooibos sensory lexicon and sensory wheel developed in this study may find application in such research, as well as in other future studies involving rooibos quality by enabling researchers to describe and differentiate between samples.

In future, the usefulness of the rooibos sensory wheel could be improved by developing definitions and obtaining reference standards for all attributes displayed on the wheel. Other modifications to the wheel may be expected as it is put to use by different role players of the industry. Analysing the sensory profiles of a number of samples from another harvesting season would add great value to the sensory wheel since this would provide an indication of the seasonal variability in the sensory quality of rooibos. This would allow for the refinement of the sensory terminology in that superfluous attributes can be deleted while new attributes can be added. Furthermore, some of the attributes may be rephrased or broken down into more apposite descriptors. The term “astringency” is a multifaceted concept which may be divided into a number of sub-qualities. Gawel *et al*. (2000), for instance, developed several groupings for astringent mouthfeel characteristics associated with red wine, e.g. “drying”, “unripe”, “harsh” and “surface smoothness”. It is likely, therefore, that the term astringency, as used in this study, is too general to describe all mouthfeel characteristics associated with a rooibos infusion. Therefore, it may be useful to analyse the variation in mouthfeel sensations and assign more suitable descriptive attributes to their sub-qualities. The term “plant-like/green”, as used in the sensory lexicon, may also encompass a number of sub-qualities that are distinct and distinguishable, and these should thus be treated as separate attributes with different definitions and reference standards.

Having developed a defined set of rooibos terminology, and having established that there is considerable variation in the sensory profiles of different batches of rooibos tea, it was determined whether

correlations could be found between certain sensory attributes and specific compounds in a rooibos infusion, with the focus being on non-volatile phenolic components and their effect on taste and mouthfeel characteristics. With the understanding gained from having analysed the relationships between composition and sensory quality of rooibos infusions, the effect of steam pasteurisation on the phenolic composition and the sensory characteristics of an infusion was investigated. Anecdotal evidence suggests that pasteurisation of rooibos leads to a softer taste and less prominent flavour, and this study is the first attempt to scientifically confirm and explain these observations.

A large variation was observed in the composition of rooibos infusions in terms of the soluble solids (SS) content, total polyphenol (TP) content and tannin content, and in the levels of 14 monomeric phenolic compounds. High quality rooibos, classified as such by expert graders, was associated with higher levels of these parameters reflecting the importance of phenolic compounds in terms of the sensory quality of rooibos. Steam pasteurisation resulted in a significant decrease in the SS and TP contents indicating that this heat treatment may affect the quality of the rooibos infusion as less phenolic compounds are extracted from the leaves. Also, significant changes in absorbance were observed which may be attributed to heat-induced structural changes in polymeric phenolic compounds.

Significant correlations were observed between certain sensory attributes and compositional parameters but these relationships were generally very weak. Since only a number of non-volatile phenolic compounds were quantified they could only be related to the basic taste modalities, sweet, bitter and sour, and to the mouthfeel attribute, astringency. Several phenolic compounds were significantly correlated either with sweet or with bitter taste characteristics. Except for aspalathin, steam pasteurisation of rooibos did not affect the levels of these monomeric phenolic compounds, and consequently, no significant differences in sweetness or bitterness were observed between unpasteurised and pasteurised rooibos samples.

Astringency, however, decreased significantly as a result of steam pasteurisation. It is likely that, during the heat treatment of rooibos leaves, thermal degradation of polymeric phenolic compounds took place which may have reduced their ability to precipitate salivary proteins, thereby leading to a reduction in astringency. Alternatively, polymerisation of phenolic compounds may have occurred, reducing the solubility of these compounds. Subsequently, less phenolic components would be extracted from the rooibos leaves which would ultimately result in a decrease in astringency. Unexpectedly, however, astringency was not related to the tannin content, which was also not affected by pasteurisation. Since the methyl cellulose precipitable (MCP) tannin assay, used for quantification of tannin-like rooibos components, did not deliver an accurate and reliable estimation of the tannin content it is possible that certain components, with astringent qualities, may not have been effectively precipitated by methyl cellulose. Also, other phenolic compounds may have interfered with the measurement. In future, it may thus be useful to analyse the efficiency, reliability and reproducibility of other tannin quantification assays which may be better able to capture the tannin-like compounds in a rooibos infusion.

