DEVELOPMENT OF QUALITY CONTROL TOOLS AND A

TASTE PREDICTION MODEL FOR ROOIBOS

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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SUMMARY

In this study quality control tools were developed for the rooibos industry, primarily to determine the quality of rooibos infusions. A considerable variation between samples of the same quality grade has been noted. As there are no guidelines or procedures in place to help minimise this inconsistency it was important to develop quality control tools, which could confront this problem. Both the sensory characteristics and phenolic composition of rooibos infusions were analysed in order to create and validate these quality control tools.

Descriptive sensory analysis was used for the development of a targeted sensory wheel and sensory lexicon, to be used as quality control tools by the rooibos industry, and to validate the major rooibos sensory profiles. In order to ensure all possible variation was taken into account, 230 fermented rooibos samples were sourced from the Northern Cape and Western Cape areas within South Africa over a 3-year period (2011-2013). The aroma, flavour, taste and mouthfeel attributes found to associate with rooibos sensory quality were validated and assembled into a rooibos sensory wheel, which included the average intensity, as well as the percentage occurrence of each attribute. Two major characteristic sensory profiles prevalent within rooibos, namely the primary and secondary profiles, were identified. Both profiles had a sweet taste and an astringent mouthfeel, however, the primary sensory profile is predominantly made up of "rooibos-woody", "fynbos-floral" and "honey" aroma notes, while "fruity-sweet", "caramel" and "apricot" aroma notes are the predominant sensory attributes of the secondary profile.

The predictive value of the phenolic compounds of the infusions towards the taste and mouthfeel attributes ("sweet", "sour", "bitter" and "astringent") was examined using different regression analyses, namely, Pearson's correlation, partial least squares regression (PLS) and step-wise regression. Correlations between individual phenolic compounds and the taste and mouthfeel attributes were found to be significant, but low. Although a large sample set (N = 260) spanning 5 years (2009-2013) and two production areas (Western Cape and Northern Cape, South Africa) was used, no individual phenolic compounds could be singled out as being responsible for a specific taste or mouthfeel attribute. Furthermore, no difference was found between the phenolic compositions of the infusions based on production area, a trend that was also seen for the sensory characterisation of rooibos infusions.

Sorting, a rapid sensory profiling method was evaluated for its potential use as a quality control tool for the rooibos industry. *Instructed sorting* was shown to successfully determine rooibos sensory quality, especially based on the aroma quality of the infusions. However, determining the quality of the infusion based on flavour quality was more difficult, possibly due to the low sensory attribute intensities. Categorisation of rooibos samples based on the two major aroma profiles i.e. the primary and secondary characteristic profiles, was achieved with *uninstructed sorting*. The potential of using sorting as a rapid technique to determine both quality and characteristic aroma profiles, was therefore demonstrated, indicating its relevance as another quality control tool to the rooibos industry.

UITTREKSEL

Gehaltebeheer hulpmiddels is as deel van hierdie studie vir die rooibosbedryf ontwikkel, hoofsaaklik om die sensoriese kwaliteit van rooibostee te bepaal. Aansienlike verskille is tussen monsters van dieselfde gehaltegraad opgemerk, primêr omdat daar in die wyer rooibosbedryf beperkte riglyne of prosedures in plek is om kwaliteitsverskille effektief te bepaal. Dit is as belangrik geag om gehaltebeheer hulpmiddels te ontwikkel om laasgenoemde probleem aan te spreek. Spesifieke gehaltebeheer hulpmiddels is dus vir hierdie studie ontwikkel en gevalideer deur die sensoriese eienskappe en fenoliese samestelling van rooibostee te analiseer.

Beskrywende sensoriese analise (BSA) is gebruik om 'n sensoriese wiel en leksikon vir die rooibosbedryf te ontwikkel en te valideer. Om alle moontlike produkvariasie te ondervang, is 230 gefermenteerde rooibos monsters afkomstig van die Noord-Kaap en Wes-Kaap areas in Suid-Afrika oor 'n tydperk van drie jaar (2011-2013) verkry. Die aroma, geur, smaak en mondgevoel eienskappe wat met rooibos se sensoriese kwaliteit assosieer, is bevestig en uiteindelik gebruik om die sensoriese wiel te ontwikkel. Die gemiddelde intensiteit en persentasie voorkoms van elke eienskap is in die wiel ingesluit. Twee belangrike "karakteristieke" sensoriese profiele wat met rooibos geassosieer word, is geïdentifiseer, nl. die primêre en sekondêre sensoriese profiele. Tipies van beide sensoriese profiele is 'n kenmerkende soet smaak en vrank mondgevoel, daarenteen bestaan die primêre sensoriese profiel hoofsaaklik uit "rooibos-houtagtige", "fynbos-blomagtige" en "heuning" aromas, terwyl "vrugtige-soet", "karamel" en "appelkoos" aromas die oorheersende sensoriese eienskappe van die sekondêre profiel is.

Die korrelasie tussen die fenoliese verbindings en die smaak en mondgevoel eienskappe van rooibos ("soet", "suur", "bitter" en "vrankheid") is ondersoek met behulp van verskillende tipe regressieontledings, nl. Pearson se korrelasie, gedeeltelike kleinstekwadrate regressie (PLS) en stapsgewyse regressie. Korrelasies tussen individuele fenoliese verbindings en die smaak en mondgevoel eienskappe was laag, maar steeds betekenisvol. Alhoewel die uitgebreide stel monsters (N = 260) verteenwoordigend was van vyf oesjare (2009-2013) en twee produksiegebiede (Wes-Kaap en Noord-Kaap, Suid-Afrika), kon geen individuele fenoliese verbindings uitgesonder word as betekenisvolle voorspellers van spesifieke smaak of mondgevoel eienskappe nie. Verder is daar ook geen verskil tussen die verskillende produksie-areas wat betref fenoliese samestelling gevind nie. Soortgelyke resultate is bevind vir die sensoriese karakterisering van rooibostee.

Sortering, 'n vinnige sensoriese profileringsmetode, is geëvalueer vir sy potensiële gebruik as 'n gehaltebeheer hulpmiddel vir die rooibosbedryf. *Gestrukteerde sortering* was suksesvol om rooibos se sensoriese kwaliteit, veral die algemene aroma kwaliteit van rooibos, te bepaal. Hierdie profileringsmetode was egter nie so suksesvol om rooibos se algemene geur, smaak en mondgevoeleienskappe te bepaal nie. Hierdie tendens kan moontlik toegeskryf word aan die betekenisvolle laer intensiteite van laasgenoemde sensoriese eienskappe.   Die kategorisering van die rooibos monsters op grond van hul karakteristieke

primêre en sekondêre sensoriese profiele is suksesvol deur middel van *ongestrukteerde sortering* bepaal. In die geheel gesien is die potensiaal van die sorteringstegniek as 'n vinnige metode om die algemene sensoriese kwaliteit, asook die karakteristieke aroma profiele van rooibos te bepaal, dus bewys. Hierdie vinnige sensoriese profileringstegniek hou dus besliste voordele in vir die rooibosbedryf as dit kom by sensoriese gehaltebeheer.

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~ "In order to succeed, your desire for success should be greater than your fear of failure." Bill Cosby ~

This thesis/dissertation is presented in the format prescribed by the Department of Food Science at Stellenbosch University. The structure is in the form of one or more research chapters (papers prepared for publication) and is prefaced by an introduction chapter with the study objectives, followed by a literature review chapter and culminating with a chapter for elaborating a general discussion and conclusion. The language, style and referencing format used are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis/dissertation represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

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CHAPTER 1

INTRODUCTION

Over the last 14 years, rooibos has been growing in popularity both locally and globally making up 10% of the global herbal tea market (Anon., 2014a). The current retail revenue of rooibos tea is worth an estimated R1.5 billion with an approximate 15 000 tons (15 million kilograms) of rooibos being harvested each year, half of which is exported to the global market (Anon., 2014a; Anon., 2014b). With harvest production up from only 8000 tons in 2004, the increased demand for this unique tea dictates that acceptable product quality is achieved and maintained at all times. The current study focuses on unpasteurised and fermented (oxidised) rooibos, and not green rooibos (unfermented), which has also seen a rapid growth in popularity amongst consumers.

Rooibos has recently been granted Geographical Indication (GI) protection, meaning that the name "rooibos" and its derivatives ("red bush", "rooitee", etc.) belong to the South African rooibos industry and are protected from use elsewhere, unless the product originates from the rooibos growing regions within South Africa (South African Rooibos Council (SARC), Clanwilliam, South Africa, September 2014). In order to obtain a GI, a product needs to "possess qualities, a reputation or characteristics that are essentially attributable to that place of origin" (Anon., 2014b). Obtaining the GI is a great achievement for this unique industry, which relies heavily on its export market, especially Europe. The granting of GI status for rooibos will have a large economic impact on the industry as well as lead to many social developments in the rooibos producing areas.

A current weakness within the rooibos industry is the inconsistency in rooibos quality due to a lack of guidelines and enforcement mechanisms (Anon., 2014a). Quality inconsistency is especially troubling when considering the international market, where the importers and consumers may not know a product is of poor quality, due to their unfamiliarity with rooibos, resulting in poor acceptance of the product by the market. According to South African export regulations, rooibos has only to have a "clean, characteristic taste and aroma" of rooibos, in order for it to be seen as acceptable for sale (Anon., 2002). This statement leaves a large amount of room for misinterpretation, as there are no accompanying descriptions pertaining to the meaning of "characteristic" rooibos tea. This could lead to miscommunication between industrial role-players, and therefore lead to rooibos teas on the market differing in quality. For the success and growth of this local industry it is of great importance that the rooibos available be of consistent quality within a quality grade, so as to increase consumer loyalty both locally and globally. It should however be acceptable that quality will vary, but this can be accommodated by quality categories.

In order to achieve the same rooibos quality, for a specific quality category, across all processors, the sensory profile of rooibos and the variation in quality needs to be understood in order to achieve a better definition of "characteristic". Koch *et al*. (2012) determined that the primary characteristic sensory

profile of rooibos is made up of "honey", "woody" and "fynbos-floral" notes accompanied by a sweet taste and subtle astringent mouthfeel. These and other attributes common to rooibos were determined and used for the creation of a generic rooibos sensory wheel and lexicon (Koch *et al.*, 2012). Sensory lexicons contain descriptors that describe the sensory attributes of a product, such as rooibos tea, and usually contain reference standards, which when created will mimic the attributes within the product (Koch *et al.*, 2012; Drake & Civille, 2002). Sensory wheels are popular quality control tools within the food industry and the creation of the rooibos sensory wheel has seen acceptance by the industry, although based on a limited scope of data (Koch *et al.*, 2012).

Whilst laying the foundation for a more scientific approach to sensory evaluation, these sensory tools developed for rooibos, were created using only the data obtained from one production season (2009) and one production area (Western Cape region, South Africa). Due to this reason, the need to increase the extent of the variation in the sample-set was imperative as this would help verify the results obtained by Koch *et al.* (2012), as well as ensure that all possible variations within rooibos are taken into account. Once a larger data set is analysed, it will be possible to validate the sensory attributes, as well as develop an updated sensory wheel and lexicon for use within the industry. Therefore the **initial aim** of this study was to determine the sensory profile of rooibos tea from two production areas, over three production seasons and four quality grades. Furthermore, it was of interest to determine the influence of production area and year on the sensory profile of the rooibos. The information of which, may allow for the marketing of niche rooibos tea products, based on unique sensory profiles that are present as a result of plant growth in the different production areas.

With the validation of these sensory tools, it will be possible to utilise them to aid in the standardisation of the grading of rooibos tea. Quality variation can be greatly decreased with the use of standardised vocabulary during the grading process. As grading processes differ between processors, standardising the vocabulary, may decrease the variation in product quality, and allow for small processors to have more success in their quality grading of the tea (Rampedi & Olivier, 2008). By using a standardised list of descriptors, all the role-players within the industry will be of the same level of understanding regarding the sensory attributes within the tea. The sensory lexicon, with its accompanying reference standards will be of great importance to the export industry, as it will allow international counterparts to be better able to understand the sensory profile of rooibos, which they may not be completely familiar with.

Sensory quality of rooibos is exhibited through aroma, flavour, taste and mouthfeel attributes. The occurrence of these attributes is dependent on the presence/concentration of both volatile (aroma) and non-volatile (taste and mouthfeel) compounds. With the focus on non-volatile components, Koch *et al.* (2013) were able to determine correlations between specific phenolic compounds and sensory attributes. Only the correlation between rutin and astringency was found to be significant. Analysing a larger sample set could possibly allow for the verification of these correlations, due to the fact that potentially more

variation is available. The taste and mouthfeel attributes; "sweet", "sour", "bitter" and "astringent" play important roles in sensory quality of rooibos. Therefore the ability to predict the intensities of these sensory attributes is important. This information could greatly help the industry to accurately predict quality, based on the phenolic composition of the rooibos. Prediction models have been developed and used with success, such as for wine (Frank & Kowalski, 1984) and dry-cured ham (Careri *et al*., 1993). A prediction model is developed using a variety of regression analysis methodologies, which allows for two data matrices to be related to one another, with the aim of interpreting and predicting data. Regression works on the theory of one variable (independent) causing or explaining the output of another variable (dependant) (T. Næs, Nofima, Norway, April 2012, personal communication). General procrustes analysis (GPA) and partial least squares regression (PLS) are popular statistical methods that have been used to determine product quality or geographic origin (Abdi, 2007; Careri *et al*., 1993; Frank & Kowalski, 1984). By having a model able to predict rooibos taste and mouthfeel attributes, one can then use this model for quality control, grading of rooibos, as well as for the rapid selection of rooibos batches for blending. Through the use of the prediction model it will be possible to ensure the standardisation of the quality of rooibos, at least in terms of taste and mouthfeel, which will be of benefit to the rooibos industry. Due to the aforementioned reason, the **second aim** of this study was to determine correlations between sensory attributes and phenolic compounds, as well as to develop a quality prediction model for the rooibos industry.

Currently descriptive sensory analysis (DSA) is the main method used when determining the sensory profile of a food product. It is also further used for quality control purposes (Murray *et al*., 2001; Lawless & Heymann, 2010). This method is time-consuming, as it involves panel training, detailed sensory analysis and substantial data analysis. DSA is a reliable method that gives very detailed sensory profiles of a product, including sensory attributes and attribute intensities. Utilised by a number of multinational product development companies, DSA is used to determine the full profile of their product ranges when doing product development, quality control or extensive quality grading. In these instances DSA data are usually combined with other types of data, e.g. chemical, microbiological or physical data, to determine the full profile, but also to ascertain which parameters should be changed during the production, product development or quality control phases. It would be an advantage to the rooibos industry, if it were possible to profile rooibos using a more rapid method than DSA, but which will result in similar results. A number of rapid sensory profiling methods, currently being used within the food and beverage industries, are available, such as sorting and projective mapping (Valentin *et al*., 2012; Cartier *et al*., 2006; Dehlholm *et al*., 2012). Although each of these methods involve the sorting or categorisation of samples, the strengths and weaknesses of each will determine their appropriateness of use with specific products. The **third aim** of the study therefore focused on determining the possibility of using the sorting method, as a reliable tool to grade rooibos based on overall sensory quality, as well as to aid in the determination of the sensory profile of rooibos. Sorting could also be used as an aid in the blending of tea. Whilst creating a blend, it is

important to ensure that each blended batch has the same sensory profile, in order to ensure consistency of quality. Its potential usefulness by the rooibos industry, especially for blending to achieve consistent quality, is thus evident.

Quality control is important within industry as it ensures a secure position on the market and loyalty from consumers. For a small and unique industry, such as rooibos, this is of the utmost importance, to ensure market growth both locally and internationally. Thus developing quality control tools, which can aid in the standardisation of rooibos grading, and resulting in the assurance of sensory quality, was the focus of this study. The aims of the study were therefore three-fold, namely **i)** to determine the sensory attributes and profiles of fermented rooibos which subsequently could be used to update, expand and validate the generic sensory wheel and lexicon; **ii)** to determine the correlations between the taste and mouthfeel attributes and the phenolic compounds within this herbal tea, the data of which would be used to develop a model able to predict the quality of rooibos tea and lastly **iii)** to determine the efficacy and reliability of using a rapid sensory method, such as sorting, to determine the sensory quality and profile of rooibos tea. The order of the chapters within this thesis is set out in the same manner as the above aims.

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CHAPTER 2

LITERATURE REVIEW

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1. SOUTH AFRICAN ROOIBOS INDUSTRY

1.1. Introduction

Aspalathus linearis, better known as rooibos, is an endemic plant in South Africa that is enjoyed as a tea. This herbal tea is popular not only for its taste and aroma, but also for the medicinal properties it exhibits (Joubert *et al.,* 2008). Rooibos has a characteristic red-brown colour that is a consequence of the "fermentation" (fermentation is an oxidation process) that the tea undergoes during production. The red-brown colour is the reason rooibos tea acquired its name "rooibos", which means "red bush" in Afrikaans (Koch, 2011; J. Basson, Rooibos Ltd, Clanwilliam, April 2012, South Africa, personal communication). This fynbos species has become popular on a globally and is currently sold in over 37 countries worldwide. These include the Netherlands, the United States of America, Japan, the United Kingdom and Germany, which made up 86% of the export market in 2011 (Joubert & De Beer, 2011). It has been stated that "rooibos appears to be headed towards becoming the second most commonly consumed beverage tea ingredient in the world after ordinary tea" (Anon., 2007).

The rooibos tea market is valued at approximately R550 million a year, and represents 10 % of the global *herbal tea* market and 0.3 % of the global *tea* market (Donnelly, 2012; Anon., 2014). The popularity of this tea, globally as well as locally, does not look like it will subside anytime soon. Within South Africa alone it is estimated that rooibos tea is consumed in more than 10.9 million households (Joubert & De Beer, 2011). The great demand for rooibos tea has allowed rooibos production to increase from 5 000 tons in 2001 to 11 500 tons in 2012 (Anon., 2014). The export volume of rooibos (approximately 6000 tons) currently exceeds the volume of rooibos consumed locally (4500 – 5000 tons) (Curnow, 2012; Anon., 2014). There has, however, been a consistent decrease in rooibos production yields, from 18 000 tons in 2008 and 2009 down to 11 500 tons in 2012. This decrease could be due to the changes in the climate, which has already been affecting rooibos crops in the rooibos producing regions (J. Basson, Rooibos Ltd., Clanwilliam, South Africa, April 2012, personal communication), as well as the fact that some farmers may not be planting as many crops as before, due to this instability.

Figure 1 Rooibos production in South Africa from 2002-2012 (Anon., 2014).

Not only is its use as conventional herbal tea popular, but there is increasing interest in the use of rooibos in the manufacture of iced teas, both locally (Food and Beverage Reporter, 2006; Anon., 2006) and in other markets (Snapple© Beverage Corp., 2012). In a bid to develop a never before seen product, a rooibos espresso called Red Espresso® was created by refining the rooibos into an espresso grind similar to that of coffee. Red Espresso® has created a new beverage category, as it is the first tea espresso ever made (Food and Beverage Reporter, 2007; Red Espresso®, 2012). The global tea market has also seen the introduction of green (unfermented) rooibos, being used in the manufacture and product development of many new products. Currently there is extensive research and development going into the creation of new variations of rooibos tea, including unflavoured green rooibos and flavoured rooibos blends (Curnow, 2012). With the world becoming more involved in the protection of the planet and its inhabitants there has been a universal increase in the demand for organically grown or fair-trade products (Nel *et al.,* 2007). Currently between 5% and 10% of rooibos is sold as certified organic rooibos (Waarts & Kuit, 2008). Although there is a global desire for organic products, the market for organic rooibos has become saturated, leading to large amounts of the organic product ending up being sold as conventional rooibos (Waarts & Kuit, 2008).

1.2. History of rooibos

In 1772 the botanist Carl Thunberg reported the use of rooibos as a beverage whilst on his travels within South Africa. Benjamin Ginsberg was also able to witness this use of *Aspalathus linearis* by the descendants of the Khoi in the early years of the 1900's when he was in the Clanwilliam area of the Western Cape, South Africa. He observed how the wild plants were harvested and processed by the chopping and crushing of the shoots, where after the leaves and stems were fermented in the hollows of stone reefs and sun-dried (Joubert & De Beer, 2011). This process provided the basis of the production process, which is still used today, although it has been tweaked for the use of modern machinery. Ginsberg started the first commercial use of rooibos in 1904, when he marketed the tea under the popular brand *"*Eleven O'Clock*"* (Joubert *et al*., 2008). During World War II there was a global shortage of Oriental teas in South Africa, which led to an increased local demand for rooibos tea (Morton, 1983; Joubert & De Beer, 2011). This presented an ideal opportunity for the growth of the rooibos market, however, after the war ended the rooibos market collapsed, mainly due to the availability of cheap coffees, Oriental teas and the declining quality of the rooibos produced (Morton, 1983; Joubert & De Beer, 2011). The production of rooibos became uneconomical between 1953 and 1954 due to the decreased demand for this herbal tea, overproduction and inconsistent quality. This led to the creation of the Rooibos Tea Control Board, formed to regulate the marketing of the tea and ensure that the quality of rooibos was consistently up to standard (Joubert *et al*., 2008). The use of this system, however, was abolished in the mid-1990's (Joubert *et al*., 2008). After the abolishment of the board it became a private firm, i.e. Rooibos Ltd. Over the years many farmers have decided to start their own companies, but Rooibos Ltd, located in Clanwilliam in the Western Cape, South Africa, still remains the biggest player in the rooibos industry (Wilson, 2005). The company

receives both fermented and fresh plant material from farmers. All processed rooibos undergoes quality analyses, i.e. chemical testing for pesticide residues and sensory testing for grading.

The Nieuwoudtville area, situated in the Northern Cape, South Africa, has recently seen the development of a rooibos processing plant to enable local rooibos farmers to have their tea processed closer to the farm (M. Baard, Nieuwoudtville Rooibos (PTY) Ltd., Nieuwoudtville, South Africa, April 2012, personal communication). The factory in Nieuwoudtville receives the majority of the tea in a fresh state from the farmers. This allows the company to control processing to ensure an end product that is up to standard. Currently, all rooibos processed at the Nieuwoudtville factory is exported (Anon., 2013a).

These processors are the major processors in each of the rooibos production areas. There are, however, small processors and small-scale farmers, within both production areas, that process and market the tea that they harvest. In total it is estimated that there are between 350-550 rooibos farmers within South Africa (Anon., 2014).

1.3. The rooibos plant

Aspalathus linearis grows mainly in the Cederberg area of the Western Cape, South Africa. This area includes the Citrusdal and Clanwilliam areas. This unique plant is also found in the Nieuwoudtville area, on the Bokkeveld plateau on the border of the Western Cape and Northern Cape. The areas used for farming purposes are indicated in **Fig. 2**. Temperature differences between these two main areas (Western Cape and Northern Cape) can be seen in **Fig. 3**, where it is clear that the Clanwilliam and Citrusdal areas (Western Cape) have higher minimum and maximum temperatures, on average, than the Nieuwoudtville area (Northern Cape) (ARC Institute for Soil, Climate and Water, South Africa). These differences can be due to the differences in altitude between the areas, as Nieuwoudtville is located on a plateau. Climatic differences may have an effect on the rooibos grown in these areas, considering the effect of climate on the composition of other plants (Tounekti *et al*., 2013, Agati *et al*., 2012). Rooibos crops are not successfully grown below a height of 450 m above sea-level and only thrive in an environment up to 900 m above sea-level (Morton, 1983).

Aspalathus linearis has needle–like leaves and yellow flowers. Some of the plants are prostrate and grow no larger than 30 cm tall whereas others can grow up to 2 m tall (Cheyney & Scholtz, 1963; Joubert *et al*., 2008). The red type of *Aspalathus linearis*, known as the "rocklands" type, is mainly used on a commercial scale (Van Der Bank *et al.,* 1995). The "rocklands" type of rooibos is again divided into two different categories namely the Nortier type, which is cultivated, and the Cederberg type, which is wild growing. The Nortier type has been improved over the years (cultivated), making it a better choice for commercial farming (Joubert *et al*., 2008). Grey and black variants of rooibos tea also exist, the marketing of which was, however, stopped in 1966 due to poor tea quality (Joubert *et al*., 2008).

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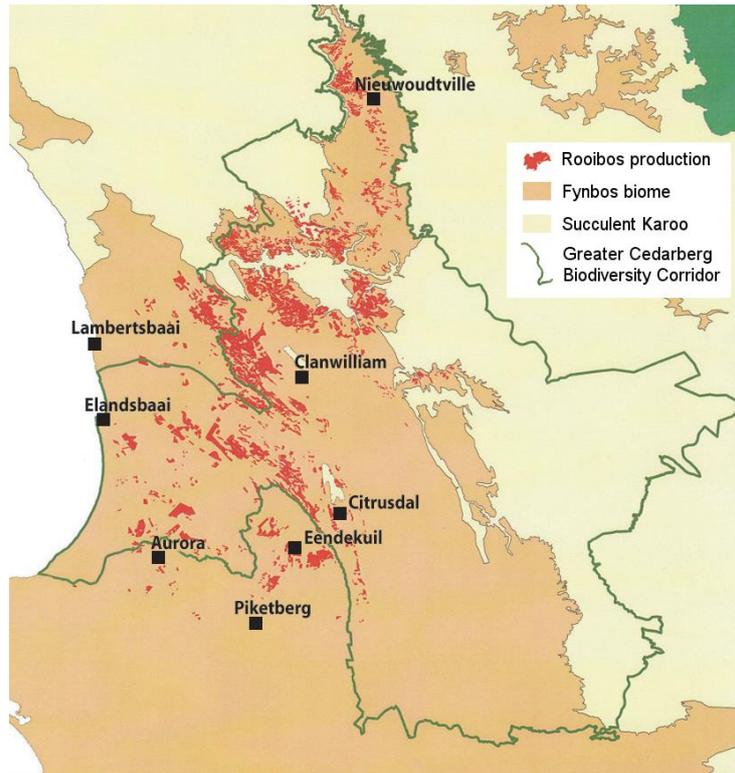
Figure 2 Map illustrating the distribution of rooibos within the rooibos producing regions of South Africa (Joubert & De Beer, 2011).
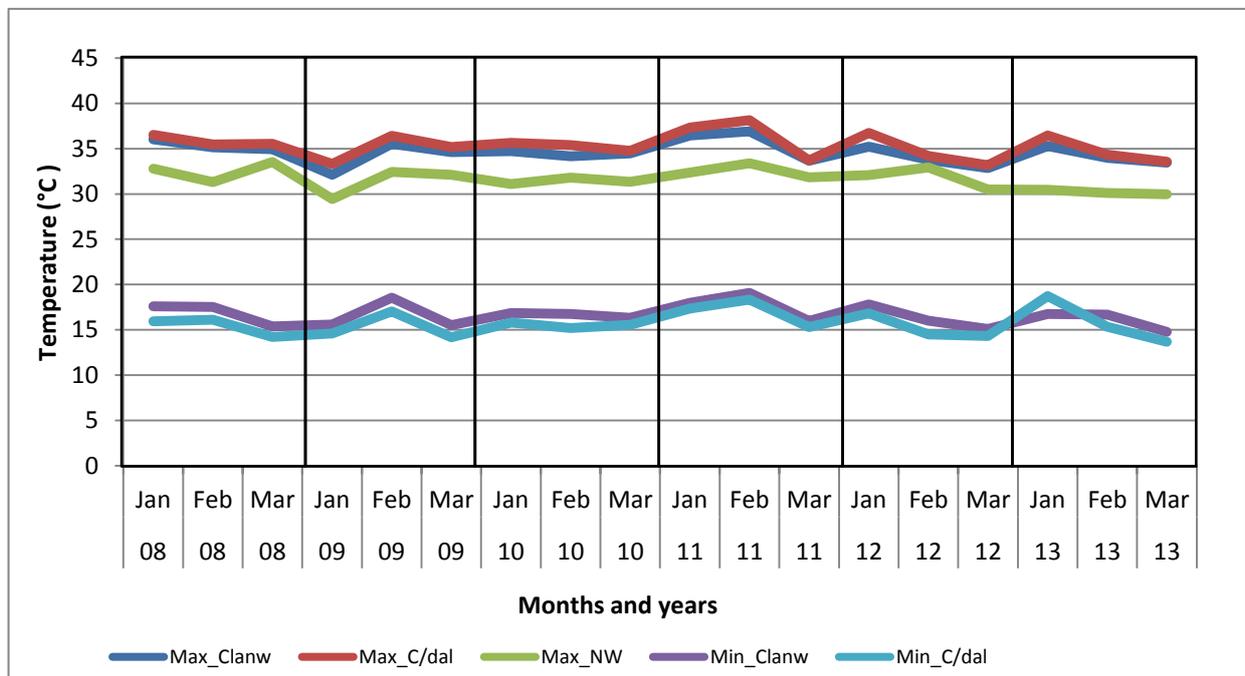
Figure 3 Temperature trends in the Clanwilliam, Citrusdal and Nieuwoudtville areas, South Africa from 2008 – 2013 (ARC Institute for Soil, Climate and Water, South Africa).

1.4. Processing of rooibos tea and the effects on tea quality

The rooibos plant is harvested during the hot summer months and the beginning of autumn, which in South Africa is from January until April (Cheyney & Scholtz, 1963). Harvesting is achieved by topping the bush to approximately 45 cm in height. The active growth of the plant should be no more than 50 cm and no flowers should be present at harvest, as this would result in a weak, *mild tasting*, lower quality tea (Joubert *et al*., 2008). Rooibos leaves and stems gain their red colour when undergoing "fermentation" (oxidation). The oxidation process is very important to ensure development of the characteristic colour, and unique aroma and flavour of rooibos (Joubert & De Beer, 2011). For fermentation the shredded plant material is placed in heaped piles for between 12 h and 24 h whilst at an ambient temperature (38°C - 42°C), thereafter the leaves are sun-dried (Joubert *et al*., 2008). Wetting and bruising of the heaped rooibos stems and leaves help to aid in the oxidation process (Joubert *et al*., 2008). When the leaves are bruised, they release polyphenols, which help to colour the stems, leading to a more uniform product (Joubert *et al*., 2008). Poor aeration of the heap leads to incomplete oxidation, which results in a tea that does not exhibit the "characteristic" attributes and is of substandard quality (Joubert *et al*., 2008).

Studies have shown that there could be an improvement in both the consistency and quality of the rooibos, if the oxidation and drying of the leaves and stems happened under controlled conditions (Joubert & De Beer, 2011; Joubert & De Villiers, 1997). A factory-based process, however, would not be feasible, because of the processing capacity required and the energy requirements for drying the tea (Joubert & De Beer, 2011). Other processing steps, such as steam pasteurisation of the dried product before bulk packaging, can have an effect on the aroma and flavour of rooibos. Koch *et al*. (2013) determined that steam pasteurisation of rooibos results in a decrease of the intensity in its aroma and flavour attributes. Pasteurisation, however, is a vital part of the processing of rooibos in order to ensure product safety.

There are numerous external factors that can also impact rooibos quality. The age of the bush when processed and the presence of young growth can affect the overall quality of the tea. It has been suggested that the area in which the rooibos is produced could affect the tea quality (Joubert & De Beer, 2011).

1.5. Quality control

Quality grading of rooibos has evolved over the years. Initially, grading was based solely on the cut, colour and aroma of the dried rooibos stems and leaves, and until 1985 no consideration was given to the infusion. A four-member panel of the Rooibos Tea Control Board were responsible for the grading procedure. To help curb the bias that could occur from the manual size grading of the leaves, a mechanical size-grading system, with sieves of different sizes, was put into place in 1965 (Joubert, 1994). Inclusion of the quality evaluation of the rooibos infusion led to the development of new quality grades, i.e. "Super", "Choice" and "Standard". Tea of a high quality was given the grade "Super" and the lowest quality tea was given the grade "Standard" (Joubert, 1994). Over the years, changes were made to this grading system and

in 1992 the grade "Selected" was added to the grading system. Three categories, (A, B, C) were later developed so that the teas could be grouped according to strong, medium or poor characteristic aromas and basic tastes.

Since the abolishment of the Rooibos Tea Control Board, each of the individual tea processing companies uses their own grading method. There are two major rooibos tea processors in South Africa, one situated in the Western Cape and the other in the Northern Cape. Within the Western Cape, the processor, receiving most of its product from producers in the Western Cape, grades the tea according to similar criteria as mentioned above. The different criteria are scored according to different weightings, and a final score is then tallied, which determines the final grade (J. Basson, Rooibos Ltd., Clanwilliam, South Africa, April 2012, personal communication). Once the tea arrives at the company, a sample is taken and sieved mechanically in order to obtain the yield, i.e. the size fraction that will be graded and eventually marketed. Both an experienced grader and a trained panel (to confirm the grade awarded) carry out the grading of the tea. The appearance of the tea leaves, in both a wet and dry state, are evaluated. Over-fermented tea leaves appear dull-brown in colour and lead to an infusion that is watery with a woody aroma (Joubert & De Villiers, 1997; J Basson, Rooibos Ltd., Clanwilliam, South Africa, April 2012, personal communication). The colour and brightness of the infusion are then evaluated, followed by the evaluation of the overall flavour of the infusion. An ideal rooibos infusion, which has been made from high quality tea, has a red-brick colour, with an orange-yellow tint where the infusion meets the edge of the cup. An under-fermented, low-quality infusion will have a brown or turbid appearance with an orange-yellow tint (Koch, 2011).

The grading system employed by the processing company in the Northern Cape of South Africa, differs from the used by to grade the tea samples from the Western Cape processor. In both companies, an experienced grader, who takes into account the aroma of the infusion and leaves (wet and dry) as well as the flavour of the infusion, does the grading of the tea. The Northern Cape processor, however, bases the quality grading on a presence or absence system, where the flavour and aroma of the infusion are rated as either being present (positive) or absent (negative), from here a final grade is calculated and added to the final grading sheet (M. Baard, Nieuwoudtville Rooibos (PTY) Ltd., Nieuwoudtville, South Africa, April 2012 & 2013, personal communication; J. Basson, Rooibos Ltd., Clanwilliam, South Africa, April 2012, personal communication).

The different grading procedures, as well as possible inconsistencies between samples of same the quality grade, present due to the lack of guidelines and enforcement mechanisms, can be a weakness in the rooibos industry. These inconsistencies in quality are seen for the aroma, taste, chemical properties, as well as the appearance of the tea. With the implementation of better guidelines and better industry training these inconsistencies can be prevented (Anon., 2014). Currently there are no specific guidelines within legislation which state how the quality of rooibos tea should be regulated. The sole regulation relating to the quality standards of rooibos states that: "All rooibos shall have the clean, characteristic taste

and aroma and clear, distinctive colour of rooibos" (Anon., 2002). No further guideline exists to explain what the term "characteristic" encompasses. The term "characteristic" taste and aroma may be familiar to the South African population, as they have spent their lives being exposed to this traditional tea, to foreign consumers and processors, however, the term "characteristic" may have a different meaning. Another important aspect to address is the difference in the interpretation of "characteristic" between the different role players within the industry, from producers to processors. In order to allow for the correct interpretation of the definitions of each of the quality grades, the definitions need to be discernable from one another. The standardisation of these terms can be achieved through the use of a sensory wheel and sensory lexicon. Recently, the initial sensory lexicon and wheel for the rooibos industry has been developed (Koch *et al.*, 2012). The wheel and lexicon, however, were created using the data gathered from only one production season (2009) and one production area (Western Cape), therefore leading to the need for validation of both the wheel and lexicon using a larger data set. By including the data of samples from a number of production years, production areas and grades, all possible variations within rooibos can be covered. This can lead to the development of a comprehensive wheel and lexicon, which can then be validated further with industry input.

1.6. Chemical composition of rooibos tea

Rooibos is well known as a caffeine-free tea and when compared to black tea (*Camellia sinensis*), it has much lower levels of tannins. Not much is known about the structure of the tannins found in rooibos tea, but procyanindin type compounds are present (Joubert & De Beer, 2011). Oxidation of the dihydrochalcones, aspalathin (unique to rooibos) and nothofagin (**Table 1**), during fermentation, leads to the formation of unidentified brown polymeric substances amongst others (Krafczyk & Glomb, 2008; Krafczyk *et al.*, 2009; Heinrich *et al.*, 2012).

Many phenolic compounds have been identified in rooibos (as reviewed by Joubert *et al.*, 2008). Recent papers by Iswaldi *et al.* (2011) and Beelders *et al.* (2012) expanded the range of phenolic compounds identified in rooibos infusions to date. Joubert *et al.* (2012) gave the first report of representative quantitative data of detectable monomeric phenolic compounds in rooibos infusions at "cup-of-tea" strength. The flavonoids, aspalathin, orientin, isoorientin and quercetin-3-*O*-robinobioside, as well as phenylpyruvic acid-2-*O*-glucoside (PPAG), a phenylpropenoic acid (present at > 5 mg/L), were present at the highest concentrations. Other compounds detected at levels > 2 mg/L were vitexin, isovitexin and hyperoside (quercetin-3-*O*-galactoside). Nothofagin, isoquercitrin (quercetin-3-*O*-glucoside), rutin (quercetin-3-*O*-rutinoside) and ferulic acid were present at > 0.9 mg/L. Joubert (1996) indicated that the amount of aspalathin and nothofagin present in the tea were dependent on the degree of oxidation of the plant material.

Apart from the potential health benefits that have been linked to the phenolic content of rooibos tea (Joubert *et al.,* 2008), the presence of these constituents is important for the taste and mouthfeel

attributes of rooibos (Joubert *et al*., 2013; Koch *et al*., 2013). PPAG has been found to associate with the "sweet" taste of the infusion (Koch *et al*., 2012), yet when tested as pure compound it was perceived as "bitter", suggesting the occurrence of taste modulation when present in the infusion (Joubert *et al*., 2013). Rutin and isoquercitrin have also been found to have a "bitter" taste when tested in water (Scharbert *et al*., 2004; Stark *et al*., 2005)

Table 1 Monomeric phenolic compounds identified in fermented *A. linearis* plant material (as reviewed by Joubert *et al.,* 2008).

General structure	Compound type, names and substituents
	Dihydrochalcone Aspalathin: R_1 = OH, R_2 = *C*-β-D-glucosyl Nothofagin: R_1 = H, R_2 = *C*-β-D -glucosyl
	Flavone Orientin: R_1 = *C*-β-D-glucosyl, R_2 = R_4 =OH, R_3 = H Iso-orientin: R_1 = H, R_2 = R_4 =OH, R_3 = *C*-β-D-glucosyl Vitexin: R_1 = *C*-β-D-glucosyl, R_2 = OH, R_3 = R_4 = H Isovitexin: R_1 = R_4 = H, R_2 = OH, R_3 = *C*-β-D-glucosyl
	Flavonol Isoquercitrin: R = *O*-β-D-glucosyl Hyperoside: R = *O*-β-D-galactosyl Rutin: R = *O*-β-D-rutinosyl Quercetin-3-*O*-β-D-robinoside: R = *O*-robinosyl
	Phenylpyruvic acid derivative 3-phenyl-2-glucopyranosyloxypropenoic acid: R = *O*-glucosyl
	Hydroxycinnamic acid and derivative 3,4,5-trihydroxycinnamic acid: R_1 = R_2 = OH; R_4 = H *p*-coumaric acid: R_1 = R_3 = H, R_2 = OH; R_4 = H Caffeic acid: R_1 = R_2 = OH, R_3 = H; R_4 = H Ferulic acid: R_1 = OCH$_3$, R_2 = OH, R_3 = H; R_4 = H Sinapic acid: R_1 = R_3 = OCH$_3$, R_2 = OH; R_4 = H Chlorogenic acid: R_1 = R_2 = OH, R_3 = H; R_4 = quinic acid
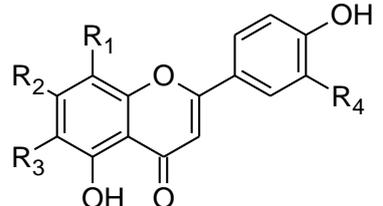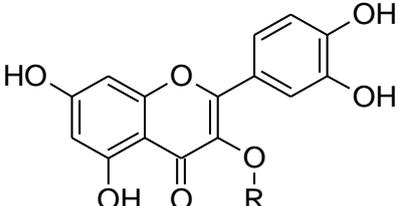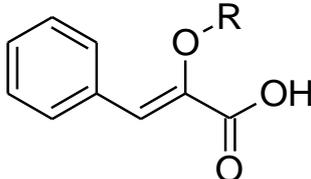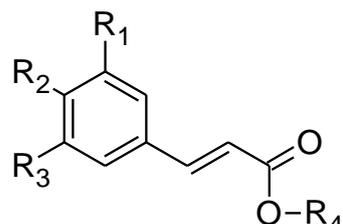
The volatile composition of rooibos includes ketones, aldehydes, alcohols, esters, hydrocarbons, phenols and ethers (Habu *et al*., 1985; Kawakami *et al*., 1993). The aroma of brewed extract has been characterised by many kinds of lactone compounds (Kawakami *et al*., 1993). Major compounds in a vacuum steam distillate of fermented rooibos were found to be guaicol, β-damascenone, dihydroactinidiolide, β-ionone, 5,6-epoxy-β-ionone, 6-methyl-3,5-heptadien-2-one, β-phenylethyl alcohol, and benzaldehyde (Habu *et al.,* 1985). The aroma profile of these compounds can be found in **Table 2**. Other major compounds included 2-phenylethanol, geranylacetone and 6-methyl-5-hepten-2-one (Kawakami *et al.,* 1993). Compounds such as *cis*-3-hexenal and *trans*-3-hexenal, associated with green/ grassy aroma, were also present in the rooibos volatile fraction (Koch, 2011). None of these compounds encompass the complete "characteristic" aroma of rooibos tea, when analysed individually. The aroma of a foodstuff is usually explained by a combination of volatiles and not one single compound (Chambers & Koppel, 2013).

Table 2 The aroma profiles of chemical compounds found in rooibos infusions.

Chemical compound	Aroma
Guaicol	Woody Smokey[a]
B-damascenone	Floral, violet[a]
Dihydroactinidiolide	Sweet, tea-like odor[b,d]
B-ionone	Rose-like[a]
5,6-epoxy-ionone	Fruity [b], floral[d]
6-methyl-3,5-heptadien-2-one	Spicy[b]
β-phenylethyl alcohol	Floral, rose/dried rose[b]
Benzaldehyde	Almond[c]

[a]Kerry Ingredients, Durban, South Africa, October 2013, Personal communication; [b]Anon., 2013b; [c]Anon., 2013c; [d]Glória *et al*., 1993.

2. SENSORY ANALYSIS OF ROOIBOS

The sensory analysis of food has been described as "the scientific method to evoke, measure, analyse and interpret responses to products as perceived through the senses of sight, smell, touch, taste and hearing" (Lawless & Heymann, 2010). Sensory analysis with regard to the grading of foods has been around since the early 1900's when producers discovered that they could ask top prices for foods that met the high standards of the consumer (Meilgaard *et al*., 1999). Since then it has been a goal of producers and researchers to determine the quality of foods through both analytical and sensory methodologies. Using descriptive sensory analysis as a research tool allows for the determination of a complete sensory profile for a specific product (Lawless & Heymann, 2010). This sensory profile can help determine the individual

attributes that are deemed important for consumer acceptance, and market potential (Hootman, 1992; Lawless & Heymann, 2010).

The importance of profiling the primary sensory attributes of a product is emphasised when a country or a group or researchers wishes to apply for the product to have a Geographical Indication (GI). When considering food products that have a Geographical Indication (GI), it is clear that the sensory characteristics of that product are of the utmost importance (Vázquez-Araújo *et al.,* 2012). These characteristics include the appearance, flavour, odour and texture of the product in question. The sensory profiling of a product helps determine the unique characteristics within this product that differentiate it from other similar foodstuffs. The definition of a Geographical Indication states that "A geographical indication is a sign used on goods that have a specific geographical origin and possess qualities, a reputation or characteristics that are essentially attributable to that place of origin" (WIPO, 2014a). By being able to define what makes the product different and unique, due not only to the geographical location, but also due to the unique sensory characteristics, brought about by location and unique processing techniques, a niche product can be created. When the GI status is given to an indigenous product, it helps to create security for the small industries involved in production and sales, and helps create a stable income for all involved whilst protecting the indigenous product itself. Rooibos meets all the requirements needed for a GI status, it grows in only one part of the world, the properties of the plant are as a result of its location and the climatic conditions there, and furthermore, there is a strong traditional knowledge about rooibos plant cultivation and harvesting due to the link that still exists between the farmers and this unique tea (WIPO, 2014b).

As a result rooibos recently obtained GI protection, ensuring that this unique product is protected and remains the property of the rooibos industry in South Africa. The name rooibos, as well as other names associated with this tea such as "rooitee", "red bush" and "rooibosch" to name only a few, are protected from being used to market rooibos, unless it comes from the rooibos growing region in South Africa. The GI protection will have major socio-economic benefits for the rooibos communities, and will help the rooibos industry to grow (Sapa, 2014). Obtaining the GI will also help ensure consistency in the high quality rooibos produced, as the GI will contain specific production guidelines to aid the farmers and processors (WIPO, 2014b). Tourism to the rooibos growing areas could also flourish as a result of the GI, due to the marketing of the tea, which will bring money into these small communities. Blends of rooibos will also be more controlled now the GI is in place, due to at least 80 % of the blend needing to be rooibos in order for it to be labelled as such (WIPO, 2014b). With more control over the sale of rooibos, the farmers and processors can now reap more benefits from the unique plant that they work with.

Sensory methods are split into two categories, namely discriminant and descriptive. The methods in each of these categories are different, and are specifically adapted for the distinctive needs of the researcher. Discriminant methods are used when the researcher wishes to distinguish one product from another, for example for market research or product development (Piggott *et al.,* 1998). The discriminant

methods will not be discussed further here, as it is not the method of analysis chosen for this study. Descriptive methods are used when the presence or intensity of certain attributes must be determined (Piggott *et al.,* 1998). This information is also useful when trying to determine the main 'drivers of liking' of a product, and therefore aid in the success of the product on the market (Måge *et al.,* 2012).

A panel of well-trained tasters is usually used to conduct sensory profiling analyses, however, new methods of analysing foodstuffs that do not require a human element have also been developed. Technologies that are now in place, allow for the accurate measurement of human responses to different foodstuffs, e.g. the electronic nose or electronic tongue. These technologies ensure the minimisation of any biasing effects regarding brand identity or any other influencing information. There is, however, evidence that descriptive sensory analysis carried out by a panel of trained judges provide valid and reliable results, especially in terms of sensory attributes, as perceived by the human senses (Lawless & Heymann, 2010).

2.1. Descriptive sensory analysis

The use of descriptive analysis is of the utmost importance when a comprehensive profile of the attributes of a single product, or the comparison between different products is required (Lawless & Heymann, 2010). Descriptive analysis is regularly used in the product development field. The most important characteristic of descriptive analysis is that it allows for the determination of the relationship between the chemical and descriptive sensory profile, of a product or range of products (Murray *et al.,* 2001). Having the knowledge of the desired characteristics of a product, the producers can know where improvements to the n processes or formulae are needed in order to maintain consumer satisfaction (Murray *et al.,* 2001). Descriptive analysis is able to provide the researcher with both qualitative and quantitative data regarding the product (Murray *et al.,* 2001; Carlucci & Monteleone, 2001). The *qualitative* part of descriptive analysis is defined by the descriptive terms or attributes that describe the full sensory profile of the product (Carlucci & Monteleone, 2001). The *quantitative* component is the measure of the intensity or degree to which the attribute is present in the product (Carlucci & Monteleone, 2001). There are many different methods that are incorporated under descriptive analysis, of which the Flavour Profile Method (FPM®), Texture Profile Method (TPM®), Quantitative Descriptive Analysis (QDA® - Descriptive Sensory Analysis) and Free-Choice profiling are just a few (Murray *et al.,* 2001). A well-trained panel is usually required when conducting descriptive analysis research. Training helps to ensure that the panel members are both consistent and reproducible in terms of the results that they produce when analysing the samples (Lawless & Heymann, 2010). The main reason behind training the panel is the development of a list of descriptors for the product in question (Lawless & Heymann, 2010; Murray *et al.,* 2001; Piggott *et al*., 1998). Through training, the panel leader can ensure that all the panellists are able to understand the terms correctly, and confirm that they are all in agreement when it comes to the chosen descriptors (Lawless & Heymann,

2010). A pre-existing list, created by another panel, can be adopted for use in analysing similar products (Murray *et al.,* 2001), which can help reduce the time needed for the creation of a new list of terms.

The terms used to describe the different attributes on the list are called "descriptors". These descriptors must be able to describe the different attributes that are present in the food product and should enable the panel to distinguish clearly between the different sensory attributes (Lawless & Heymann, 2010). If a number of samples are evaluated, intensity scales can be used to help differentiate between the samples, using one scale per attribute.

There are a number of guidelines that should be followed regarding the creation of a list of descriptors. If these guidelines are adhered to, then a list of superior quality and ease-of-use can be created. The descriptors must discriminate between the different attributes in a clear manner (Lawless & Heymann, 2010; Murray *et al.,* 2001). These descriptors should also be non-redundant, meaning that the terms used do not overlap or are not similar in meaning (Lawless & Heymann, 2010). This enables the descriptors to be mutually independent which in turn means that the panel will not be unproductive when analysing the product. Unproductiveness can occur when the panel are unable to distinguish clearly between the different attributes, as a result of the descriptors being unclear (Lawless & Heymann, 2010). An important aspect of the training period is to eliminate as many redundant terms as possible, this, however, is not always possible and reporting discrepancies to the panel leader during the testing phase is essential (Drake & Civille, 2002). If the panel leader and the panel feel that the redundant descriptor should be removed or replaced, it can either be taken off the list or replaced with a better fitting descriptor (Lawless & Heymann, 2010). When a descriptor list ends up being long, with a large number of attributes on it, then the panel and panel leader should make sure that there are no redundancies and that all the terms present are necessary for the accurate evaluation of the product in question (Murray *et al.,* 2001).

Furthermore, to ensure that the descriptors used are clear, the panel must also ensure that the terms used are singular, rather than a combination of several different terms (Lawless & Heymann, 2010). To allow for ease of use and clear understanding, the terms used to describe the attributes should be in their most basic form, and terms more suited for the marketing side of the industry should be avoided (Lawless & Heymann, 2010). An example given by Lawless & Heymann (2010) that was found to lead to confusion, is the description 'creaminess', used to describe a product. It has the effect of possibly being perceived as either the 'fatty-mouthfeel' given by the product or the 'smoothness' of the product. These differences in the interpretation of the attribute can cause problems for the panel members when evaluating the samples. Lawless & Heymann (2010) suggested that instead of using the word 'creaminess' which can be understood in a number of different ways, the terms such as 'fatty mouthfeel' or 'smoothness' should rather be used. These descriptors are simple to understand and lead to no confusion arising with regards to meaning (Lawless & Heymann, 2010). The simplicity of the attributes used, aids the researcher when sourcing reference standards, i.e. actual samples depicting or illustrating specific sensory

attributes. The more complex the attributes the harder it will be to find a reference standard that can mimic the attribute exactly.

Reference standards are used along with the descriptor list to aid the panellists in having a better and clearer understanding of the different attributes discovered in the product (Lawless & Heymann, 2010; Murray *et al.,* 2001). Reference standards can be both qualitative and quantitative in nature (Murray *et al.,* 2001). By having reference standards accompany the descriptor list, the panel are better able to understand the boundaries of each of the given attributes. Therefore it becomes less difficult to understand the terms when analysing the samples (Lawless & Heymann, 2010).

Reference standards usually form part of flavour lexicons, i.e. a document indicating i) a list of descriptors; ii) a verbal description or definition of the respective sensory descriptors; and iii) a physical reference standard illustrating the specific sensory attribute in question. The use of reference standards along with the lexicon will help panels to understand the terms within the lexicon in a much clearer manner (Lawless & Heymann, 2010). Reference standards can also be quantified in terms of intensity scale values. When a sensory lexicon was developed for green tea, a scale of intensity was incorporated for the respective reference standards associated with green tea attributes (Lee & Chambers*,* 2006). This quantitative frame gives the panellists boundaries that they can make use of to compare the sample that they are assessing (Muñoz & Civille, 1998). It is suggested that a reference standard be made to represent the highest intensity of the attribute, so that the panel are able to compare the sample to this reference and evaluate it accordingly (Muñoz & Civille, 1998).

Descriptive sensory analysis (DSA) was originally developed in the 1970's to help correct some of the problems that were encountered through the use of the Flavour Profiling Method (FPM®) (Murray *et al.,* 2001). DSA is a "generic" method used by researchers worldwide, which makes use of a trained panel to analyse samples. During the training phase of DSA, the panellists, usually between 8 and 12 persons, come to a consensus on the language or descriptors that are to be used for describing the product, in other words a sensory lexicon (Drake & Civille, 2002; Lawless & Heymann, 2010). As mentioned previously, this is an important part of the process, and can be time–consuming. Not only are the panel members responsible for determining the descriptors, and therefore the reference standards to be used, they are also in charge of determining the order in which the attributes shall appear on the attribute list that will be used when analysing the product. Once the lexicon is finalised, there are trial evaluations performed to ensure that both the list of the descriptors, and the accompanying reference standards are appropriate and are understood correctly by the panellists. Trial evaluations also allow for determining the most appropriate terms that will be used to describe the product being analysed (Carlucci & Monteleone, 2001). The terms that receive the highest values when scoring the product will be the attributes that are important to the product profile and will be included in the final list (lexicon), as these are seen to be the most relevant to the product (Carlucci & Monteleone, 2001). Determination of these key attributes (primary attributes) is important when trying to understand the 'drivers of consumer liking' of a product

and to effectively compare products or product ranges. It is, however, important to include all the attributes when compiling the profile of a product.

