SENSORY AND PHENOLIC PROFILING OF *CYCLOPIA* SPECIES (HONEYBUSH) AND OPTIMISATION OF THE FERMENTATION CONDITIONS

Karin Alicia Theron

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Department of Food Science

Stellenbosch University

Supervisor: **Prof. E. Joubert**

Co-supervisor: **M. Muller**

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DECLARATION

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Karin Alicia Theron

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ABSTRACT

The sensory profiles, phenolic composition and colour of honeybush infusions, prepared from six *Cyclopia* species (*C. sessiliflora, C. longifolia, C. genistoides, C. intermedia, C. subternata* and *C. maculata*), were determined to establish the variation between species. The results of the sensory study were used to create a honeybush sensory wheel and lexicon. The "characteristic" sensory profile of honeybush tea can be described as a combination of floral, sweet, fruity and plantlike flavours with a sweet taste and a slightly astringent mouthfeel. Sensory results indicated that the species could be divided into three distinct groups; group A (*C. sessiliflora, C. intermedia* and *C. genistoides)*, group B (*C. longifolia* and *C. subternata)* and group C (*C. maculata*). Group A was associated with fynbos floral, fynbos sweet and plantlike attributes, group B with rose geranium and fruity sweet attributes and group C with woody, boiled syrup and cassia/cinnamon attributes. Gas chromatography-olfactometry analysis of the *C. maculata* aroma fraction indicated that the spicy note of its aroma could possibly be explained by the high concentration of the volatile component eugenol. However, none of the aroma impact volatiles had a specific cassia/cinnamon note.

Large variation in the composition of the honeybush infusions was revealed through the quantification of the soluble solids, total polyphenol and individual monomeric polyphenolic compounds, as well as the absorbance ("colour"). Infusions of *C. genistoides, C. longifolia* and *C. sessiliflora* had the highest soluble solids and total polyphenol content, as well as the highest absorbance values. Only mangiferin, isomangiferin, hesperidin and compound C (unidentified compound) were detected in all six *Cyclopia* species. *Cyclopia genistoides, C. longifolia* and *C. sessiliflora,* in order of prominence, contained the highest concentration of both mangiferin and isomangiferin whereas *C. genistoides* and *C. maculata* contained the highest hesperidin content. The bitter taste present in certain *Cyclopia* species appeared to be due to a high mangiferin content, however, compounds such as isomangiferin and compound C might also have played a role.

The effect of fermentation (oxidation) temperature (80°C and 90°C) and time (8 h, 16 h, 24 h and 32 h) of *C. genistoides, C. subternata* and *C. maculata* on the sensory characteristics of their infusions was also investigated. Fermentation for longer than 8 h resulted in an increase in positive sensory attributes and a decrease in negative sensory attributes rather than the formation of new sensory attributes. A fermentation temperature/time combination of 80°C/24 hours or 90°C/16 h was required for *C. genistoides, C. subternata* and *C. maculata*. Fermenting *C. genistoides* at 90°C would result in a honeybush infusion with slightly less rose geranium notes whereas *C. subternata* can be fermented at either 80°C or 90°C, depending on whether floral or apricot jam notes are desired. *Cyclopia maculata* should preferably not be fermented at 90°C due to an increase in negative sensory attributes (hay/dried grass and green grass). Fermentation reduced the soluble solids content, total polyphenol content, colour and concentration of individual polyphenolic compounds. Changes in the taste and mouthfeel of honeybush tea could be attributed to changes in the polyphenolic composition caused by the high temperature oxidation. Mangiferin associated with the bitter taste of *C. genistoides,* while in *C. subternata* astringency may be partly attributed to the mangiferin and isomangiferin content. The study substantiated the need for further research on the contribution of the major phenolic compounds towards the taste and mouthfeel of *Cyclopia* species.

UITREKSEL

Die sensoriese profiel, fenoliese samestelling en kleur van heuningbostee, berei van ses *Cyclopia* spesies *(C. sessiliflora, C. longifolia, C. genistoides, C. intermedia, C. subternata* en *C. maculata*), is bepaal ten einde die mate van variasie vas te stel. Die resultate van die sensoriese studie is gebruik om ‘n sensoriese wiel en leksikon vir heuningbostee te ontwikkel. Die “karakteristieke” sensoriese profiel van heuningbostee kan beskryf word as ‘n kombinasie van blomagtig, soet, vrugtig en plantagtige geure met ‘n soet smaak en ’n effense frankheid. Sensoriese resultate het aangedui dat die spesies in drie groepe verdeel kon word; groep A (*C. sessiliflora, C. intermedia* and *C. genistoides),* groep B (*C. longifolia* and *C. subternata)* en groep C (*C. maculata*). Groep A is met fynbos blom, fynbos-soet en plantagtige geure geassosieer, groep B met roos geranium en vrugtige-soet geure en group C met houtagtige, gekookte stroop en kassia/kaneel geure. Gaschromatografie-olfaktometrie analises van *C. maculata* se aroma fraksie het getoon dat die speseryagtige aroma moontlik as gevolg van die hoë konsentrasie van die vlugtige komponent, eugenol, kon wees. Geen van die aroma-impak vlugtige verbindings het egter ‘n spesifieke kassia/kaneelagtige noot gehad nie.

Groot variasie in die samestelling van heuningbostee ten opsigte van die inhoud van oplosbare vastestowwe, totale polifenole en monomeriese fenoliese verbindings, asook die absorbansie (“kleur”) is aangetoon. Heuningbostee berei van *C. genistoides, C. longifolia* en *C. sessiliflora* het die hoogste oplosbare vastestowwe en totale polifenol inhoud, asook die hoogste absorbansie waardes gehad. Slegs mangiferien, isomangiferien, hesperidien en verbinding C (ongeïdentifiseerde verbinding) is in al ses *Cyclopia* spesies geïdentifiseer. *Cyclopia genistoides, C. longifolia* en *C. sessiliflora,* in volgorde van belangrikheid, het die hoogste konsentrasie van beide mangiferien en isomangiferin gehad teenoor *C. genistoides* en *C. maculata* wat die hoogste hesperidien konsentrasie gehad het. Die bitter smaak teenwoordig in sekere *Cyclopia* spesies blyk moontlik as gevolg van die hoë mangiferien inhoud te wees, hoewel komponente soos isomangiferien en komponent C dalk ook ‘n rol mag speel.

Die effek van die fermentasie temperatuur (80°C en 90°C) en tyd (8 h, 16 h, 24 h en 32 h) van *C. genistoides, C. subternata* en *C. maculata* op die sensoriese eienskappe van heuningbostee is ondersoek. Fermentasie vir langer as 8 h het tot ‘n toename in positiewe sensoriese eienskappe en afname in negatiewe sensoriese eienskappe gelei eerder as die ontstaan van nuwe sensoriese eienskappe. Om heuningbostee met ‘n optimum sensoriese profiel te verkry is ‘n fermentasie temperatuur/tyd kombinaise van 80°C/24 h of 90°C/16 h nodig vir *C. genistoides, C. subternata* en *C. maculata*. *Cyclopia genistoides* wat by 90°C gefermenteer word sal minder van die roos geranium note bevat, terwyl *C. subternata* by 80°C of 90°C gefermenteer kan word, afhangende of ‘n blomagtige of ‘n appelkooskonfyt noot verlang word. Fermentasie by 90°C word nie aanbeveel *C. maculata* nie as gevolg van die toename van sekere negatiewe sensoriese eienskappe (hooi/droe gras aroma en -geur en groen gras aroma). Fermentasie het die inhoud van oplosbare vastestowwe, totale polifenole, individuele polifenoliese verbindings, asook kleur verminder. Veranderinge in die smaak en mondgevoel van heuningbostee kon toegeskryf word aan die veranderinge in die polifenoliese inhoud as gevolg van die hoë temperatuur oksidasie. Mangiferien is met die bitter smaak van *C. genistoides* geassosieer, terwyl mangiferien and isomangiferien moontlik deels frankheid in *C. subternata* veroorsaak. Die studie het die noodsaaklikheid vir verdere navorsing op die bydrae van die hoof fenoliese verbindings tot die smaak en mondgevoel van *Cyclopia* spesies gestaaf.

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Chapter 1

INTRODUCTION

The foliage and stems of the indigenous *Cyclopia* shrub is used to prepare a traditional South African sweet, honeylike herbal tea known as honeybush tea (Du Toit *et al.,* 1998). The *Cyclopia* shrub grows localised in the coastal districts and the mountainous areas of the Cape Floristic Region. Honeybush tea is believed to be first used by the Bushmen and Khoisan for medicinal purposes; later Dutch and British settlers began to use it as a substitute for their regular tea (*Camellia sinensis*). To date, more than 20 *Cyclopia* species has been identified but mainly three of these are currently being utilized commercially (Joubert *et al.,* 2011). The demand for honeybush tea, nationally and internationally, has increased substantially over the last decade with exports currently exceeding 200 tons and this growth is expected to continue (Joubert *et al.,* 2011). Honeybush tea is currently exported to 18 countries with the Netherlands being the top importer followed by Germany, the United Kingdom (UK), Poland and the United States of America (USA). Until recently, exports consisted mainly of *C. intermedia, C. genistoides* and *C. subternata,* however, as the demand began exceeding the supply, the focus, due to necessity, shifted to include other *Cyclopia* species, such as *C. sessiliflora, C. longifolia* and *C. maculata*.

The only descriptors, additional to sweet and honeylike, used to describe the flavour of honeybush tea were flowery, fruity, grassy and burnt (Du Toit & Joubert, 1998; Du Toit & Joubert, 1999). According to the Foodstuffs, Cosmetics and Disinfectants Act (Anon., 2002) honeybush tea refers to the product obtained from the leaves, flowers and stems of the *Cyclopia* genus. There is no reference to specific *Cyclopia* species nor is it required to indicate *Cyclopia* species on the packaging. Only certain brands, mostly those found in speciality shops or up-market farm stalls, consists of one *Cyclopia* species (Joubert *et al.,* 2011). Most products are a mixture of two or more *Cyclopia* species or blends of honeybush and rooibos (*Asphalatus linearis*) and other indigenous South African plants and fruits (Joubert *et al.,* 2008). The South African quality standards for the export of honeybush tea according to the Agricultural Product Standards Act (Anon, 2000) state that, in terms of taste and aroma, honeybush "must have the clean characteristic taste and aroma of honeybush and that it shall be free from any foreign flavours and odours which detrimentally effect the characteristics of the product". However, neither the "characteristic" taste and aroma nor what is considered to be foreign and detrimental to the taste and aroma of the product are defined. Variation in sensory quality due to differences in localities, environmental conditions, processing parameters and the inherent species differences is not taken into account. Consequently, the lack of standardised terminology with which to describe the characteristic honeybush flavour, as well as the fact that no information is available with regards to the differences between different *Cyclopia* species results in considerable variation in the sensory profiles of teas currently being sold in the market place as honeybush tea. This could lead to detrimental consequences as a consistent supply of high quality tea with consistent flavour profiles cannot be ensured without this information.

According to literature it is the oxidative chemical reaction, more commonly referred to as "fermentation", which is responsible for the characteristic taste and aroma of honeybush tea (Du Toit & Joubert, 1999). However, the fermentation conditions, ranging from 70°C/60 h for *C. intermedia* to 80-85°C/18-24 h for other *Cyclopia* species, currently employed by the industry is based on research done on

C. intermedia and *C. buxifolia* (previously classified as *C. maculata*) (Joubert *et al.,* 2011). In the case of *C. intermedia,* 70°C/60 h represents the optimum fermentation temperature-time regime. Fermentation at 80-85°C/18-24 h represents a trade-off between cost and optimum quality (E. Joubert, ARC Infruitec-Nietvoorbij, Stellenbosch, personal communication). It is thus quite possible that the conditions employed by the industry are not necessarily optimum for producing honeybush tea from *Cyclopia* species, other than *C. intermedia*. Higher/lower temperatures and shorter/longer fermentation periods might be required to obtain the characteristic honeybush flavour, depending on the species. Once again the need for a set of standardised terms to describe the characteristic honeybush flavour as well as specific negative attributes associated with honeybush is highlighted. Without these sensory descriptors it is very difficult to identify and describe tea of inferior sensory quality.

The continuing increase in international demand for honeybush tea may lead to an interest in production of honeybush tea in other countries which would pose a major treat to the South African honeybush industry. The development of a geographical indication (GI) for honeybush might be essential in the future (Blanchard Oritz, 2006; Blanchard *et al.,* 2006). GI is a label that is reserved for products which acquire the characteristic and defining qualities as a result of their geographical location (Grazioli, 2002) and it enables producers to distinguish their product based on its specific origin-related characteristics. However, in order to establish a GI for honeybush the product needs to be described - emphasising the need for a list of sensory descriptors describing the typical flavour and mouthfeel attributes of honeybush tea and to determine the extent of similarity, but also dissimilarity in the sensory attributes of different species.

Sensory lexicons and sensory wheels are often used in the industry or research-related environments to describe the sensory attributes associated with certain food and beverage products (Drake & Civille, 2002). A sensory lexicon consists of a set of words describing the sensory attributes of a product along with definitions and/or reference standards for clarification whereas a sensory wheel is a simple graphical representation of the sensory lexicon. These tools are used to standardise the terminology used to discuss the sensory properties related to a certain product and have been reported to increase communication between different role players in the industry. The successful application of these sensory tools by a number of industries, i.e. wine (Noble *et al*., 1987; Gawel *et al*., 2000), beer (Meilgaard *et al*., 1979) and tea (*Camellia sinensis*) (Bhuyan & Borah, 2001), suggests that the development of a sensory lexicon and wheel would make a valuable contribution to the honeybush industry. These tools would enable the industry to compare the sensory profiles of different *Cyclopia* species as well as facilitate communication between the different role players in the industry. Such tools could also be used to describe the effect a number of different factors, such as processing conditions and geographical origin, have on the sensory characteristics of honeybush tea, identify niche markets, as well as determine which *Cyclopia* species can be blended to retain a certain flavour profile.

A list of sensory descriptors may be used to investigate the relationship between chemical/instrumental data and the sensory quality as the sensory terms have direct application to the multitude of compounds present in a food or beverage product (Drake & Civille, 2002). Non-volatile compounds are detected by taste whereas volatile compounds are detected by the sense of smell (Dutta *et al.,* 2003). Flavour is the result of the combination of the basic tastes and specific aroma characteristics that arise from the volatile components which enter the nasal passages though the nose and the back of the mouth (Jackson, 2009; Ross, 2009). The volatile compounds in honeybush tea and their relationship to aroma have been studied extensively by Le Roux *et al.* (2008) and Cronje (2010). However, research on the

relationship between taste and the non-volatile compounds in honeybush tea is limited to the study Reichelt *et al.* (2010) which identified hesperetin as a flavour modulating compound with sweet enhancing properties. Ley *et al.* (2005) and Ley (2008) reported that the honeybush flavanone, eriodictyol, possesses bitter masking properties.

Sour taste is usually caused by small, soluble, inorganic cations, however, it has been reported that certain phenolic acids have acidic or sour taste characteristics (Huang & Zayas, 1991; Peleg & Noble, 1995). It is thus quite possible that the sour taste in honeybush tea may be related to *p*-coumaric or shikimic acid which has been identified in *Cyclopia* species (Ferreira *et al.,* 1998; Kamara *et al,* 2003; Kamara *et al.,* 2004; Jackson, 2009). On the other hand, bitter taste and astringency are elicited by flavonoids, such as flavanols and flavonols (Lesschaeve & Noble, 2005). Based on the proposition made by McManus *et al.* (1981), that for a phenolic compound to elicit an astringent sensation it must possess two adjacent hydroxyl groups, it can be postulated that the xanthones, mangiferin and isomangferin, and the flavanone, eriocitrin, might be responsible for astringency in honeybush tea. Identifying specific constituents in honeybush infusions responsible for the basic tastes, specifically bitter taste, and astringency, may be useful for many reasons. It could be used as indicators of the quality and sensory properties of honeybush tea which would facilitate the development of a prediction model with which its sensory properties/quality could be estimated by analysing its chemical composition. These taste properties are also crucial with regards to the development of value-added products such as drinks, dairy products and food bars.

The objectives of this study were thus to evaluate the flavour of honeybush tea and to identify differences and similarities between the different *Cyclopia* species used to produce honeybush tea and to develop a defined set of descriptors, in the form of a sensory lexicon and wheel, for honeybush tea. The focus fell on *C. maculata*, recently identified for commercialisation, and gas chromatography-olfactometry (GC-O) was undertaken in an attempt to identify specific compounds responsible for the aroma of *C. maculata*. Correlation between specific sensory attributes and polyphenolic compounds were evaluated in order to determine whether specific polyphenolic compounds in the honeybush infusion could be linked to certain taste and mouthfeel attributes associated with honeybush tea. The effect of different fermentation temperature and time combinations on the flavour profile and the polyphenolic composition of three *Cyclopia* species, i.e. *C. genistoides, C. subternata* and *C. maculata*, were evaluated in order to determine the optimum fermentation conditions for each species.

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Chapter 2

LITERATURE REVIEW

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1. INTRODUCTION

The dramatic growth in the consumption of herbal teas by health conscious consumers seeking products associated with health and wellness coupled with the commercial success of rooibos tea has led to an increase in the demand for honeybush tea, both locally and internationally (Joubert *et al.,* 2011). Honeybush tea has become internationally recognized as a substitute for ordinary tea (*Camellia sinensis*) due to its associated biological properties. Research has shown that honeybush tea possess antioxidant (Joubert *et al*., 2008a), antimutagenic (Marnewick *et al.,* 2000; Marnewick *et al.,* 2003), phyto-estrogenic (Verhoog *et al.,* 2007a; Verhoog *et al.,* 2007b) and antimicrobial (Coetzee *et al.,* 2008) properties. This chapter gives an overview of honeybush tea; its history, botanical description, geographical distribution, chemical composition and the current state of the industry. The processing methods, aroma, taste, mouthfeel and flavour physiology, perception and analysis, and the sensory methods employed for the estimation of tea quality are also reviewed.

2. HONEYBUSH (*CYCLOPIA* SPECIES)

2.1. History

Honeybush tea is believed to have first been used by the native tribes in the Cape as a treatment for coughs and upper respiratory systems associated with infection although there are no published reports confirming this (Du Toit *et al.,* 1998). The earliest mention of the honeybush plant was in 1705 in a taxonomic script (Kies, 1951) and in 1772 the name “honingtee” (Dutch) was first recorded by C. Thunberg, a Swedish botanist (De Lange, 2002). However, it was not until 1808 that the specific species referred to in this literature was classified and named *C. genistoides* (Greenish, 1881). In 1815 C. Latrobe mentions the use of “tea-water” in the Langkloof (Latrobe, 1818), an area rich in honeybush. A few years later, Bowie (1830) reported the use of the honeybush plant as an expectorant in chronic catarrh and pulmonary tuberculosis. According to Marloth (1925) the infusion was used by colonists as a stomachic and to alleviate heartburn and nausea. Other species such as *C. vogelii* (renamed *C. subternata*) (Watt and Breyer-Brandwijk, 1962), *C. latifolia* and *C. longifolia* (Marloth, 1913; Marloth, 1925) were also used to prepare honeybush tea. Marloth (1925) first noted the regional use of specific species, probably due to their prevalence in those localities, i.e. *C. genistoides* was used in the Cape Peninsula whereas *C. subternata* was used in Caledon (Overberg) and George areas. Despite the fact that the first branded honeybush tea “Caspa Cyclopia tea” appeared on the South African market in the 1960s the honeybush industry remained dormant until the 1990s when the commercial success of rooibos led to renewed interest in honeybush tea. The South African National Botanical Institute (SANBI, Kirstenbosch) and the Agricultural Research Council (ARC) initiated projects to investigate the commercial cultivation, processing and health-promoting properties of honeybush (Joubert et al., 2008b). The complete history with regards to the development of a formal honeybush tea industry can be found in Joubert *et al.* (2011).

2.2. Botanical description

The *Cyclopia* plant belongs to a very distinct genus of the tribe Podalyrieae and is classified as a member of the Fabaceae (Schutte, 1995). To date more than 20 species of *Cyclopia* have been described in literature (Kies, 1951; Bond & Goldblatt, 1984; Schutte, 1995), however, only a few of these are used for the commercial production of honeybush tea (Joubert *et al.,* 2011). *Cyclopia* plants have woody stems with a relatively low leaf-to-stem ratio and are normally 1.5 m high, but these bushes can reach a height of up to 3 m (Bond & Goldblatt, 1984; Welgemoed, 1993). The leaves are trifoliate while the leaf shape varies between the different *Cyclopia* species – from pubescent, narrow leafed (*C. genistoides*) to flattened (*C. intermedia* and *C. subternata*) (Levyns, 1920; Marloth, 1925; Kies, 1951; Bond & Goldblatt, 1984). *Cyclopia* species can be divided into two distinct groups depending on which fire-survival strategy the plant utilises; reseeders (killed by fire and re-established from seeds) or resprouters (survive fire and sprout from the woody base) (Van Wyk, 2008). During the flowering period the *Cyclopia* bushes can easily be recognised in the field by their deep-yellow flowers with indented calyxes and their sweet, honeylike scent (Du Toit *et al.,* 1998). Flowering usually occurs in the spring (September and October), with the exception of *C. sessiliflora* which flowers during the late autumn or early winter (May and June) (Joubert *et al.,* 2011).

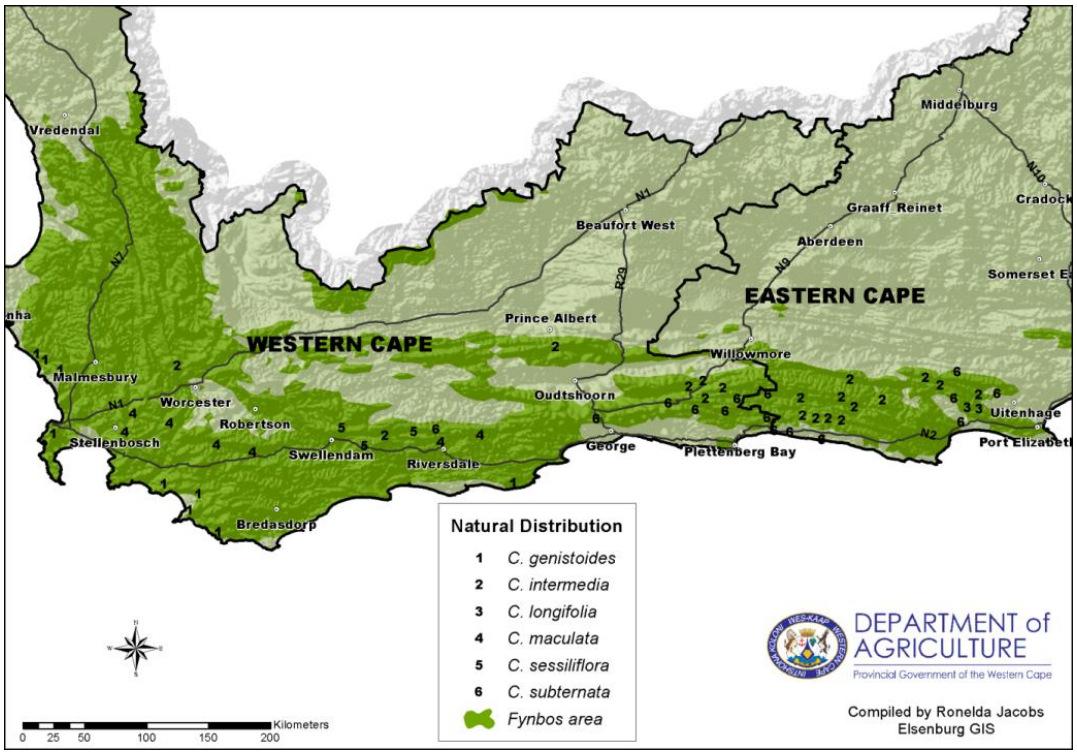
2.3. Geographical distribution

The indigenous *Cyclopia* species forms part of the Cape Floristic Region (CFR), which falls in the Western and Eastern Cape Provinces of South Africa (Du Toit *et al.,* 1998). The CFR is the smallest and richest of the world's six floral kingdoms, with more than 8700 plant species of which an astonishing 68% are endemic only to this area (Turpie *et al.,* 2003). The honeybush shrub grows in the coastal districts of the Western and Eastern Cape (Fig. 1) (Du Toit *et al.,* 1998). Unlike rooibos tea (*Aspalathus linearis)* which is prepared from a single species, honeybush tea is prepared from a variety of *Cyclopia* species found in different climatic regions of South Africa. The tea is therefore not only known as honeybush tea to the local inhabitants, but has different descriptive names according to their habitat and appearance (Table 1). Most *Cyclopia* species prefer to grow on the sandy and cooler southern slopes of the mountain ranges, with the exception of *C. genistoides* which can be found on the flat and sandy coastal areas (Du Toit *et al.,* 1998; Joubert et al., 2008b). *Cyclopia longifolia* is endangered and only a few plants are found in the wild, however, the potential to cultivate this species, along with *C. maculata* and *C. sessiliflora*, is currently under investigation (Joubert *et al.,* 2011)

In the past, harvesting activities has led to the degradation and depletion, and even the extinction of many *Cyclopia* populations (Du Toit *et al*., 1998). The fear of over-exploitation of the natural *Cyclopia* populations, due to the growing interest in honeybush tea, has led to the establishment of commercial plantations to lessen the pressure on the natural *Cyclopia* populations. In terms of cultivation, *C. subternata* and *C. genistoides* are the two main species used (De Lange & von Mollendorff, 2006). The cultivation of these species is localized to the area between the Overberg and the Langkloof area, with approximately 200 ha under cultivation. *Cyclopia subternata* grows mainly on sandy loam soil in valleys in the Langkloof, the Waboomskraal area near George and the Riversdal area whereas *C. genistoides* grows naturally in the coastal sandy areas from the west coast to Mossel Bay and therefore plantations have been established in the Overberg and Mossel Bay/Albertinia areas. *Cyclopia intermedia* is not ideal for cultivation as it can only

be harvested every second to third year making it uneconomical to cultivate for commercial purposes (Joubert *et al.,* 2011).

a)



b)

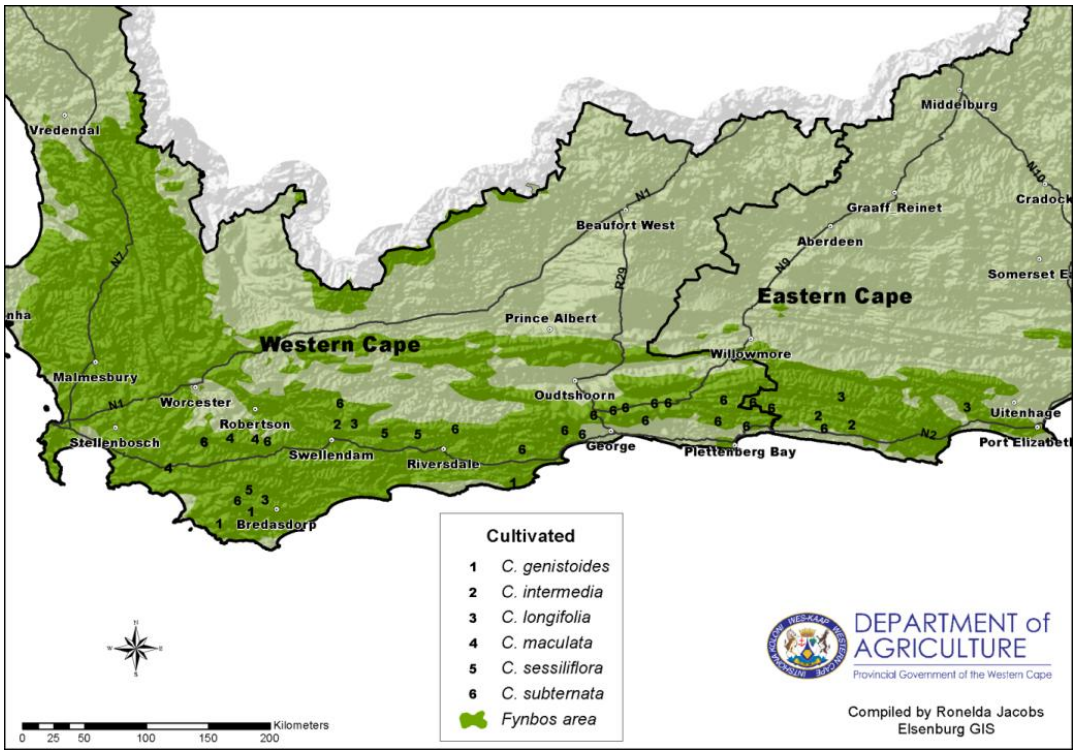


Figure 1 Natural (a) and cultivated distribution (b) of *C. genistoides, C. intermedia, C. longifolia, C. maculata, C. sessiliflora* and *C. subternata* (Joubert *et al.,* 2011).

Table 1 General information on the six *Cyclopia* species with commercial and potential commercial value

Species	Descriptive name	Distribution	Soil type	Flowering period	Comments
Ses	Heidelbergtee	Langeberg and Warmwaterberg mountains in the southern region (Rare/localised)	Well-drained loamy, sandy soils	May/June	Good tea quality, favourable growth form, slow growing, harvest close to the ground
Lon	No known descriptive name	Van Stadens River mountains near Port Elizabeth	Moist, sandy soils along the banks of the river	September/October	Tea quality unknown, harvest close to the ground
Gen	Kustee/ Heuningtee/ Overbergtee	Malmesbury – Darling area, the hills and mountains on the Cape Peninsula and Cape Flats, Grabouw, Kogelberg, Betty's Bay, Hermanus, Bredasdorp, De Hoop, Swellendam and eastwards to Albertina in the southern region	Sandy soils	August/September	Good quality tea, excellent growth form, harvest close to the ground
Int	Bergtee/ Kougabergtee	Witteberg, Anysberg, Swartberg, Touwsberg, Rooiboerg, Kammanassie, Kouga, Baviaanskloof, Langeberg, Outeniqua, Tsitsikamma, Van Stadens mountains (most widespread)	Rocky, loamy, sandy soils	September	Very good quality tea, slow growing, harvest close to the ground, possibly drought resistant
Sub	Vleitee	Widely distributed along the coastal mountain ranges (Tsitsikama, Outeniqua and Langeberge) where it occurs on the southern slopes	Well-drained, stony, loamy soils	September	Very good quality tea, vigorous grower, producing relatively thick shoots, harvest knee-high
Mac	Vleitee/ Genadendaltee	Along riverbanks and streams in the south-western and southern region	Wet, peaty soils	August/September	Tea quality unknown, vigorous growth, thick shoots, harvest knee-high

Compiled from Schutte (1997), De Lange & von Mollendorff (2006), and Joubert *et* al. (2011). Ses = *C. sessiliflora,* Lon = *C. longifolia,* Gen = *C. genisoitdes;* Int = *C. intermedia,* Sub = *C. subternata,* Mac = *C. maculata.*

2.4. Chemical composition

Honeybush is a caffeine-free (Greenish, 1881) herbal infusion with a very low tannin content (Marloth, 1925; Terblanche, 1982) containing numerous polyphenolic compounds which have the ability to act as antioxidants and have anticarcinogenic and phyto-oestrogenic properties (Joubert et al., 2008b; Joubert *et al.,* 2009). Approximately 30% of the total polyphenol content and 4.34% of the soluble solids of fermented *C. buxifolia* is tannin (Du Toit & Joubert, 1998b). The tannin is of the proanthocyanidin type (Marnewick *et al.,* 2005). A cup of honeybush tea was reported to contain 0.59 µg/mL fluoride and 20.5 µg/mL Ca (Touyz & Smith, 1982; Malik *et al.,*2008). The earliest reports of the chemical composition of the *Cyclopia* plant date back to 1870 and 1881, where the presence of unknown and unidentified substances was mentioned (Watt & Breyer-Brandwijk, 1962). It was only recently, in 1996, when De Nyschen *et al.* (1996) identified the three major constituents in the leaves of *Cyclopia* species as a xanthone C-glycoside (mangiferin) and two O-glycosides of the flavonones (hesperetin and isosakuranetin). Analysis to date did not shown the presence of isosakuranetin (Joubert *et al.,* 2011)

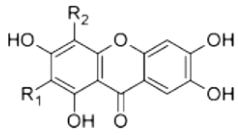
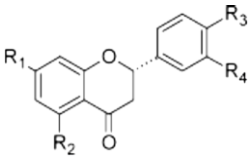
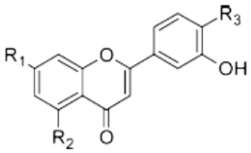
In processed leaves and stems of *C. intermedia* species Ferreira *et al.* (1998) identified xanthones (mangiferin and isomangiferin) along with the inositil (+)-pinitol, luteolin, the hydroxycinnamic acid 4-coumaric acid, five isoflanones (formononetin, afrormosin, calycosin, pseudobaptigen and fujikinetin), four flavonones (hesperitin, hesperidin, naringenin and eriodictyol) and three coumestants (medicagol, flemichapparin and sophorocoumestan). A few years later Kamara *et al.* (2003; 2004) identified additional flavonoids including hydroxyphenylethanol tyrosol and a couple 4-O-glycosyl derivatives, five glycosylated flavonols (including monoglucosylated kaempferols), four flavanones, two isoflavones (including wistin) and two flavones. These studies were, however, limited to *C. intermedia* (fermented) and *C. subternata* (unfermented). Joubert *et al.* (2008b) also identified a number of unknown compounds in *Cyclopia* species. Table 2 summarises the secondary metabolites identified in the plant material of *Cyclopia* species.