The only compound that was significantly correlated with astringency was rutin, one of the major rooibos flavonoids which has an extremely low detection threshold (Scharbert *et al*., 2004). The correlation coefficients for quercetin and aspalathin, however, were only slightly lower indicating that these compounds may also contribute to astringency. However, steam pasteurisation reduced only the aspalathin concentration, and not that of rutin, quercetin or other phenolic compounds, and consequently, the significant reduction in astringency may, to a large extent, be the result of heat-induced changes in aspalathin.

Furthermore, steam pasteurisation also significantly decreased the intensity of most of the aroma attributes. This can be attributed to the loss of volatile compounds, especially those associated with "green" aroma notes which generally have lower boiling points than volatiles associated with pleasant aromas, e.g. floral, fruity or sweet notes. By reducing the intensities of "green" and "woody" flavours and aromas, as well as the astringency of rooibos infusions, steam pasteurisation may increase consumer acceptability of rooibos, especially of low quality rooibos. However, because of the importance of volatile compounds for rooibos flavour it may be recommended that changes in volatile aroma compounds due to steam pasteurisation be quantified using gas chromatography, and then linked specifically to concurrent changes in the sensory quality of rooibos infusions.

The poor correlations between sensory attributes and compositional parameters suggest that other compounds, which were not quantified in this study, have a meaningful impact on the sensory characteristics of rooibos infusions. These include **polymeric phenolic oxidation products** that are formed during fermentation of rooibos plant material. Although some studies have analysed the formation of such brown oxidation products (Krafczyk & Glomb, 2008; Krafczyk *et al*., 2009) no information is available on their sensory characteristics. Rooibos infusions also contain other phenolic components (Rabe *et al*., 1994) that exhibit bitter or astringent taste properties. **(+)-Catechin**, for instance, is also present at low concentrations in rooibos tea (Krafczyk & Glomb, 2008) and may contribute to the slightly bitter taste and subtle astringency of rooibos infusions (Wang *et al*., 2000; Lesschaeve & Noble, 2005). A range of phenolic acids is present in rooibos (Rabe *et al*., 1994). ***p*-Hydrobenzoic acid**, for example, has been reported as having bitter and acidic taste qualities (Peleg & Noble, 1995). Both **protochatecuic acid** and ***p*-coumaric acid** have been described as bitter and astringent at detection thresholds of 30 and 48 µl/l, respectively, while **ferulic acid** was found to have a sour taste at 90 µl/l (Maga & Lorenz, 1973; Huang & Zayas, 1991) or a bitter and smoky taste at a threshold of 62 µl/l (Work & Camire, 1996). Although the detection thresholds of these compounds are quite high, synergistic effects may occur between combinations of phenolic acids which can result in a decrease in their individual detection thresholds (Shahidi & Naczk, 1995) leading to an increase in their sensory impact.

The important contribution of certain **amino acids** to tea taste and tea quality has been pointed out in studies on black tea flavour (Nakagawa, 1975; Le Gall *et al*., 2004; Shu *et al*., 2006 Wang & Ruan, 2009; Wang *et al*., 2010), and it is well known that amino acids are often associated with bitter or sweet-tasting properties (Kawai & Hayakawa, 2005). Furthermore, black tea **polysaccharides** were found to impart a

certain “maltiness” to the taste of black tea (Millin *et al*., 1969). These studies reflect the potentially significant effect of amino acids and polysaccharides on the sensory quality of rooibos tea infusions. To verify whether their impact is indeed meaningful these compounds must be characterised and quantified, which has not been attempted so far.