The final testing phase of a product, during DSA, is not performed in a group manner; instead the panellists are separated into isolation taste booths, where they are unable to be influenced by another panellist (Carlucci & Monteleone, 2001; Lawless & Heymann, 2010). An unstructured line scale is usually given to the panellists for each of the attributes being evaluated (Murray *et al.,* 2001; Lawless & Heymann, 2010). The analyses can be performed on a computer using data capturing software packages such as Compusense® *five* (Compusense®, 2012). Using a computerised system enables the data to be collected and analysed with ease. The panellists evaluate each of the different attributes on an individual numerical scale that is anchored (Lee & Chambers, 2007). Usually the scale is anchored with 0 on the lower end and 100 on the higher end. The use of words as anchors is also sometimes used, where "none" would appear on the lower end and "extremely" would appear on the higher end (Lee & Chambers, 2007; Powers, 1984).

There are many parameters that need to be adhered to when analysing specific products. By adhering to these parameters, researchers can ensure that the product is in the correct state to be evaluated, and that there has been no effect from outside factors, that can skew the results. In an experiment on rooibos, primarily to determine the full sensory profile of different batches of commercial rooibos tea, Koch *et al.* (2012) indicated that it was of the utmost importance to keep the infusion warm and at a constant temperature. This ensured that the aroma and flavour attributes within the infusion were not compromised, as noted when the infusion begins to cool. In order to ensure that the temperature was controlled throughout the preparation and evaluation process, the flasks, as well as the mugs used, were pre-heated. During the evaluation process itself, the mugs containing the infusions were kept in scientific water baths at a constant temperature of 65°C. The mugs containing the infusion were also covered with a plastic lid to prevent loss of aroma (Koch, 2011). Knowledge of the product before testing is therefore essential to ensure that the sensory profile, and therefore the results are not compromised in any way.

In a competitive industry it is of the utmost importance that producers know the sensory characteristics of their products. DSA can be used to describe the nature and intensity of the characteristics that may differentiate a product from competitors. DSA is known to give reliable, consistent and detailed information (Cartier *et al*., 2006). There are, however, certain flaws associated with using DSA as the preferred method of analysis. The first uncertainty about the use of DSA is the fact that the panellists have to divide their perceptions into independent sensory dimensions (Cartier *et al*., 2006). DSA can also be quite time-consuming, due to the requirement of both the training and testing phases, and can therefore be regarded as an uneconomical procedure, especially within industry where time is of the essence (Cartier *et al*., 2006; Chollet *et al*., 2011; Lawless & Heymann, 2010). The use of a method that is completely language based, such as DSA, can also lead to problems with comprehension and agreement amongst the panellists. The achievement of the latter, however, is essential to ensure that the testing is

carried out correctly (Chollet *et al*., 2011). In spite of the flaws of DSA, it remains the sensory analysis method of choice, when detailed and precise information on the product profile is needed, or differences between samples or products must quantified. The ability to obtain accurate and reliable quantitative information, as well as a descriptive sensory profile gives this method an advantage over many others (Cartier *et al.*, 2006).

2.2. Statistical analyses of sensory data

Analysis of the data obtained from the sensory analysis tests is essential to the success of the research. The data gathered from the sensory panel are always seen as a three-way data table. This three-way table has the *assessors*, *samples* and *attributes* representing the three different "ways" (Luciano & Næs, 2009) and needs to be taken into account in order to analyse the data correctly. This is especially important when looking at the similarities and differences between both the panellists and the different samples (Luciano & Næs, 2009).

When analysing the data, at least one of these dimensions (ways) is removed prior to the final analysis, due to the averaging of the results over the assessors. This is done to try and simplify the data for easier analysis, but by doing so, it becomes difficult to obtain the information about the individual data amongst the assessors (Dahl *et al.,* 2008). Principal Component Analysis (PCA) and Parallel Factor Analysis (PARAFAC) are methods that have been developed to try and eliminate the aforementioned effect. These statistical methodologies give the researcher information about the relationships amongst the assessors and amongst the samples, but can be complicated to use (Dahl *et al.,* 2008). The PARAFAC model takes into account that the panellists have different sensitivities towards different variables and allows for better handling of the variations in the scale and the variability between the assessors (Bro *et al.,* 2008). PCA, however, is based on the assumption that all the panellists are on the same level of ability, meaning that they are all seen as good and do not exhibit any individual differences (significant) within their individual data (Bro *et al.,* 2008).

The panel can also be judged on the consistency of their results by re-analysis of each of the samples either in duplicate or triplicate. The results gathered from the different analyses, allows the panel leader to determine whether more training is needed or, determine whether the descriptors were easy enough to understand so that the panel could discriminate between the attributes with ease (Lawless & Heymann, 2010). The panel leader needs to be sure that his/her panel can perform at the highest level, especially when creating a sensory lexicon and sensory wheel. In order to determine which of the panellists are not performing, the panel leader can use Principal Component Analysis (PCA) and Cluster Analysis (CA) to analyse the assessors' performances (Sinesio *et al.,* 1993). It has been suggested that the complexity of a product can influence the reliability of the panel used. Research done by Bitnes *et al.* (2009) showed that there was only a minor decline in the reliability of the panellists when there was an increase in the complexity of the product. When analysing the panel there are some methods that outperform others.

The correlation plot, for example, is best used to determine how an individual panellist uses the scale when assessing the samples; this method takes into account each of the attributes. Eggshell plots, however, are best used when attempting to determine the differences between the panellists (Tomic *et al.,* 2007).

After pre-processing the data using the aforementioned methods, the final dataset is usually analysed using Analysis of Variance (ANOVA) or appropriate multivariate techniques such as PCA (Lawless & Heymann, 2010). When using ANOVA the data are usually represented graphically, often using spider diagrams (Murray *et al.,* 2001). ANOVA also helps to give an indication of whether the terms, chosen during the training phase to describe each of the attributes, are discriminating (Wolters & Allchurch, 1994). When the attribute is clearly discriminating amongst the samples then it can be deemed appropriate for the testing phase. ANOVA also allows the panel leader to determine that there are no significant differences amongst the results obtained for the same attribute after replicate testing. If the difference is not significant then the attribute is discriminating (Wolters & Allchurch, 1994). This is an important test to use when creating a sensory lexicon, as it will help with the creation of a clear and discriminating list of attributes.

From the information above it can be concluded that much research needs to be done by the panel leader and researchers before deciding upon an appropriate statistical analysis method to use. The method must be able to analyse the data in a way that will be beneficial to the answers that they seek.

2.3. Sensory lexicon

A sensory lexicon is an important tool within the food industry. A sensory lexicon usually consists of a list of sensory descriptors used to describe the sensory attributes found within a specific product, a description or definition for each of the respective terms, as well as an actual sample or reference standard illustrating the sensory attributes in question.

Sensory lexicons have been used within many industries to help describe and discriminate amongst products within the same product category (Drake & Civille, 2002). They have been developed for honey (Galán-Soldevilla *et al*., 2005), green tea (Lee & Chambers, 2007), almonds (Civille *et al.,* 2010), spices (Lawless *et al.,* 2012), turrón (Vázquez-Araújo *et al.,* 2012), pawpaw pulp (Brannan *et al.,* 2012), etc. Sensory lexicons are also used in industry to profile new products, during product developmental stages or to assist with the quality control of certain products (Drake & Civille, 2002). The usefulness of the lexicon within numerous industries has enabled the development of clear communication between all the role players in each of these industries. The sensory lexicon gives the researchers an organised view of the vocabulary from which they evaluate the product. Without a standardised lexicon researchers are unable to create a sensory wheel, which is an easy-to-use and graphical representation of the descriptors used for describing a particular product (Lawless *et al.,* 2012). The sensory lexicon can be used to define or fully categorise a new or existing product, or a product that has a protected status (Vázquez-Araújo *et al.,* 2012). In the pawpaw industry a sensory lexicon has been used successfully to assist the growers, by aiding them

in the selection of superior varieties for the fresh-markets and other varieties for processing (Duffrin & Pomper, 2006). Within the green tea industry there has been great success in the production and use of the sensory lexicon. The flavour lexicon developed for green tea, is made up of 31 flavour attributes along with reference standards (Lee & Chambers, 2007). Sensory lexicons have also recently been developed in South Africa, for both rooibos and honeybush teas (Koch *et al.,* 2012; Theron *et al*, 2014).

2.3.1 Development of a sensory lexicon

The development of a sensory lexicon follows the same techniques mentioned previously for the descriptive analysis of a food product (Drake & Civille, 2002). To ensure that the terms used are both descriptive and discriminating the samples used should be obtained from a broad and representative sample set (Lawless & Heymann, 2010). This in essence means that the sample-set should contain samples that can cover all the possible attributes available for this particular product. In order to achieve a sample set with a broad range of attributes, the samples must be collected over a large production area or over different production seasons. By having a sample set that is representative of the whole range of attributes, an accurate and concise sensory lexicon can be produced (Lawless & Heymann, 2010). When creating the flavour lexicon for green tea, Lee & Chambers (2007) used 138 green tea samples sourced from nine countries, which allowed for a broad range of attributes to be tapped into when creating the flavour lexicon.

The reference standards chosen to accompany the sensory lexicon can either be qualitative or quantitative, or sometimes both. A *qualitative reference* (allows panellist to correctly understand the written descriptive terms) is required for every term on the lexicon whereas a *quantitative reference* (intensity reference) is usually only applied to specific attributes (Drake & Civille, 2002; Muñoz & Civille, 1998).

The reference standards chosen can be of either food or chemical origin. When creating a lexicon that can be used on a global scale, it is important to ensure that the reference standards chosen are also available globally (Drake & Civille, 2002). This is especially important when working with seasonal and indigenous products. For this reason most panels decide on using chemical reference standards (Drake & Civille, 2002). In some instances the chemical that mimics a particular attribute is often added to a neutral base of the product being analysed. This allows the panellist to understand the attribute as it appears in the product (Noble *et al.,* 1984). An example would be the addition of a small amount of anise extract to a neutral base wine to mimic the liquorice aroma that can be present in certain wines (**Table 3**; Noble *et al.,* 1987). These references are extremely important in the training of a descriptive panel or when conducting day-to-day quality control within industry. It is therefore of the utmost importance that reference standards are of top quality and that they can be used all-year-round on a global scale (Drake & Civille, 2002). Reference standards can be further used to create a flavour kit. A flavour kit allows for a

standardised collection of reference standards to be created and used in the training of panels, graders and industry personnel.

Table 3 Lexicon indicating wine aroma terminology including the reference standards associated with each of the attributes (Noble *et al.,* 1987).

Principal or 1st-tier term	2nd-tier term	3rd-tier term	Base Wine	Reference composition
Floral	Floral	Linalool	W	1 mg (drop) linalool/100 mL white wine
		Orange blossom	W	Crushed orange blossoms
		Rose	W/R	1 mg 2-phenylethanol/150 mL white wine or crushed petals of one rose
		Violet	R	Petals from 10 crushed violets
		Geranium	R	Piece of ripped geranium leaf (10 mm x 10 mm)
Spicy	Spicy	Cloves	W/R	Soak one whole clove for 10-20 min and remove
		Black pepper	R	2-3 grains ground black pepper
		Liquorice, anise	W/R	1 drop anise extract/50 mL wine
Fruity	Citrus	Grapefruit	W	5 mL juice and small piece peel of fresh fruit
		Lemon	W	5 mL juice and small piece peel of fresh fruit
	Berry	Blackberry	R	1-2 crushed fresh or frozen blackberries

2.4. Sensory wheel

The sensory wheel is a graphical representation of the information provided by the sensory lexicon. The use of the sensory wheel has seen great success within many industries, most notably the wine industry. Noble *et al.* (1984) developed the wine aroma wheel to help aid communication between the different members of the wine industry. The wine aroma wheel saw a greatly positive response, not only from members of the industry but also from wine consumers and writers (Noble *et al.,* 1987). The wine aroma wheel designed by Noble *et al.* (1984) is depicted in **Fig. 4**.

Over the years, researchers have been developing sensory wheels as a simpler and more easy-to-use version of the sensory lexicon. This enables all industry personnel to use the information in a way that is both quick and easy to understand, without them having to be sensory scientists. The wine industry is a good example where the use of the sensory wheel benefits the process. By using the wheel cellar workers are able to fully understand the flavour defects that the wine-maker describes to them, without there being any misinterpretation along the way. By using the sensory wheel the defects can be understood much easier and can therefore be prevented (Noble *et al.,* 1984).

Figure 4 Wine aroma wheel developed by Noble *et al.* (1984).

The sensory wheel is usually made up of different tiers, with the outer tier giving a broad description of the attributes. The inner tier contains descriptions that are more detailed and are associated with the outer tier of the wheel. This format was applied in the creation of a sensory lexicon for South African brandies (Jolly & Hattingh, 2001). Descriptors captured in the brandy flavour wheel were further split into positive and negative attributes. The positioning of the attributes within the wheel allows for a clear and rapid understanding (Jolly & Hattingh, 2001). Differentiating between the positive and negative attributes within the sensory wheel, allows for better understanding of the attributes in question. It also allows for use of the wheel for quality control purposes, where it is important to discern between attributes that have either a negative or positive contribution to the product. The terms that are used to describe similar aromas or flavours can be grouped accordingly, mainly to prevent the appearance of redundant terms (Noble *et al.,* 1984). Terms such as "musty" and "mouldy" for rooibos tea, for instance, are often interpreted as the same sensory attribute, and are therefore grouped together as "musty/mouldy", so as to prevent misinterpretation (Koch *et al*., 2012).

Sensory wheels are not only based on the flavour and aroma attributes of a product, but they can also be based on the mouthfeel attributes that present themselves when tasting the product. The development of the mouthfeel wheel by Gawel *et al.* (2000), illustrates this. The mouthfeel wheel was developed with the intention of covering all of the mouthfeel attributes experienced when tasting red wine. The most important attribute present was the *astringent* mouthfeel sensation, which remains in the mouth of the assessor. The topic of astringency is, however, very broad and many opinions exist as to the exact cause of this sensation.

Recently, sensory wheels have been developed for both the rooibos and honeybush industries. These sensory wheels for honeybush and rooibos are depicted in **Fig. 5** and **Fig. 6**, respectively. For the development of a sensory wheel for honeybush, 58 samples of different *Cyclopia* species, collected from producers and from research sample sets, were analysed for primary and secondary sensory attributes using a trained panel (Theron *et al.*, 2014). Thirty-two (32) descriptors, based on flavour, taste and mouthfeel, were used to compile the honeybush sensory lexicon and wheel. The sensory wheel (**Fig. 5**) was made up of nine primary attributes (aroma and flavour), i.e. "floral", "fruity", "plant-like", "nutty", "spicy", "sweet-associated", "chemical", "vegetative" and "earthy". The basic taste modalities and the mouthfeel attribute, astringency, brings the total number of wedges up to ten. These primary attributes were separated into positive and negative classes, and again divided further into more specific secondary terms in the inner tier (Theron, 2012). Although samples of different *Cyclopia* (honeybush) species were analysed, only one, generic sensory lexicon and wheel were assembled for honeybush (Theron, 2012).

In the rooibos sensory wheel developed by Koch *et al.* (2012; **Fig. 6**), the outer tier contains both the positive and negative attributes. These make up the primary descriptors of the sensory attributes. The second tier contains terms that are more detailed descriptors, i.e. a range of attributes describing each of the primary descriptors in the 1st tier. A total of nine primary attributes (aroma and flavour) make up the 1st tier, including; "spicy", "floral", "woody", "fruity", "sweet", "earthy", "micro", "chemical" and "vegetative". These are accompanied by the taste and mouthfeel primary attributes namely "mouthfeel", which is split into positive and negative detailed attributes, and "basic" taste attributes. The inner tier or 2nd tier, contained more detailed descriptions of each of the primary attributes, bringing the total number of terms to 27 (Koch *et al.*, 2012)

2.5. Standardisation of the sensory lexicon and sensory wheel

In order for the sensory lexicon to be ready for use by industry, it needs to be standardised and validated. Similarly, a sensory wheel should also be standardised and any inaccuracies should be identified and rectified. When creating an aroma wheel for wine, a questionnaire, pertaining to the wine terminology, was sent to over 100 individuals that were involved in either the wine industry or wine research (Noble *et al.,* 1984). These individuals sent back their responses and the feedback gathered was used to standardise the wine terminology. After a few years of use within the industry, further appropriate feedback was received and the wine aroma wheel and lexicon were changed accordingly (Noble *et al.,* 1987). With the development of the mouthfeel wheel by Gawel *et al.* (2000) for the wine industry, it was important that industry input was obtained, given that mainly researchers involved in wine analysis developed the wheel. From the feedback gathered the researchers were able to successfully make the appropriate changes to the wheel, allowing both the lexicon and wheel to be standardised and prepared for use within the industry (Gawel *et al.,* 2000).

The rooibos sensory wheel and sensory lexicon were developed after the analyses of 69 rooibos samples harvested from mainly one production area and during one production season. In order to ensure that both the sensory lexicon and sensory wheel are standardised for use within the rooibos industry, sensory profiling tests need to be done on a wider range of samples, i.e. samples from different harvest years and different harvest areas. The use of such a large sample set will ensure that a large degree of sample variation is captured and it will guarantee that as many attributes as can be found within the rooibos tea are gathered. This will allow for a more accurate evaluation of the sensory drivers within this herbal tea (Koch *et al.,* 2012).

A valid, standardised sensory wheel and lexicon for rooibos will be of great benefit to the rooibos industry. Not only will these tools assist researchers with the determination of the final list of "characteristic" attributes present in rooibos tea, in order to obtain a comprehensive description of the sensory profile of the product, but also help standardise current grading methods used within the rooibos industry. Processors and graders will have a better understanding of the different positive and negative attributes found in this herbal tea and thus know what sensory attributes to look for when assessing tea quality. Ultimately, the aim with the development of a valid, standardised sensory wheel and lexicon for rooibos is provide industry with tools that can help to set uniform sensory criteria for the production of rooibos with consistent quality (Koch *et al.,* 2012).
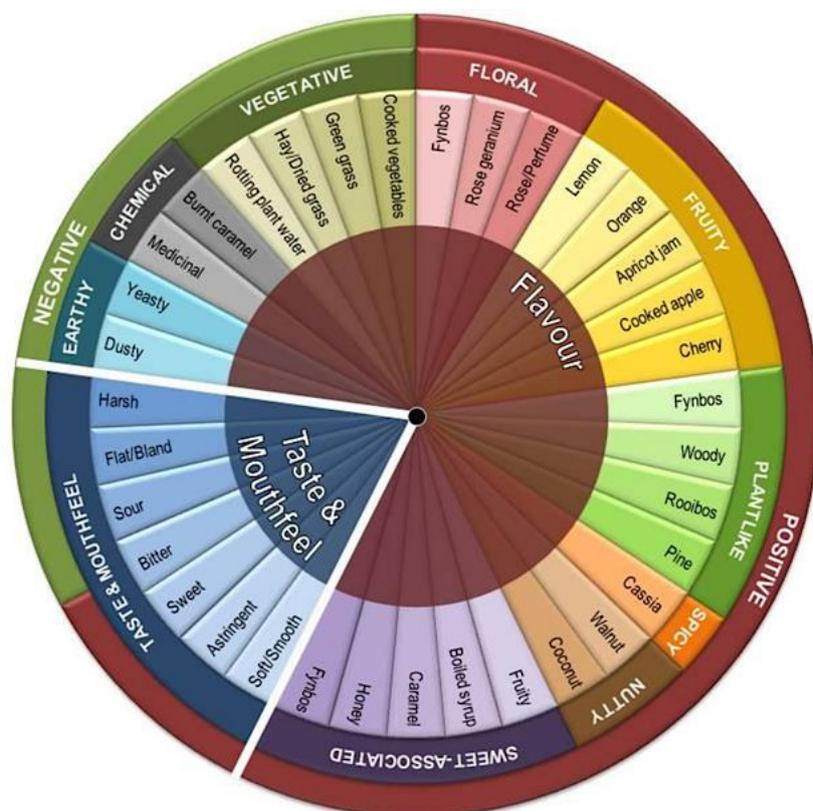
Figure 5 Honeybush sensory wheel comprising 28 flavour and 7 taste and mouthfeel terms that describe the sensory attributes of 58 honeybush tea infusions (Theron, 2012).
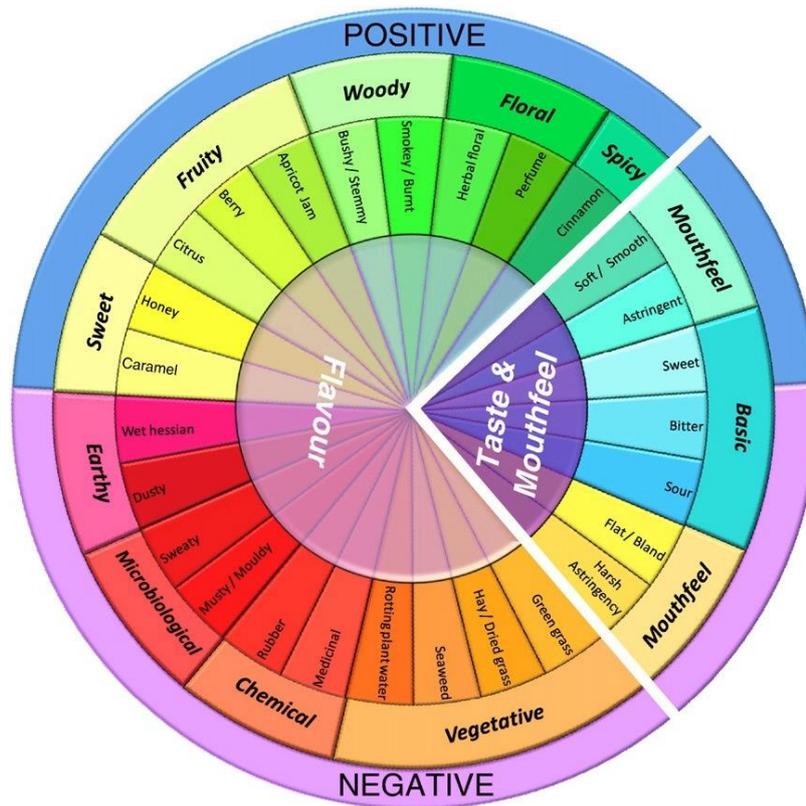
Figure 6 Rooibos sensory wheel including 27 terms, developed after the sensory analysis of 69 rooibos samples (Koch *et al.,* 2012).

2.6. Sorting technique as alternative to DSA

The sorting technique has been suggested as a rapid replacement method for the mapping of sensory data (Cartier *et al*., 2006). Sorting is currently being used in the food industry as a simple and efficient way of gathering important sensory data, mainly to classify samples into different groupings. When using this technique, the panel creates groups of products that are deemed to have similar attributes (Courcoux *et al.,* 2012; Abdi *et al*., 2007; Lelièvre *et al*., 2008). An advantage of this is that, sorting appeals to the cognitive responses that we use in everyday life, and therefore no previous training is required (Valentin, 2012). So far the sorting technique has been used on a large variety of foods such as cheeses (Lawless *et al*., 1995), jellies (Tang & Heymann, 1999) and red wine (Gawel *et al*., 2001).

Sorting can be simply the grouping of samples based on similarities (Chollet *et al*., 2011), at which point the researcher can stop the testing entirely as they will have the results they need. However, in many cases this initial grouping step is followed by a step in which the panellists are asked to describe each of the groups they have created, by indicating the relevant sensory terms (descriptors) (Chollet *et al*., 2011). When sorting samples, the panel are either free to sort as they wish, or are directed to sort the samples based on specific criteria, i.e. sorting according to the sensory quality of the samples or in the answer to a specific question (Valentin, 2012). The first objective when using a directed sorting procedure is to decide whether a trained or untrained panel will be used (Valentin, 2012). When choosing a panel for use with the

sorting technique, the researcher must choose the members according to the question that the researcher wishes to be answered. This is well illustrated by research done on wine. The question posed to a panel comprising wine connoisseurs, wine experts (such as wine makers), novices and trained sensory panellists was "whether the wine in front of the panellist is a ready-to-drink wine or whether it needs to be placed in a cellar and allowed to mature over time?" The results showed that the use of an untrained panel, in this particular case, was in fact not the best decision. The novice panellists did not have an extensive wine knowledge as the wine makers did, who unsurprisingly, produced the best results as they were able to answer the question posed to them correctly (Valentin, 2012).

In recent years a substantial body of research has been done on the use of sorting techniques. These have included research on the stability of the sorting maps produced (Blancher *et al*., 2012), the choice of panel members and panel size (Cartier *et al*., 2006; Blancher *et al*., 2012), the sample set size and type of samples suitable for use with the sorting technique (Cartier *et al*., 2006). The sorting method, as an example of rapid sensory techniques, has also been compared with other rapid techniques (Valentin *et al*., 2012; Varela & Ares, 2012; Nestrud & Lawless, 2009).

Research has also shown that the sorting technique, when carried out by a trained panel, can result in the production of a product map that is similar to the one that is produced when using DSA (Cartier *et al*., 2006). It has been suggested that the sorting procedure should be performed by a trained panel so that a rough description of the product can be established before continuing with DSA training and testing, as this will save time and therefore money (Cartier *et al*., 2006). When untrained panellists carry out sorting, the results obtained are reasonably similar to those obtained by the trained panel. This means that when there are time constraints and a trained panel are unable to be sourced, the researchers can use an untrained panel in their place with the achievement of similar results, although they will not be able to continue DSA after the initial sorting, as a trained panel will therefore be needed. In research untrained panels were found to produce consistent product maps over time, which shows that there may not be the need for repetition when using the sorting technique.

Researchers have, however, begun to question whether or not the sorting maps produced from using the sorting procedure are stable enough (Blancher *et al*., 2012). Although there is the ability to carry out this sensory technique using an untrained panel, Blancher *et al.* (2012) feels that there is a need to assess the reliability of the sorting. The results of the sorting task are deemed reliable and the map stable if the researchers produce the same, or a similar map when conducting the experiment again. This type of testing would have to be done using the same panel, same stimuli and the same directions given to the panellists (Blancher *et al*., 2012). Bootstrapping is a technique developed to test the stability of the sorting map and eliminates the need for the panel to reassess the samples, as the variability of the maps can be simulated from within the data (Blancher *et al*., 2012). This technique draws confidence ellipses around the products on the map, to aid in the interpretation of the results (Dehlholm, *et al*., 2012), if the ellipses are far apart and do not overlap, the products in question can be regarded as different (Blancher *et al*., 2012).

R_V coefficients, introduced by Escoufier in 1973, are commonly applied to the data obtained from DISTATIS, in order to evaluate the similarity between two configurations i.e. replications (Abdi, 2007; Louw, 2014). R_V coefficient values are represented between 0 and 1. It has been found in literature that an R_V coefficient from as low as 0.4 up to 0.7 indicates sufficient similarity between bi-plots (Louw, 2014). When faced with a low RV, however, it is important to understand the complexity of the product being tested. Looking at the bi-plots can give further indication of the reason for the low R_V values, as well as the reasons for the similarities between repetitions (T. Næs, Nofima, Norway, May 2014, personal communication).

It has been suggested that the sorting task be used to select products or samples before undertaking another test, such as DSA or consumer testing (Chollet *et al.*, 2011). This way the initial number of samples to be tested can be decreased to include only the samples deemed to be the most appropriate for the study. The sorting technique seems to be a real contender to take over from DSA for the evaluation of certain products. An advantage of using the sorting method it that it does not require a quantitative scoring system and there is no forced agreement amongst the panellists. Another of the main traits contributing to the popularity of this procedure is the rapidity with which the analyses can be performed, which is much faster than the DSA method (Cartier *et al.*, 2006; Abdi *et al.*, 2007). This rapid analysis does not allow for panel fatigue that can often have a major effect on the results (Cartier *et al.*, 2006). The ability to evaluate a larger samples set than DSA, is another aspect that appeals to both researchers and the food industry, as a simple result can be achieved whilst saving both time and money (Cartier *et al.*, 2006; Abdi *et al.*, 2007).

There are, however, challenges in the sorting method as with most other methods available. The number of samples that can be evaluated accurately at one time has still not been definitively decided upon. Cartier *et al.* (2006) has suggested that the number of samples to be analysed in one batch be limited to between 6 and 15 samples. The fact that all the samples have to be presented at the same time limits the number that can be analysed at once (Chollet *et al.*, 2011). The types of samples and nature of the samples needs to be assessed in detail before using the sorting technique. It has been suggested that if the samples have a delicate and unstable chemical or physical profile then analysis by the sorting technique is not the best choice, due to all the samples having to be presented at the same time. For example a product that needs to remain cool, i.e. ice cream, can become compromised during the analysis. In this case it is up to the researcher to ensure the samples are packaged specially or the conditions surrounding the samples are controlled (Cartier *et al.*, 2006). If this is not possible DSA or another form of analysis will need to be considered. The sorting technique is not recommended when very detailed and precise information is required. The use of the sorting technique gives the researchers qualitative information rather than quantitative information and is not recommended when researchers wish to quantify the differences between products (Cartier *et al.,* 2006; Chollet *et al.*, 2011).

The number of panellists required to carry out the sorting technique and to obtain a stable set of results, is still unknown (Blancher *et al.*, 2012). Chollet *et al.* (2011) demonstrated that sorting of beer

required more assessors (approximately 20 assessors) than DSA (10-15 assessors). Currently there is still debate as to the recommended number of panellists required to achieve a stable result, and may be dependant of the type of product being sorted.

The use of the sorting technique to determine the shelf-life stability of a product is not recommended. The nature of the test set-up, where samples are positioned relative to one another, is not correct, for shelf life testing, as the goal is not to compare samples to one another, but rather to determine if a product has maintained the required attributes for product freshness (Cartier *et al*., 2006). When deciding upon an analysis method, researchers need to take into account the products being analysed and the results they wish to obtain before deciding on the method of analysis. Both DSA and sorting have advantages and disadvantages, which allow them to be suited and unsuited to certain tasks.

The sorting technique is a much faster and time saving alternative to the DSA method when the researcher is looking for information that is not necessarily *extremely* detailed. The way the sorting task can be used to single out samples deemed most appropriate for further testing in detail can be of interest to the rooibos industry. The rapid sorting of rooibos infusions into groups based on the quality of the samples could help graders to rapidly sort production batches. Further analysis of the samples, when more detail is required, can then be performed on the samples that have been grouped into the different groupings according to their similarity in quality or sensory profiles. **Table 4** indicates comparisons between the sorting method and the DSA method.

Table 4 Comparison between DSA and the sorting method.

DSA[a,b,c,d]	Sorting[e,f,g]
Trained panel (10-15)	Trained/Untrained panel (20)
Training required	No training required
Time consuming	Rapid method
High cost	Low cost (rapid)
Quantitative data	No quantitative data
ANOVA and PCA plots	MDS/DISTATIS and CA plot
Complex	Easy to understand
Descriptors provided	Descriptors not provided (own criteria)

[a]Carlucci & Monteleone, 2001; [b]Lawless & Heymann, 2010; [c]Murray *et al.,* 2001; [d]Piggott *et al.,* 1998; [e]Abdi *et al*., 2007; [f]Chollet *et al*., 2011; [g]Cartier *et al*., 2006; [h]Lelièvre *et al*., 2008.

3. AROMA, FLAVOUR AND BASIC TASTES AND MOUTHFEEL

Flavour is defined as the overall sensation experienced due to the interaction of taste, odour and texture upon food consumption (Belitz *et al*., 2009). As the results of interactions between compounds, flavour can be divided into both taste (non volatile compounds) and odours (volatile compounds). Non-volatile compounds interact with the taste buds on the tongue causing the sensation of sweet, sour and bitterness

(Belitz *et al*., 2009). The taste, mouthfeel and aroma characteristics of products, as perceived by the human senses, are what determine their acceptance by consumers. These characteristics can also be an indication of the quality and freshness of the product. For example, the rancidity of a product can be determined through its aroma or taste. Defining individual volatile compounds, as having specific aroma characteristics can be difficult, and requires the use of Gas chromatography-olfactometry (GC-O). The presence of taste and mouthfeel attributes of a plant foodstuff can be attributed to the presence of phenolic compounds (Bravo, 1998), and as such, will be discussed in further detail within this chapter.

3.1. Oral physiology

The taste receptors cells (TRC) that allow perceiving of the five basic modalities, i.e. sweet, sour, bitter, salty and umami (sodium glutamate), are located taste buds within the mouth (Jackson, 2002). Although the epiglottis and soft palate are host to a few taste buds, primarily, the taste buds are located on the tongue (Herness & Gilbertson, 1999). Each taste bud is made up of between 50 and 100 individual cells gathered together to form the papillae structure of the taste bud. The taste cells are long and slender and stretch from the basal lamina to the apical region. Nerve fibres that enter from the base of the taste bud are responsible for transmitting information to the brain (Herness & Gilbertson, 1999).

3.1.1. Bitter taste

The bitter taste of foodstuffs is innate and leads to the triggering of stereotypical behavioural outputs. The presence of a bitter taste usually leads to the rejection of a foodstuff (Meyerhof *et al*., 2010). Bitter taste is also responsible for the protection of animals from the consumption of foodstuffs that are toxic or contain substances that can be harmful (Ley, 2008). Bitter compounds can occur in many different variations from alkaloids such as quinine, the terpenoids, flavonoids and higher peptides, amongst others (Ley, 2008).

Humans are able to perceive a large number of compounds as bitter. Bitter compounds in food are detected by a specific subset of TRC. They are characterised by TASTE 2 receptors (T2R), which are part of the family of G-protein receptors, encoded to detect the bitter taste in foods. To date, 25 bitter receptors have been identified (Meyerhof *et al*., 2010). Within the human mouth there are receptors that only recognise a single or a very small number of compounds, and others that are able to respond to a great number. Bitter compounds have different capacities in which they can stimulate T2R's. Approximately 50% of the compounds investigated by Meyerhof *et al.* (2010), stimulated only the human T2R, while the other half were able to stimulate from between 2 to 15 receptors (Meyerhof *et al*., 2010). Bitter compounds present in foodstuffs are able to activate various T2R's when they appear in differing concentrations (Meyerhof *et al*., 2010). Small structural differences in the chemical structure of some compounds can lead to a change in bitter threshold or the manifestation of different taste attributes (Ley, 2008). Aspalathin and nothofagin, two dihydrochalcones found within rooibos infusions, were found to associate significantly with the "bitter" attribute.

3.1.2. Sweet taste

Sweet receptors allow for the recognition of foods that are nutritionally rich (Zhang *et al.*, 2003). There are a number of sweet molecules such as, sugars, amino acids, proteins and peptides (Temussi, 2007). It has been suggested that the taste receptor cells type 1 (T1R1) and taste receptor cells type 2 (T1R2) coupled proteins, combine with taste receptor cells type 3 (T1R3) forming a hetero-dimeric sweet receptor (Li *et al.*, 2002; Zhang *et al.*, 2003). By changing the combinations of the T1R's, they could function as both sweet and umami taste receptors (Li *et al.*, 2002). T1R2 and T1R3 are able to recognise both natural and synthetic sweeteners individually, whereas a combination of T1R1 and T1R3 can recognise the umami taste of L-glutamate (Li *et al.*, 2002). Modelling studies showed that the sweet taste receptor, T1R2–T1R3, has multiple active sites, explaining why both small and large molecules can interact with the taste receptor to induce a sweet taste (Temussi, 2007). Taste has been found to increase the apparent intensity of different aromas (Valentin *et al.*, 2006). The odour responsible for the enhancement in the taste sensation of a product needs to be perceptually similar to the taste (Small & Prescott, 2005). Djordjevic *et al*. (2004) found that sweet taste was enhanced by the simultaneous presentation of sweet smelling odours such as strawberry aroma. Koch *et al*. (2013) discovered that although PPAG is perceived as bitter when analysed alone (Joubert *et al*., 2013) it correlated significantly with the "sweet" taste attribute within rooibos infusions. Aspalathin, originally thought to impart sweetness to rooibos, was found to have a low and non-significant correlation with the "sweet" attribute during the study (Koch *et al*. 2013).

3.1.3. Sour taste

Organic acids generate the sour taste of foodstuffs, and their presence generally causes people to avoid ingesting the said product or ingesting excessive amounts of the product, due to the unpleasant sour taste. The excessive ingestion of acids can cause unnecessary stress and overloading on the internal mechanisms that are responsible for keeping the acid-base concentration in the body balanced (Chaudhari & Roper, 2010). Foods that have become spoiled over time also become acidic and as a result, the body is conditioned to avoid foods that are sour in taste (Chaudhari & Roper, 2010). Over the years a number of different cell types, mechanisms and receptors have been suggested, as being responsible for the sour taste that arises when eating certain foodstuffs (Chandrashekar *et al.,* 2006). More recently, there have been great developments in this area, with PKD2L1, a member of the TRP ion-channel family, being named as the TRC responsible for sour taste (Chandrashekar *et al.,* 2006; Huang *et al*., 2006). A study done on animals that did not possess the PDK2L1 taste receptor cells indicated that these animals were unable to respond to sour taste stimuli (Huang *et al*., 2006). Koch *et al.* (2013) found no correlations between the "sour" attribute and the phenolic compounds within the rooibos infusions tested.

3.1.4. Astringency

Sensory responses vary greatly between different individuals. The sensation of astringency on the human palate has been defined as a complex group of sensations, involving the drying of the oral surface and the tightening and puckering sensations of the mucosa and the muscles around the mouth (Dinnella *et al.,* 2011; Luck *et al.*, 1994). Astringency has often been associated with the sensations of bitterness and/or sourness (Green, 1993). These associations can lead to confusion about the exact sensory profile or characteristics of the astringency attribute (Green, 1993). Astringency comes from the Latin word *ad stringere*, which means, "to bind". The ability of compounds to bind with and cross-link proteins allows them to be called astringent (Green, 1993). These cross-linking proteins lead to the dehydration of the mouth, which causes the perceptions of dryness and astringency (Guest *et al.*, 2008). Cross-linking of the polypeptides occurs due to the exposure of the phenolic groups on the surface of the polyphenols, which causes aggregation and as a result precipitation and an astringent mouthfeel (Jöbstl *et al.*, 2004). The exact method that results in the cross-linking of the proteins is not yet evident to researchers (Guest *et al.*, 2008). The most likely answer is that these astringents have an effect on the lubricating capacity of the saliva (Green, 1993; Monteleone *et al.*, 2004; Guest *et al.*, 2008). The cross-linking of the nucleoproteins causes them to precipitate out of saliva leaving a less viscous and less lubricating fluid. Proteins that have been precipitated are now free to adhere to the dentition and the mucosa where they form a sticky residue. This sticky residue, coupled with the reduced lubrication within the mouth, increases the coefficient of friction between the mucosal surfaces (Green, 1993). Tannins are water-soluble polyphenolic compounds found in plant foodstuffs, which vary in molecular size and complexity (Chung *et* al., 1998). Tannins tend to bind to proteins, and it is thought that the salivary proteins bind well to tannins. This binding leads to the aggregation of protein-tannin complexes, which can lead to an increase in friction and therefore lead to the astringent mouthfeel feeling (McRae *et al.*, 2011). The increase in the friction can be due to the interaction between the tannins and the oral epithelial proteins or an interaction with the taste receptors, although the exact mechanism responsible for astringency is unknown (McRae *et al.*, 2011). Being able to perceive astringency differs between individuals due to saliva flow, viscosity and protein composition differing amongst individuals, which have been found to have a significant effect on astringency (McRae, *et al.*, 2011). Although there are theories behind the entire mechanism of the workings of astringency, there have not been sufficient details gathered to prove these (Monteleone *et al.*, 2004). Tests done have shown that astringency in the mouth builds up over repeated exposures, and the astringency of wine and beer was found to increase over 3-5 exposures (Green, 1993). The phenolic compound PPAG, present in rooibos infusions, has been linked to astringency when isolated and analysed as a single compound (Joubert *et al.*, 2013). When analysed within rooibos infusions, however, it was linked with a sweet taste, indicating that other phenolic compounds present in the infusion, may have a masking or modulating effect on this compound. Due to the chemical complexity of rooibos, it can be possible that the presence of astringency can be due to interactions between a number of different compounds (Koch, 2011).

4. PREDICTION MODEL FOR ROOIBOS

The main aim in researching and potentially developing a prediction model for rooibos is to predict and determine the chemical drivers responsible for the taste and mouthfeel attributes. Prediction models have become popular in many different branches of the food industry. The ability to predict certain aspects of production or food quality can help speed up processes and save the industry valuable time and money. Prediction models are built using the regression analysis of the gathered data. Regression analysis allows for two data matrices or tables to be related to each other, the purpose of which is to allow for the prediction and interpretation of data (T. Næs, Nofima, Norway, April 2012, personal communication). Regression works on the concept of one variable, being the cause of the changes or outcome of another variable. For example, an independent variable X causes and explains the output of the dependant variable Y, as illustrated **Fig. 8** (T. Næs, Nofima, Norway, April 2012, personal communication).
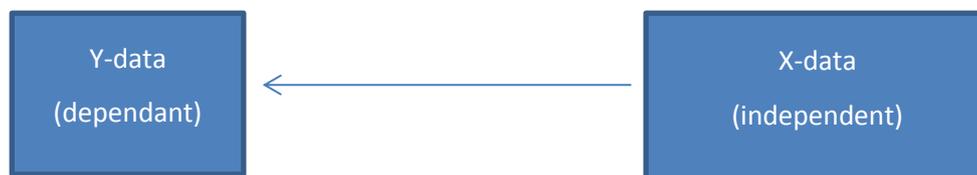
Figure 8 Pictographic description of a prediction model.

Simple linear regression can be used if only one X variable is needed to predict one Y variable. It is, however, not as easy as using one variable to predict another. There are often a number of variables that work together to influence an outcome. In this instance this means that there would be more than one X variable necessary to predict the Y variable, i.e. there are a number of variables that influence the appearance of one characteristic. More often than not this is the case, especially within sensory science, as most products are chemically complex and made up of many different compounds. In these instances, the researcher can use multivariate regression to develop a prediction model and interpret the data. The problem that arises with using multivariate regression, is the unstable regression equations that can occur due to there being X variables with a high correlation. There has, however, been methods developed to eliminate these complications namely, step-wise regression, partial least squares regression (PLS) or principal component regression (PCR). When relating chemistry data to sensory data (DSA), PLS can be used for the analysis (T. Næs, Nofima, Norway, April 2012, personal communication). PLS is used when one wishes to predict a set of dependent variables (e.g. taste and mouthfeel attributes) from a large set of independent variables (e.g. chemical data) (Abdi, 2007).

The use of a prediction model is something that many industries have undertaken in order to help them predict the quality of their product (Careri *et al.,* 1993; Frank & Kowalski, 1984). When carrying out production, the manufacturer wants to ensure consistency in the quality of the product being produced. By ensuring a high quality product is produced consistently, customer loyalty will increase (Van Boekel, 2008). A prediction model gives the ability to address certain aspects within the manufacturing process, and

determines the role they play in the quality of the product. An example is the taste quality of a rooibos infusion. Rooibos containing a high intensity of bitter, sour and astringency, seen as negative attributes, will be of a low quality. Therefore being able to determine the cause of the high intensities of these attributes is of importance to the industry, as it allows for the determination of rooibos quality as well as possibly aid in finding reasons as to why these intensities are so high.

4.1. Development of a prediction model

Considering rooibos tea, it is important to try and develop a prediction model that can predict the quality (taste and mouthfeel) of the infusion, based on a rapid method of analysis. Such a model could be valuable to the industry, as it would clarify the contribution of non-volatile constituents (phenolic compounds) to taste and astringency. This means that the industry can have a standardised method for quality evaluations, which could save time and money, both of which are valuable to industry, in the long term. From the development of a prediction model for rooibos, it is hoped that there are prominent chemical compounds, that when present, indicate that specific attributes will be present as a result. Due to the complexity of the chemical make-up of rooibos, it will be unlikely that there is only one chemical compound responsible for a specific attribute. This is where complications may arise, when developing the model, as the final sensory attributes present are not due to the presence of only one chemical compound but rather due to an interaction between a number of different compounds.

The prediction model will be an important tool in helping to determine the effects that specific phenolic compounds may have on the taste and mouthfeel attributes found within rooibos infusions. Researchers will be able to determine if there are any chemical drivers for specific sensory attributes (taste and mouthfeel). Some phenolic compounds have been found to have an effect on the taste or mouthfeel of the tea. Koch (2011) found that the flavonoid rutin, also known as quercetin-3-*O*-rutinoside, was responsible for an astringent mouthfeel when consuming rooibos infusions. Sweetness and bitterness, were found to associate with a number of non-volatile compounds including aspalathin (bitter) and PPAG (sweet) (Koch, 2011).

The astringent attribute is not necessarily perceived as a negative attribute within all food products. Black tea, for example, is recognisable due to its astringency (Koch *et al.,* 2012). Koch (2011) determined that quercetin and aspalathin, in rooibos infusions, associated with astringency, these associations were, however, not significant. It is hoped that clearer and more defined relationships between the phenolic compounds and the sensory attribute may be determined.

4.2. Success of a prediction model in other industries

The use of regression modelling to predict quality has been seen in a number of industries. The development of a prediction model does, however, become more difficult the higher the number of parameters. Model building has been successful when applied to Italian-type dry-cured ham (Careri *et al.,*

1993). During the study done on these hams, five regression models were developed which showed the relationships between the taste and odour components, and the compositional and non-volatile compounds, found in the samples. Both sensory and chemical tests were done on the Italian-type dry-cured hams in order to obtain the data necessary to determine the relationships (Careri *et al.,* 1993). The data gathered was analysed using the principles of Generalised Procrustes Analysis (GPA) and Partial Least Squares analysis (PLS).

Within the wine industry the use of PLS regression led to the determination of wine quality and geographic origin. The PLS method allowed both the individual and overall sensory scores to be predicted from the chemical composition of the wine (Frank & Kowalski, 1984). The study showed that the chemical data obtained, contained sufficient information to aid in the prediction of the geographic origin of the wine. The individual sensory parameters, as well as the overall quality of the wines, were also determined. From the data gathered in this study, it was suggested that a model being developed should be able to predict, not only the overall quality of a product, but also be able to predict the individual parameters (Frank & Kowalski, 1984). This study highlighted the importance of the PLS method in to development of a prediction model. Being able to make use of several blocks of data containing multiple measurements, and extracting the important information from this, is what the PLS method does well. From this stand point it can be used in the prediction of many response variables simultaneously (Frank & Kowalski, 1984).

Principal Component Analysis (PCA) classified Chinese tea samples according to the origin of the tea, and its quality (Liu *et al.,* 1987). In this study PCA and cluster analysis were applied to the data collected from the chemical analysis of the samples. The chemical tests were done to determine the content of cellulose, hemicellulose, lignin, polyphenols, caffeine and amino acids of the tea samples. This was done to discover the reason behind the sensory differences between the teas, and to determine how to recognise teas from different areas. The main idea behind doing these tests was to determine the relationship between the chemical composition of the tea and its subsequent quality (Liu *et al.,* 1987). The quality of the tea was graded by tea experts and based on the taste of the infusion; therefore the quality was based on non-volatile compounds. The use of hierarchal clustering allowed the researchers to discover that the information on the quality and the category of the tea is present in the results of the chemical analysis. Once the data were analysed and placed in the hierarchal order it was further analysed using PCA. By using the PCA plots, the researchers were able to distinguish between the different categories of the tea, as well as the different varieties within the categories (Liu *et al.,* 1987).

Numerous quality prediction methods have been developed using solely chemical information from specially designed tests. Some of these tests are based on the use of electronic machinery that analyses the samples and does not use a trained human panel for results (Chen *et al*., 2008; Ivarsson *et al*., 2001; Laureati *et al*., 2010; Tudu *et al*., 2009; Bhondekar *et al*., 2010; Legin *et al*., 1997; Dutta *et al*., 2003; Bhuyan & Borah, 2001; Hall *et al*., 1988). These tests include capillary electrophoresis, electronic tongue and lipid

membrane taste sensors (Liang *et al.,* 2005). The use of these non-human tests has not been used widely within commercial tea production (Liang *et al.,* 2005).

Their value for prediction of rooibos quality has not been investigated to date, however, greater understanding of the factors (and chemical constituents), contributing to quality of rooibos is required before such research could be attempted

5. SUMMARY

Rooibos tea has grown in popularity not only globally but also locally. The increase in popularity can be attributed to it being chosen as the healthier choose by consumers due to this herbal tea being both low in tannins and caffeine-free. The expanding rooibos export market emphasises the importance of ensuring that both the consumers and bulk purchasers of rooibos have a standardised sensory terminology, which they can refer to when purchasing or working with rooibos.

With no concrete and encompassing regulations for the quality of rooibos, there has been a lack of standardised specifications for determining the quality of the tea. This means that currently, tea processors are using their own methods and following their own specifications when it comes to determining the quality, and therefore the final grade of rooibos tea. The methods and specifications for the different processors may not be flawed, but due to lack of standardisation between processors, teas of differing quality but equal grade are marketed. These differences can lead to confusion amongst consumers and can eventually lead to customer dissatisfaction. Current regulations state that rooibos "shall have the clean, characteristic taste and aroma and clear, distinctive colour of rooibos" (Anon., 2002). There are no further descriptors on as to what the term "characteristic" incudes. Koch *et al*. (2012) determined, through the use of sensory analysis of rooibos infusions, that there are attributes that lend themselves to creating a "characteristic" profile for rooibos tea. This characteristic profile is described as "honey, woody and fynbos-floral aroma with a slightly sweet taste and a subtle astringent mouth feel" (Koch *et al*., 2012). These characteristics, along with the red-brick colour of the infusion of rooibos, are what make rooibos so unique. It is therefore important to ensure that quality standards are adhered to, so that consumers are able to consume a high quality rooibos tea each time they purchase this unique beverage.

The rooibos sensory wheel and sensory lexicon developed by Koch *et al.* (2012), needs to be standardised and validated, as samples were collected from only one production area and year. More research needs to be done on a larger data set, so as to allow all possible variation within rooibos to be included. Variation can be present in the samples due to production season, production area, etc., and these need to be taken into account in order to validate the result.

The development of a prediction model for the rooibos industry will allow for the prediction of rooibos quality, based on the intensity of the taste and mouthfeel attributes, which are known to have a major effect on the overall quality grade of the infusion. By determining the phenolic compounds responsible for these attributes, better insight into the relationships between phenolic compounds may be determined. Processing procedures that may have an effect on the concentration of these phenolic compounds may then be controlled in a stricter manner, so as to ensure the production of high quality rooibos infusions.

 The use of rapid methods for determining sensory profiles of products is gaining popularity within the food industry (Varela & Ares, 2012). Sorting is a popular method used for obtaining non-quantitative

information about food products. The sorting method can be used within the rooibos industry as a rapid way in which to grade rooibos batches based on the aroma, flavour, taste and mouthfeel quality of these infusions. The use of rapid methods, as a grading tool, will greatly benefit small-scale farmers, who do not have access to the knowledge and tools that the larger processors do. In this way they can also ensure consistency in the quality of tea produced, and can be competitive on the rooibos tea market.

The use of quality control tools will be of great benefit to the rooibos industry. Using the tools will allow for consistency of quality between samples of the same quality grade to be achieved. It also allows for greater understanding and communication between different role-players within the rooibos industry, therefore improving and standardising aspects of the grading process.

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CHAPTER 3

Sensory profile of rooibos originating from the Northern Cape and Western Cape and the development of quality control tools

TABLE OF CONTENTS

ABSTRACT

Aspalathus linearis is cultivated in the Western Cape and Northern Cape provinces of South Africa for the production of rooibos tea. A total of 230 samples, spanning the two production areas, three production seasons and four quality grades, were gathered to ensure that all possible variations within rooibos were included in the analysis. The samples were analysed using descriptive sensory analysis (DSA) to evaluate a total of 38 aroma, flavour, taste and mouthfeel attributes. Results confirmed the primary characteristic profile of rooibos, previously profiled as containing "rooibos-woody", "fynbos-floral" and "honey" aroma and flavour attributes, with a slightly "sweet" taste and an "astringent" mouthfeel. In the present study the "hay/dried grass" note appeared in more than 90% of the rooibos samples and therefore forms part of the "characteristic" profile of this unique herbal tea. Another distinct profile, showing "fruity-sweet", "caramel" and "apricot" notes was evident. Although not as common as the primary characteristic profile of rooibos, this secondary characteristic profile appeared to be present in samples from both production areas. No clear differences between production areas were seen, but production seasons produced clustering. High-quality (high grade) samples associated with positive attributes such as those responsible for the "characteristic" rooibos profiles, whereas the low-quality (low grade) samples largely associated with the negative attributes, including "green" and "musty/mouldy" notes. Based on the comprehensive data set, a revised sensory wheel and sensory lexicon could be developed. These quality control tools can be used by industry to aid in the grading and marketing of rooibos tea.

1. INTRODUCTION

The herbal tea made from the endemic South African fynbos plant, *Aspalathus linearis* (Burm. F) Dahlg. (Fabaceae), is more commonly known as rooibos tea, or on some international markets, as "redbush" or "red" tea. According to the 2013 export data, supplied by the South African Rooibos Council (SARC), Germany (45%) dominated the market, followed by the United Kingdom (12%), Japan (11%), the Netherlands (9%), and the United States of America (7%). The remaining 16% were exported to 33 countries, including India, Sri Lanka and China.

With the continued growth in the popularity of rooibos on the international market, the need to understand and to profile the aroma and flavour of the tea has become essential to ensure effective quality control and to exploit niche markets. Koch *et al.* (2012) developed a sensory lexicon and wheel to address this need, as these sensory tools can help to standardise terminology and improve the understanding of rooibos quality, i.e. recognise the attributes responsible for rooibos quality. In order to develop the sensory wheel and lexicon, samples of rooibos produced in the Western Cape province of South Africa were sourced during the 2009 production season (Koch *et al.*, 2012).