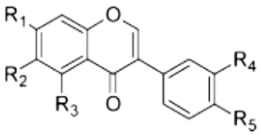
The total polyphenol content of honeybush tea extracts has been shown by Joubert *et al.* (2008b) to differ between the different *Cyclopia* species and to decrease with fermentation of the raw honeybush plant material. The total polyphenol content of *C. genistoides* was least effected by fermentation followed by *C. sessiliflora,* whereas *C. intermedia* and *C. subternata* retained less than 50% of its total polyphenol content. The lower total polyphenol content after fermentation of the raw plant material is attributed to the decrease in the xanthone as well as the flavonoid contents.

All *Cyclopia* species, analysed to date, contain the three major polyphenolic compounds (mangiferin, isomangiferin and hesperidin) (Joubert et al., 2008b). In a study by De Beer & Joubert (2010) only mangiferin, isomangiferin, hesperidin and compound B, an unidentified flavanone-glycosides, could be identified in all *Cyclopia* species analysed, namely *C. genistoides, C. intermedia, C. sessiliflora* and *C. subternata.* They showed that extracts of fermented plant material contain lower concentrations of specific compounds than the unfermented plant material. Unfermented *C. genistoides* contained the highest concentration of both mangiferin and isomangiferin followed by *C. sessiliflora, C. intermedia* and *C. subternata* (Joubert *et al.,* 2008b). The hesperidin content was very similar between the four *Cyclopia* species but *C. sessiliflora* had the highest concentration and *C. subternata* the lowest. However, previously Joubert *et al.* (2003; 2008b) found that *C. intermedia* contained the most and *C. sessiliflora* the least hesperidin. Compound B was present in all *Cyclopia* species analysed but it was significantly higher in *C. sessiliflora* compared to the other species. In fermented *C. subternata* only trace amounts of compound B

could be identified. Eriocitrin was present in all the *Cyclopia* species except *C. genistoides.* The highest concentration was present in *C. subternata* and *C. sessiliflora,* followed by *C. intermedia.* However, it is mentioned that there was a problem with co-elution in *C. sessiliflora* which could have led to overestimation. Compound D (an eriodictyol-glucoside) was only present in unfermented *C. subternata* and *C. intermedia.* Unfermented *C. subternata, C. intermedia* and, interestingly, fermented *C. sessiliflora* contained compound E (unidentified hydroxycinnamic acid derivative). Compound F was detected in unfermented and fermented C*. subternata* and *C. sessiliflora* as well as unfermented *C. intermedia*. Eriodictyol, luteolin and narirutin were not quantified as they were either not detected, present only as traces or co-eluted with other compounds. Joubert *et al.* (2003) showed that *C. genistoides* (Overberg type) contain more mangiferin and less hesperidin than *C. genistoides* (West Coast type). Also, it showed that the mangiferin content varied with the harvesting date.

Table 2 Secondary metabolites identified in the plant material of *Cyclopia* species (Joubert et al., 2008b)

Structure	Compound type, names and substituents
	**Xanthone** Manigferin[a]: R_1 = *C*-β-D-glucosyl, R_2 = H Isomangiferin[a]: R_1 = H, R_2 = *C*-β-D-glucosyl
	**Flavanone** Hesperidin[a,c]: R_1 = *O*-rutinosyl; R_2 = OH, R_3 = OCH_3, R_4 = OH Hesperetin[a]: R_1 = R_2 = OH, R_3 = OCH_3, R_4 = OH Eriocitrin[c]: R_1 = *O*-rutinosyl, R_2 = R_3 = R_4 = OH Eriodictyol[a]: R_1 = R_2 = R_3 = R_4 = OH Narirutin[c]: R_1 = *O*-rutinosyl, R_2 = R_3 = OH, R_4 = H Naringenin[a]: R_1 = R_2 = R_3 = OH, R_4 = H Prunin[b]: R_1 = *O*-rutinosyl, R_2 = R_3 = OH, R_4 = H Naringenin-5-*O*-rutinoside[b]: R_1 = R_3 = OH, R_2 = *O*-rutinosyl, R_4 = H Eriodictyol-5-*O*-glucoside[b]: R_1 = R_3 = R_4 = OH, R_2 = *O*- β-D-glucosyl Eriodictyol-7-*O*-glucoside[b]: R_1 =*O*- β-D-glucosyl, R_2 =R_3 =R_4 =OH
	**Flavone** Luteolin[a,c]: R_1 = R_2 = R_3 = OH Diosmetin[b]: R_1 = R_2 = OH, R_3 = OCH_3 5-Deoxyluteolin[c]: R_1 = R_3 = OH, R_2 = H Scolymoside[c]: R_1 = *O*-rutinosyl, R_2 = R_3 = OH



Isoflavone

Formononetin[a]: R_1 = OH, R_2 = R_3 = R_4 = H, R_5 = OCH_3

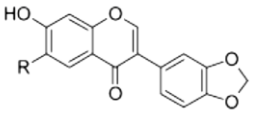
Formononetin diglucoside[b]: R_1 = *O*-α-apiofuranosyl-(1'''→6'')-β-D-glucopyranosyl, R_2 = R_3 = R_4 = H, R_5 = OCH_3

Afrormosin[a]: R_1 = OH, R_3 = R_4 = H, R_2 = R_5 = OCH_3

Calycosin[a]: R_1 = R_4 = OH, R_2 = R_3 = H, R_5 = OCH_3

Wistin[b]: R_1 = *O*-β-D-glucosyl, R_3 = R_4 = H, R_2 = R_5 = OCH_3

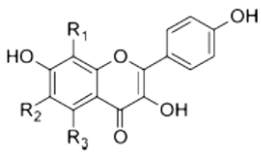
Orobol[c]: R_1 = R_3 = R_4 = R_5 = OH, R_2 = H



Methylinedioxyisoflavone derivative

Pseudobaptigenin[a]: R = H

Fujikinetin[a]: R = OCH_3



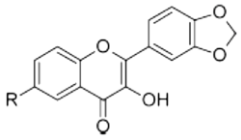
Flavonol

Kaempferol-5-*O*-glucoside[b]: R_1 = R_2 = H, R_3 = *O*-β-D-glucosyl

Kaempferol-6-*C*-glucoside[b,c]: R_1 = H, R_2 = *C*- β-D-glucosyl, R_3 = OH

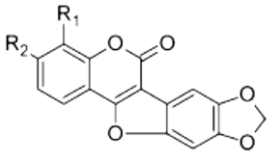
Kaempferol-8-*C*-glucoside[b]: R_1 = *C*- β-D-glucosyl, R_2 = H, R_3 = OH

Kaempferol-8-*C*-glucoside[b]: R_1 = *C*- β-D-glucosyl, R_2 = H, R_3 = OH



Methylinedioxyflavonol derivative

3',4'-Methylenedioxyflavonol diglucoside[b]: R = *O*-α-apiofuranosyl-(1'''→6'')-β-D-glucopyranosyl

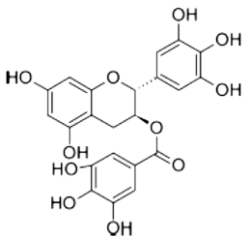


Coumestan

Medicagol[a]: R_1 = H, R_2 = OH

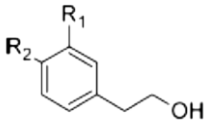
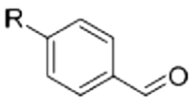
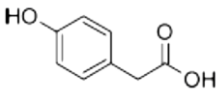
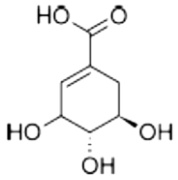
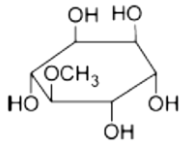
Flemichapparin[a]: R_1 = H, R_2 = OCH_3

Sophoracoumestan B[a]: R_1 = OCH_3, R_2 = OH



Flavan-3-ol

(−)-epigallocatechin gallate[c]

	**Phenylethanol derivative** Tyrosol[b]: R_1 = H, R_2 = OH 3-Methoxy-tyrosol[b]: R_1 = OCH_3, R_2 = OH 4-Glucosyltyrosol[c]: R_1 = H, R_2 = *O*- β-D-glucosyl Phenylethanol diglucoside[b]: R_1 = *O*-α-apiofuranosyl-(1''→6')-β-D-glucopyranosyl, R_2 = H
	**Benzaldehyde derivative** Benzaldehyde diglucoside[b]: R = *O*-α-apiofuranosyl-(1''→2')-β-D-glucopyranosyl
	**Phenolic carboxylic acid** *p*-Coumaric acid[a]
	**Organic acid** (±)-Shikimic acid[c]
	**Inositol** (+)-Pinitol[a,c]

[a] Ferreira *et al.* (1998), [b] Kamara *et al.* (2003), [c] Kamara *et al.* (2004)

2.5. The industry

McKay and Blumberg (2007) reported that the overall herbal tea consumption has been increasing at an annual rate of 15-20% per year. This dramatic growth is fuelled primarily by health conscious consumers seeking products that will help them live longer, feel better and stay healthier. Honeybush tea has become internationally recognized as a substitute for ordinary tea (*Camellia sinensis),* mainly due to its high antioxidant potential, and the honeybush industry has the potential to emulate the success achieved by the rooibos industry (Joubert *et al.,* 2011).

In the early eighties, exporting honeybush tea to the United States of America (USA) was investigated, but a lack of supply prevented exporting (Viljoen, 1994). Due to the inconsistent quality of honeybush tea a number of studies was conducted in order to develop a standardized processing method with adequate control to ensure that honeybush tea of constant good microbial and sensory quality are

produced (Du Toit *et al.,* 1998). However, quality problems still exist and could severely harm the reputation of the relatively new and still developing honeybush industry.

The export market of honeybush has grown from 50 to 200 tonnes over the past ten years (Fig. 2) and currently the supply cannot meet the demand (Joubert *et al.,* 2011). The Netherlands are currently the top importer of honeybush tea with a share of almost 45%, followed by Germany, the United Kingdom (UK), Poland and the USA (Table 3). Most of the tea produced (95%) is sold in bulk to overseas clients. Until recently, exports consisted mainly of *C. intermedia* (as well as *C. genistoides* and *C. subternata*), however, as the demand for honeybush tea increased, both locally and internationally, the demand began exceeding the supply and the focus, due to necessity, shifted to other *Cyclopia* species, such as *C. sessiliflora, C. longifolia* and *C. maculata*. Due to the fact that honeybush has huge potential in the herbal tea market, is unique to South Africa, can be grown organically and can be used in value-added food products, medicinal products and cosmetics, the rapid growth of the industry is expected to continue (Joubert *et al.,* 2011).

Table 3 Top importers of Honeybush tea in 2010 (S. Snyman, South Africa, Rooibos Council, 2011, personal communication)

	Country	Total (kg)	Percentage
1	Netherlands	55 841	43.98%
2	Germany	40 285	31.73 %
3	UK	9 900	7.80%
4	Poland	8 010	6.31%
5	USA	6 291	4.95%

USA = United States of America, UK = United Kingdom. Other countries: Sri Lanka (1.2%), Austria (0.95%), Australia (0.71%), Russia (0.71%), Canada (0.65%), Italy (0.26%), India (0.24%), Norway (0.14%), Lithuania (0.13%), Japan (0.08%), New Zealand (0.07%), China (0.05%) and Hong Kong (0.05%).

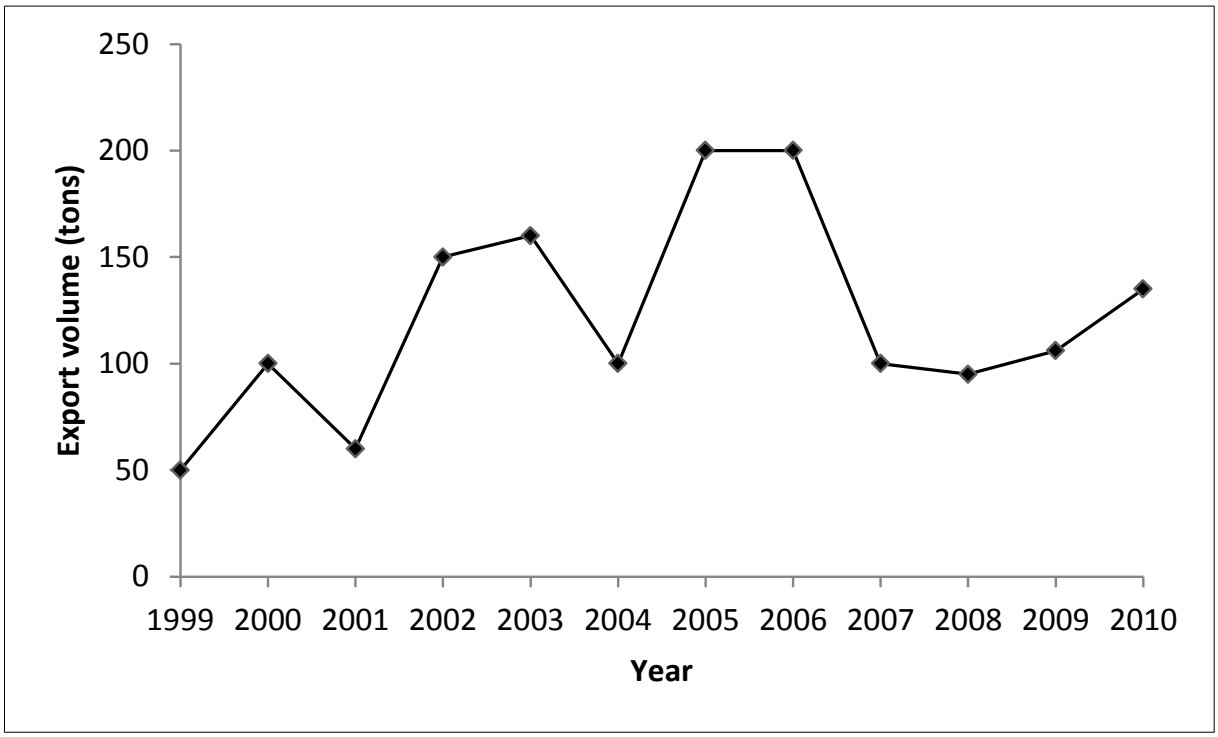


Figure 2 Total exports of honeybush tea from 1999 to 2010 (Joubert *et al.,* 2011).

Although honeybush has been known to South Africans for centuries it is only during the last few years that honeybush tea has appeared on our supermarket shelves (Joubert *et al.,* 2011). Products such as mixtures of honeybush and rooibos and other indigenous South African plants and fruits and ice teas are now available (Joubert et al., 2008b). Although certain brands, mostly those found in specialty shops or up-market farm stalls consists of only one *Cyclopia* species, most of the honeybush products are a mixture of two or more *Cyclopia* species.

The continuing increase in the international demand for honeybush tea may lead to an interest in the production of honeybush tea in other countries which would pose a major threat to the South African honeybush industry (Joubert *et al.,* 2011). The development of a geographical indication (GI) for honeybush might also be useful, however, the industry is currently still too small (Blanchard Oritz, 2006; Blanchard *et al.,* 2006). A GI is a label that is reserved for products which acquire the characteristic and defining qualities as a result of their geographical location (Grazioli, 2002). It enables producers to distinguish their product based on its specific origin-related characteristics. However, in order to establish a GI for honeybush the product needs to be described - emphasising the need for a list of sensory descriptors describing the typical flavour and mouthfeel attributes of honeybush tea and to determine the extent of similarity, but also dissimilarity in the sensory attributes of different species.

3. PROCESSING OF HONEYBUSH

3.1. Harvesting

Sprouters (*C. genistoides, C. intermedia* and *C. sessiliflora)* can be harvested 2 to 3 years after planting, depending on the soil and climate (Viljoen, 2001). The sprouters are cut back to soil level to stimulate the formation of new shoots from the rootstock. After the first harvest *C. genistoides* can be harvested annually whereas *C. intermedia* and *C. sessiliflora* can only be harvested every second or third year (Joubert *et al.,* 2011). The non-sprouter *C. subternata* is a relatively fast grower and can be harvested annually. It is harvested by cutting the shoots back to between 30 and 50 cm above the ground. About a third of the active growth should remain on the plant as too severe pruning places stress on the plant causing dieback (Joubert *et al.,* 2011). Bushes previously harvested tend to provide more coarse material due to their thicker stems (Du Toit *et al.,* 1998). The lifespan of a sprouter is at least 10 years whereas the lifespan of non-sprouters is about 7 to 8 years (Joubert *et al.,* 2011).

Harvesting of the *Cyclopia* shrub was traditionally done during the flowering period (either May or September, depending on the species (Du Toit *et al.,* 1998), but due to the increase in demand many farmers extended the harvesting period (Welgemoed, 1993). The presence of the flowers is believed to improve the distinctive sweet, honey-like flavour of the tea but a study by Du Toit and Joubert (1999) indicated that material processed without the presence of flowers still delivers an acceptable product. Therefore, today the raw plant material is harvested in the summer to late autumn before flowering occurs, as flowering places the plant under unnecessary stress (Du Toit *et al.,* 1998). *C. genistoides* is usually harvested during November and March whereas *C. subternata* is normally harvested in April to June (Joubert *et al.,* 2011).

3.2. Cutting

Harvested tea should be processed as soon as possible, but processing is often delayed for days (Du Toit *et al.,* 1998). Mechanised fodder cutters or tobacco cutters are used to cut the plant material into small pieces (2-3 mm in length) to disrupt the cellular integrity as this facilitates fermentation. Poor cutting equipment can lead to coarse material with unappealing white pieces of stem in the final product which in turn influences the quality of the tea (Du Toit & Joubert, 1998b). In the manufacture of honeybush tea, both the leaves and the stems are used which leads to a relatively low moisture content (<50%) for the fresh plant material. The relatively low moisture content obviates the need for a withering stage as is used in black tea (*Camellia sinensis)* manufacture.

3.3. Pre-treatment

Cut plant material is pre-treated with cold water (pre-wetted) as this treatment results in more uniformly brown-coloured tea leaves with improved infusion characteristics (Du Toit & Joubert, 1998b). This treatment decreases the presence of uncoloured bits of stems in the final product. Additionally, pre-treatment results in honeybush infusions with better flavour. The characteristic red-brown dark-brown leaf colour develops faster in water treated material as some of the oxidisable matter is extracted to the surface, rendering it more accessible to oxygen.

3.4. Fermentation

“Fermentation” refers to the chemical oxidation step during processing needed for the formation of the sought-after brown colour and sweet, honey-like flavour of honeybush tea (Du Toit & Joubert, 1998b). Traditionally, fermentation heaps (Marloth, 1909; Marloth, 1925) or baking ovens (Hofmeyer & Phillips, 1922) were used for fermentation, followed by sun-drying (Du Toit *et al.,* 1998). These traditional processing methods did not allow for control of the processing parameters and problems with mould and bacterial growth, as well as under- and unfermented tea resulted in honeybush tea of poor quality (Du Toit & Joubert, 1998a). More than a decade ago these problems were addressed by Du Toit and Joubert (1999) and rotation drums, similar to those conceptualised for rooibos processing (Joubert & Müller, 1997), and elevated temperatures (> 60°C) were introduced in order to eliminate microbial contaminants and to produce tea of consistent high quality (Du Toit, 1997).

Optimally fermented honeybush tea has a characteristic sweet taste often described as flowery, fruity or honey-like, while insufficiently fermented tea has a grassy taste and aroma (Du Toit & Joubert, 1998b). Poor fermentation conditions and slow drying can result in tea with a musty off-flavour. Studies by Du Toit (1997) on *C. intermedia* and *C. buxifolia* (previously classified as *C. maculata*) have shown that fermentation temperature and time affected the quality of honeybush tea. Longer fermentation periods (60-72 h) were necessary when fermentation was carried out at lower temperatures (60 and 70°C), whereas fermentation was completed after 36 h at 90°C. However, the fermentation period at elevated temperatures was more critical and good control is necessary to prevent the occurrence of flat liquors with a burnt taste (Du Toit *et al.,* 1998). Most of the compounds that enhance the brown colour of the leaves are formed

during the first 48 hours of the fermentation process thus prolonging the fermentation period will not markedly contribute to the colour development of the tea leaves (Du Toit & Joubert, 1998b).

Today, the industry employ fermentation conditions ranging from 70°C/60 h for *C. intermedia* to 80-85°C/18-24 h for other *Cyclopia* species, such as *C. genistoides, C. subternata* and *C. maculata,* whereas the ARC ferments honeybush, for research purposes, at either 80°C/24h or 90°C/16 h (E. Joubert, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, 2010, personal communication).

3.5. Drying

It is believed by traditional processors that the appearance of the final product is improved by sun-drying (Du Toit *et al.,* 1998). However, research has shown that the drying method used (sun-drying vs. controlled drying in a tunnel) did not significantly influence the quality of honeybush tea (Du Toit, 1997). The characteristic aroma and taste as well as mouthfeel of honeybush tea are formed during fermentation and the relative short drying time does therefore not significantly influence the quality of honeybush tea (Du Toit & Joubert, 1998a). Only the aroma of honeybush tea (*C. intermedia)* is influenced by the drying temperature and it seems that lower temperatures (40-50°C) produce tea with superior aroma properties compared to tea dried at higher temperatures (70°C). Drying temperatures also do not significantly affect the colour, soluble solids, total polyphenol or flavonoid contents of *C. intermedia* and *C. genistoides* tea infusions (Du Toit & Joubert, 1998a). Currently, honeybush tea is dried at 40°C for 6 hours under artificial conditions (Joubert *et al.,* 2011).

3.6. Sieving

Honeybush tea is traditionally a very coarse product which contributed to the belief that the unrefined product has certain health given properties (Du Toit *et al.,* 1998). The tea was therefore often sold as a mixture of short stems and leaves. The final product was put through an electrically driven, cylindrical sieve with a 6.5 mm aperture screen, to remove all the pieces thicker than a match stick (Viljoen, 1994). However, the export market demanded a finer product, necessitating further improvements to the sieving process (Du Toit *et al.,* 1998). Today, the dried tea is sieved (200 g/30 sec) using a mini-sifter and the <12>40 mesh fraction collected for experimental purposes whereas five classes (extra coarse cut, regular coarse cut, regular fine cut, super fine cut, dust 1 and other cut) exist according to the Agricultural Product Standards Act (Act no. 119 of 1990; Anon., 2000).

4. QUALITY CONTROL

4.1. Tea grading systems

Grading systems are used to enable standardization and commercialization of a food product by improving control over its overall quality and thereby increasing consumer satisfaction (Feria-Morales, 2002). To develop such systems quality parameters needs to be identified, defined and measured. It is crucial that these methods are reliable, quick, simple, scientifically validated and correlated to the way that consumers perceive product quality.

Tea (*Camellia sinensis)* is assessed by tea tasters which observe the appearance of the dry and the infused tea leaves (Sinija & Mishra, 2008). The tasters are primarily concerned with the liquor colour, aroma and taste of the brewed tea liquor. Similarly, rooibos tea of the major rooibos processors is currently graded by experienced tasters into seven different grades (AA, A, B, C, D, E or F) based on the appearance of the dry and wet tea leaves and the appearance and flavour of an infusion prepared according to a standard protocol (Koch, 2011).

Tea researchers have made many attempts to explain tea quality and its quality attributes chemically and physically to develop equipment to replace sensory assessment (Sinija & Mishra, 2008). In the last decade progress has been made, especially in terms of black tea (*Camellia sinensis*). Capillary electrophoresis, the electronic tongue and a lipid membrane taste sensor have been successfully applied to black tea quality estimation (Legin *et al.,* 1997; Horie & Kohata, 1998; Ivarsson *et al.,* 2001a*;* 2001b). Chemical compositions and liquor colours were used to estimate the quality of black (*Camellia sinensis*) and pu-erh teas (Liang *et al*., 2003). However, these techniques have not been used widely in commercial practices of tea production and marketing and sensory evaluation still remains the most popular and effective method for determining tea quality.

Currently no universal grading system is in place for the evaluation of honeybush tea which could lead to teas of inferior quality being sold in the market place. The fact that different species are used interchangeably further complicates this matter. A similar grading process to that employed in the rooibos industry could greatly improve the quality of honeybush tea.

4.2. Regulatory control

According to the Foodstuffs, Cosmetics and Disinfectants Act “honeybush tea” means the product obtained from the leaves, flowers and stems of the *Cyclopia* genus (Anon., 2002). The quality standards, in terms of taste and aroma, for the export of honeybush tea are defined by the Agricultural Product Standards Act (Act no. 119 of 1990). The regulation states that “honeybush must have the clean characteristic taste and aroma of honeybush and that it shall be free from any foreign flavours and odours which detrimentally affect the characteristics of the product” (Anon, 2000). No sensory descriptors are provided by the regulations. Regulatory control of honeybush tea, similarly to rooibos, is limited to pesticide residues and microbial contamination (Joubert *et al*., 2008b). Each tea manufacturer exercises their own set of standards in terms of cut size, colour and flavour. No specifications exist for the total polyphenol content or the antioxidant activity, in spite of their importance in terms of marketing health promoting properties of honeybush tea.

5. TASTE AND MOUTHFEEL

5.1. Oral physiology

The four basic taste modalities (sweet, sour, bitter, and salty) are perceived within the oral cavity by the taste buds that are distributed on the tip and edges of the tongue within a variety of projections known as papillae (Jackson & Linskens, 2002; Worobey *et al.,* 2006). Humans have three types of functional taste papillae: fungiform (mushroom shaped located at the front of the tongue), foliate (appearing as parallel rows of ridges and valleys located at side of the tongue) and circumvallate (button-shaped located at the back of the

tongue). The circumvallate papillae are sensitive to bitter substances, the folate papillae are sensitive to sour materials and the fundiform papillae preferentially detect sweet compounds (Jackson & Linskens, 2002).

Polarized, neuroepithelial taste receptor cells (TRCs) in clusters of 50 to 150 are organized into taste buds in each papillia (Beidler, 1978). Each of these taste buds is composed of a series of elongated taste receptor cells that are arranged around a central core with the top of the taste bud exposed to the oral cavity where the microvilli of the TRCs make contact with saliva and tastants (Akabas, 1990; Worobey *et al.,* 2006). It is this configuration which permits taste molecules dissolved in saliva to gain access to the taste cells (Worobey *et al.,* 2006). Additional taste buds are also found on the soft palate and epiglottis, at the back of the throat. Although TRCs are not neurons the contact between these cells and sensory fibres has the morphological characteristics of chemical synapses. In addition, TRCs are electrically excitable cells with voltage-gated Na^+, K^+ and Ca^{2+} channels capable of generating action potentials. Besides TRCs the oral cavity also houses mechanoreceptors (MRs) which appear to be of greater importance for astringency perception (Weiffenbach, 1993; Trulsson & Essick, 1997). Unlike TRCs, MRs are neurons classified according to the size and character of their receptive field (Kaas, 2004) as either Type I (small and distinct receptive fields) or Type II (large, diffuse receptive fields) (Jacobs *et al.*, 2002). MRs are further classified as rapidly adapting (RA) receptors which respond during the dynamic phase of stimulus application or slowly adapting (SA) receptors which respond to both dynamic and static force applications.

The salivary glands are under collaborative parasympathetic (acetylcholine) and sympathetic (noradrenaline) control via the efferent (secreto-motor) fibers of the facial and glossopharyngeal nerves (Garrett, 1967; Garrett and Kidd, 1993). There are three pairs of major salivary glands (parotid, submaxillary/submandibular and sublingual exocrine) (Nieuw Amerongen & Veerman, 2002) that secrete saliva (containing proteins and enzymes) into the oral cavity, where they provide lubrication and initiate the process of digestion (Dawes & Wood, 1973; Young & Schneyer, 1981). Salivary protein composition varies greatly between individuals (0.9-7 mg/mL) (Jenzano *et al.,* 1986; Lu & Bennick, 1998). Recently, Xie *et al.* (2005) has identified 437 proteins in saliva but only the proline-rich proteins (PRPs), histatins (HRPs), α-amylase, lactoferrin and mucins are implicated in the sensation of astringency (Yan & Bennick, 1995; Lu & Bennick, 1998; de Freitas & Mateus, 2001a; 2001b; Gambuti *et al.,* 2006; Condelli *et al.,* 2006).

5.2. Taste

Taste buds respond to the four classic basic tastes (Worobey *et al.,* 2006). Specific receptors G-protein coupled receptors (GPCRs) localized to the surface of the taste cells have been identified for sweet and bitter (Brandbury, 2004; Li *et al.,* 2002; Ugawa *et al.,* 2003; Worobey *et al.,* 2006). Zhang *et al.* (2003) showed that responses to all sweet and bitter stimuli require two signalling molecules; the T1Rs and T2Rs. Additional pathways may modulate sweet or bitter taste reception but do not, themselves, trigger a taste response.

5.2.1. Sweet

Sweet taste is mediated by a family of three GPCRs (the T1Rs) that combine to generate at least two heteromeric receptors: T1R2 and T1R3 associate to function as a broadly tuned sweet receptor, while T1R1

and T1R3 form a universal L-amino acid sensor (Li *et al.,* 2002). It is generally accepted that hydrogen bonding is at the origin of sweet taste chemoreception (Mathlouthi & Portmann, 1990). The mechanism involves the presence of a pair, AH-B, of donor and acceptor of hydrogen bonds established with the corresponding B-AH unit of the receptor as well as a hydrophobic group called a "γ-centre" situated opposite to the AH-B couple.

5.2.2. Bitter

Bitter tastants are detected by members of an unrelated family of about 30 different GPCRs, with the T2Rs on the apical membrane of the TRCs located in the taste buds (Adler *et al.,* 2000; Chandrashekar *et al.,* 2000; Matsunami *et al.,* 2000). Most T2Rs are co-expressed in the same subset of taste receptor cells of the tongue and palate epithelium suggesting that these cells are capable of responding to a broad array of bitter compounds (Adler *et al.,* 2000). T2Rs may also function as heteromeric receptors to accommodate the great chemical diversity of bitter tastants (Zhang *et al.,* 2003). Depending on the species, vertebrate genomes contain between three T2R genes in chickens and up to 50 in amphibians with only about 25 in the human genome which raises the question as to how humans can perceive such a large number of chemically diverse substances as bitter with such a limited number of receptors (Shi & Zhang, 2009; Meyerhof *et al.,* 2010).

Unfortunately it is mostly the potential beneficial phytonutrients, such as polyphenolic acid derivates, flavonoids, isoflavones, terpenes and glucosinolates which are described as bitter (Drewnowski & Gomez-Carneros, 2000). Low molecular weight polymers (flavonoids) tend to be bitter whereas the higher molecular-weight polyphenols (plant tannins) are more likely astringent. Bitter molecules occur in many variations; the strongest and most important representatives are certain alkaloids, terpenoids and flavonoids (Ley, 2008). Bitterness of tea (*Camellia sinensis*) is generally due to the presence of catechins, saponin, caffeine and amino acids (Drewnowski & Gomez-Carneros, 2000).

Besides the extreme wide structural range of bitterness, it is surprising that the bitter taste is very specific to isomers of similar molecular structure (Ley, 2008). Very small structural variations can change the taste profile or strongly influence the threshold. As an example, the hesperetin rutinoside (hesperidin) is tasteless but the positional isomer hesperetin neohesperidoside (neohesperedin) is strongly bitter. Due to the wide variations of the structural basis of bitter tasting molecules it is difficult to generalize the molecular requirements. Nevertheless, there have been several attempts to correlate structural elements with bitter taste to understand how taste perception works. According to Belitz and Wieser (1985) a bitter molecule needs a polar group and a hydrophobic moiety. However, the spatial distribution of the two structural features seems to be much more important as even a small structural change can cause dramatic differences in taste attributes (Ley, 2008).

In the last few years the problem of bitter-tasting food products has surfaced again due to the demand for healthier food and beverage products (Ley, 2008). Bitter taste is a major problem due to its negative hedonic impact on ingestion (Drewnoswki & Gomez-Carneros, 2000; Drewnoswki, 2001). Compounds such as certain polyphenols used for the fortification of functional food can cause serious taste deficiencies and reduced consumer demand for such products (Eckert & Riker, 2007). Bitter tasting compounds can be diminished, both in raw materials and finished products, by a variety of different methods such as breeding plants to obtain less bitter varieties, optimization of fermentation of protein-containing raw

materials and debittering of citrus by precipitation or enzymatic degradation of naringin (Saha & Hayashi, 2001). The addition of sugar or other sweeteners can be used to mask bitterness however this method fails for nonsweet applications (Cano *et al.,* 2000). The addition of proteins (e.g. milk) can also be used to debitter products such as coffee or tea but other taste and aroma qualities are altered significantly (Ley *et al.*, 2005).

There is no literature available with regards to bitter taste in honeybush infusions, however, according to E. Joubert (ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, 2010, personal communication) there appears to be a problem with a bitter taste in *C. genistoides.* This could possibly be due to the high concentration of polyphenolic compounds present in this particular *Cyclopia* species.

5.2.3. Sour

Sour taste is detected by the activation of ion channels that reside within the taste cell membrane (Herness & Gilbertson, 1999; Li *et al.,* 2002; Ugawa *et al.,* 2003; Brandbury, 2004; Worobey *et al.,* 2006). Sour taste appears to results from either the passage of H^+ ions through amiloride-sensitive Na^+ channels or from the blockade of K^+ channels, which are normally open at resting potential. Sour taste is usually caused by small, soluble, inorganic cations, however, it has been reported that certain phenolic acids have acidic or sour taste characteristics (Huang & Zayas, 1991; Peleg & Noble, 1995).