The importance of volatile compounds in relation to flavour perception is well recognised and it has even been suggested that it is the number and the ratio of volatile components that determine the quality of tea (Dutta *et al*., 2003). More than 120 **volatiles** have been identified in rooibos tea extracts (Habu *et al*., 1985; Kawakami *et al*., 1993). Based on some of their aroma descriptors (Chapter 2, Tables 8 and 9) a number of volatile compounds can be identified which are likely to be responsible for the rooibos aroma and flavour attributes, “floral”, “caramel”, “sweet” (fruity sweet), “woody”, “hay-like” and “green”. Green or grassy aroma is associated with under-fermented tea (Joubert & De Villiers, 1997) and has been attributed to *cis*-3-hexenal and *trans*-3-hexenal, whereas floral and woody notes may be associated with β-ionone (Joubert & De Villiers, 1997). Rooibos also contains a number of volatiles which have been described as having caramellic aroma qualities, e.g. 5-methyl furfural, 2-acetyl furan and furfural (The Good Scent Company, 2010). These may, therefore, be responsible for the strong caramel flavour that was perceived in a number of rooibos samples in this study. However, the relationships between these volatiles and specific flavour/aroma attributes have not yet been verified with gas chromatography olfactometry (GC-O). Accurately evaluating the aroma impact of each of these compounds and their effect on the sensory quality of rooibos infusions would necessitate a comprehensive olfactory study, taking into account the concentration and detection threshold of each volatile compound.

With regard to all of the above it can be concluded that rooibos tea taste, flavour and aroma attributes arise from an interplay of components, each of which has the potential to influence the flavour of a tea infusion. For future studies on the sensory characteristics of rooibos it is recommended that an alternative approach be followed that would generate a more comprehensive picture of rooibos taste and flavour. This approach should be based on dose-over-threshold (Dot) values (i.e. the ratio of the concentration and the detection threshold of a compound), which are a useful reflection of the sensory impact of each specific component. However, in order to calculate the Dot values, detection thresholds must first be established for the major components in a rooibos infusion including aspalathin, nothofagin and other phenolic components, as well as polymeric oxidation products, amino acids, polysaccharides and volatile compounds. Once the detection thresholds and concentrations of all of these compounds have been determined, Dot values can be calculated. Finally, the flavour and taste impact of those compounds with the largest Dot values can be confirmed by means of reconstitution and omission experiments as described by Scharbert and Hofmann (2005). With the findings obtained from such studies it may then be possible to formulate a prediction model based on the chemical composition of a rooibos infusion, which could be used to predict its sensory quality.

Lastly, it should be considered that the elucidation of rooibos flavour may become even more confounding by interactions that may take place between non-volatile and volatile compounds. Certain volatiles, for example, that are associated with floral, caramel, honey and fruity aroma notes, may increase the in-mouth perception of sweet taste related to certain non-volatile compounds, whereas a green aroma may reduce the intensity of the sweet taste and increase that of sour taste. Also, research on flavour of food and beverage products has shown that the dose-response relationship between flavour components and human intensity ratings is non-linear. Such factors would not be taken into account when predicting the sensory quality of rooibos with a prediction model, and highlight again the complexity and intricacy revolving around flavour analysis. It is evident from this study that, in terms of the molecular analysis of rooibos flavour, only the tip of the iceberg has been uncovered.

This study scientifically demonstrated for the first time that the variation in the sensory characteristics of a variety of rooibos batches is more extensive than may have been anticipated. Rooibos tea samples with specific and pleasant flavours, e.g. caramel or fruity notes, were identified, disclosing a potential niche market for rooibos that may exploit its natural variation in sensory characteristics. It was also shown that the impact of steam pasteurisation on rooibos flavour may be either beneficial or detrimental with regards to its sensory characteristics, depending on the original sensory profile prior to pasteurisation. In future, it could be investigated whether steam pasteurisation may be used as a means of manipulating the flavour characteristics of certain batches of rooibos. Furthermore, tools were developed to facilitate the characterisation and description of the rooibos sensory characteristics in a way that can be understood even by those unfamiliar with the product. The rooibos sensory lexicon and sensory wheel will not only be able to improve the understanding and communication of different rooibos attributes, but may also form the foundation of many future studies involving sensory-related aspects of rooibos.

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ADDENDA

ADDENDUM 1

A summary of the 69 rooibos samples used for sensory analysis, their production areas and quality grading parameters