The wheel developed by Koch *et al.* (2012) contained 27 flavour, taste and mouthfeel attributes, including both positive and negative attributes. The most frequently occurring of these (N = 14) were

chosen for inclusion in the sensory lexicon. The lexicon included a definition of each of the attributes, accompanied by a list of reference standards (Koch *et al.,* 2012). Descriptive sensory analysis (DSA) showed that the sensory attributes responsible for the "characteristic" rooibos profile were "honey", "rooibos-woody" and "fynbos-floral" notes (aroma and flavour) coupled with a "sweet" taste and an "astringent" mouthfeel. These attributes were found to be present in a majority, if not all, of the rooibos samples tested during that time. Samples could be differentiated based on quality, as defined by the grading system, used by the major rooibos tea marketing company. Low grade (low-quality) and high-grade (high-quality) samples could be differentiated, but not those of in-between quality. Low-grade samples were found to have prominent "green", "hay-like" and "musty" aroma and flavour notes with a "bitter" or "sour" taste. The high-grade samples, however, had "honey", "woody", "floral" and "caramel" aroma and flavour notes with a slightly "sweet" taste (Koch *et al.*, 2012).

Anecdotal evidence suggests that rooibos tea quality depends on factors such as: the presence of young growth, the age of the bush, the cultivation area and climatic conditions, in addition to processing (Joubert, 1994). The overall quality of rooibos can therefore vary from year to year, as is observed in the varying number of production batches receiving a high-quality grading each year (J. Basson, Rooibos Ltd., Clanwilliam, South Africa, April 2012, personal communication). The effect of production area and production season on the sensory profile of rooibos has not yet been defined scientifically.

The aims of this study were, to confirm the "characteristic" profile of rooibos tea, and to determine whether production area and season have an effect on this profile. To achieve these aims samples were procured from two production areas, i.e. Western Cape and Northern Cape, South Africa, as well as from three production seasons, and were analysed using DSA. Determination of the "characteristic" and distinct rooibos profiles allowed for the validation of the rooibos sensory wheel and lexicon.

2. MATERIALS AND METHODS

2.1. Rooibos samples

A total of 230 rooibos samples from individual production batches were sourced from April to June in 2011, 2012 and 2013, from both the Western and Northern Cape provinces of South Africa. On arrival at the laboratory, the samples of each production season were assigned a unique code reflecting the grade and production area, where NC and WC indicate Northern Cape and Western Cape, respectively. The fermented, unrefined and unpasteurised samples ranged from 500 g to 1 kg. The samples represented four quality grades, A, B, C and D, as graded by the two processing facilities. The number of samples sourced per grade, area and season are summarised in **Table 1**. A complete list of the entire set of rooibos samples, including the grading details, is provided in **Addendum A (Table A1 & Table A2)**.

Each sample (400 g) was sieved for 1.5 min at 190 rpm, to remove any dust (< 40 mesh) or coarse material (> 10 mesh), using a SMC mini-sifter (JM Quality Services, Cape Town, South Africa). The sieved

samples were stored in sealed glass-jars at room temperature during the analysis; thereafter the samples were moved into a cold storage area (4°C).

Similar to the study of Koch *et al.* (2012) a control sample was used during both the training and testing phases of the DSA. The control sample, initially blended in 2009, was created through the blending of six B grade rooibos samples, representing different production batches and originating from the Western Cape (Koch *et al.,* 2012). This control sample was used continuously over the period from 2009 − 2013. This was done in order to maintain consistency, as it served as a fixed point to which all other rooibos samples could be compared, thereby allowing the panellists to calibrate their sensory perception at the start of each training and testing session.

2.2. Sample preparation

The rooibos infusions were prepared as described by Koch *et al.* (2012) by pouring freshly boiled, distilled water (1000 g) onto 19.3 g of unpasteurised rooibos leaves. After the infusion was stirred for approximately 5 s, it was covered and left to infuse for 5 min, where after it was strained through a fine mesh tea strainer into a pre–warmed thermos flask (1000 mL). Approximately 100 mL of infusion was then poured into each of the required number of white porcelain cups, which were covered with a plastic lid to ensure that no evaporation or loss of volatiles took place. Each rooibos sample was prepared three times per day, so that there was a "fresh" infusion of each of the samples for each of the replicates.

During preparation of the rooibos infusions it was essential that the temperature of the infusions was kept as constant as possible at all times. Similarly to Koch *et al.* (2012), a number of different actions were taken to ensure the temperature of the infusions was at no point compromised. Stainless steel thermos flasks, used to aid in the maintenance of the constant temperature of the infusions, were pre-heated prior to the addition of the infusion. The white porcelain mugs were also pre-heated (in an industrial convection oven (Hobart, USA) at 70°C) prior to the addition of the infusion. A consistent infusion temperature was not only essential during the preparation stage but also during the training and testing phases, so as not to compromise the sensory quality and attributes of the infusions. This was achieved by placing the filled cups in scientific water-baths (SMC, Cape Town, South Africa) set at 65°C, where they were kept throughout the analysis period.

2.3. Descriptive sensory analysis

2.3.1 Panel training

The panellists were chosen according to availability and their experience in sensory analysis. The majority of the panellists took part in the previous rooibos study by Koch *et al.* (2012). A total of 10 female panellists participated in the study in 2011 and 2012 and 9 female panellists in 2013.

Training of the panel was done in accordance with the consensus method set out by Lawless & Heymann (2010), and Koch *et al.* (2012). At the start of the training phase, the panellists were informed of

the objectives and outlines of the current study, and were re-familiarised with the training methods and protocol involved in DSA. When analysing a sample, the panel was instructed to remove the sample from the water-bath, remove the plastic lid and swirl the infusion several times before analysing the aroma. The taste and mouthfeel attributes were analysed by directing the panellists to suck up a mouthful of the infusion off a rounded tablespoon, as opposed to sipping the tea as one usually would. Tea is sucked up into the mouth so that the liquid is drawn into the back of the mouth, whilst breathing in. This action draws the tea aroma up to the olfactory nerve located in the nose, allowing one to identify the aromas present in the tea. The aromas within tea are associated with the volatile compounds. With this procedure the volatile compounds are therefore picked up by the olfactory receptors, unlike the non-volatile compounds, which give rise to the taste and mouthfeel attributes (Owour, 2003). The panel was directed to swallow not expectorate the infusion, and to cleanse their palates between samples with water biscuits and distilled water.

The control sample was used to calibrate the sensory perception of the panel at the start of each training and testing session. This sample embodied a rooibos with the perfect balance between positive and negative attributes and represented a "characteristic" cup of rooibos tea. During the testing phase the control sample was not analysed as it was merely used as a frame of reference.

Other rooibos reference standards were also used during the training phase to familiarise judges with the sensory attributes in question. Rooibos samples exhibiting a high intensity of a specific attribute were chosen as reference standards.

The descriptors and definitions for each of the attributes were adjusted, where necessary, during the training phase. These changes ensured that the definitions used were both clear and concise. Any attributes found not to be important to the rooibos profile, or not frequently present in the samples, were removed from the initial list. The final list of attributes used in the DSA training and testing periods is summarised in **Table 2**.

2.3.2 Analysis of rooibos infusions

Once the training of the panel was completed, the panellists moved on to the testing phase, which entailed scoring the intensities of the attributes of each sample. This was done using the Compusense® *five* program (Compusense, Guelph, Canada). The panellists rated the intensities of 17 aroma attributes, 17 flavour attributes, 3 taste attributes and 1 mouthfeel attribute, of the rooibos samples being tested. Rating of the intensity of each attribute is conducted on a unstructured line-scale, where the panellist gives each attribute an intensity rating of between 0 (not detectable) and 100 (extremely high intensity). The testing took place over a 15-day period, during each year, with seven samples being tested in triplicate each day. Between each testing session, the panel was required to take a 10-min break; this allowed for the panel to rest and limited panel fatigue.

The samples, labelled with 3-digit codes for blind testing, were presented to each of the panellists in water-baths. The presentation order was randomised, and specific to each of the panellists. The control sample was, however, labelled the same for each panellist and identified as such, so that it would serve as a fixed point.

2.4. Statistical procedures

A complete block design was used and the data were analysed using various appropriate statistical methods. The performance of the panellists was determined using PanelCheck software (Version 4.1.0, Nofima Mat, Norway). Reliability of the panel was determined from the data gathered during the testing period, which was subjected to test-retest analysis of variance (ANOVA), using SAS® software version 9.2 (SAS Institute, Cary, NC, USA). The normality of the residuals was determined using the Shapiro-Wilk test (Shapiro & Wilk, 1965). When necessary, outliers were identified and removed until the data were normally distributed. Least significant difference (LSD; p = 0.05) was calculated to determine if there were significant differences between the attributes based on the grade of the samples, as well as the season and area of production. XLSTAT (Version 2014.01.02, Addinsoft, France) was used to create principal component analysis (PCA) plots, as well as discriminant analysis (DA) plots to visualise the relationship within or between the samples based on different quality grades, production areas and seasons, as well as between the respective attributes.

3. RESULTS

3.1. Determination of the differences between rooibos from the Western Cape and Northern Cape production areas based on differing production seasons and sensory profiles

Fig. 1 illustrates the association between the all rooibos samples and the sensory attributes. The DA plot (**Fig.1 (a)**) depicts the samples as they are plotted in relation to one another based on the sensory profile that each of the samples portrays, with PC 1 (Factor 1) explaining 81.5% of the variance and PC 2 18.5% of the variance. According to **Fig. 1(a)** there is a definite split between the samples with the Western Cape 2011 (WC11) and Northern Cape 2011 (NC11) samples grouping together to the right-hand side of the DA plot, across the PC 1 (Group 1). The Western Cape 2012 (WC12) and Northern Cape 2012 (NC12) samples (Group 2) are situated close together, however, they also lie in a seemingly close association with the Western Cape 2013 (WC13) and Northern Cape 2013(NC13) samples (Group 3) to the left side of PC 1. Although both Group 2 and Group 3 lie close to one another on PC 1, there is some split on PC 2 with the 2013 samples being scattered across the top part of PC 2, and the 2012 and 2011 samples lying across PC 2 in the lower left and right quadrants, respectively. Within each of these groupings it is clear that there is no distinct split between the production areas, but that the split is rather according to the production season.

When the DA plot is viewed in combination with the PCA plot (**Fig. 1(b)**), associations between the samples and the sensory attributes can be determined. The WC12, NC12, WC13 and NC13 samples lie in close association with "fynbos-floral" aroma, as well as the "bitter" taste attribute, which was found to be more prominent in samples of these years than for the WC11 and NC11 samples. The WC11 and NC11 samples, however, were found to have higher intensities of the "rooibos-woody" and "honey" aromas, as well as "sweet" taste. These mean values are summarised in **Table 3** and **Table 4**.

In order to determine if there are any defining sensory characteristic differences between samples from different production areas within the same production season, as well as determine if area plays a role in the occurrence of the primary and secondary characteristic profiles, further analysis was done.

For each of the production areas and seasons, a scatterplot was drawn up indicating the intensities of the sensory attributes in conjunction with the percentage occurrence of the attributes in the respective sample sets. The scatter plots were compared according to the area and season groupings that were prominently seen in **Fig. 1(a)**, i.e. WC12 and NC12. Due to the separation based on the different production seasons, it was important to compare the results from the two areas within each production season, to try further narrow down any differences between the production areas.

Fig. 2 illustrates the percentage of samples that exhibit each of the sensory attributes (y-axis) and the average intensity of these attributes in the respective samples (x-axis), for the rooibos samples collected during the 2011 production season from the Western Cape area. "Rooibos-woody" aroma and flavour (mean intensity of approx. 40), "fynbos-floral" aroma and flavour (mean intensity of more than 15), "hay/dried grass" aroma and flavour (mean intensity of more than 10), "honey" aroma (mean intensity = 25), "sweet" taste (mean intensity = 24) and "astringent" mouthfeel (mean intensity = 24) attributes were found in 100% of these rooibos infusions. "Fruity-sweet" aroma, present in 96% of the samples, had a mean intensity of 18 out of 100. **Fig. 3** depicts the samples from the 2011 production season for the Northern Cape. Here again it can be noted that the majority of the attributes present in 100% of the WC11 samples were indeed present in 100% of the NC11 samples, with the exception of the "hay/dried grass" aroma, which was present in only 94% of the samples. The "fruity-sweet" aroma (mean intensity < 15), for this particular production season, was found to be present in 100% of the NC11 samples. The attributes present in 100% of the NC11 samples, are present in 100% of the WC11, and have similar intensities to that of WC11 with "rooibos-woody" notes (aroma and flavour) (mean intensities > 41), "fynbos-floral" notes (mean intensities > 17), "honey" aroma (mean intensity = 23), "sweet" taste (mean intensity = 24) and "astringent" mouthfeel (mean intensity = 24). "Hay/dried grass" flavour (mean intensity = 10), although regarded as a negative attribute, was present in a low intensity in 100% of the samples. The "hay/dried grass" aroma (mean intensity = 12), however, was present in 94% of the samples tested. The sub-profiles for both WC11 and NC11 do not contain many attributes in common, although "green" aroma is seen in more than 40% of the samples for both areas.

In 2012 the number of attributes tested increased from the 24 attributes tested in 2011, to 38 attributes. **Fig. 4** illustrates these attributes according to the intensity and occurrence values for the WC12 samples. All of the samples contained the "rooibos-woody" notes (mean intensities > 33), followed by "fynbos-floral" notes (mean intensities > 23) and "astringent" mouthfeel (mean intensity = 23). The remaining attributes found in 100% of the samples were present in lower intensities; these included "sweet" taste (mean intensity = 20), followed by "honey" aroma (mean intensity = 17) and "hay/dried grass" notes (mean intensities of > 13). A "fruity-sweet" aroma (mean intensity = 9) was present in more than 80% of the samples. The negatively associated "green" aroma (mean intensity = 6) was present in more than 55% of the samples. "Caramel" aroma (mean intensity = 8) was detected in more than 60 % of the samples. **Fig. 5** depicts the attributes present in 100% of the NC12 samples; these include 8 of the total 38 attributes tested. The "rooibos-woody" notes again were detected in 100% of the samples, and in the highest intensities of all the attributes (> 32). The other attributes present in 100% of the samples were, in decreasing intensity; "fynbos-floral" notes (mean intensities of < 24), "astringent" mouthfeel (mean intensity = 23), "sweet" taste (mean intensity = 20), "honey" aroma (mean intensity = 16) and "hay/dried grass" flavour (mean intensity = 14). Two attributes, one of them found in 100% of the WC12 samples, were found to be present in 98% of the NC12 samples. These include the "fruity-sweet" (mean intensity = 9) and "hay/dried grass" (mean intensity = 14) aromas. Both the WC12 and NC12 samples contain the above-mentioned attributes in similar intensities (**Table 3** and **Table 4**). The sub-profiles of the 2012 samples of both areas contain similar attributes, found to be present in more than 40% of the rooibos samples, but at differing intensities. The sub-profiles both include "caramel", "green" and "apricot" aromas, as well as the "fruity-sweet" flavour.

Results of the WC13 samples are depicted in **Fig. 6**. "Rooibos-woody" aroma (mean intensity = 37) and flavour (mean intensity = 35), along with the "fynbos-floral" and "hay/dried grass" notes with mean intensities of more than 25 and 11, respectively, as well as "honey" aroma (mean intensity = 19), "sweet" taste (mean intensity = 21) and "astringent" mouthfeel (mean intensity = 26) are present in 100% of the samples. The sub-profile contains "caramel" aroma (mean intensity = 10) detected in 88% of the samples, "fruity-sweet" aroma (mean intensity = 5) in 55% of the samples and "green" aroma (mean intensity < 5) in less than 40% of the samples. The profile for the NC13 rooibos samples is illustrated in **Fig. 7** where "rooibos-woody" notes were again present at the highest mean intensity score of 34. The other attributes in 100% of the samples were "fynbos-floral" notes (mean intensity of > 20), "astringent" mouthfeel (mean intensity = 26), "sweet" taste (mean intensity = 21) and "honey" aroma (mean intensity = 18). The "hay/dried grass" notes, were again present in 100% of the 2013 rooibos samples. The "caramel", "green" and "fruity-sweet" aromas make up the sub-profile of the NC13 samples, and are present in more than 50% of the samples. Again, similar intensities were seen for the attributes in WC13 and NC13 samples, both groups with the exclusion of the "apricot" aroma from the sub-profile.

As indicated in the above-mentioned results, the rooibos infusions of both production areas appear to give rise to two sensory profiles, i.e. a "rooibos-woody", "fynbos-floral" and "honey" profile, and secondary a "caramel", "fruity-sweet", and "apricot" profile. Significant correlations (p < 0.05) were found for the attributes within each of the profile groupings for 2011-2013. It must be noted that during the 2011 testing period a number of attributes were not included in the sensory analyses. However, the Northern Cape results from 2012-2013 show a significant correlation (p < 0.05) between the "rooibos-woody" and "fynbos-floral" aromas (r = 0.578) and "rooibos-woody" and "honey" aromas (r = 0.478). The "caramel" and "fruity-sweet" aromas were also significantly (p < 0.05) and moderately associated (r = 0.649). The "apricot" aroma was found to correlate strongly and significantly (p < 0.05) with the "fruity-sweet" aroma (r = 0.848) and with the "caramel" aroma (r = 0.771). Furthermore, "cooked apple" aroma correlated strongly and significantly (p < 0.05) with "spicy" aroma (r = 0.880). The Western Cape samples from 2012-2013 saw similar associations although the correlation coefficients are lower in value.   "Fynbos-floral" aroma correlated significantly (p < 0.05) with "rooibos-woody" aroma (r = 0.464) and "honey" aroma (r = 0.444), while "fruity-sweet" correlated significantly (p < 0.05) and moderately with "caramel" aroma (r = 0.575) and strongly with "apricot" aroma (r = 0.852).   Another significant correlation (p < 0.05) was between "apricot" aroma and "caramel" aroma, which were moderately correlated (r = 0.600).

Table 5 depicts the percentage of samples from both production regions, from the 2012 and 2013 production seasons that fits into either the primary characteristic profile or the secondary characteristic profile. The primary characteristic rooibos profile is, made up of attributes, previously found to be present in 100% of rooibos samples, although without the presence of higher than average intensities for the negative attributes. The attributes prominent in this profile include the "rooibos-woody", "fynbos-floral" and "honey" aromas in high intensities, coupled to low intensities of the other positive and negative attributes.   The secondary characteristic profile includes the "caramel", "fruity-sweet" and "apricot" aromas, again in higher than average intensities, with low negative attribute intensities. The WC11 and NC11 samples were not included in these groupings, as they were not tested for all the attributes present and therefore would not give accurate profile results. Overall, 57% of the 2012 and 2013 samples from the Western Cape fitted into the primary characteristic profile, whereas the Northern Cape samples represented 61%. The secondary characteristic profile was represented to a lesser degree, with 9% of the Western Cape samples and 15% of the Northern Cape samples exhibiting this profile. The remainder of the rooibos samples, did not fit exactly into the criteria for either of the profiles, and therefore do not make up the remainder of the percentages.

3.2. Determination of the relationship between the sensory attributes and the sample quality grades

Fig. 8 and **Fig. 9** illustrate the relationship between the samples (graded according to quality) and the association these samples have with the sensory attributes, for the 2012 and 2013 production seasons.

Samples of these seasons were tested for the entire 38 attributes. As the samples are graded according to different methods by the respective processors from each production area, it was important to compare the samples from the same area, so as to better determine the role of the sensory attributes in the final quality grade of the samples.

Fig. 8 (b), the scores plot, illustrates the different quality grades in association with one another for the 2012 and 2013 production season, for the Western Cape. The scores plot (**Fig. 8 (b)**), illustrates the scattering of the A, B and C grade samples across PC 1, meaning they associate with both the negative and positive attributes in the loadings plot(**Fig. 8(a)**). The D grade samples lie predominantly to the left of PC 1 and associate therefore with the negative attributes in the loadings plot (**Fig. 8(a)**). A small number of A grade samples associate with the positive attributes seen to the right of PC 1 in **Fig. 8(a)**.

Fig. 9(b) depicts the samples from the 2012 and 2013 production seasons, from the Northern Cape, showing the position of the different graded samples in association with each other. The A grade samples mostly lie to the right of the scores plot in the top quadrant. The B grade and C grade samples, however, are scattered across PC 1, with a majority on the left of PC 1. Although found on both sides of PC 1, the D grade samples lie predominantly on the left of PC 1. The loadings plot (**Fig. 9(a)**), depicts the sensory attributes, and when analysed in conjunction with the PCA scores plot, indicates the relationship between the quality grades and sensory attributes. The majority of the B, C and D grade samples, lying on the left of PC 1, associate with the negative attributes, found to the left of PC 1 in the loadings plot. The A grade samples predominantly associate with the positive attributes in the top right-hand quadrant of the PCA loadings plot (**Fig. 9(a)**).

3.3. Significant trends and interactions amongst production seasons, production areas and quality grades of different rooibos samples for each of the sensory attributes

Due to the inconsistencies in the number of A grade and D grade samples received each year, and the different procedures used for grading by the different rooibos processors, it was deemed a better choice to only analyse the B and C grade samples in further detail. These samples form the bulk of production and are expected to be of a more similar quality. Significant interactions between production seasons, areas and grades are summarised in **Table 6** and **Table 7** for the aroma attributes of the respective B and C grade rooibos samples. Here significant interactions, for certain factor combinations, are highlighted. For a selected number of factor combinations, bar graphs illustrating the mean values and the least significant difference values are presented. These bar graphs serve to aid in determining any trends that may occur for the different interactions. **Fig. 10(a)** illustrates that the area X season interaction shows no clear patterns with regards to either the production seasons or areas, this illustrates that neither production area and nor production season resulted in significantly higher aroma intensities of these attributes. The year X grade interaction (**Fig. 10(b)**) depicts no trend for the "fynbos-floral" or the "green" aromas, indicating that

these attributes were affected by both the production season and grade. For the season interactions, **Fig 10(c)**, a significant difference between the production seasons is illustrated for the "honey" aroma.

No clear conclusions from the plots can thus be drawn, showing that the differences, albeit small differences in the mean intensity values for the respective aroma attributes, are not based on the production area alone and are most likely due to a combination of the area and season interactions.

4. DISCUSSION

The South African legislation regarding rooibos tea is not clear when outlining the standards of quality and vague terminology is used. The regulation states, "all rooibos should have the clean, characteristic taste and aroma of rooibos" (Anon., 2002). This statement does not give any clear and definitive indication as to the exact profile of a typical rooibos tea. In order to ensure that all role players within the rooibos industry are able to adhere to this regulation, they must all be able to have the same level of understanding about what exactly constitutes the "characteristic taste and aroma of rooibos".

In 2009 Koch *et al.* (2012) analysed rooibos samples, sourced from the Western Cape region, using DSA as a research method. The samples tested represented four quality grades (A, B, C and D). It was found that the "characteristic" rooibos flavour could be described as a combination of "honey", "woody" and "floral" notes with a slightly "sweet" taste and subtle "astringency". Differences in the sensory characteristics between and within different quality grades were established. Low-quality tea was 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, as well as a "sweet" taste. 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 occurring descriptors were selected to compile a rooibos sensory lexicon consisting of 14 flavour, taste and mouthfeel attributes along with a definition and physical reference standard for each term (Koch, 2011; Koch *et al.*, 2012).

In order to develop a valid sensory wheel and accompanying lexicon for the South African rooibos industry, it is vitally important to base the decisions made on a large data set spanning a number of production seasons, primarily to ensure that all possible variations are captured in the data set. By conducting the present study on samples collected during three production seasons (2011 – 2013) and two production regions (Western Cape and Northern Cape), it was possible to determine whether the respective production regions resulted in specific, unique sensory profiles and whether production season affects the sensory profile of rooibos. The inclusion of four quality grades of rooibos also enabled the determination of the significant positive and negative sensory attributes associated with rooibos quality.

Descriptive sensory analysis (DSA) was used to determine the full sensory profile of the entire sample set (Lawless & Heymann, 2010: Koch *et al.,* 2012). The results also led to the development of a revised sensory wheel and lexicon for rooibos, i.e. quality control tools that allow for the evaluation of products in a consistent manner.

4.1. Sensory profiles of rooibos from the Northern Cape and Western Cape and the differences between these profiles based on production season and production area

Previous sensory analyses of rooibos (Koch et al., 2012) focused only on profiling rooibos produced in the Western Cape region during the 2009 production season. Since potential variation introduced by production season and production area was not taken into account, further investigation was deemed necessary to validate results. As already indicated, for the present study the sample set was expanded to include several production seasons (2011 – 2013), as well as rooibos produced in the Northern Cape region in addition to that produced in the Western Cape region.

The initial analysis was conducted using discriminant analysis (DA). This multivariate technique has a dual function, i.e. classification and separation; however, in research DA is mostly used for its classification function (Lawless & Heymann, 2010). Within the DA plot, three clear groupings were formed from the full set of samples (2011 – 2013). The split, as indicated in the results, was based on the production season and not the production area. This leads to the conclusion that the production season plays a greater role in the final sensory profile of the rooibos than the production area. There are a number of factors that may be responsible for these differences, including climatic differences, seen mainly by changes in the temperature and rainfall patterns from year to year (Archer *et al.,* 2009). Joubert *et al.* 2012) demonstrates differences in the phenolic composition from year to year. The differences between the climatic conditions of the two production areas seem to play only a minimal or negated role in the sensory profiling of the rooibos, when compared to the yearly climatic changes. Changes in climate, whether it is a decrease or increase in rainfall or the presence of extreme events (droughts), are already having a significant effect on the crops in this area (Gérard, 2010). The climatic changes occurring in both the rooibos producing regions will not only influence the yields of the crops, but possibly also the quality of the final product. Initial research has shown that UV affects the accumulation of phenolic compounds (Schreiner, *et* al., 2012) and water stress can lead to an increase in flavonoids (Hernández *et al.*, 2006). It is vitally important that these climate changes and the effect thereof on rooibos yield and ultimate product quality and sensory profile be researched further.

After testing the comprehensive sample set, the present study indicated that "rooibos-woody" aroma and flavour, "fynbos-floral" aroma and flavour, "honey" aroma, "sweet" taste and "astringent" mouthfeel were present in 100% of the samples, irrespective of the region of origin. The "astringent" attribute, when present in high intensities can have a negative impact on the quality of rooibos, however, when not detectable, the infusion is found to be insipid, therefore when present at a mild intensity, it adds

to the characteristic profile of the tea. "Hay/dried grass" notes were present in 90% to 100% of the samples from both regions and at differing intensities. The attribute "hay/dried grass" is definitely viewed by industry as a negative attribute (Personal communication, workshop with industry to validate the rooibos sensory wheel, 21 November 2013). However, when present in lower intensities, e.g. at intensities below 15/100, this negative attribute could possibly be viewed as not having a negative impact on the overall profile of rooibos. This view should, however, be tested for validity.

Sub-profiles, also emerged from samples collected in 2012 and 2013, indicating that regardless of the production area, "caramel" or "fruity-sweet" aroma was present in more than 40% of the samples. "Apricot" aroma was also found to be present in the sub-profile, although sometimes in a lower intensity and percentage occurrence than the "caramel" and "fruity-sweet" aromas. As indicated in the results, there are significant associations between these attributes for both production areas. The attributes found in 100% of the rooibos samples, from both production areas, therefore are indicative of the primary characteristic profile of rooibos tea. The primary characteristic profile is thus "rooibos-woody" and "fynbos-floral" notes, with a "honey" aroma, "sweet" taste and an "astringent" mouthfeel, often coupled with the slight flavour or aroma of "hay/dried grass". The sub-profile lends itself to the occurrence of a secondary characteristic profile for rooibos tea. This secondary characteristic profile includes the "fruity-sweet" and "caramel" aromas, often combined with an "apricot" aroma. With the exclusion of the WC11 and NC11 samples, as they were not tested for all aroma attributes, the data set for 2012 and 2013 sufficiently represents the variation over production areas and production seasons. Most of the Northern Cape samples (61.4%) fall under the primary characteristic rooibos profile, whereas only 14.45% represent the secondary characteristic profile. The Western Cape samples represent the primary profile with 57% of the samples, and 9.35% of the samples fall under the secondary profile. These values are similar to those obtained for the samples from the Northern Cape, although slightly lower in value. In order to be considered as a match to the different rooibos profiles, the samples needed to exhibit the intensities of the attributes, within certain criteria. For the primary characteristic profile, the samples needed to contain the "rooibos-woody", "fynbos-floral" and "honey" aromas at an intensity of more than 30, 20 and 15, respectively. For the negative attributes, they all needed to be present at an intensity of less than 10, whereas "hay/dried grass" needed to be below an intensity of 15. The secondary characteristic profile adhered to the same rules for the negative attributes, as for the primary profile. Additionally, the secondary characteristic profile required that the "apricot", "fruity-sweet" and "caramel" aromas all be present at an intensity of greater than 10. If all the criteria were met, then the sample was added to the respective profile group, either primary or secondary. The samples that did not meet all the criteria for each of the profiles, were not labelled as having either a prominent primary or secondary characteristic profile.

Overall, samples harvested during the same production season, regardless of the production area, exhibited similar intensities for the sensory attributes. No distinct differences between the regions were

observed; leading to the conclusion that plant growth within either rooibos production region, does not affect the sensory profile of rooibos. Therefore, the development of production region-specific sensory wheels is not justified for the rooibos industry.

4.2. Relationship between sensory profiles and quality grades

Production processes can have an influence on the overall sensory quality of rooibos tea. The processing skills developed by the rooibos producers, as well as the "uncontrolled" nature of the process, can have an important effect on the quality of the tea that is produced (Koch *et al.,* 2013; Joubert & Schulz, 2006). Processing steps that affect the quality of rooibos include the "oxidation", drying and steam pasteurisation. Samples analysed in the present study were not steam pasteurised as quality grading by the companies that supplied the samples for the present study takes place before this process.

Rooibos samples are not graded solely based on the aroma or flavour of the infusion, but grading includes other criteria, often deemed of lesser importance to the sensory profile, such as the appearance of wet and dry leaves, and the colour of the infusion (Koch, 2011; M. Baard, Nieuwoudtville Rooibos (PTY) Ltd., Nieuwoudtville, South Africa, April 2012, personal communication; C. Cronje, Rooibos Ltd., Clanwilliam, South Africa, April 2013, personal communication; J. Basson, Rooibos Ltd., Clanwilliam, South Africa, April 2012, personal communication).

The criteria, according to which rooibos are graded, differ between the two rooibos processing companies. Both take into account the aroma of the wet leaves, the flavour of the cup (infusion), the colour of the infusion, and the density of the tea. However different grading methods are used. The one company uses a weighted system and a trained panel to analyse the tea before assigning a grade and the other uses a presence or absence (positive or negative) system, in order to reach the outcome. For the weighted system, criteria are assigned a percentage to calculate their contribution to the final quality grade. The criteria deemed more important are weighted higher and therefore contribute more to the final grade. For the positive or negative system, the main criteria, the aroma and flavour of the infusions, are scored according to the attribute being positive (+) and pleasing or negative (-) if unpleasant. From here additional criteria are taken into account and a grade is calculated accordingly (Koch, 2011; M. Baard, Nieuwoudtville Rooibos (PTY) Ltd., Nieuwoudtville, South Africa, April 2012, personal communication; C. Cronje, Rooibos Ltd., Clanwilliam, South Africa, April 2013, personal communication; J. Basson, Rooibos Ltd., Clanwilliam, South Africa, April 2012, personal communication).

Four quality grades are usually assigned; A grade depicts excellent quality, whereas D grade is given to a batch of tea with a number of poor quality attributes. Due to the grading methods differing between rooibos producers, and different quality assessing panels, the samples may have the same quality grade, but differ when it comes to their overall quality. This can lead to inconsistencies between the quality of the grades given to the teas, by the different tea producers, and therefore result in irregularities within the industry.

When analysing data and interpreting PCA plots, one would assume that samples of the same grade should be grouped closely to one another on the PCA scores plot, primarily because they should have reasonably similar sensory profiles. D grade samples should lie apart from A grade samples as these grades are not expected to have similar sensory profiles. Furthermore, A grade samples usually contain higher intensities of the positive attributes and the D grade samples higher intensities of the negative attributes, therefore their predominant profiles should lie apart on a PCA plot. The B and C grade samples are expected to lie closer to one another on a PCA plot as these samples are expected to have reasonably similar profiles, i.e. a mixture of both positive and negative sensory attributes.

From the data gathered, PCA was carried out on the WC12, NC12, WC13 and NC13 samples. For the Northern Cape samples (2012 – 2013) the majority of the A grade samples lay on the opposite side to the majority of the D grade samples, indicating differences between the sensory profiles of these samples. The B and C samples lay scattered across PC 1, an indication that these samples contain both the positive and negative attributes, in seemingly equal intensities. The separations between the samples, amongst the quality grades, however, are not clear, and there are definite overlaps, due to similarities between the samples. The Western Cape samples (2012-2013) also lay scattered over PC 1, with no clear separation between the different quality grades, especially the A grade samples, a majority of which associate with the negative attributes and D grade samples. These discrepancies can be explained by the fact the industry assigns grades not solely based on the aroma, flavour and mouthfeel of the infusion, as mentioned previously, however, the A grade samples from both areas, should exhibit the same associations with the positive attributes and little to no association with the negative attributes (Koch, 2011; M. Baard, Nieuwoudtville Rooibos (PTY) Ltd., Nieuwoudtville, South Africa, April 2012, personal communication; C. Cronje, Rooibos Ltd., Clanwilliam, South Africa, April 2013, personal communication; J. Basson, Rooibos Ltd., Clanwilliam, South Africa, April 2012, personal communication).

4.3. Development of sensory quality control tools for rooibos industry

A sensory wheel and lexicon, as quality control tools, would be used in the determination of food quality. Sensory wheels and lexicons have been developed for use within many sectors of the food industry and have seen great success, such as for blueberry juice (Bett-Garber & Lea, 2013), pawpaw puree (Brannan *et al*., 2012), turrón (Vázquez-Araújo *et al*., 2011), honey (Stolzenbach *et al.,* 2011), green tea (Lee & Chambers, 2006), floral honey (Galán-Soldevilla *et al*., 2005), cheddar cheese (Drake *et al*., 2001), fish (Warm *et al.,* 2000) wine (Noble *et* al., 1987) and beer (Meilgaard *et al.,* 1979), to name but a few.

Sensory wheels and lexicons can be used successfully during processing operations, where it is necessary to compare product quality between different production sites. This has already been illustrated in rooibos research (Koch *et al.,* 2012). A sensory wheel is essentially a list of sensory attributes organised in a graphical format and made up of different tiers (Drake & Civille, 2002). Each sensory descriptor is defined or described in more detail within the lexicon. Each descriptor is accompanied by a description of a

"recipe" for creating the physical reference standards, which are either chemical-based or food-based, and which will mimic the descriptor in question (Drake & Civille, 2002; Talavera-bianchi *et al.*, 2009). The sensory wheel is an easy to use, rapid quality control tool, which can aid the graders, exporters or consumers in differentiating clearly between the sensory attributes associated with rooibos, and possibly help with standardising the grading method. If used in conjunction with the sensory wheel, lexicons can provide precise definitions of each of the attributes. The reference standards within the lexicon can be used to obtain a clearer understanding of the attributes, as well as for training personnel.

As mentioned, Koch *et al*. (2012) developed an initial sensory wheel for rooibos. The rooibos sensory wheel was created by selecting 20 flavour, 3 taste and 4 mouthfeel attributes and grouping these terms together to form a logical, convenient and user-friendly overview of the sensory descriptors associated with rooibos (see **Chapter 2**). The most frequently occurring descriptors were selected to compile a rooibos sensory lexicon, consisting of 14 flavour, taste and mouthfeel attributes along with a definition and physical reference standard for each term (Koch *et al.*, 2012).

When developing encompassing and reliable sensory wheels, it is vitally important to base the final product on a large sample set that covers all possible sample variation. The sensory results of the present study indicated that there was substantial variation in the occurrence and intensity of the respective sensory attributes, in the samples sourced from the two production regions from 2011 – 2013. This warranted the further development and refinement of the generic sensory wheel developed by Koch *et al.* (2012).

As previously mentioned, it was hoped that a sensory wheel and lexicon could be developed for each of the rooibos production areas, showing the sensory profile differences between samples from each area. It was found, however, that there are no significant differences in the sensory profiles of the respective areas. Instead of region-specific sensory wheels, aroma and flavour attributes were captured in separate wheels and provisions were made for the intensities of the attributes. The first wheel contains 17 aroma attributes (**Fig. 11a**) (both positive and negative), whereas the second wheel contains 17 flavour attributes (**Fig. 12a**) (both positive and negative), as well as the 3 taste and 1 mouthfeel attributes. Each of the "slices" within the wheel represents the average intensity of that attribute, i.e. the wider the slice, the higher the intensity of the attribute and *vice versa.* Accompanying each of the sensory wheels are bar graphs (**Fig. 11(b & c)**; **Fig. 12(b, c & d)**), representing the percentage occurrence of each of the attributes in the total group of samples. The newly developed wheels each contain 3 tiers, with the outer tier indicating which of the attributes are positive or negative. The second tier contains the primary sensory attributes; there are 10 primary aroma attributes, whereas the flavour wheel contains 9 primary attributes. The innermost tier is made up of the names of the sensory attributes.

The inclusion of an intensity scale within a sensory lexicon, was done by Vázquez-Araújo *et al*. (2011), where the reference standards for each attribute (at differing intensities) were accompanied by an intensity score of between 0 (none) and 15 (extremely strong). In this way the industry personnel are able

to better understand the characteristics of an attribute at both a low or extremely high intensity. The format of the newly developed rooibos wheel, i.e. indicating intensity and occurrence of a comprehensive list of sensory attributes are therefore in line with trends for other tools. The indication of intensity and occurrence makes the newly developed sensory wheels for rooibos more comprehensive and thus highly applicable to the rooibos industry. Including reference standards, representing different intensities for each attribute, should be researched for the rooibos lexicon, as it could be a useful tool for training rooibos industry personnel as well as future sensory panellists. The sensory lexicon developed for rooibos by Koch *et al.* (2012) (**Table 8**) was updated to reflect the changes in the newly developed wheels (**Table 9**). Finally, the rooibos sensory wheels and lexicon were validated using direct input from industry during a workshop (Stellenbosch University, 21 November 2013). Preliminary reference standards were also tested, with industry input, and the list is included in the sensory lexicon (**Table 9**).

The newly developed sensory wheels and lexicon for rooibos were designed to incorporate all possible variation within the rooibos species, i.e. production season, area of production and quality grade differences. These wheels will enable all members of the rooibos industry to be on the same level of understanding when grading rooibos tea batches and applying quality control measures. These new industry tools will also assist in product development and marketing endeavours, especially on a global level (Drake & Civille, 2002). Within research, the standardised, validated terminology can be used to calibrate descriptive sensory analysis panels (Noble, *et al.*, 1984; Noble *et al.*, 1987) and compare the efficacy of panels at different research locations (Aparicio & Morales, 1995).

5. CONCLUSIONS

The South African regulation regarding rooibos quality states, "all rooibos should have the clean, characteristic taste and aroma of rooibos" (Anon., 2002). This statement is unclear and open to misinterpretation. It is vitally important that all industry role players within the rooibos industry have the same level of understanding about what exactly constitutes the "characteristic taste and aroma" of rooibos. This study was undertaken to address this limitation. A comprehensive rooibos sample-set was sourced from both production areas over a three-year period to include all possible variation.

The results indicated that 100 % of the samples from both production areas exhibit the aroma attributes from the primary characteristic profile, i.e. "rooibos-woody", "fynbos-floral" and "honey" aroma, "sweet" taste and a slight "astringent" mouthfeel. However, in order to be classified as having a primary characteristic profile the samples needed to contain higher than average intensities of the above-mentioned attributes. In this case more than 50 % of the samples from both areas have a prominent primary characteristic profile. On average only between 9 % and 15 % of the samples, from both areas, exhibited a prominent secondary characteristic profile, with attribute intensities above average for "caramel", "fruity-sweet" and "apricot" aroma notes. This result, i.e. rooibos tea with a prominent fruity character, could open up the opportunity for marketing niche products especially on a global level.

The study also resulted in the development, updating and verification of sensory wheels and an accompanying lexicon for the rooibos industry. Both types of revised sensory tools will allow for the evaluation of rooibos based on a uniform manner, which will prove essential for the success of the South African export and local rooibos industry.

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Table 1 The number of samples sourced, representing each quality grade, each of the production areas (Western Cape and Northern Cape) and the production seasons, 2011 – 2013 (N = 230).

Areas	Year	Grades				Totals
		A	B	C	D	
Western Cape (WC), South Africa	2011	6	6	6	6	24
	2012	18	20	20	6	64
	2013	10	15	16	2	43
Northern Cape (NC), South Africa	2011	6	6	4	-	16
	2012	4	26	8	3	41
	2013	3	26	9	4	42

Table 2 List of attributes used during descriptive sensory analysis (DSA), of rooibos infusions, accompanied by a list of descriptors used during the training phase.

Primary attribute	Attribute	Description
Floral	Fynbos-floral	The unique, somewhat sweet floral aromatics associated with fynbos[a] vegetation
Woody	Rooibos woody	Aromatics associated with dry bushes, stems and twigs of the rooibos vegetation
	Apricot	An aromatic associated with apricots
Fruity	Cooked apple	Sweet aromatics associated with cooked apples or apple pie
	Citrus	The sour/sweet aroma associated with citrus fruit
	Fruity-sweet	An aromatic associated with the sweet/sour smell of non-specific fruits
Sweet-associated	Honey	Aromatics associated with the sweet fragrance of fynbos honey
	Caramel	Sweet aromatics characteristic of molten sugar or caramel pudding
Spicy	Spicy	Aromatics associated with sweet spice primarily cinnamon
	Hay/dried grass	Slightly sweet aromatics associated with dried grass or hay
	Green grass/(Plant-like[b])	Aromatics associated with freshly cut grass
Vegetative	Rotting plant water	Aromatics associated with the rotting aroma of stagnant flower water
	Seaweed	Aromatics associated with seaweed that has been lying in the sun
	Burnt caramel	Aromatics associated with burnt sugar or burnt caramel
Chemical	Medicinal/rubber	Aromatics associated with band- aids or rubber bands
Earthy	Dusty	Earthy aromatics associated with dust from a gravel road or ground
Micro	Musty/mouldy	Mouldy aromatics associated with mildew, damp cellars or wet cardboard

[a]Fynbos is natural shrubland vegetation occurring in the Western Cape, South Africa.
[b]"Plantlike/green" and "grassy" were grouped together under one attribute during descriptive analysis.

Table 3 Mean intensity values for aroma attributes for each production season (2011-2013) and area (Western Cape and Northern Cape).

Attributes	Mean intensity values					
	WC11	NC11	WC12	NC12	WC13	NC13
Fynbos-floral	21.13	22.53	25.90	25.39	27.00	26.51
Rooibos-woody	40.08	43.07	33.96	32.60	36.90	35.83
Honey	25.11	23.03	17.57	16.36	18.86	18.18
Fruity-sweet	17.97	13.39	9.02	11.00	5.50	5.99
Apricot	NT[a]	NT	5.84	8.27	3.46	3.38
Cooked apple	NT	NT	1.50	1.17	0.34	0.75
Citrus	NT	NT	0.06	0.17	0.10	0.11
Caramel	7.64	6.64	7.66	11.55	10.19	8.77
Spicy	2.69	1.14	2.01	1.74	0.58	1.00
Hay/dried grass	15.61	11.69	13.57	11.27	11.41	11.36
Green	6.99	7.73	6.18	7.24	5.01	6.33
Musty/mouldy	3.14	5.01	1.89	1.48	3.55	3.01
Burnt caramel	1.68	1.76	2.00	3.28	1.14	1.10
Medicinal/rubber	3.22	5.52	3.30	1.96	2.80	1.80
Dusty	NT	NT	1.85	1.21	1.10	1.03
Rotting plant water	NT	NT	3.16	1.43	3.17	2.10
Seaweed	NT	NT	0.35	0.67	0.45	0.96

[a]NT indicates the attributes that were not tested.

Table 4 Mean intensity values for flavour, taste and mouthfeel attributes for each production season (2011-2013) and area (Western Cape and Northern Cape).

Attributes	Mean intensity values					
	WC11	NC11	WC12	NC12	WC13	NC13
Fynbos- floral	14.77	16.87	23.77	23.94	21.89	21.98
Rooibos-woody	40.18	41.05	34.08	33.37	35.08	34.84
Honey	4.39	4.48	2.67	2.30	2.27	1.90
Fruity-sweet	3.50	2.23	3.96	4.75	1.14	1.08
Apricot	NT[a]	NT	1.15	2.12	0.42	0.32
Cooked apple	NT	NT	0.24	0.07	0.02	0.08
Citrus	NT	NT	0.01	0.02	0.02	0.01
Caramel	2.18	1.65	2.55	4.18	1.58	1.19
Spicy	0.45	0.23	0.82	0.58	0.11	0.23
Hay/dried grass	11.82	9.91	14.93	13.78	12.75	12.51
Green	2.26	3.43	3.39	4.11	3.59	3.91
Medicinal/rubber	1.73	2.58	0.61	0.28	0.80	0.20
Musty/mouldy	NT	NT	0.65	0.80	0.66	0.45
Burnt caramel	NT	NT	1.33	0.39	0.23	0.09
Dusty	NT	NT	0.75	0.47	0.19	0.32
Rotting plant water	NT	NT	1.34	0.36	1.05	0.40
Seaweed	NT	NT	0.02	0.34	0.07	0.27
Sweet taste	24.12	23.65	20.06	19.89	20.81	20.79
Sour taste	3.85	2.09	3.20	3.61	4.28	4.18
Bitter taste	1.48	1.32	2.45	2.11	3.18	3.27
Astringent	24.23	24.46	22.88	22.88	25.73	25.86

[a]NT indicates the attributes that were not tested.

Table 5 Breakdown of the two sensory profiles found in rooibos infusions, namely the primary characteristic profile and the secondary characteristic profile. The percentage occurrence of attributes of the respective profiles was calculated for the respective production seasons and areas, i.e. only if present in above-average intensities.

Sensory profiles	Production season (calculated for both NC & WC)		Production areas (calculated for both 2012 & 2013)	
Primary Characteristic profile (Fynbos-floral, rooibos-woody, honey)	2012	44.7%	Western Cape	57.0%
	2013	80.0%	Northern Cape	61.4%
Secondary characteristic profile (Apricot, fruity-sweet, caramel)	2012	18.0%	Western Cape	9.3%
	2013	3.5%	Northern Cape	14.4%

Table 6 Interactions between the factors and factor combinations present in the study (production area, production season and quality grade), and the aroma attributes, of the B and C grade rooibos samples (2011-2013). The significant interaction, of the largest combination of factors for each of the attributes, is highlighted in <mark>yellow</mark>.

Factors	Aroma attributes										
	Fynbos-floral	Rooibos-woody	Honey	Fruity-sweet	Caramel	Spicy	Hay/dried grass	Green	Musty/mouldy	Burnt caramel	Medicinal/rubber
Area	0.01	0.02	< .0001	0.99	0.72	0.59	0	0	0.70	0.04	0.73
Season	< .001	< .001	<mark>< .0001</mark>	< .0001	0.05	0	0	0.04	< .0001	< .0001	0.01
Area x Season	0.15	<mark>0</mark>	0.06	<mark>0</mark>	<mark>0</mark>	<mark>0.04</mark>	<mark>0</mark>	0.28	<mark>0.01</mark>	<mark>0.04</mark>	< .0001
Grade	0.01	0.09	0.36	0.48	0.82	0.76	0.91	0.43	0.03	0.05	0
Area x Grade	0.34	0.29	0.36	0.09	0.88	0.79	0.58	0.79	0.79	0.41	0.02
Season x Grade	<mark>0</mark>	0.14	0.18	0.33	0.50	0.53	0.42	<mark>0.01</mark>	0.36	0.29	< .0001
Area x Season x Grade	0.33	0.15	0.22	0.16	0.17	0.33	0.26	0.82	0.38	0.11	<mark>< .0001</mark>

Table 7 Interactions between the factors and factor combinations present in the study (production area, production season and quality grade), and the aroma attributes only tested in 2012 and 2013, of the B and C grade rooibos samples. The significant interaction, of the largest combination of factors for each of the attributes, is highlighted in **yellow**.

Factors	Aroma attributes					
	Apricot	Cooked apple	Citrus	Dusty	Rotting plant water	Seaweed
Area	0.27	0.26	0.65	0.04	0	0
Season	< .0001	0.04	0.66	0.09	0.68	0.22
Area x Season	0.01	0.46	0.51	0.43	0.03	0.12
Grade	0.84	0.41	0.61	0.63	0.96	0.58
Area x Grade	0.91	0.92	0.33	0.46	0.72	0.30
Season x Grade	0.85	0.31	0.60	0.41	0.99	0.16
Area x Season x Grade	0.83	0.48	0.79	0.54	0.61	0.28

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Table 8 Aroma attributes that made up the sensory profile of rooibos along with a detailed description of each of those attributes as published by Koch *et al.* (2012).

Attributes	Definitions
Herbal floral	The unique, somewhat sweet aromatics associated with flowers of the fynbos[a] vegetation
Woody	Aromatics associated with the dry bushes, stems and twigs of the fynbos vegetation
Honey	Aromatics associated with the sweet fragrance of fynbos honey
Caramel	Sweet aromatics characteristic of molten sugar or caramel pudding
Apricot jam	An aromatic associated with the sweet smell of fruit especially apricot jam and berries
Plantlike/green[b]	Slightly sour aromatics characteristic of freshly cut green leaves or plant material
Grassy[b]	Aromatics associated with freshly cut grass
Hay/dried grass	Slightly sweet aromatics associated with dried grass or hay
Dusty[c]	Earthy aromatics associated with wet hessian or wet cardboard
Musty[c]	Mouldy aromatics associated with mildew or damp cellars

[a]Fynbos is natural shrubland vegetation occurring in the Western Cape, South Africa.
[b]"Plantlike/green" and "grassy" were grouped together under one attribute during descriptive analysis.
[c]"Dusty" and "musty" were grouped together under one attribute during descriptive analysis.

Table 9 Rooibos sensory lexicon, containing upgraded attribute names and descriptions. The list of reference standards included is preliminary, and needs to be further researched, prior to use within industry (Personal communication, workshop with industry experts to validate the rooibos sensory lexicon, 21 November 2013).

Primary	Attributes	Description	Reference standards[b]
Floral	Fynbos-floral	The unique, somewhat sweet aromatics associated with fynbos[a] vegetation	β-damascenone (140 µL/L)
Woody	Rooibos-woody	Aromatics associated with dry bushes, stems and twigs of the rooibos vegetation	2-acetyl-5-methylfuran (50 µL/L)
Fruity	Apricot	Aromatics associated with apricot jam	Deltadodecalactone (15 µL/L)
	Baked apple	Sweet aromatics associated with cooked apples or apple pie	Hexyl acetate (60 µL/L);
	Citrus	The sweet aroma associated with ripe oranges	Orange terpenes(10 µL/L)
Sweet-associated	Fruity-sweet	Aromatics associated with the sweet/sour smell of non-specific fruit	Geranyl isovalerate (80 µL/L)
	Honey	Aromatics associated with the sweet fragrance of fynbos honey or *Alyssum* blossoms	"Honey-like" flavour (100 µL/L)
	Caramel	Sweet aromatics characteristic of caramelized sugar	"Caramellic" flavour (40 µL/L)
Spicy	Sweet spice	Aromatics associated with sweet spice	Cinnamaldehyde (50 µL/L)
Vegetative	Hay/dried grass	Slightly sweet aromatics associated with dried grass or hay	4-dihydrocoumerin (150 µL/L)
	Green grass	Aromatics associated with freshly cut grass	(Z)-3-hexen-1-ol (70 µL/L)
	Rotting plant water	Aromatics associated with the rotting aroma of old flower water	NA[c]
	Seaweed	Aromatics associated with seaweed that has been lying in the sun	NA
Chemical	Burnt caramel	Aromatics associated with burnt sugar or burnt caramel	NA
	Medicinal/Rubber	Aromatics associated with Band-Aids® or burnt rubber	4-ethylphenol (50µL/L)
Earthy	Dusty	Earthy aromatics associated with dust from a gravel road or ground	NA
Microbiological	Musty/mouldy	Mouldy aromatics associated with mildew, damp cellars or wet hessian	NA

[a]Fynbos is natural shrubland vegetation occurring in the Western Cape, South Africa.