5.3. Mouthfeel

5.3.1. Astringency

Astringency can be described as a rough, dry, puckering feeling experienced after the consumption of certain types of fruits and beverages that are rich in plant-based polyphenols (Bate-Smith, 1954; Gawel, 1998; Scharbert *et al.,* 2004). Astringency, unlike the taste sensations, is not confined to a specific region of the mouth but has been defined as a diffuse surface phenomenon, characterized by a loss of lubrication which takes about 20 seconds to develop fully (Lee & Lawless, 1991; Gawell *et al.,* 2000). This loss of lubrication causes dryness of the oral surface and a tightening and puckering sensation of the mucosa and muscles around the mouth (Breslin *et al.,* 1993). Unlike taste the molecular and physiological mechanisms underlying astringency have not been definitively elucidated (Bajec & Pickering, 2008). It is far from clear whether astringency is best regarded as a single perceptual phenomenon or as a composite term encompassing a number of subtle tactile sensations. According to Bajec and Pickering (2008) most of the literature agrees with the hypothesis that astringency is a tactile stimulus arising from configurational changes of salivary proteins and their loss of lubricating properties.

5.3.2. Astringent compounds

There are four groups of true astringent compounds: salts of multivalent metallic cations, dehydrating agents, mineral and organic acids, and polyphenols (Joslyn & Goldstein, 1964). Tannins are the primary source of astringency in foods and beverages (Bate-Smith, 1954; Joslyn& Goldstein, 1964; Arnold *et al.,* 1980; Courregelongue *et al.,* 1999). Tannins can be categorized as either condensed (composed of

proanthocyanidins) or hydrolysable (composed of galloyl and hexahydroxyldiphenoyl esters) (Haslam & Lilley, 1988; Bennick, 2002). Traditionally astringent polyphenols have been defined as intermediately sized, having molecular weights of 500-3000 Da (Bakker, 1998; Lesschaeve & Noble, 2005), but smaller compounds, such as flavan-3-ol monomers, dimers and trimers, have also been shown to be able to elicit astringency (Nasih *et al.,* 1993; Peleg *et al., 1998).* It is generally accepted that the greater the degree of polymerization and molecular weight of an astringent compound the greater its ability to precipitate proteins (Bate-Smith, 1973) and its perceived intensity (Arnold *et al.,* 1980; Peleg & Noble, 1999). According to McManus *et al.* (1981) for a phenolic compound to elicit an astringent sensation it must possess two adjacent hydroxyl groups. It is thus quite possible that the soft astringent taste of honeybush could be due to the xanthones, mangiferin and isomangferin, and the flavanone eriocitrin. The tannin content of honeybush could also influence the astringency.

5.3.3. Polyphenol-protein binding

Astringency caused by polyphenolic compounds is due to the formation of salivary protein-polyphenol complexes (Monteleone *et al.*, 2004). The primary reaction leading to astringency is the aggregation and the subsequent precipitation of proteins and mucins by the cross-linkingsurface-exposed phenolic groups (Kallithraka *et al.,* 1998; Lu & Bennick, 1998; Charlton *et al.,* 2002). Saliva is produced by the salivary glands, consisting mainly of PRPs (Azen & Maeda, 1988). There are three groups of PRPs (basic, acidic and glycosylated). It is the basic PRPs which appear to be responsible for the complexation of polyphenols (Hagerman & Butler, 1981; Lu & Bennick, 1998). The way in which polyphenols bind to the PRPs can be divided into three stages (Fig. 4) (Charlton *et al.,* 2002; Jöbstl *et al.,* 2004). Firstly, the binding of multiple multidentate polyphenols to several sites on the protein causes the previously randomly coiled protein to coil around the polyphenol, making the protein more compact. Secondly, the polyphenol fractions of the protein-phenol complexes cross-link to polyphenol bridges and create protein dimers, and finally, the dimers aggregate to form large complexes and precipitate.

The protein-binding ability of polyphenols is well documented, and has been demonstrated with a variety of proteins besides salivary PRPs. These include casein (Luck *et al*., 1994; Jöbstl *et al*., 2004), gelatin (Oh *et al*., 1980; Hagerman & Butler, 1981; Yokotsuka & Singleton, 1995; Siebert *et al*., 1996; Edelmann & Lendl, 2002), bovine serum albumin (Hagerman & Butler, 1980a; 1980b; 1981), haemoaglobin (Bate-Smith, 1973), pectin (Hayashi *et al*., 2005), and HRPs (Nauratoet *al*., 1999; Yan & Bennick, 1995). More recently data have been presented indicating that mucins (Monteleone *et al.,* 2004; Condelli *et al.,* 2006), lactoferrin and α-amylase are also capable of polyphenol-binding (de Freitas & Mateus, 2001a; 2001b; Gambuti *et al.,* 2006), and that, along with the PRPs and HRPs, these proteins are involved with the sensation of astringency. The greater affinity of larger polymerized polyphenols for PRPs, and vice versa, has been attributed to the multidentate nature of polyphenols, which allows a single polyphenols to bind multiple residues of the protein (Baxter *et al.,* 1997; Charlton *et al.,* 2002; Jöbstlet *al.,* 2004).

In the case of hydrolysable tannins, the affinity of tannin-protein binding is directly related to the degree of galloylation (Baxter *et al.,* 1997; Charlton *et al.,* 2002). Protein-tannin complexes have been described as both soluble and insoluble, and recent data suggests that complex solubility is dependent on a number of variables. Hagerman and Robbins (1987) and Luck *et al*. (1994) showed that under optimal protein:polyphenol ratios and pH conditions, protein-polyphenol complexes are insoluble. However, in the

presence of excess protein the protein-polyphenol complexes are soluble as there is not enough tannin present to sufficiently crosslink proteins and form aggregates (Luck *et al.,* 1994). These findings suggest that the stability of polyphenol-protein complexes depends not only on the environmental conditions of the reaction (Hagerman & Robbins, 1987; Kawamoto & Nakatsubo, 1997), but also on the types of polyphenol and protein used.

Studies have confirmed that for condensed tannins, hydrogen bonding is the driving force of the interaction (Oh *et al*., 1980; Hagerman & Butler, 1980a, 1980b; 1981; Hagerman *et al*., 1998; Simon *et al*., 2003), but in some cases, it appears that hydrophobic interactions may be the basis for the complexation of tannins with protein (Luck *et al*., 1994; Baxter *et al*., 1997; Hagerman *et al*., 1998; Charlton *et al.,* 2002; Jöbstl *et a*l., 2004). Hagerman et al. (1998) suggest that polyphenol polarity is the main predictor of the type of association that will occur between polyphenols and proteins (i.e. hydrogen bond vs. hydrophobic interaction), with polar polyphenols forming hydrogen bonds and nonpolar polyphenols forming hydrophobic interactions.

5.4. Taste analysis

The oldest and most successful method used for taste analysis is by simply tasting the food product (Ley, 2008). In the past tea quality has been assessed by many different analytical tools, such as high-performance liquid chromatography (Valera *et al.,* 1996; Zuo et al., 2002), gas chromatography (Togari *et al.,* 1995), capillary electrophoresis (Horie *et al.,* 1997) and plasma atomic emission spectrometry (Herrador & Gonzalez, 2001). These methods, however, are time-consuming and the results are often inconsistent with sensory results, as tea quality is very complex (Chen *et al.,* 2008). More recently, electronic tongue technology, an application of multi-sensor systems which is applied particularly to analyse liquid phase foodstuffs, coupled with multivariate data analysis, has proved to be an extremely powerful analytical tool applied widely in foodstuffs and beverages (Hayashi *et al.,* 2007; Chen *et al.,* 2008). Electronic tongues have been used successfully for discrimination, classification, quality control and process monitoring. It is low cost, easy-to-handle measurement set-up and rapid compared to well-established analytical methods such as liquid chromatography and spectroscopy, but no sensors are available for some analytes and sometimes it is not sufficiently selective.

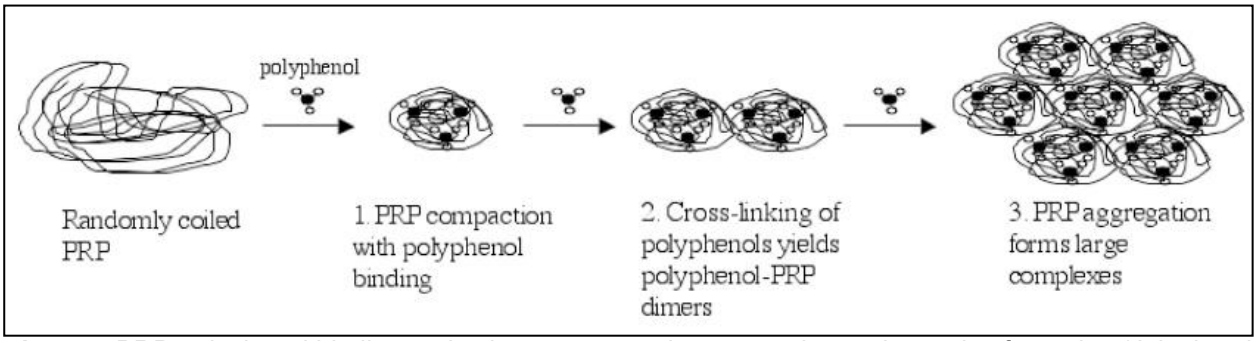


Figure 4 PRP-polyphenol binding and subsequent protein aggregation and complex formation (Jöbstl*et al.,* 2004)

6. AROMA

6.1. Odour perception

Odour perception takes place in the upper part of the nasal cavity located beneath the cribriform plate, known as the regio olfactoria (olfactory sensory epithelium) (Fig. 5; Rothe, 1988; Negoias *et al.*, 2008). This part of the nasal mucosal contains millions of receptors although it is only 10 cm^2 in area. There are three principle types of cells: 1) olfactory receptors, 2) sustentacular cells and 3) basal cells. Olfactory receptors detect, decode and transmit the sensory information about quality and intensity of odour whereas sustentacular cells add land-produced mucopolysaccharide to the mucus layer on the epithelial surface. On the other hand, basal cells seem to be stem cells becoming active in the course of normal cell turnover (Rothe, 1988).

There are two possible ways for odorous substances to reach the regio olfactoria; one way is with the breath of air steam via the nasal cavity (orthonasal olfaction) and the other is via the nasopharynx connecting the mouth with the nasal cavity during chewing and swallowing (retronasal olfaction) (Fig. 5) (Rothe, 1988; Worobey *et al.,* 2006). In both of these cases volatiles pass the regio olfactoria where after sorption in the mucus layer over the active surface the stimulus sets off an electrical signal within the smelling cells which are fitted with six to eight micro hairs per cell (Rothe, 1988). This signal is conducted via the cribriform plate to the bulbus olfactorius (olfactory bulb) in the front brain. The path of the information to the odour field of the brain, its registration, method of storage and the comparison with impressions received and stored in the brain earlier is unknown. Approximately 500-750 odour receptor genes have been identified in humans yet we are able to perceive thousands of individual odour molecules (Worobey *et al.,* 2006). It is thus believed that different combinations of receptors are employed in combination to encode a complex aroma.

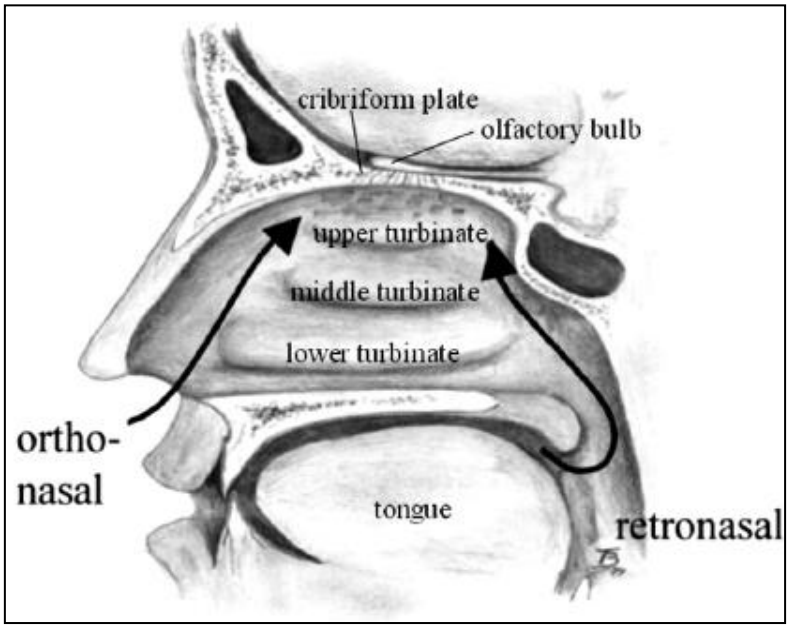


Figure 5 Schematic of orthonasal and retronasal olfaction (Negoias *et al.*, 2008).

6.2. Odour active compounds

The volatile fraction of food products consist of many compounds, however, only a small number of these compounds are of significance in determining the aroma (Grosch, 1993). A major task in flavour chemistry is thus to distinguish between the odour active compounds and the less odorous or odourless compounds present. Volatile organic compounds (VOCs) have different odour activities that can be ascribed to three important properties of a compound: 1) the absolute threshold, 2) the intensity of the compound as a function of its concentration (psychometric function) and 3) its quality (Delahunty *et al.,* 2006). Due to these different properties only a few compounds in a complex aroma mixture contributes to the overall aroma (Delahunty *et al.,* 2006). Additionally, aromas of different qualities can mask or suppress one another. Compounds with similar qualities can blend and produce a new aroma. Certain compounds present in concentrations below their odour threshold, or which has no odour activity when assessed individually, can contribute to the aroma when they are in a mixture (Delahunty *et al.,* 2006).

Gas-chromatography-olfactometry (GC-O) is used to determine the odour activity of volatile organic compounds in samples and to assign a relative importance to each of these compounds (Delahunty *et al.,* 2006). GC detector response cannot be used as an accurate representative of odour activity due to the large variation in odour thresholds and psychometric functions of odour-active compounds, however, human assessors can be trained to indicate for each compound eluting from the GC whether a odour is present, the duration of the odour activity and the quality and intensity of the perceived odour. Three types of GC-O techniques have been developed: 1) detection frequency (DF) (Linssen *et al.,* 1993; Pollien *et al.,* 1997; van Ruth, 2004); 2) dilution to threshold (Acree *et al.,* 1984; Ullrich & Grosch, 1987; van Ruth, 2004) and 3) perceived intensity (Miranda-Lopez *et al.,* 1992; Da Silva *et al.,* 1994; Guichard *et al.,* 1995; Étiévant *et al.,* 1999; Étiévant, 2002; van Ruth, 2004).

The DF method uses a group of assessors which are required to sniff the eluate and indicate when they detect an odorant (Pollien *et al.,* 1997; van Ruth, 2001; van Ruth & O'Connor, 2001). The compounds which are detected more frequently are concluded to have a greater relative importance and this is assumed to be related to actual odour intensity perceived at the concentration of the compound present in the sample (van Ruth & O'Connor, 2001). Flavour dilution (FD) factors serve to rank key aroma compounds in order of their potency (van Ruth & O'Connor, 2001).

In dilution analysis an extract is diluted and each dilution is sniffed until there are no longer any odours detected (van Ruth, 2004). The last dilution at which a compound is detected is a measure for its odour potency. Intensity methods measure the odour intensity of a compound in the GC effluent. Dilution threshold methods quantify the odour potency of a compound based upon the ratio of its concentration to its odour threshold in air (van Ruth, 2004). The significant contribution of each individual odorant to the overall aroma can be determined by calculating its odour activity value (OAV) or its relative flavour activity (RFA) (Grosch, 1994). The OAV is the ratio of the actual concentration of the compound in the sample to its odour threshold. Compounds with higher OAVs contribute more to the overall aroma (Guth & Grosch, 1999). Since the calculation of OAVs is difficult the determination of threshold values for compounds are problematic (Schieberle *et al.,* 1993). RFA can be calculated as an alternative parameter to OAV (Schieberle *et al.,* 1993).

6.3. Volatile compounds present in honeybush

The volatile fraction of unfermented and fermented *C. genistoides* has been studied in detail using headspace gas chromatography with mass spectrometry (HS-GC-MS) and GC-O detection by Cronje (2010). According to these studies the same volatile compounds were present in both fermented and unfermented honeybush but major qualitative differences were observed. These results are similar to those obtained for the effect of processing techniques on the volatile organic compounds present in black tea (*Camellia sinensis*) (Ravichandran & Parthiban, 1998).

Le Roux *et al.* (2008) reported that the volatile fraction of unfermented *C. genistoides* consisted mainly of saturated and unsaturated alcohols, aldehydes and methyl ketones whereas the volatile fraction of the fermented *C. genistoides* consisted mainly of terpenoids. The specific individual compounds identified in the volatile fraction of unfermented and fermented *C. genistoides* along with the aroma descriptors associated with these compounds are summarised in Table 5. The major constituent in unfermented plant material of *C. genistoides* is 6-methyl-5-hepten-2-one which can be described as an oily, green grass, herbaceous compound. Other compounds in relatively high concentrations are linalool, limonene, hexenal, α-terpineol, 3,5-octadien-2-one, geranyl acetone, β-cyclocitral, dihydroactinidiolide, geraniol and trans-furanoid linalool oxide. Linalool is the major constituent present in the aroma of fermented *C. genistoides* can be descibred as a refreshing, light, clean, floral compound. Other compounds present are α-terpineol, 6-methyl-5-hepten-2-one, geraniol, nerol, limonene, *trans*-furanoid linalool oxide, hexenal and *cis*-furanoid linalool oxide.

Table 5 Odour descriptions of volatile components in the aroma of unfermented and fermented *C. genistoides* compiled by Cronje (2010)

Compound	**Unfermented** Area %	**Fermented** Area %	**Aroma Descriptors**
Hexanal	**4.08**	1.76	Fatty, green grass
6-methyl-5-hepten-2-one	**54.07**	14.17	Oily, green grass, herbaceous
Limonene	**4.60**	3.15	Citrus, sweet, orange, lemon
3,5-octadien-2-one	**2.42**	0.50	-
trans-furanoid linalool oxide	0.93	**2.29**	Sweet-woody, floral-woody-earthy
cis-furanoid linalool oxide	0.81	**1.67**	Sweet-woody, floral-woody-earthy
6-methyl-3,5-heptadien-2-one	**1.43**	-	Warm spicy cinnamon-like
Linalool	10.68	**35.94**	Refreshing, light, clean, floral
α-terpineol	3.75	**17.30**	Fragrant, floral, sweet lilac
β-cyclocitral	**1.47**	0.25	Minty, fruity, green
Nerol	0.34	**3.49**	Sweet, floral
Geraniol	0.96	**10.80**	Sweet, floral, rose, fruity
Geranyl acetone	**2.33**	0.59	Floral, sweet-rosy, slightly green
Dihydroactinidiolide	**1.02**	0.16	Sweet, floral, tobacco

The values shown in bold are higher in either unfermented or fermented honeybush tea, respectively.

The aroma descriptors associated with the compounds present in higher concentrations in the unfermented honeybush are mainly citrus-like, herbaceous, camphoraceous and in some cases, green and woody whereas the aroma descriptors associated with the compounds present in higher concentrations in fermented honeybush are mostly sweet, floral, fruity, and in some cases, woody (Cronje, 2010). Thus the compounds can be divided into two distinct groups based on their aroma descriptors: "green" and "floral". The compounds associated with "green" tend to be higher in the unfermented honeybush volatile fraction whereas those compounds associated with the "floral" descriptors tend to be higher in the fermented volatile fraction.

Cronje (2010) also identified the volatile organic compounds present in unfermented and fermented *C. intermedia.* The eight most important odour active compounds identified in this species and the aromas associated with these compounds can be viewed in Table 6. The major constituent in unfermented plant material of *C. intermedia* is linalool. Other compounds in relatively high concentrations are geraniol, (*E*)-β-damascenone, α-terpineol, 6-methyl-5-hepten-2-one and terpinolene. In the fermented *C. intermedia* the concentration of terpinolene, (*E*)-β-damascenone and (*E*)-β-ionone has decreased whereas linalool, geraniol, α-terpineol and nerol increased dramatically. Additionally, the concentration of 6-methyl-5-hepten-2-one has also increased slightly. Linalool, geraniol, α-terpineol and nerol are therefore expected to contribute to the unique aroma of fermented honeybush whereas (*E*)-β-damascenone and terpinolene contribute to the aroma of unfermented *C. intermedia*.

Cronje (2010) identified an array of odour-active compounds present in *C. subternata*. The most intense odour active compounds identified can be viewed in Table 7. According to Cronje (2010) based on the DF method linalool, (*E,Z*)-2,6-nonadienal, *(E)*-2-nonenal and (*E*)-β-damascenone could be the most intense individual contributors to the unique aroma of *C. subternata*. Also, (*E*)-β-damascone, (*E*)-β-ionone, 3,4-dehydro-β-ionone and 2,3-dehydro-γ-ionone can also be considered as important contributors to the aroma of *C. subternata.*

Table 6 Most important odour active volatile organic compounds in unfermented and fermented *C. intermedia* identified by HS-GC-MS compiled by Cronje (2010)

Compound	**Unfermented Area %**	**Fermented Area %**	**Aroma Description**
6-Methyl-5-hepten-2-one	2.7494	**2.8289**	Oily-green, pungent-herbaceous, grassy, with fresh and green-fruity notes
Terpinolene	**2.702**	0.5617	Sweet-piney, oily
Linalool	13.158	**28.878**	Refreshing, floral-woody
α-Terpineol	4.1208	**8.7939**	Floral, sweet, lilac-type
Nerol	0.8906	**2.8329**	Fresh, sweet-rosy
Geraniol	6.7717	**13.8964**	Sweet, floral, rose
(*E*)-β-Damascenone	**4.4832**	1.0417	Woody, sweet, fruity, earthy, green-floral
(*E*)-β-ionone	**2.2513**	1.5161	Warm, woody, fruity, raspberry-like; resembles cedarwood

The values shown in bold are higher in either unfermented or fermented honeybush tea, respectively.

Table 7 Most intense odour-active compounds identified in fermented *C. subternata* aroma (Cronje, 2010)

Compound	Aroma Descriptors	% Area	DF (%)
(*E*)-β-Damascenone	Woody, sweet, fruity, earthy green-floral	0.607	100
Linalool	Refreshing, floral-woody	23.954	100
***(E,Z)*-2,6-Nonadienal**	Green-vegetable, cucumber, violet leaf	0.223	100
(*E*)-2-Nonenal	Green, cucumber, aldehydic, fatty	0.128	100
(E)-β-damascone	Fruity (apple-citrus), tea-like, minty	0.607	93
Geraniol	Sweet, floral, rose, citrus-like	25.344	93
(E)-β-Ionone	Warm, woody, fruity, raspberry-like	3.061	87
Component C178	Not available	0.061	60
3,4-Dehydro-β-ionone	Ionone-damascone, saffron-like, fruity, leathery	0.104	87
2,3-Dehydro-γ-ionone	Not available	0.247	87
(7E)-Megastigma-5,7,9-trien-4-one	Tea-like, spicy and resembling dried fruit	0.002	60
epi-α-Cadinol	Herbaceous, woody	0.061	60
epi-α-Muurolol	Herbaceous, slightly spicy	0.034	60
10-epi-γ-Eudesmol	Woody, floral, sweet	0.117	40
(E,E)-2,4-Decadienal	Fried, waxy, fatty, orange-like	0.035	33

The most intense contributors to aroma of honeybush are indicated in bold. DF = Detection frequency

Cronje (2010) also compared four *Cyclopia* species with two species originating of different areas [*C. genistoides* (Albertinia), *C. genistoides* (Pearly Beach), *C. intermedia, C. longifolia, C. subternata* (Bredasdorp) and *C. subternata* (Genadendal)]. Based on the results obtained, Cronje (2010) concluded that the four species are qualitatively very similar but there are important quantitative differences between the samples (Table 8). Quantitative differences between species from different areas also existed.

Linalool occured in relative same concentrations in all six samples analysed, with the highest concentration in both the *C. genistoides* samples (Cronje, 2010). (*E,Z*)-2,6-nonadienal and (E)-nonenal were present in smaller quantities in both the *C. genistoides* samples and the latter is also lower in *C. intermedia*. The concentration of geraniol was relatively low in the *C. subternata* (Genadendal) sample whereas (*E,E*)-2,4-decadienal was present in much larger quantities than in the other five samples. Component C178 was especially prominent in *C. genistoides* (Albertinia), *C. intermedia* as well as *C. subternata* (Genadendal). (*E*)-β-damascenone were more prominent in *C. genistoides* (Pearly beach) whereas (*E*)-β-damascone was more prominent in *C. intermedia*. 2,3-dehydro-γ-ione was present in higher concentrations in *C. subternata* (Bredasdorp), *C. genistoides* (Pearly beach)and *C. longifolia*. *C. subternata* (Bredasdorp), *C. subternata* (Genadendal) and *C. longifolia* had higher relative quantities of *(E)-β-ionone* whereas 10-*epi*-γ-eudesmol was prominent in *C. intermedia*. *Epi*-α-cardinoland *epi*-α-muurolol was present in more or less the same relative concentrations in most of the samples, but markedly less in *C. genistoides* (Albertinia). It is thus stands to reason that each of these *Cyclopia* samples have slightly different aromas as their volatile fraction consists of different concentrations of specific volatile compounds.

Table 8 Comparison of the relative concentrations (% Area) of the most intense odour active compounds identified in six *Cyclopia* species (Cronje, 2010)

Compound	**Aroma descriptor**	***C. genistoides***		***C. intermedia***	***C. longifolia***	***C. subternata***	
		Albertinia	**Pearly beach**			**Bredasdorp**	**Genadendal**
Linalool	Refreshing, light, clean, floral	29.38	**31.7**	28.88	19.67	23.95	17.41
(*E,Z*)-2,6-Nonadienal	Green-vegetable, cucumber, violet-leaf	0.07	0.11	0.12	**0.22**	**0.22**	0.17
(*E*)-2-Nonenal	Green, cucumber, aldehydic, fatty	0.05	0.07	0.11	**0.12**	**0.13**	0.09
Geraniol	Sweet, floral, rose	12.43	22.5	13.9	**27.61**	25.34	5.1
Component C178	Not available	0.37	0.08	**0.42**	0.09	0.06	**0.42**
(E)-β-Damascenone	Woody, sweet, fruity, earthy, green-floral	0.67	**1.37**	1.04	0.72	0.61	0.5
(E)-β-Damascone	Fruity (apple-citrus), tea-like, minty notes	0.24	0.4	**0.74**	0.48	0.25	0.45
2,3-Dehydro-γ-ionone	Not available	0.04	0.2	0.09	**0.3**	0.25	0.11
3,4-Dehydro-β-ionone	Ionone-damascone, saffron-like, fruity, leathery	0.16	0.04	0.13	0.12	0.1	**0.46**
(E)-β-Ionone	Warm, woody, fruity, raspberry-like	1.43	0.84	1.52	2.5	**3.06**	2.99
10-epi-γ-Eudesmol	Woody, floral, sweet	0.06	0.02	**0.59**	0.1	0.12	0.22
epi-α-Cadinol	Herbaceous, woody	0.01	**0.078**	0.063	0.061	0.061	0.064
epi-α-Muurolol	Herbaceous, slightly spicy	0.007	**0.045**	0.043	0.029	0.034	0.034
(7E)-Megastigma-5,7,9-trien-4-one	Tea-like, spicy, dried fruit	0.0011	0.0017	<0.001	<0.001	**0.0018**	0.0014

The highest value for each compound is indicated in bold.

6.4. Aroma of honeybush tea

Cronje (2010) investigated eight different honeybush samples [*C. genistoides* (Albertinia), *C. genistoides* (Pearly Beach), *C. genistoides x C. intermedia*, *C. intermedia*, *C. longifolia, C. subternata* (Bredasdorp), *C. subternata* (Genadendal, flowers absent) and *C. subternata* (Genadendal, flowers present)] in terms of their sensory aroma attributes. Quantitative descriptive analysis was used to evaluate the eight samples with regards to honeybush, sweet, plantlike, rooibos and lemon, as well as Earl Grey (bergamot) aroma. All the samples had a moderately strong honeybush and sweet aroma.

Cyclopia longifolia was found to have the strongest honeybush aroma whereas *C. subternata* (Genadendal, flowers absent) had the sweetest aroma (Cronje, 2010). Additionally, Cronje (2010) reported that during training the judges indicated that *C. subternata* (Genadendal, with flowers) had a prominent pot-pourri aroma. The plantlike, rooibos, lemon and Earl Grey aromas were more subtle and were not equally strongly associated with the samples. *Cyclopia genistoides* (Pearly Beach) as well as *C. subternata* (Bredasdorp) had a plantlike aroma. *Cyclopia subternata* (Bredasdorp), as well as *C. longifolia* had a slight rooibos aroma, whereas *C. genistoides* (Pearly Beach), *C. genistoides* (Albertinia) and *C. subternata* (Bredasdorp) had a slight lemon aroma. Only *C. genisoitdes* (Albertinia) had a notable Earl Grey aroma.

Multivariate statistical analysis was used to highlight patterns and correlations and revealed interesting correlation between certain compounds and sensory aroma attributes (Cronje, 2010). (*E)*-β-damascenone and linalool were significantly negatively correlated to sweet aroma whereas 3,4-dehydro-β-ionone and component C178were significantly positively correlated to sweet aroma. (*E*)-β-ionone correlated significantly to honeybush aroma whereas geraniol, 2,3-dehydro-γ-ionone, (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal correlated significantly to rooibos aroma. (*7E*)-Megastigma-5,7,9-trine-4-one postiviely correlated to plant aroma whereas 10-*epi*-γ-eudesmol correlated negatively to plant aroma. Additionally, (*E)*-β-damascone and (*E,E*)-2,4-decadienal correlated negatively to lemon aroma and *epi*-α-cardinol and *epi*-α-muurolol negatively correlated to Earl Grey aroma.

Cronje (2010) concluded that honeybush and sweet aroma could be classified as generic aromas (i.e. typical of all honeybush species) whereas the other four aromas (Earl Grey, lemon, plantlike and rooibos) can be classified as species-specific aromas. It was also suggested that further research to profile the sensory attributes of each *Cyclopia* species is needed in order to develop a valid flavour lexicon for honeybush tea.

6.5. Aroma analysis

Sensory analysis is considered to be the ultimate method to measure aroma of food products as chemical and instrumental procedures lack the acuity of the human senses and the ability to integrate perceptions (Aparicio *et al.,* 1996). However, sensory analysis with a human panel can be inaccurate, laborious and very time consuming due to adaptation, fatigue, infection and state of mind (Duta *et al.,* 2003).

An electronic nose (EN) can be a better alternative to conventional methods for tea tasting and quality monitoring during production process. EN systems are based on inexpensive, non-specific solid-state sensors, which are sensitive to gasses that are emitted by tea samples. Furthermore, once an EN has been “trained”, it does not require a skilled operator and can potentially obtain the results in under a minute. Duta *et al.* (2003) have successfully used a metal oxide sensor (MOS) based EN to analyse tea samples

which were manufactured under different processing conditions. The MOS-EN is capable of discriminating between the different flavours of different fermented teas (over-fermented, over-fired, under-fermented etc.).

Bhattacharyya *et al.* (2007) proposed a new electronic nose-based approach for monitoring tea aroma during the fermentation process. As part of this study 81 tea fermentation cycles have been experimented with and most of the EN readings accurately matched with colometric as well as human panel data. Bhattacharyya *et al. (*2007) concluded that an EN can definitely be used for monitoring of volatile emission patterns during black tea fermentation processes with a very high degree of accuracy, reliability and repeatability. Yu & Wang (2007) used an EN to classify LongJing green-tea into different quality grades and 90% of all the tea samples analysed were classified correctly.

7. FLAVOUR

Flavour is one of the most important qualities of foodstuffs and plays a major role in consumer acceptance (Aparicio *et al.,* 2006). It is a complex combination of gustation (sense of taste by the tongue), olfaction (sense of smell by the nose) and trigeminal (sense of irritation) sensations which may additionally be influenced by tactile, thermal, painful and/or kinaesthetic effects (Dutta *et al.,* 2003; Tournier *et al.*, 2007). Non-volatile compounds are detected by taste whereas volatile compounds are detected by the sense of smell (Dutta *et al.,* 2003). The sense of olfaction plays the most important role in the overall flavour perception.The exact mechanism of flavour perception have not yet been elucidated due to a number of reasons: 1) flavour perception involves a wide range of stimuli; 2) chemical compounds and food structures that activate the flavour sensors change as the food is eaten; and 3) the individual modalities interact in a very complex way (Taylor & Roberts, 2004).

There is not much literature available describing the flavour of honeybush tea. In most research articles honeybush is described as having a “characteristic honeybush” flavour (Du Toit & Joubert, 1998a). According to Du Toit and Joubert (1998b) the attributes considered to be important for this characteristic flavour of honeybush is a sweet taste and aroma combined with a mild astringent taste. Additionally terms such as flowery, fruity, honeylike, grassy and musty has been used to describe the flavour of honeybush tea. However to date, no extensive research has been done on the flavour of honeybush tea nor has the differences in terms of flavour of the different species used for processing been described.

8. SENSORY ANALYSIS

Sensory analysis can be defined as a scientific discipline used to evoke, measure, analyse and interpret reactions to characteristics of foods and beverages as they are perceived by the senses (Stone & Sidel, 1993). Sensory evaluation is generally considered to be the ultimate method to measure flavour quality of food products as chemical and instrumental procedures lack the acuity of the human senses and the ability to integrate perceptions (Aparicio *et al.,* 1996).