Batch number	Sample number	Grade	Sample code	Producer	Production area	Cut	Dry Appearance	Wet Appearance	Infusion colour	Flavour	Total Grade value
579	1	A	A1	JJ THIART	Suid Bokveld	1	8.0	8.0	8.0	7.0	76
580	1	B	B1	JAL	Noord Bokveld	1	7.3	7.3	7.0	7.0	71
594	1	C	C1	IZANTI	Agter Pakhuys	2	6.0	6.0	6.0	6.3	61
585	1	D	D1	CJ DIXON	Agter Pakhuys	2	4.3	5.8	6.0	5.5	53
710	2	A	A2	JDM HANEKOM	Citrusdal Berg	1	9.3	7.7	7.0	8.0	79
664	2	B	B2	HR BOSMAN	Bo Rivier	2	7.0	7.0	7.0	6.7	69
677	2	C	C2	F OBERHOLSTER	Agter Pakhuys	2	6.0	6.5	6.3	5.3	58
716	2	D	D2	MOUTON BROERS	Eendekuil	2	5.0	5.3	7.0	5.0	54
824	3	A	A3	PAPKUILSFONTEIN	Suid Bokveld	1	8.0	7.0	7.0	7.8	75
789	3	B	B3	AMW BOERDERY	Jakkalsvlei	2	7.3	6.7	7.0	6.7	69
758	3	C	C3	HB LAING	Grootfontein	1	7.5	6.5	6.0	6.0	64
755	3	D	D3	JP BERGH	Zeekoevlei	1	4.8	5.8	6.0	5.3	55
902	4	A	A4	WUPPERTHAL	Wupperthal	2	8.0	8.0	7.0	7.7	77
900	4	B	B4	KRAALBOSVLAK	Citrusdal Berg	2	7.0	6.7	7.0	6.3	67
888	4	C	C4	GR GROBBELAAR	Eendekuil	2	5.5	6.0	7.0	6.0	61
932	4	D	D4	J MARITZ	Ouberg	1	5.0	5.2	5.0	5.0	50
953	5	A	A5	JA BRAND	Nardouwsberg	1	7.4	7.0	8.0	7.4	75
950	5	B	B5	WA NEL	Zeekoevlei	1	6.6	6.2	8.0	6.8	71
963	5	C	C5	JHG DU PLESSIS	Aggenbagskraal	2	6.6	6.4	6.0	6.0	62
962	5	D	D5	ZEEKOEIVLEI	Redelinghuys	1	5.2	5.0	6.0	5.2	54
1112	6	A	A6	A BAKKER	Biedouw Berg	2	8.8	8.0	7.0	7.8	80
1080	6	B	B6	CPJ SMITH	Nardouwsberg	2	7.3	7.0	7.0	7.0	71
1065	6	C	C6	JJP PRINS	Gifberg	2	6.0	7.0	6.0	6.3	62
1076	6	D	D6	HG LANGEVELDT	Eendekuil	2	4.7	4.7	6.0	3.7	46
1113	7	A	A7	JFG LAMBRECHTS	Nardouwsberg	1	8.0	8.0	8.0	7.3	77
1122	7	B	B7	GC GRIB	Citrusdal Berg	3	7.3	7.3	7.0	6.7	71
1142	7	C	C7	JCJ COETZEE	Nardouwsberg	1	6.0	6.5	6.0	6.0	61
1134	7	D	D7	JU GROBBELAAR	Eendekuil	1	5.0	5.0	7.0	4.0	52
1194	8	B	B8	NUWEDAM	Gifberg	2	7.0	6.8	7.0	6.8	69
1175	8	C	C8	PW VISSER	Sandberg	2	6.0	5.5	7.0	6.0	62
1176	8	D	D8	FG COETZEE	Grootfontein	2	5.0	5.0	8.0	4.5	54
1225	9	B	B9	JJ MARAIS	Citrusdal Berg	2	7.0	7.0	7.0	7.0	70
1261	9	C	B9	WAC STEENKAMP	Urionskraal	1	6.7	6.3	8.0	6.3	69