[b] The reference standards indicated, were added to a neutral rooibos infusion, which served as a base. These reference standards are preliminary and further research into more suitable reference standards needs to be done before they can be used within industry. Suppliers of these flavours and chemicals is included in **Addendum A (Table A3)**

[c] Suitable reference standards for these specific attributes were not successfully determined, and thus not included in this preliminary list.
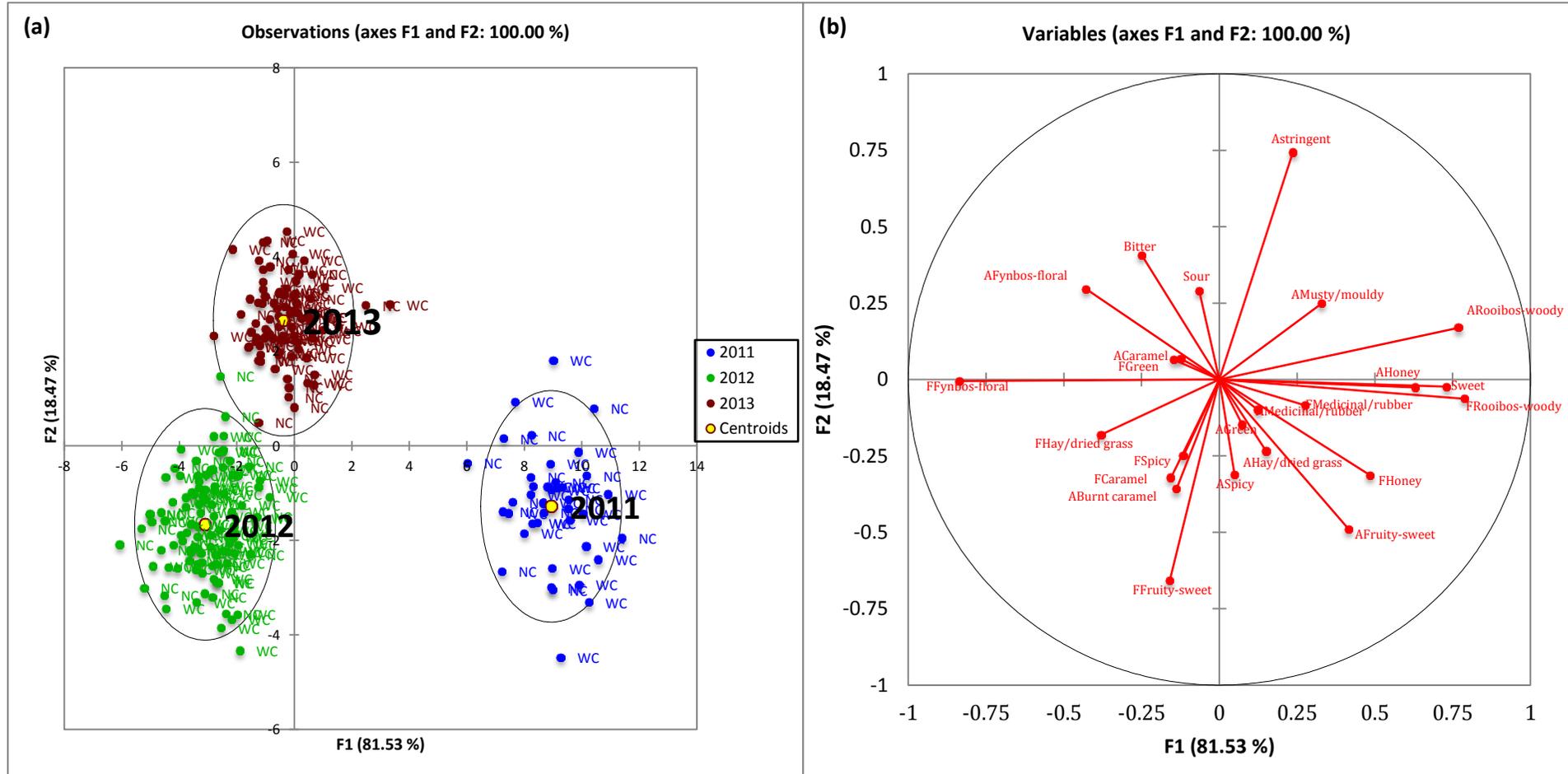
Figure 1 DA plot **(a)** of the samples from 2011 – 2013. The loadings plot **(b)** shows the sensory attributes taken into account in the DA plot. The letters "A" and "F" in front of the attribute names refer to the "aroma" and "flavour" attributes, respectively. The taste and mouthfeel attributes are written as-is.
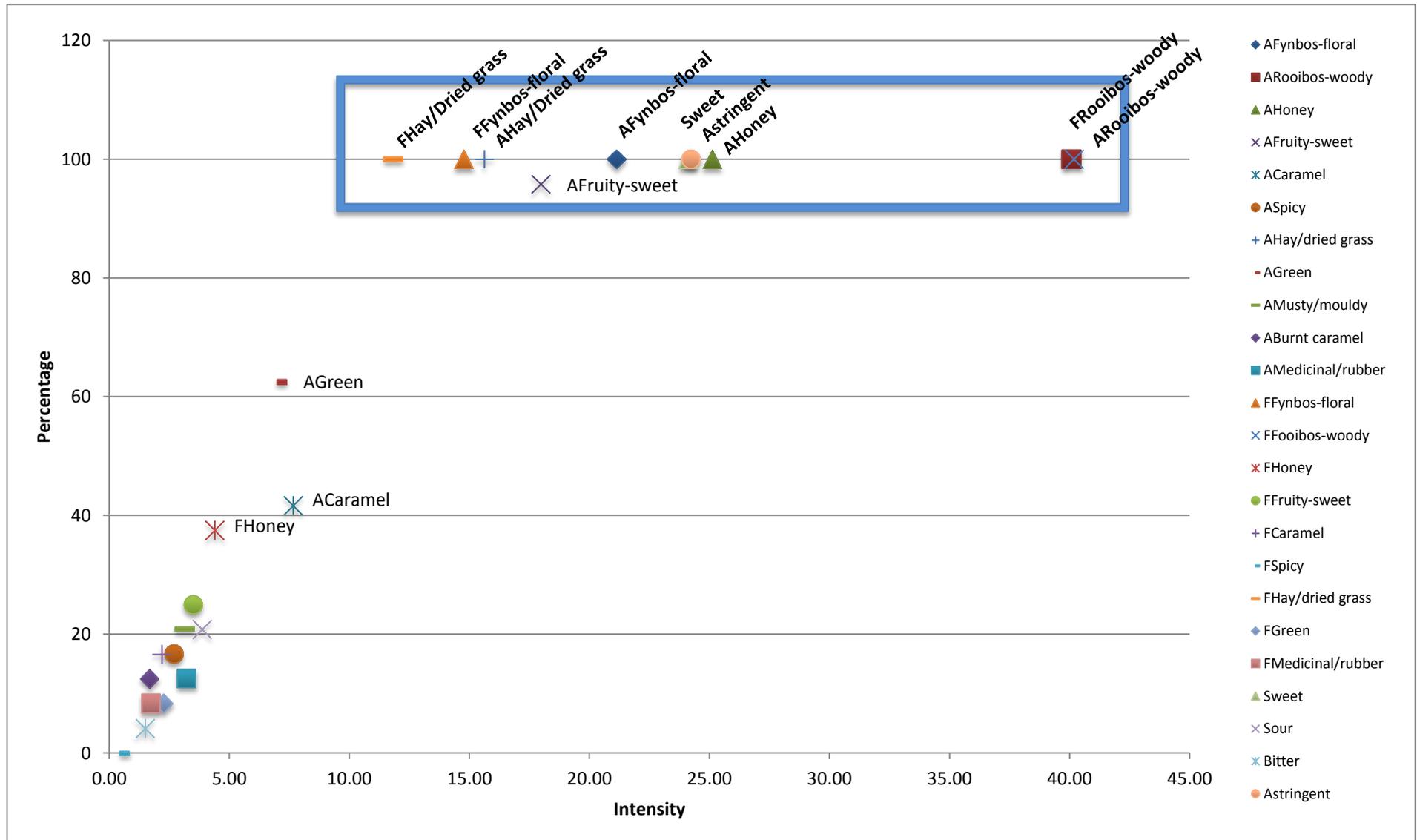
Figure 2 A scatter plot illustrating the mean intensities of the *full range* of attributes, as well as the percentage of samples exhibiting a specific attribute for the 2011 production from the Western Cape area. The "A" and "F" in front of the attributes refer to the "aroma" and "flavour" attributes, respectively. The taste and mouthfeel attributes are written as-is.
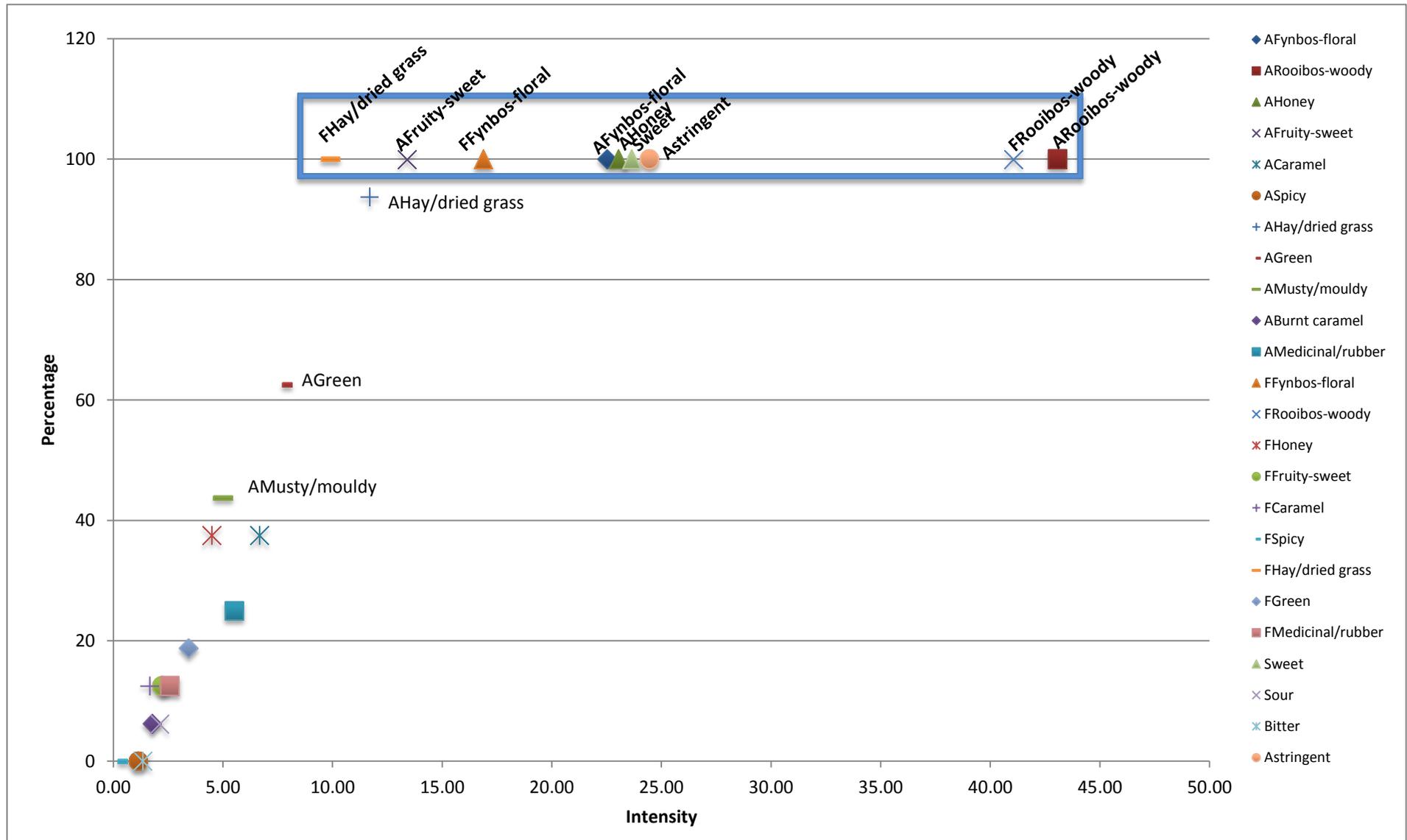
Figure 3 A scatter plot illustrating the mean intensities of the *full range* of attributes, as well as the percentage of samples exhibiting a specific attribute for the 2011 production from the Northern Cape area. The "A" and "F" in front of the attributes refer to the "aroma" and "flavour" attributes, respectively. The taste and mouthfeel attributes are written as-is.
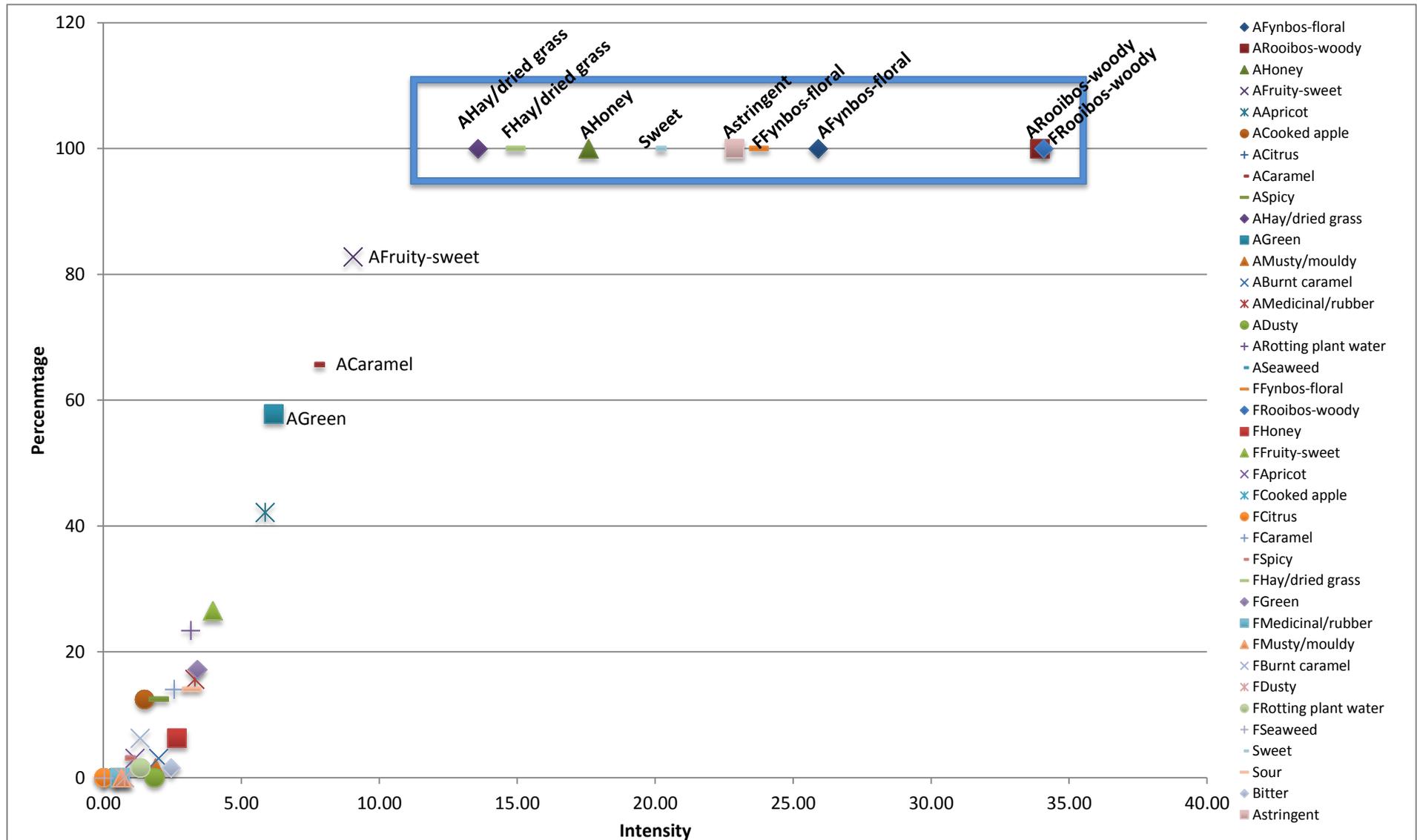
Figure 4 A scatter plot illustrating the mean intensities of the *full range* of attributes, as well as the percentage of samples exhibiting a specific attribute for the 2012 production from the Western Cape area. The "A" and "F" in front of the attributes refer to the "aroma" and "flavour" attributes, respectively. The taste and mouthfeel attributes are written as-is.

Figure 5 A scatter plot illustrating the mean intensities of the *full range* of attributes, as well as the percentage of samples exhibiting a specific attribute for the 2012 production from the Northern Cape area. The "A" and "F" in front of the attributes refer to the "aroma" and "flavour" attributes, respectively. The taste and mouthfeel attributes are written as-is.
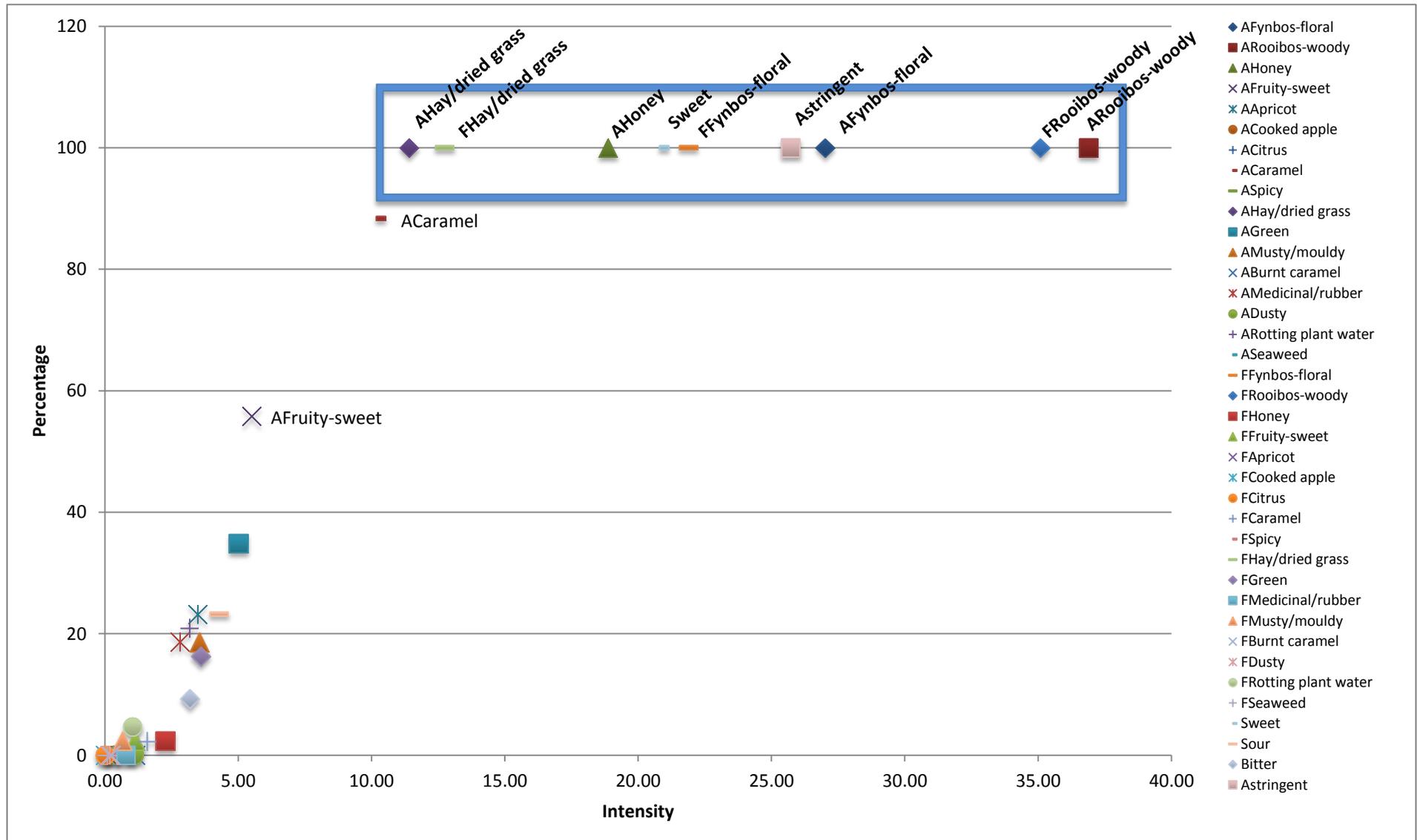
Figure 6 A scatter plot illustrating the mean intensities of the *full range* of attributes, as well as the percentage of samples exhibiting a specific attribute for the 2013 production from the Western Cape area. The "A" and "F" in front of the attributes refer to the "aroma" and "flavour" attributes, respectively. The taste and mouthfeel attributes are written as-is.
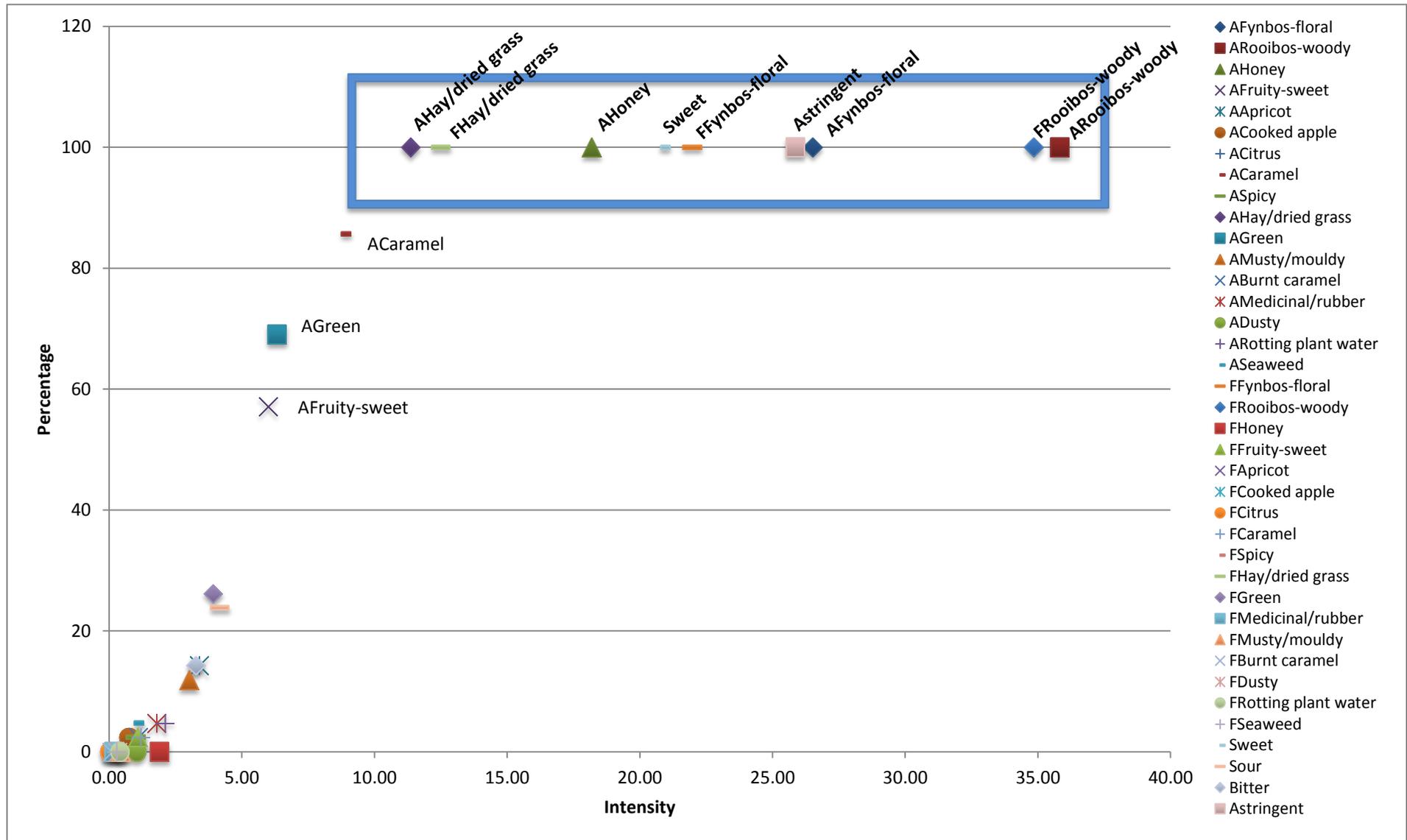
Figure 7 Scatter plot illustrating the mean intensities of the *full range* of attributes, as well as the percentage of samples exhibiting a specific attribute for the 2013 production season from the Northern Cape area. The "A" and "F" in front of the attributes refer to the "aroma" and "flavour" attributes, respectively. The taste and mouth feel attributes are written as-is.

Figure 8 Loadings plot **(a)** showing the ***full range*** of aroma, flavour, taste and mouthfeel attributes for the Western Cape samples in 2012 and 2013. The letters "A" and "F" in front of the attributes refer to the "aroma" and "flavour" attributes, respectively. The taste and mouthfeel attributes are written as-is. The scores plot **(b)** illustrates the spread of the samples with the Grade A samples coloured in **green**, Grade B in **red**, Grade C in **blue** and Grade D in black.

Figure 9 Loadings plot **(a)** showing the ***full range*** of aroma, flavour, taste and mouthfeel attributes for the Northern Cape samples in 2012 and 2013. The letters "A" and "F" in front of the attributes refer to the "aroma" and "flavour" attributes, respectively. The taste and mouthfeel attributes are written as-is. The scores plot **(b)** illustrates the spread of the samples with the Grade A samples coloured in **green**, Grade B in **red**, Grade C in **blue** and Grade D in black.

Figure 10 The mean intensity values for the different aroma attributes, exhibiting a significant association to the production season, production area, and grade combinations. **(a)** Indicates the season x area combination, **(b)** indicates the season x grade combination and **(c)** represents the production seasons.
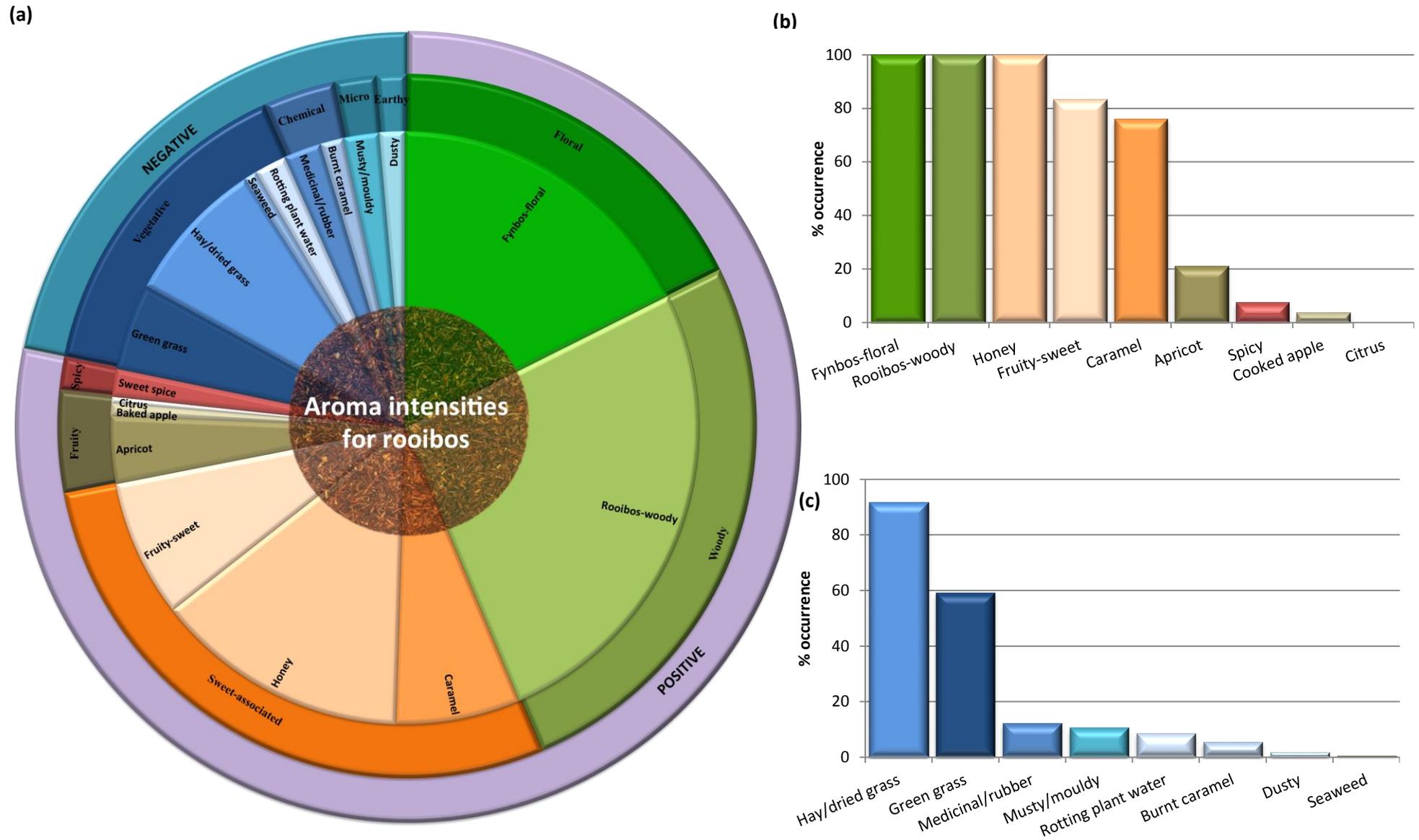
Figure 11 (a) Rooibos sensory wheel depicting the mean intensities of the aroma attributes. Graphs **(b)** and **(c)** illustrate the average percentage that each attribute appeared in the rooibos infusions.
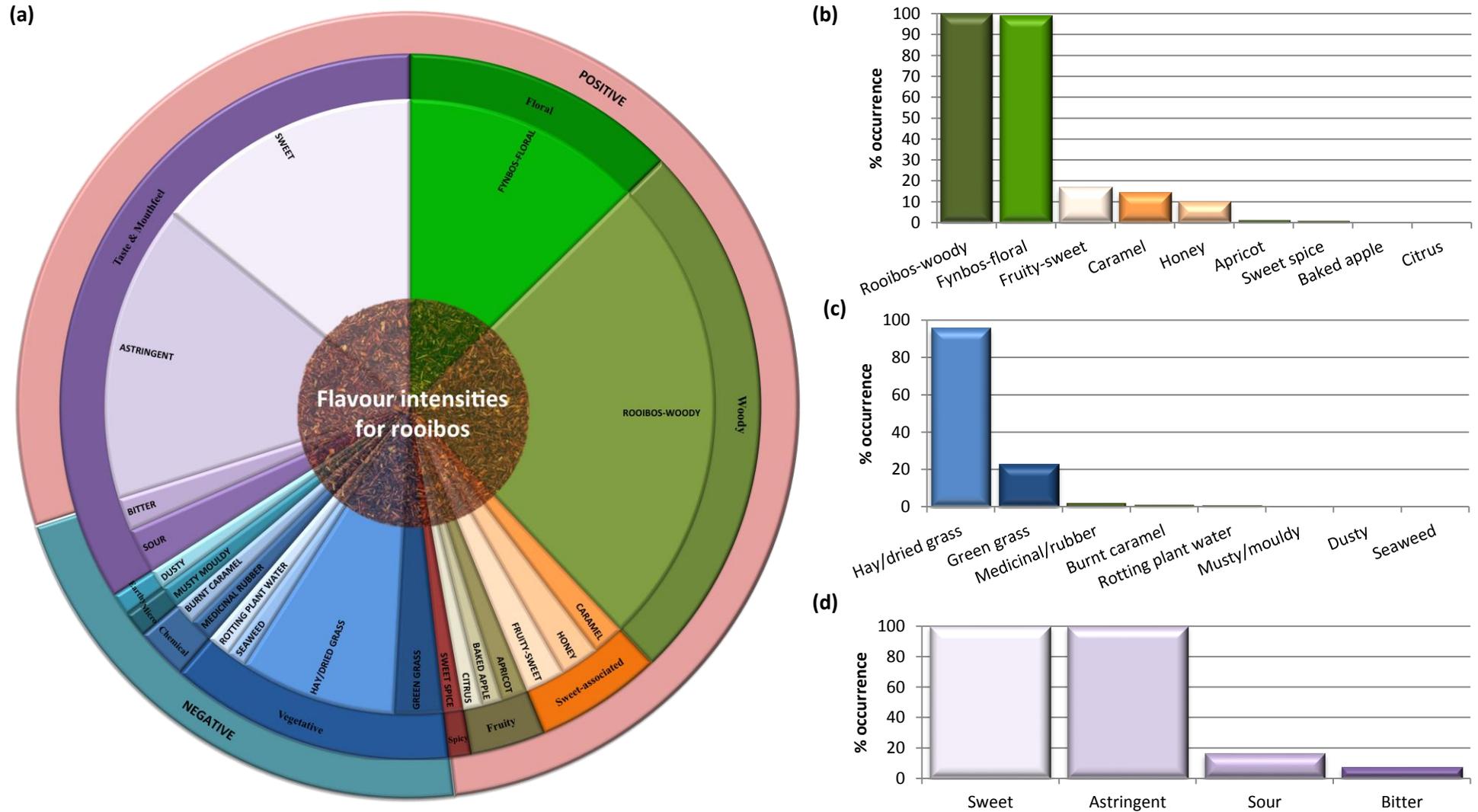
Figure 12 (a) Rooibos Sensory Wheel depicting the mean intensities of the flavour, taste and mouthfeel attributes. Graphs **(b), (c), (d)** illustrate the average percentage that each attribute appeared in the rooibos infusions.

CHAPTER 4

Relation of individual phenolic compounds and selected taste and mouthfeel attributes in rooibos

TABLE OF CONTENTS

ABSTRACT

The initial aim of the current study was to identify rooibos phenolic compounds that correlate with the taste and mouthfeel attributes ("sweet", "sour", "bitter" and "astringent") of the infusion. Once this was achieved, focus was directed towards developing an improved prediction model, in order to be able to determine the intensity of the afore-mentioned attributes based on the presence of specific phenolic compounds present in a rooibos infusion as a previous prediction model was based on a relativly small set of samples (N = 69) (Koch *et al.*, 2012). A large sample set (N = 260) of fermented, unpasteurised rooibos, spanning the production seasons 2009 – 2013 from the two production areas (Western Cape and Northern Cape, South Africa) was used. For quantification of the major phenolic constituents of rooibos infusions RP-HPLC-DAD analysis was carried out. The latter and sensory (taste and mouthfeel) data (**Chapter 3**) were subjected to multivariate analyses (PLS, PCA and DA) to determine associations between the phenolic compounds and sensory attributes. Additionally, step-wise regression analysis was performed to determine whether the phenolic compounds could be used as predictors of the intensity of the sensory attributes. Results indicated that there were no differences between the phenolic content of samples from the two production areas. However, variations in the phenolic contents between production seasons were observed. The stepwise regression led to a prediction model able to predict 47% and 42% of the intensity of the "sweet" and "astringent" attributes, respectively. In the case of "sour" and "bitter", the prediction model was only able to predict less than 30% of the intensity.

1. INTRODUCTION

Polyphenols are abundant in plant foods and beverages, including teas, and are partly responsible for the sensory (taste and mouthfeel attributes) and beneficial properties of these products (Bravo, 1998). Bitter taste and/or astringent mouthfeel are associated with phenolic compounds. Catechins, found in green and black teas, are usually both bitter and astringent (Yu *et al.,* 2014). According to Narukawa *et al.* (2010) bitter taste and astringent mouthfeel increases with an increase in the concentration of the catechins.

Astringency is largely a tactile sensation (Green, 1993), and it has been suggested that astringency is not a single sensation, but rather the result of a number of sensations or "sub-qualities" occurring simultaneously (as reviewed by Bajec & Pickering, 2008; Gawel 1998). Dryness, roughing and puckering are the most frequently occurring mouthfeel sensations (Payne *et al.*, 2009). It has long been thought that the sensation of astringency is primarily due to the de-lubrication of saliva. This mechanism, however, is no longer thought to be the main cause of astringency. Instead, it has been suggested that astringency may also be due to the binding of polyphenols such as procyanidins or procyanidin-protein complexes to the oral epithelial cells (Payne *et al.*, 2009). Due to considerable variations in the composition and flow of saliva from one individual to another, the perceived intensity, quality and persistence of astringency, may differ between individuals (Gawel, 1998). The structure and molecular size of polyphenols can determine their astringency (Bajec & Pickering, 2008; McRae & Kennedy, 2011), and the latter could be modulated by

pH and the presence of carbohydrate polymers in foods or beverages (Kallithraka *et al.*, 1997; Troszyńska *et al.*, 2010). Furthermore, minor modifications to polyphenol structure, within a class of compounds, could affect bitter intensity (Narukawa *et al.*, 2010: Narukawa *et al.*, 2011). Polyphenols react differently, with some activating certain bitter taste receptors (Soares *et al.*, 2013), whilst others mask bitter taste (Ley *et al.*, 2005).

The major phenolic compounds present in rooibos tea include a phenolic acid, monomeric flavonoids such as dihydrochalcones, flavanones, flavones and flavonols, and a phenylpropenoic acid derivative (Beelders *et al.*, 2012). Aspalathin, a dihydrochalcone-*C*-glucoside, is unique to rooibos. Previous studies on fractions and pure compounds indicated that they could potentially contribute to the overall taste of a rooibos beverage through their direct impact on bitterness and/or astringency (Reichelt *et al.*, 2010; Joubert *et al.*, 2013). Sensory and chemical analysis of rooibos samples, collected in the Western Cape region, South Africa during the 2009 production season (Koch *et al.*, 2013), showed that bitterness of the infusion correlated weakly, but significantly, with aspalathin and its 3-deoxy analogue, nothofagin, as well as the flavanol/flavone aglycones, quercetin, luteolin and chrysoeriol. These aglycones have been found to be present in very low quantities in rooibos infusions (Joubert *et al.*, 2012), which could explain the weak correlations. Several glycosides, including flavone oxidation products of aspalathin (orientin, isoorientin) and nothofagin (isovitexin), two quercetin glycosides, and a luteolin-o-glucoside showed a weak, but significant correlation with sweetness. The phenylpyruvic acid-2-*O*-glucoside (PPAG) also associated with the "sweet" taste of the infusion (Koch *et al.*, 2013). Yet, when tested as a pure compound solubilised in water, PPAG was perceived as bitter, suggesting that taste modulation may occur when present in the rooibos infusion (Joubert *et al.*, 2013).

Koch *et al.* (2013) were unable to establish significant correlations between rooibos flavonoids and astringency, except for rutin, which has a very low oral threshold (0.0006 mg/L) (Scharbert *et al.*, 2004). In spite of the lack of correlation, Koch *et al.* (2013) postulated that the compounds are likely to contribute, to some extent, to the mouthfeel sensation of rooibos. Given that the threshold values for astringent compounds, i.e. rutin, isoquercitrin (0.33 mg/L) and hyperoside (0.19 mg/L), were much lower than their average content in a rooibos infusion, resulting in dose-over-threshold (DOT) values (> 1), they could be expected to have an impact on astringency (Joubert *et al.*, 2012; Stark *et al.*, 2005; Scharbert *et al.*, 2004). By definition values of > 1 indicates a significant influence on taste with larger values indicating an even greater contribution to taste (Scharbert & Hoffman, 2005).

The phenolic composition of rooibos has been found to vary depending on subspecies, production season, processing and quality grade (Joubert, 1996; Joubert *et al.*, 2012; Joubert *et al.*, 2013; Stanimirova *et al.*, 2013; Van Heerden *et al.,* 2003). Although not studied to date, it has already been observed that changes in the climatic conditions in the Western Cape and Northern Cape rooibos growing areas of South Africa are having an effect on the rooibos crops (Gérard, 2010). Stress due to environmental factors, including water deficit, has been shown to affect accumulation of polyphenols in plants (Yaginuma *et* al.,

2002; Hernández *et al*., 2006; Cheruiyot *et al.,* 2007; Tattini *et al*., 2000; Schreiner *et al*., 2012). The effects of season (year) and quality grade on the sensory attributes of rooibos infusions have already been demonstrated (**Chapter 3**). Furthermore, it has been found that the higher the phenolic compound content, the higher the quality grade of rooibos (Joubert *et al*., 2012). Ferulic acid has been singled out as the phenolic compound that could act as a rooibos quality indicator, as it has been found to be present in high quantities in low quality rooibos tea (Stanimirova *et al*., 2013).

The aim of the present study was to confirm the contribution of individual rooibos compounds to the taste and mouthfeel of rooibos infusions, as previously found for a limited sample set, collected during one production season and produced in Western Cape only (Koch *et al.,* 2013). To validate the results of Koch *et al.* (2013) a comprehensive sample set comprising samples differing in quality and spanning over five production seasons were used. Furthermore, the samples originated from two production areas, Western Cape and Northern Cape, primarily to ensure major sources of potential variation in composition are taken into account. The data were used to develop a prediction model for the sensory characteristics (taste and mouthfeel) based on phenolic content.

2. MATERIALS AND METHODS

2.1. Chemicals

The phenolic standards were obtained from Extrasynthese (Genay Cedex, France), Roth (Karlsruhe, Germany), Sigma-Aldrich (St Louis, MO, USA) and Fluka (Sigma-Aldrich) as described by Beelders *et al*. (2012). PPAG was isolated and supplied by the Post-Harvest & Wine Technology Division of the Agricultural Research Council of South Africa (ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa). Both aspalathin and nothofagin were obtained from the PROMEC unit of the Medical Research Council of South Africa (Cape Town, South Africa). HPLC grade water was prepared by purification of deionised water using a Milli-Q academic water purification system (Millipore, Milford, MA, USA). HPLC gradient-grade acetonitrile was purchased from Merck (Darmstadt, Germany). Analytical grade reagents, i.e. ascorbic acid, dimethyl sulfoxide and glacial acetic acid, were obtained from Sigma-Aldrich.

The following notations are used in the tables and figures to indicate the compounds: ASP (aspalathin), NOTH (nothofagin), PPAG (phenylpyruvic acid glucoside), ISOORI (isoorientin), ORI (orientin), FerulicA (ferulic acid), QROB (quercetin-3-*O*-robinobioside), VIT (vitexin), HYP (hyperoside), RUT (rutin), ISOV (isovitexin) and ISOQ (isoquercitrin). Samples of the different production seasons and areas are indicated in figures, tables and text by the area (WC for Western Cape or NC for Northern Cape), followed by the production season (e.g. WC11 for 2011 samples from the Western Cape area).

2.2. Rooibos samples

The rooibos samples were sourced during the 2009 to 2013 production seasons from the Northern Cape (N = 129) and 2011 to 2013 from the Western Cape (N = 131) regions. The samples encompassed the quality

grades A, B, C and D (**Table 1**). All the samples were unpasteurized and represented the particle size fraction < 10 and > 40 mesh as described in **Chapter 3**.

2.3. Sample preparation

The infusions, prepared for quantitative descriptive analysis as described in Chapter 3, were used for HPLC analysis. A 200 ml aliquot of each infusion was filtered through Whatman No.4 filter paper and allowed to cool to room temperature. Aliquots of the filtrate were transferred into 2 ml microfuge tubes and stored at -18°C until required for HPLC analysis.

2.4. Quantification of individual phenolic compounds by high performance liquid chromatography (HPLC)

The samples were analysed using the high-resolution HPLC-DAD method developed by Beelders *et al.* (2012). The major phenolic compounds found in rooibos infusions were quantified using an Agilent 1200 series instrument (max pressure 400 bar; Agilent, Santa Clara, CA, USA), equipped with a quaternary pump, auto sampler, column thermostat and diode-array detector (standard 13 µL flow cell, 10 mm path length). The separation was achieved on a Zorbax SB-C18 column (100 x 4.6 mm i.d., 1.8 µm particle size, Agilent), protected by an inline filter and 5.0 µm SB-C18 guard column (Agilent), all thermostatted at 37°C. The flow rate was maintained at 1.0 mL/min and a multilinear gradient was performed as follows: 10% B (0−2 min), 10−14.8% B (2−19 min), 14.8−36.8% B (19−34 min), 36.8−100% B (34−37 min), 100% B isocratic (37−42 min), 100−10% B (42−45 min), and 10% B (45−50 min), with solvents A and B being 2% (m/v) acetic acid in water and acetonitrile, respectively.

Stock solutions of the phenolic standards (ca 1 mg/mL) were prepared in dimethyl dioxide and standard mixtures were prepared, containing 0.5 mg/mL ascorbic acid to prevent oxidative degradation. A six-point standard curve of all standards was prepared for quantification. The dihydrochalcones and PPAG were quantified at 288 nm, while the flavones, flavonols and ferulic acid were quantified at 350 nm. The sample aliquots were defrosted before analysis and ascorbic acid was added at 0.9 mg/ml. Both the standards and samples were filtered through 0.22 µm hydrophilic PVDF filters (Millipore, Milford, MA, USA) prior to injection.

2.5. Statistical procedures

SAS® Software (Version 9.2, SAS institute Inc., Cary, USA) and XLStat (Version 2014.01.02, Addinsoft, France) were used for the respective univariate and multivariate analyses. The chemical and sensory data were subjected to analysis of variance (ANOVA) and several multivariate analysis methodologies, i.e. discriminant analysis (DA), principal component analysis (PCA) and partial least squares regression (PLS) to determine the association between the taste and mouthfeel attributes and the phenolic compounds (Abdi, 2007; Jolliffe, 2002). Pearson's correlation analysis was performed to determine the correlation between

individual phenolic compounds and specific sensory attributes. Stepwise regression was performed on the rooibos samples in order to build a model able to predict the dependent variable (taste and mouthfeel) of an infusion, based on the independent variables (individual phenolic compounds). Step-wise regression analysis selects individual phenolic compounds that make a significant contribution to the model developed, in order to predict the individual dependent variables ("sweet", "bitter" and "sour" taste modalities, as well as "astringency"). The purpose was to select a subset of independent variables (predictors) that predict a dependent criterion. Predictors are added and removed, in a stepwise manner, until the highest model R^2 is achieved. When two predictors are significantly and highly correlated to each other and to a dependent variable, the model only selects one of the predictors to be present in the model. The aim is to select a subset of predictors so that the resulting regression model is simple, yet has a good predictive ability (Snedecor & Cochran, 1989).

3. RESULTS

3.1. Phenolic content and sensory intensities

For the present study aglycones were excluded, due to only trace quantities being present in most infusions, while data for quercetin-3-*O*-robinobioside and ferulic acid were obtained, in addition to the other compounds present as determined by Koch *et al*. (2013). Trace quantities of the aglycones were also detected in rooibos infusions analysed by Joubert *et al.* (2012). **Table 2** summarizes the minimum, maximum, mean and standard deviation values of the phenolic compounds and the sensory attributes (taste and mouthfeel) of the full sample set (N = 260). Within **Table 2** there is also an indication of the astringency threshold values for PPAG, ferulic acid, hyperoside, rutin and isoquercitrin in water. According to **Table 2** large variation was observed in the content of the phenolic compounds. Several compounds varied from not detected (0 mg/L) to being present in significant quantities (aspalathin, nothofagin, hyperoside, rutin, isoquercitrin and ferulic acid), for example aspalathin with a mean value of 10.4 mg/L varied from not detected to > 50 mg/L. Based on the mean values, isoorientin and orientin were present in the highest quantities (> 23 mg/L). Other major compounds were PPAG, aspalathin and quercitrin-3-*O*-robinobioside (> 10 mg/L). The minor compounds, nothofagin, ferulic acid, vitexin, isovitexin, hyperoside, rutin and isoquercitrin were present in concentrations less than 5 mg/L. In contrast, limited variation was observed (ca. 10%) in the four palate attributes, especially given the fact that an intensity scale of 0 to 100 was used (**Table 2**). The two major attributes, "sweet" and "astringent", both had mean intensity values higher than 20. "Sour" and "bitter" scored mean intensity values less than 5. Given the intensity scale, intensity values of 20 are low, while intensity values of 5 are barely perceptible.

3.2. Association between phenolic compounds and potential trends due to production area or season

Fig. 1 illustrates the association between the samples and their phenolic compounds. In the DA plot (**Fig. 1(a)**) the position of the samples are plotted in relation to one another, based on the phenolic composition of the infusions. There is reasonable split in the samples with NC09, NC10, NC11 and WC11 samples lying in close association with one another (Group 1). Two other groupings are also evident with NC12 and WC12 forming Group 2 and NC13 and WC13 forming Group 3. Combined with the PCA plot (**Fig. 1(b)**) associations between the phenolic compounds and samples are indicated. Isoquercitrin, isoorientin and quercetin-3-*O*-robinobioside quantities indicated a trend to be higher in association with majority of the samples, except NC13 and WC13 in Group 3, which did not indicate this trend. The latter samples contained the highest levels of other compounds, especially PPAG, nothofagin and isovitexin. The individual phenolic content averaged over quality grades for the respective productions seasons and areas, summarised in **Table 3**, support these trends. **Table 4** summarises the phenolic content according to quality grade, averaged over production seasons and area. Significantly higher levels of a number of compounds were present in Grade A samples than the Grade D samples, with differences between grade B and C samples less defined. Considering the specific compounds did not differ, quality grade did not significantly affect aspalathin and ferulic acid content of the infusions. PPAG, isoorientin and orientin contents were not significantly different in A, B and C grade samples, but were significantly higher than in D grade samples. The flavonol glycosides, quercetin-3-*O*-robinobioside, hyperoside and rutin contents showed a similar trend, with A grade samples containing higher levels than the Grade B samples. Grade D samples had the lowest content of these flavonol glycosides, although their contents were not significantly lower than that of the Grade C samples. Vitexin and isovitexin showed the same trend with the Grade A and D samples having the highest and lowest contents, respectively. The isoquercitrin contents of Grade A, B and C samples were not significantly different.

Fig. 2 indicates the association between all samples, based on phenolic content and taste and mouthfeel attributes. In this case separation of the samples, split according to production season, rather than production area, is again evident. NC09, NC10, NC11 and WC11 samples (Group 1) clustered predominantly in the top right quadrant of the DA plot (**Fig. 2(a)**), associating with "sweet" taste, as indicated by the PCA plot (**Fig. 2(b)**). NC12 and WC12 (Group 2) samples lie in both the upper and lower left quadrants of the DA plot, while NC13 and WC13 (Group 3) samples clustered in the lower right quadrant. "Bitter", "sour" and "astringent" associated with the Group 3 samples, while Group 2 does not strongly associate with the sensory attributes.

For further insight into these associations principal component analysis was carried out on the 2012, and 2013 samples as these two production seasons represented large samples sets (N = 105 for 2012 and N = 85 for 2013). **Fig. 3** illustrates the 2012 samples in association with phenolic composition and sensory attributes. According to the PCA scores plot (**Fig. 3(a)**), no clear distinction between the Northern

Cape and Western Cape samples could be observed. Similarly, the 2013 samples also did not separate into groups based on production area (**Fig. 4(a)**). Furthermore, for both production seasons no grouping based on grading was evident. In this instance no clear-cut conclusions can thus be made relating production area and quality to specific compounds and/or sensory attributes.

3.3. Prediction of sensory attributes, based on phenolic composition, using regression analyses

Three methods, Pearson's correlation, PLS and step-wise regression analysis, were applied to the data in order to determine the predictive value of the phenolic compounds towards specific sensory attributes.

The results of the Pearson's correlation analysis are summarized in **Table 5**. Low, significant positive correlation ($p < 0.05$) can be observed between "sweet" and the compounds, isoorientin ($r = 0.188$), vitexin ($r = 0.129$) and isovitexin ($r = 0.162$). Similarly, low significant positive correlations ($p < 0.05$) between "sour" and PPAG ($r = 0.196$), aspalathin ($r = 0.259$), nothofagin ($r = 0.193$), vitexin ($r = 0.168$), hyperoside ($r = 0.189$), isovitexin ($r = 0.208$) and isoquercitrin ($r = 0.308$) were observed. "Bitter" correlates with PPAG ($r = 0.127$), aspalathin ($r = 0.150$), nothofagin ($r = 0.276$), orientin ($r = 0.158$), ferulic acid ($r = 0.167$), quercetin-3-*O*-robinobioside ($r = 0.194$), vitexin ($r = 0.170$), hyperoside ($r = 0.261$) and isovitexin ($r = 0.217$). Significant correlations between the mouthfeel attribute, "astringency" and aspalathin ($r = 0.131$), nothofagin ($r = 0.237$), orientin ($r = 0.146$), ferulic acid ($r = 0.138$), quercetin-3-*O*-robinobioside ($r = 0.151$), vitexin ($r = 0.340$), hyperoside ($r = 0.249$) and isovitexin ($r = 0.425$) were noted. The respective compounds, giving the highest correlation with a sensory attribute, was isoquercitrin, nothofagin and isovitexin for "sour", "bitter" and "astringent" respectively, with r ranging between 0.2 and 0.4. PLS was also conducted to determine the association between individual sensory attributes and the full range of phenolic compounds. This resulted in four PLS plots, illustrated in **Fig. 5**, indicating that the individual sensory attributes are not strongly associated with any specific phenolic compounds.

As alternative to Pearson's correlation analysis and PLS, step-wise regression analysis was evaluated as a method, primarily because it offers the advantage to determine the simultaneous contribution of the phenolic compounds to predict the variation in a sensory attribute. The results are summarized in **Table 6**.

The model R^2 values for the attributes, "sweet" and "astringent", present in highest intensities in the infusions (see **Table 2**), were 0.471 and 0.423, respectively. The other two attributes, "sour" and "bitter", present in low intensities in the infusions (see **Table 2**), had substantially lower model R^2 values ($R^2 < 0.3$). For "sweet", the compounds isoorientin, orientin, nothofagin, isovitexin, quercetin-3-*O*-robinobioside, rutin and hyperoside explained 47% of the variation in the intensity of this attribute. Isovitexin, isoorientin, hyperoside and isoquercitrin explained 42% of the variation in the "astringent" attribute. In the case of the "sour" attribute only 29.6% of the variance is explained by isoquercitrin, rutin, isovitexin, isoorientin, aspalathin, quercetin-3-*O*-robinobioside and nothofagin. Nothofagin, hyperoside, aspalathin and isoquercitrin explained only 23% of the variation in the "bitter" attribute. According to step-

wise regression analysis isoorientin, nothofagin and isoquercitrin correlated with three sensory attributes. All other compounds, except orientin and ferulic acid, correlated significantly with two sensory attributes. Interestingly, nothofagin correlated significantly with both "sweet" and "bitter".