8.1. Sensory lexicons

A sensory lexicon is simply a set of terms used to describe the aroma, flavour, taste and/or mouthfeel of a specific product (Drake & Civille, 2002). Such a lexicon usually also includes reference standards and

definitions for each term for clarification. They are used as a tool for documenting and describing sensory perception of a selected food product. The major steps in setting up a sensory lexicon include collecting a product frame of reference, generating appropriate descriptors, reviewing reference standards and examples, and developing a final list of descriptors. Once a sensory lexicon is developed it can be used to record and define product flavour and to compare products as well as interface with consumer liking and acceptability as well as chemical flavour data.

In order to generate a reliable sensory lexicon several aspects should be taken into account: attribute intensities must be anchored consistently; terms must be precise, clear and appropriately defined; reference standards provided; and the terms must be discriminating, descriptive, relevant and non-redundant (Meilgaard *et al.,* 1999; Drake & Civille, 2002).

A variety of different sensory lexicons for different products have already been developed with the use of quantitative descriptive analysis (Table 9). Sensory lexicons developed for tea is limited to canned teas (Cho *et al.,* 2005), green tea (Lee & Chambers, 2007; Lee *et al.,* 2008) and more recently rooibos tea (*Aspalathus linearis*) (Koch, 2011). No attempt has yet been made to set up a sensory lexicon for honeybush tea.

Table 9 Examples of sensory lexicons developed for various food and beverage products

Product	**Reference**
Wine	Noble *et al.,* 1987; Gawel *et al.,* 2001; Mirarefi *et al.,* 2004; Preston *et al.,* 2008
Whiskey	Lee *et al.,* 2001
Distilled beverages	Mc Donnell *et al*., 2001
Cheese	Bárcenas *et al.,* 1999; Murray & Delahunty, 2000; Drake *et al.,* 2001; Rétiveau *et al*., 2005;
Bread	Lotong *et al.*, 2000
Tea	Lee & Chambers, 2007; Lee *et al.,* 2008; Hongsoongnern & Chambers IV, 2007; Koch, 2011 Cho *et al.,* 2005; Yau & Huang, 2000
Yerba Mate(South America beverage)	Santa Cruz *et al.,* 2002
Tomatoes	Hongsoongnern & Chambers IV, 2008
Yoghurt	Coggins *et al.,* 2008
Coffee	Seo *et al*., 2009
Almonds	Civille *et al.,* 2010
Honey	Galán-Soldevilla *et al*., 2005; Lorente *et al.,* 2008
Puree	Duffin & Pomper, 2006
Fruit	Wismer *et al*., 2005; Le Moigne *et al*., 2008

8.2. Sensory wheels

Standardized industry-specific terminology is usually presented in a wheel format with certain descriptors coupled to reference standards (Piggot & Jardine, 1979; Meilgaard, 1982; Noble *et al.,* 1987; Piggot & Mowat, 1991). In essence, a sensory wheel is thus a simplified graphical representation of a sensory lexicon. A sensory wheel can consists of only flavour, aroma or mouthfeel attributes or a combination of these attributes. A sensory wheel usually consists of two levels: 1) general/basic terms situated near the centre and 2) more specific descriptive attributes situated on the outer ring of the circle (Lawless & Heymann 2010). Additionally, the descriptors are often divided into two categories; positive and negative (Jolly & Hattingh, 2001; Koch, 2011).

Sensory wheels are used extensively in both the wine (Noble et al., 1987; Gawel et al., 2000) and the beer (Meilgaard et al., 1979) industry is to evaluate aroma, flavour, taste and mouthfeel and to facilitate communication among the different role players in the respective industries and the consumer (Noble *et al.,* 1987). After the success of the so-called “Wine Aroma Wheel” a sensory wheel focusing only on mouthfeel sensations associated with red wine has even been developed in order to accurately define and describe mouthfeel (Fig. 6) (Gawel et al., 2000). Additionally, a number of articles describing the development of sensory wheels for a wide variety of different food products, such as fish, fruit, whiskey, brandy and honey, have been published (Table 10).

In terms of tea, a flavour wheel for black tea (Fig. 7) and rooibos tea (Fig. 8) has been developed for use by processors, graders, extract producers and flavour companies (Bhuyan & Borah, 2001; Koch, 2011). The black tea sensory wheel consist of 22 terms describing the odour, taste and mouthfeel of black tea and are divided into eight categories: 1) Aromatic, fragrant and sweet; 2) Overfired; 3) Poor; 4) Baggy, papery and smokey, 5) Sour; 6) Mouthfeel; 7) Metallic; and 8) Fullness. Each of these categories are used to group together specific groups of adjectives describing the specific odour, taste and mouthfeel of black tea (Bhuyan & Borah, 2001). On the other hand, the rooibos tea sensory wheel is divided into two groups: positive (13 terms) and negative (14 terms) to describe the sensory attributes associated with rooibos tea (Koch, 2011). The sensory wheel contains terms describing the flavour, taste and mouthfeel of rooibos tea and can be grouped into 11 categories: 1) Sweet; 2) Fruity; 3) Woody; 4) Floral; 5) Spicy; 6) Mouthfeel; 7) Basic; 8) Vegetative; 9) Chemical; 10) Micro; and 11) Earthy.

The honeybush sensory wheel could be used as an aid during honeybush tea evaluation and for comparing and monitoring the quality and consistency of honeybush tea as well as profiling new and competitive products within the tea industry. Furthermore, the honeybush sensory wheel together with the reference standards will enable effective training of those involved in the honeybush tea industry. At the same time it would be a very useful tool to use for communication between researchers, the industry, marketing personnel and the consumer.

Table 10 Examples of sensory wheels developed for various food and beverage products

Product	Reference
Fish	Warm *et al*., 2000
Kiwifruit	Wismer *et al.*, 2005
Pawpaw fruit puree	Duffin & Pomper, 2006
Whiskey	Lee *et al.,* 2001
Wine	Gawel *et al.,* 2000
	Mirarefi *et al*., 2004
	Noble *et al*., 1984
Brandy	Jolly & Hattingh, 2001
Beer	Meilgaard *et al*., 1979
Honey	Piana *et al*., 2004
Tea	Bhuyan & Borah, 2001
	Koch, 2011

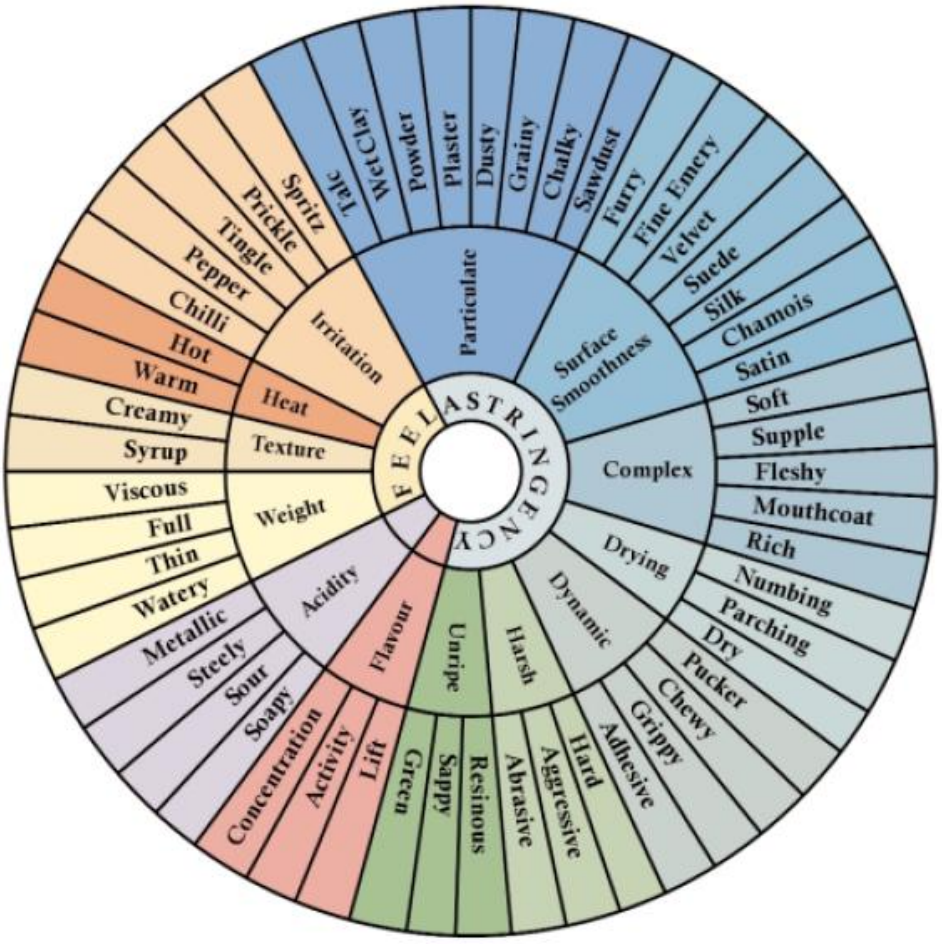


Figure 6 A mouthfeel wheel to describe the mouthfeel characteristics of red wine (Gawel et al., 2000)

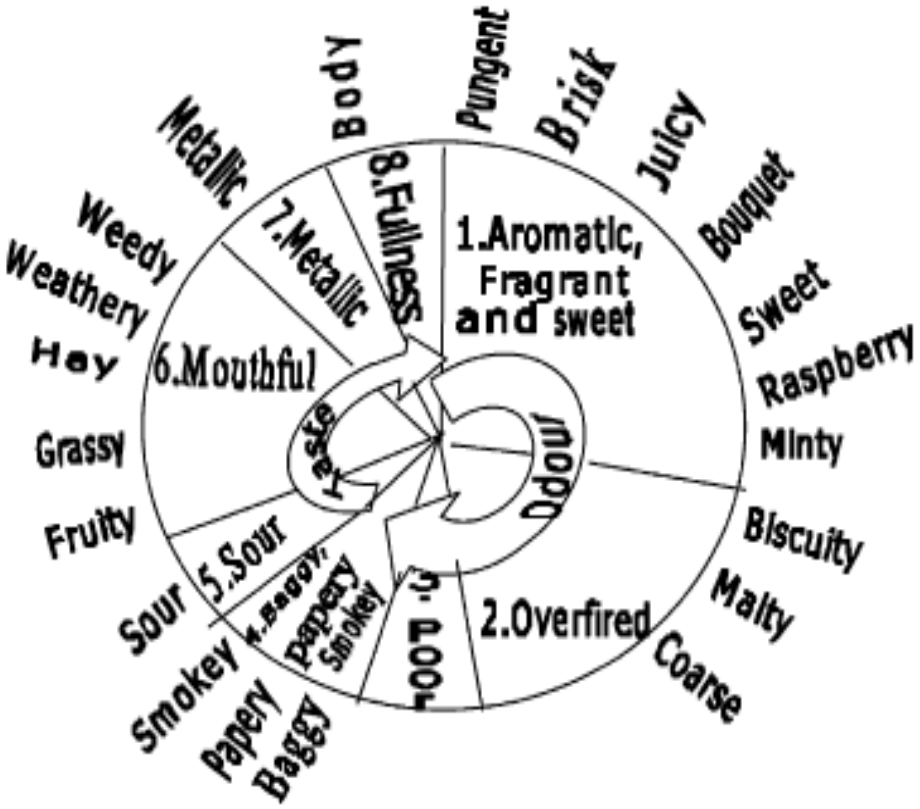


Figure 7 Flavour wheel used for black tea (Bhuyan & Borah, 2001)

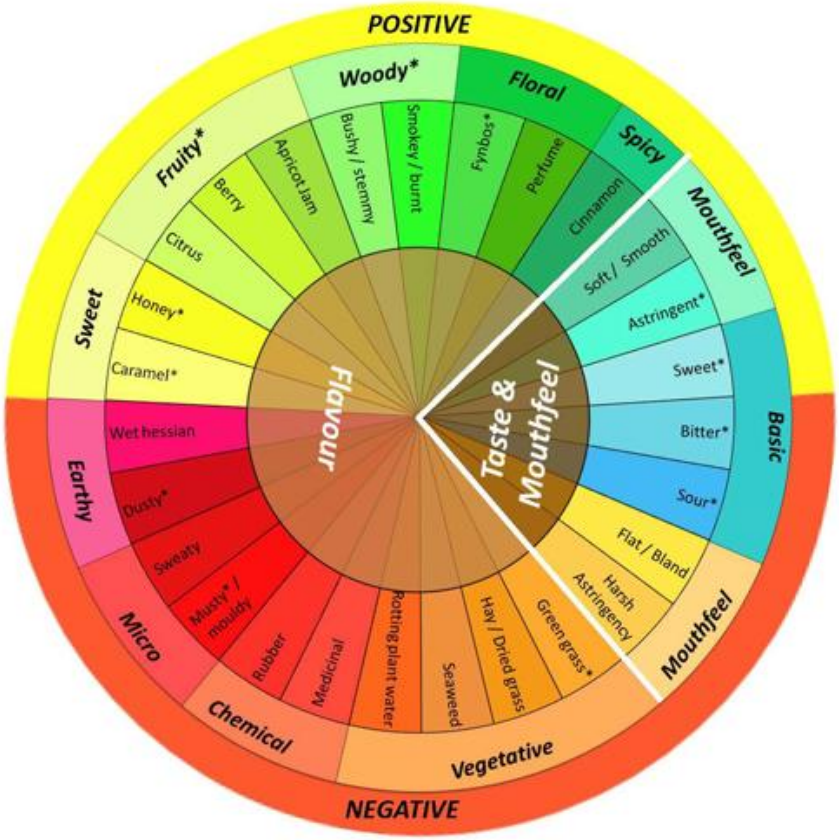


Figure 8 Flavour wheel developed for rooibos tea (Koch, 2011)

9. CONCLUSION

Honeybush tea is a traditional South African beverage which is caffeine-free, has a low tannin content and contains a rage of polyphenolic compounds responsible for the health benefits associated with it. It is also these compounds that might be responsible for the taste and mouthfeel characteristics of honeybush tea. It has been shown that there are both quantitative and qualitative differences in the polyphenolic composition as well as the volatile fraction of the different *Cyclopia* species. It is thus expected that the species will have different sensory profiles.

Despite the recent growth in the demand for honeybush tea the descriptors used to describe the flavour and taste of honeybush infusions is limited to only a handful of descriptors. According to regulations with regards to the export of honeybush tea, it must have the clean characteristic taste and aroma of honeybush and that it shall be free from any foreign flavour and odours which detrimentally effect the characteristics of the product. However, the characteristics taste and aroma is not defined, nor what is considered to be foreign and detrimental to the taste and aroma of the product. Variation in sensory quality due to differences in localities, environmental conditions, processing parameters and the inherentspecies differences are also not taken into account. Consequently, the lack of standardised terminology with which to describe the characteristic honeybush flavour, as well as the fact that no information is available with regards to the differences between different *Cyclopia* species indicates that there is considerable variation in the sensory profiles of teas currently being sold in the market place as honeybush tea. This could lead to detrimental consequences as there is no way to ensure a consistent supply of high quality tea with unchanging flavour profiles without this information.

A honeybush flavour lexicon and wheel would be tools that could aid in implementing a honeybush grading system. Flavour lexicons and wheels have been implemented by a number of industries and have been reported to increase communication between the different role players. A flavour lexicon is a set of descriptors coupled with definitions and reference standards whereas a flavour wheel is simple graphical representation of the flavour lexicon. Due to the simple and convenient nature of the flavour wheel it may prove useful to honeybush processors, extract manufactures and flavour companies.

One of the most influential factors with regards to the flavour of honeybush tea is the oxidative chemical reaction referred to as fermentation. It is this process which is responsible for the development of the sweet honeylike flavour of honeybush tea. However, the conditions currently employed by the industry is based on research done on *C. intermedia* and might thus not apply to all *Cyclopia* species. It is thus necessary to re-examine the temperature-time fermentation combinations in order to determine the optimum fermentation conditions for each species.

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Chapter 3

SENSORY PROFILING OF *CYCLOPIA* SPECIES (HONEYBUSH TEA) AND THE DEVELOPMENT OF A HONEYBUSH SENSORY WHEEL AND LEXICON

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1. ABSTRACT

Honeybush samples produced from six *Cyclopia* species (*C. sessiliflora, C. longifolia, C. genistoides, C. intermedia, C. subternata* and *C. maculata*) and representative of different geographic areas were analysed using descriptive analysis in order to develop a sensory profile for honeybush tea. A total of 68 aroma and 51 flavour, taste and mouthfeel descriptors were generated. It was found that the "characteristic" sensory profile of honeybush tea can be described as a combination of floral, sweet, fruity and plantlike flavours with a sweet taste and a slightly astringent mouthfeel. Similarities and differences in the sensory characteristics between the different *Cyclopia* species were also established using univariate analysis, principle component analysis and discriminant analysis. Based on discriminant analysis the species could be divided into three distinct groups; group A (*C. sessiliflora, C. intermedia* and *C. genistoides)*, group B (*C. longifolia* and *C. subternata)* and group C (*C. maculata*). Group A associated with fynbos floral, fynbos sweet and plantlike attributes, group B associated with rose geranium and fruity sweet attributes and group C associated with woody, boiled syrup and cassia/cinnamon attributes. Gas chromatography-olfactometry analysis of the *C. maculata* (*C. maculata* sample no. 3) aroma fraction indicated that its spicy aroma could possibly be explained by the high concentration of the volatile component, eugenol, which is known to have a warm-spicy, dry aroma. The variation in the sensory attributes within a specific species, especially in terms of the negative sensory attributes, seems to be due to different processing conditions rather than being species specific, however, further investigation is needed to verify this. Additionally, a honeybush sensory lexicon was created by selecting 32 attributes along with a definition and reference standard for each term. A honeybush sensory wheel, comprising of 35 attributes, was also created to form a simple graphical representation of the sensory lexicon.

2. INTRODUCTION

The honeybush industry has grown from 50 to 200 export per annum tons in a period of ten years and today, the tea is exported to 25 countries with the top importers being the Netherlands, Germany, the United Kingdom (UK) and the United States of America (USA) (Joubert *et al.,* 2011). Until recently, exports consisted mainly of *C. intermedia* (as well as *C. genistoides* and *C. subternata*), however, with demand exceeding supply interest in other species, i.e. *C. sessiliflora, C. longifolia* and *C. maculata*, developed. The rapid growth of the industry is expected to continue as honeybush has huge potential in the herbal tea market, as it is unique to South Africa, can be grown organically and can be used in value-added food products, medicinal products and cosmetics (Joubert *et al.,* 2011).

Despite the positive momentum of the industry internationally it is only during the last few years that honeybush tea has appeared on local supermarket shelves (Joubert *et al.,* 2011). Products such as mixtures of honeybush and rooibos (*Asphalathus linearis*) and other indigenous South African plants, as well as ice teas are now also available (Joubert *et al.,* 2008). Although certain brands, mostly those found in specialty shops or up-market farm stalls consists of only one *Cyclopia* species, most of the honeybush products are a mixture of two or more *Cyclopia* species. Blending different *Cyclopia* species, without taking into account their different flavours, could lead to teas in the market place with inconsistent flavour profiles. Blending could also lead to a loss of the unique flavour associated with the individual species which could

possibly, in future, be used to establish niche markets. In order to do this the distinctive flavour profile of each species needs to be described.

Companies often make use of sensory lexicons and/or sensory wheels to describe the sensory attributes of food and beverage products (Drake & Civille, 2002). These tools are employed to standardize the terminology used to discuss the sensory properties of a certain product and have been reported to facillitate communication between different role players in the industry. To date no sensory descriptors, except for sweet and honeylike, have been established to describe the flavour of honeybush tea. In general the terms "characteristic" honeybush, sweet and honeylike are used to describe the flavour of honeybush tea (Du Toit & Joubert, 1999). Other descriptors, such as flowery and fruity (fermented) and grassy (un-/under-fermented) as well as burnt (over-fermented), have also been used to describe honeybush (Du Toit & Joubert, 1998; Du Toit & Joubert, 1999; Le Roux *et al.,* 2008; Cronje, 2010). Honeybush tea must have a clean characteristic taste and aroma of honeybush and that it shall be free from any foreign flavour and odours which detrimentally affect the characteristics of the product according to the Agricultural Product Standards Act for the export of honeybush tea (Anon, 2000). However, the "characteristic" flavour is not defined nor what is considered to be foreign and detrimental to the flavour of the product. The different localities, environmental conditions, processing parameters and the inherent species differences and its effect on the sensory quality of honeybush tea are not taken into account. Consequently, the lack of standardised terminology with which to describe flavour of honeybush tea, as well as the fact that no information is available with regards to the differences between different *Cyclopia* species indicates that there is considerable variation in the sensory profile of commercial honeybush tea.

In view of these commercial challenges, the study was conducted to characterise the sensory attributes associated with honeybush in order to properly define and describe the flavour instead of referring to a non-specific term such as "characteristic" honeybush flavour. By analysing the sensory characteristics of a broad range of honeybush samples from six *Cyclopia* species the characteristic honeybush flavour and the variation between the six species were established. Additionally, the odour active compounds present in the aroma of a representative *C. maculata* sample were determined in order to gain a better understanding of the spicy character this specific *Cyclopia* species revealed. The sensory attributes associated with honeybush tea were used to create a sensory lexicon and a sensory wheel to facilitate improved communication between producers, processors, researchers and marketers regarding the flavour of honeybush tea.

3. MATERIALS AND METHODS

3.1. Honeybush samples

A total of 58 honeybush tea samples, representing six *Cyclopia* species, i.e. *C. sessiliflora, C. longifolia, C. genistoides, C. intermedia, C. subternata* and *C. maculata,* were collected. Each of the samples of a species differed either in terms of the batch, geographical area, producer, harvesting date, processing conditions or size fraction to capture as much potential sensory variation as possible. Batches represented either different bushes and/or independently processed pooled plant material. *Cyclopia sessiliflora* and *C. longifolia* consisted of seven samples each, whereas the other species consisted of eleven samples each (Table 1).

Table 1 Illustration of the experimental design

	Samples					
Rep	*C. sessiliflora*	*C. longifolia*	*C. genistoides*	*C. intermedia*	*C. subternata*	*C. maculata*
1	Ses 1	Lon 1	Gen 1	Int 1	Sub 1	Mac 1
2	Ses 2	Lon 2	Gen 2	Int 2	Sub 2	Mac 2
3	Ses 3	Lon 3	Gen 3	Int 3	Sub 3	Mac 3
4	Ses 4	Lon 4	Gen 4	Int 4	Sub 4	Mac 4
5	Ses 5	Lon 5	Gen 5	Int 5	Sub 5	Mac 5
6	Ses 6	Lon 6	Gen 6	Int 6	Sub 6	Mac 6
7	Ses 7	Lon 7	Gen 7	Int 7	Sub 7	Mac 7
8			Gen 8	Int 8	Sub 8	Mac 8
9			Gen 9	Int 9	Sub 9	Mac 9
10			Gen 10	Int 10	Sub 10	Mac 10
11			Gen 11	Int 11	Sub 11	Mac 11

Rep = Replication, Ses = *C. sessiliflora,* Lon = *C. longifolia,* Gen = *C. genistoides,* Int = *C. intermedia,* Sub = *C. subternata,* Mac = *C. maculata.*

The complete list of samples can be found in Addendum A. Statistically, the six *Cyclopia* species each represented a treatment whereas each sample of a species was considered an independent replication.

3.1.1. Preparation of fermented material

Samples not obtained from commercial processors were processed on laboratory scale. Different batches were harvested from different locations in the Western Cape Province of South Africa during 2010 and 2011. The shoots from each batch (±13 kg) were cut to 2-3 mm lengths using a mechanised fodder cutter, divided into four equal parts (1.5 kg each) and placed into stainless-steel containers. Deionised water (250 mL) was added to the shredded plant material and thoroughly mixed before sealing the containers with aluminium foil. The plant material was fermented at either 80°C for 24 h or 90°C for 16 h in preheated temperature-controlled laboratory ovens (CAL 3200; CAL Controls Ltd., UK). The containers were removed and dried to a moisture content below 10% by thinly spreading the contents onto 30 mesh stainless steel drying racks and placing it in a temperature-controlled dehydration tunnel (Continental Fan Works, Parow, South Africa) at 40°C for 6 h. The dried tea was sieved (200 g/30 s) using a mini-sifter and the <12>40 mesh fraction collected. The fractions were stored in sealed glass jars at room temperature until needed.

3.2. Sample preparation

3.2.1. Preparation of infusion

Boiled (100°C) distilled water (900 g) was poured onto 11.25 g dry plant material and left to infuse for a period of 5 min where after it was poured through a fine-mesh aluminium tea strainer into a 1 L stainless steel thermos flask (Woolworths, South Africa). The herbal infusion (ca. 100 mL) was served in white porcelain mugs covered with a plastic lid to prevent evaporation and the consequent loss of volatiles.

3.2.2. Temperature maintenance

In order to keep the temperature of the tea as constant as possible to ensure effective sensory analysis a number of measures were taken as proposed by Koch (2011). Firstly, the thermos flasks were filled with boiling water to heat the inner surface, secondly, the tea mugs were preheated in an industrial forced-convection oven (Hobart CSD 1012) set to 70°C and thirdly, the infusions were kept warm in a scientific waterbath (SMC, Cape Town) with the temperature regulator set to 65°C throughout the sensory analysis process.

3.3. Descriptive analysis

3.3.1. Assessors

A total of nine female assessors were selected to participate in this study. The assessors were selected based on both availability and interest. All of the assessors who took part in this study had extensive experience with sensory analysis of a wide range of different food products. Although none of the assessors had any previous experience with honeybush tea, most of the judges had extensive experience in rooibos tea analysis.

3.3.2. Training

The sensory panel was trained according to the consensus method as described by Lawless and Heymann (2010). Before analysing the samples, the panel was given a short summary of the objectives of the study, as well as instructions on the analysis procedure. The aroma of the tea samples was analysed first by removing the plastic lid and swirling the sample cup several times. Thereafter, the flavour, taste and mouthfeel of the tea were evaluated by sucking, and not sipping, a mouthful of the tea infusion from a round tablespoon. Additionally, the assessors were requested to cleanse their palates between each sample using water and unsalted water biscuits (Carr, UK). A total of 24 one-hour sessions were used for training the assessors. During each one-hour session 4 to 6 of the 58 honeybush samples were analysed and the panel generated aroma, flavour, taste and mouthfeel descriptors. Aroma was defined as the fragrance perceived through orthonasal analysis, flavour as the retronasal perception within the mouth and taste describes the basic taste modalities whereas mouthfeel described the tactile sensation that occurred in the oral cavity after drinking a sip of tea (Ross, 2009).

Descriptors which best described the aroma, flavour, taste and mouthfeel of the samples were generated during an open discussion led by the panel leader. Relationships and redundancies among the descriptors were discussed in order to select the most recurring sensory attributes. At this point, a reference standard for each attribute was introduced to the panel to clarify the meaning of each descriptor. In each session the panel was also given a honeybush sample (*C. intermedia*) as a control sample.

A total of 68 aroma and 51 flavour, taste and mouthfeel descriptors were generated during the training period. A complete list of these descriptors can be viewed in Addendum B. This list was reduced to a total of 28 aroma, 23 flavour and 3 taste descriptors and 1 mouthfeel descriptor based on whether they were relevant, unambiguous and non-redundant (Table 2). The maximum and minimum intensity values for

each attribute were discussed and compared to the attribute intensity values that had been established for the control honeybush sample. Those attributes which were not present in the control sample were compared to samples with the highest intensity value for the specific attribute. A score sheet containing the final list of descriptors together with a 10 cm unstructured line scale, as well as the maximum intensity values for each attribute, was developed (Addendum C). This score sheet was used during the last few training sessions to familiarise the panel with the layout and procedure.

Table 2 Final aroma, flavour, taste and mouthfeel attributes used for descriptive analysis

Aroma attributes	**Descriptors**
Floral	Fynbos floral, Rose geranium, Rose/Perfume
Fruity	Lemon, Orange, Cooked apple, Apricot jam, Cherry
Plantlike	Plantlike, Woody, Rooibos, Pine
Sweet	Fruity sweet, Boiled syrup, Caramel, Honey, Fynbos sweet
Spicy	Cassia/Cinnamon
Nutty	Walnut, Coconut
Negative	Dusty, Yeasty, Medicinal, Burnt caramel, Rotting plant water, Hay/Dried grass, Green grass, Cooked vegetables

Flavour, taste and mouthfeel attributes	**Descriptors**
Taste	Sweet, Sour, Bitter
Mouthfeel	Astringent
Floral	Fynbos floral, Rose geranium, Rose/Perfume
Fruity	Lemon, Orange, Cooked apple, Apricot jam, Cherry
Plantlike	Plantlike, Woody, Rooibos, Pine
Spicy	Cassia/Cinnamon
Nutty	Walnut, Coconut
Negative	Dusty, Yeasty, Medicinal, Burnt caramel, Rotting plant water, Hay/Dried grass, Green grass, Cooked vegetables

3.3.3. Scaling

After the training was completed the assessors were requested to use the score sheets to rate the intensities of the 28 aroma, 23 flavour and 3 taste attributes, as well as 1 mouthfeel attribute for each of the 58 samples during 9 sessions spread over a period of two weeks. Each sample was analysed only once as a sample within a species represents a replication. Two sessions were conducted per day during which 8 to 12 samples were analysed. The assessors were requested to take a 10 min break between each session to avoid panel fatigue. Samples were labelled with three-digit codes and presented to each assessor in a randomized order. The control honeybush sample was labelled as such so that it could be identified by the assessors and used for comparison.

3.4. Gas chromatography-olfactometry

Sample preparation and gas chromatography-olfactometry (GC-O) were carried out as described by Cronje (2010). An infusion of *C. maculata* (Mac3, Addendum A) was prepared by adding boiling distilled water (130 mL) to 20 g of the dry leaves and stems (<12>40 mesh sieved fraction) in an insulated flask, sealing it immediately and allowing the tea to infuse for 15 min while swirling the contents of the flask periodically after which the leaves and stems were removed by filtering. For each analysis 50 mL of the infusion was transferred to a 100 mL glass bottle with adapted cap, sealed and incubated at 50°C for 30 min, after which the headspace volatiles of the infusion were enriched by means of a SEP60 at 50°C for 17 h.

GC-O was performed on a Carlo Erba HR gas chromatograph with a split/splitless injector and an FID operated at 220°C and 250°C, respectively. The capillary column was connected to a glass effluent splitter with two deactivated fused silica tubing outlets of equal lengths conducting the column effluent to the FID and a sample collection device. Medical air was purified by passing it through a column of activated charcoal and humidified by bubbling the air at about 20 mL/min through a wash bottle containing clean deionised water at room temperature. The humidified air conduit was connected to the leg of a small funnel that was formed using a glass blower's torch in such a way as to allow the assessor to smell the column effluent without breathing in too much of the ambient air. One of the fused silica aromas of the effluent splitter was inserted through a small hole into the leg of the funnel, mounted on the side of the GC in such a position and at such a height as to subject the assessor to as little physical strain as possible.

The sorbed volatiles were thermally desorbed from the SEP at an injector temperature of 230°C without cryotrapping and were analysed on a capillary column (glass, 40 m x 0.25 mm) coated with 0.25 µm of PS-0890OH (DB-5 equivalent) using a temperature program of 2°C/min from 40°C to 280°C. Helium was used as carrier gas at a linear flow velocity of 28.6 cm/s, measured at an oven temperature of 40°C. The injector was operated in the split mode with a split flow of 10 mL/min.

Members of a panel of 8 volunteer assessors were required to sniff the GC effluent and results were reported according to the detection frequency method. In order to prevent sensory fatigue each assessor was required to sniff the effluent for 35 min after which a second person took over and sniffed for the remaining 35 min of the analysis. Each person participated in the sniffing of both the first and the second 35 min session during two consecutive analyses. Assessors verbally announced when they were able to smell a compound as it eluted from the GC and each positive response was marked on the chromatogram at the corresponding retention time. The total number of panel members able to positively detect an aroma at a specific retention time was expressed as a percentage of the total number of assessors. A compound was considered to be aroma active if it was positively detected by at least 50% of the assessors (Áslaug & Rouseff, 2003).

3.5. Development of a sensory wheel and lexicon

A honeybush tea sensory lexicon was created by selecting 32 flavour, taste and mouthfeel attributes based on whether they were relevant, unambiguous, non-redundant and non-hedonic along with a definition. By trial and error a number of reference standards were developed and included in the sensory lexicon. Additionally, a honeybush tea sensory wheel was created as a simple graphical representation of the

sensory lexicon consisting of the same 32 attributes along with three additional mouthfeel descriptors (Harsh, Flat/Bland and Soft/Smooth).

3.6. Data analysis

The data for each individual sensory attribute rated for each sample by all assessors were collected and analysed using various statistical techniques. *PanelCheck*Software (Version 1.4.0, Nofima Mat, Norway) was used to monitor the performance of the panel. Panel reliability was determined by subjecting the data to test-retest analysis of variance (ANOVA) using SAS® software (Version 9.2, SAS Institute Inc, Carry, USA). The Shapiro-Wilk test was used to test for non-normality of the residuals (Shapiro & Wilk, 1965). In the event of significant non-normality ($p \leq 0.05$) outliers were identified and removed until the data were normally distributed. The student t-least significant difference (LSD) was calculated at the 5% significance level to compare treatment means.