1276	9	D	D9	BURGER BROERS	Bo Rivier	1	4.0	5.0	6.0	5.0	51
1302	8	A	A8	SA DE BEER	Grootfontein	1	8.5	7.3	7.0	7.5	75
1305	10	B	B10	HC DE BEER	Aggenbagskraal	2	7.7	7.0	7.0	6.7	71
1318	10	C	C10	WK BERGH	Ouberg	2	6.0	5.8	7.0	5.8	61
1306	10	D	D10	C VISSER	Aurora	2	4.0	5.0	6.0	5.0	49
1389	11	B	B11	PA HUISAMEN	Nardouwsberg	2	7.0	7.0	7.0	6.5	68
1426	11	C	C11	FERDBURG	Eendekuil	2	6.0	6.0	7.0	6.0	62
1424	11	D	D11	JH V/D MERWE	Zeekoevlei	2	3.7	4.3	7.0	5.3	51
1459	12	B	B12	AJJ KOOPMAN	Suid Bokveld	2	6.8	6.0	7.0	6.5	66
1451	12	C	C12	JD NIEUWOUDT	Grootfontein	2	6.0	6.0	7.0	6.0	62
1432	12	D	D12	BAIEVLEI BOERDERY	Ouberg	1	4.8	4.8	7.0	4.5	53
1504	13	B	B13	JEA TRUST	Aggenbagskraal	2	8.5	8.0	7.0	6.5	74
1495	13	C	C13	GJ VAN WYK	Nardouwsberg	2	6.3	6.0	7.0	5.0	59
1481	13	D	D13	JCH BOERDERY	Gifberg	2	4.4	5.4	6.0	5.0	51
1514	14	B	B14	N SLINGER	Nardouwsberg	1	7.0	6.0	7.0	6.7	68
1511	14	C	C14	PS OLIVIER	Aurora	2	5.0	5.3	7.0	5.3	56
1516	14	D	D14	F KOTZE	Suid Bokveld	2	4.0	5.3	7.0	4.3	49
1566	15	B	B15	DE HANGEN BOERDERY	Nardouwsberg	1	7.0	7.0	7.0	7.0	70
1562	15	C	C15	SKURFKOP BOERDERY	Grootfontein	1	6.0	6.0	7.0	6.0	63
1553	15	D	D15	JA LOUW	Noord Bokveld	1	4.3	5.3	7.0	4.0	51
1570	1	AA	AA1	A BAKKER	Biedouw Berg	1	9.3	9.3	8.0	8.3	85
1576	16	B	B16	DE HANGEN	Nardouwsberg	1	7.0	7.3	7.0	6.3	68
1571	16	C	C16	G VAN ZYL	Sandberg	2	6.3	6.0	6.0	6.0	61
1585	17	B	B17	DUIKERFONTEIN	Nardouwsberg	2	7.0	7.0	7.0	7.0	70
1588	17	C	C17	OM BERGH	Ouberg	2	7.0	7.0	6.0	6.0	64
1616	18	C	C18	JC HUISAMEN	Nardouwsberg	1	6.0	6.0	7.0	6.0	63
1596	19	C	C19	WJ NIEUWOUDT	Agter Pakhuys	1	6.0	6.0	7.0	6.0	63
1606	16	D	D16	XZEE02	Redelinghuys	2	5.0	6.0	6.0	5.0	53
1626	17	D	D17	SFV VAN GEEMS	Aurora	1	6.0	7.0	6.0	4.0	53
1635	20	C	C20	SA V/D WESTHUIZEN	Gifberg	1	7.0	6.0	6.0	6.0	62
1642	18	D	D18	W STEMMET	Kobee Berg	2	5.0	5.3	6.0	5.3	54
1396	18	B	B18	SF NIEUWOUDT	Gifberg	1	7.3	7.0	7.0	6.3	68
1453	19	B	B19	EJ DE BEER	Aggenbagskraal	2	6.5	6.5	7.0	6.5	66
1515	20	B	B20	AI CARSTENS	Nardouwsberg	2	7.0	6.3	7.0	7.0	69
1545	19	D	D19	ORNATE TRADING	Aurora	1	4.7	5.7	7.0	4.7	55
1534	20	D	D20	WA NEL	Zeekoevlei	2	5.0	5.0	6.0	5.0	52

Cut: 1 = fine 2 = short 3 = long

ADDENDUM 2

An example of the score sheet developed for
Quantitative descriptive analysis (QDA)