4. DISCUSSION

The phenolic content of rooibos tea has previously been shown to vary greatly between production batches (Joubert *et al.,* 2012). Not only are plants propagated from seedlings, resulting in inherent variation, but processing is not controlled and standardised (Joubert & Schulz, 2006). Although not studied for rooibos, environmental factors such as climate and soil type are known to affect biosynthesis of plant polyphenols. Their role has been described by numerous studies on other plants (Tounekti *et al*., 2013; Agati *et al*., 2012; Hernández *et al*., 2011). The samples for the present study were collected from the two production areas over several production seasons to capture variation possibly caused by environment and season. The samples originated from different farms and/or plantations to allow for maximum variation. The results as indicated in **Tables 2** and **3** support this variation. In spite of expected segregation according to production area (average temperatures were lower in the Northern Cape area than the Western Cape area) (ARC, Agrimetric Services, 2013), no grouping according to production area was evident when using phenolic composition (as basis), or a combination of phenolic composition and sensory attributes. Year effects, however, were evident for phenolic composition and the combination of composition and sensory attributes. This would suggest that variation in climate from year to year was greater than climate difference and other environmental factors between these two production areas.

The variation observed in the intensity values of sensory attributes was relatively small, especially considering the 100-point intensity scale used. Given that "sour" and "bitter" scored mean intensity values less than 5 (i.e. barely perceptible) they could be considered of little importance in rooibos. "Sour" is usually indicative of "over-fermentation" and poor quality rooibos tea (Joubert, 1994), whereas "bitter" is most probably related to the polyphenols of rooibos. Roland *et al*. (2013) identified several structural features, present in rooibos flavonoids that could activate bitter taste receptors. The phenylpyruvic acid glucoside, PPAG, dissolved in water and tasted at ambient temperature, has been shown to have a slight bitter taste at 0.4 mg/L (Joubert *et al.,* 2013). This concentration is vastly lower than the concentration found in the present study, yet "bitter" intensity was scored extremely low. This phenomenon could be the result of masking. Ley *et al*. (2005) demonstrated that some flavanones (homoeriodictyol and eriodictyol) have significant bitter masking properties without affecting the taste of the product. Although not quantified, the presence of eriodictyol *C*-glucosides, oxidation intermediates of aspalathin, have been demonstrated in rooibos infusions (Beelders *et al.,* 2012; Iswaldi *et al.,* 2011) and these compounds could potentially affect this taste modality. Other factors that may affect taste perception include the concentration of the compounds/stimuli and temperature (Talavera *et al.,* 2007).

The major attribute, "sweet" taste, present at an intensity higher than 25 on a 100-point intensity scale, could also be linked to non-volatile compounds. Rooibos contains limited amounts of sugars, including oligosaccharides. "Fermentation", the oxidation process essential for formation of the flavour and colour of rooibos, could affect the enzymatic release of monomers (Coetzee *et al.,* 2014). Uncontrolled conditions during "fermentation" could therefore also impact on compounds resulting in sweet taste. Some flavonoids, even if not "sweet" tasting, have been found to have a sweet-enhancing effect on sugar (Ley *et al*., 2008). Flavonoids in rooibos have not yet been studied for this sweet-enhancing property.

"Astringency", the other major palate attribute, scored a similar low intensity as "sweet" taste. Astringency is an important attribute of rooibos, eliciting a slightly dry mouthfeel. When not perceived, the rooibos infusion is usually described as "flat". High intensities are also not desirable from a quality point of view as it results in a harsh, puckering mouthfeel. Koch *et al.* (2012) showed low quality rooibos associated with higher levels of astringency. In spite of the low concentrations of ferulic acid, hyperoside, rutin and isoquercitrin in the rooibos infusions, all of these compounds, except ferulic acid, have extremely low astringency threshold values (**Table 2**). PPAG, in addition to a "bitter" taste, also imparted a harsh astringent mouthfeel when tested in water at room temperature (Joubert *et al.,* 2013). The polymeric fraction of rooibos infusions has not been well characterised, but it is known that an irregular procyanidin type heteropolymer, containing (−)-epicatechin chain extending units, as well as (+)-catechin, and (+)-catechin as a terminal unit is present (Marais *et al.,* 1998). Very low concentrations of the dimer, procyanindin B3, and the trimer, bis-fisetinidol-(4β,6:4β,8)-catechin, are also present in fermented rooibos (Ferreira *et al.,* 1995). Several studies have demonstrated "astringent" and "bitter" sensations for catechins and procyanidins (Kielhorn & Thorngate, 1999; Peleg *et al*., 1999; Hugnagel & Hofmann, 2008a; Hufnagel & Hofmann 2008b; Lesschaeve & Noble, 2005)

The predictive value of the compounds towards the sensory attributes was investigated using a number of regression analyses. In view of the inherent slightly "sweet" and subtle "astringent" nature of rooibos shown by Koch *et al.* (2012), and confirmed by the present study (**Chapter 3**), prediction of these attributes is important.

In order to determine whether individual phenolic compounds are able to predict the presence of a taste or mouthfeel attribute, Pearson's correlation, PLS and step-wise regression analysis were conducted. None of these methods clearly indicated phenolic drivers of taste and mouthfeel attributes, in spite of the large data set used. This can be attributed to the natural variation in plant material on the one hand, and importantly, the "small" range of the intensity of the sensory attributes. Comparing the results to those of Koch *et al.* (2013), similarities were found with regards to certain phenolic compounds correlating with specific attributes. Isoorientin and orientin were seen to correlate with the "sweet" attribute by Koch *et al*. (2013), and through the step-wise regression model. There were however no similarities in the results obtained by Koch *et al*. (2013) and the prediction model for the attributes "astringency" and "bitter". The attribute "sour" was found to correlate with aspalathin and nothofagin in both studies. Although these

similarities were found, the correlations were not strong and therefore these compounds cannot be the sole phenolic compounds responsible for these attributes. However, these attributes can be used in further research as possible "predictors" of the above taste and mouthfeel attributes. The regression models were unable to take into account every factor (relationships of phenolics compounds; modulating effects) that plays a role in the manifestation of the taste and mouthfeel attributes of the infusions.

Given the results, it is clear that the range of sensory intensities should be substantially expanded to allow for better differentiation of attribute intensities. Furthermore, knowledge of the sensory qualities of the compounds is vital to ensure that taste-active compounds are considered for modelling. Then other statistical models such as multi-block analysis could be investigated as methods to predict the intensity of the taste and mouthfeel attributes.

5. CONCLUSIONS

The present study was undertaken to determine the phenolic constituents of rooibos infusions that contribute to its taste and mouthfeel. For this reason a large sample set was sourced from the two production areas, spanning a number of production seasons and quality grades. In spite of the large sample set, no area effect could be established and it was found that production seasons contribute to large variation in quantitative phenolic composition. Similar clustering was found in **Chapter 3**, indicating that the phenolic composition in the rooibos may have an influence on the sensory profile, and that the clustering was not necessarily due to a panel effect. The study identified "potential" candidate predictors, but further studies are required to confirm their predictive ability. This would require knowledge of the sensory quality/ties of the candidate predictors. It could also be of interest to pursue the expansion of DSA and targeted statistical methodologies to improve prediction based on composition.

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Table 1 A log of the rooibos samples encompassing all grades (A, B, C, D) spanning from 2009 - 2013 for the Western Cape and Northern Cape (N = 260).

Areas	Year	Grades				Totals
		A	B	C	D	
Western Cape (WC), South Africa	2011	6	6	6	6	24
	2012	18	20	20	6	64
	2013	10	15	16	2	43
Northern Cape (WC), South Africa	2009	6	6	-	-	12
	2010	6	6	6	-	18
	2011	6	6	4	-	16
	2012	4	26	8	3	41
	2013	3	26	9	4	42

Table 2 Minimum, maximum, mean and standard deviation for the respective sensory attributes (scored on a 100 point scale) as well as phenolic compounds (mg/L in water) for the full dataset (N = 260). Theoretical astringency threshold values for specific compounds are indicated. These threshold values, where available, were obtained from literature.

Variables[e]	Minimum	Maximum	Mean	Standard deviation	Theoretical threshold values for astringency (mg/L in water)
Sweet taste	16.417	27.379	21.363	2.189	
Sour taste	0.417	10.643	3.568	1.756	
Bitter taste	0.000	6.979	2.366	1.457	
Astringent	20.267	28.625	24.268	1.835	
PPAG	1.646	18.537	11.845	2.610	0.4[b]
ASP	0.000	51.544	10.414	7.419	
NOTH	0.000	17.881	2.698	1.805	
ISOORI	12.428	36.836	26.728	3.969	
ORI	11.851	31.708	23.084	3.137	
FerulicA	0.000	6.994	2.233	0.874	13[a] (Puckering)
QROB	0.358	34.520	16.182	6.916	
VIT	2.229	7.278	4.641	0.719	
HYP	0.000	12.324	4.353	2.189	0.19[cd]
RUT	0.000	12.595	3.355	2.705	0.0006[c]
ISOV	1.964	6.767	4.430	0.731	
ISOQ	0.000	12.021	2.865	2.063	0.33[d]

[a] Hufnagel & Hofmann (2008); [b] Joubert *et al*. (2013); [c] Scharbert *et al*. (2004); [d] Stark *et al*. (2005). [e] The following notations are used in the tables and figures to indicate the compounds: ASP (aspalathin), NOTH (nothofagin), PPAG (phenylpyruvic acid glucoside), ISOORI (isoorientin), ORI (orientin), FerulicA (ferulic acid), QROB (quercetin-3-*O*-robinobioside), VIT (vitexin), HYP (hyperoside), RUT (rutin), ISOV (isovitexin) and ISOQ (isoquercitrin).

Table 3 Mean values (mg/L) and least significant differences (LSD) for phenolic compounds[a] for different production season and area combinations.

Area x Season	PPAG	ASP	NOTH	ISOORI	ORI	FerulicA	QROB	VIT	HYP	RUT	ISOV	ISOQ
WC 2011	10.98[c]	12.85[a]	2.43[bc]	26.92[ab]	22.24[a]	2.37[ab]	16.23[b]	4.29[c]	4.66[a]	2.32[d]	4.42[cd]	2.37[bc]
WC 2012	11.64[bc]	11.17[ab]	2.55[bc]	26.25[b]	22.82[ab]	2.031[bc]	17.44[ab]	4.32[c]	4.49[ab]	4.06[b]	4.0[e]	2.97[b]
WC 2013	12.48[ab]	14.01[a]	4.03[a]	26.52[b]	23.46[a]	2.40[ab]	15.70[b]	4.72[b]	5.06[a]	2.69[cd]	4.74[ac]	2.95[b]
NC 2009	10.97[c]	6.22[c]	1.37[d]	27.68[ab]	22.50[ab]	2.82[a]	17.21[ab]	4.73[b]	4.27[ab]	2.90[bd]	4.45[bd]	1.70[c]
NC 2010	10.97[c]	12.41[a]	2.67[bc]	28.91[a]	23.62[a]	1.64[c]	20.28[a]	5.19[a]	5.12[a]	5.17[a]	4.67[ab]	4.57[a]
NC 2011	9.40[d]	5.54[c]	1.88[cd]	26.08[b]	21.68[b]	2.11[bc]	11.99[c]	4.62[bc]	4.05[c]	2.37[d]	4.32[bd]	2.08[bc]
NC 2012	11.77[bc]	8.30[bc]	2.17[bcd]	26.97[ab]	23.42[a]	2.22[b]	14.26[bc]	4.47[cb]	3.44[bc]	2.43[d]	4.10[de]	2.88[b]
NC 2013	13.62[a]	8.45[bc]	2.92[b]	26.36[b]	23.72[a]	2.44[ab]	16.14[b]	5.16[a]	4.34[ab]	4.02[bc]	5.0[a]	2.79[bc]
LSD (P=0.05)	1.26	3.89	0.96	2.13	1.70	0.47	3.55	0.36	1.15	1.36	0.35	1.11

The superscript letters indicate whether the phenolic content was the same or differed significantly according to the area x year. If the superscript letters differ, it indicates a significant difference between those particular season x area combinations within the columns. [a]The notations used in **Table 3** are explained in **Table 2**.

Table 4 Mean values (mg/L) and least significant differences (LSD) for phenolic compounds[a] for different quality grades (A, B, C and D).

Grades	PPAG	ASP	NOTH	ISOORI	ORI	FerulicA	QROB	VIT	HYP	RUT	ISOV	ISOQ
A	12.31^a	10.40^a	2.38^a	27.48^a	$23.33a$	2.012^a	19.78^a	4.64^a	5.33^a	4.57^a	4.43^a	3.44^a
B	12.11^a	11.00^a	2.94^a	27.12^a	23.49^a	2.36^a	16.18^b	4.73^a	4.34^b	3.31^b	4.51^a	2.79^{ab}
C	11.63^a	9.41^a	2.69^a	26.24^a	22.88^a	2.19^a	14.23^{bc}	4.60^{ab}	3.83^{bc}	2.71^{bc}	4.38^{ab}	2.64^{ab}
D	9.84^b	10.67^a	2.38^a	24.16^b	20.88^b	2.30^a	12.47^c	4.34^b	3.40^c	2.31^c	4.14^b	2.42^b
LSD (p=0.05)	0.92	2.84	0.70	1.56	1.23	0.34	2.60	0.27	0.83	0.99	0.26	0.81

The superscript letters indicate whether the phenolic content was the same or differed significantly according to grades within columns. If the superscript letters differ, then there is a significant difference between specific grades. [a]The notations used in **Table 4** are explained in **Table 2**.

Table 5 Pearson correlation plot for the taste, mouthfeel and phenolic compounds[a] found in the rooibos infusions (N=260) from the Western Cape and Northern Cape spanning the years 2009 – 2013.

Variables[a]	Sweet	Sour	Bitter	Astringent	PPAG	ASP	NOTH	ISOORI	ORI	FerulicA	QROB	VIT	HYP	RUT	ISOV	ISOQ
Sweet	**1**	**-0.138**	**-0.488**	-0.014	-0.079	-0.055	**-0.146**	**0.188**	0.016	-0.066	0.011	**0.129**	-0.037	0.070	**0.162**	0.077
Sour	**-0.138**	**1**	**0.155**	**0.243**	**0.196**	**0.259**	**0.193**	0.070	0.093	**-0.193**	0.087	**0.168**	**0.189**	0.004	**0.208**	**0.308**
Bitter	**-0.488**	**0.155**	**1**	**0.577**	**0.127**	**0.150**	**0.276**	0.043	**0.158**	**0.167**	**0.194**	**0.170**	**0.261**	0.102	**0.217**	0.010
Astringent	-0.014	**0.243**	**0.577**	**1**	0.105	**0.131**	**0.237**	0.083	**0.146**	**0.138**	**0.151**	**0.340**	**0.249**	0.078	**0.425**	0.013
PPAG	-0.079	**0.196**	**0.127**	0.105	**1**	**0.320**	**0.335**	**0.550**	**0.598**	**-0.209**	**0.536**	**0.505**	**0.480**	**0.428**	**0.558**	**0.541**
ASP	-0.055	**0.259**	**0.150**	**0.131**	**0.320**	**1**	**0.835**	**0.420**	**0.371**	**-0.300**	**0.599**	**0.247**	**0.625**	**0.502**	**0.380**	**0.571**
NOTH	**-0.146**	**0.193**	**0.276**	**0.237**	**0.335**	**0.835**	**1**	**0.370**	**0.379**	**-0.175**	**0.401**	**0.325**	**0.432**	**0.411**	**0.473**	**0.399**
ISOORI	**0.188**	0.070	0.043	0.083	**0.550**	**0.420**	**0.370**	**1**	**0.953**	**-0.134**	**0.631**	**0.781**	**0.439**	**0.565**	**0.803**	**0.501**
ORI	0.016	0.093	**0.158**	**0.146**	**0.598**	**0.371**	**0.379**	**0.953**	**1**	-0.085	**0.595**	**0.825**	**0.426**	**0.538**	**0.822**	**0.465**
FerulicA	-0.066	**-0.193**	**0.167**	**0.138**	**-0.209**	**-0.300**	**-0.175**	**-0.134**	-0.085	**1**	**-0.256**	-0.062	**-0.163**	**-0.400**	-0.047	**-0.580**
QROB	0.011	0.087	**0.194**	**0.151**	**0.536**	**0.599**	**0.401**	**0.631**	**0.595**	**-0.256**	**1**	**0.520**	**0.898**	**0.779**	**0.551**	**0.757**
VIT	**0.129**	**0.168**	**0.170**	**0.340**	**0.505**	**0.247**	**0.325**	**0.781**	**0.825**	-0.062	**0.520**	**1**	**0.391**	**0.502**	**0.918**	**0.438**
HYP	-0.037	**0.189**	**0.261**	**0.249**	**0.480**	**0.625**	**0.432**	**0.439**	**0.426**	**-0.163**	**0.898**	**0.391**	**1**	**0.562**	**0.466**	**0.716**
RUT	0.070	0.004	0.102	0.078	**0.428**	**0.502**	**0.411**	**0.565**	**0.538**	**-0.400**	**0.779**	**0.502**	**0.562**	**1**	**0.500**	**0.685**
ISOV	**0.162**	**0.208**	**0.217**	**0.425**	**0.558**	**0.380**	**0.473**	**0.803**	**0.822**	-0.047	**0.551**	**0.918**	**0.466**	**0.500**	**1**	**0.445**
ISOQ	0.077	**0.308**	0.010	0.013	**0.541**	**0.571**	**0.399**	**0.501**	**0.465**	**-0.580**	**0.757**	**0.438**	**0.716**	**0.685**	**0.445**	**1**

Values in bold are different from 0 with a significance level alpha=0.05.

[a] The notations used in **Table 5** are explained in **Table 2.**

[*] Significant correlations between the taste and mouthfeel attributes are highlighted in yellow.

Table 6 Step-wise regression model showing the percentage variation that the phenolic compounds[a] are able to explain within each of the three taste modalities, as well as the mouthfeel attribute, astringency.

	Summary of stepwise selection										
	Sweet taste			Sour taste		Bitter taste			Astringent mouthfeel	
Step	Variable entered[a]	Variable removed	Model R-Square	Variable entered	Model R-Square	Variable entered	Variable removed	Model R-Square	Variable entered	Model R-Square
1	ISOORI		0.0353	ISOQ	0.0949	NOTH		0.0764	ISOV	0.1809
2	ORI		0.3289	RUT	0.1758	FerulicA		0.1242	ISOORI	0.3702
3	VIT		0.3795	ISOV	0.205	HYP		0.1562	HYP	0.3828
4	NOTH		0.4144	ISOORI	0.2356	ASP		0.2029	ISOQ	**0.4226**
5	ISOV		0.4335	ASP	0.2577	ISOQ		0.2303		
6		VIT	0.4323	QROB	0.2748		FerulicA	**0.23**		
7	QROB		0.4483	NOTH	**0.296**					
8	RUT		0.4589							
9	HYP		**0.4714**							

[a]The notations used in **Table 6** are explained in **Table 2**.

[*] The final model R[2] values are highlighted in yellow.
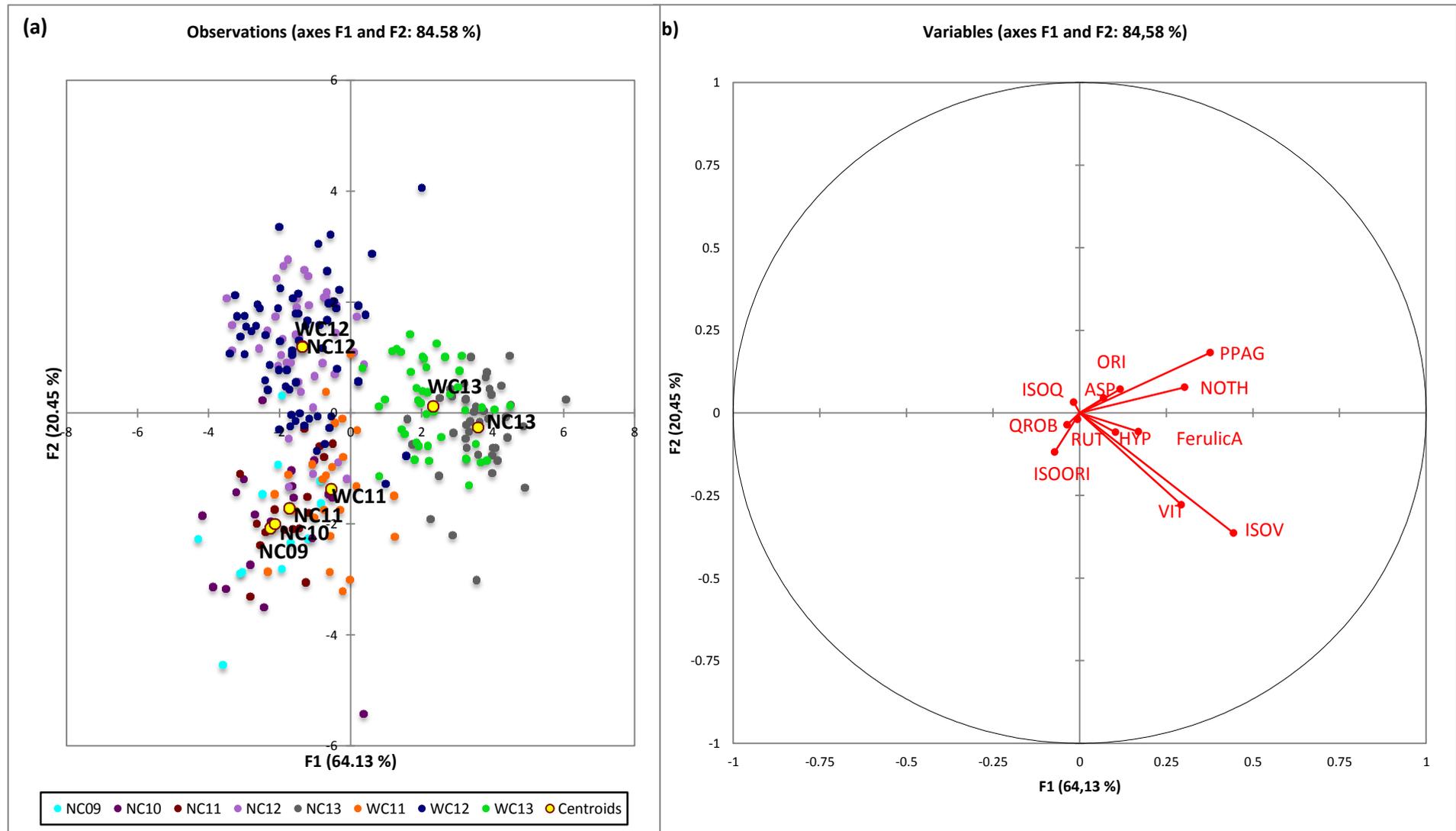
Figure 1 Discriminant analysis plot (DA) **(a)** illustrating the position of the samples (N = 260) separated according to production season (2009 – 2013) and area (Western Cape and Northern Cape). The principal component analysis (PCA) loadings plot **(b)** showing the position of the phenolic composition in relation to one another. The notations for the phenolic compounds used are explained in **Table 2**. The notations for the production areas are WC for Western Cape or NC for Northern Cape, followed by the production season (e.g. WC11 indicates the 2011 samples from the Western Cape).
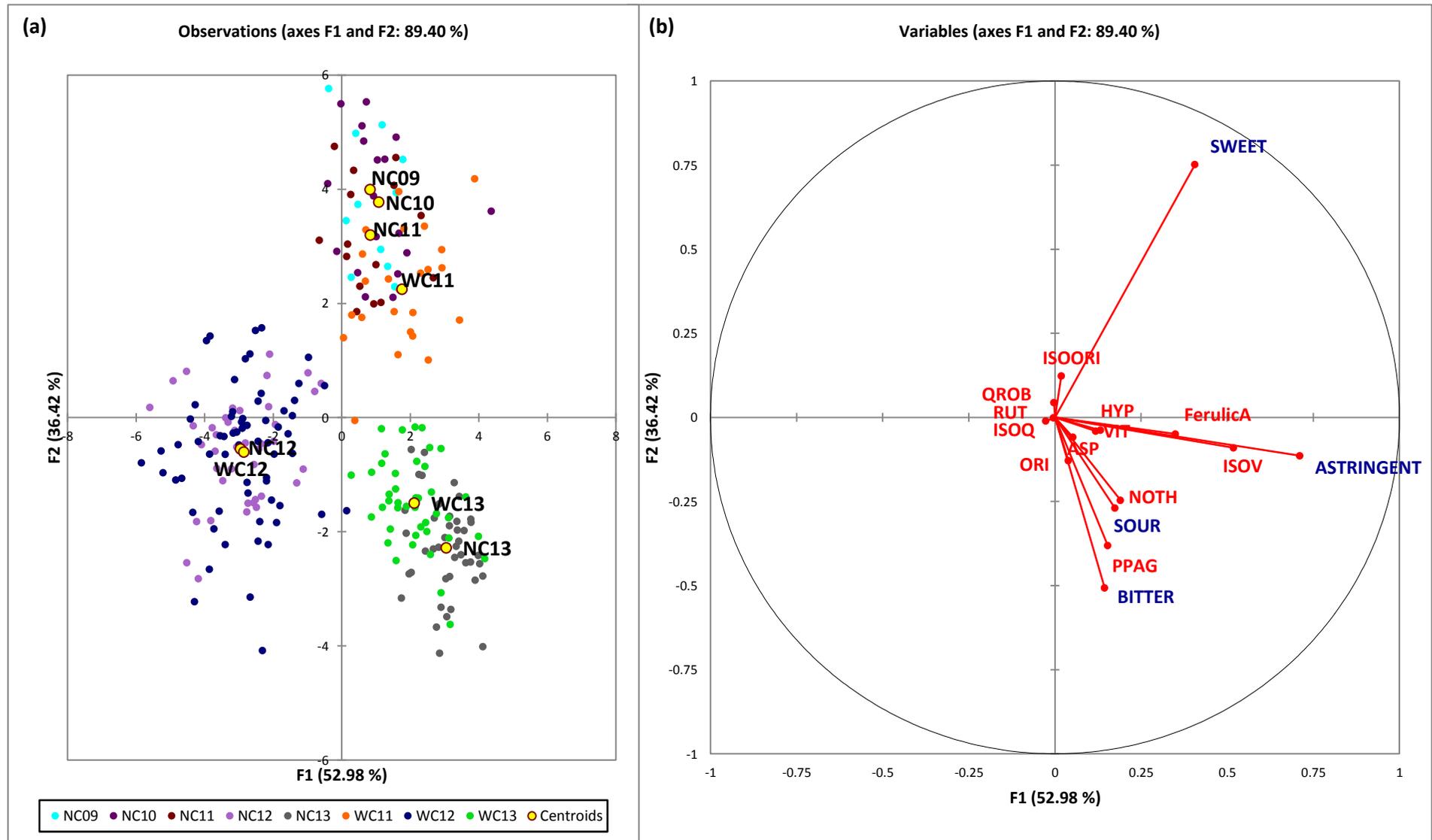
Figure 2 DA plot **(a)** illustrating the position of the samples (N = 260) separated according to production season (2009 – 2013) and area (Western Cape and Northern Cape). The PCA loadings plot **(b)** showing the association of the phenolic composition and sensory characteristics (taste attributes and astringency). The notations for the phenolic compounds are explained in **Table 2**. The notations for the production season and area are indicated in **Fig. 1**.
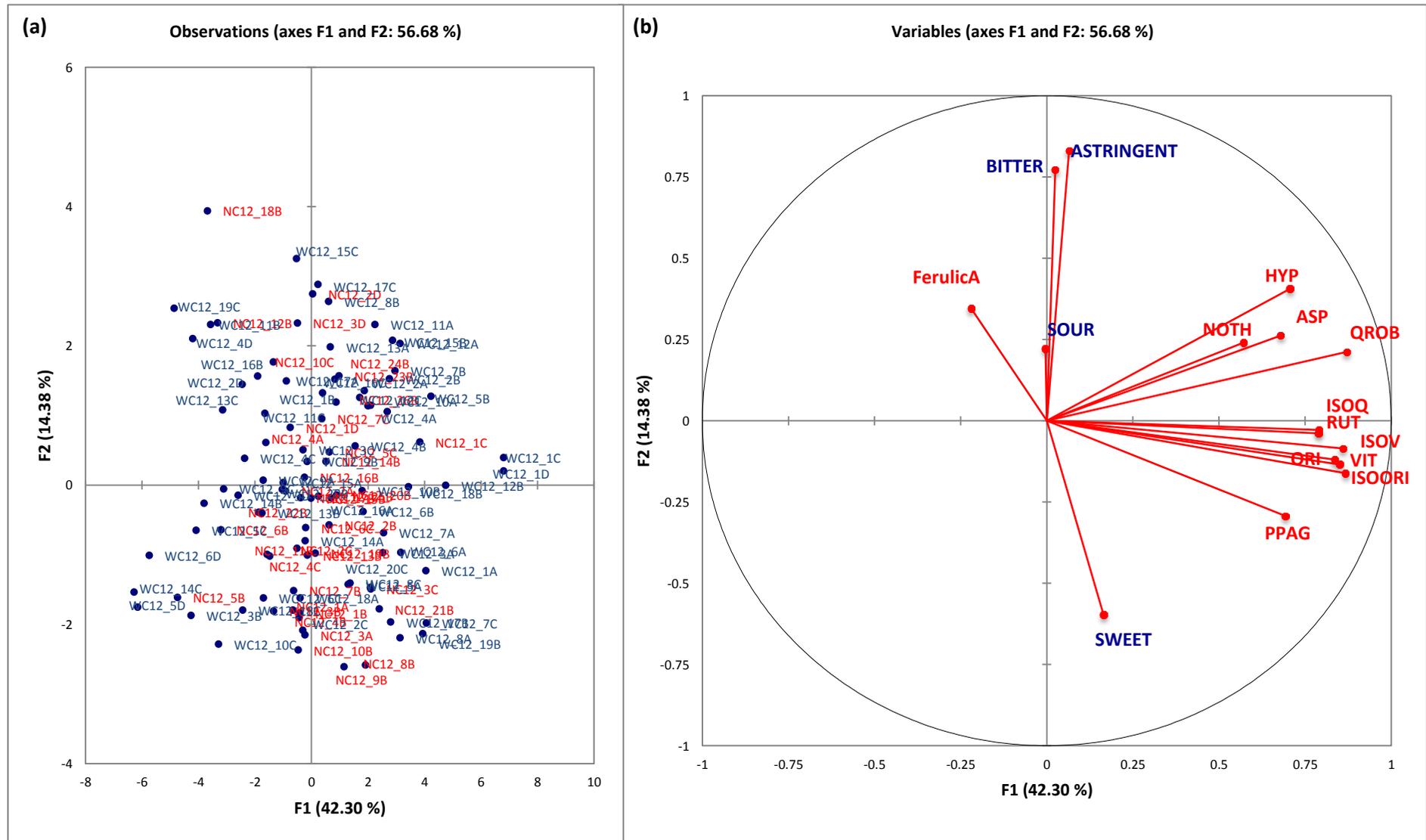
Figure 3 PCA scores plot **(a)** showing the position of the 2012 rooibos samples from the Western Cape and Northern Cape area and the relation of these samples with one another. The PCA loadings plot **(b)** illustrates the relationship between the phenolic compounds and the taste and mouthfeel attributes. The notations for the phenolic compounds are explained in **Table 2**. The notations for the production season and area are indicated in **Fig. 1**, given here with the addition of the sample code (e.g. WC12_3B is the Western Cape sample 3B from 2012).
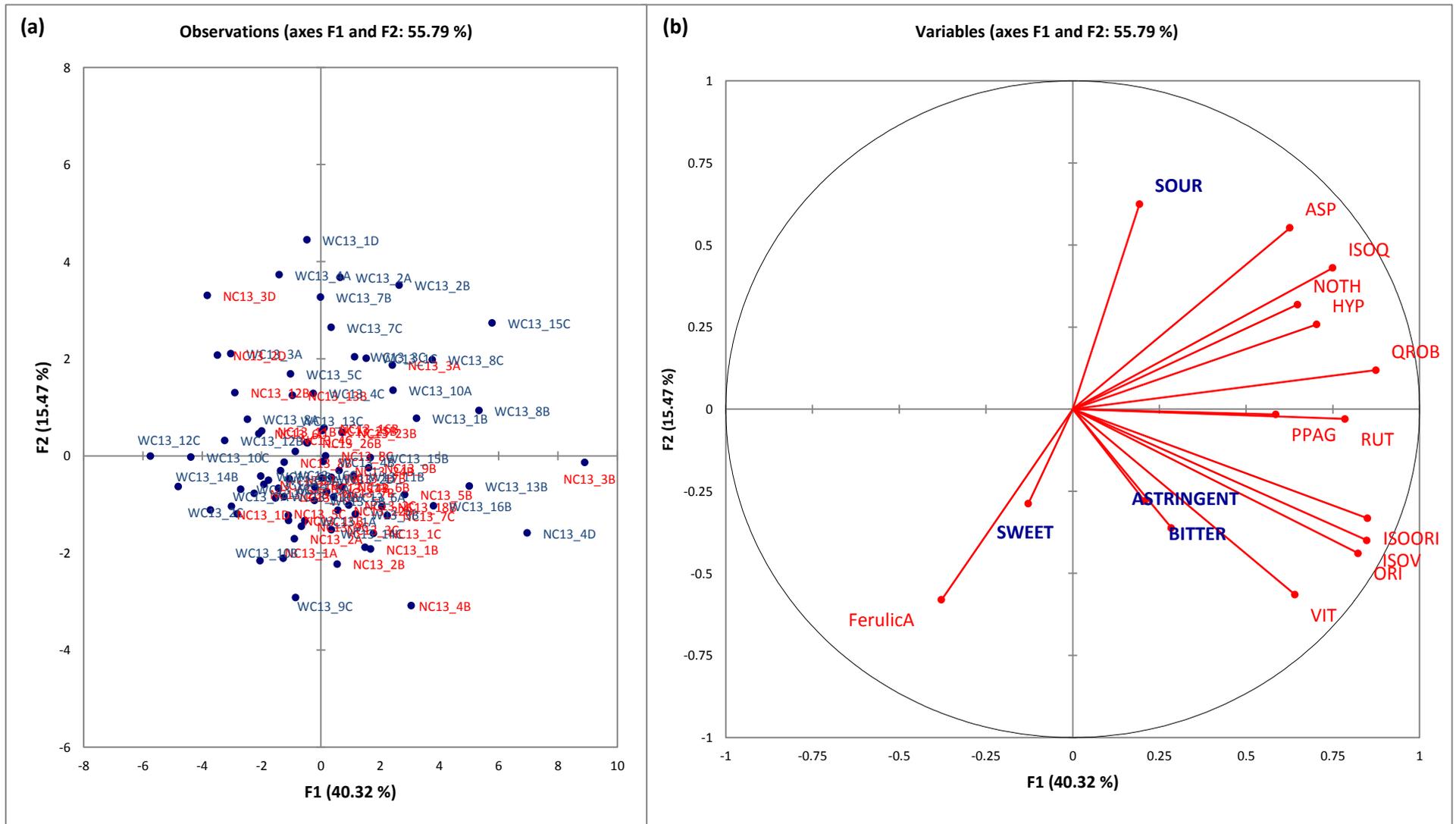
Figure 4 PCA scores plot **(a)** showing the position of the 2013 rooibos samples from the Western Cape and Northern Cape area and the relation of these samples with one another. The PCA loadings plot **(b)** illustrates the relationship between the phenolic compounds and the taste and mouthfeel attributes. The notations for the phenolic compounds are explained in **Table 2**. The notations for the production season and area are indicated in **Fig. 1**, given here with the addition of the sample code (e.g. WC13_3B is the Western Cape sample 3B from 2013).
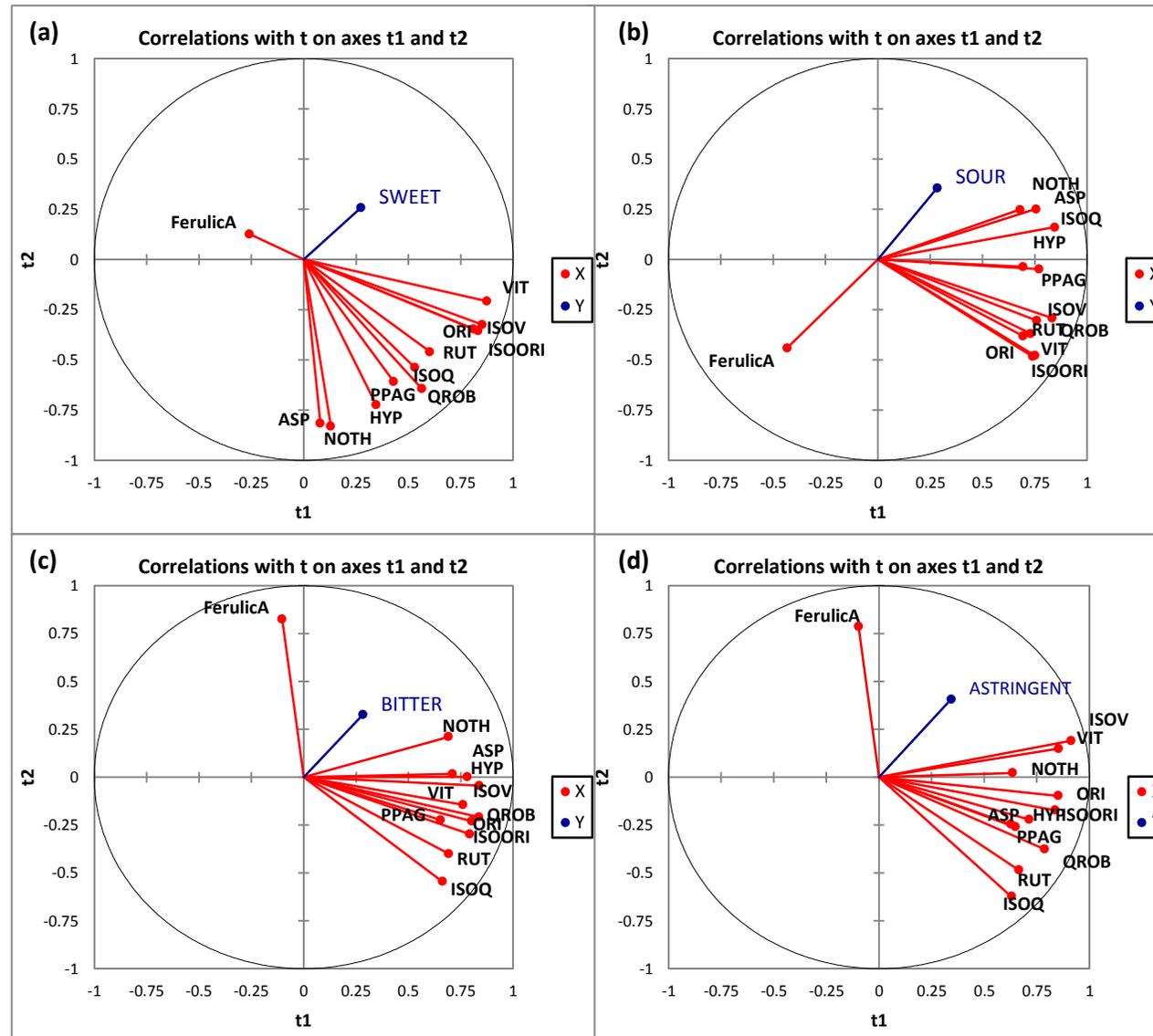
Figure 5 PLS regression plots showing the correlations between the phenolic compounds and the sweet **(a)**, sour **(b)**, bitter **(c)** and astringent **(d)** attributes, respectively. The notations for the phenolic compounds used are explained in **Table 2**.

CHAPTER 5

Development of a quality control tool for the rooibos industry: a rapid profiling method of sensory quality

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ABSTRACT

Rapid sensory methods are being researched as alternatives to the more time-consuming methods such as descriptive sensory analysis (DSA). For this study, the main aim was, through the use of **instructed sorting**, to determine the potential use of sorting when evaluating rooibos infusions for *overall sensory quality*. The second aim was to categorise a range of samples representing the characteristic primary and secondary *aroma profiles* of rooibos, using **uninstructed sorting**. Both methods were followed by a descriptive task, so as to allow for comparison with previously obtained DSA results. A selection of fermented rooibos samples were categorised according to *overall sensory quality* (i.e. high quality, medium quality and low quality) of the infusions using **instructed** sorting. The samples of a high quality needed to exhibit zero, or very few negative attributes at low intensities (intensity < 5), whereas the low quality samples needed to contain these negative attributes at intensities higher than the average intensity of each negative attribute. The medium quality samples, however, were viewed as having neither a prominent positive or negative profile, but rather a combination of both positive and negative attributes each with an average intensity. Using the rapid method of sorting, it was determined that it is possible to discern between samples of a high and low **aroma** quality with ease. Sorting of medium quality samples, as well as sorting based on the **palate** (**taste and mouthfeel**) quality proved more of a challenge, leading to less conclusive results. The inclusion of the descriptive task verified the results from **Chapter 3**, stating that high quality samples associate with "fynbos-floral", "honey" and "rooibos-woody" aroma notes whereas the low quality samples associate with "hay/dried grass", "green grass" and "rotting plant water" aroma notes. **Instructed** sorting was therefore demonstrated as a screening tool to determine *overall sensory quality* of rooibos, prior to further quality analyses. By applying it, the large variation between samples of the same quality grade can be minimised. **Uninstructed** sorting, performed on samples representing the primary "characteristic" profile and the secondary "characteristic" profile of rooibos, indicated the ability of the panel to discern between the profiles based on the *aroma profile* of the samples. As with DSA, it was found that the primary profile was represented by "honey", "rooibos-woody" and "fynbos-floral" aroma notes, whereas the secondary profile associated with "caramel", "fruity-sweet" and "apricot" aroma notes, assigned during the descriptive task. These results confirmed the potential of this method to aid in the profiling of rooibos for blending and marketing purposes. If used in conjunction with the already developed sensory lexicon and sensory wheel, both **instructed** and **uninstructed** sorting can be used as quality control tools, to help standardise the quality grading method, as well as minimise quality and profile variation within blended rooibos samples.

1. INTRODUCTION

Within industry the determination of sensory quality plays a vital role in quality control, i.e. to determine profile maps but also to ensure that product quality is consistent. Currently, descriptive sensory analysis (DSA) is one of the most extensively used tools to determine the full sensory profile of a product, both

qualitatively and quantitatively (Lawless & Heymann, 2010). In the current study DSA has thus been used to characterise rooibos in terms the full sensory profile (**Chapter 3**). This method is, however, time consuming and there has been a trend towards the development and use of methods that are able to give reliable results in a more rapid manner, so as to save time and therefore cost (Kemp *et al.*, 2009; Louw *et al.,* 2013).

One of the major motivations behind the development of rapid techniques is to provide industry with valid tools for sample screening and quality control (Varela & Ares, 2012). An example of one of these newly developed methods, gaining in popularity, is **sorting** (Blancher *et al.*, 2007; Cartier *et al.*, 2006; Chollet *et al*., 2011). In principal, sorting is a classification technique that has been widely used in both psychology and sociology (Varela & Ares, 2012). The main aim of sorting is to measure the overall degree of similarity or dissimilarity between various samples. This is determined by the manner in which these samples are sorted into different groups (Varela & Ares, 2012). Sorting is simple and quick to execute, and can be either **uninstructed**, i.e. the panel is not given any sorting guidelines, or **instructed**, i.e. the panel is provided with the definitions or guidelines according to which they must sort the samples (Chollet *et al.*, 2011; Courcoux *et al*., 2012; Lelièvre *et al*., 2008). Samples that are sorted into the same group do not all represent that group equally and may not contain all the characteristics that the other samples exhibit, however, these samples will share more similar attributes with one another than with the samples within another grouping (Ballester *et al.*, 2008). Once the products have been sorted into different groupings, it is possible for the researchers to analyse the data as is, or this rapid method can be extended by the addition of a descriptive task. In the latter case, the panel of judges are instructed to assign a number of descriptors that best describe each of the groupings of samples that were formed during the sorting task, providing a concise understanding of the sensory drivers of quality (Chollet *et al.,* 2011).

The number of assessors required to produce stable sorting results is not clearly indicated in literature, and remains a much-discussed topic when conducting a sorting task. Between 8 and 22 panellists (Abdi *et al.,* 2007; Deegan *et al*., 2010; Chollet *et al.* 2011) have been used to perform sorting on different products, although Blancher *et al*. (2012) indicated that the stability of the results tends to be influenced more by the efficacy of the sorting task itself, and less so by the number of panellists. It has also been indicated that the results of the sorting task can be influenced by the experience of the panel performing the sorting task. Both trained and untrained panellists have been used to perform sorting tasks (Cartier *et al*., 2006; Chollet *et al*., 2011; Louw *et al*., 2013). It has been found that results obtained from trained panellists (expert) tend to be more in agreement, and more comparable to conventional profiling (DSA) (Chollet *et al*., 2011; Lelièvre *et al*., 2008), than that obtained from naïve judges, leading to more stable maps being produced by trained assessors (Blancher *et al*., 2012; Louw *et al*., 2013).

As indicated, there are a number of advantages associated with the sorting technique; however, like most sensory analysis techniques there are also a few disadvantages. It is important to remember when performing any sensory technique, that the testing method needs to be chosen, not only with the

required output in mind, but also the stability and nature of the product in question. In cases when products exhibit complex or possibly unstable characteristics, it is important to ascertain whether the sorting task is the best choice, or whether the task should be carried out in a controlled environment and on a smaller scale (Varela & Ares, 2012).

Sorting data can be analysed using multidimensional scaling (MDS) or DISTATIS and correspondence analysis (CA) (Chollet *et al.*, 2011). MDS leads to the production of spatial or pictorial representations indicating similarities and dissimilarities between samples based on the distance between the samples (Deegan *et al.,* 2010; Abdi *et* al., 2007). The one limitation of the MDS method is the loss of the differences between the individual judges, due to the pooling of the sorting data (Lawless *et al.,* 1995). DISTATIS, however, takes into account the data from each individual assessor involved in the sorting task (Abdi *et al.*, 2007) and from the resultant plots one can ascertain whether the samples are similar or dissimilar. Thereafter, CA can be used to evaluate the similarity between products, when the sorting task is accompanied with a descriptive assignment (Cadoret *et al.*, 2009). Repeating the sorting task can test the reliability of sorting data. Consistent results have been achieved when using both trained and untrained assessors to perform the sorting task (Chollet *et al*., 2011; Cartier *et al*., 2006). Ward's cluster analysis can be performed on the DISTATIS and CA plots, to give further insight into the groupings of the samples, based on more than 2 dimensions (M. Kidd, Stellenbosch University, Stellenbosch, South Africa, May 2014, personal communication).

Quality grading of rooibos is done according to criteria set up by the respective rooibos processors. The grading of rooibos can be based on a combination of leaf colour (wet and dry), cup (infusion) colour and sensory attributes of the infusion. The colour of the infusion and leaf, the aroma as well as the overall palate attributes (flavour, taste and mouthfeel) of the infusion, give the grader an insight into the fermentation process and ultimately the quality of the final product. Under-fermented or over-fermented rooibos leads to a lower grade tea (J. Basson, Rooibos Ltd., Clanwilliam, South Africa, April 2012, personal communication). A problem regarding the grading of rooibos is that there is no uniform quality grading system in place that can ensure the reliable grading of rooibos into quality grades. Therefore each processor assigns the quality grade of the tea based on the criteria they deem to be the most important. These criteria can differ between processors, therefore leading to large variation between samples given the same final quality grade. Variation due to the lack of a standardised grading procedure can be prevented with the use of standardised grading criteria.

Several tools have been developed to aid in standardising rooibos sensory quality grades based on sensory quality. The first draft of the sensory wheel and lexicon for rooibos (Koch *et al.,* 2012) has been updated and verified (see **Chapter 3**), the use of which could lead to a decrease in variation between samples of the same quality grade, across processors. In addition to the use of these tools, it would be beneficial to develop a reliable and rapid quality grading method for use by smaller processors or small-scale individual farmers, to ensure consistency in rooibos quality grades.

In view of the above, the aim of this study was two-fold: The first aim was to determine the potential of sorting when evaluating rooibos infusions for **overall sensory quality** (i.e. high quality, medium quality and low quality) using **instructed** sorting. The second aim was to categorise a range of samples representing the **characteristic aroma profiles of rooibos** (i.e. primary and secondary) using **uninstructed** sorting. All sorting tasks were followed up with a descriptive task and replicated to test for consistency.

2. MATERIALS AND METHODS

2.1. Rooibos samples

The rooibos samples used for the sorting were selected from the large sample-set, spanning five production years (2009-2013).

Samples were selected to represent the two production areas for **instructed** sorting according to **overall sensory quality**, i.e. the Northern Cape and the Western Cape, South Africa. A total of 12 samples were selected from each area. Each set of 12 samples was selected to represent three quality groupings, high quality, medium quality and low quality (4 samples per quality sub-group), based on the full range of positive and negative attributes associated with each sample, as previously determined by DSA (refer to **Chapter 3**). All the samples used contained both positive and negative attributes, although the ratio of positive and negative attributes differed, making the sample either high, medium or low in quality. For this study, the criteria that had to be met for each of the quality groupings (high, medium and low) were decided upon by the researchers and were based on the intensities and presence of the positive and negative attributes, and were not based on the original quality grading given by industry. A high quality sample needed to contain virtually zero or a very low intensity (intensity < 5) of perceptible taints or negative attributes. For a sample to be low quality it had to contain a high number of taints or negative attributes (intensity higher than average for each attribute; unpleasant), whereas a medium quality sample needed to contain a near equal ratio of both positive and negative attributes, at an average intensity for each attribute. **Table 1** summarises the details of the selected samples indicating their quality grades as decided based on above-mentioned criteria, sourced from a collection of Western Cape samples, whereas **Table 2** indicates the Northern Cape samples and the chosen quality grades.

For **uninstructed** sorting 12 samples were selected to represent the **characteristic rooibos aroma profiles**. The primary profile consists of predominant "rooibos-woody", "fynbos-floral", and "honey" aroma attributes, whereas the secondary profile consists of predominant "fruity-sweet", "caramel" and "apricot" aroma attributes. The total group of 12 samples represented the two production areas with 6 samples per area. Splitting the 6 samples from each area again, 3 samples represented the primary profile and the other 3 samples represented the secondary profile (**Table 3**). Although negative attributes are present in the rooibos samples, the samples chosen for the **characteristic aroma profiles** contained these attributes at intensities below average (intensity < 10), so as to have little effect on the overall aroma profile. The sample infusions were prepared and served according to the protocol stipulated in **Chapter 3**.

2.2. Descriptive sensory analysis (DSA)

Data previously obtained from descriptive sensory analysis (**Chapter 3**; Koch *et al*., 2012) were used for the analyses. Each sample was analysed in triplicate in three consecutive sessions on one day using the same trained panel (N = 9), previously used for DSA. Data were captured using Compusense® *five* software (Compusense, Guelph, Canada).

2.3. Sorting methodology

2.3.1 Instructed sorting to test for overall sensory quality of rooibos sourced from different production regions

Instructed sorting involves giving the panel of assessors a set of instructions that they need to adhere to during the sorting task. These instructions state the sensory criteria that need to be considered when conducting the task, the maximum number of groupings to be made, as well as the maximum number of samples allowed per grouping (See **Addendum B; Fig. B14**).

In the case of the **instructed sorting** of rooibos samples according to ***overall sensory quality***, the panel was instructed to group the samples into 3 groups based on the sensory quality. The criteria were high quality with minimal perceptible taints, i.e. negative attribute (intensity < 5), low quality with a high number of perceptible taints (intensity higher than average), and medium quality with an equal ratio of positive and negative attributes (intensity below average). These criteria are based on the perceivable aroma and palate intensities, as analysed by each panellist. Each of the groups formed were to contain no more than six samples. For the descriptive task assessors were instructed to provide no more than five descriptors to describe each of their groupings, so as to substantiate the reason for grouping the samples as such (See **Addendum B**; **Fig. B14**).

On each testing day, the panellists were presented with two samples sets, each consisting of 12 samples (labelled A – L), in two consecutive sessions (session 1 & session 2). In session 1 the panellists were asked to analyse the samples based on the **aroma** attributes, while in session 2, sorting was based only on the **palate** attributes (flavour, taste and mouthfeel). A 10-minute break was given to the panel between the sessions, primarily to reduce panel fatigue. The sample codes (A - L) were randomised across the samples during each session, ensuring that the position of the samples differed each time they were presented to the panellists i.e. sample B for repetition 1 (rep 1) would be sample L for repetition 2 (rep 2), etc. Two infusions, one for each session (**aroma** and **palate**), were made for each sample so that the panel was ensured that each analysis took place using a new sample.

Each of the sample sets was analysed in duplicate. Aroma (rep 1) and palate (rep 1) were analysed on the first day, followed by the second replications of aroma and palate on the following day, for each of the production areas. Day 1 and 2 entailed the sorting of the Western Cape samples, while the Northern Cape samples were analysed on day 3 and 4.

2.3.2 Uninstructed sorting to test for characteristic rooibos profiles

The panellists were required to sort the samples into groups based on the *similarity of characteristic aroma profiles.* They were given no instructions pertaining to the groupings in which they needed to sort the samples. The only guideline that needed to be adhered to was, that the panellists were to place no more than 6 samples in a single group and the maximum of number of groups was limited to six. For the additional descriptive task, it was again important that the panellists provided no more than five descriptors for each of the sample groupings (See **Addendum B; Fig. B15**)

The panellists received the samples in the same manner as during the instructed sorting. Two replicates of uninstructed sorting of the *aroma* profiles were conducted on Day 5 (rep 1 and rep 2) to determine panel consistency.