Principal component analysis (PCA) using the correlation matrix was conducted using XLStat (Version 7.5.2, Addinsoft, New York, USA) and used to visualize the relationship between the samples and their attributes. Discriminant analysis (DA) was used to differentiate between the six *Cyclopia* species. In order to profile the *Cyclopia* species more effectively the data were split into two subsets, i.e. positive attributes and negative attributes.

4. RESULTS AND DISCUSSION

4.1. Sensory attributes

According to Vannier *et al.* (1999) efficient sensory profiling requires the reduction of the number of sensory terms to about 10 to 20 sensory descriptors, however, Stone & Sidel (1985) and Wolters (1994) warned against the dangers of working with a strongly reduced set of descriptors. According to these authors sensations for which no adequate descriptors are available are ascribed to different existing descriptors by different assessors, thus not only losing their specific information but also making the existing descriptors unclear and ambiguous. For these reasons, the list of 119 descriptors generated during training was reduced to 28 aroma, 23 flavour and 3 taste attributes, as well as 1 mouthfeel attribute by grouping together similar terms. This eliminated redundancies and disregarded those attributes that were perceived in only a limited number of samples to facilitate efficient sensory profiling.

To investigate the relative importance of the major sensory attributes, graphs of the percentage of samples that exhibit each attribute plotted against its average intensity and graphs that show the average intensity range of each individual attributes were used. These graphs gave an indication of the perceived intensity of the sensory attributes in an infusion, as well as their prevalence amongst the samples. The aroma attributes, fynbos sweet, fynbos floral and woody, obtained the highest scores, followed by fruity sweet, apricot jam and plantlike. These six aroma attributes were perceived in at least 80% of the honeybush tea samples (Fig. 1). However, considering the large average intensity ratings for cassia/cinnamon aroma (higher than 50 out of a 100), this attribute should also be considered important (Fig. 2). Rose geranium, fynbos sweet, fynbos floral, fruity sweet, plantlike and apricot jam aroma also obtained a relatively high average intensity scores with a maximum score of more than 25 out of 100. In terms of the

negative aroma attributes, only hay/dried grass aroma was perceived in more than 50% of the samples and the highest maximum average intensity was 33 out of 100 for both hay/dried grass and burnt caramel.

The flavour attributes, fynbos floral, woody and plantlike, obtained the highest scores (Fig. 1). The flavour attributes followed a very similar pattern to that of the aroma attributes, however, they had lower maximum intensity scores. Cassia/cinnamon flavour obtained the largest range of average intensity scores, from 0 to more than 30 out of 100 (Fig. 2). Rose geranium, rose/perfume, woody and plantlike flavours obtained a relatively large average intensity scores with a maximum score of more than 20 out of 100. In terms of the negative flavour attributes, only hay/dried grass flavour was perceived in more than 50% of the samples and none of the negative flavour attributes obtained a maximum average intensity score of more than 20 out of 100, with hay/dried grass and burnt caramel having the highest maximum intensity scores.

Sweet taste and astringency were perceived in all 58 of the honeybush tea samples and these are the only two attributes, except for fynbos floral flavour, which obtained a minimum intensity score of more than zero. Astringency had a very low maximum intensity score whereas sweet taste had a relatively high maximum intensity score. This indicated that astringency was present in all samples but at very low intensities and that all samples were considered sweet, although, some less than others. Bitter and sour taste were detected in more than 90% of the honeybush tea samples, however, at low levels. Bitter taste had a slightly higher maximum intensity score compared to sour taste.

Even though the rest of the sensory attributes occurred in less than 80% of the samples, and at lower average intensities, this does not necessarily mean that they should be disregarded. These attributes are those which may be more species-specific. Also, attributes rated low in intensity can still contribute significantly to the aroma or flavour of the tea, especially the negative sensory attributes. Conversely, those attributes, for example cassia/cinnamon, which had extremely high maximum intensity scores, but were only present in a relatively small number of samples, cannot be seen as “characteristic” of honeybush tea.

It can thus be concluded that the “characteristic” sensory profile of honeybush tea can be described as a combination of floral, sweet, fruity and plantlike flavours with a sweet taste and a slight astringent mouthfeel. The most detrimental negative attributes appeared to be hay/dried grass and burnt caramel aroma and flavour.

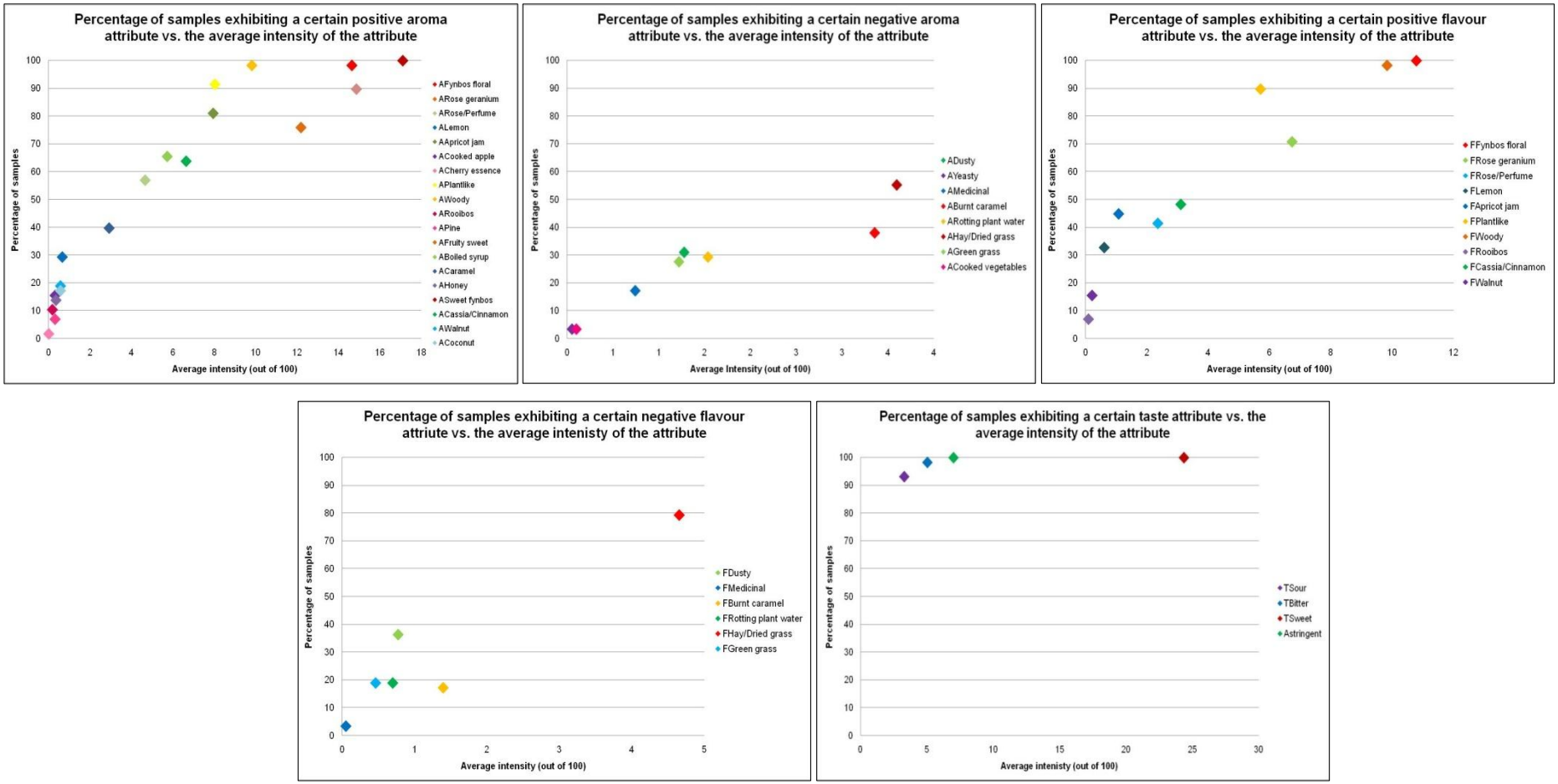

Figure 1 Scatter plots showing the percentage of samples exhibiting a certain attribute vs. the average intensity of the specific attribute. “A”, “F” and “T” in front of an attribute refer to aroma, flavour and taste attributes, respectively.

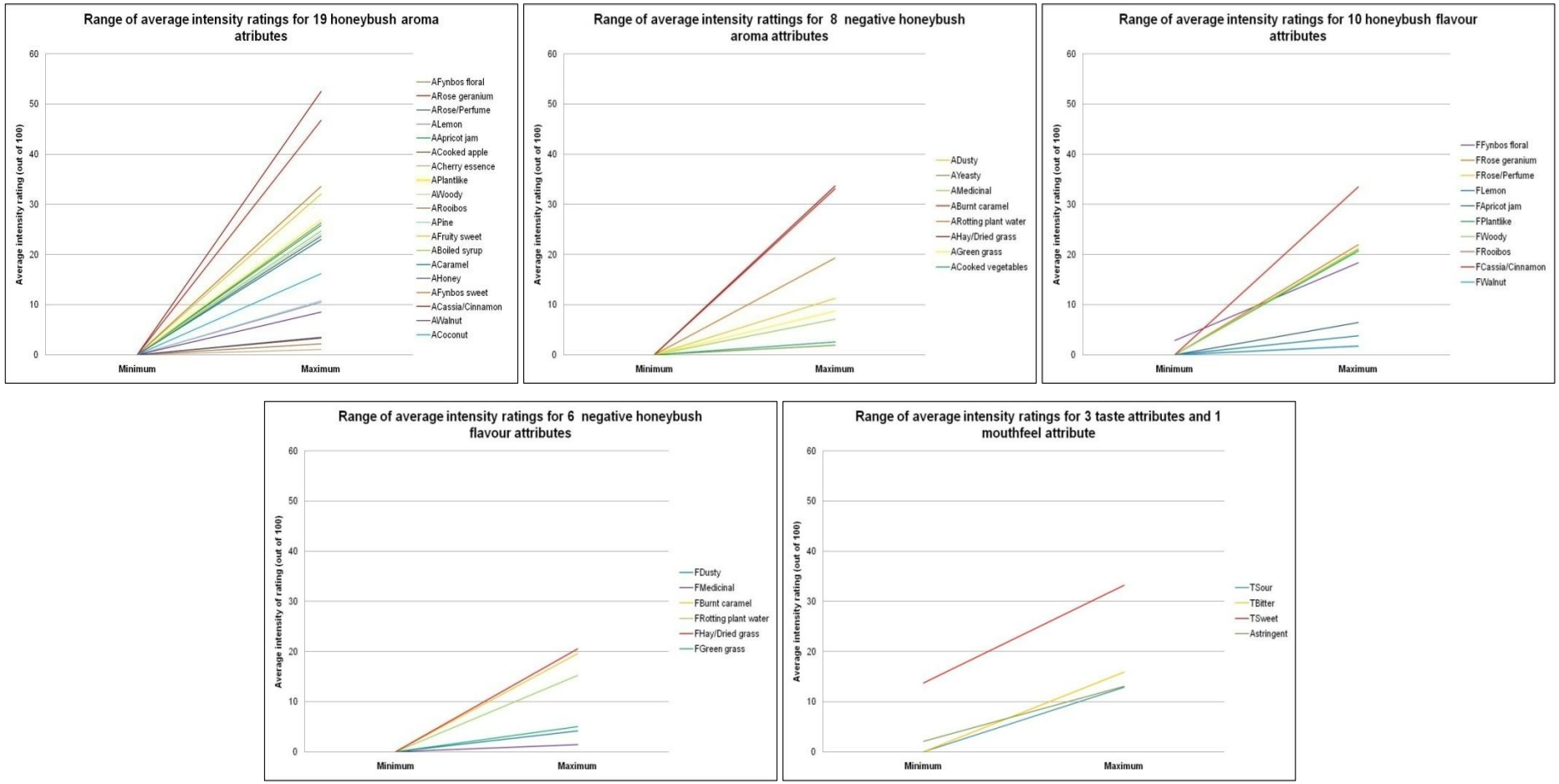

Figure 2 Minimum and maximum average intensity ratings (averages over 9 judges and 58 samples) for each attribute. “A”, “F” and “T” in front of an attribute refer to aroma, flavour and taste attributes, respectively.

4.2. Relationship between sensory attributes

PCA plots are commonly used to display the relationship between sensory attributes and individual samples or to indicate whether certain sensory attributes are redundant and may be reduced to a simplified set of terms to prevent different attributes from being used to describe the identical sensory characteristic. PCA plots are also used to demonstrate whether correlations exist between an aroma attribute analysed by nose (orthonasal) and flavour attribute analysed by mouth (retronasal).

The PCA loadings plot (Fig. 3a) displays the positioning of and association between the sensory attributes associated with the honeybush samples analysed. Most of the negative attributes are located on the right side of the plot whereas the positive sensory attributes are located mostly on the left side of the PCA loadings plot. However, a few positive sensory attributes, such as cassia/cinnamon, woody, coconut and boiled syrup, are located at the top on the right hand side of the plot. The corresponding scores plot (Fig. 3b) reflects the positioning of the 58 honeybush tea samples analysed relative to each other. It is important to remember that although certain sensory attributes might seem to be highly correlated on a PCA loadings plot this is not always the case. It is possible that attribute groupings may arise from a general tendency of certain attributes to change in a similar way over a large group of samples (Wolters & Alchurch, 1994; Talavera-Bianchi *et al.,* 2010). It is for this very reason that it is often useful to also examine the correlation coefficients.

4.3.1. Positive sensory attributes

Based on the PCA loadings plot (Fig. 3a) most of the orthonasal (ON) and retronasal (RN) attributes are closely associated with one another indicating that these notes are perceived similarly on the nose, as well as in the mouth, but there are a few attributes (lemon, plantlike and woody) which lie further apart. Based on the correlation coefficients (Table 4), there were significant correlations ($p \leq 0.05$) and strong positive correlations ($r > 0.7$) between the aroma and flavour attributes for rose geranium ($r = 0.864$), rose/perfume ($r = 0.720$) and cassia/cinnamon ($r = 0.951$). The correlation coefficients for fynbos floral ($r = 0.535$), lemon ($r = 0.392$), apricot jam ($r = 0.365$), plantlike ($r = 0.547$), woody ($r = 0.60$) and walnut ($r = 0.432$) aromas and flavours were much weaker but they are nevertheless significant ($p \leq 0.05$). The correlation coefficients thus show that there was in fact a significant correlation between lemon, plantlike and woody aroma and flavour. However, although rooibos woody aroma and flavour lie close to each other on the PCA plot there was in fact no significant correlation between them ($r = 0.173$). The reason for this is not clear.

The mechanism of perception of aromas and flavours is different; aromas are perceived through the nose whereas flavours are detected in the mouth by transportation of the stimulus from the back of the throat up to the olfactory receptors in the nasal cavity (Ross, 2009). Because of the different mechanisms the sensory perception of certain attributes perceived by RN and ON analysis may differ. This has been shown by Aubrey *et al.* (1999) who examined the differences between the sensory profiles of wines by ON and RN evaluation. The results for ON and RN analysis were correlated for most of the descriptors, however, a few attributes, notably fruity notes, seemed to be more effectively evaluated by the nose. Similar results were obtained during this study as the fruity notes present in honeybush tea, namely lemon and apricot jam aroma, were not perceived very strongly by RN analysis.

In terms of the relationship between the aroma and flavour attributes and the taste and mouthfeel attributes (Table 5, Table 6) there were quite a few significant correlations, however, none of them were very strong ($r > 0.7$). The correlation coefficient between lemon aroma and sour taste ($r = 0.440$) and plantlike aroma and sour taste (0.447) indicate that there is a significant correlation ($p \leq 0.05$) between these two aroma attributes and sour taste. Similarly, there was a significant positive correlation ($p \leq 0.05$) and a significant negative correlation between plantlike flavour and sour ($r = 0.420$) and sweet ($r = -0.447$) taste, respectively. Although, sour and bitter taste, as well as astringency are often considered as negative sensory attributes these attributes were considered as positive sensory attributes during this study as there was very little variation between the samples with regards to these attributes. Also, the attribute intensities of these attributes were at levels contributing positively to the flavour and mouthfeel of the samples. It was only in a few samples that the intensities were high enough to be considered as negative.

4.3.2. Negative sensory attributes

Based on the PCA loadings plot (Fig. 3a) most of the ON and RN negative attributes are closely associated with one another indicating that these notes are perceived similarly and equally strong on the nose, as well as on the tongue. However, there are two attributes (dusty and medicinal) which lie further apart than the others on the PCA loadings plot. Based on the correlation coefficients (Table 7) there was a significant ($p \leq 0.05$) and strong ($r > 0.7$) positive correlation between burnt caramel ($r = 0.869$) and rotting plant water ($r = 0.752$) aroma and flavour. Additionally, there was a significant ($p \leq 0.05$) and moderate ($r > 0.5$) correlation between yeasty ($r = 0.599$), hay/dried grass ($r = 0.323$) and green grass ($r = 0.453$) aroma and flavour. As indicated by the PCA loadings plot no significant correlation existed between the aroma and flavour attributes for medicinal ($r = 0.155$) and dusty ($r = 0.025$). On the other hand, cooked vegetables aroma and flavour, were close together on the PCA loadings plot, however, based on the correlations coefficient ($r = -0.030$) there was in fact no significant and notable relationship between them.

In terms of the relationship between the negative aroma and flavour attributes and the taste and mouthfeel attributes (Table 8) there were a number of significant correlations ($p \leq 0.05$) but none of them were very strong. Burnt caramel, rotting plant water and cooked vegetables attributes appeared to associate with sour, bitter and astringency. Additionally, these negative aroma attributes revealed a negative correlation with sweet taste. Hongsoongnern and Chambers (2008) found that certain attributes such as musty/earthy, astringent and bitter were intrinsically associated with the green character of many products. Similarly, there seems to be a distinct association between plantlike or green attributes (rotting plant water and cooked vegetables) and negative taste (sour and bitter) and mouthfeel (astringent) attributes. Additionally, there also seem to be an association between burnt caramel and these negative taste and mouthfeel attributes.

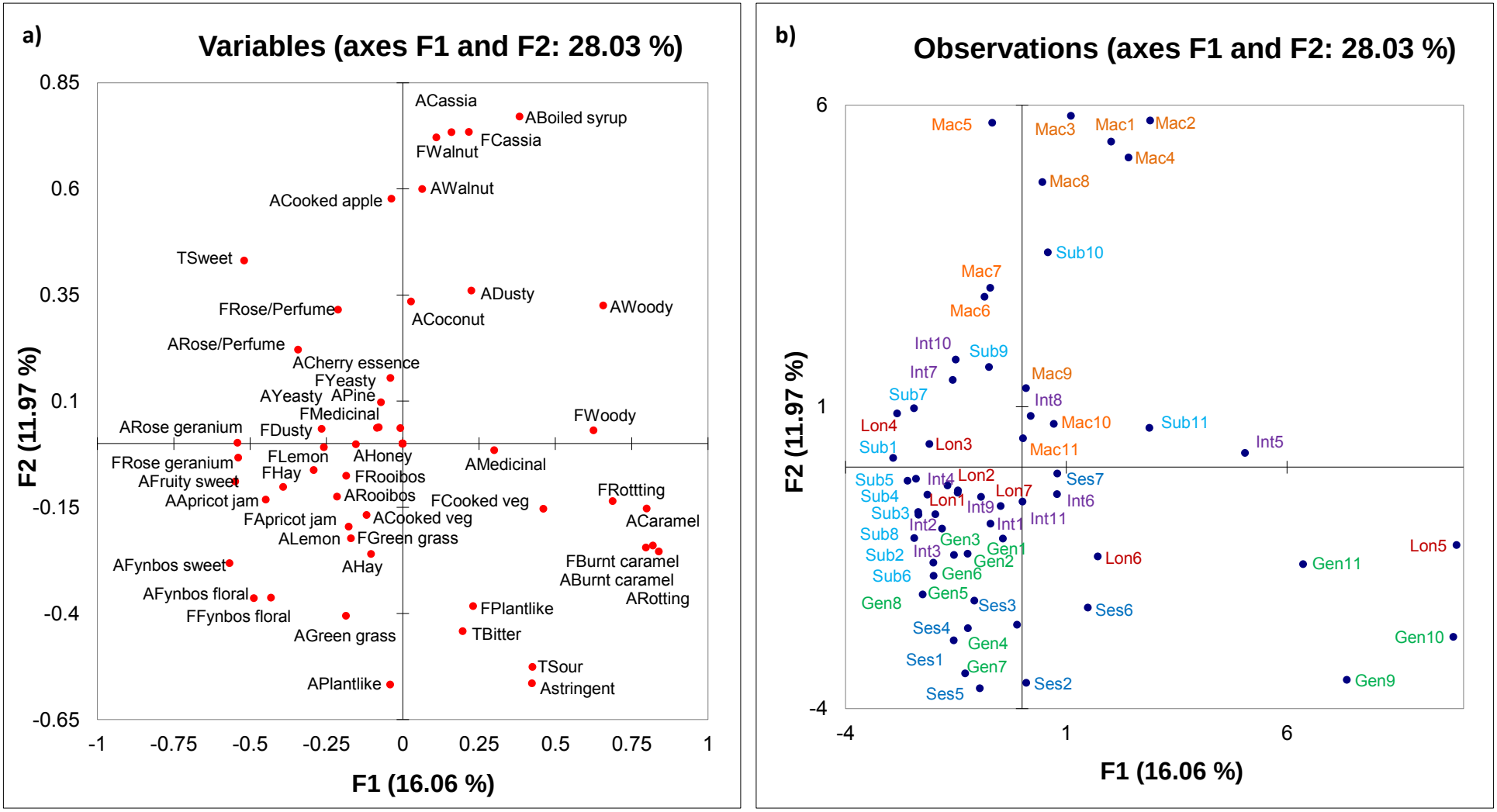

Figure 3 a) PCA loadings plot showing the positioning of both positive and negative sensory attributes. The letters "A", "F" and "T" in front of the attributes refer to aroma, flavour and taste attributes, respectively. Cassia = Cassia/Cinnamon, Rotting = Rotting plant water, Hay = Hay/Dried grass, Cooked veg = Cooked vegetables. b) PCA scores plot showing the positioning of the 58 honeybush tea samples. The abbreviations Ses, Lon, Gen, Int, Sub and Mac in the scores plot refer to the specific *Cyclopia* species; *C. sessiliflora, C. longifolia, C. genistoides, C. intermedia, C. subternata* and *C. maculata*, respectively.

Table 4 Pearson's correlation coefficients (r) illustrating the relationship between aroma and flavour attributes

Variables	AFynbos floral	ARose geranium	ARose/ Perfume	ALemon	AApricot jam	APlantlike	AWoody	ARooibos	ACassia/ Cinnamon	AWalnut
FFynbos floral	**0.535**									
FRose geranium		**0.864**								
FRose/Perfume			**0.720**							
FLemon				**0.392**						
FApricotjam					**0.365**					
FPlantlike						**0.547**				
FWoody							**0.604**			
FRooibos								0.173		
FCassia/Cinnamon									**0.951**	
FWalnut										**0.432**

Positive significant correlations ($p < 0.05$) are indicated in purple. Correlations above 0.7 are indicated in red. The letters "A" and "F" before the attribute descriptors refer to aroma and flavour attributes, respectively.

Table 5 Pearson's correlation coefficients (r) illustrating the relationship between aroma and taste and mouthfeel attributes

Variables	AFynbos floral	ARose geranium	ARose/ Perfume	ALemon	AApricot jam	ACooked apple	ACherry essence	APlantlike	AWoody	ARooibos
TSour	0.001	-0.217	**-0.382**	**0.440**	-0.250	-0.209	-0.118	**0.447**	0.030	-0.121
TBitter	-0.027	-0.023	0.129	0.016	0.210	-0.219	-0.002	0.078	0.076	0.108
Astringent	0.062	-0.207	**-0.305**	0.076	-0.208	**-0.282**	0.028	0.216	0.205	-0.110
TSweet	0.139	**0.297**	**0.344**	-0.038	0.189	0.188	0.256	-0.196	-0.216	0.049

Variables	APine	AFruity sweet	ABoiled syrup	ACaramel	AHoney	AFynbos sweet	ACassia/ Cinnamon	AWalnut	ACoconut
TSour	0.015	**-0.312**	-0.226	**0.325**	**-0.262**	-0.061	-0.106	-0.224	-0.243
TBitter	-0.112	0.148	-0.143	0.206	0.122	-0.047	**-0.374**	**-0.321**	-0.138
Astringent	-0.001	-0.149	-0.216	**0.330**	**-0.286**	0.002	-0.234	-0.217	-0.147
TSweet	0.008	**0.325**	0.112	**-0.322**	0.110	0.161	0.037	0.256	0.182

Positive significant correlations ($p < 0.05$) are indicated in purple, whereas negative significant correlations ($p < 0.05$) are indicated in green. The letters "A" and "T" before the attribute descriptors refer to aroma and taste attributes.

Table 6 Pearson's correlation coefficients (r) illustrating the relationship between flavour and taste and mouthfeel attributes

Variables	FFynbos floral	FRose geranium	FRose/ Perfume	FLemon	FApricot jam	FPlantlike	FWoody	FRooibos	FCassia	FWalnut
TSour	0.005	**-0.259**	**-0.287**	0.138	-0.157	**0.420**	0.256	-0.172	-0.125	-0.220
TBitter	-0.124	-0.022	0.088	0.068	0.063	0.081	0.125	-0.098	**-0.349**	-0.241
Astringent	0.133	-0.199	-0.238	-0.112	0.007	**0.344**	0.255	-0.150	-0.222	**-0.373**
TSweet	0.217	0.241	**0.325**	0.059	**0.287**	**-0.447**	-0.113	-0.050	0.072	0.176

Positive significant correlations ($p < 0.05$) are indicated in purple, whereas negative significant correlations ($p < 0.05$) are indicated in green. The letters "F" and "T" before the attribute descriptors refer to flavour and taste attributes.

Table 7 Pearson's correlation coefficients (r) illustrating the relationship between negative aroma and flavour attributes

Variables	ADusty	AYeasty	AMedicinal	ABurnt caramel	ARotting plant water	AHay/Dried grass	AGreen grass	ACooked vegetables
FDusty	0.025							
FYeasty		**0.599**						
FMedicinal			0.155					
FBurnt caramel				**0.869**				
FRottting plant water					**0.752**			
FHay/Dried grass						**0.323**		
FGreen grass							**0.453**	
FCooked vegetables								-0.030

Positive significant correlations ($p < 0.05$) are indicated in purple. Correlations above 0.7 are indicated in red. The letters "F" and "A" before the attribute descriptors refer to aroma and flavour attributes.

Table 8 Pearson's correlation coefficients (r) illustrating the relationship between negative aroma or flavour attributes and taste and mouthfeel attributes

Variables	ADusty	AYeasty	AMedicinal	ABurnt caramel	ARotting plant water	AHay/Dried grass	AGreen grass	ACooked vegetables
TSour	-0.013	-0.189	0.001	**0.365**	**0.362**	0.170	0.235	0.040
TBitter	-0.149	-0.161	0.103	**0.371**	0.237	0.104	-0.013	-0.068
Astringent	-0.050	0.017	0.173	**0.434**	**0.382**	-0.044	0.220	0.050
TSweet	-0.040	0.216	0.026	**-0.393**	**-0.419**	-0.125	-0.030	-0.029
Variables	**FDusty**	**FYeasty**	**FMedicinal**	**FBurnt caramel**	**FRottting plant water**	**FHay/Dried grass**	**FGreen grass**	**FCooked vegetables**
TSour	-0.060	-0.169	-0.156	**0.345**	**0.342**	-0.218	-0.004	0.110
TBitter	-0.071	-0.124	-0.116	**0.300**	0.118	0.196	-0.034	**0.277**
Astringent	-0.107	-0.119	0.025	**0.473**	0.166	0.011	0.209	**0.377**
TSweet	0.184	0.169	0.072	**-0.414**	**-0.314**	-0.100	-0.090	**-0.307**

Positive significant correlations ($p < 0.05$) are indicated in purple, whereas negative significant correlations ($p < 0.05$) are indicated in green. The letters "A","F" and "T" before the attribute descriptors refer to aroma, flavour and taste attributes.

4.3. Segmentation of the sensory profile of *Cyclopia* species

Discriminant analysis was used to generate a perceptual map of the six *Cyclopia* species in order to identify groupings and to determine which of the sensory attributes are responsible for these groupings. The DA plot can be viewed in Fig. 4. The DA plot indicates that the sensory attributes could effectively identify three groupings based on the sensory attributes; group A (*C. sessiliflora, C. genistoides* and *C. intermedia)*, Group B (*C. longifolia* and *C. subternata*) and Group C (*C. maculata)* (Fig. 4).

In order to determine which sensory attributes caused these groupings PCA scores and loadings plots were created, of the positive (including the basic taste attributes and mouthfeel attribute) and negative sensory attributes separately, in order to investigate sample patterns. The relationship between the samples and the positive sensory attributes can be viewed in Fig. 5 whereas the relationship between the samples and the negative sensory attributes can be viewed in Fig 6. Bar graphs indicating the differences in terms of aroma, flavour, taste and mouthfeel and negative attributes between the six *Cyclopia* species can be viewed in Figs. 7 to 10, respectively.

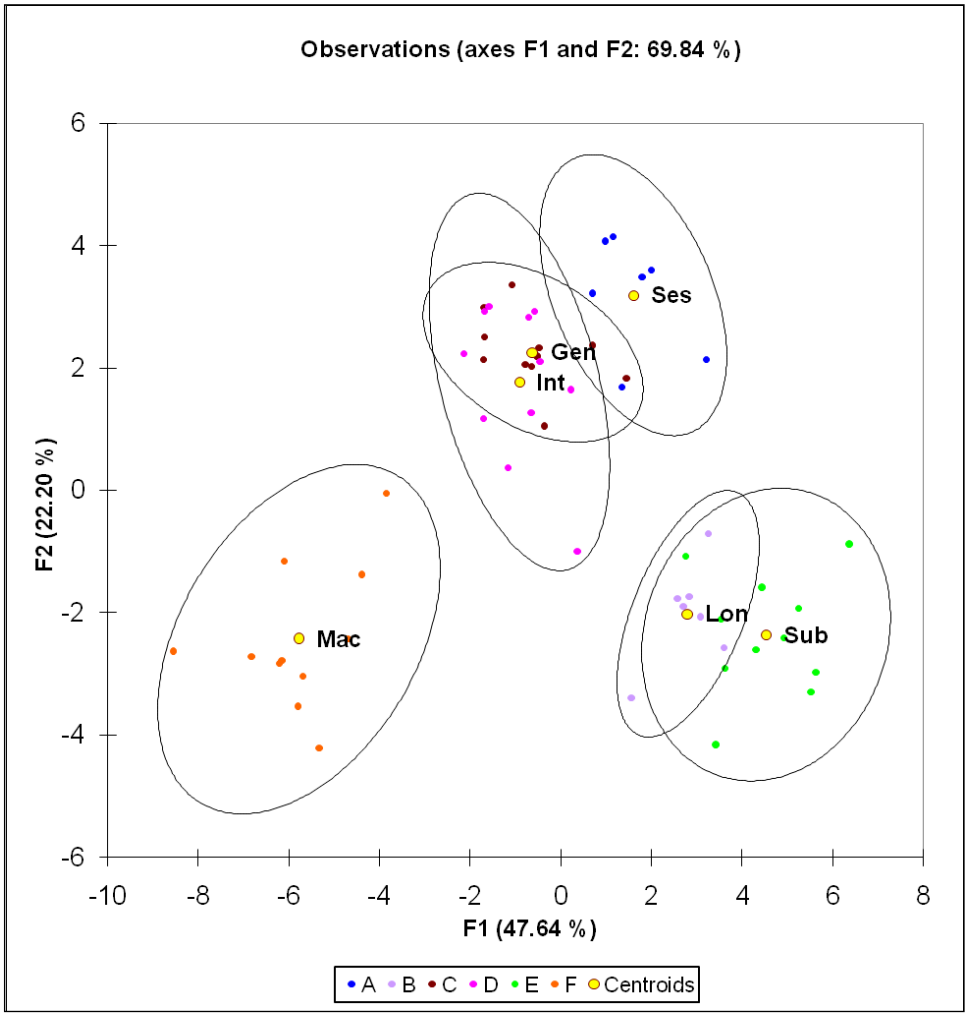

Figure 4 DA plot illustrating groupings.The abbreviations Ses, Lon, Gen, Int, Sub and Mac in the plot refer to *C. sessiliflora, C. longifolia, C. genistoides, C. intermedia, C. subternata* and *C. maculata,* respectively.

4.3.1. Group A

The first group, group A, consisted of three *Cyclopia* species namely,*C. sessiliflora, C. genistoides* and *C. intermedia.* These species associate with the same basic sensory attributes. They are located at the top, mostly to the left, of the PCA scores plot (Fig. 5a). This reflects a strong association with attributes such as fynbos sweet, fynbos floral, lemon, plantlike, sour, bitter and astringent (Fig. 5b). There are a few samples situated to the right of the plot associating with caramel and woody attributes. Although these species have similar sensory characteristics there were subtle differences.