QUESTIONNAIRE FOR TRAINED PANEL – ROOIBOS **Sample Code_____**

JUDGE NO	Judge name	TEST SESSION ___ Date_____

AROMA ATTRIBUTES	**Rooibos Floral** None 0--------100 Prominent	0 |_____| 100
	Rooibos Woody None 0--------100 Prominent	0 |_____| 100
	Rooibos Honey None 0--------100 Prominent	0 |_____| 100
	Sweet (General / Fruity sweet) None 0--------100 Prominent	0 |_____| 100
	Plant-like / Green None 0--------100 Prominent	0 |_____| 100
	Hay / Dried grass None 0--------100 Prominent	0 |_____| 100
	Caramel None 0--------100 Prominent	0 |_____| 100
	Dusty / Musty None 0--------100 Prominent	0 |_____| 100
	Other (e.g. green grass, seaweed, citrus, fruity, medicinal) _____	0 |_____| 100
	_____	0 |_____| 100
FLAVOUR, TASTE AND MOUTHFEEL ATTRIBUTES	**Rooibos Floral** None 0--------100 Prominent	0 |_____| 100
	Rooibos Woody None 0--------100 Prominent	0 |_____| 100
	Plant-like / Green None 0--------100 Prominent	0 |_____| 100
	Hay / Dried grass None 0--------100 Prominent	0 |_____| 100
	Caramel None 0--------100 Prominent	0 |_____| 100
	Sweet None 0--------100 Prominent	0 |_____| 100
	Astringent None 0--------100 Prominent	0 |_____| 100
	Sour None 0--------100 Prominent	0 |_____| 100
	Bitter None 0--------100 Prominent	0 |_____| 100
	Other (Specify) _____	0 |_____| 100
	_____	0 |_____| 100

ADDENDUM 3

The complete list of descriptive terms that were generated by the assessors during QDA training

Aroma attributes			
Fruit	**Sugar sweet**	**Woody**	**Musty / Earthy**
Fruity - berry	Caramel	Woody	Musty – (museum / cellar)
Fruity - sweet	Burnt sugar	Woody - fresh	Musty – sweet
Citrus	Hot butter	Bushy	Hessian
Citrus – floral	Pudding	Bushy - dry	Wet hessian
Citrus blossom	Molasses	Bushy - wild	Wet carpet
Orange blossom	**Vegetative - dried**	Stemmy	Sweaty
Jam	Hay	**Burnt**	Dusty
Jam - cooking	Hay - wet	Burnt	Rotting flower water
Jam - apricot	Dried grass	Smokey	Compost
Berry	Straw	Roasted	**Off-taints**
Raspberry	Lucerne	Roasted twig/fynbos	Medicinal
Pomegranate	Tobacco	**Spicy**	Chlorine
Raisin	**Vegetative - green**	Spicy	Chemical
Floral	Plant-like	Pungent	Rubber
Floral	Green	Incense	Fish oil / fishy
Floral - sweet	Green plant	Cinnamon	**Other**
Floral - fynbos	Green leaves	**Sea-like**	Bland
Fynbos	Wet plant / grass	Sea-like	Flat
Perfume	Green - oily	Coastal	Soft
Perfume - sweet	Grassy	Beach	Sour
Soapy	Green grass	Seaweed	Milky
Honey	Sweet grass		Fragrant
Honey	Green tea		
Honeysuckle	Bamboo		
Sweet Alyssum flower	Herbaceous		
	Herbal		

Flavour, taste and mouthfeel attributes			
Fruit	**Vegetative - dried**	**Woody**	**Basic**
Citrus	Hay	Woody	Bitter
Citrus - floral	Hay - wet	Bushy	Salty
Fruity	Straw	Stok / Stemmy	Sour
Sweet – raisin	Lucerne		Sweet
Floral	**Vegetative - green**		Astringent
Floral	Plant-like		Flat
Floral - sweet	Green	**Other**	Bland
Soapy	Green plant	Medicinal	Round
Sugar sweet	Green leaves	Metallic	Soft / Smooth
Caramel	Grassy	Flower water	Subtle
Honey	Green grass		
Honey	Cut grass		
	Aloe		
	Herbal		

ADDENDUM 4

Suppliers of compounds used as Reference Standards in the QDA training and compilation of the rooibos sensory lexicon

Compound	Supplier
α-ionone	Fluka, Sigma-Aldrich, Steinheim, Germany
Cis-3-hexenol	Fluka, Sigma-Aldrich, Steinheim, Germany
Citric acid	Sigma, Sigma-Aldrich, Steinheim, Germany
Caffeine	Fluka, Sigma-Aldrich, Steinheim, Germany
Alum (aluminum potassium sulfate)	Sigma-Aldrich, Steinheim, Germany
Sucrose	Huletts, South Africa

ADDENDUM 5

Comparison between PCA loadings and scores plots including and excluding luteolin (Lut), chrysoeriol (Chrys) and quercetin (Quer)