2.4. Panel of assessors

The same panel was used for both the **instructed** and **uninstructed** sorting tasks. This panel consisted of 12 panellists, all with extensive experience of analysing rooibos quality, i.e. knowledge of the full range of positive and negative aroma, flavour, taste and mouthfeel attributes associated with rooibos quality. The same panellists were responsible for the DSA of the full sample set (**Chapter 3**), however, none of the panellists had prior experience of **instructed** or **uninstructed** sorting. The panellists used for the two sorting experiments could thus be regarded as experts and thus trained judges.

2.5. Statistical procedures

Principal component analysis (PCA) was performed on the DSA data, averaged over the assessors (Lawless & Heymann, 2010). DISTATIS was used to analyse both the **instructed** and **uninstructed** sorting data (Abdi *et al.,* 2007). This method takes into account the data from each assessor involved in the sorting task and the resulting plots indicated whether the 12 samples could be categorised into different groupings. Correspondence analysis (CA) was used to evaluate the similarity of samples, based on the descriptors assigned to the samples during the descriptive task (Cadoret *et al.,* 2009). R_V coefficients were calculated to measure the similarity between product configurations (Abdi *et al*., 2007). The R_V coefficients range from between 0 and 1 and the closer the values are to 1, the more similar the groupings on the plots (Nestrud & Lawless, 2009). Agglomerative hierarchal clustering (AHC) was then performed on the DISTATIS plots. This method produces tree configurations indicating similarities and differences between samples (Giacalone *et al*., 2013). Ward's cluster analysis was used on the data in order to create the clusters of samples deemed similar through their sensory characteristics (De Saldamando *et al.,* 2013). This method allows for the relationships between samples to be viewed on more than two principal components or dimensions. All data analyses were performed using the STATISTICA program (Statistica 10, StatSoft Inc., Tulsa, Oklahoma, USA).

3. RESULTS

The sorting technique was evaluated both for **instructed**, and **uninstructed** sorting. For the **instructed** task the panel was asked to sort the selected rooibos samples based on ***overall sensory quality.*** Here the aim was to determine whether it was possible to rapidly discern between high quality, medium quality and low quality rooibos based on **aroma** or **palate** attributes. In contrast, **uninstructed** sorting was conducted to determine whether an expert panel of judges could group rooibos samples according to the similarity or dissimilarity of ***characteristic aroma profiles***. During this study it was discerned that fermented rooibos, for the most part, could be split between two different aroma profiles, the first being the primary characteristic profile and the other being the secondary characteristic profile. The attributes included in each of these profiles were determined in **Chapter 3**.

After the sorting analysis was complete, the samples were assigned specific codes for the purpose of data analysis. These codes were based on the profile or quality for which the sample was initially selected to represent during the sorting task. These codes are used in the respective **tables** and **figures** to represent the samples. For the sorting according to ***overall sensory quality***, the samples were given the codes "H", "M" and "L", to indicate high quality, medium quality and low quality, respectively (**Tables 1 & 2**). The samples numbered from 1-4 indicate the samples from the Western Cape, i.e. H1 is the first high quality sample from the Western Cape. Northern Cape samples were given the number coding from 5-8, i.e. M8 is a medium quality sample from the Northern Cape. With the experiment on the ***characteristic rooibos aroma profiles,*** i.e. the primary and secondary characteristic profiles, the samples were given a code relating to the profile grouping, as well as the production area of that sample. The ***characteristic aroma profile*** samples were given the codes "P" and "S", indicating the primary and secondary profiles, respectively. To indicate production area, the samples were given an "N" for the Northern Cape and "W" for the Western Cape, i.e. PN represents a primary profile sample from the Northern Cape. In addition to the profile and area codes, the samples were numbered from 1-3 for each particular profile and area combination, i.e. PN1 represents the first sample from the Northern Cape fitting the primary characteristic profile, whereas SN3 indicates that it is the third sample from the Northern Cape, represents the secondary characteristic profile.

3.1. Instructed sorting to test for *overall sensory quality* of rooibos sourced from two production regions

Table 1 includes details of the Western Cape samples used during the **instructed** sorting, the overall sensory quality designation of each sample, the year of production, and assigned code for easy recognition on the plots.

The DISTATIS plots, **Fig. 1(a & b)** to **Fig. 4(a & b)**, focus on the samples of both the Western Cape and Northern Cape regions, sorted using an **instructed** sorting technique. As mentioned, the samples were to be sorted into groupings based on the samples having a high, medium or low **overall sensory quality**

(aroma and palate). Each of the sorting sessions was carried out in duplicate, in order to determine the repeatability of the method, primarily with regard to its potential use as a rapid grading tool.

Fig. 1(a) and Fig. 1(b) depict the data of the two replicates for the samples from the Western Cape, sorted according to **aroma** quality. Focusing on the DISTATIS plots alone, it is clear that for the first two dimensions (PC 1 & PC 2) the samples do not lie in the same positions for rep 1 and rep 2. There are, however, similarities in the way in which the samples group together, the high quality samples tend to be located to the left and the low quality samples to the right of the plots. In order to verify the clustering of the samples, Ward's cluster analysis was performed and the results for the two replications are depicted in Fig. 1(c) and Fig. 1(d), respectively. The linkage distance chosen as the point for determining clusters is decided upon by selecting the major or most prominent link in the plot, whilst taking the results of the DISTATIS plot into account (M. Kidd, Stellenbosch University, Stellenbosch, South Africa, May 2014, personal communication). Fig. 1(c) depicts the clusters formed for the first replication; however, the clusters formed were calculated using the first four dimensions, and not only two, as was the case for the corresponding DISTATIS plot. Examining an increased number of dimensions increases the ability to correctly ascertain the formation of groupings of samples, as some of the samples may in fact be lying on a different dimension, which is not clear when just considering only the first two dimensions (M. Kidd, Stellenbosch University, Stellenbosch, South Africa, May 2014, personal communication). Fig. 1(c) illustrates that the samples of rep 1 are split into 3 groupings at the major linkage distance of 0.8. Group 1 is made up of the samples H2, H3, H1 and H4. Group 2 contains the samples M1, M2, L4 and M3. The third group (Group 3) contains samples which are the most different from Group 1, and which were sorted furthest from the first group. Samples L1, L3, L2 and M4 form Group 3. Fig. 1(d) for rep 2 illustrates the same clustering of the samples at the 0.8 linkage distance. Although the positions of the samples may have changed slightly, the samples within each of the groupings remained the same. These quality groupings illustrated in the cluster analysis plots can also be clearly seen in Fig. 1(a) and Fig. 1(b), therefore verifying the groupings seen in the DISTATIS plots. The R_V coefficient between rep 1 and rep 2 (Fig. 1(a & b)) was greater than 0.5 (R_V = 0.66), which indicates a moderate consistency between the two replications (Table 4).

The samples from the Northern Cape (Table 2) were also sorted based on the quality of the **aroma** attributes of the samples. This again was done in duplicate, with the results of rep 1 depicted in Fig. 2(a) and rep 2 in Fig. 2(b). Applying Ward's cluster analysis for the first replication it is clear that the samples separated into three groups at the major linkage distance of 0.8 (Fig. 2(c)). These groupings are explained based on the first five components, as opposed to the first two components as is seen on the corresponding DISTATIS plot (Fig. 2(a)). Fig. 2(c) clearly indicates the three groupings, which can also be seen in Fig. 2(a). Group 1 is made up of samples L7, L8, L5 and L6. The second grouping (Group 2) only contains two samples, namely samples H6 and H8, which are seen to lie close to one another (Fig. 2(a)). The third and largest group (Group 3) contains the samples M7, M6, H5, M5, H7 and M8. Although only

explaining 2 components, it is clear that the groupings are separated in a similar fashion on the corresponding DISTATIS plot (**Fig. 2(a)**). For the second replication (**Fig. 2(b)**), the clustering of samples on the DISTATIS plot, as explained by the first 2 principal components, is not quite obvious. However, when viewing **Fig. 2(d)**, it becomes clear that there is an obvious split between the samples into two distinct groupings at the major linkage distance of 1.0. The decision regarding the appropriate linkage distance is decided upon by taking the DISTATIS plot, as well as product knowledge into account (M. Kidd, Stellenbosch University, Stellenbosch, South Africa, May 2014, personal communication). This grouping of samples, as explained by the first four principal components (**Fig. 2(d)**), is quite similar to the diffuse grouping illustrated in **Fig. 2(b)**. The first and largest of the groups contains samples H5, H6, L8, M8, M5, H8, H7, M6 and M7. The second grouping is a lot smaller and contains only three samples namely, L5, L7 and L6. What is clear from these DISTATIS plots is that the samples in rep 1 do not lie in the same position as the samples in rep 2, i.e. in rep 1 the low quality sample L8 is grouped together with the other low quality samples, whereas in rep 2 sample L8 was clearly grouped together with the other medium and high quality samples. This can also be seen through the low R_V coefficient of less than 0.5 ($R_V = 0.4$) in **Table 4**, indicating the difference in the groupings within these two DISTATIS plots, further confirming the observation that there is a low replication correlation.

What is thus clear from **Fig. 1** and **Fig. 2**, both illustrating the aroma quality of a range of rooibos samples from the Western Cape and Northern Cape respectively, is that it is possible to sort rooibos samples according to *aroma quality*. This is especially true for the samples sourced from the Western Cape region where it was possible to group the samples according high, medium and low quality (**Fig. 1 (a & b)**).

Fig. 3(a) and **Fig. 3(b)** depict the samples of rep 1 and rep 2 from the Western Cape that were sorted according to the quality of their **palate** profiles, i.e. flavour, taste and mouthfeel attributes. The DISTATIS plots show a possible three groupings in each of the plots, but the samples forming each of these groupings are not in exactly the same positions. Again it must be remembered that these groupings are explained over two dimensions, and therefore various samples that may appear to lie together, may in fact be lying on a different dimension and may consequently not be grouped together. For the verification of the groupings, Ward's cluster analysis was also performed. In **Fig. 3(c)**, the results for rep 1 can be seen, described over 5 components. It is clear that, at a linkage distance of 0.8 the samples split into 3 groupings. Group 1 contains the samples, L4, H3, L2 and H2. Group 2 and group 3 contain H1, M1, M3 and H4 and M2, M4, L1 and L3, respectively. The groupings of the samples for rep 2 are illustrated in **Fig. 3(d)**. At a linkage distance of 0.8 the samples are split into 3 clear groupings. Group 1 contains, H2, M1, H3 and H4. Group 2 contains M3, M2, H1, L4 and L2, while Group 3 contains only 3 samples, namely L3, L1 and M4. The samples are not grouped in a similar manner when comparing rep 1 with rep 2, as a majority of the samples in rep 2 lie in a different position, and therefore grouping, compared to the first replication. The R_V coefficient for the two DISTATIS plots (**Fig. 3(a & b)**) (rep 1 and rep 2) is slightly low at a value below 0.5 ($R_V = 0.45$), indicating a low similarity correlation between replications (**Table 4**).

The samples from the Northern Cape were also sorted and analysed according to the quality of the **palate** quality of the samples. The DISTATIS plot depicted in **Fig. 4(a)** (rep 1) indicates clear potential quality groupings of samples, whereas the groupings are not as defined for rep 2 (**Fig. 4(b)**). In the latter case the samples appear scattered across the plot, with potential groupings being less obvious. This again can be due to the DISTATIS plots only illustrating the groupings on 2 dimensions, and therefore it is important to perform Ward's cluster analysis to determine or verify the clusters that were formed. For rep 1, **Fig. 4(c)**, it is clear that at a linkage distance of 0.8, the samples split into 3 groupings. The first and largest of the groupings, Group 1, contains L5, H8, H5, M7, and M6. Group 2 is the smallest of the groupings and contains the samples, M5, H7 and M8. The final grouping, Group 3, is made up of H6, L6, L7 and L8. The groupings are easily identifiable as individual groupings within the DISTATIS plot. **Fig. 4(d)** indicates that the samples are split into two groupings, at a linkage distance of 0.8. These groupings were previously not as clearly defined when looking only at the DISTATIS plot. Ward's cluster analysis took into account 4 dimensions and not only 2. Group 1 contains M6, H5, M5, H7, H8, M8 and H6. Group 2 contains 5 samples, namely M7, L5, L6, L7 and L8. From these results it is clear that the samples within the respective groupings are not similar. This result is substantiated by the fact that no R_V coefficient could be calculated between these plots, indicating that the results from the sorting were not reproducible.

Again it is clear from **Fig. 3** and **Fig. 4,** both illustrating the palate quality of a range of rooibos samples from the Western Cape and Northern Cape respectively, is that it is difficult to sort rooibos samples according to **palate** quality**.** This is especially true for the samples sourced from the Western Cape region where it was not possible to group the samples according high, medium and low quality (**Fig. 3(a & b)**).

The correspondence analysis plots (CA) (**Fig. 5(a & b)**), based on **aroma** quality of the Western Cape samples, contain the samples in relation to one another, as well as to the sensory descriptors that were assigned by the panel to represent these groupings. The positioning of the samples in **Fig. 5(a)** indicated that the samples previously chosen to represent high quality, H4, H3, H2 and H1, lie close together, and in a close association with the attributes "honey", "caramel", "fynbos-floral" and "rooibos-woody". The sample L4, of low quality, is however, also positioned close to these positive attributes. The medium and low quality samples are situated separately from the high quality samples positioned on the right of the plot. These samples appear to lie in close association to the negative attributes, "hay/dried grass", "rotting plant water", "musty/mouldy" and "burnt caramel". There is a definite split within the map along the first dimension, between the high quality samples on the one side, and the medium quality and low quality samples on the other side. **Fig 5(b)** gives the CA plot of rep 2. This plot indicates that a split along dimension 1 is not as defined as in the first replication. It can be noted, however, that the samples H1, H2, H3 and H4 (high quality) associate with the "honey", "rooibos-woody", "fynbos-floral" "caramel" notes. The samples M1 and L4 also lie in a close association with the "rooibos-woody" attribute. The attribute "apricot" appeared in the descriptions given by the panel during this replication, whereas it was absent for

the first replication. Sample M4 lies close to the "apricot" attribute. Again the medium and low quality samples lie closer to the negative attributes, than the high quality samples.

Fig. 6(a) and **Fig. 6(b)** depict the CA plots for the two replications, illustrating the ***overall aroma quality*** of the samples sourced from the Northern Cape. **Fig. 6(a)**, replication 1, indicates that the samples previously selected as high quality and medium quality samples lie in a close association to one another, as well as to the positive attributes, "caramel", "apricot", "rooibos-woody", "honey" and "fynbos-floral". These samples are also clearly separated from the low quality samples along dimension 1, where the low quality samples, lie to the left of the first dimension and are associated with the negative attributes, "hay/dried grass", "green grass/plant like" and "rotting plant water". For the second replication (**Fig. 6(b)**), it again is clear that the high and medium quality samples are separated from the low quality samples along dimension 1. Again, the positive attributes associate with the medium and high quality samples, while the low quality samples associate with the negative attributes.

Fig. 7(a) and **Fig. 7(b)** give a representation of the results obtained from the sorting of the Western Cape samples based on the **palate** quality attributes. What can be seen in both instances is the separation of the positive and negative attributes along dimension 1. The high quality samples are again separated from the lower quality attributes, although the split is not as well defined as for the aroma plots (**Fig. 5(a)** and **Fig. 5(b)**).

For the sorting of the samples from the Northern Cape, based on ***palate*** quality, it can be seen in **Fig. 8(a)** for rep 1 that the medium and high quality samples lie in a close association, surrounding the positive attributes. H6, however, is seen to lie close to the low quality samples, and therefore the negative attributes. For the second replication (**Fig. 8(b)**), the high and medium quality samples lie more to the right of the plot, again close to the positive attributes, whereas the low quality samples lie closer to the negative attributes. Sample M7, in this instance, lies in a close proximity to the negative attributes. The CA plots clearly show that there is a separation between the high and low quality samples, as well as a separation between the positive and negative attributes.

3.2. Uninstructed sorting to test for the characteristic sensory profile of rooibos

Details of the samples selected for the **uninstructed** sorting, or free sorting experiment, are summarised in **Table 3.** The samples were selected from a large pool of production samples according to different prominent ***characteristic rooibos aroma profiles*** (primary vs. secondary) and the respective attributes that make up these profiles. The production region, as well as an assigned code for easy recognition on the plots, is included in **Table 3**.

During this free sorting experiment, the samples were analysed in duplicate and the analysis was based only on the ***characteristic aroma profile*** of the samples. The DISTATIS plots give an indication of how the panel sorted the samples into groupings according to the different aroma profiles. From **Fig. 9(a),** for rep 1, it appears that apart from a few small groupings there is no distinct pattern. Ward's cluster

analysis was again performed in order to determine and verify the groupings seen in the DISTATIS plot. Using the first 4 dimensions, the samples were found to obviously split at a linkage distance of 1.0 into two groupings (**Fig. 9(c)**). The first grouping contains 7 samples whereas the second contains 5. The first group is made up of 5 samples, having the primary characteristic rooibos profile, while the remaining two samples to fit the secondary characteristic profile. The second group contains 4 samples fitting the secondary profile and 1 sample fitting the primary profile, i.e. PN2. For the second replication, it is clear from the DISTATIS plot (**Fig. 9(b)**), that the samples are split along the first dimension, with one group of samples on the left of the plot and a possibility of two separate groupings to the right. With the help of the Ward's cluster analysis based on the first 4 dimensions, it became clear that the samples were split into two groupings, at a linkage distance of 1.6.

Both the CA plots, for rep 1 (**Fig. 10(a)**) and rep 2 (**Fig. 10(b)**), indicate that the attributes were split clearly along the 1st dimension, and according to the different profiles that they represented. To the left of the plot lie the secondary attributes, "caramel", "fruity-sweet" and "apricot" accompanied by the negative attribute "burnt caramel". The primary characteristic attributes ("fynbos-floral", "rooibos-woody" and "honey") are located on the right of the plot and are accompanied by the negative attributes "hay/dried grass", "medicinal", "rotting plant water" and "green grass/plant-like". In rep 1 and rep 2 (**Fig. 10(a)** and **Fig. 10(b)**), samples PN2, SN2 and SN3 lie in association with the "caramel" and "burnt caramel" attributes. SW1 lies in association with "apricot" in rep 1, but in rep 2 it lies much further from this attribute. Sample SW3, in rep 2, associates closely with both the "apricot" and "fruity-sweet" attributes, whereas in rep 1 it lies far from these attributes. Samples PW3 and PW1, in rep 1, associate closely with "medicinal" and "hay/dried grass", respectively, but in the plot for rep 2 these samples lie closer to the primary characteristic attributes and the "hay/dried grass" attribute. The primary characteristic attributes were closely associated with samples, PN3, PW2, SN1, PN1, SW2 in both rep 1 and rep 2, although SN1 lies closer to "hay/dried grass" in rep 2.

3.3. Comparison of DSA and sorting results

The DSA results (**Addendum B; Table B11 & Table B12**) of the samples used in the **uninstructed** sorting experiments were also compared statistically to the sorting results, primarily to establish the efficacy of both sorting and DSA as profiling methodologies. The ***characteristic aroma profiles*** of each of the samples can be seen in **Table 3**. Note that sample PW2 was not included in the latter data analysis. Although sample PW2 was sorted during the sorting experiments, it was only used as a control sample in DSA and thus never scored.

Fig. 11(a) depicts the DISTATIS data for the **uninstructed** sorting (rep 1), with the exclusion of PW2. The samples were grouped according to the ***"characteristic" aroma profiles***. The samples, indicated on the left of the 1st dimension, fit the primary characteristic profile, with the exception of samples SW2 and SN1. The second grouping, to the right, is made up of samples representing the secondary characteristic profile,

with the exception of the PN2. For the second replication (**Fig. 11(b)**), the same groupings were seen. It is therefore possible to separate samples based on the ***primary/secondary characteristic aroma profile*** of the samples

The PCA bi-plot, averaged over the three replications of DSA (**Fig. 12**), illustrates the association of the samples, as well as the association of samples and aroma attributes. The PCA bi-plot (**Fig. 12**) shows that the samples defined as belonging to the primary characteristic group (**Table 3**) do not necessarily associate exclusively with the attributes that are prominent of this group. PW3 and PN1 associate with the "fynbos-floral", "rooibos-woody" and "honey" aroma attributes, whereas PN2 associates with "fruity-sweet", with PN3 and PW1 associate with the negative attributes "green" and "hay/dried grass". The samples for the secondary characteristic profile, SW2, SN2, SW1 and SW3, associated with the "caramel" and "fruity-sweet" attributes, whereas SN3 and SN1 associated with the "green" and "hay/dried grass" attributes. The R_V coefficients between the CA plots and the DSA plot are not significant and low, being 0.32 and 0.29 respectively (**Table 5**), indicating a low association between the CA and DSA. Although this value is low, it does not rule out the reliability of the sorting sample when compared to DSA.

4. DISCUSSION

There are several indications that the sorting technique has been used successfully in the food industry (Valentin *et al*., 2012). Sorting has been used in the categorisation of a number of food products including, red wine (Gawel *et al*., 2001), cheese (Lawless *et al*., 1995), jellies (Tang & Heymann, 1999), beer (Chollet *et al*., 2011) and yoghurts (Saint Eve *et al*., 2004). Whether it is to determine the consistency of product quality (Chollet *et al*., 2011) or the position of a product relative to a competitive product (Chollet *et al*., 2011), the sorting task can yield insightful results. In research it is also a useful technique as it is an easy-to-use, rapid profiling tool giving the researcher an overview of results that can then be further researched using more detailed methods such as DSA.

Although the sorting task does not generate highly detailed information, it allows researchers to obtain a rapid understanding of the type of results expected and can aid in determining the direction that needs to be followed to obtain more detailed results. The addition of a descriptive task to the sorting technique allows researchers to better understand the reason behind each of the groupings (Cartier *et al*., 2006).

In some instances product experts are used when conducting sorting, however, consumers can also be used. Experts or previously trained panellists are usually used when it is important to group samples according to industry-established, broad-based sensory or quality attributes. In contrast, consumers are usually used when the aim is to determine how the general consumer would view, classify or describe a group of samples. The sorting technique can furthermore either be **instructed** or **uninstructed**. In the former the panellists use a predefined set of terms or categories to sort the samples (Cartier *et al*., 2006). In the latter instance no directions are given to the group of panellists when classifying or grouping the

samples. **Uninstructed** sorting is also known as free sorting (Valentin *et al*., 2012) and in this instance the primary main aim is to ascertain all possible groupings of samples within a larger sample set (Chollet *et al*., 2011).

In our study the sorting task was evaluated to determine whether sorting could be used as a possible tool in the quality grading of rooibos. For this reason, sorting of samples according to pre-determined quality grades using **instructed** sorting was investigated. Furthermore **uninstructed** or free sorting was also investigated, primarily to ascertain whether there are other sensory profiles associated with rooibos than the primary profile as indicated in **Chapter 3.** In order to ensure that the sorting method would perform correctly when used by industry, it was important to determine the stability of the method whilst testing rooibos and determine whether the data obtained from the sorting task is comparable to that obtained from DSA. If the sorting data proved stable and produced similar results to that of DSA, it would mean that it is possible to discern between the quality of rooibos based on the common **aroma** and **palate** attributes using **instructed** sorting. It would furthermore indicate whether a panel would be able to separate samples into groupings based on the *characteristic aroma profiles* fitting either the primary characteristic or secondary characteristic profiles, without being instructed to do so.

The ability to perform these sorting tasks correctly, could lead to the development of a sorting method to assist in the screening of rooibos samples as a first step in the grading process. The use of a standardised method would be a great asset to the rooibos industry, as it would be a way to standardise at least one aspect of the industry. A rapid technique that could save time and therefore also costs would have greater chance of acceptance by industry.

4.1. Instructed sorting to test the *overall aroma* and *palate quality* of rooibos sourced from different production regions

Instructed sorting was conducted using samples from the two production regions, i.e. Western Cape and Northern Cape. Both sample sets had to be sorted by a panel of expert judges in consecutive sessions according to aroma, as well as palate attributes associating with high quality, medium quality and low quality rooibos. All analyses were replicated to test for consistency of results.

According to Abdi *et al*. (2007), DISTATIS plots are able to show the similarities and differences between samples based on how they are grouped during the sorting task. DISTATIS is a combination of the statistical methods MDS and STATIS, although DISTATIS, in comparison to MDS, allows for individual panellist data to be taken into account (Abdi *et al*., 2007). The results obtained allows the researcher an opportunity to view the manner in which the panellists view the similarities or dissimilarities between products, and the latter usually provide further data for more targeted data analyses. Therefore once the samples are grouped and a DISTATIS plot is drawn up, the resulting clusters can be determined. The distances between the samples are a representation of the similarities between the samples. The closer

the samples, the more similar the samples are thought to be and the further apart the samples, the more different the samples tend to be.

The respective DISTATIS plots (**Fig. 1** and **Fig. 2**) produced from the sorting of the rooibos samples from the Western Cape and Northern Cape based on **aroma** quality yielded similar results in terms of the overall outcome. In both cases it was clear that there was a separation along the 1st dimension, separating the high quality samples from the low quality samples. With the assistance of Ward's cluster analysis, using at least four dimensions, it was possible to verify the sample groupings and therefore substantiate the split between the samples based on *overall aroma quality*. By including more dimensions in Ward's cluster analysis, the correct groupings of the samples can be determined, as certain correlations/relationships between samples are lost when only looking at the samples on a two-dimensional DISTATIS plot. Ward's cluster analysis combines similar objects together, ensuring that the overall within-cluster variation is kept to a minimum (Mooi & Sarstedt, 2011). The clusters obtained can be determined from a hierarchal dendrogram or tree diagram. When determining the number of clusters, it is important to remember that knowledge of the product in question is important, as this can help determine whether the number of clusters obtained make sense (Mooi & Sarstedt, 2011). In this study Ward's cluster analysis helped to verify the groupings of the rooibos samples, and therefore indicate the similarities/dissimilarities between them. From the groupings it was clear that the high quality and low quality samples were not similar as they were situated far apart on the DISTATIS plots. The medium quality samples, on the other hand, were found to associate with the low quality samples, but in more instances with the high quality samples. It was expected that the medium quality rooibos samples would not be easily discernible from the other two categories of rooibos quality due to the mixed nature of its aroma profile, i.e. a mix of positive and negative aroma attributes.

With the inclusion of the descriptive task, it was possible to determine the reason behind the grouping of specific rooibos samples sourced from both production areas using CA plots. Although the CA plots appear to be similar to the DISTATIS plots, the DISTATIS plots do not take the inclusion of the descriptors into consideration. The high quality samples from both the Western Cape and Northern Cape were seen to associate with the positive aroma attributes "fynbos-floral", "rooibos-woody", "honey" and "caramel". For the samples from the Northern Cape it was also found that, in addition to the attributes already mentioned, the attributes "fruity-sweet" and "apricot" aroma also associated with the high quality rooibos samples. In contrast, the low quality samples were seen to associate with the negatively associated aroma attributes. These include the "green grass/plant-like", "rotting plant water", and "musty/mouldy" and "hay/dried grass" aroma attributes. Several of the low quality Northern Cape samples also tended to associate with a "medicinal" aroma, a negative aroma attribute.

In view of the above, instructed sorting seems to be viable when rooibos samples need to be categorised as low quality or high quality based on **aroma** quality, especially when the sorting task is accompanied with a descriptive step. Our results have shown that the sorting task is, however, less

effective when medium quality samples need to be clustered based only on overall **aroma** quality. In order to correctly categorise these samples based on quality, and the inclusion of the sorting task relating to the taste and mouthfeel quality of the samples, may give greater insight into the sample quality, as these attributes can be indicators of quality.

In contrast with the above, it was not as easy to determine the ***overall palate quality*** of rooibos using the sorting task. The present study (**Chapter 3**) and Koch *et al.* (2012) showed that, because of the low intensities of the flavour attributes and very little variation in the taste and mouthfeel intensities within rooibos, it is often not easy to recognise these attributes or to distinguish between samples. It is thought that this may have influenced the results from the sorting task (**Fig. 3** and **Fig. 4**). Although there is a separation between the high quality and low quality samples based on the palate attributes, the split is not as clearly defined as for the **aroma** quality, indicating that the panel had difficulty in grouping the samples. With the low intensities of palate attributes, it is often not possible to pick out a defining attribute, making it hard to profile the sample.

Overall it can be said that it is possible to use the sorting method to rapidly separate samples of a high quality and samples of a low quality, based to a greater extent on the **aroma** attributes and a lesser extent on the **palate** attributes. It is therefore possible to consider the sorting task as a tool to aid grading, based mainly on the **aroma** quality of the rooibos. The focus of quality grading needs to be concentrated on the **aroma** quality and less on the **palate** quality, due to the low intensities of the **flavour** attributes making interpretation difficult. The taste and mouthfeel attributes, although similar in their intensities, can be an indicator of quality and therefore if used in combination with the sorting based on **aroma** quality, could provide better grouping of medium quality samples. By being able to split the samples rapidly into high and low quality groupings, the grader will be able to screen the samples prior to further analysis, thereby speeding up the overall grading process. In order to ensure consistency, the grader can sort the samples according to a selected list of aroma attributes from the sensory lexicon or sensory wheel (**Chapter 3**). These attributes should be characteristic/defining of a high quality or low quality rooibos aroma. The list of aroma attributes could include intensity scores i.e. "very", "a little", "medium" and "not", which would allow the graders to quantify the attributes and therefore be better equipped to place the samples into different quality groupings (Lelièvre *et al.*, 2008; Chollet *et al.*, 2011). This will be especially helpful for the placement of the medium quality samples, which are hard to categorise because of the similarity these samples have with both the high and low quality samples. Once the screening has been done, the grader will now have an insight into the **aroma** quality and possibly the ***overall quality grading***, before beginning further analyses.

4.2. Uninstructed sorting to test the *characteristic aroma profile* of rooibos

Two **aroma profiles** for rooibos were determined (**Chapter 3**; Koch *et al.*, 2012), i.e. the primary characteristic profile and the secondary characteristic profile. It was found that rooibos samples generally

predominantly exhibit one of these profiles. The primary profile is a rooibos with a "honey", "rooibos-woody" and "fynbos-floral" aroma, which has become synonymous with this herbal tea within South Africa, although it was previously undefined prior to the study by Koch *et al*. (2012). The secondary profile includes aroma attributes not typically found to be present in high intensities within rooibos but which, when present in high intensities, create a secondary characteristic rooibos aroma profile. This profile includes the "fruity-sweet", "caramel" and "apricot" aromas.

The ***aroma profiling*** of the samples was done using **uninstructed** sorting. The panel were only asked to sort the samples according to the ***aroma profiles*** of the samples by placing the samples they deemed similar into groups. In addition, the panel had to justify the groupings by providing a list of descriptors to describe the ***aroma profile*** of the respective groupings. The DISTATIS plots, and Ward's cluster analysis, clearly show a differentiation between the primary characteristic and secondary characteristic samples.

The samples illustrating the primary characteristic profile tend to cluster together, similarly the samples illustrating the secondary characteristic profile are seen to form another cluster, although it must be noted that three of the samples do not lie in the intended profile grouping, as seen and determined for DSA (**Fig. 12**). Samples PN2, SN1 and SW2, lie in association with attributes, which were not found to dominate the profile of each of these samples during DSA (**Fig.12**; **Addendum B; Table B11 & Table B12**). SW2, however, was given a high "rooibos-woody" intensity (intensity = 38) during DSA, which may have had an effect on the overall **aroma profile**, although accompanied by high intensities of the secondary characteristic profile attributes. Samples PN2 and SN1, however, did not display any obvious indications as to why they were sorted into the opposite profile's for which they were originally chosen to represent (by researchers), namely the primary characteristic and secondary characteristic profiles, respectively. Although there are samples that lie in the opposite group than the one for which they were originally chosen, it was seen that the formation of clusters were the same in each replication, showing that the panel were consistent. It was also clear that the samples split according to the ***aroma profile*** of the samples and not by the production area. These results were also seen in **Chapter 3**, indicating it is not possible to split the samples based only on sensory characteristics, into different production areas.

CA done on the sorting task data yielded further information to help verify the attributes responsible for the primary and secondary profiles. The samples chosen to represent the primary profile were said to associate with "fynbos-floral", "honey" and "rooibos-woody" aromas. Samples displaying the secondary characteristic profile were found to associate with "caramel", "apricot" and "fruity-sweet" aromas. There were also samples that associated with negative attributes; these attributes may have been present in very low intensities. Although the characteristic attributes representing the primary and secondary profiles are based on prominent positive attributes, there are often negative attributes present within rooibos, though often at low intensities. Therefore these negative attributes do not associate with a specific characteristic sensory profile, but can rather be present at low intensities within either of the

rooibos aroma profiles. However, in our study the negative aroma attributes, "hay/dried grass", "medicinal" and "green-grass/plant-like" indicated in the CA plots associated more with the primary characteristic profile, whereas the sensory attribute "burnt caramel" associated more with the secondary profile.

The results obtained thus indicate that it is possible to discern between a rooibos exhibiting either a primary or secondary characteristic profile, based on the aroma attributes. Although it is not possible to determine the intensity of the negative attributes, it is clear that these attributes were only seen a small number of the samples, and that there was a stronger presence of the positive attributes. The positive identification of the attributes associated with the samples, chosen for the sample set from each of the profiles, helps to further verify these groupings as being separate from one another and both prominent within rooibos tea. As it is possible to sort samples based on an entire ***aroma profile*** it may in fact be possible to use this method as a means of screening or sorting samples for blends, based on a brief given by a customer.

4.3. Determining the stability and reproducibility of sorting as test methodology

Once it was determined that the sorting method can group rooibos according to the aroma and palate quality, it was important to determine how reproducible the results were. The sorting task was carried out in duplicate to determine if the task was reproducible, i.e. gave the same results each time it was performed. This was determined using the R_V coefficient, introduced by Escoufier in 1973 (Abdi, 2007; Blancher *et al.,* 2007; Dehlholm *et al.*, 2012). The use of R_V coefficients is common when using STATIS and DISTATIS (Abdi *et al.*, 2007). The R_V coefficient indicates the similarity between the sorting plots; in this case the DISTATIS plots. Where matched, the R_V values will be between 0 and +1, and the closer the R_V is to 1, the stronger the similarity is between the two methods or replications (M. Kidd, Stellenbosch University, Stellenbosch, South Africa, May 2013, personal communication; Abdi, *et al.*, 2007).

The DISTATIS plots were compared first to determine whether the samples were sorted in a similar manner over the replications. Sorting the Western Cape samples, based on **aroma** quality, yielded an R_V value of 0.66 ($p < 0.05$), which shows that the panel were moderately able to repeat the task and yield the same results (**Fig. 1(a & b)**; **Table 4**). Considering **palate** quality, an R_V value of 0.45 ($p < 0.05$) was achieved for the Western Cape samples, indicating a low level of similarity between repetitions (**Fig. 3(a & b)**; **Table 4**). Sorting of the Northern Cape samples based on **aroma** quality and **palate** quality did not yield a match and thus no R_V values could be determined. In this case the panel could not reproduce the task with the same results. Due to the complexity of sorting the medium quality samples, these samples seemed to group differently with the high quality samples, during each replication. This could explain the reason for no correlation between the plots, as the groupings of the samples completely changed. The sorting carried out on the ***aroma profiles*** of the rooibos samples yielded an R_V of 0.72 ($p < 0.05$) between the DISTATIS

plots (rep 1 and rep 2), the highest value achieved during the task (**Fig. 9(a & b)**; **Table 4**). This again verifies the ability to separate rooibos samples based on the ***aroma profile*** of the samples.

The CA plots for rep 1 and rep 2 of the Western Cape samples had an R_V value of 0.79 (p < 0.05) while that of the Northern Cape samples gave an R_V of 0.80 (p < 0.05). It is clear that, although the DISTATIS plots may not have yielded high R_V values, the panel were consistent when assigning the attributes to the samples. These results mean that the panel placed the same attributes with the respective groupings each time, demonstrating that rooibos samples can be profiled in a reproducible manner.

Different quality batches of rooibos are often blended, to obtain a tea that meets the quality and aroma profile specified by marketers or for the needs of international customers. The use of sorting is favourable for the industry, as it allows for the rapid comparison of blended rooibos samples, to ensure consistent results after each new blend is created, as well as allowing for the rapid screening of rooibos batches based on quality. Ensuring little or no variation between blended samples, will allow for more consistency in the quality of the blends being marketed. The ability to profile rooibos as either primary characteristic or secondary characteristic reliably, will allow for the possible development of niche markets for rooibos. With this method being easy to understand and implement, small processors or small-scale farmers can use it effectively as a means to better differentiate their products from competitors, as well as position their products in a clearer manner on the market.

4.4. Comparison of DSA and sorting as profiling methodologies

It was important to determine whether or not the same results achieved in the sorting task, were also achieved during DSA. This was accomplished using the results from the **uninstructed** sorting. DSA was carried out over 3 replications, therefore it was important to determine the average of these results in order for a successful comparison with the data within the two CA plots developed from the sorting data. In addition, for the comparison to be possible, sample PW2 (control) had to be removed from the analysis, as it was not previously analysed during the DSA. CA plots were chosen for the comparison, instead of DISTATIS plots, as they contain descriptors, which can be compared to the descriptors obtained from DSA. The results from DSA show that the samples chosen to represent the primary profile, associate with one another and to the "fynbos-floral", "rooibos-woody" and "honey" aroma attributes on the bi-plot, although two of the samples (PW1 and PN3) associated more with the negative attributes present. The samples from the secondary profile lay in a closer association with one another than the primary profile samples, and with the "caramel" and "fruity-sweet" attributes. Comparison of DSA and sorting is therefore possible, as similar results were achieved. However, it is important to note that different methods were used to obtain the results, and therefore the data obtained are not the same and can be difficult to compare. This can result in the plots not being comparable on a numerical level (R_V) (T. Næs, Nofima, Norway, May 2014, personal communication). Results mirroring these predictions were noted, with non-significant R_V values of 0.32 and 0.29 being achieved for CA (rep 1) vs. DSA and CA (rep 2) vs. DSA, respectively. Although these

results do not indicate a good match between the plots, it can visually be seen that the samples on the PCA bi-plot (**Fig. 12**) associate in a similar manner to the samples on the CA plots (**Fig. 10(a & b)**), in relation to the aroma attributes. Only a small number of aroma attributes are present in the PCA bi-plot (**Fig. 12**). During certain years of DSA testing, not all the aroma attributes currently on the sensory wheel were analysed quantitatively. Therefore the comparison between DSA and the **uninstructed** sorting method could only be carried out on the aroma attributes tested throughout the 5-year period, and the influence of the other aroma attributes on the comparison can thus not be determined.

It can therefore be said that similar results to DSA can be achieved when using the sorting method, combined with a descriptive task. Again, however, it must be noted that DSA takes into account many more factors than the sorting task and is a much more detailed approach, leading to the data differing slightly. It was possible to distinguish between the primary characteristic and secondary characteristic samples on the PCA bi-plot, as well as on the CA plots, and similar interactions between the samples and attributes were achieved.

5. CONCLUSIONS

The aim of the study was to determine whether it was possible to use sorting, as a rapid and reliable method for determining the ***overall sensory quality*** and ***characteristic aroma profiles*** of rooibos infusions. It was important that the results obtained were comparable to the data obtained during DSA, which yields a more detailed sensory profile.

The results indicated that the trained panel were able to sort the samples based on ***overall sensory quality*** (**instructed sorting**)*,* as well as ***characteristic aroma profiles*** (**uninstructed sorting**). Although the R_V coefficients between the sorting data (DISTATIS and CA) and the DSA data were low and not significant, which can be expected when comparing different methods, the overall pattern of data was seen to be similar. This is an indication that sorting and DSA can achieve similar results, although the sorting data is much less detailed. More research into the reliability and consistency of these methods needs to be done, in order to determine their reliability over a period of time. Basing the possible screening and profiling of rooibos on the **aroma** attributes within the infusion, is advisable, as obtaining results based on the low intensity **flavour** attributes proved difficult and inconclusive. Taste and mouthfeel attributes are often indicators of quality, although very little variation is seen between the attribute intensities. The addition of these attributes to the results from the **aroma** quality sorting can help yield clearer results about the ***overall sensory quality***. The use of sorting within the rooibos industry can lead to more consistency within the different **quality** grades and blends, furthermore it allows for possible further development of these and other rapid methods, such as polarised sensory positioning (PSP), into complete grading tools for smaller processors and small-scale farmers.

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Table 1 Rooibos samples sourced from the Western Cape region used for instructed sorting of overall sensory quality (aroma and palate).

Quality designation	Year of production	Sample code	Assigned code
High quality	2013	1A	H1
High quality	2009	5A	H2
High quality	2009	AA	H3
High quality	2009	Control	H4
Medium quality	2011	4B	M1
Medium quality	2012	13C	M2
Medium quality	2011	2A	M3
Medium quality	2013	7C	M4
Low quality	2011	6D	L1
Low quality	2012	6D	L2
Low quality	2013	1D	L3
Low quality	2009	14D	L4

Quality designation: Deducted from DSA data set (**Chapter 3**). A high quality sample contained virtually zero or a very low intensity (intensity < 5) of perceptible taints or negative attributes. The low quality samples contained a high number of taints or negative attributes (intensity > 10), whereas a medium quality sample contained a near equal ratio of both positive and negative attributes, at below average intensity for each attribute.

Sample code: Represents different grades as assigned by industry, based on in-house quality grading (**Chapter 3**).

Control sample: This sample was prepared by blending six batches of Grade B rooibos samples (provided by Rooibos Ltd., Clanwilliam, South Africa) and was used in DSA (**Chapter 3**) as a control sample. The sensory attributes of this control sample were considered to be representative of the sensory profile typically associated with rooibos.

Assigned code: The samples were assigned a code for easy interpretation during data analysis. The "H" represents a sample of high quality, the "M" represents a sample of medium quality and the "L" represents a sample of low quality. The numbers 1 – 4 represent the samples from the Western Cape. These are based on the **aroma** and **palate** quality of the samples, and assigned based on the DSA results. For DSA data for aroma see **Addendum B** (**Table B1 & Table B2**) and for palate quality see **Addendum B** (**Table B5, Table B6 & Table B7**).

Table 2 Rooibos samples sourced from the Northern Cape region used for instructed sorting of overall sensory quality (**aroma** and **palate**).

Quality designation	Year of production	Sample code	Assigned code
High quality	2013	1A	H5
High quality	2012	3A	H6
High quality	2012	11B	H7
High quality	2011	3B	H8
Medium quality	2009	1A	M5
Medium quality	2011	5A	M6
Medium quality	2010	4A	M7
Medium quality	2012	1A	M8
Low quality	2010	3C	L5
Low quality	2011	4C	L6
Low quality	2012	2D	L7
Low quality	2013	4D	L8

Quality designation: Deducted from DSA data set (**Chapter 3**). A high quality sample contained virtually zero or a very low intensity (intensity < 5) of perceptible taints or negative attributes. The low quality samples contained a high number of taints or negative attributes (intensity > 10), whereas a medium quality sample contained a near equal ratio of both positive and negative attributes, at below average intensity for each attribute.

Sample code: Represents different grades as assigned by industry, based on in-house quality grading (**Chapter 3**).

Assigned code: The samples were assigned a code for easy interpretation during data analysis. The "H" represents a sample of high quality, the "M" represents a sample of medium quality and the "L" represents a sample of low quality. The numbers 5 – 8 represent the samples from the Northern Cape. These are based on the **aroma** and **palate** quality of the samples, and assigned based on the DSA results For DSA data for aroma see **Addendum B** (**Table B3 & Table B4**) and for palate quality see **Addendum B** (**Table B8, Table B9 & Table B10**).

Table 3 Rooibos samples, representing the rooibos profiles (primary and secondary), sourced from the Western Cape and Northern Cape regions for uninstructed sorting of characteristic *aroma profiles* of rooibos.

Profile	Sensory profile	Year of production	Company	Sample code	Assigned code
Primary profile	Floral, woody, honey	2009	Northern Cape	1A	PN1
	Floral, woody, honey	2013	Northern Cape	10B	PN2
	Floral, woody, honey	2013	Northern Cape	11B	PN3
	Floral, woody, honey	2012	Western Cape	1A	PW1
	Floral, woody, honey	2009	Western Cape	Control	PW2
	Floral, woody, honey	2009	Western Cape	5A	PW3
Secondary profile	Caramel	2012	Northern Cape	10B	SN1
	Apricot, caramel	2013	Northern Cape	3A	SN2
	Caramel	2013	Northern Cape	4D	SN3
	Fruity-sweet, caramel	2012	Western Cape	5D	SW1
	Caramel	2012	Western Cape	4A	SW2
	Caramel	2013	Western Cape	1C	SW3

Control sample: This sample was prepared by blending six batches of Grade B rooibos samples (provided by Rooibos Ltd., Clanwilliam, South Africa) and was used in DSA (**Chapter 3**) as a control sample. The sensory attributes of this control sample were considered to be representative of the sensory profile typically associated with rooibos.

Assigned code: The samples were assigned a code for easy interpretation during data analysis. The "P" represents a sample with a primary profile and the "S" represents a secondary profile. The second letter "N" or "W" represents the Northern Cape and Western Cape, respectively. These are based on the *aroma* profile and production areas of the samples, obtained from the DSA results (**Addendum B; Table B11 & Table B12**).

Table 4 R_V coefficients comparing results of the instructed sorting (DISTATIS plots), where infusions were compared according to *overall sensory quality* (aroma, as well as palate attributes). For both aroma and palate quality the samples were analysed in duplicate (rep 1 and rep 2). The results of the uninstructed sorting (DISTATIS plots) according to *aroma profile*, rep 1 and rep 2, were also compared.

Plot 1[a]	Plot 2[a]	R_V coefficient	p-value
Quality Western Cape(Rep 1;Aroma)	Quality Western Cape (Rep 1; Palate)	0.45	0.02
Quality Western Cape(Rep 1; Aroma)	Quality Western Cape (Rep 2; Aroma)	0.66	0.00
Quality Western Cape(Rep 1; Aroma)	Quality Western Cape (Rep 2; Palate)	0.45	0.02
Quality Western Cape(Rep 1; Palate)	Quality Western Cape (Rep 2; Aroma)	0.36	0.06
Quality Western Cape(Rep 1; Palate)	Quality Western Cape (Rep 2; Palate)	0.45	0.02
Quality Western Cape (Rep 2; Aroma)	Quality Western Cape (Rep 2; Palate)	0.65	0.00
Quality Northern Cape (Rep 1; Aroma)	Quality Northern Cape (Rep 1; Palate)	0.46	0.02
Quality Northern Cape (Rep 2; Aroma)	Quality Northern Cape (Rep 2; Palate)	0.58	0.00
Aroma profile (Rep 1; Aroma)	Aroma profile (Rep 2; Aroma)	0.72	0.00

[a] All the plots being compared to determine the R_V coefficient are DISTATIS plots.

Quality: Quality refers to the samples that were sorted according to ***overall sensory quality***, based on either the **aroma** or **palate** (flavour, taste and mouthfeel) quality profiles.

Aroma profile: Aroma profile refers to the samples sorted according to the ***characteristic sensory profiles***, which were based on the **aroma profile** of the samples.

Table 5 R_V coefficients comparing results of uninstructed sorting (DISTATIS and CA plots) and DSA, without the control sample (PW2). Samples were sourced from both production regions and the infusions thereof were compared in terms of ***characteristic sensory profiles*** associated with rooibos, and analysed in duplicate.