Cyclopia sessiliflora had the highest average score for fynbos floral aroma and flavour but it did not differ significantly ($p > 0.05$) from *C. intermedia* (Figs. 7 and 8). *Cyclopia genistoides* differed significantly ($p \leq 0.05$) from *C. sessiliflora* in terms of fynbos floral aroma but not with regard to fynbos floral flavour. *Cyclopia intermedia* had the second highest average score for rose geranium aroma and flavour, even though this is not one of the main attributes associated with group A, whereas *C. sessiliflora* and *C. genistoides* both had very low average scores. *Cyclopia sessiliflora* had a significant stronger ($p \leq 0.05$) lemon aroma compared to *C. genistoides* and *C. intermedia* but there was no significant difference in the lemon flavour of the species. *Cyclopia genistoides* had the highest average score for apricot jam aroma whereas *C. sessiliflora* had the lowest average score for apricot jam aroma. There was no significant difference between the species in terms of apricot flavour but *C. genistoides* had the highest average score. *Cyclopia sessiliflora* had a significant stronger ($p \leq 0.05$) plantlike aroma and flavour compared to *C. genistoides* and *C. intermedia*. *Cyclopia sessiliflora* had the highest average score for fynbos sweet and differed significantly from *C. genistoides,* however, it did not differ significantly ($p > 0.05$) from *C. intermedia.*

Cyclopia intermedia had the highest average score of the three species for sweet taste followed by *C. sessiliflora* (Fig. 9). There was no significant difference ($p > 0.05$) between these two species in terms of sweetness, however, *C. genistoides* was significantly ($p \leq 0.05$) less sweet than *C. intermedia. Cyclopia sessiliflora* had a significantly stronger ($p \leq 0.05$) sour taste compared to *C. intermedia,* however, there was no significance difference ($p > 0.05$) between *C. sessiliflora* and *C. genistoides. Cyclopia genistoides* had a significantly ($p \leq 0.05$) higher score compared to both *C. sessiliflora* and *C. intermedia* for bitter taste. Also, *C. genistoides* was significantly ($p \leq 0.05$) more astringent compared to *C. intermedia*. However, there was no significant difference in astringency between *C. genistoides* and *C. sessiliflora.* As it is the same species (*C. genistoides*) exhibiting the highest average score for both bitterness and astringency the question arises whether theses attributes were confused by the assessors as it has been reported by Lea and Arnold (1978) that astringency and bitterness may often be confused or seen as the same attribute by assessors.

Cyclopia intermedia had the highest average score of the three species for dusty aroma, however, it did not differ significantly ($p > 0.05$), nor was there a significant difference ($p > 0.05$) in terms of dusty flavour (Fig. 10). *Cyclopia intermedia* had a significantly stronger ($p \leq 0.05$) yeasty aroma but once again, this difference was not detected by retronasal analysis. Additionally, *C. intermedia* had the highest average score for medicinal aroma and differed significantly ($p \leq 0.05$) from *C. sessiliflora*. In terms of medicinal flavour, *C. intermedia* differed significantly ($p \leq 0.05$) from both *C. sessiliflora* and *C. genistoides. Cyclopia genistoides* had the highest burnt caramel flavour followed by *C. intermedia* and differed significantly from *C. sessiliflora. Cyclopia genistoides* differed significantly ($p \leq 0.05$) from both *C. sessiliflora* and *C. intermedia* in terms of burnt caramel flavour. There was no significant difference ($p > 0.05$) between the three species in terms of hay/dried grass aroma but *C. genistoides* had the highest average score. However, in terms of

hay/dried grass flavour *C. genistoides* differed significantly from both *C. sessiliflora* and *C. intermedia. Cyclopia sessiliflora* had a significantly stronger ($p \leq 0.05$) green grass aroma. In terms of flavour there was no significant difference ($p > 0.05$) between the three species; *C. sessiliflora* had the highest average score.

In conclusion, *C. sessiliflora* associated with "green" (plantlike and green grass) and lemon attributes, as well as sour taste, *C. genistoides* associated with apricot jam aroma and a slight bitter taste and *C. intermedia* appears to associate with more floral (rose geranium) notes and appears to be slightly sweeter compared to the other two species. *Cyclopia intermedia* appears to have a problem with negative attributes such as yeasty and medicinal attributes whereas *C. genistoides* appears to have a problem with burnt caramel and hay/dried grass attributes and *C. sessiliflora* with green grass. Based on the PCA plot of the negative sensory attributes (Fig. 6a and b) it appears for *C. genistoides* and *C. intermedia* as if all the samples located near negative attributes were samples obtained from industry for which the processing conditions were not available (Addendum A). The negative attributes could thus be due to over- or even under-fermentation instead of being associated with a specific species. It is very likely that the burnt caramel associated with *C. genistoides* was as a result of over-fermentation, This specific negative attribute has been reported by Du Toit and Joubert (1999) to be present in tea prepared from *C. intermedia* which was over-fermented. In the case of *C. sessiliflora*, it might be possible that although the tea was prepared according to the standard fermentation conditions used by the Agricultural Research Council (ARC) to ferment honeybush (80°C/24 h or 90°C/16h) that the tea might have been under-fermented resulting in the "green" character as the fermentation conditions currently used are based on research done on *C. intermedia* (Du Toit & Joubert, 1999) and not *C. sessiliflora.* Also, it is possible that the panel did not completely understand the difference between plantlike and green grass which could have led to the higher green grass mean score. However, it is quite possible that the green character might be species-specific.

4.3.2. Group B

Group B, consisted of two *Cyclopia* species namely, *C. longifolia* and *C. subternata.* It can thus be assumed that these species associate with the same basic sensory attributes. These species are located at the bottom, mostly to the left, of the PCA scores plot (Fig. 13a). This reflects a strong association with attributes such as rooibos, apricot jam, rose geranium, fruity sweet, rose/perfume and sweet taste (Fig. 13b). Additionally, there are a few *C. longifolia* at the top on the left side of the PCA plot associating with woody and caramel attributes and a few *C. subternata* samples situated at the bottom on the right side of the plot associating with attributes such as walnut and coconut. Although these two species have similar sensory characteristics there are also a few differences.

Cyclopia longifolia had the lowest average score for fynbos floral aroma and differed significantly ($p \leq 0.05$) from *C. subternata,* however, there was no significant difference ($p > 0.05$) between the two species in terms of fynbos floral flavour (Figs. 7 and 8). *Cyclopia subternata* had the highest average score for both rose geranium aroma and flavour but there was no significant difference ($p > 0.05$) between the two species. *Cyclopia longifolia* had a higher average score for apricot jam aroma compared to *C. subternata,* however, there was no significant difference ($p > 0.05$) and both species had very similar average scores for apricot jam flavour. Both *C. longifolia* and *C. subternata* had very low average scores for plantlike aroma and flavour but *C. subternata* had the lowest average score for plantlike aroma whereas *C. longifolia* had the lowest average score for plantlike flavour. *Cyclopia subternata* and *C. longifolia* had the two highest average

scores for fruity sweet aroma and relatively low average scores for fynbos sweet. *Cyclopia subternata* had a higher average score for coconut aroma compared to *C. longifolia* however there was no significant difference ($p > 0.05$).

There was no significant difference ($p > 0.05$) between the two species in terms of sweetness, bitterness, sour taste or astringency (Fig. 9). *Cyclopia subternata* had a slightly higher average score for sweetness and a slightly lower score for sour taste and bitterness. The average score values for astringency for the two species were very similar.

Cyclopia subternata had a higher average score for dusty aroma, however, there was no significant difference ($p > 0.05$) and there was no difference between the two species in terms of dusty flavour (Fig. 10). *Cyclopia longifolia* had quite a high average score for burnt caramel aroma and flavour compared to *C. subternata,* however, there was no significance difference ($p > 0.05$). Although there was no significant difference ($p > 0.05$) in terms of rotting plant water aroma, *C. longifolia* had a higher average score. *Cyclopia longifolia* had a significantly higher ($p \leq 0.05$) average score for rotting plant water flavour compared to *C. subternata.* There was no significant difference ($p > 0.05$) between the two species in terms of hay/dried grass aroma and flavour; in both cases *C. longifolia* had a higher average score.

Based on these results it is clear that there are very little differences between these two species in terms of aroma, flavour, taste and mouthfeel. The only difference was the fact that *C. longifolia* had a significantly ($p \leq 0.05$) lower fynbos floral aroma. Furthermore, *C. longifolia* seemed to have had higher average scores for all negative attributes compared to *C. subternata* although none of them were significant. *Cyclopia longifolia* have a problem with rotting plant water flavour. This could be due to the fact that there were a few samples in the *C. longifolia* sample set which were commercial samples for which we did not have any processing information and the fact that some of the samples were fermented at 70°C for 60 h. As mentioned previously the latter might not be the most suitable fermentation conditions for this specific species as it is based on a study done on *C. intermedia* by Du Toit and Joubert (1999). A smaller sample set was also used for *C. longifolia* compared to *C. subternata* which could have caused these slightly higher average scores for the negative attributes. Furthermore, there were a limited number of samples present which were fermented according to the standard fermentation conditions used by the ARC.

4.3.3. Group C

Group C, consisted of only one *Cyclopia* species namely, *C. maculata.* This indicates that this species had very different sensory attributes compared to the other *Cyclopia* species analysed. *Cyclopia maculata* is located at the bottom on the right side of the PCA scores plot (Fig. 13a). This reflected a strong association with attributes such as cassia/cinnamon, boiled syrup, walnut, cooked apple and coconut (Fig. 13b).

Cyclopia maculata had one of the lowest average scores for fynbos floral aroma and the lowest average score for fynbos floral flavour (Fig 7 and 8). Similarly, *C. maculata* also had one of the lowest average scores for rose geranium aroma and flavour. Although there was no significant difference between the rose/perfume aroma of the six species, *C. maculata* had the highest average score for rose/perfume flavour. *Cyclopia maculata* only differed significantly ($p \leq 0.05$) from *C. sessiliflora* in terms of this attribute. *Cyclopia maculata* had a significant stronger ($p \leq 0.05$) cooked apple aroma than all of the species, except for *C. intermedia.* However, the cooked apple flavour was undetectable. *Cyclopia maculata* had one of the lowest average scores for plantlike aroma and flavour. Even though *C. maculata* had the highest average

score for both woody aroma and flavour, *C. maculata* only differed significantly ($p \leq 0.05$) from *C. longifolia* in terms of woody aroma. *Cyclopia maculata* had a relatively high average score for fruity sweet aroma. *Cyclopia maculata* had a significantly higher ($p \leq 0.05$) average score for boiled syrup aroma compared to the rest of the species. *Cyclopia maculata* had one of the lowest average scores for fynbos sweet. *Cyclopia maculata* had a significant stronger ($p \leq 0.05$) cassia/cinnamon spicy aroma and flavour compared to the other species. *Cyclopia maculata* had a significantly stronger ($p \leq 0.05$) walnut aroma and flavour compared to the other species. Also, *C. maculata* had one of the highest average scores for coconut aroma, however, it did not differ significantly ($p \leq 0.05$) from any of the other species. *Cyclopia maculata* had one of the highest average scores for sweet taste and the lowest scores for sour taste, bitter taste and astringency (Fig. 9). C*yclopia maculata* had the highest average score for dusty aroma although the difference was only significant ($p \leq 0.05$) with regard to *C. longifolia* and *C. genistoides* (Fig. 10). There did not seem to be any other specific negative attribute present in *C. maculata* except for hay/dried grass aroma and flavour for which *C. maculata* had quite a high average score.

In summary *C. maculata* is thus not considered to be a very floral species, however, in comparison to the other species associated relatively strongly with a spicy cassia/cinnamon aroma and flavour. Although it appears as if there is a fruity sweet note present, the overall sweetness was described as boiled syrup. *Cyclopia maculata* seemed to also have a cooked apple, walnut and coconut character. The presence of this spicy cassia/cinnamon note was unexpected as neither honeybush tea nor *C. maculata* specifically have previously been described as a spicy tea. Cinnamon-like or spicy volatile compounds (6-methyl-3,5-heptadien-2-one, 4-Acetyl-1-methyl-cyclohexene, (+)-*p*-Menth-1-en-9-al, eugenol and (7*E*)-Megastigma-5,7,9-trien-4-one) have, however, been identified in a number of *Cyclopia* species (Le Roux *et al.,* 2008; Cronje, 2010) and it was postulated that one or more of these volatiles may be responsible for the spicy note. In order to identify which compound/combination of compounds are responsible for this characteristic aroma note, a representative spicy *C. maculata* sample (Mac3) was selected and analysed using GC-O. A list of the odour active compounds identified in *C. maculata* (Mac3) and the aroma attributes associated with these compounds are provided in Table 9. The sensory attributes associated with *C. maculata* (Mac3) can be viewed in Fig. 11.

The prominent spicy note could possibly be attributed to the high relative concentration of eugenol (Table 9). Eugenol is described as having a warm spicy, dry aroma and it is the only volatile component identified in Mac3 associated with a spicy aroma. Eugenol has previously been identified as an odour active compound of *C. genistoides (*Le Roux *et al.,* 2008) and *C. subternata* (Cronje, 2010). In Mac3 it was more prominent, comprising more than 2% of the total volatile fraction (% of area) compared to less than 0.1% for *C. genistoides* and *C. subternata*.

The floral notes present in *C. maculata* are extremely low, however, this could perhaps be due to the overwhelming spicy note which might have caused lower scores to be awarded for floral notes by the assessors. It is difficult to evaluate more subtle attributes if one sensory attribute is particularly strong. A number of odour active volatile compounds associated with floral, fruity, woody and sweet aromas were identified (Table 9). Unknown compound C178, (*E*)-β-ionone and (*E,E*)-2,4-decadienal have previously been linked to the sweet aroma in *C. subternata* (Cronje, 2010). (*E*)-β-ionone, which has a woody floral aroma, was linked to the typical honeybush aroma (Cronje, 2010). It is thus not surprising that these volatile compounds were also present in the infusion of Mac3, described as very sweet and woody.

The pine and nutty notes detected in this particular sample by descriptive analysis could be linked to quite a few odour active compounds identified in Mac3. Both (*E,E)*-2,4-heptadienal and (*E)*-2-octenal are described as nutty, whereas pinolene is described as having a sweet-piney aroma. The only volatile component identified in *Cyclopia* species associated with a coconut aroma to date is (*R)-o*ctan-5-olide; this compound was not identified as one of the aroma active compounds present in Mac3. Possibly, the coconut aroma could be due to one of the unidentified components or one of the components for which no aroma descriptors was available.

There are quite a number of odour active components associated specifically with green aroma notes which were not detected by descriptive analysis. This could be due to the overwhelming spicy aroma as well as the prominent green character of some of the other *Cyclopia* species to which *C. maculata* was compared to during descriptive analysis.

Although certain compounds can thus be linked to specific aroma notes it is important to realise that aromas of different qualities can mask or suppress one another and compounds with similar qualities can blend and produce a new aroma and certain compounds present in concentrations below their odour threshold or which has no odour activity when assessed individually can contribute to the aroma when they are in a mixture (Delahunty *et al.,* 2006).

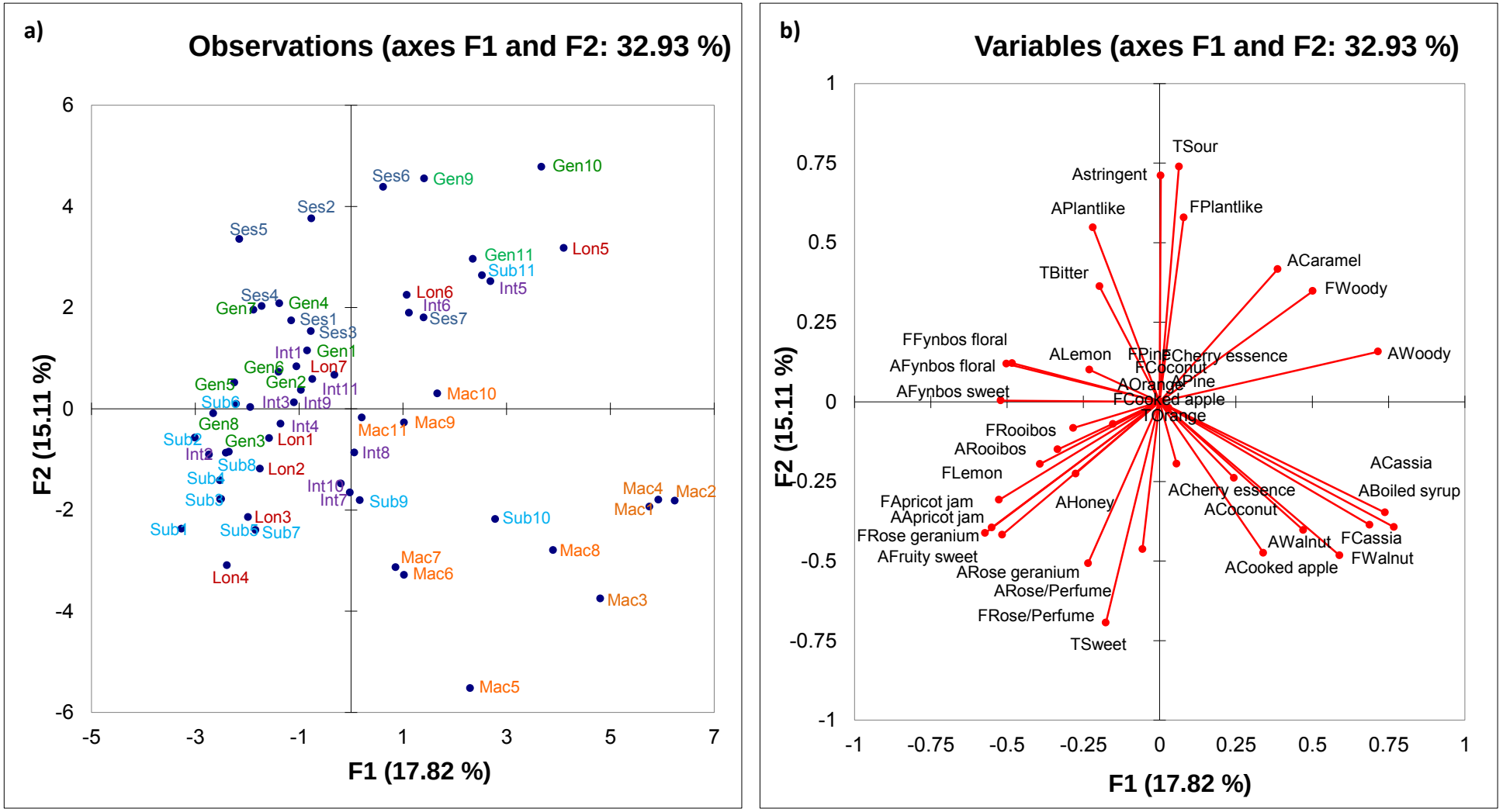

Figure 5 a) PCA scores plot showing the positioning of the 58 honeybush tea samples. The abbreviations Ses, Lon, Gen, Int, Sub and Mac in the scores plot refer to the specific *Cyclopia* species; *C. sessiliflora, C. longifolia, C. genistoides, C. intermedia, C. subternata* and *C. maculata,* respectively. b) PCA loadings plot showing the positioning of the positive, taste and mouthfeel sensory attributes. The letters "A", "F" and "T" in front of the attributes refer to aroma, flavour and taste attributes, respectively. Cassia = Cassia/Cinnamon.

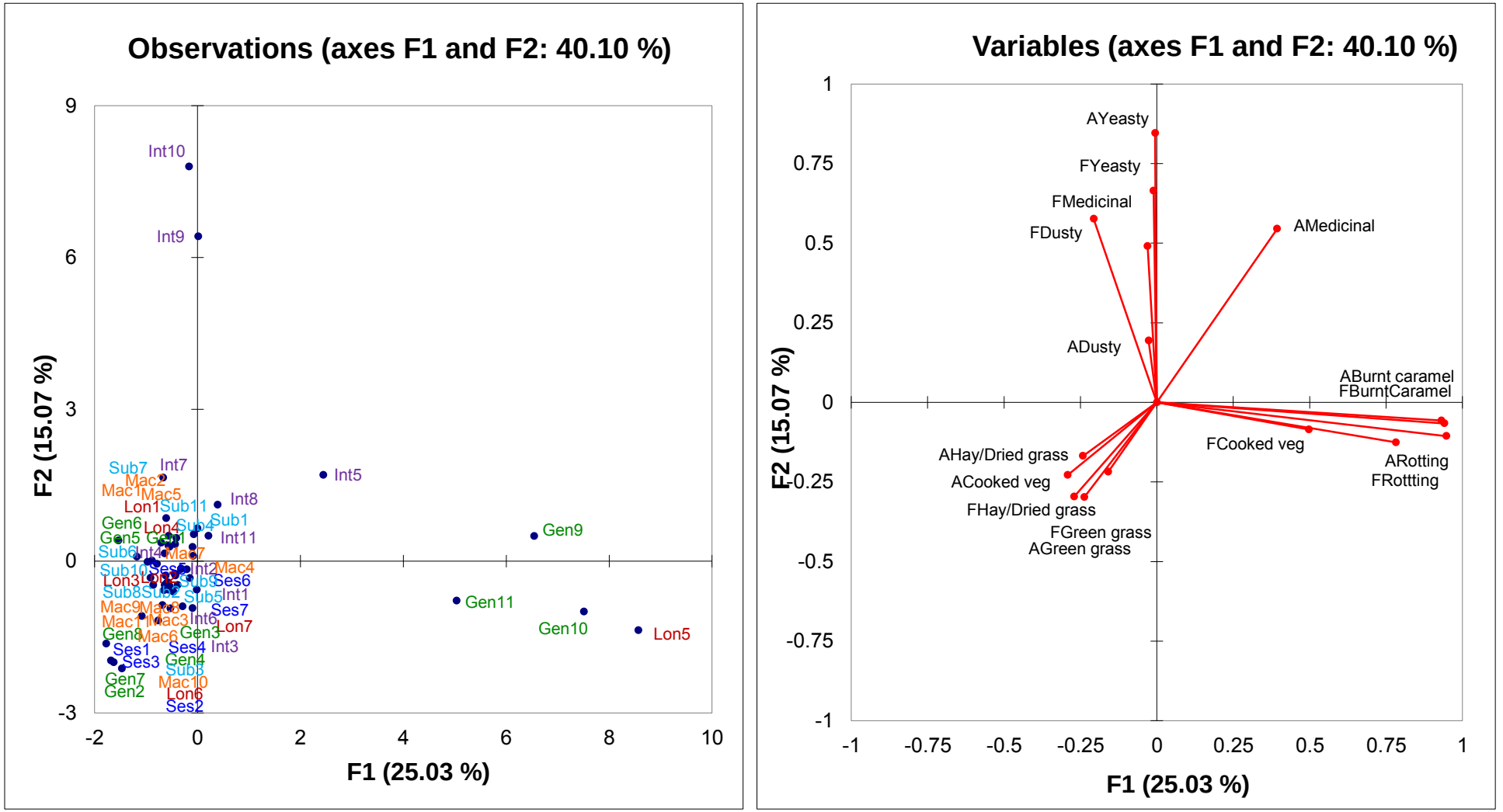

Figure 6 a) PCA scores plot showing the positioning of the 58 honeybush tea samples. The abbreviations Ses, Lon, Gen, Int, Sub and Mac in the scores plot refer to the specific *Cyclopia* species; *C. sessiliflora, C. longifolia, C. genistoides, C. intermedia, C. subternata* and *C. maculata,* respectively. b) PCA loadings plot showing the positioning of the negative sensory attributes. Rotting = Rotting plant water, Cooked veg = Cooked vegetables. The letters "A" and "F" in front of the attributes refer to aroma and flavour attributes, respectively.

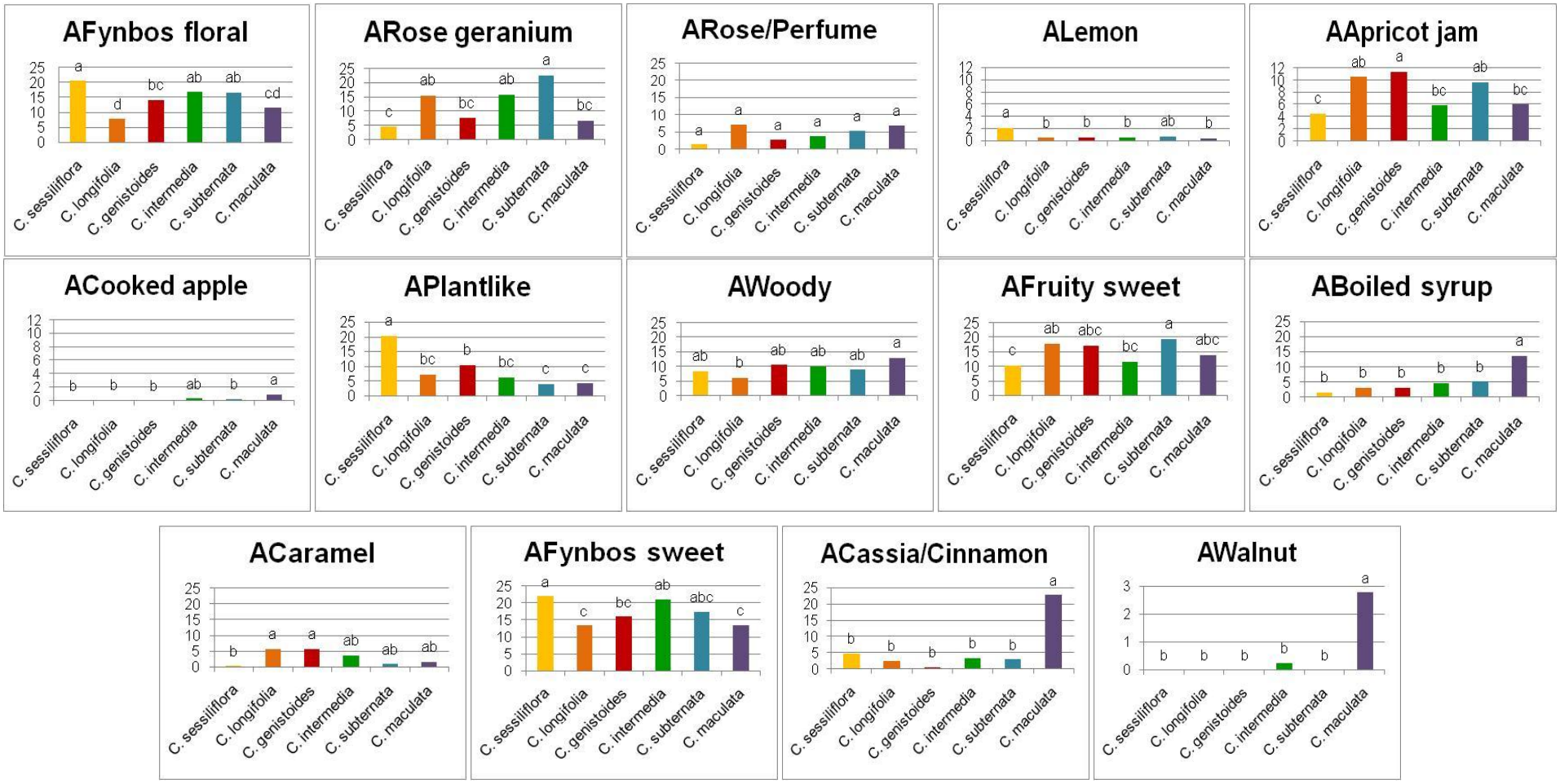

Figure 7 Average attribute intensities for aroma attributes present in the *Cyclopia* species. Bars with different alphabetical letters are significantly different from each other ($p \leq 0.05$). The letter "A" in front of the attribute name refers to aroma. Attributes with a mean score of less than 5 and which did not differ significantly are not shown.

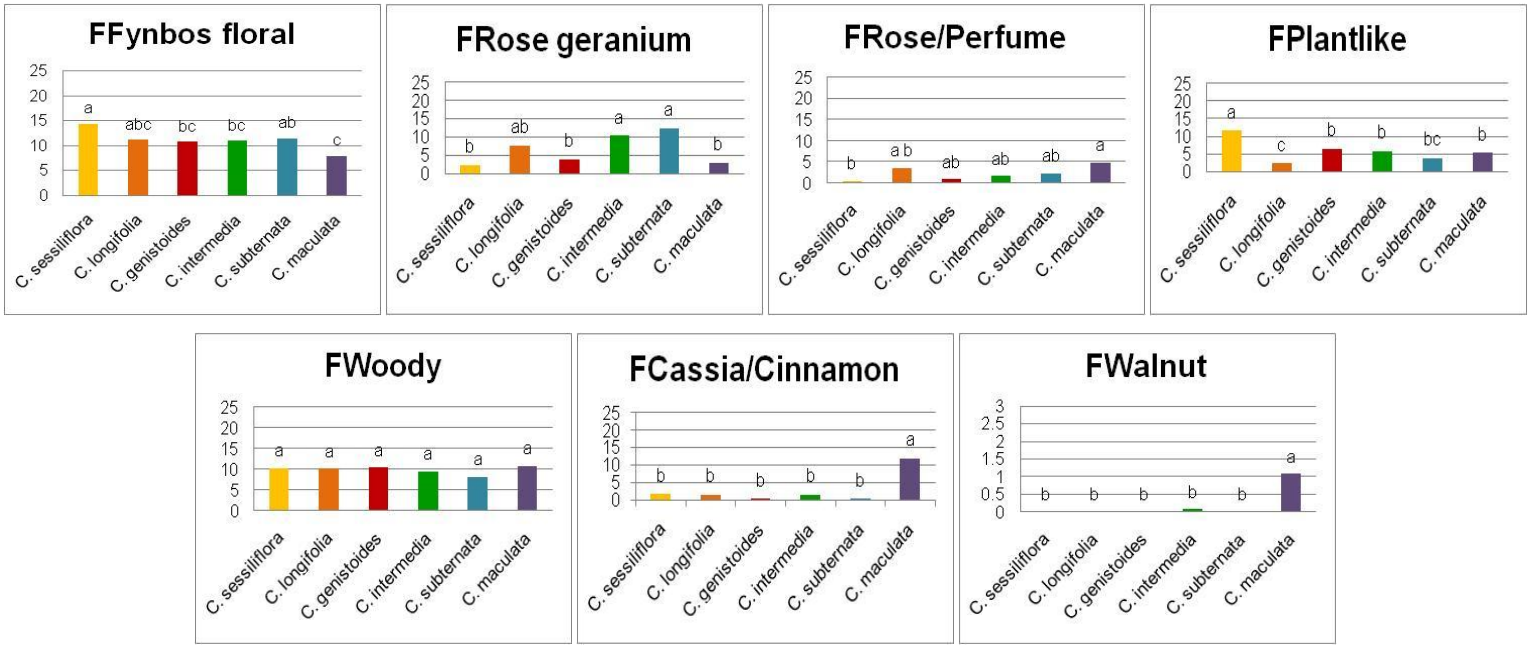

Figure 8 Average attribute intensities for flavour attributes present in the *Cyclopia* species. Bars with different alphabetical letters are significantly different from each other ($p \leq 0.05$). The letter "F" in front of the attribute name refers to flavour. Attributes with a mean score of less than 5 which did not differ significantly are not shown.

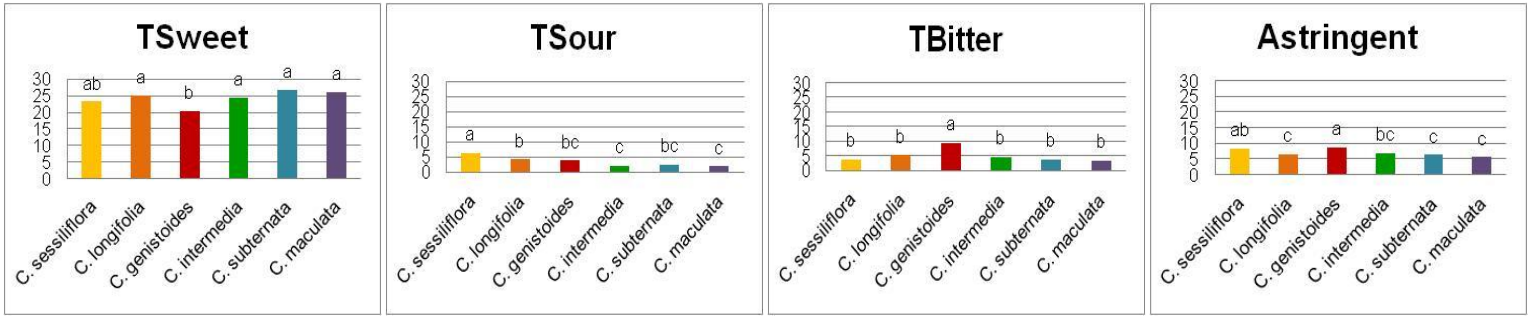

Figure 9 Average attribute intensities for the taste and mouthfeel attributes present in the *Cyclopia* species. Bars with different alphabetical letters are significantly different from each other ($p \leq 0.05$). The letter "T" in front of the attribute name refers to taste.