Plot 1	Plot 2	R_V coefficient	p-value
Aroma profile DISTATIS (Rep 1) [a]	CA plot aroma profile(Rep 1) [a]	0.84	0.001
Aroma profile DISTATIS (Rep 1) [a]	Aroma profile DISTATIS (Rep 2) [a]	0.66	0.001
Aroma profile DISTATIS (Rep 1) [a]	CA plot aroma profile (Rep 2) [a]	0.61	0.001
Aroma profile DISTATIS (Rep 1) [a]	DSA aroma averaged over 3 sessions [a]	0.32	0.12
Aroma profile DISTATIS (Rep 2) [a]	CA plot aroma profile(Rep 2) [a]	0.86	0.001
Aroma profile DISTATIS (Rep 2) [a]	DSA aroma averaged over 3 sessions [a]	0.29	0.16
Aroma profile DISTATIS (Rep 2) [a]	CA plot aroma profile(Rep 1) [a]	0.79	0.001

[a] The sample PW2 (control sample) was not included during the comparison of the DISTATIS plots, CA plots and DSA plots. Due to this sample not being scored during the DSA, there are no values relating to the intensity of the attributes within this sample, making it non-usable for the DSA bi-plot.
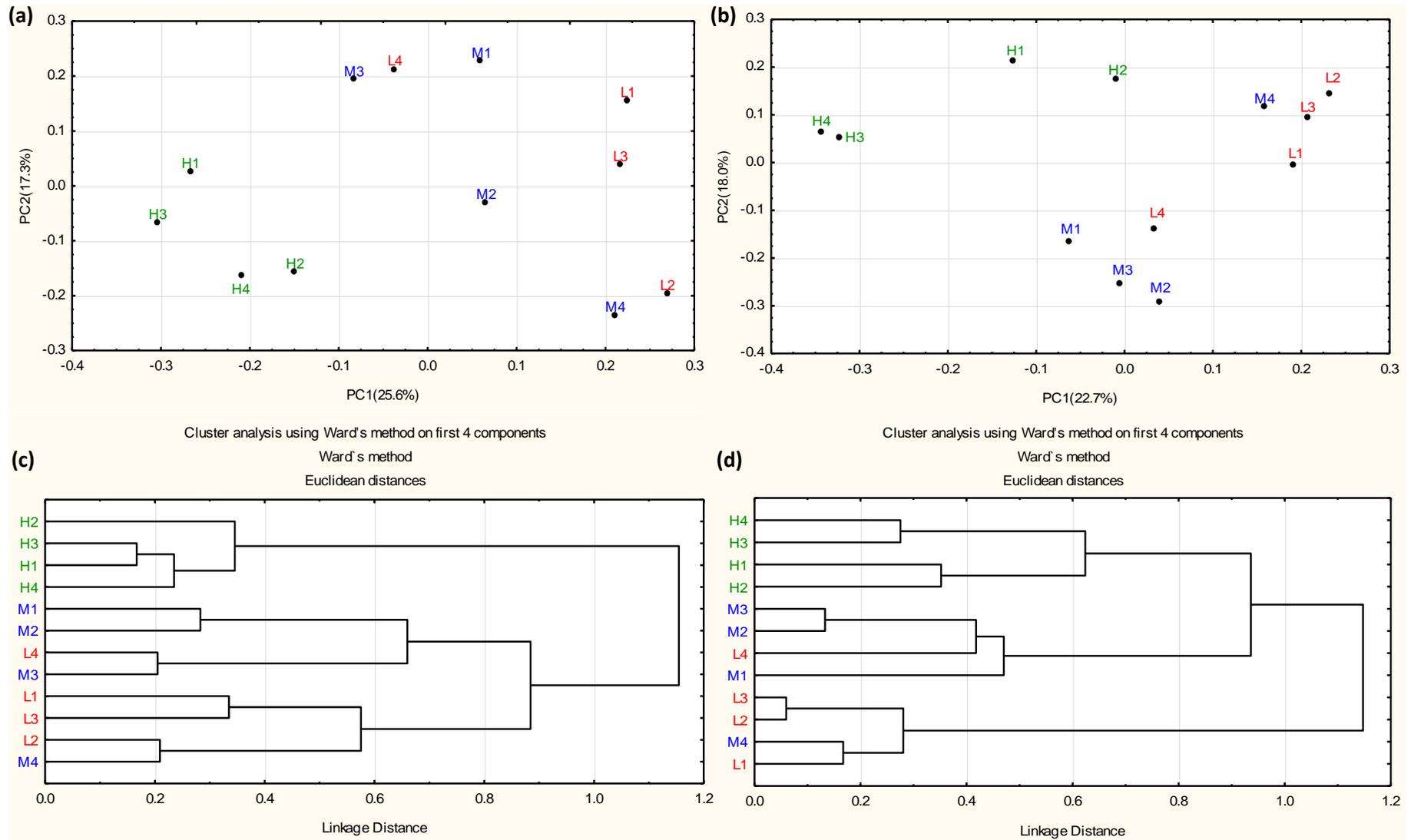
Figure 1 Instructed sorting based on *overall sensory quality*: DISTASTIS plots showing the position of rooibos samples from the Western Cape, sorted according to their *aroma* quality profile (a) rep 1, (b) rep 2. The **green** samples indicate the high quality samples, **blue** indicates the medium quality samples and **red** the low quality samples. The Ward's cluster analysis plots indicate groupings for (c) rep 1 and (d) rep 2. The samples are represented the same as for the DISTATIS plots.
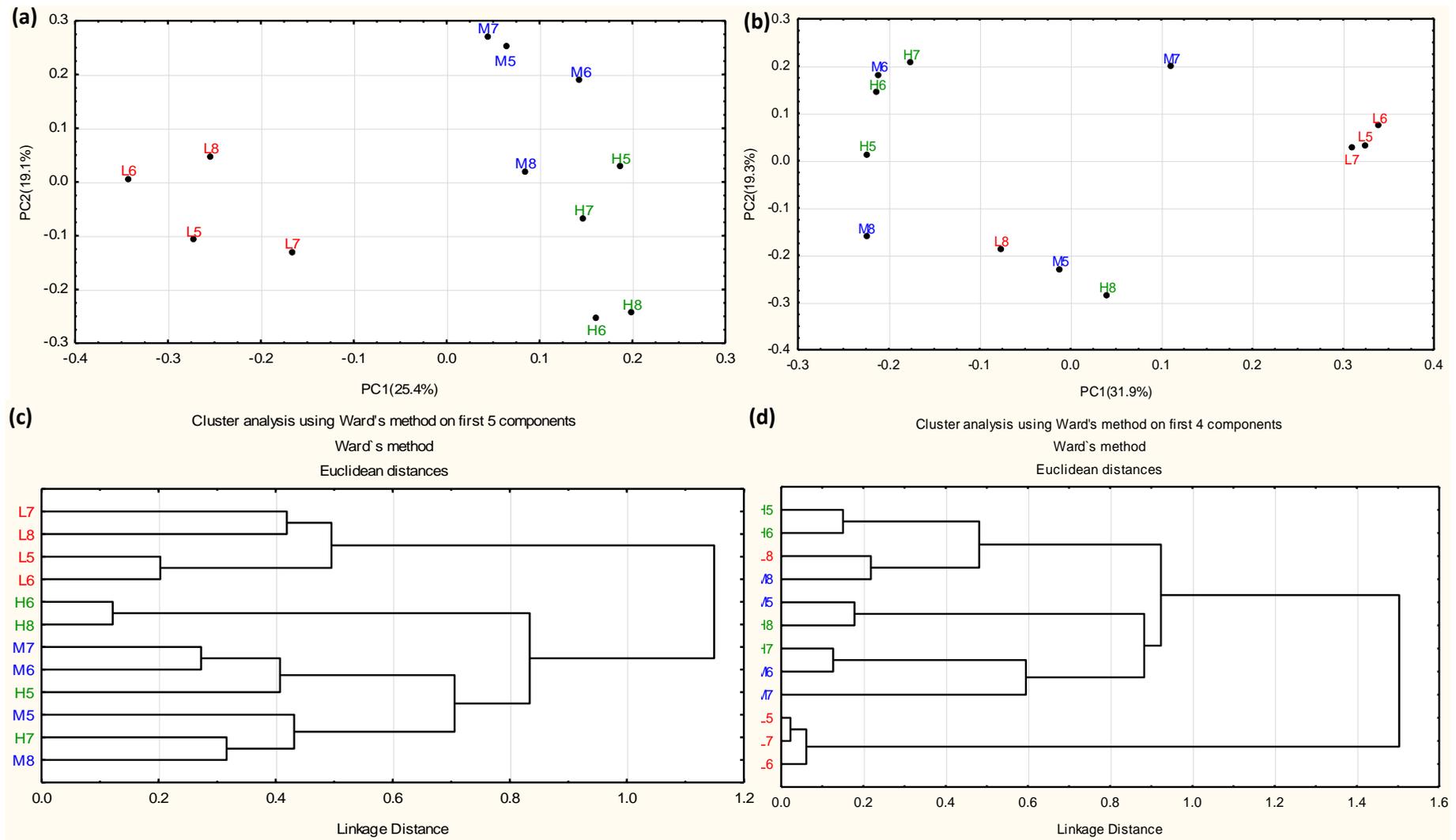
Figure 2 Instructed sorting based on *overall sensory quality*: DISTASTIS plots showing the position of rooibos samples from the Northern Cape, sorted according to their *aroma* quality profile (a) rep 1, (b) rep 2. The **green** samples indicate the high quality samples, **blue** indicates the medium quality samples and **red** the low quality samples. The Ward's cluster analysis plots indicate groupings for (c) rep 1 and (d) rep 2. The samples are represented the same as for the DISTATIS plots.
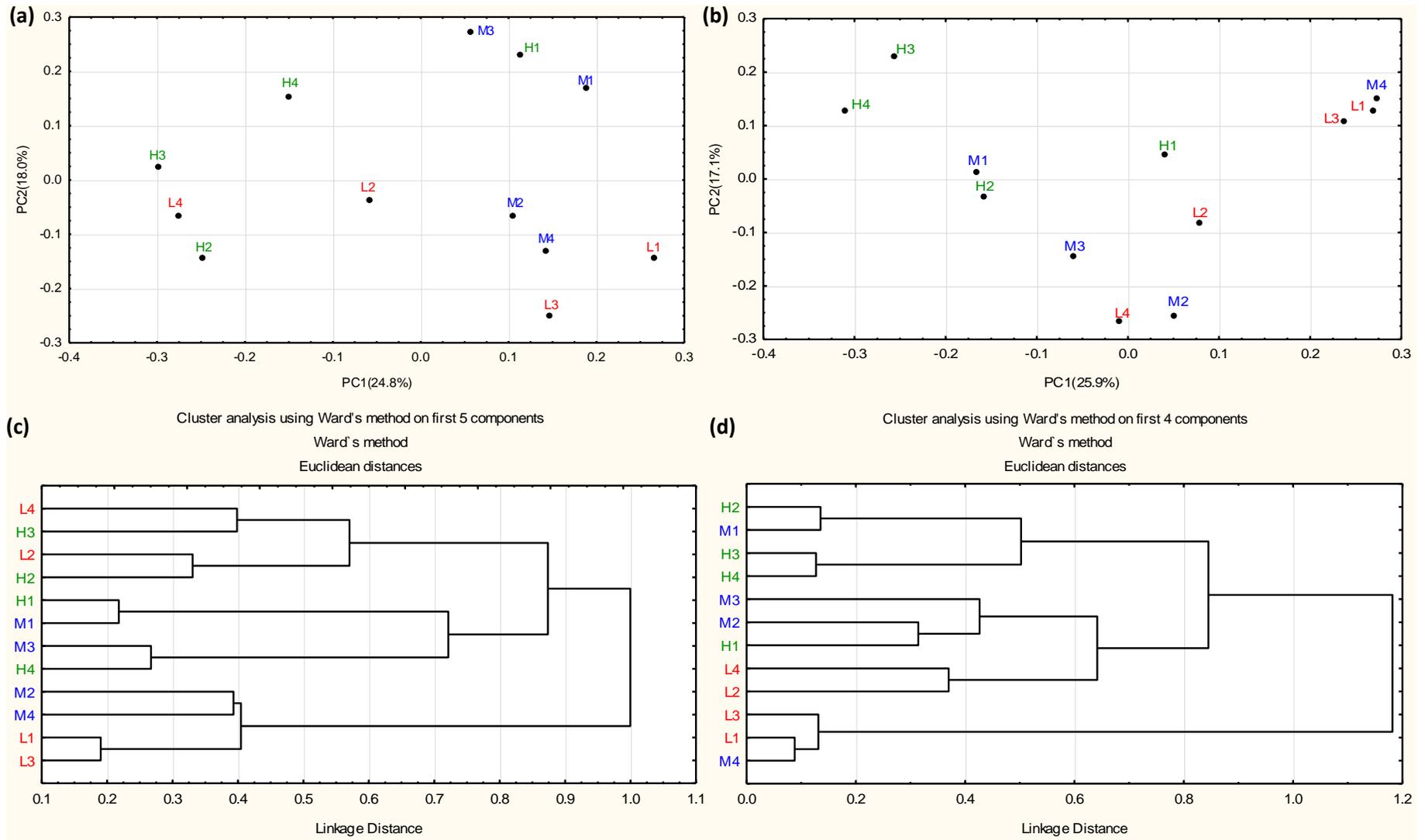
Figure 3 Instructed sorting based on *overall sensory quality*: DISTASTIS plots showing the position of rooibos samples from the Western Cape, sorted according to their *palate* quality profile (a) rep 1, (b) rep 2. The **green** samples indicate the high quality samples, **blue** indicates the medium quality samples and **red** the low quality samples. The Ward's cluster analysis plots indicate groupings for (c) rep 1 and (d) rep 2. The samples are represented the same as for the DISTATIS plots.
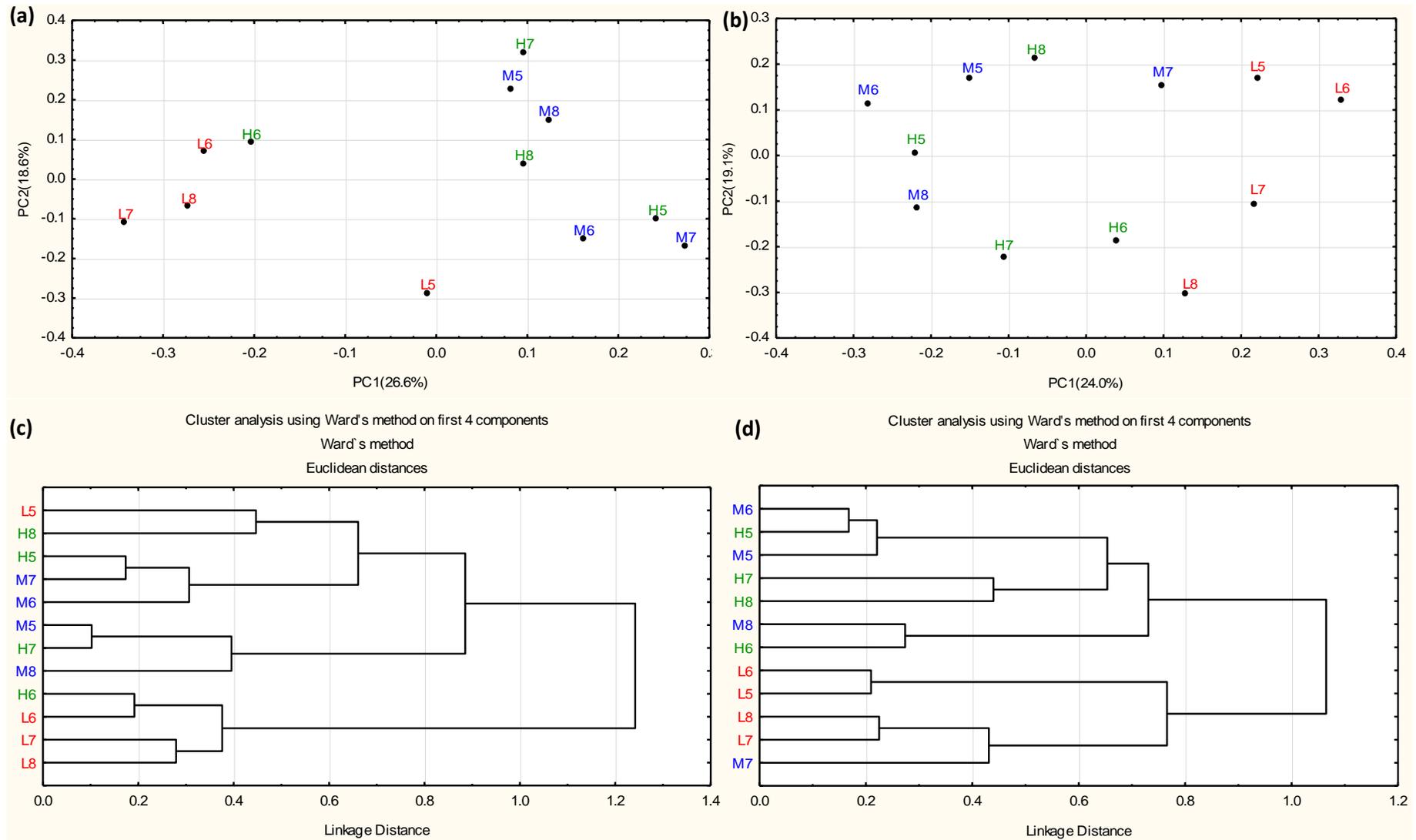
Figure 4 Instructed sorting based on *overall sensory quality*: DISTASTIS plots showing the position of rooibos samples from the Northern Cape, sorted according to their *palate* quality profile (a) rep 1, (b) rep 2. The **green** samples indicate the high quality samples, **blue** indicates the medium quality samples and **red** the low quality samples. The Ward's cluster analysis plots indicate groupings for (c) rep 1 and (d) rep 2. The samples are represented the same as for the DISTATIS plots.

(a) Include condition: "descriptor count">9.0

(b) Include condition: "descriptor count">=16.0
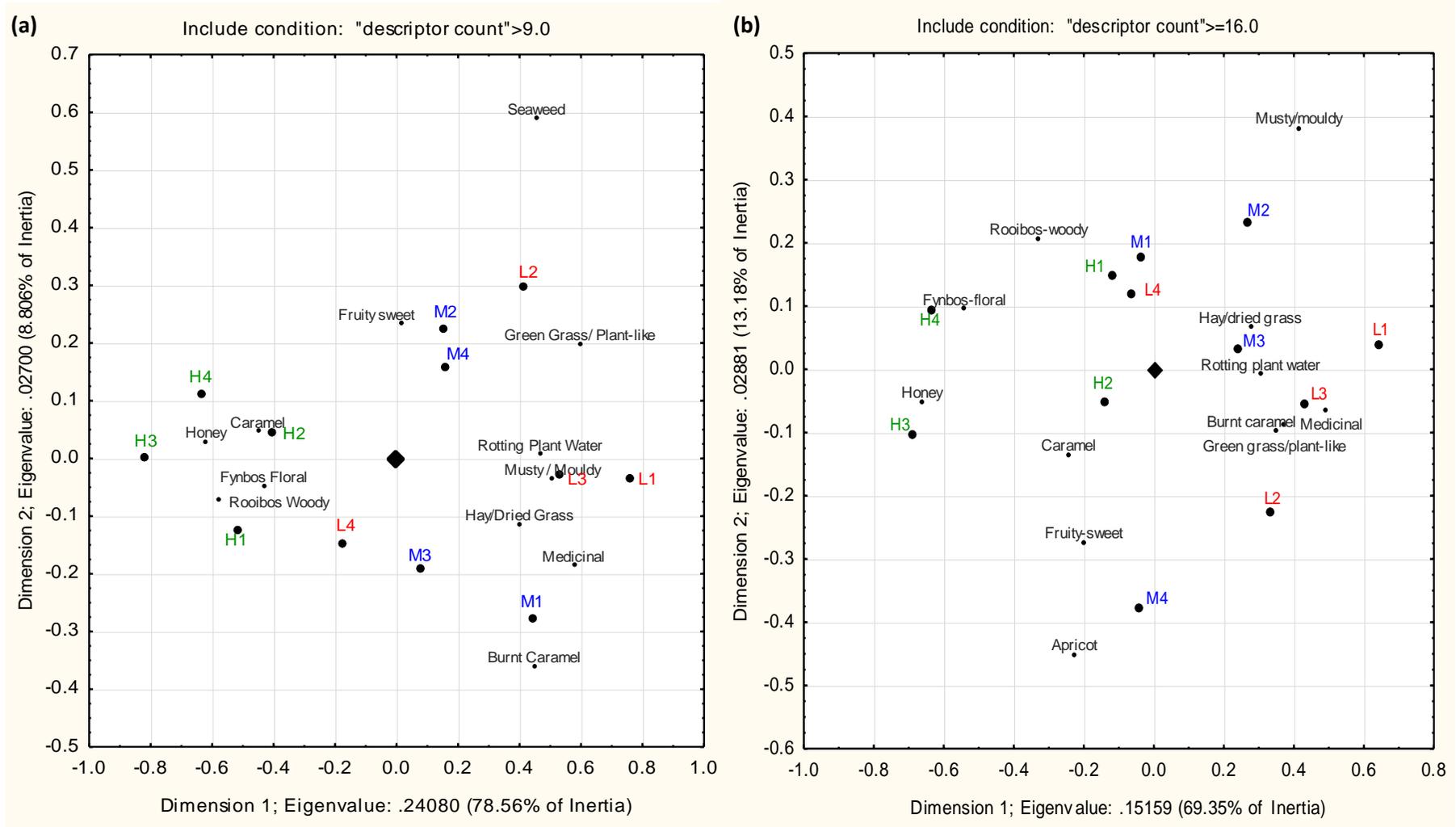
Figure 5 Instructed sorting based on *overall sensory quality*: CA plots indicating the position of rooibos samples from the Western Cape, sorted according to their *aroma* quality profile (a) rep 1, (b) rep 2. The **green** samples indicate the high quality samples, blue indicates the medium quality samples and **red** the low quality samples.

(a) Include condition: "descriptor count">=15.0

(b) Include condition: "descriptor count">=21.0
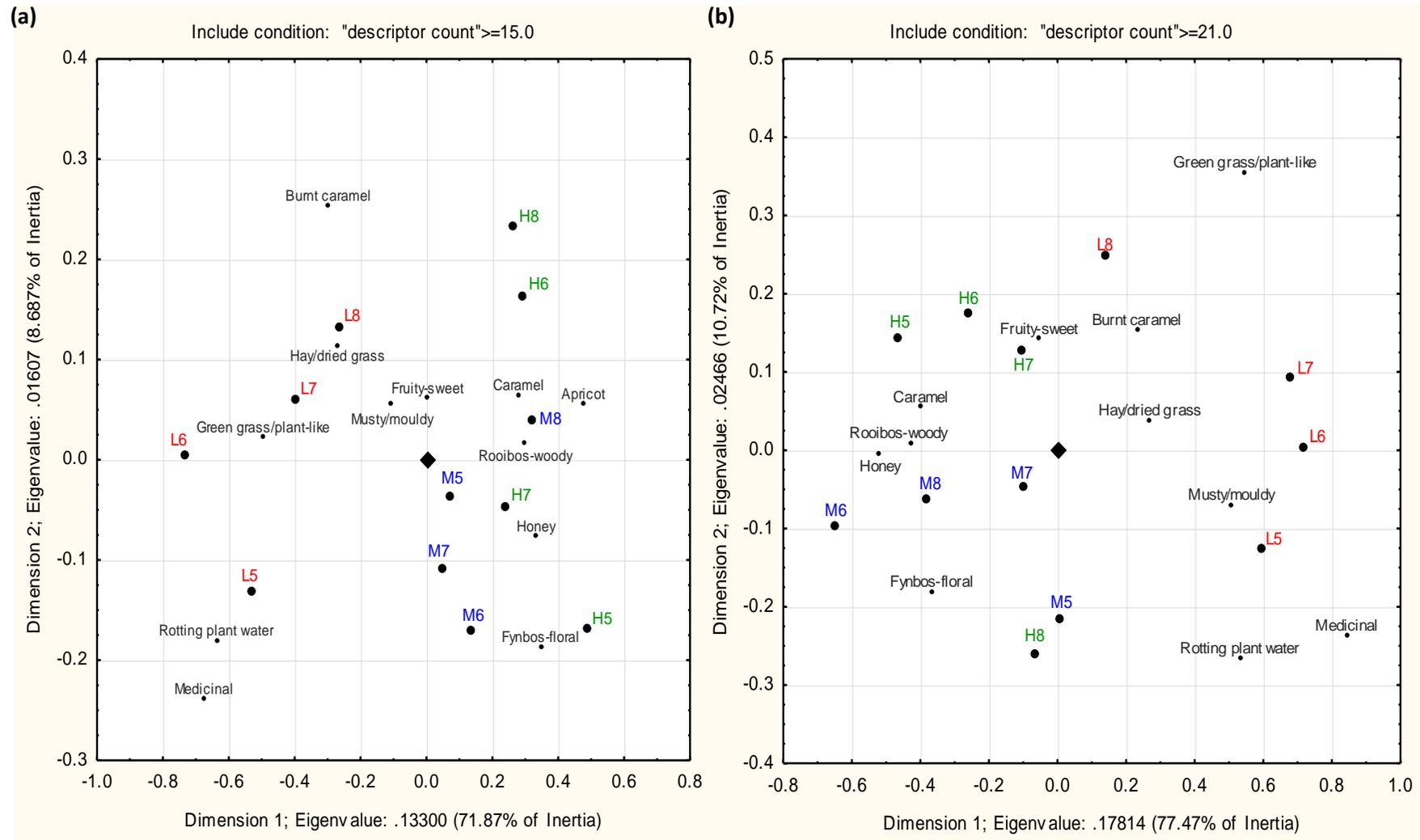
Figure 6 Instructed sorting based on *overall sensory quality*: CA plots indicating the position of rooibos samples from the Northern Cape, sorted according to their *aroma* quality profile (a) rep 1, (b) rep 2. The **green** samples indicate the high quality samples, blue indicates the medium quality samples and **red** the low quality samples.
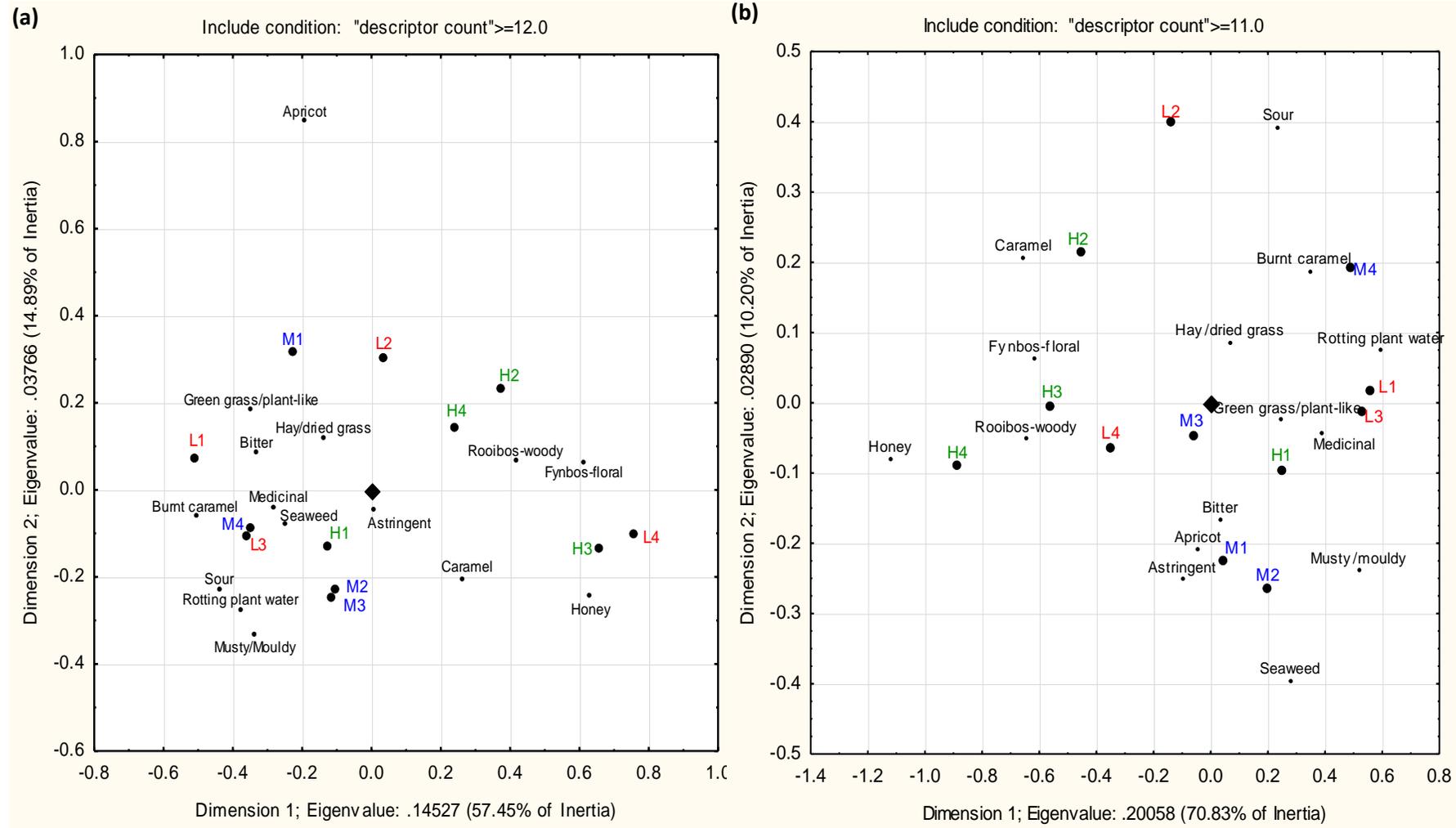
Figure 7 Instructed sorting based on *overall sensory quality:* CA plots indicating the position of rooibos samples from the Western Cape, sorted according to their *palate* quality profile (a) rep 1, (b) rep 2. The **green** samples indicate the high quality samples, blue indicates the medium quality samples and **red** the low quality samples.
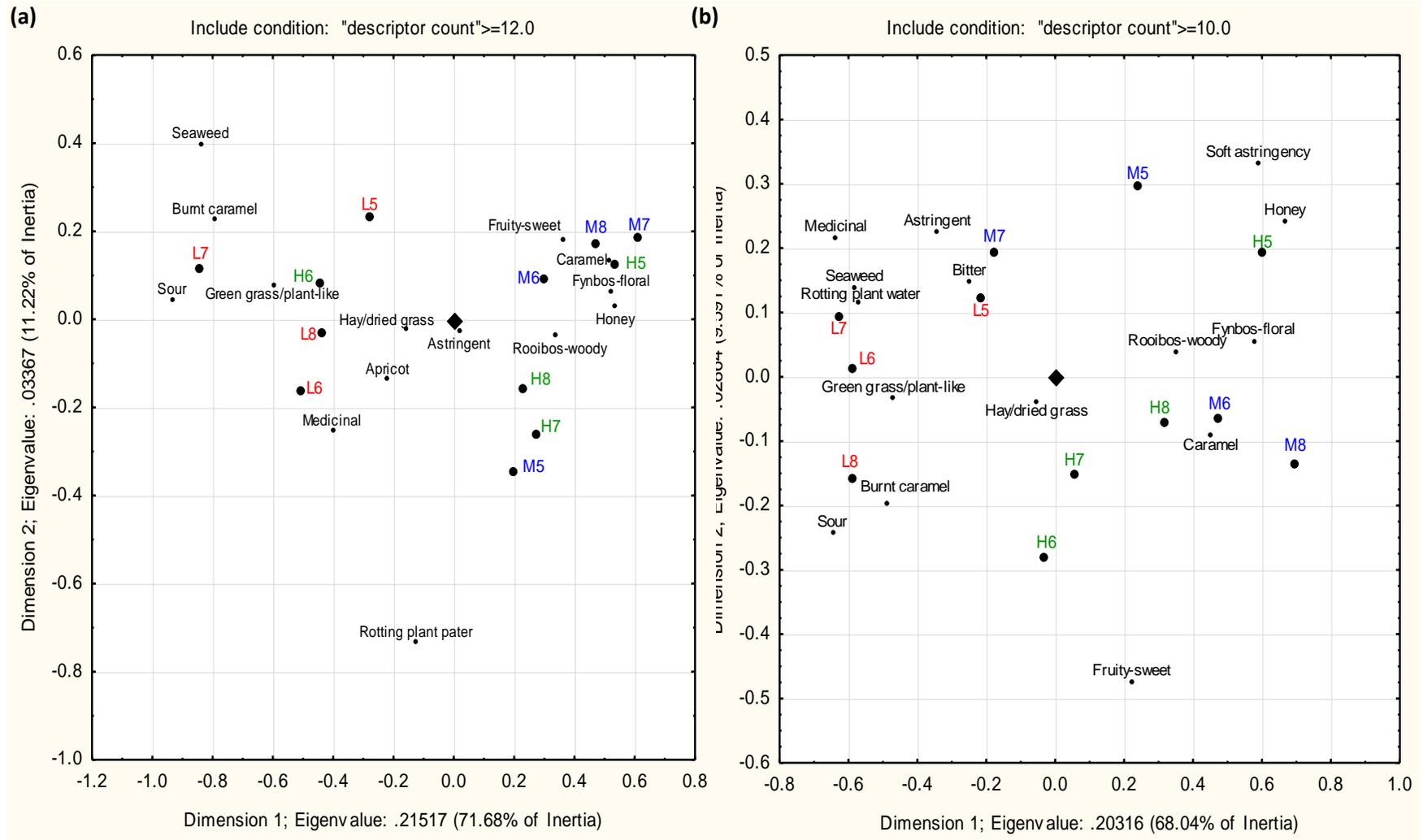
Figure 8 Instructed sorting based on *overall sensory quality*: CA plots indicating the position of rooibos samples from the Northern Cape, sorted according to their *palate* quality profile (a) rep 1, (b) rep 2. The **green** samples indicate the high quality samples, blue indicates the medium quality samples and **red** the low quality samples.
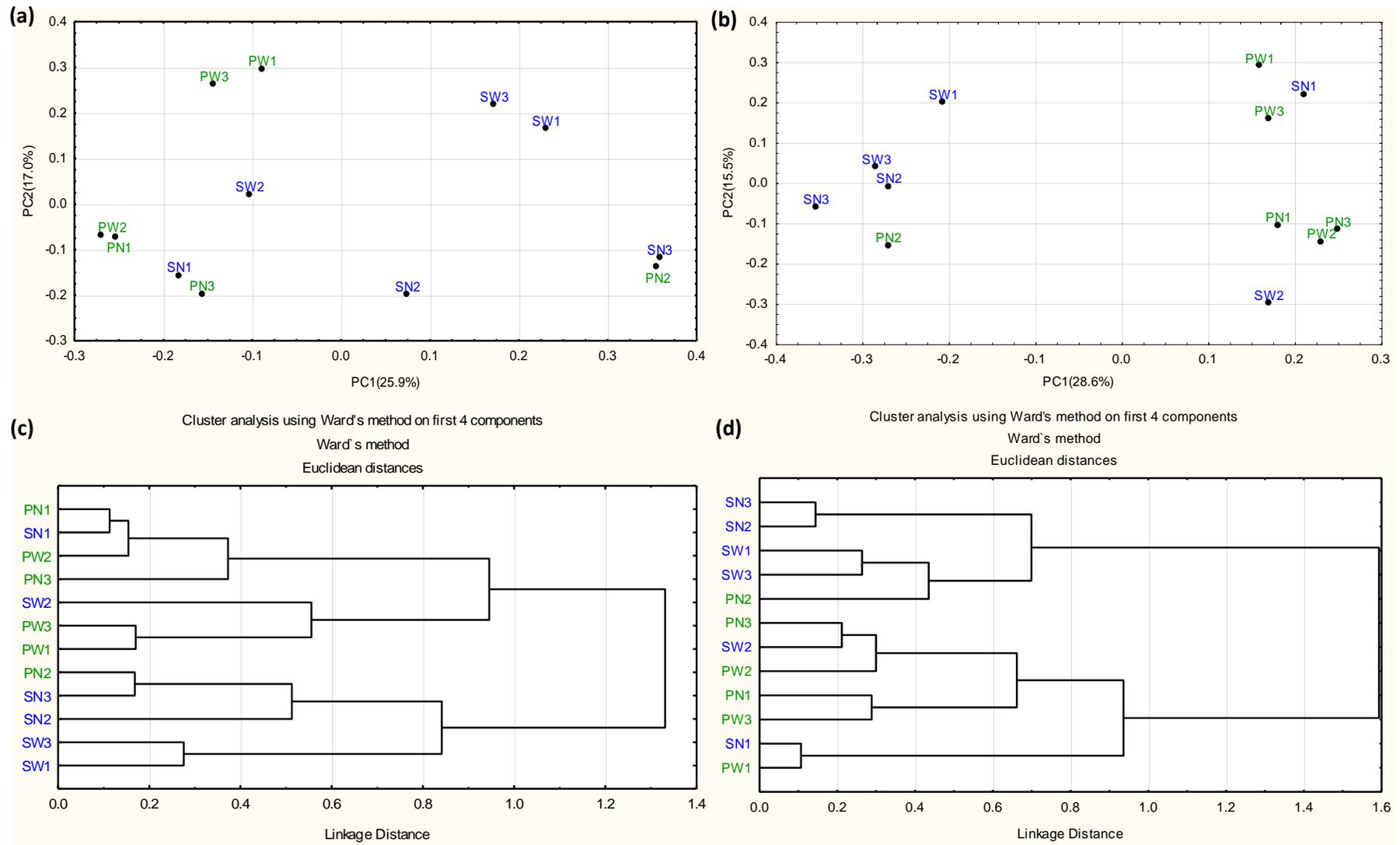
Figure 9 Uninstructed sorting based on *characteristic rooibos profiles*: DISTASTIS plot showing the position of rooibos samples from the Northern Cape and Western Cape, sorted according to their characteristic *aroma profile* (a) rep 1, (b) rep 2. The **green** samples indicate the samples that fit the primary profile and the **blue** indicates the secondary profile. The Ward's cluster analysis plots indicate groupings for (c) rep 1 and (d) rep 2. The samples are represented the same as for the DISTATIS plots.
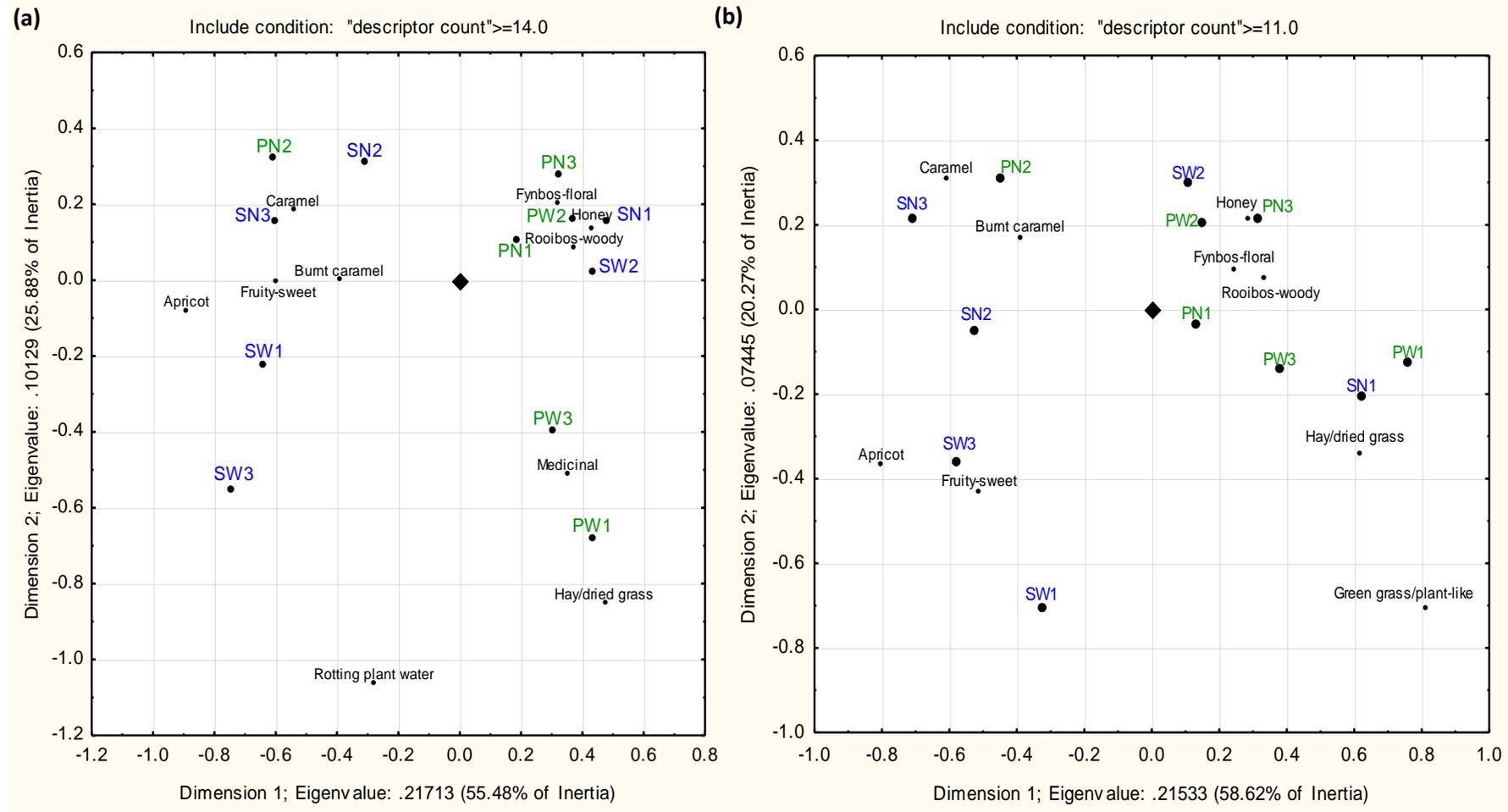
Figure 10 Uninstructed sorting based on *characteristic rooibos profiles*: CA plots indicating the position of rooibos samples from the Western Cape and Northern Cape, sorted according to their *aroma profile* (a) rep 1, (b) rep 2. The **green** samples indicate the primary profile; **blue** indicates the secondary profile.
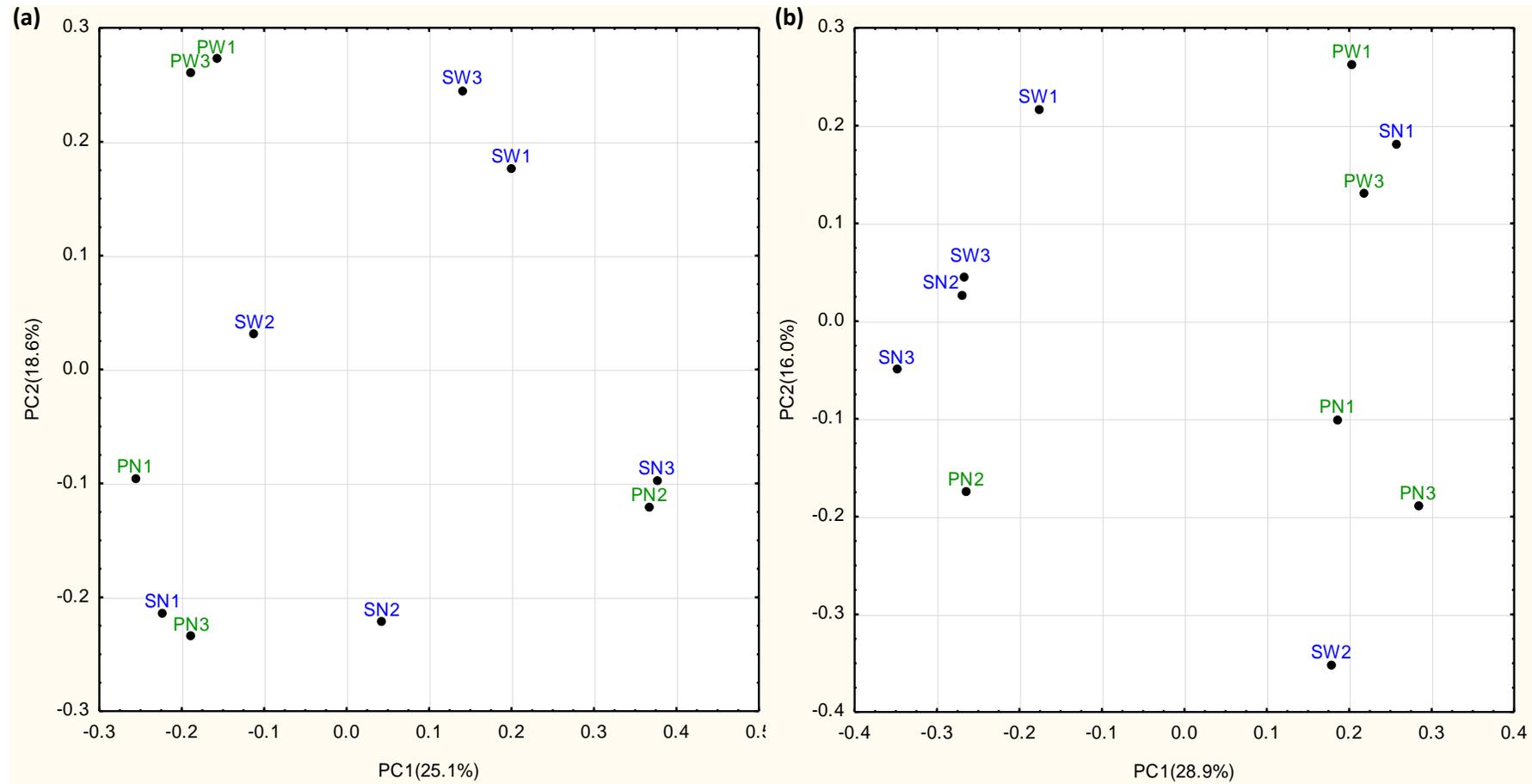
Figure 11 Uninstructed sorting based on *characteristic rooibos profiles*: DISTASTIS plot showing the position of rooibos samples from the Northern Cape and Western Cape, excluding sample PW2 (control) sorted according to their aroma profile (a) rep 1, (b) rep 2. The **green** samples indicate the samples that fit the primary profile and the **blue** indicates the secondary profile.
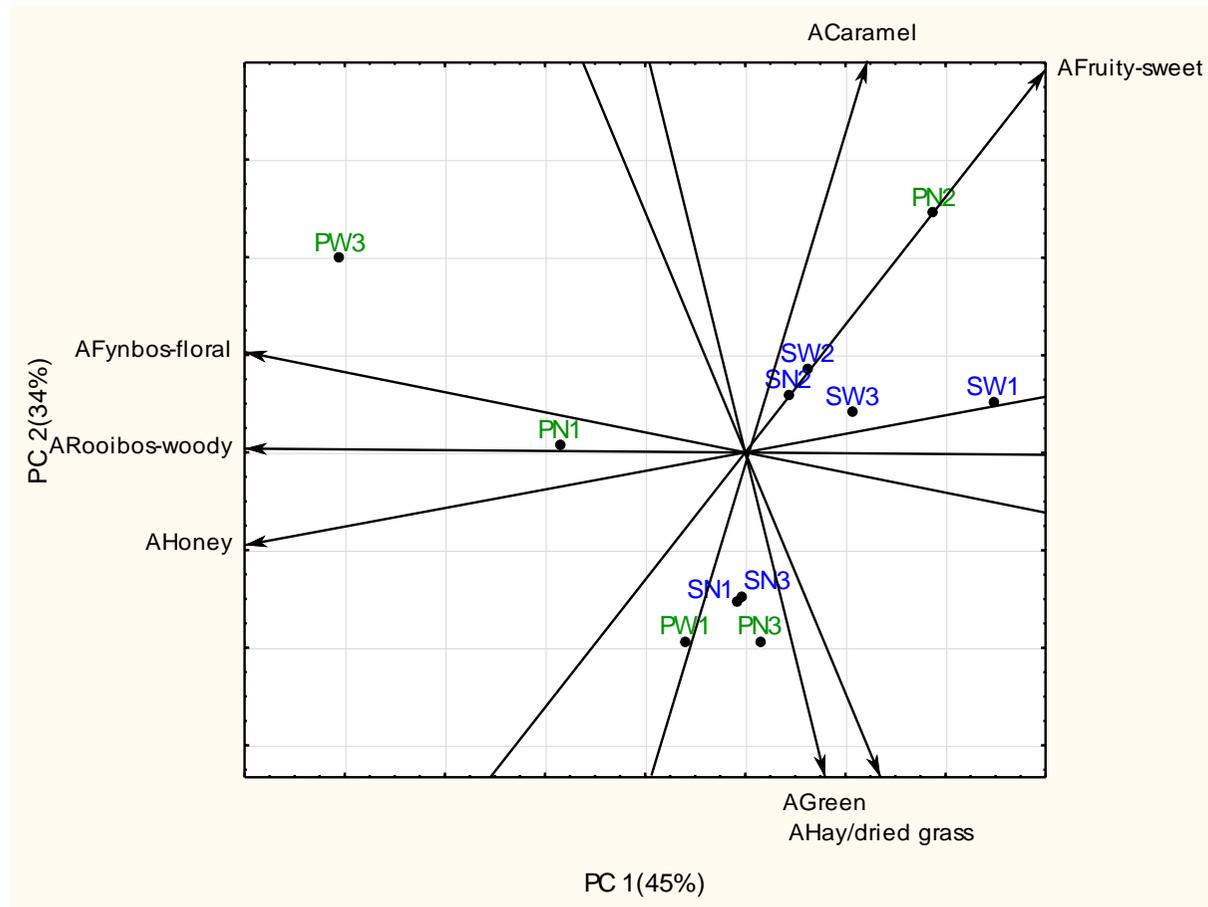
Figure 12 PCA bi-plot showing the results obtained during DSA of the samples used for the ***uninstructed*** sorting of the ***characteristic rooibos aroma profiles***, with the exclusion of sample PW2 (control). The **green** samples indicate the samples that fit the primary profile and the **blue** indicates the secondary profile.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

Product quality encompasses meeting the expectations laid down by the consumer (Van Boekel, 2008). Therefore ensuring that a product consistently meets the quality standards set for that specific foodstuff is of the utmost importance, guaranteeing not only customer loyalty but also company growth. Consistent product quality can be achieved through the creation of tools, based on sensory or chemical data of specific foodstuffs and the use of these tools to aid in quality control (Van Boekel, 2008; Lawless & Civille, 2013).

Rooibos is endemic to South Africa and grows in the fynbos biome region in the Western Cape and Northern Cape, within and surrounding the Greater Cederberg Biodiversity Corridor (Joubert & De Beer, 2011). The rooibos industry is worth an estimated R550 million (per year), with approximately 15 000 tons of rooibos being harvested each year (Donnelly, 2012; Curnow, 2012). There are between 350 and 550 rooibos farmers within South Africa, whom process the tea themselves (small-scale farmers) or send their tea for processing to a large rooibos processor (Anon., 2014a). Processing of rooibos, such as the fermentation of cut tea shoots, takes place in an open-air environment and therefore the parameters cannot be controlled (Joubert & Schulz, 2006). Mostly fermented rooibos is produced and exported, although unfermented "green" rooibos is growing in popularity, due to its high antioxidant levels (Joubert & De Beer, 2011).

Recent acquisition of a geographical indication (GI) for rooibos is a tremendous achievement for this relatively small industry. By obtaining a GI the rooibos industry in South Africa are now better able to control the sales of this herbal tea not only locally, but also internationally. The GI status has significant socioeconomic benefits and will lead to area development and the improvement of livelihoods (Anon., 2014). The rooibos industry is sure to grow as a result of the GI status, allowing not only market expansion but also growth within the rooibos regions (WIPO, 2014). Guidelines should be in place to monitor the production of rooibos to ensure that there is no variation between products of the same quality (WIPO., 2014). With the need to ensure consistency in the production of good quality tea, comes the need to have procedures in place to ensure that these goals are attainable.

Currently, the grading process of rooibos is not standardised, meaning each processor or small-scale farmer grades the tea according to their own criteria primarily based on, leaf colour, infusion colour, the flavour, taste and mouthfeel of the infusion, etc. The criteria deemed the most important by these role-players could differ, leading to variation in product quality. Variations in sensory quality are not only due to grading differences but can also be influenced by production area or climatic conditions, neither of which are taken into account during grading and the impact of which therefore remains unknown. Without the standardisation of the grading process, or at least the grading tools, a consistent supply of high quality

rooibos, from different producers, cannot be ensured or maintained. Healthy lifestyle trends have been on the rise for a number of years, and with it the popularity of herbal teas. With numerous herbal and "health" teas flooding the market, it is important that rooibos tea be able to distinguish itself from the rest. This can be achieved through marketing based on the unique sensory profile of the tea, accompanied by the reliance of high quality in addition to its numerous reported health benefits, such as anti-carcinogenic and anti-inflammatory properties (Joubert & De Beer, 2011). Therefore the sensory profile of rooibos needs to be validated using reliable sensory analysis and industry expertise.

Apart from the prominent red-brick colour of this South African herbal tea, the sensory profile of rooibos is what epitomises its popularity. Currently legislation states that rooibos "shall have the clean, characteristic taste and aroma and clear, distinctive colour of rooibos" (Anon., 2002). With no specific guidelines pertaining to the "characteristic" sensory profile of rooibos, it was almost impossible know what this term encompassed and as a result it became increasingly difficult to ensure consistency between rooibos samples of the same sensory quality, when processed by different processors and farmers. Koch *et al*. (2012) determined that, based on a large sample set from a single production year and area (N = 69), the "characteristic" sensory profile of rooibos meant that rooibos has "woody", "fynbos-floral" and "honey" aroma and flavour notes with a "sweet" taste and a subtle "astringent" mouthfeel. The development of a sensory lexicon and wheel, which accompanied these findings, were welcomed by the industry. It became clear that, in order to improve the reliability of the initial results, it was important that the sensory lexicon and wheel be updated and validated using results obtained from a larger number of samples, i.e. a sample set encompassing a larger degree of variation. Lawless & Heymann (2010) stated that, for the attributes to be both descriptive and discriminating, the samples used needed to be from a large data set able to cover all possible sample variations.

With the use of validated quality control tools comes the ability to significantly assist in the standardisation of quality. Until now, each of the rooibos processors uses their own grading methods, in order to determine the quality of the rooibos, as well as the final quality grade. This is where smaller processors or small-scale farmers struggle to ensure consistency between samples of the same quality. For the future growth and stability of the small role players in the rooibos industry, or even the newly established large processors it is necessary to provide them with the tools and methods needed to allow them to have as much impact on the market as the major, more established companies.

Phenolic compounds are abundant in plant foods and have numerous potential health benefits (Bravo, 1998). Within rooibos infusions, it has been found that phenolic compounds are responsible for the taste and mouthfeel characteristics of the tea (Joubert *et al*., 2013; Koch *et al.,* 2013). These attributes play an important role in the quality perception of rooibos; therefore determining the non-volatile compounds that drive these attributes can be very useful to industry. Understanding the importance of particular phenolic compounds, as contributors to the sensory profile, can be important for future harvests and for use as indicators of quality. Koch *et al*. (2013) were able to establish weak, but significant, correlations

between the phenolic compounds and the taste and mouthfeel attributes. With the correlations all being low (r < 0.5), there was an indication that the presence of each of the taste and mouthfeel characteristics could not be attributed to individual phenolic compounds (Koch *et al.*, 2013). Further insight into these relationships using a larger data set, might provide stronger correlations, due to increased sample variation.

The objectives of this study were, therefore, to determine the sensory profile of rooibos by defining the significant sensory attributes responsible for the sensory characteristics of rooibos in the form of a validated sensory wheel, to determine the influence of specific factors such as production area and production season on the sensory profiles, to determine the phenolic drivers of the basic taste and mouthfeel attributes of rooibos through the creation of a prediction model and, furthermore, to determine the possibility of using rapid sensory profiling methods, instructed and uninstructed sorting, as reliable methods to determine rooibos quality and sensory profiles, respectively.

Increasing the number of samples (N = 230), and production years (2011, 2012 & 2013) for rooibos of different quality grades (A, B, C, D), produced in the Western Cape and Northern Cape, ensured that all possible variation within rooibos was taken into account, so that the findings could be used with confidence by the industry. Production area (Western Cape and Northern Cape) was, however, found to have no effect on the sensory quality or profile of the tea, as opposed to production years, which were found to have an effect on the tea quality and profiles. Although it can be said that the production area does not have an effect on the sensory profile of rooibos, more intensive testing would need to be carried out on controlled rooibos plots within in each area, so as to investigate soil and climate, to further substantiate that production area has no effect on sensory aspects of rooibos. Production years, however, did have an effect on the rooibos sensory profile; leading to the conclusion that climate may play a crucial role in the profile development of this commercial product. Changes in climate have already been documented in areas known for rooibos tea cultivation (Archer, 2009). Climate during growth is expected to affect the composition of the plant, seen through large phenolic variation in samples harvested from different production seasons (Joubert *et al.*, 2012), while prevalent environmental conditions (temperature, rainfall and humidity, etc.) during open air processing will also affect tea quality (Joubert & De Villiers, 1997).

The primary characteristic sensory profile of rooibos was validated and determined to be, "rooibos-woody", "fynbos-floral" and "honey" aroma and flavour notes, accompanied by a "sweet" taste and a slight "astringent" mouthfeel, thereby confirming the results obtained by Koch *et al*. (2012). These attributes were found to be present in 100% of the samples, irrespective of the production area. The average intensities for "rooibos-woody" and "fynbos-floral" were high, being 37 and 25 respectively. "Honey" was lower at an average intensity of 20, whereas "sweet" and "astringent" were 21.5 and 24.3, respectively. An attribute that became of particular interest was the "hay/dried grass" attribute (average intensity = 12.5), which was found in more than 90% of the rooibos samples. This result also indicated that "hay/dried grass" is important to the primary characteristic profile of rooibos although it is often viewed as a negative

attribute. It was, therefore determined, after consultation with industry, that at low intensities (intensity < 12.5), this attribute can be deemed as part of the primary characteristic profile of rooibos, and is important to its unique aroma and flavour, whereas at high intensities (intensity > 12.5) this attribute becomes unpleasant and leads to tea being of a low quality.

The preliminary lexicon and sensory wheel were recreated and validated, with input from industry, to reflect the results that arose from using the larger sample set. The sensory lexicon was updated with an increase in the number of aroma and flavour attributes from 10 to 17, the restructuring of the attribute definitions, as well as the inclusion of new reference standards. The attributes "baked apple", "fruity-sweet", "spicy", "rotting plant water", "seaweed", "burnt caramel" and "medicinal/rubber" were added to the list of attributes, and were included in the sensory wheel. The sensory wheel was updated to include these changes. Unlike the previous sensory wheel, the updated rooibos sensory wheel contains the average intensities of each of the attributes, depicted through the thickness of each slice in the wheel, and is accompanied by bar graphs indicating the percentage occurrence of these attributes. Due to the inclusion of the intensities to the wheel, it was not possible to depict each of the attributes accurately within one wheel; therefore it was necessary to split the sensory wheel into a rooibos aroma wheel and a rooibos flavour, taste and mouthfeel wheel. The number of attributes within the aroma wheel is 17 and the flavour taste and mouthfeel is made up of 21 attributes. A number of attributes previously contained within the rooibos sensory wheel, which contained 27 attributes (Koch *et al.*, 2012), were removed. Attributes such as "perfume", "wet hessian" and "sweaty", etc., were removed as they were found to be redundant and uncommon to rooibos.

These sensory tools can be used both in the profiling and quality control of rooibos. When incorporated into the grading process, the use of the sensory tools can ensure that the graders, across different processors, are able to correctly understand the meaning of each attribute responsible for the sensory quality of rooibos. By using standardised vocabulary, better communication will arise, leaving no possibility for misinterpretation. Better communication ensures that the quality of rooibos, produced at different processors or farms, will be of consistent quality when on the market, which is important for consumer loyalty and product growth. Providing international marketing companies with a validated list of attributes, accompanied by reference standards and a user-friendly sensory wheel, will lead to better understanding and communication with the suppliers and consumers, as well as benefit the marketing of rooibos on the international market.