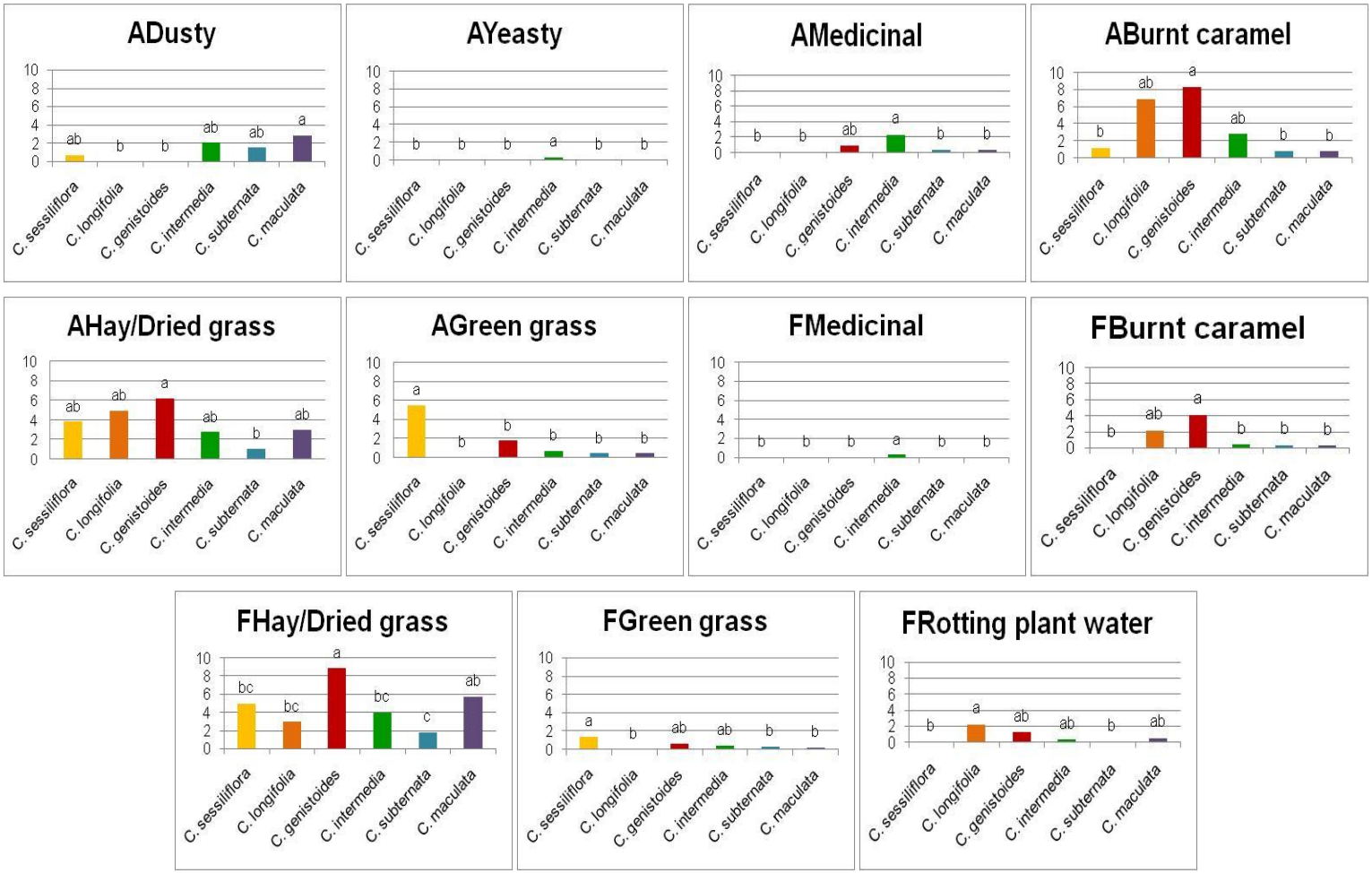

Figure 10 Average attribute intensities for the negative aroma and flavour attributes present in the *Cyclopia* species. Bars with different alphabetical letters are significantly different from each other ($p \leq 0.05$). The letter "A" and "F" in front of the attribute name refers to aroma and flavour, respectively. Attributes with a mean score of less than 5 which did not differ significantly are not shown.

Table 9 Odour active compounds and aroma descriptors associated with each compound identified by means of GC-O in the volatile fraction of Mac3

Compounds	Detection frequency (%)	Area %	Aroma descriptor*
(E)-2-nonenal	100	0.71	Green, cucumber, aldehydic and fatty
(*E*)-β-damascenone	100	3.71	Woody, sweet, fruity, earthy green-floral
(*E*)-β-damascone	100	1.5	Fruity (apple-citrus), tea-like with slight minty notes
(*E*)-β-ionone	100	9.91	Woody, floral
(*E,E*)-2,4-nonadienal	100	1.17	Fatty-soapy
(*E,E*)-3,5-octadien-2-one	100	0.94	Fatty, fruity, mushroom
(*E,E,Z*)-2,4,6-nonatrienal	100	0.73	Oat-flake like
(*E,Z*)-2,6-nonadienal	100	0.96	Green-vegetable, cucumber or violet leaf
1-octen-3-ol	100	0.40	Mushroom
2,3-dehydro-γ-ionone	100	0.06	Tobacco-like
2-methylbutanoic acid	100	0.05	Cheesy, sweaty, sharp
3-methylbutanoic acid	100	0.04	Acid acrid, cheesy, unpleasant
6-methyl-6-(5-methylfuran-2-yl)-heptan-2-one	100	0.25	
A benzyl ester	100	0.10	
Nonanal	100	1.15	Floral
Bovolide	100	0.64	Celery- and lovage-like, fruity and pleasant
Eugenol	100	2.30	Warm-spicy, dry
Hexanal	100	7.65	Fatty-green grassy odour
Hotrienol	100	0.65	Floral, fruity
m/z 163 unknown compound	100	0.06	
Phenylacetaldehyde	100	0.21	Floral, Lilac
Piperonal	100	0.06	Floral
Unknown C178	100	0.66	
Unknown C269	100	0.07	
Unknown *m/z* 91 compound	100	0.32	
(*E,E*)-2,4-decadienal	87.5	0.29	Deep fried

Table 9 continued

Compounds	Detection frequency (%)	Area %	Aroma descriptor*
2-phenylethanol	87.5	0.29	Mild, warm, rose-honey-like
6-methyl-5-hepten-2-one	87.5	23.26	Oily-green, pungent-herbaceous, grassy, with fresh and green-fruity notes
Safranal	87.5	0.82	Herbaceous (saffron)
2-ethyl-3-methylmaleimide	75	0.04	
cis-linalooloxide	75	0.17	Sweet floral, green, fruity
Geraniol	75	22.86	Sweet, floral, rose
Linalool	75	8.41	Refreshing, floral-woody
p-anisaldehyde	75	0.14	Sweet, floral, "hay-like"
Unknown oxo-edulan type compound	75	0.08	
α-terpineol	75	3.72	
(*E,E*)-2,4-heptadienal	62.5	4.01	Fatty, nutty, hay, fishy
2,3-dehydro-α-ionone	62.5	0.30	
trans-calamenene	62.5	0.45	
Unknown compound	62.5	0.01	
Unknown compound	62.5	0.03	
Unknown compound	62.5	0.01	
Unknown *m/z* 135 compound	62.5	0.07	
Unknown *m/z* 83 compound	62.5	0.13	
(*E*)-2-octenal	50	0.59	Burnt, mushroom, fatty, nutty
(*E,Z*)-2,4-decadienal	50	0.04	Deep fried
3-hydroxy-α-damascone	50	0.05	
Calamenene-1,11-epoxide	50	0.06	
Geranylformate	50	0.28	Fresh, green-rosy, fruity
Terpinolene	50	0.18	Sweet-piney, oily
Unknown C162	50	0.06	
Unknown compound	50	0.22	
β-selinene	50	0.07	

*Compiled from Cronje (2010), Linssen *et al.* (1993), Petka *et al. (*2006), Venkateshwarlu *et al.* (2004), Wang *et al.* (2008), Morales *et al.* (1995), Sanz *et al*.,(2002).

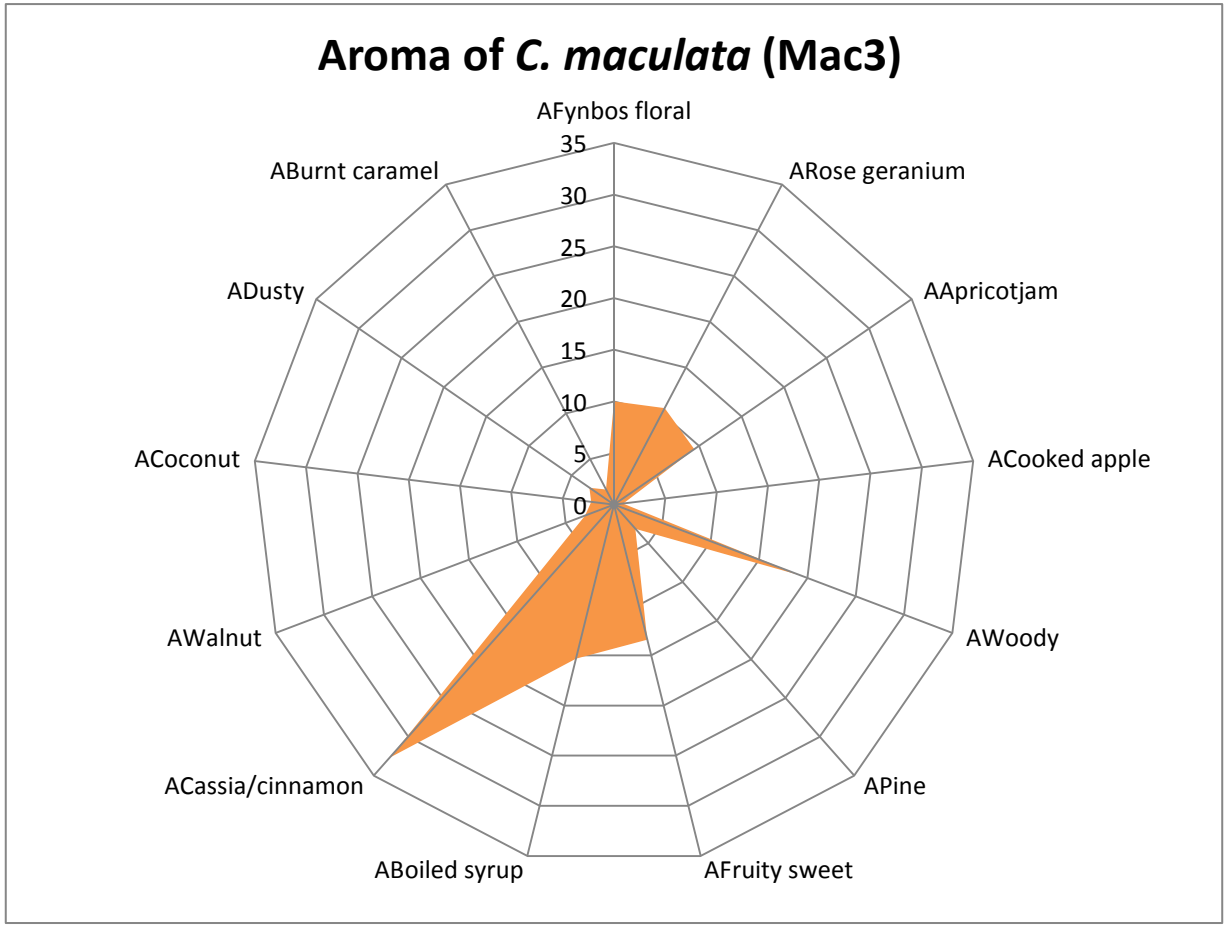

Figure 11 Spider plot reflecting the mean scores for the aroma attributes associated with *C. maculate* (Mac3) as determined by descriptive analysis. The letter "A" in front of the sensory attribute refers to aroma.

4.4. The sensory wheel

A sensory wheel for honeybush was created by selecting 28 flavour and 7 taste and mouthfeel attributes to form a simple graphical representation of the sensory lexicon. As the basic taste modalities (sweet, sour and bitter), as well as mouthfeel (astringency) usually form part of most sensory wheels these four descriptors were also included on the sensory wheel. The 35 terms were assembled to form a three-tiered wheel consisting of ten sectors: floral, fruity, spicy, nutty, sweet, taste and mouthfeel, earthy, chemical and vegetative (Fig. 12). The two classes of attributes (positive and negative) are located on the outer tier and the generic terms used to group together a certain class of adjectives are located in the middle tier, whereas the more specific attributes are located in the inner tier. The terms were also grouped together based on whether it was taste and mouthfeel or flavour attributes.

Aroma and flavour wheels, as well as mouthfeel wheels, have been developed for a variety of food and beverage products such as fish (Warm *et al.,* 2000), kiwifruit and pawpaw fruit puree (Wismer *et al.,* 2005; Duffrin & Pomper, 2006), whisky (Lee *et al.,* 2001), brandy (Jolly & Hattingh, 2001), wine (Gawel *et al.,* 2000; Mirarefi *et al.,* 2004; Noble *et al.,* 1984), beer (Meilgaard *et al.,* 1979) and honey (Piana *et al.,* 2004). Sensory wheels have also been developed for black tea (*Camellia sinensis*; Bhuyan & Borah, 2001) and rooibos tea (*Aspalathus linearis*; Koch, 2011). A number of first- and second-tier descriptors such as floral (rose, perfume, geranium, lilac, orange blossom, violets), fruity (citrus, berry, apricot, stewed fruit), sweet (honey, caramel, toffee) and green (green grass, hay, vegetative, herbaceous) are often used to describe these products.

The sensory wheel developed for honeybush in this study can be used as a communication tool between researcher institutions, industry and marketing companies. Additionally, it can be used for comparing and monitoring the quality and consistency of honeybush tea as well as profiling new and competitive products within the tea industry. It should be noted that this was the first attempt to develop a sensory wheel for honeybush and although the list of descriptors is useful and comprehensive for the sensory characterisation of honeybush, the list of terms may be incomplete as it is based on a sample size of only 58 honeybush tea samples. It is thus expected that some adjustments will need to be made in future.

It would be beneficial to eventually develop flavour wheels tailored for each *Cyclopia* species as not all the descriptive terms currently included on the sensory wheel necessarily apply to all *Cyclopia* species. For example, the spicy (cassia/cinnamon), nutty (walnut) and fruity (cooked apple) notes was only present in one *Cyclopia* species, *C. maculata.* These terms would therefore not be applicable to the other *Cyclopia* species.

4.5. The sensory lexicon

The honeybush tea sensory lexicon developed during this study is shown in Table 10. The lexicon comprises a descriptive term together with a definition and a reference standard for each term. Attributes determined orthonasally and retronasally were grouped together as flavour terms and mouthfeel (astringency) were grouped with the three taste modalities (sweet, bitter and sour) as taste terms.

Obtaining suitable qualitative reference standards that accurately exemplify specific attributes is a challenging task because of the unique sensory characteristics of each food and beverage product. A book of standardised definitions and reference standards was compiled by Civille and Lyon (1996), however, the

proposed definitions and references are not always well-suited to the product being evaluated. The process of selecting appropriate references can thus not be limited to a literature study but requires time-consuming examination of a wide range of products and chemicals until the most suitable substance has been identified.

Although references can be qualitative or quantitative, only qualitative reference standards were used during this study. These references are essential when developing sensory lexicons since they allow for clarification of the terminology for future use or comparison. Attribute intensities were rated in relation to the control honeybush sample, or to the samples exhibiting the most prominent or the weakest intensities of a specific attribute when the attribute was absent in the control (Munoz & Civille, 1998).

The importance of reference standards, i.e. to assist in the understanding of flavour terminology, has been noted by a number of authors (Noble *et al.,* 1984; Wolters, 1994; Munoz & Civille, 1998; Drake & Civille, 2002). In this study it was necessary to assign specific definitions and reference standards to each descriptor to facilitate communication as most of the sensory attributes had a specific meaning within the context of honeybush tea. Using a food, chemical or any other substance that communicate the concept of specific attributes to the assessors increases their understanding and improves the clarity of attribute terminology (Drake & Civille, 2002). Meilgaard *et al.* (1979) suggested reference standards to define beer flavour terminology but these standards were prepared using a single compound in a beer base. One of the major problems with this approach is the inability of a single compound to validly reproduce a complex aroma characteristic. An alternative approach is a commercial sensory kit consisting of flavour essences, however, these essences can be extremely unstable and degrade readily. For both of these systems of defining reference standards major problems result from the difficulty in handling of the standards. To provide standards which have consistent, characteristic aromas at suitable intensities Noble *et al.* (1987) suggested that reference standards should be prepared using foodstuffs available throughout the world during most of the seasons. For this reason most of the reference standards chosen for this study to represent honeybush flavours are readily available foodstuffs. Where no appropriate flavour, essence or foodstuff could be determined honeybush tea prepared from a specific *Cyclopia* species is suggested. The standardised terminology can facilitate improved communication among the different role players in the industry. These descriptors could facilitate both the definition and protection of the reputation of high quality products from inferior ones, as well as encourage successful promotion of honeybush tea based on its unique sensory qualities. In future research honeybush tea can be described using this set of objectively determined flavour attributes. Given the many different *Cyclopia* species available, the number of attributes was not reduced to 10 to 20 attributes as suggested by Vannier *et al.* (1999) as it seemed appropriate to maintain the integrity of the present lexicon so that a larger pool of attributes would be available to choose from when conducting a study on a specific *Cyclopia* species. This being said, the list of terms may be incomplete and should be continually expanded as necessary.

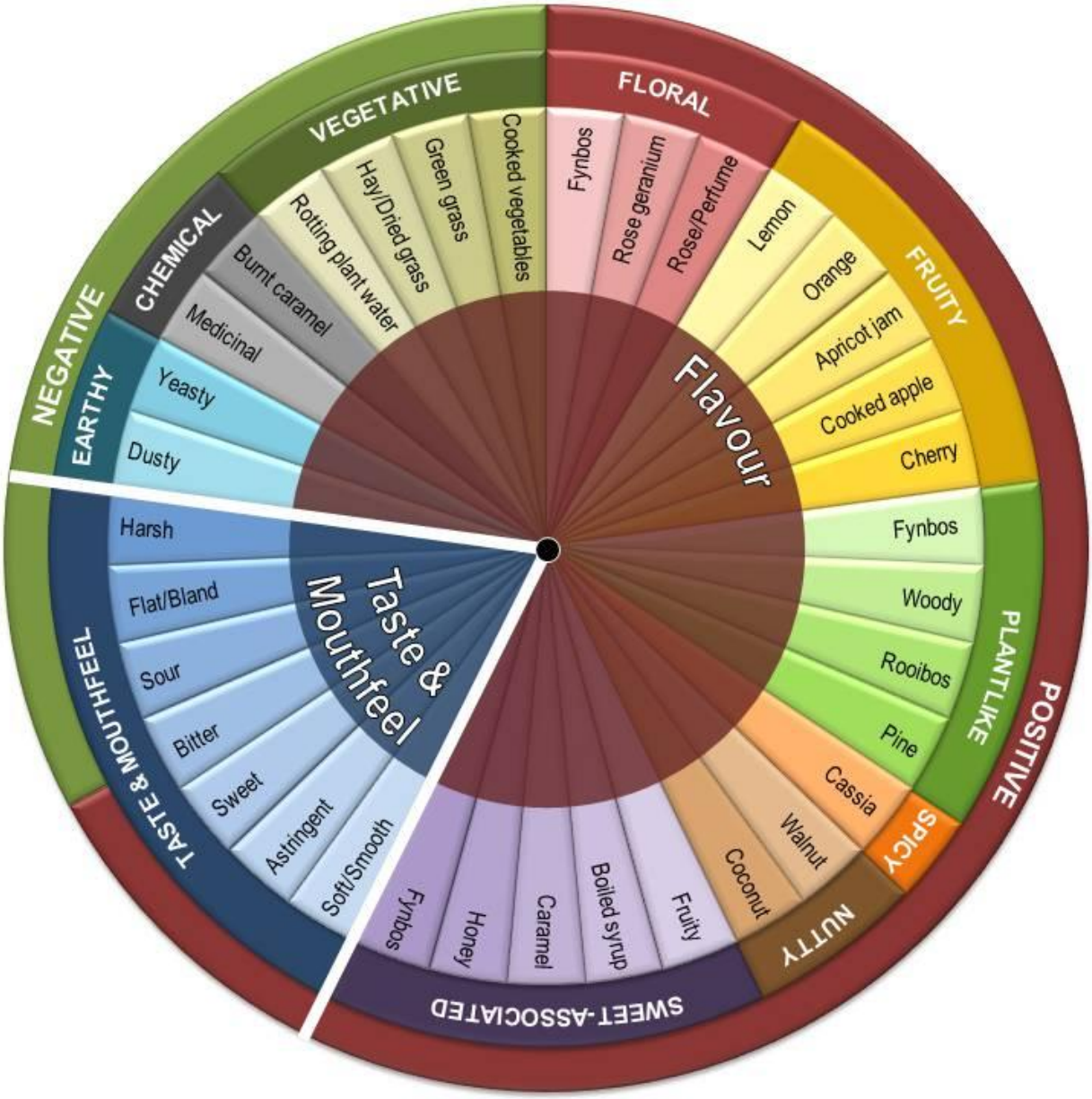

Figure 12 Sensory wheel comprising 28 flavour and 7 taste and mouthfeel terms that describe the sensory attributes of the 58 honeybush tea infusions.

Table 10 Sensory lexicon describing flavour and mouthfeel characteristics of honeybush infusions analysed by descriptive analysis

1st Tier Attribute	2nd Tier attribute	Definition	Reference Standard
FLORAL AROMA	Fynbos floral*	Floral aroma note associated with the flowers of fynbos vegetation	Honeybush tea prepared from *C. intermedia* (3 g/100 mL)
	Rose geranium	Floral aroma note associated with the rose geranium plant	Fresh rose geranium leaf (10 mm x 10 mm)/Rose geranium oil (0.005%)
	Rose/Perfume	Floral aroma note associated with rose petals	Crushed petals of one rose[a]
FRUITY AROMA	Lemon	Aromatic associated with general impression of fresh lemons	Lemon juice (5%)
	Orange	Flavour reminiscent of orange peel	Orange flavour (0.01%)
	Apple, cooked	The flat, slightly sour aroma and flavour of cooked apples	Apple Puree (2.5 g/100 mL)
	Apricot jam	Sweet flavour reminiscent of apricot jam	Superfine apricot jam (15 g/100 mL hot water)
	Cherry	Fruity aroma note associated with cherry essence	Cherry essence (0.005%)
PLANTLIKE AROMA	Plantlike*	Slightly sour aromatic characteristic of freshly cut fynbos plant material	Honeybush tea prepared from *C. sessiliflora* (3 g/100 mL)
	Woody*	Aromatic associated with dry bushes, stems and twigs of the fynbos vegetation	Honeybush tea prepared from *C. maculata* (3 g/100 mL)
	Rooibos	Aromatic associated with dry bushes, stems and twigs of *Aspalathus linearis*(rooibos)	FTNF Rooibos Extract (2%)[b]
	Pine	Aroma reminiscent of pine needles	Fresh pine needles
SWEET AROMA	Fruity sweet	Sweet aromatic reminiscent of non-specific fruit especially berries and apricot jam	Superfine apricot jam and strawberry jam (5 g each/100 mL hot water)[b]
	Boiled syrup	Aroma note associated with boiled syrup	Golden syrup (10 g/100 mL hot water)
	Caramel	Sweet aromatic characteristic of molten sugar or caramel pudding	Caramel, natural flavour (0.4%)[b]
	Honey	Aromatics associated with the sweet fragrance of fynbos honey	Wild flower honey[b]
	Fynbos*	Aroma note reminiscent of the fynbos plant	Honeybush tea prepared from *C. intermedia* (3 g/100 mL)
SPICY AROMA	Cassia/ Cinnamon	The sweet woody spicy aromatic of ground cinnamon/cassia bark	Soak cinnamon/cassia bark in water overnight[a]
NUTTY AROMA	Walnuts	Aroma note associated with fresh (not rancid) walnuts	Freshly chopped walnuts[c]
	Coconut	Aromatic associated with desiccated coconut	Desiccated coconut
NEGATIVE AROMA	Dusty	Earthy aromatic associated with wet hessian or wet cardboard	Old, dry tree bark (*Jacaranda mimosifolia)* (1 piece/100 mL hot water, infuse for 5 min, filter)[a]
	Medicinal	Aromatic characteristic of band-aid, disinfectant-like (phenolic)	Place a Band-aid adhesive bandage in a petri dish and cover[d]
	Rotting plant water	Slightly sour aromatic characteristic of rotting plant water	Grass (*Pennisetum clandestinum)* (30 shredded blades/100 mL hot water, store 1 week, filter)
	Hay/Dried grass	Slightly sweet aromatic associated with dried grass or hay	Dried grass (*Pennisetum clandestinum)*[b]
	Green grass	Aromatic associated with freshly cut green grass	*Cis*-3-hexen-1-ol (0.005%)/Green grass (*Pennisetum clandestinum)* 1 shredded 20 mm blade of fresh green grass (*Pennisetum clandestinum*)[e]
	Cooked vegetables	An overall aroma note associated with canned/cooked vegetables	Brine from canned green beans (5%)[f]
	Burnt caramel	Aromatic associated with blackened/acrid carbohydrates	Caramel, natural flavour (0.4%)
TASTE AND MOUTHFEEL	Sweet	Fundamental taste sensation of which sucrose is typical	Sucrose (0.1%)[g]
	Sour	Fundamental taste sensation of which citric acid is typical	Citric acid (0.035%)[g]
	Bitter	Fundamental taste sensation of which caffeine is typical	Caffeine solution (0.03%)[g]
	Astringent	The drying, puckering sensation on the tongue and other mouth surfaces	Alum solution (0.05%)[e]

*For certain sensory attributes (fynbos floral, plantlike, woody and fynbos sweet) no adequate reference standard has yet been found and honeybush tea prepared from specific *Cyclopia* species are recommended for these attributes. Suppliers of the chemicals/products are given in Addendum D. All reference standards prepared using distilled water. [a]Civille & Lyon (1996), [b]Koch (2011),[c]Heisserer & Chambers (1993), [d]Lee & Chambers (2007),[e]Galan-Soldeville *et al.*(2005),[f]Preston *et al.*(2008), [g]ISO 5496:1992.

5. CONCLUSION

The "characteristic" sensory profile of honeybush tea can be described as a combination of floral, sweet, fruity and plantlike flavours with a sweet taste and a slightly astringent mouthfeel. Using DA the six *Cyclopia* species analysed could be divided into three distinct groups based on their sensory properties; group A (*C. sessiliflora, C. intermedia* and *C. genistoides)*, group B (*C. longifolia* and *C. subternata)* and group C (*C. maculata*). Species in the same group had very similar sensory characteristics and it would thus be possible to blend these species together without altering the typical flavour associated with these species. It should, however, be kept in mind that even within a group there are subtle differences between the species. Group A associated with fynbos floral, fynbos sweet and plantlike attributes, group B associated with rose geranium and fruity sweet attributes and group C associated with woody, boiled syrup and cassia/cinnamon attributes. The spicy aroma of *C. maculate* could be explained by the high concentration of the volatile component eugenol which is known to have a warm-spicy, dry aroma. Also, the floral, pine, nutty and woody notes could be explained by the odour active compounds present in *C. maculata.* More research is needed to verify this as these results are based on one *C. maculata* sample.

The variation in the sensory attributes within a specific species, especially in terms of the negative sensory attributes, seems to be due to different processing conditions rather than being species-specific. Most of the samples associated with negative sensory attributes were commercial samples and not necessarily optimally processed. This could have resulted in the emergence of the negative sensory attributes. The fermentation conditions used during processing was not provided for most of the commercial samples and those that were, indicated that the tea was fermented at 70°C for 60 hours or 80-85°C for 16-24 hours. These fermentation conditions are not necessarily optimum fermentation conditions for all the *Cyclopia* species. It is thus highly possible that some of the latter samples might have been over- or even under-fermented. However, further investigation is needed to verify this observation. The honeybush sensory lexicon as well as the sensory wheel consisting of flavour, taste and mouthfeel attributes may well find invaluable application in future research, panellist training, quality control and marketing. These tools are crucial in standardizing the sensory quality of honeybush tea and could assist in identifying niche markets for specific *Cyclopia* species in future.

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CHAPTER 4

THE POLYPHENOLIC COMPOSITION OF SIX *CYCLOPIA* SPECIES AND ITS RELATIONSHIP TO TASTE AND MOUTHFEEL

TABLE OF CONTENTS

1. ABSTRACT

Quantification of the levels of soluble solids, total polyphenol and individual monomeric polyphenolic compounds, as well as the absorbance of the infusions prepared from different batches of six *Cyclopia* species revealed that large variation exist within and between the different *Cyclopia* species. Factors such as locality, climate, soil condition and variation in processing conditions could contribute to this variation. Infusions of *C. genistoides, C. longifolia* and *C. sessiliflora* had the highest soluble solids and total polyphenol content, as well as the highest absorbance values. Only mangiferin, isomangiferin, hesperidin and compound C were detected in all six *Cyclopia* species. Infusions of *C. genistoides, C. longifolia* and *C. sessiliflora* in order of prominence contained the highest concentration of both mangiferin and isomangiferin whereas *C. genistoides* and *C. maculata* contained the highest hesperidin content. These species would thus be ideal for production of extracts containing high levels of these phytochemicals. The bitter taste present in certain *Cyclopia* species appears to be due to the high mangiferin content, however, compounds such as isomangiferin and compound C might also play a role. Hesperidin, considered to be tasteless, also correlated significantly with bitter taste. The impact of other constituents present in honeybush, such as amino acids, polysaccharides, volatile components, their interaction and their influence on the flavour should be considered in future research in order to gain more insight into the relationship between the sensory characteristics and the chemical composition of honeybush tea.

2. INTRODUCTION

Flavour is one of the most important qualities of food products and mainly determines whether a food product is accepted or rejected by the consumer (Dattatreya *et al.,* 2002). Flavour is determined by the result of the combination of the basic tastes and more specific flavour characteristics that arise from the volatile components which enter the nasal passages though the nose and the back of the mouth (Jackson, 2009; Ross, 2009). The four basic taste modalities (sweet, sour, bitter and salty) are perceived within the oral cavity by the taste buds and are associated with components such as sugars, polysaccharides, alcohols, acids, phenolics and nucleic acids (Jackson & Linskens, 2002; Worobey *et al.,* 2006; Jackson, 2009). On the other hand, astringency is a diffuse surface phenomenon characterized by a loss of lubrication which causes dryness of the oral surfaces and a tightening and puckering sensation (Lee & Lawless, 1991; Breslin *et al.,* 1993; Gawell *et al.,* 2000). Sour taste in food products is caused by small, soluble, inorganic cations or organic acids (Ramos Da Conceicao Neta *et al.,* 2007; Jackson, 2009) whereas bitter taste and astringency are elicited by flavonoids, such as flavanols and flavonols (Lesschaeve & Noble, 2005). Unfortunately, it is polyphenolic compounds with their unique biological activities which are responsible for the many health benefits associated with honeybush tea (Joubert *et al.,* 2008a).

Aroma is defined as the fragrance perceived through orthonasal analysis and is determined by the volatile fraction (Ross, 2009). The volatile fraction of food products consist of many compounds however, only a few of these compounds are of significance in determining the aroma (Grosch, 1993). These compounds are known as odour active compounds (Delahunty *et al.,* 2006). Aromas of different qualities can mask or suppress one another, compounds with similar qualities can blend and produce a new aroma

and certain compounds present in concentrations below their odour threshold or which has no odour activity when assessed individually can contribute to the aroma when they are in a mixture (Delahunty *et al.,* 2006).

The identification of specific compounds present in honeybush tea and the variation between the different *Cyclopia* species can be useful for many reasons. Components which influence honeybush flavour could serve as an indication of the quality and flavour characteristics of the herbal infusion. This would make it possible to distinguish between high and low quality tea based on the levels of specific components. Also, if certain flavour impact compounds could be linked to taste and mouthfeel characteristics certain sensory characteristics of a honeybush infusion can possibly be predicted by the levels of these specific compounds.

Joubert *et al.* (2008b) and De Beer & Joubert (2010) investigated the qualitative and quantitative differences in the polyphenolic composition of hot water extracts of fermented *Cyclopia* species. These studies were limited to *C. subternata, C. intermedia, C. genistoides* and *C. sessiliflora.* The volatile components and its effect on the aroma of honeybush infusions (*C. genistoides, C. intermedia* and *C. subternata*) have been studied extensively by Cronje (2010). To date, only hesperetin and eriodictyol of the *Cyclopia* polyphenols have been linked to taste. Hesperetin has been identified as a flavour modulating compound with sweet enhancing properties (Reichelt *et al.,* 2010a; 2010b) whereas eriodictyol possesses bitter masking properties (Ley *et al.,* 2005; Ley, 2008). Both hesperetin (Joubert *et al.,* 2003) and eriodictyol (De Beer & Joubert, 2010) are found in low quantities in honeybush extracts. Ley (2008) reported hesperidin, present in honeybush, as tasteless and its positional isomer, neohesperidin, as strongly bitter.

This study was conducted in order to establish whether significant correlations exist between certain chemical/instrumental parameters and the sensory characteristics of honeybush infusions prepared from six *Cyclopia* species (*C. sessiliflora, C. longifolia, C. genistoides, C. intermedia, C. subternata* and *C. maculata*). For this reason batches were selected to include large variation in composition. The focus of the study was on non-volatile compounds and their link to taste and mouthfeel. The variation in the levels of soluble solids, total polyphenol and phenolic compounds as well as spectrophotometric colour measurements were related to the sensory profiles of the infusions prepared from the different *Cyclopia* species.

3. MATERIALS AND METHODS

3.1. Samples

The honeybush infusions used for this study (n = 57) were the same as those used for descriptive analysis described in Chapter 3.

3.2. Sample preparation

An aliquot (100 mL) of each infusion prepared for descriptive analysis (Chapter 3) was filtered through Whatman No. 4 filter paper and allowed to cool. The soluble solids content was determined and the remaining part of the infusion transferred into several microfuge tubes (2 mL) and stored in a freezer at -18°C until required for further analysis.