The reference standards used in the sensory lexicon were chosen from a large number of chemical aroma mixtures and individual chemical compounds. These were then tested and partially validated with feedback from the rooibos industry. Although these reference standards may, at this point in time, not be able to exactly mimic the sensory attributes in question, the list of these reference standards provide a base for further research and development of a rooibos aroma kit. These quality control tools will be very

important for the growth of the industry, especially now with the GI in place, as interest in rooibos will increase, and be accompanied by growth in the expectation of consistent quality.

An aspect that became clear throughout the sensory, and data analyses was the emergence of a secondary rooibos profile. Although containing attributes from the primary characteristic profile of rooibos, these samples appeared to have higher than average intensities of "caramel", "fruity-sweet" and "apricot" notes. These attributes when grouped together were given the title secondary characteristic profile, as this profile does not occur as commonly as the primary characteristic profile. No explanation for the differences in these profiles could be determined, based on area alone, and more research into the drivers of these specific attributes would need to be done. The use of gas chromatography-olfactometry (GC-O) would be of use as it is able to determine the volatile compounds responsible for the aroma attributes within the infusions. Preliminary GC-O work done on rooibos infusions, determined that rooibos contains high levels of damascenone and guaicol, among others, these compounds usually associate with floral and woody aromas, respectively (N. Wiltshire, Kerry Ingredients, Durban, South Africa, October 2013, personal communication). Another volatile compound found within rooibos was eugenol. It is most likely responsible for the "spicy" aroma of rooibos, as was found for honeybush (*Cyclopia spp*.) by Theron *et al*. (2014), where it was responsible for the high spicy aroma of a *Cyclopia maculata* sample. Further GC-O work, on samples exhibiting specific aroma attributes or profiles, will help in the identification of the volatile constituents responsible for the unique aroma's found in rooibos infusions. Profiling rooibos as either primary characteristic or secondary characteristic allows for the expansion of the market with the creation of a niche market for the secondary characteristic rooibos.

Once validated, the taste and mouthfeel attributes were further analysed, this time on a phenolic level. Using a larger data set than for DSA, spanning a five year period, two production areas and four quality grades (N = 260), it was possible to determine the variation, or lack thereof, between the samples, and determine the phenolic drivers of the taste and mouthfeel attributes. The results indicated that the phenolic variation between the samples was not as a result of area but rather the result of production season. The same trend was seen for the sensory results, indicating that both the sensory profile and the phenolic composition of the rooibos are influenced by climate. Climate has been known to affect the biosynthesis of polyphenols in plants (Tounekti *et al*., 2013; Agati *et al*., 2012), and further research on controlled rooibos crops may be able to determine the exact effects climate has on the quality of rooibos as a result. Although a large number of samples were analysed statistically, using partial least squares (PLS), step-wise regression and Pearson's correlation analysis (Abdi, 2007; Snedecor & Cochran, 1989), the results obtained could not clearly indicate which of the phenolic compounds were responsible for any of the taste and mouthfeel attributes. The lack of definitive correlations between specific compounds and the attributes could be due to natural variation within the plant material, lack of variation between the core phenolic compounds within rooibos, the "narrow" range of the intensities of the respective sensory attributes, as well as the combined effect of several compounds, all of which are not taken into account

during the analysis. None of the above-mentioned regression methods can take every factor that may have an effect on the taste and mouthfeel attributes into account, such as the relationships between compounds or their modulating effects (Joubert *et al*., 2013; Soares *et al*., 2013). Therefore it would be advisable to pursue the use of other methods such as multiblock analysis (Multiblock-PLS, Parallel and orthogonalised-PLS (PO-PLS), and sequential and orthogonalised-PLS (SO-PLS)) to be able to predict the intensities of taste and mouthfeel attributes (Næs *et al*., 2013). Multiblock analysis can improve the interpretability of multivariate models and is useful when a large number of variables are available for analysis (Westerhuis *et al*., 1998). Expansion of the intensity scale for the taste and mouthfeel attributes could also lead to clearer variation being discovered between the samples. In the present study the intensity range used for the taste and mouthfeel attributes is small (intensity between 0 and 25 on a 100-point intensity scale), meaning that the results obtained for the samples imply little variation between the samples, although the perceived differences in the profiles are large. With the expansion of the scale by making use of the whole scale (intensity 0 to 100), thereby creating larger intensity differences between samples deemed dissimilar based on a particular attribute, it will be possible to better define the variation between the samples.

Rapid methods have been gaining popularity within the food industry as a rapid and reliable alternative to DSA, for the categorisation of products based on their sensory profiles (Varela & Ares, 2012). Sorting is one of these methods, that has been used for obtaining non-quantitative information about different food products, such as beer (Chollett *et al*., 2006), breakfast cereals (Cartier *et al*., 2006) and drinking waters (Falahee & MacRae, 1997). The sorting method, both instructed and uninstructed, used in the current study with great success. *Instructed sorting* was successfully used in the separation of rooibos samples based on overall sensory quality. By being instructed to sort the samples based on high, medium and low aroma quality, determined by the presence of positive and negative attributes, the panellists were able to differentiate between the samples based on sensory quality. Sorting according to medium sensory quality proved difficult, due to the samples containing near equal intensities of positive and negative attributes. Therefore it was determined that it is possible to use instructed sorting as a means to categorise samples based on *aroma* quality. Sorting according to flavour yielded results that were less specific, and distinct differences between samples based on sensory quality was not as clear. The lack of separation between the quality groupings, based on flavour could be due to the low intensities at which the flavour attributes are present in the rooibos infusions, making it difficult to separate high and low quality samples. Instructed sorting, as a tool for quality control, has not been used often within the food industry (Chollett *et al*., 2006), however, the results indicated the potential of incorporating this process into quality grading of rooibos. *Uninstructed sorting* was evaluated for its potential use as a rapid method to profile rooibos based on the aroma profile of the infusions. Results indicated that the panel sorted the samples into groupings based on the primary characteristic and secondary characteristic profiles, without being instructed to sort for these specific profiles. The appearance of these characteristic profiles again verified

results obtained during the DSA. Therefore it is possible to use uninstructed sorting to profile rooibos infusions based on aroma profiles alone within an industry setup.

With the combined use of the sensory wheel, sensory lexicon and sorting method, it would be possible to not only profile rooibos but also determine sample quality based on aroma. This would prove useful during the grading process, as a rapid means to screen the samples according to infusion quality prior to further grading analyses. Ensuring that each blend created meets the specified criteria each time is important to ensure quality consistency. By using the sorting method, it would be possible to compare a newly blended sample to a reference sample, to determine similarities or differences in a rapid manner. Using sorting along with other quality control tools, can mean the development of a grading method for small processors and small-scale farmers, which would improve quality consistency and grading techniques. The current sorting method does not allow for the clear sorting of samples based on flavour quality, due to low flavour attribute intensities. The inclusion of taste and mouthfeel attributes to the sorting process prior to grading (screening), however, may prove beneficial. These attributes have been known to affect the overall sensory quality of rooibos, and their inclusion can aid in the clarification of the quality groupings made.

Numerous criteria are taken into account when grading rooibos, therefore, in order for the complete grading process based on aroma and flavour to be achieved, another method able to incorporate the overall quality aspects of the tea needs to be considered. The use of polarized sensory positioning (PSP) and optimized descriptive profile (ODP) should be researched in the future as rapid methods to aid in the grading process. PSP allows for the comparison of each of the tea samples to a fixed reference sample, meaning that a sample depicting a specific quality grade or profile can be used as a base to which future samples can be compared (Varela & Ares, 2012). The use of ODP allows for the determination of quantitative information, but is achieved in a more rapid manner than DSA. This method also includes the use of reference standards during analysis, and the samples are scaled according to intensity, using the references as the extremes on either side of the scale (Silva *et al*., 2012). By incorporating rapid methods into the grading of rooibos, or by basing grading on these methods entirely, small processors or farmers could use these methods successfully with the assurance that quality will be standardised.

This study was successful in the recreation and validation of both the rooibos sensory wheel and lexicon. These easy-to-use tools can be incorporated seamlessly into the current grading procedures, already in place for the grading of rooibos tea samples, so as to ensure consistency between samples of the same quality grade, which is especially important now that the GI is in place. Use of these quality control tools will also ensure better communication amongst members of the rooibos industry, thus improving and standardising the grading procedure. The incorporation of attribute intensities into the sensory wheel, as well as the inclusion of percentage occurrence bar graphs, is a new concept and was well received after discussion with the rooibos industry. Through the inclusion of this additional information, the grader will have a better idea as to what to expect with regards to each attribute. Accompanying the use of the wheel

with the reference standards from the sensory lexicon, will further the understanding of each individual attribute. Although not possible to determine the phenolic drivers of the taste and mouthfeel attributes within rooibos infusions, indication as to possible "predictor" attributes was determined. The use of other regression methods may be able to better indicate the phenolic compounds responsible for these attributes. Further analysis on the phenolic compounds themselves is suggested, as greater understanding of the relationships between samples and possible modulating effects will make the determination of "predictor" compounds easier to achieve. Sorting was successfully identified as a possible rapid method, to be used for the screening of rooibos infusions, prior to further quality analyses. It was found that greater and more reliable results would be achieved if this screening were based on the aroma quality, as well as the taste and mouthfeel attributes of the rooibos infusion, rather than the flavour quality. Furthermore, two prominent rooibos profiles, namely the primary and secondary characteristic profiles were defined using both DSA and sorting. The occurrence of these profiles will allow for greater marketing opportunities for the rooibos industry, and will aid in the growth of this unique local industry.

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ADDENDA

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ADDENDUM A
Sensory profile of rooibos originating from the Northern Cape and Western Cape and the development of quality control tools

Table A1 Details on the rooibos samples from the Western Cape (2011-2013) including the grading criteria and scores given by during quality grading by the processors.

						Sample and Grading Detail											
Production area	Year	Grade	Batch	Sample code	Cut	Dry appearance		Wet appearance		Infusion clarity		Taste		TOTAL		MOISTURE %
WC	2011	A	1	WC11_1A	NA	NA[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	A	2	WC11_2A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	A	3	WC11_3A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	A	4	WC11_4A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	A	5	WC11_5A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	A	6	WC11_6A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	B	1	WC11_1B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	B	2	WC11_2B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	B	3	WC11_3B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	B	4	WC11_4B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	B	5	WC11_5B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	B	6	WC11_6B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	C	1	WC11_1C	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	C	2	WC11_2C	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	C	3	WC11_3C	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	C	4	WC11_4C	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	C	5	WC11_5C	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	C	6	WC11_6C	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	D	1	WC11_1D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	D	2	WC11_2D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	D	3	WC11_3D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	D	4	WC11_4D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	D	5	WC11_5D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2011	D	6	WC11_6D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WC	2012	A	1	WC12_1A	SHORT	8	8.7	8	8	8	8	8	8.3	80	83	7.88
WC	2012	A	2	WC12_2A	SHORT	8	8	8	8	8	8	8	8	80	80	7.96

WC	2012	A	3	WC12_3A	FINE	9	9	8	8	8	8	8	8	82	82	8.03
WC	2012	AA	4	WC12_4AA	FINE	9	9	8	9	7	8	8	9	79	87	8.2
WC	2012	A	5	WC12_5A	FINE	9	8.5	8	8	7	7	8	8	79	78	8.16
WC	2012	A	6	WC12_6A	SHORT	9	9	9	9	8	7	8	8	84	82	8.48
WC	2012	A	7	WC12_7A	SHORT	8	8	8	7.5	8	7.5	9	7.5	84	77	8.32
WC	2012	A	8	WC12_8A	SHORT	8	8	8	8	8	8	8	8.3	80	81	8.75
WC	2012	A	9	WC12_9A	SHORT	8	8	8	8	7	8	8	8	78	80	8.12
WC	2012	AA	10	WC12_10AA	SHORT	9	9.3	9	9	7	8	8	9	82	89	7.63
WC	2012	A	11	WC12_11A	SHORT	9	9	10	7.7	8	7	9	7	89	77	8.27
WC	2012	A	12	WC12_12A	FINE	9	9	8	8.3	8	8	8	8	82	82	8.85
WC	2012	A	13	WC12_13A	FINE	9	9	8	8.7	7	8	8	8	79	83	7.26
WC	2012	A	14	WC12_14A	SHORT	9	9	8	9	8	8	8	7.7	83	83	7.88
WC	2012	A	15	WC12_15A	FINE	8	8.3	8	8.5	7	8	8	7.3	77	78	8.16
WC	2012	A	16	WC12_16A	FINE	9	9	7	8	8	8	8	8	81	82	8.36
WC	2012	A	17	WC12_17A	FINE	7	7.5	7	7	7	8	7	7.5	70	76	6.34
WC	2012	A	18	WC12_18A	FINE	8	8	8	8	6	8	7	8	70	80	7.22
WC	2012	B	1	WC12_1B	FINE	7	7	7	6.7	7	7	7	6.7	70	69	7.94
WC	2012	B	2	WC12_2B	FINE	8	6.7	7	7	7	7	7	6.3	72	67	8.52
WC	2012	B	3	WC12_3B	FINE	7	7	7	6.7	7	7	7	7	70	70	6.98
WC	2012	B	4	WC12_4B	FINE	7	7	6	6.5	7	7	7	7	69	70	7.49
WC	2012	B	5	WC12_5B	FINE	8	7.8	7	7	7	7	7	7	72	72	7.8
WC	2012	B	6	WC12_6B	SHORT	7	7	7	7	7	7	7	7	70	70	6.86
WC	2012	B	7	WC12_7B	FINE	7	7	7	7	8	7	7	7	73	70	8.95
WC	2012	B	8	WC12_8B	SHORT	8	8	7	8	7	7	7	6.7	73	73	9.03
WC	2012	B	9	WC12_9B	FINE	6	6.3	6	7	6	6	6	7	60	66	7.28
WC	2012	B	10	WC12_10B	FINE	7	7	7	7	7	7	7	6.5	70	68	7.42
WC	2012	B	11	WC12_11B	FINE	7	7	6	7	7	7	7	6	69	66	8.94
WC	2012	B	12	WC12_12B	FINE	7	7	6	7.5	7	7	7	7.5	69	73	7.08
WC	2012	B	13	WC12_13B	FINE	8	-	7	-	7	-	7	-	72	-	9.28
WC	2012	B	14	WC12_14B	FINE	8	8	7	7	6	6	6	6.3	65	66	6.2
WC	2012	B	15	WC12_15B	FINE	7	7	6	6.7	7	7	7	7	69	70	6.22
WC	2012	B	16	WC12_16B	FINE	7	7	7	7	7	7	7	6.5	70	68	8.49
WC	2012	B	17	WC12_17B	FINE	8	8	7	6.5	6	6	7	7	69	69	6.94
WC	2012	B	18	WC12_18B	FINE	8	8	7	7	7	7	7	6	72	68	6.08

WC	2012	B	19	WC12_19B	FINE	7	7	7	7.5	7	7	7	6	70	67	6.82
WC	2012	B	20	WC12_20B	FINE	7	7	6	6.3	6	6	6	6.7	62	65	8.26
WC	2012	C	1	WC12_1C	FINE	6	6	6	6	6	6	6	7	60	64	7.06
WC	2012	C	2	WC12_2C	FINE	7	6.3	6	6.7	6	6	6	6.3	62	63	9.58
WC	2012	C	3	WC12_3C	FINE	6	6	6	6	6	6	6	6.7	60	63	6.58
WC	2012	C	4	WC12_4C	-	6	6.5	7	7	6	6	6	6.5	61	64	6.96
WC	2012	C	5	WC12_5C	FINE	6	6	6	6	6	6	6	6	60	60	8.35
WC	2012	C	6	WC12_6C	FINE	6	6	6	6	6	6	6	6	60	60	7.6
WC	2012	C	7	WC12_7C	FINE	6	6	6	6	6	6	7	6	64	60	6.84
WC	2012	C	8	WC12_8C	FINE	7	6	7	7	7	7	7	7	62	60	5.34
WC	2012	C	9	WC12_9C	FINE	6	6	6	6	7	6	6	6	63	60	8.05
WC	2012	C	10	WC12_10C	FINE	6	6	6	6	6	6	5	6	56	60	8.46
WC	2012	C	11	WC12_11C	FINE	6	6	6	6	6	6	6	6	60	60	4.54
WC	2012	C	12	WC12_12C	FINE	6	5.5	6	6	7	6	6	6	63	59	8.38
WC	2012	C	13	WC12_13C	FINE	6	6	6	7	6	6.5	6	6	60	63	6.12
WC	2012	C	14	WC12_14C	FINE	5	5.5	5	6	7	6	6	6	60	59	8.16
WC	2012	C	15	WC12_15C	FINE	6	6	6	6	6	6	6	6	60	60	7.23
WC	2012	C	16	WC12_16C	SHORT	6	6	6	6	6	6	6	6	60	60	7.36
WC	2012	C	17	WC12_17C	FINE	6	6	6	6	7	7	6	6	63	63	8.88
WC	2012	C	18	WC12_18C	FINE	6	6.5	6	6.3	6	6	6	6.3	60	63	7.72
WC	2012	C	19	WC12_19C	FINE	6	6	6	6	7	6	6	5	63	56	10.68
WC	2012	C	20	WC12_20C	FINE	6	6	6	6	6	6	6	6	60	60	9.45
WC	2012	D	1	WC12_1D	INE	5	5	5	5	6	6	4	4	49	49	9.69
WC	2012	D	2	WC12_2D	FINE	5	5	6	5	6	7	5	4.5	54	54	9.69
WC	2012	D	3	WC12_3D	FINE	5	5	6	6	6	6	5	5	54	54	8.94
WC	2012	D	4	WC12_4D	FINE	5	5	5	5	6	6	5	5	53	53	9.14
WC	2012	D	5	WC12_5D	LONG	5	5	6	5.7	5	5	5	5.3	51	52	6.88
WC	2012	D	6	WC12_6D	FINE	4	5	6	6	6	6	4	3	48	46	9.36
WC	2013	A	1	WC13_1A	Fine	7	8	6	7	7	7	7	8	69	76	5.18
WC	2013	A	2	WC13_2A	Fine	8	9	7	8	7	8	7	7	72	78	6.02
WC	2013	A	3	WC13_3A	Short	7	8	7	8	6	8	7	7	68	76	6.48
WC	2013	A	4	WC13_4A	Fine	8	8	8	8	7	7	7	8	73	77	7.32
WC	2013	A	5	WC13_5A	Fine	7	7	7	7	7	8	8	8	74	77	7.4

WC	2013	A	6	WC13_6A	Fine	9	9	7	7	8	8	8	8	81	81	6.4
WC	2013	A	7	WC13_7A	Fine	7	7	7	7	8	8	7	8	73	77	7.87
WC	2013	A	8	WC13_8A	Short	8	8	8	8	7	7	8	8	78	78	6.84
WC	2013	A	9	WC13_9A	Short	8	8	7	7	7	7	7	8	73	77	7.16
WC	2013	A	10	WC13_10A	Fine	10	9	8	8	7	8	8	8	81	82	8.1
WC	2013	B	1	WC13_1B	Fine	7	7	7	7	7	7	6	6	66	66	5.44
WC	2013	B	2	WC13_2B	Fine	7	7	7	7	7	7	7	7	70	70	7.62
WC	2013	B	3	WC13_3B	Fine	8	8	6	6	7	7	7	6	71	67	4.7
WC	2013	B	4	WC13_4B	Fine	7	7	7	7	7	7	7	7	70	70	6.54
WC	2013	B	5	WC13_5B	Fine	8	8	6	7	7	7	7	7	71	72	6.38
WC	2013	B	6	WC13_6B	Fine	7	7	6	6	7	7	7	7	69	69	6.02
WC	2013	B	7	WC13_7B	Fine	8	8	7	7	6	6	7	7	69	69	6.32
WC	2013	B	8	WC13_8B	Fine	6	6	6	6	7	7	7	7	67	67	6.35
WC	2013	B	9	WC13_9B	Fine	6	7	6	6	8	8	6	7	66	72	6.63
WC	2013	B	10	WC13_10B	Fine	6	6	6	6	7	7	7	7	67	67	7.11
WC	2013	B	11	WC13_11B	Short	7	7	6	6	7	7	7	7	69	69	6.13
WC	2013	B	12	WC13_12B	Fine	7	8	6	7	7	7	7	7	69	72	7.86
WC	2013	B	13	WC13_13B	Fine	6	6	6	6	7	7	7	7	67	67	6.9
WC	2013	B	14	WC13_14B	Fine	7	7	6	7	7	8	7	7	69	73	7.72
WC	2013	B	15	WC13_15B	Short	8	8	7	7	7	7	7	7	73	73	7.24
WC	2013	B	16	WC13_16B	Fine	7	7	7	7	7	7	6	7	66	70	6.98
WC	2013	C	1	WC13_1C	Fine	6	6	6	6	6	6	6	6	60	60	8.12
WC	2013	C	2	WC13_2C	Fine	5	5	6	6	7	7	6	6	61	61	8.14
WC	2013	C	3	WC13_3C	Fine	7	7	6	6	6	6	6	6	62	62	6.14
WC	2013	C	4	WC13_4C	Fine	6	6	6	6	6	6	6	6	60	60	6.4
WC	2013	C	5	WC13_5C	Short	6	6	6	6	6	6	6	7	60	64	6.56
WC	2013	C	6	WC13_6C	Fine	6	6	6	6	6	6	6	6	60	60	8.6
WC	2013	C	7	WC13_7C	Fine	6	6	6	6	6	6	6	6	60	60	6
WC	2013	C	8	WC13_8C	Fine	6	6	6	6	7	6	6	6	63	60	5.74
WC	2013	C	9	WC13_9C	Fine	5	5	6	6	7	7	6	6	61	61	4.24
WC	2013	C	10	WC13_10C	Fine	6	6	6	6	7	7	6	6	63	63	9.78
WC	2013	C	11	WC13_11C	Fine	6	6	6	6	6	6	6	6	60	60	5.3
WC	2013	C	12	WC13_12C	Fine	5	5	5	6	6	6	6	6	57	58	6.92

WC	2013	C	13	WC13_13C	Fine	7	6	6	6	6	6	6	6	62	60	9.62
WC	2013	C	14	WC13_14C	Fine	6	6	6	6	7	7	6	6	63	63	6.58
WC	2013	C	15	WC13_15C	Fine	6	6	6	6	6	6	6	6	60	60	4.8
WC	2013	C	16	WC13_16C	Fine	7	7	7	7	6	6	6	6	63	63	7.36
WC	2013	D	1	WC13_1D	Fine	5	6	4	6	5	5	5	5	49	53	7.9
WC	2013	D	2	WC13_2D	Fine	4	5	5	5	6	6	5	5	51	53	6.45

[a] Grading details for these particular batches of rooibos were not received.

Table A2 Details on the rooibos samples from the Northern Cape (2011-2013) including the grading criteria and scores given by during quality grading by the processors.

					Sample and Grading Details					
Company	Year	Grade	Batch	Sample	Weight (g)	Cut Size	Sensory code	Leaf colour code	Cup colour code	Density
NC	2011	A	1	NC11_1A	>500 g	NA	NA	NA	NA	NA
NC	2011	A	2	NC11_2A	>500 g	NA	NA	NA	NA	NA
NC	2011	A	3	NC11_3A	>500 g	NA	NA	NA	NA	NA
NC	2011	A	4	NC11_4A	>500 g	NA	NA	NA	NA	NA
NC	2011	A	5	NC11_5A	>500 g	NA	NA	NA	NA	NA
NC	2011	A	6	NC11_6A	>500 g	NA	NA	NA	NA	NA
NC	2011	B	1	NC11_1B	>500 g	NA	NA	NA	NA	NA
NC	2011	B	2	NC11_2B	>500 g	NA	NA	NA	NA	NA
NC	2011	B	3	NC11_3B	>500 g	NA	NA	NA	NA	NA
NC	2011	B	4	NC11_4B	>500 g	NA	NA	NA	NA	NA
NC	2011	B	5	NC11_5B	>500 g	NA	NA	NA	NA	NA
NC	2011	B	6	NC11_6B	>500 g	NA	NA	NA	NA	NA
NC	2011	C	1	NC11_1C	>500 g	NA	NA	NA	NA	NA
NC	2011	C	2	NC11_2C	>500 g	NA	NA	NA	NA	NA
NC	2011	C	3	NC11_3C	>500 g	NA	NA	NA	NA	NA
NC	2011	C	4	NC11_4C	>500 g	NA	NA	NA	NA	NA
NC	2012	A	1	NC12_1A	394,70 g	1	A	2	3	340ml/100g
NC	2012	A	2	NC12_2A	NA	NA	NA	NA	NA	NA
NC	2012	A	3	NC12_3A	NA	NA	NA	NA	NA	NA
NC	2012	A	4	NC12_4A	NA	NA	NA	NA	NA	NA
NC	2012	B	1	NC12_1B	450,75 g	1	B	3	3	375ml/100g
NC	2012	B	2	NC12_2B	859,44 g	3	B	3	3	345ml/100g
NC	2012	B	3	NC12_3B	433,01 g	1	B	2	3	370ml/100g
NC	2012	B	4	NC12_4B	512,46 g	1	B	2	2	340ml/100g
NC	2012	B	5	NC12_5B	428,81 g	1	B	1	3	350ml/100g
NC	2012	B	6	NC12_6B	513,13 g	1	B	3	3	345ml/100g

NC	2012	B	7	NC12_7B	511,90 g	1	B	2	3	340ml/100g
NC	2012	B	8	NC12_8B	465,30 g	1	B	2	2	340ml/100g
NC	2012	B	9	NC12_9B	521,25 g	1	B	3	3	350ml/100g
NC	2012	B	10	NC12_10B	520,71 g	1	B	1	2	350ml/100g
NC	2012	B	11	NC12_11B	876,31 g	1	B	3	4	340ml/100g
NC	2012	B	12	NC12_12B	350,96 g	3	B	3	3	340ml/100g
NC	2012	B	13	NC12_13B	520,1 g	1	B	2	1	360ml/100g
NC	2012	B	14	NC12_14B	522,15 g	3	B	3	2	360ml/100g
NC	2012	B	15	NC12_15B	519,37 g	1	B	2	3	340ml/100g
NC	2012	B	16	NC12_16B	516,82 g	1	B	2	3	340ml/100g
NC	2012	B	17	NC12_17B	508,78 g	1	B	2	4	350ml/100g
NC	2012	B	18	NC12_18B	511,89 g	1	B	2	2	350ml/100g
NC	2012	B	19	NC12_19B	389,45 g	1	B	2	3	340ml/100g
NC	2012	B	20	NC12_20B	365,93 g	1	B	2	3	350ml/100g
NC	2012	B	21	NC12_21B	462,57 g	1	B	2	3	340ml/100g
NC	2012	B	22	NC12_22B	385,62 g	1	B	2	3	355ml/100g
NC	2012	B	23	NC12_23B	454,54 g	1	B	2	4	360ml/100g
NC	2012	B	24	NC12_24B	447,95 g	1	B	2	4	340ml/100g
NC	2012	B	25	NC12_25B	511,48 g	1	B	1	3	335ml/100g
NC	2012	B	26	NC12_26B	430,43 g	1	B	2	2	330ml/100g
NC	2012	C	1	NC12_1C	435,13 g	1	C	2	3	340ml/100g
NC	2012	C	2	NC12_2C	511,79 g	1	C	2	2	340ml/100g
NC	2012	C	3	NC12_3C	517,70 g	1	C	2	2	340ml/100g
NC	2012	C	4	NC12_4C	504,14 g	2	C	2	3	340ml/100g
NC	2012	C	5	NC12_5C	440,28 g	1	C	2	3	345ml/100g
NC	2012	C	6	NC12_6C	416,22 g	1	C	2	3	370ml/100g
NC	2012	C	7	NC12_7C	489,13 g	1	C	2	3	335ml/100g
NC	2012	C	10	NC12_10C	520,13 g	1	C	2	2	350ml/100g
NC	2012	D	1	NC12_1D	423,97 g	1	D	2	2	370ml/100g
NC	2012	D	2	NC12_2D	519,17 g	1	D	2	3	370ml/100g
NC	2012	D	3	NC12_3D	517,66 g	1	D	1	2	370ml/100g
NC	2013	A	1	NC13_1A	>500 g	NA	NA	NA	NA	NA
NC	2013	A	2	NC13_2A	>500 g	NA	NA	NA	NA	NA

NC	2013	A	3	NC13_3A	>500 g	NA	NA	NA	NA	NA
NC	2013	B	1	NC13_1B	>500 g	1	B	3	3	NA
NC	2013	B	2	NC13_2B	>500 g	1	B	3	4	NA
NC	2013	B	3	NC13_3B	>500 g	1	B	3	4	NA
NC	2013	B	4	NC13_4B	>500 g	1	B	2	3	NA
NC	2013	B	5	NC13_5B	>500 g	1	B	4	4	NA
NC	2013	B	6	NC13_6B	>500 g	1	B	3	4	NA
NC	2013	B	7	NC13_7B	>500 g	1	B	4	3	NA
NC	2013	B	8	NC13_8B	>500 g	1	B	2	3	NA
NC	2013	B	9	NC13_9B	>500 g	1	B	3	3	NA
NC	2013	B	10	NC13_10B	>500 g	1	B	2	3	NA
NC	2013	B	11	NC13_11B	>500 g	1	B	3	3	NA
NC	2013	B	12	NC13_12B	>500 g	1	B	2	3	NA
NC	2013	B	13	NC13_13B	>500 g	1	B	3	3	NA
NC	2013	B	14	NC13_14B	>500 g	1	B	3	4	NA
NC	2013	B	15	NC13_15B	>500 g	1	B	3	4	NA
NC	2013	B	16	NC13_16B	>500 g	1	B	3	4	NA
NC	2013	B	17	NC13_17B	>500 g	1	B	3	4	NA
NC	2013	B	18	NC13_18B	>500 g	1	B	3	3	NA
NC	2013	B	19	NC13_19B	>500 g	1	B	3	3	NA
NC	2013	B	20	NC13_20B	>500 g	1	B	3	4	NA
NC	2013	B	21	NC13_21B	>500 g	1	B	3	3	NA
NC	2013	B	22	NC13_22B	>500 g	1	B	3	3	NA
NC	2013	B	23	NC13_23B	>500 g	1	B	3	4	NA
NC	2013	B	24	NC13_24B	>500 g	1	B	3	4	NA
NC	2013	B	25	NC13_25B	>500 g	1	B	3	4	NA
NC	2013	B	26	NC13_26B	>500 g	1	B	3	4	NA
NC	2013	C	1	NC13_1C	>500 g	1	C	3	4	NA
NC	2013	C	2	NC13_2C	>500 g	1	C	3	4	NA
NC	2013	C	3	NC13_3C	>500 g	1	C	3	4	NA
NC	2013	C	4	NC13_4C	>500 g	1	C	3	3	NA
NC	2013	C	5	NC13_5C	>500 g	1	C	3	4	NA
NC	2013	C	6	NC13_6C	>500 g	1	C	2	3	NA

NC	2013	C	7	NC13_7C	>500 g	1	C	2	3	NA
NC	2013	C	8	NC13_8C	>500 g	1	C	4	4	NA
NC	2013	C	9	NC13_9C	>500 g	1	C	2	2	NA
NC	2013	D	1	NC13_1D	>500 g	1	D	2	3	NA
NC	2013	D	2	NC13_2D	>500 g	1	D	2	2	NA
NC	2013	D	3	NC13_3D	>500 g	1	D	2	2	NA
NC	2013	D	4	NC13_4D	>500 g	1	D	3	4	NA

[a] Grading details for these particular batches of rooibos were not received.

Table A3 Suppliers of compounds used as preliminary reference standards in the DSA training and compilation of the rooibos sensory lexicon.

Compounds	Suppliers
β-damascenone	Kerry Ingredients, Durban, South Africa
2-acetyl-5-methylfuran	Kerry Ingredients, Durban, South Africa
Deltadodecalactone	Kerry Ingredients, Durban, South Africa
Hexyl acetate	Kerry Ingredients, Durban, South Africa
Orange terpenes	Kerry Ingredients, Durban, South Africa
Geranyl isovalerate	Kerry Ingredients, Durban, South Africa
"Honey-like" flavour	Givaudan South Africa (PTY) Ltd, Johannesburg, South Africa
"Caramellic" flavour	Givaudan South Africa (PTY) Ltd, Johannesburg, South Africa
Cinnamaldehyde	Kerry Ingredients, Durban, South Africa
4-dihydrocoumerin	Kerry Ingredients, Durban, South Africa
(Z)-3-hexen-1-ol	Kerry Ingredients, Durban, South Africa
4-ethylphenol	Kerry Ingredients, Durban, South Africa

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ADDENDUM B
Relation of individual phenolic compounds and selected taste and mouthfeel attributes in rooibos

Table B1 DSA aroma intensity data (out of 100) for the positive attributes, for the Western Cape samples used for *instructed sorting,* based on aroma quality.

Production area	Sample	Year	ARooibos-woody	AFynbos-floral	AHoney	AFruity-sweet	AApricot	ACaramel	ACooked apple	ASpicy	ACitrus
WC	AA	2009	38.6	30.3	17.8	13.2	NA[a]	23.2	NA	NA	NA
WC	5A	2009	48.9	39.6	35.3	5.9	NA	18.6	NA	NA	NA
WC	14D	2009	42.3	29.1	26.3	8.4	NA	8.8	NA	NA	NA
WC	2A	2011	43.5	32.3	32.7	19.3	NA	4.4	NA	1.0	NA
WC	4B	2011	45.1	20.7	27.3	9.9	NA	2.8	NA	2.4	NA
WC	6D	2011	36.7	7.8	11.8	4.3	NA	4.6	NA	0.7	NA
WC	13C	2012	33.3	26.5	19.7	5.1	2.1	3.7	0.7	0.3	0.0
WC	6D	2012	30.8	27.6	15.7	4.1	2.2	1.2	1.8	4.1	0.7
WC	1A	2013	42.6	26.4	25.9	1.0	0.9	6.9	0.0	0.6	0.0
WC	7C	2013	36.5	26.6	15.7	14.9	10.9	19.0	0.0	0.4	0.9
WC	1D	2013	32.5	24.4	16.9	11.4	5.1	4.8	1.4	0.9	0.0

[a] Descriptive sensory analysis was not done for these specific attributes during this particular year of analysis.

Table B2 DSA aroma intensity data (out of 100) for the negative attributes, for the Western Cape samples used for *instructed sorting,* based on aroma quality.

Production area	Sample	Year	AHay/dried grass	AGreen	ARotting plant water	ABurnt caramel	ADusty	AMusty/ mouldy	AMedicinal/ rubber	ASeaweed
WC	AA	2009	1.7	2.6	NA[a]	NA	0.0	NA	NA	NA
WC	5A	2009	2.2	1.9	NA	NA	0.0	NA	NA	NA
WC	14D	2009	10.9	14.6	NA	NA	1.0	NA	NA	NA
WC	2A	2011	14.5	3.4	NA	0.0	NA	0.0	0.0	NA
WC	4B	2011	12.6	8.1	NA	0.8	NA	1.1	1.5	NA
WC	6D	2011	18.3	8.8	NA	9.1	NA	8.4	39.2	NA
WC	13C	2012	12.8	3.0	3.0	2.9	4.7	2.4	2.8	0.0
WC	6D	2012	12.9	11.5	2.7	0.5	4.3	3.6	13.8	1.2
WC	1A	2013	11.3	2.7	2.6	0.0	0.8	2.2	6.5	0.0
WC	7C	2013	9.4	5.7	0.7	4.1	0.4	1.5	1.8	0.0
WC	1D	2013	22.0	11.7	2.0	0.0	0.9	2.7	0.2	0.0

[a] Descriptive sensory analysis was not done for these specific attributes during this particular year of analysis.

Table B3 DSA aroma intensity data (out of 100) for the positive attributes, for the Northern Cape samples used for *instructed sorting,* based on aroma quality

Production area	Sample	Year	ARooibos-woody	AFynbos-floral	AHoney	AFruity-sweet	AApricot	ACaramel	ACooked apple	ASpicy	ACitrus
NC	1A	2009	45.88	31.25	31.82	19.45	NA[a]	3.87	NA	4.57	NA
NC	4A	2010	45.55	25.17	31.63	19.58	NA	6.98	NA	6.07	NA
NC	3C	2010	35.93	19.58	14.68	8.43	NA	3.97	NA	3.32	NA
NC	5A	2011	45.12	29.40	31.62	7.13	NA	1.17	NA	0.80	NA
NC	3B	2011	37.30	22.55	19.18	25.22	NA	2.95	NA	0.33	NA
NC	4C	2011	41.88	14.73	19.28	5.47	NA	2.03	NA	0.00	NA
NC	1A	2012	31.02	26.85	14.00	16.95	17.31	21.50	8.43	8.13	0.00
NC	3A	2012	32.95	25.18	14.22	10.48	8.75	16.37	2.65	4.92	0.00
NC	11B	2012	37.14	28.19	23.63	8.93	3.55	7.37	0.00	1.02	0.00
NC	2D	2012	30.34	23.91	16.33	4.85	2.03	6.58	0.33	0.33	2.00
NC	3A	2013	35.23	29.08	14.73	21.31	17.50	13.35	0.00	0.00	1.91
NC	4D	2013	41.48	32.25	17.23	11.52	10.67	25.21	0.75	1.23	0.00

[a] Descriptive sensory analysis was not done for these specific attributes during this particular year of analysis.

Table B4 DSA aroma intensity data (out of 100) for the negative attributes, for the Northern Cape samples used for *instructed sorting,* based on aroma quality

Production area	Sample	Year	AHay/dried grass	AGreen	ARotting plant water	ABurnt caramel	ADusty	AMusty/ mouldy	AMedicinal/ rubber	ASeaweed
NC	1A	2009	8.20	2.32	NA[a]	0.95	NA	0.00	1.32	NA
NC	4A	2010	9.33	1.93	NA	1.47	NA	0.35	0.87	NA
NC	3C	2010	10.30	20.63	NA	0.00	NA	7.17	13.74	NA
NC	5A	2011	9.23	4.42	NA	0.00	NA	2.53	2.17	NA
NC	3B	2011	11.12	16.60	NA	0.52	NA	2.82	1.73	NA
NC	4C	2011	15.73	10.72	NA	8.33	NA	7.63	28.37	NA
NC	1A	2012	5.57	2.38	0.00	3.24	0.33	0.67	0.00	0.00
NC	3A	2012	7.25	4.25	0.88	3.48	0.42	0.84	0.52	1.02
NC	11B	2012	12.62	5.00	0.64	2.28	1.98	0.03	1.42	1.00
NC	2D	2012	11.20	18.63	2.91	4.60	0.42	4.48	10.41	0.33
NC	3A	2013	5.94	1.15	0.00	0.40	0.85	1.00	0.40	0.00
NC	4D	2013	5.19	4.79	0.71	6.09	0.00	1.25	0.21	0.00

[a] Descriptive sensory analysis was not done for these specific attributes during this particular year of analysis.

Table B5 DSA flavour intensity data (out of 100) for the positive attributes, for the Western Cape samples used for *instructed sorting,* based on palate quality

Production area	Sample	Year	FRooibos-woody	FFynbos-floral	FHoney	FFruity-sweet	FApricot	FCaramel	FCooked apple	FSpicy	FCitrus
WC	AA	2009	37.8	7.6	NA	NA	NA	2.2	NA	NA	NA
WC	5A	2009	41.9	10.2	NA	NA	NA	5.8	NA	NA	NA
WC	14D	2009	38.6	8.2	NA	NA	NA	0.0	NA	NA	NA
WC	2A	2011	42.2	17.5	7.9	1.6	NA	0.3	NA	0.0	NA
WC	4B	2011	42.2	16.5	3.1	1.5	NA	0.3	NA	0.3	NA
WC	6D	2011	34.3	8.4	1.0	0.8	NA	1.0	NA	0.4	NA
WC	13C	2012	35.3	23.4	3.9	3.3	0.3	1.7	0.0	0.0	0.0
WC	6D	2012	31.9	26.8	2.2	0.7	0.0	0.3	0.0	0.3	0.0
WC	1A	2013	40.0	18.1	6.0	0.0	0.0	1.7	0.0	0.0	0.0
WC	7C	2013	34.6	21.7	2.0	7.0	2.8	4.2	0.0	0.0	0.0
WC	1D	2013	29.3	17.5	1.6	3.0	1.0	0.3	0.7	0.0	0.0

[a] Descriptive sensory analysis was not done for these specific attributes during this particular year of analysis.

Table B6 DSA flavour intensity data (out of 100) for the negative attributes, for the Western Cape samples used for *instructed sorting,* based on palate quality

Production area	Sample	Year	FHay/dried grass	FGreen	FRotting plant water	FBurnt caramel	FDusty	FMusty/ mouldy	FMedicinal/ rubber	FSeaweed
WC	AA	2009	6.8	1.2	NA	NA	NA	NA	NA	NA
WC	5A	2009	2.7	0.0	NA	NA	NA	NA	NA	NA
WC	14D	2009	13.6	8.7	NA	NA	NA	NA	NA	NA
WC	2A	2011	10.1	0.0	NA	NA	NA	NA	0.1	NA
WC	4B	2011	9.3	1.7	NA	NA	NA	NA	0.0	NA
WC	6D	2011	9.4	7.3	NA	NA	NA	NA	24.0	NA
WC	13C	2012	15.9	2.1	1.5	1.3	3.6	1.1	5.9	0.0
WC	6D	2012	12.0	7.7	2.1	0.3	0.8	0.3	0.3	0.0
WC	1A	2013	13.2	2.0	2.5	0.0	0.0	0.4	3.3	0.0
WC	7C	2013	11.3	4.3	0.3	1.2	0.0	0.0	0.0	0.0
WC	1D	2013	20.8	6.6	0.8	0.0	0.0	1.3	0.3	0.0

[a] Descriptive sensory analysis was not done for these specific attributes during this particular year of analysis.

Table B7 DSA taste and mouthfeel intensity data (out of 100), for the Western Cape samples used for *instructed sorting,* based on palate quality

Production area	Sample	Year	Sweet	Sour	Bitter	Astringent
WC	AA	2009	20.7	1.4	2.2	12.2
WC	5A	2009	17.9	1.4	3.1	19.2
WC	14D	2009	13.1	1.7	5.3	21.4
WC	2A	2011	25.3	2.3	1.4	23.0
WC	4B	2011	24.7	3.1	0.8	24.6
WC	6D	2011	19.1	2.1	3.8	27.0
WC	13C	2012	18.2	3.3	3.1	23.7
WC	6D	2012	23.0	3.9	1.7	21.2
WC	1A	2013	19.9	3.0	5.6	28.3
WC	7C	2013	20.8	9.3	1.3	25.5
WC	1D	2013	19.3	7.7	1.3	25.3

Table B8 DSA flavour intensity data (out of 100) for the positive attributes, for the Northern Cape samples used for *instructed sorting,* based on palate quality

Production area	Sample	Year	FRooibos-woody	FFynbos-floral	FHoney	FFruity-sweet	FApricot	FCaramel	FCooked apple	FSpicy	FCitrus
NC	1A	2009	42.25	18.38	3.48	2.88	NA[a]	1.17	NA	1.59	NA
NC	4A	2010	46.68	17.82	9.00	3.42	NA	1.07	NA	0.27	NA
NC	3C	2010	36.80	14.50	2.60	0.67	NA	1.00	NA	0.00	NA
NC	5A	2011	39.60	19.18	7.13	1.52	NA	0.00	NA	0.00	NA
NC	3B	2011	39.77	18.33	5.57	3.78	NA	0.83	NA	0.00	NA
NC	4C	2011	42.16	9.12	2.30	0.78	NA	0.50	NA	0.00	NA
NC	1A	2012	32.98	24.93	2.68	10.20	1.41	9.98	1.88	4.38	0.00
NC	3A	2012	32.97	22.89	3.17	6.05	3.22	6.50	0.00	1.74	0.00
NC	11B	2012	35.35	27.02	3.78	5.20	0.00	3.52	0.02	0.27	0.00
NC	2D	2012	31.25	23.10	2.88	2.47	0.67	2.00	0.00	0.00	0.00
NC	3A	2013	34.38	25.50	1.60	6.31	3.00	3.90	0.00	0.37	0.43
NC	4D	2013	38.69	24.27	0.67	4.23	3.37	6.43	0.00	0.57	0.00

[a] Descriptive sensory analysis was not done for these specific attributes during this particular year of analysis.

Table B9 DSA flavour intensity data (out of 100) for the negative attributes, for the Northern Cape samples used for *instructed sorting,* based on palate quality

Production area	Sample	Year	FHay/dried grass	FGreen	FRotting plant water	FBurnt caramel	FDusty	FMusty/ mouldy	FMedicinal/ rubber	FSeaweed
NC	1A	2009	4.95	0.00	NA[a]	NA	NA	NA	0.53	NA
NC	4A	2010	7.65	0.50	NA	NA	NA	NA	0.00	NA
NC	3C	2010	8.30	12.53	NA	NA	NA	NA	5.93	NA
NC	5A	2011	5.43	0.50	NA	NA	NA	NA	0.00	NA
NC	3B	2011	11.63	4.36	NA	NA	NA	NA	1.15	NA
NC	4C	2011	13.72	4.92	NA	NA	NA	NA	13.59	NA
NC	1A	2012	10.67	0.52	0.00	1.17	1.52	0.64	0.00	0.00
NC	3A	2012	14.03	3.31	0.43	1.82	0.40	0.31	0.00	0.00
NC	11B	2012	12.24	1.37	0.33	0.33	0.00	0.00	0.00	0.00
NC	2D	2012	16.00	14.05	0.33	2.00	0.00	0.25	1.35	0.00
NC	3A	2013	9.71	1.10	0.00	0.35	0.00	0.31	0.00	0
NC	4D	2013	9.17	2.46	0.00	1.50	0.00	0.56	0.00	0

[a] Descriptive sensory analysis was not done for these specific attributes during this particular year of analysis.

Table B10 DSA taste and mouthfeel intensity data (out of 100), for the Northern Cape samples used for *instructed sorting,* based on palate quality

Production area	Sample	Year	Sweet	Sour	Bitter	Astringent
NC	1A	2009	24.58	1.60	0.45	22.40
NC	4A	2010	23.35	3.30	3.82	27.98
NC	3C	2010	22.72	3.71	0.93	24.68
NC	5A	2011	23.00	1.15	0.83	23.80
NC	3B	2011	23.78	2.33	1.95	25.78
NC	4C	2011	21.50	0.93	2.50	28.37
NC	1A	2012	21.68	3.05	1.03	21.22
NC	3A	2012	18.88	3.00	0.52	22.52
NC	11B	2012	19.28	2.70	0.98	22.05
NC	2D	2012	16.42	5.97	5.00	23.67
NC	3A	2013	22.00	5.81	1.23	23.90
NC	4D	2013	22.21	4.75	3.06	25.25

Table B11 DSA aroma data for the samples chosen for the *uninstructed sorting*, according to aroma profile.

Production area	Sample	Year	ARooibos-woody	AFynbos-floral	AHoney	AFruity-sweet	AApricot	ACaramel	ACooked apple	ASpicy	ACitrus
WC	5A	2009	49	40	35	6	NA[a]	19	NA	NA	NA
WC	1A	2012	32.57	22.33	15.83	11.60	7.22	7.16	5.93	4.40	0.00
WC	4A	2012	38.48	28.76	13.88	19.00	12.02	14.02	2.97	4.78	0.00
WC	5D	2012	31.43	22.15	10.14	20.72	25.31	14.73	0.00	1.30	0.00
WC	1C	2013	25.77	39.25	8.44	0.96	0.38	14.46	15.60	19.33	0.00
NW	1A	2009	45.88	31.25	31.82	19.45	NA	3.87	NA	4.57	NA
NW	10B	2012	33.62	26.10	12.83	26.63	25.09	24.13	1.21	2.33	2.14
NW	3A	2013	35.23	29.08	14.73	21.31	17.50	13.35	0.00	0.00	1.91
NW	10B	2013	39.37	30.81	24.35	8.42	2.06	6.41	0.44	0.38	0.21
NW	11B	2013	35.52	28.25	17.63	8.69	1.63	9.26	3.20	2.10	0.00
NW	4D	2013	41.48	32.25	17.23	11.52	10.67	25.21	0.75	1.23	0.00

[a] Descriptive sensory analysis was not done for these specific attributes during this particular year of analysis.

Table B12 DSA aroma data, for the negative attributes, for the samples chosen for the *uninstructed sorting*, according to aroma profile.

Production area	Sample	Year	AHay/dried grass	AGreen	ARotting plant water	ABurnt caramel	ADusty	AMusty/ mouldy	AMedicinal/ rubber	ASeaweed
WC	5A	2009	2	2	NA[a]	NA	0	NA	NA	NA
WC	1A	2012	16.66	2.58	4.82	0.68	4.05	3.71	4.90	0.30
WC	4A	2012	8.31	2.68	0.83	2.74	0.77	0.50	2.53	0.00
WC	5D	2012	7.50	4.18	1.47	5.91	0.33	0.00	1.46	0.62
WC	1C	2013	9.67	3.73	2.40	1.13	1.61	0.29	1.94	0.00
NW	1A	2009	8.20	2.32	NA	0.95	1.32	0.00	NA	NA
NW	10B	2012	7.60	1.82	0.55	4.16	0.00	0.00	0.00	0.33
NW	3A	2013	5.94	1.15	0.00	0.40	0.85	1.00	0.40	0.00
NW	10B	2013	9.98	4.73	0.29	0.79	2.00	4.85	0.27	0.21
NW	11B	2013	10.60	8.60	0.31	0.75	0.68	2.44	0.00	0.00
NW	4D	2013	5.19	4.79	0.71	6.09	0.00	1.25	0.21	0.00

[a] Descriptive sensory analysis was not done for these specific attributes during this particular year of analysis.

Figure B14 An example of a questionnaire for the sorting of rooibos samples according to quality.

ROOIBOS SORTING – QUALITY (WESTERN CAPE)

Day 1 –Monday, 10 June 2013

SESSION 1   Instructed sorting of rooibos tea samples according to AROMA

Please read through the instructions <u>thoroughly</u> and do not hesitate to ask if you encounter any difficulties during the process.

INSTRUCTIONS

- You have been presented with 12 Rooibos samples labelled from A to L.

- The samples represent **3 different categories** based on the **AROMA QUALITY**

- Please sort the samples according to the **3X QUALITY profiles** associated with the rooibos samples. This is provided in Table 1 below.

  ➢ You are allowed to **smell** the samples as many times as you like and in any order.

  ➢ On the large A3 paper that is provided, place the samples that have a similar aroma profile in **three groups only**. Each group may contain **no more than 6 samples**.

  ➢ Once you have placed all samples in one of the 3 groups, use the **table** provided on the **separate A4 page** to indicate which samples you have placed into which group.

  ➢ Then please use the **quality attributes provided in Table 1** and any **additional attributes** you would like to add to describe the aroma profile of each group. **Do not use more than 5 attributes** to describe each group.

  ➢ **NOTE**: Please try to work as quickly as possible to **prevent the samples from cooling down** too much. Place samples back in the waterbath while you are the smelling the other samples.

Table 1 Quality profiles of rooibos tea		
Group 1	*Group 2*	*Group 3*
1. No perceptible taints	1. Marginal/borderline taints	1. Tainted samples
2. Positive attributes dominant	2. Slightly tainted	2. Taints dominant
3. Excellent quality	3. Acceptable quality	3. Poor quality

 * **No samples contain zero taints**

NAME_____

Group	Samples						AROMA attributes associated with the three groups		
	Complete the table below by indicating which samples you have placed in the three respective groups. Then please write the major **AROMA** attributes associated with each group in the columns on the right.								
1							1.		4.
							2.		5.
							3.		
2							1.		4.
							2.		5.
							3.		
3							1.		4.
							2.		5.
							3.		

Thank you for your participation and valuable input.

Figure B15 An example of a questionnaire for the sorting of rooibos samples according to aroma profile

ROOIBOS SORTING – FOR PROFILE (WESTERN CAPE & NORTHERN CAPE)

Day 5 – Thursday, 20 June 2013

SESSION 2   Uninstructed sorting according to AROMA

Please read through the instructions <u>thoroughly</u> and do not hesitate to ask if you encounter any difficulties during the process.

INSTRUCTIONS

- You have been presented with **12 rooibos samples** labelled from A to L.

- Please sort the samples according to the **SIMILARITY OF THEIR <u>AROMA PROFILES</u>**

  - ➢ You are allowed to **smell** the samples as many times as you like and in any order.

  - ➢ On the large A3 paper that is provided, group together the samples that have a similar aroma profile

  - ➢ You may form **as many groups as you wish, but NOT MORE THAN 6 GROUPS.**

  - ➢ Each group may contain **as many samples as you like**

  - ➢ Once you have assigned all samples to a group, use the **table** provided on the **separate A4 page** to indicate which samples you have grouped together

- Then please write down the major **aroma attributes associated with each of the sample groups**. D**o not use more than 5 attributes** to describe the aroma characteristics of each group.

  - ➢ **NOTE**: Please try to work as quickly as possible to **prevent the samples from cooling down** too much. Place samples back in the waterbath while you are the smelling the other samples.

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Group	Complete the table below by indicating which samples you have placed in which group. Then please write down the major **AROMA** attributes associated with each group in the column on the right.						AROMA attributes associated with each group	
	Samples							
1							1.	4.
							2.	5.
							3.	
2							1.	4.
							2.	5.
							3.	
3							1.	4.
							2.	5.
							3.	
4							1.	4.
							2.	5.
							3.	
5							1.	4.
							2.	5.
							3.	
6							1.	4.
							2.	5.
							3.	