3.3. Chemicals

The reagents required for the quantification of the total polyphenol content were Folin-Ciocalteu's phenol reagent (Merck, Cape Town, South Africa), anhydrous sodium carbonate (Saarchem, Gauteng, South Africa) and gallic acid (Sigma Aldrich, St. Louis, USA). Chemicals required for high performance liquid chromatography-diode-array detection (HPLC-DAD) analysis were 99.8% formic acid (BDH, VWR International, Poole, UK), HPLC FarUV gradient grade acetonitrile (BDH) and ascorbic acid (Sigma-Aldrich). Mangiferin (Mg) and naringenin (Nar) were supplied by Sigma-Aldrich whereas hesperidin (Hd), hesperetin (Ht), luteolin (Lut), eriocitrin (ErioTrin), narirutin (NarRut) and eriodictyol (Erio) were supplied by Extrasynthese (Genay, France). A Modulab Water Purification System (Continental Water Systems Corporation, San Antonio, TX, USA), containing in sequence carbon, reverse osmosis and deioniser cartridges, was used for preparation of laboratory grade water which was further treated with a Milli-Q academic water purifier (Millipore, Bedford, MA, USA) to prepare HPLC grade water for preparation of the mobile phase.

3.4. Soluble solids content

The soluble solids (SS) content of the infusions was determined gravimetrically by evaporating 20 mL aliquots of the filtrate of the honeybush infusion, in triplicate, to dryness on a steam bath (Merck) in pre-weighed nickel moisture dishes, followed by oven drying at 100°C for 1 h. The moisture dishes were allowed to cool in a desiccator before re-weighing. The results were expressed in mg/L infusion.

3.5. Total polyphenol content

The total polyphenol (TP) content of the honeybush infusions was determined using a Biotek Synergy HTmultiplate reader (Biotek Instruments, Winooski, USA) as described by Arthur *et al.* (2011). After defrosting the filtrate sample at room temperature the sample was diluted to obtain a soluble solids content of between 0.2 and 0.3 mg/mL in order to obtain absorbance values within the range of the calibration curve. Gallic acid was used to prepare a calibration curve ranging from 1 mg/L to 10 mg/L in the final reaction volume. Twenty µL of each standard, sample and assay control (deionised water) were transferred in triplicate into a clear 96-well flat bottom plate (Greiner Bio-one, LASEC, Cape Town, South Africa). Folin-Ciocalteu's reagent (10 x diluted; 100 µL) and sodium carbonate solution (7.5% w/v; 80 µL) were added followed by mixing using an EppendorfMixMate (Merck). The plates were incubated at 30°C for 2 hours in a temperature-controlled laboratory oven whereafter the absorbance was measured at 765 nm. The TP content was expressed as mg gallic acid equivalents (GAE)/L infusion.

3.6. Individual polyphenolic compounds

An Agilent 1200 system comprising of a quaternary pump, autosampler, on-line degasser, column oven and diode-array detector (Agilent Technologies Inc., Santa Clara, USA) with Chemstation 3D LC software was used for HPLC-DAD analysis. A Zorbac Eclipse XDB-C18 column (150x4.6 mm, 5 µm particle size, Agilent) was used for separation of the compounds at 30°C with acetonitrile (A) and 0.1% formic acid (B) as the

solvents. The flow rate was set to 1 mL/min and the following solvent gradient was used: 0-6 min (12%), 7 min (18%), 14 min (25%), 19 min (40%), 24 min (50%) and 29 min (12%) as described by De Beer & Joubert (2010). Aliquots of stock solutions of each compound were defrosted at room temperature *ca.* half an hour before preparing the working solutions (Mix 1 to 5) (Table 1). The standard mixtures and infusions were filtered using 4 mm Millex-H.V. hydrophilic PVDF 0.45 µm syringe filter devices (Millipore, Bedford, USA) and 2 mL disposable PP syringes into wide-necked HPLC autosampler vials with shell-style inserts and closed with screw caps. Each standard mixture (1-5) was injected at 10 µL and standard mixture 1 also at 15 and 20 mL in order to set up an appropriate standard concentration curve for each compound. The concentration of the compounds in the different mixtures and the correlation coefficients for each of the calibration curves are summarised in Table 1. For the infusions an injection volume of 10 µL was used. Retention times and spectral characteristics were used for peak identification and peak area integrations were performed using Chemstation software. The peak areas of compounds were quantified at 288 nm [compound C (unknown compound), compound D (possibly an eriodictyol glucoside), eriocitrin, compound B (possibly a flavanone glucoside), narirutin, hesperidin, eriodictyol, compound F (possibly a flavanone glucoside), naringenin and hesperetin] and 320 mm [mangiferin, isomangiferin, compound E (unidentified hydroxycinnamic acid derivative), compound A (possibly scolymoside) and luteolin]. Quantification of all compounds, except for compounds A to F and isomangiferin, was carried out using the appropriate standard curves. Compounds A and E were quantified as luteolin equivalents whereas compounds B to D and F were quantified as hesperidin equivalents. Isomangiferin was quantified by means of an absorbance ratio in terms of mangiferin (D. De Beer, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, 2010, personal communication).

3.7. Absorbance as measure of colour

Spectrophotometric measurements of each sample were carried out using a Biotek Synergy HT multiplate reader. After defrosting and mixing the filtrate on a vortex mixer, 100 µL were transferred in triplicate into wells of a clear 96-well flat-bottom microplate (Greiner Bio-one) followed by thorough mixing of the well contents for 30 seconds using an Eppendorf MixMate. Absorbance of the infusion was measured at 10 nm intervals ranging from 370 to 700 nm. Using Gen5 Secure software (Biotek Instruments), values for the integral of the absorbance spectrum across the wavelength range of 370 to 570 nm were obtained, i.e. the Area Under the Curve (AUC) reflecting the “total colour” of the sample. Normalised AUC (AUCnorm) was calculated based on the average SS content of all the samples.

Table 1 Composition of standard mixtures 1 to 5 and correlation coefficients for each calibration curve

	Concentration of compounds in standard mix (µg/mL)							
Standard mix	**ErioTrin**	**NarRut**	**Hd**	**Erio**	**Nar**	**Ht**	**Mg**	**Lut**
Mix 5	0.69	0.26	1.22	0.29	0.27	0.28	3.64	0.14
Mix 4	22.35	8.32	39.59	9.27	8.74	9.19	118.21	4.55
Mix 3	37.82	14.08	66.99	15.69	14.78	15.55	200.05	7.70
Mix 2	61.89	23.04	109.62	25.68	24.19	25.44	327.36	12.60
Mix 1	85.96	32.00	152.25	35.67	33.60	35.33	454.67	17.50
R^2 of calibration curve	0.99995	0.99995	0.99995	0.99995	0.99995	0.99995	0.99992	0.99995

3.8. Data analysis

The data were subjected to analysis of variance (ANOVA) using SAS® softwatre (Version 9.2, SAS Institute Inc, Cay, USA). The Shapiro-Wilk test was used to test for non-normality of the residuals (Shapiro &Wilk, 1965) and in the event of significant non-normality ($p \leq 0.05$) outliers were identified and removed until the data were normally distributed. Principal component analysis (PCA) was performed using XLStat (Version 7.5.2, Addinosoft, New York, USA) to visualize the relationship between the samples and their composition.

4. RESULTS

The mean, standard deviation (SD), minimum, maximum and range values for SS, TP, AUC, AUCnorm and the individual polyphenolic compounds of all the honeybush infusions analysed are summarised in Table 2 whereas the mean values for SS, TP, AUC, AUCnorm and the individual polyphenolic compounds of each individual *Cyclopia* species are summarised in Table 3. The variation between the samples within each species can be viewed in Addendum E. The association between the chemical/instrumental parameters as well as between the individual honeybush samples are displayed on the principal component analysis (PCA) loadings and scores plots (Fig. 1a, b). Table 4 shows the correlation coefficients for the chemical/instrumental variables. Typical chromatograms for the infusions of fermented *C. sessiliflora, C. longifolia, C. genistoides, C. intermedia, C. subternata* and *C. maculata* are shown in Fig. 3.

4.1. Soluble solids content

The average soluble solids content of the infusions differed considerably between the species ranging from 658.33 to 2891.67 mg/L (range of 2233.33 mg/L) (Table 2). The normalised soluble solids content (based on the exact mass of the tea leaves used during preparation of the infusion) gave similar results indicating that the small variation in the mass did not have a significant effect on the soluble solids content of the tea infusions and this parameter was thus excluded during further analysis. The soluble solids content of the infusions of the different species followed the order - *Cyclopia genistoides* ≥ *C. longifolia* ≥ *C. sessiliflora* ≥ *C. subternata* ≥ *C. intermedia* ≈ *C. maculata* (Table 3). Similar results are reflected by multivariate analysis as the soluble solids content is situated on the right hand side of the PCA loadings plot (Fig. 1a) associating predominantly with three of the *Cyclopia* species namely, *C. sessiliflora, C. longifolia* and *C. genistoides* (Fig. 1b). The SS content correlated significantly with the TP ($r = 0.906$), mangiferin ($r = 0.762$) and isomangiferin ($r = 0.873$) contents, as well as with the AUC ($r = 0.907$) (Table 4). Weak correlations with SS content ($r < 0.6$; $p \leq 0.05$) were observed for narirutin, hesperidin and compound C.

4.2. Total polyphenol content

The average total polyphenol content of the infusions was 298 mg GAE/L with a range of 579.72 mg GAE/L (Table 2). Thus, there was considerable variation between the infusions in terms of the total polyphenol content with a maximum of 616.98 and a minimum as low as 37.25 mg GAE/L. Based on the TP content two distinct groups could be identified, i.e. group 1 consisting of *C. sessiliflora, C. longifolia* and *C. genistoides* with the higher mean total polyphenol content and group 2 consisting of *C. intermedia, C. subternata* and *C.*

maculata with the lower mean total polyphenol content (Table 3). On the PCA loadings plot (Fig. 1a) the total polyphenol content is situated very close to the soluble solids content and thus associated with *C. genistoides, C. sessiliflora* and *C. longifolia* (Fig. 1b). The TP content strongly correlated with the SS (r = 0.906) and isomangiferin (r = 0.803) contents and AUC (r = 0.875) (Table 4). Correlation of mangiferin and compound C with total polyphenol content was less than 0.7. Hesperidin, eriocitrin and compound B gave correlations with TP of less than 0.4 ($p \leq 0.05$).

4.3. Absorbance as measure of colour

The average AUC value was 59.02 with a range of 121.92 (Table 2). Similarly to the TP content the species formed two distinct groups, i.e. *C. sessiliflora, C. longifolia* and *C. genistoides* had the higher AUC values while the lower values were observed for *C. intermedia, C. maculata* and *C. subternata* (Table 3). On the PCA loadings plot (Fig. 1a) AUC is situated close to both the SS and TP content and associates with *C. genistoides, C. sessiliflora* and *C. longifolia* (Fig. 1b). The AUC correlates strongly with the SS (r = 0.907), TP (r = 0.875), mangiferin (r = 0.810) and isomangiferin (r = 0.886) contents (Table 4). However, taking into account the variation in the SS content and the effect it has on the AUC value normalisation of AUC values (based on the average SS content of all the samples analysed) was done. However, normalisation did not have a huge effect on the AUC values (Fig. 2). The values lowered slightly, but, the relative differences between the species remained constant. This indicates that the differences in the absorbance values reflect differences in the hue of the colour and not simply the differences in the SS content. Three distinct groups formed with *C. sessiliflora* and *C. genistoides* having the highest AUCnorm values, followed by *C. longifolia, C. intermedia* and *C. maculata* and *C. subternata* having the lowest AUCnorm value (Table 3).

4.4. Individual polyphenolic compounds

All the polyphenolic compounds, except compounds A, B and F, were located on the right side of the PCA loadings plot (Fig. 1a) reflecting a relationship with *C. genistoides, C. longifolia* and *C. sessiliflora* (Fig. 1b). Compounds A and B reflected an association with *C. subternata* whereas compound F reflected an association with *C. intermedia* and *C. maculata* (Fig. 1a,b). Isomangiferin strongly correlated with both mangiferin (r = 0.908) and compound C (r = 0.722) whereas eriocitrin strongly correlated with compound A (r = 0.716) and compound B (r = 0.821; $p \leq 0.05$; Table 4).

Only four of the polyphenolic compounds (mangiferin, isomangiferin, hesperidin and compound C) were detected in all six *Cyclopia* species (Table 3). The mangiferin and isomangiferin contents of *Cyclopia genistoides* were the highest ($p \leq 0.05$) followed by *C. longifolia* and *C. sessiliflora* with *C. intermedia, C. subternata* and *C. maculata* having the lowest contents. *Cyclopia genistoides* and *C. maculata* contained the highest concentration of hesperidin followed by *C. longifolia* whereas *C. sessiliflora, C. intermedia* and *C. subternata* had the lowest concentration. The content of compound C in infusions of *C. genistoides* and *C. sessiliflora* was significantly ($p \leq 0.05$) higher than that of the other species. Their contents did not differ significantly ($p > 0.05$). The lowest content was observed for *C. maculata*.

Eriocitrin was not detected in *C. genistoides* and *C. intermedia (*Table 3). *Cyclopia sessiliflora* and *C. subternata* had the highest eriocitrin content followed by *C. longifolia.* Eriocitrin was detected in *C. maculata* at low concentration. Narirutin was only detected in one of the six *Cyclopia* species, namely *C.*

longifolia, while compound A was only detected in *C. longifolia* and *C. subternata.* *Cyclopia subternata* contained approximately three times as much narirutin than *C. longifolia.* Compound B was detected in the same two species as well as *C. sessiliflora*. *Cyclopia sessiliflora* had the highest concentration of compounds B followed by *C. subternata* and *C. longifolia.* Compound F was only present in *C. intermedia*.

4.5. The relationship between the chemical composition and sensory attributes

Sour taste significantly ($p \leq 0.05$) correlated with the SS ($r = 0.363$), TP ($r = 0.373$), isomangiferin ($r = 0.315$), compound B ($r = 0.261$) and compound C ($r = 0.301$) contents and AUC ($r = 0.446$; $p \leq 0.05$). The strongest correlation between bitter taste and composition was for mangiferin ($r = 0.740$). Furthermore, the SS ($r = 0.530$), TP (0.455), isomangiferin ($r = 0.623$), hesperidin ($r = 0.299$) and compound C ($r = 0.445$) contents and AUC ($r = 0.632$) also correlated significantly but to varying extents with bitter taste. A significant but weak negative correlation was observed between eriocitrin and bitter taste ($r = -0.292$). There was no compositional parameter which had a significant positive correlation with sweetness, however, significant negative correlations between sweet taste and SS ($r = -0.485$), TP ($r = -0.463$), mangiferin ($r = -0.568$), isomangiferin ($r = -0.544$) and compound C ($r = -0.271$) contents and AUC ($r = -6.24$) were observed (Table 5). A general trend can be identified; all those compositional parameters strongly correlating with bitter taste correlates negatively with sweet taste. There was no strong correlation between astringency and the compositional parameters, however, weak, but significant, correlations of astringency with SS ($r = 0.337$), TP ($r = 0.278$), mangiferin ($r = 0.392$) and isomangiferin ($r = 0.384$) contents, as well as AUC ($r = 0.466$) were observed.

Table 2 Average values, standard deviation, minimum, maximum and range for the concentrations of SS, TP and the individual polyphenolic compounds of the honeybush infusions analysed, as well as AUC and AUCnorm values.

	SS	SSnorm	TP	AUC	AUCnorm	Mg	IsoMg	ErioTrin	NarRut	Hd	Comp A	Comp B	Comp C	Comp F
Mean	1799.60	1801.29	298.64	59.02	56.54	48.71	23.13	2.07	0.11	6.90	2.40	1.78	9.65	0.22
SD	577.66	577.59	156.95	27.34	12.48	58.91	18.79	2.50	0.42	4.30	4.81	2.94	12.88	0.66
Minimum	658.33	657.75	37.25	15.64	28.67	0.00	1.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	2891.67	2889.10	616.98	137.56	93.03	201.03	66.25	7.38	1.80	17.54	18.80	10.24	51.91	3.88
Range	2233.33	2231.35	579.72	121.92	64.35	201.03	64.41	7.38	1.80	17.54	18.80	10.24	51.91	3.88

SD = Standard deviation; SS = Soluble solids, SSnorm = SS normalised, TP = Total polyphenol, AUC = Area under the curve (370 – 570 nm), AUCnorm = AUC normalised, Mg = Mangiferin; IsoMg = Isomangiferin; ErioTrin = Eriocitrin; NarRut = Narirutin; Hd = Hesperidin; Comp A = Compound A; Comp B = Compound B; Comp C = Compound C; Comp F = Compound F. All chemical/instrumental analysis provided in mg/L except for SSnorm, AUC, AUCnorm and TP (mg GAE/L).

Table 3 Mean values for the concentrations of SS, TP and the individual polyphenolic compounds for each *Cyclopia* species

	Means																							
Species	**SS**		**TP**		**AUC**		**AUCnorm**		**Mg**		**IsoMg**		**ErioTrin**		**NarRut**		**Hd**		**Comp A**		**Comp B**		**Comp C**		**Comp F**	
Ses	1957.10	bc	425.98	a	71.15	a	63.88	a	42.43	bc	29.55	b	5.07	a	0.00	b	5.85	bc	0.00	c	6.86	a	24.02	a	0.00	b
Lon	2301.00	ab	385.33	a	74.00	a	53.71	b	64.31	b	35.30	b	2.87	b	0.95	a	7.97	ab	3.03	b	1.58	c	6.03	b	0.00	b
Gen	2383.20	a	428.10	a	89.45	a	65.29	a	150.63	a	47.95	a	0.00	c	0.00	b	9.99	a	0.00	c	0.00	c	24.02	a	0.00	b
Int	1400.00	d	218.83	b	48.28	b	55.54	b	17.40	d	13.60	c	0.00	c	0.00	b	4.43	c	0.00	c	0.00	c	3.91	b	1.17	a
Sub	1611.70	cd	230.55	b	37.73	b	41.44	c	2.44	d	5.24	c	4.82	a	0.00	b	3.02	c	10.72	a	4.04	b	2.37	b	0.00	b
Mac	1384.20	d	180.87	b	43.39	b	54.22	b	18.45	cd	13.89	c	1.06	c	0.00	b	10.16	a	0.00	c	0.00	c	1.44	b	0.00	b

Data marked with different letters in the same column were significantly different at p=0.05. Ses = *C. sessiliflora;* Lon = *C. longifolia*; Gen = *C. genistoides*; Int = *C. intermedia*; Sub = *C. subternata;* Mac = *C. maculata;* SS = Soluble solids; TP = Total polyphenol; AUC = Area under the curve (370 – 570 nm); AUCnorm = AUC normalised; Mg = Mangiferin; IsoMg = Isomangiferin; ErioTrin = Eriocitrin; NarRut = Narirutin; Hd = Hesperidin; Comp A = Compound A; Comp B = Compound B; Comp C = Compound C; Comp F = Compound F. All chemical/instrumental analysis provided in mg/L except for AUC, AUCnorm and TP (mg GAE/L).

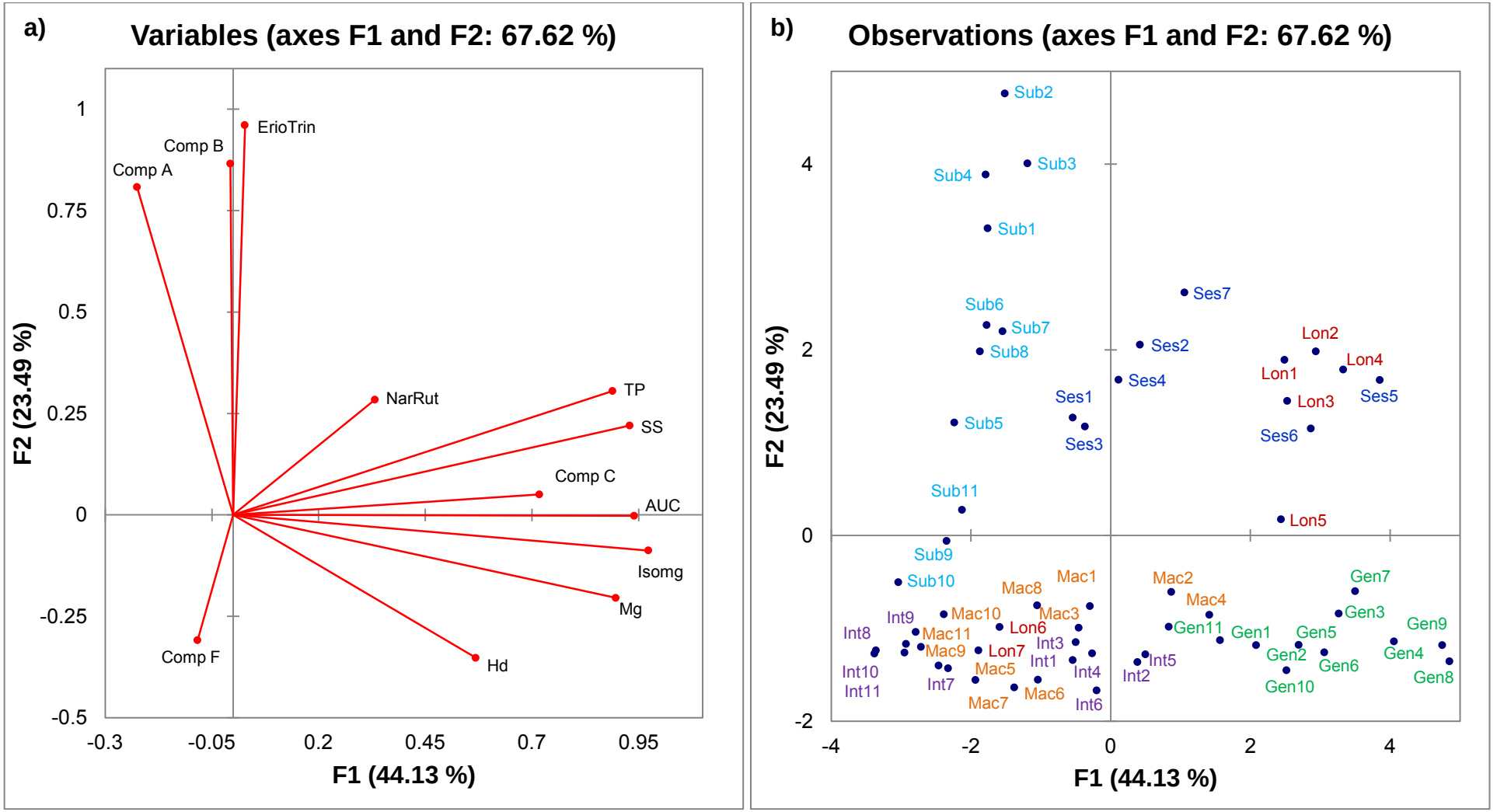

Figure 1 a) PCA loadings plot showing the positioning of the chemical/instrumental parameters. SS = Soluble solids; TP = Total polyphenol; AUC = Area under the curve (370 – 570 nm); Mg = Mangiferin; IsoMg = Isomangiferin; ErioTrin = Eriocitrin; NarRut = Narirutin; Hd = Hesperidin; Comp A = Compound A; Comp B = Compound B; Comp C = Compound C; Comp F = Compound F. b) PCA scores plot showing the positioning of the 72 honeybush samples. Ses = *C. sessiliflora;* Lon = *C. longifolia*; Gen = *C. genistoides*; Int = *C. intermedia*; Sub = *C. subternata;* Mac = *C. maculata.*

Table 4 Correlation coefficients for the chemical/instrumental variables

Variables	SS	TP	AUC	AUCnorm	Mg	IsoMg	ErioTrin	NarRut	Hd	Comp A	Comp B	Comp C	Comp F
SS	**1**												
TP	**0.906***	**1**											
AUC	**0.907***	**0.875***	**1**										
AUCnorm	0.467*	0.554*	**0.759***	1									
Mg	**0.762***	0.698*	**0.810***	0.593*	**1**								
IsoMg	**0.873***	**0.803***	**0.886***	0.645*	**0.908***	**1**							
ErioTrin	0.23	0.315*	0.03	-0.223	-0.221	-0.053	**1**						
NarRut	0.386*	0.325*	0.241	0.056	0.19	0.375*	0.271*	**1**					
Hd	0.448*	0.354*	0.540*	0.465*	0.488*	0.516*	-0.251	0.049	**1**				
Comp A	0.053	0.018	-0.207	-0.498*	-0.280*	-0.302*	**0.716***	0.143	-0.370*	**1**			
Comp B	0.126	0.295*	0.014	-0.127	-0.22	-0.109	**0.821***	0.018	-0.282*	0.536*	**1**		
Comp C	0.591*	0.608*	0.552*	0.420*	0.655*	**0.722***	0.057	0.007	0.215	-0.225	0.165	**1**	
Comp F	-0.014	-0.001	0.022	0.078	-0.14	-0.06	-0.301*	-0.098	-0.189	-0.181	-0.221	-0.105	**1**

*Values marked with an asterix are significantly different from 0 with a significance level of p = 0.05. Values in bold are higher than ± 0.7. SS = Soluble solids; TP = Total polyphenol; AUC = Area under the curve (370 – 570 nm); AUCnorm = AUC normalised; Mg = Mangiferin; IsoMg = Isomangiferin; ErioTrin = Eriocitrin; NarRut = Narirutin; Hd = Hesperidin; Comp A = Compound A; Comp B = Compound B; Comp C = Compound C; Comp F = Compound F.

Table 5 Correlation coefficients (r) for the taste and mouthfeel attributes and the chemical/instrumental variables

Variables	SS	TP	AUC	Mg	IsoMg	ErioTrin	NarRut	Hd	Comp A	Comp B	Comp C	Comp F
TSour	0.363*	0.373*	0.446*	0.237	0.315*	0.225	-0.094	0.092	-0.088	0.261*	0.301*	-0.080
TBitter	0.530*	0.455*	0.632*	**0.740***	0.623*	-0.292*	0.074	0.299*	-0.201	-0.242	0.445*	-0.009
TSweet	-0.485*	-0.463*	-0.624*	-0.568*	-0.544*	0.112	0.033	-0.157	0.228	0.135	-0.271*	-0.109
TAstringent	0.337*	0.278*	0.466*	0.392*	0.384*	-0.049	-0.083	0.043	-0.189	0.028	0.214	0.044

*Values marked with an asterix are significantly different from 0 with a significance level of p=0.05. Values in bold are higher than ± 0.7. SS = Soluble solids content, TP = Total polyphenol content, AUC = Area under the curve (370 – 570 nm), Mg = Mangiferin, IsoMg = Isomangiferin, ErioTrin = Eriocitrin, Comp B = Component B, NarRut = Narirutin, Hd = Hesperidin, Comp A = Component A, Comp C = Component C, and Comp F = Component F.

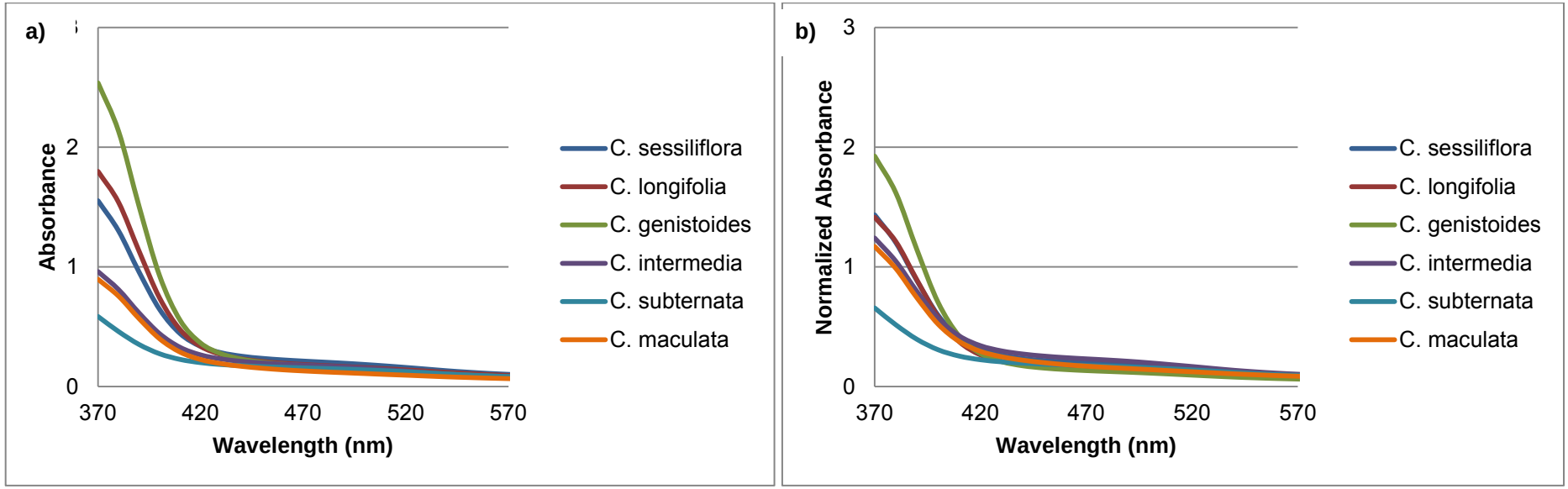

Figure 2 Absorbance values for *Cyclopia* species (*C. sessiliflora, C. longifolia, C. genistoides, C. intermedia, C. subternata* and *C. maculata*). a) Average values and b) normalised according to the soluble solids content.

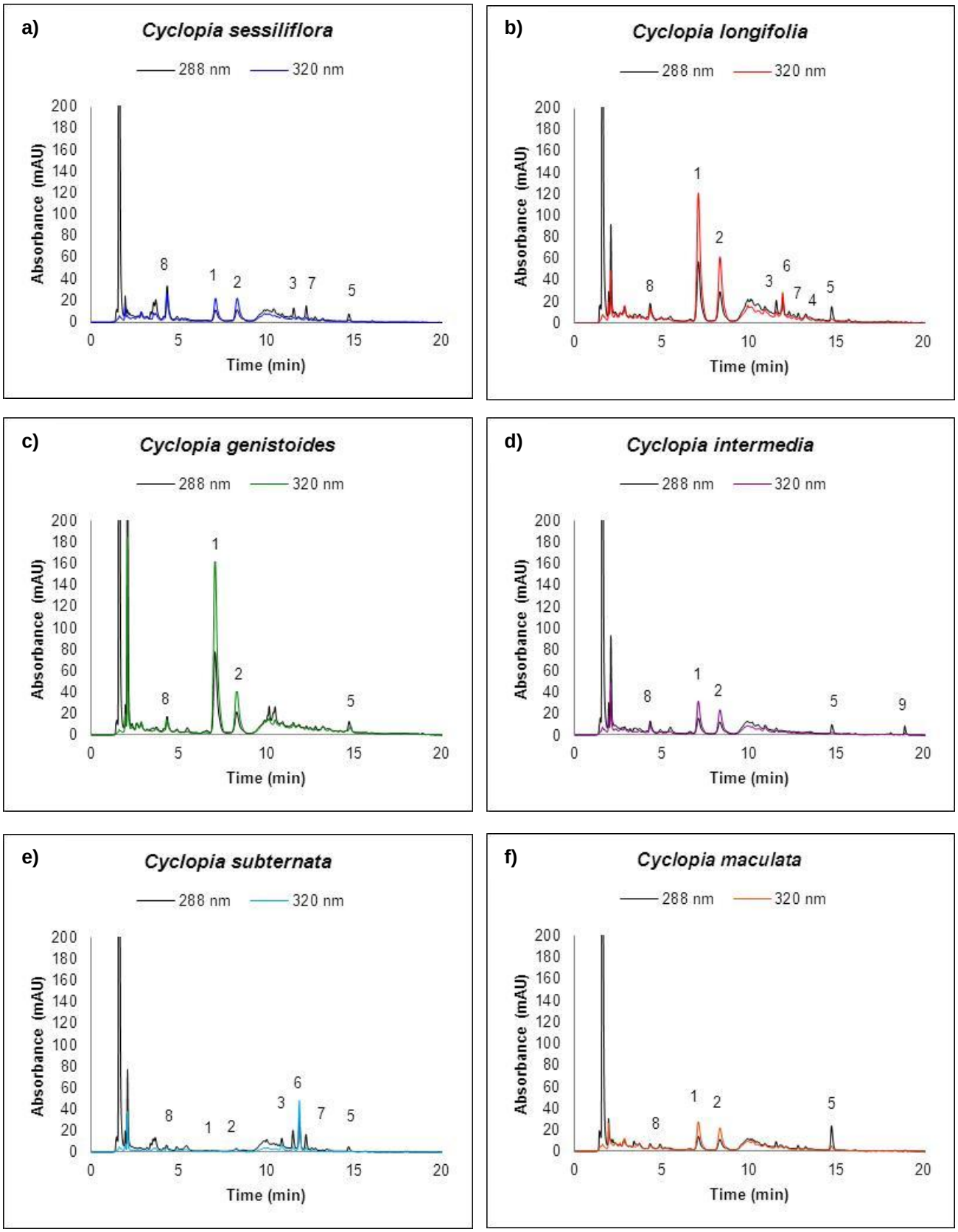

Figure 3 HPLC-DAD profiles for fermented *C. sessiliflora* (a), *C. longifolia* (b), *C. genistoides* (c), *C. intermedia* (D), *C. subternata* (E) and *C. maculata* (F). 1 = Mangiferin, 2 = Isomangiferin, 3 = Eriocitrin, 4 = Narirutin, 5 = Hesperidin, 6 = Compound A, 7 = Compound B, 8 = Compound C, 9 = Compound F.

5. DISCUSSION

5.1. Variation in the chemical composition

The large variation in the SS, TP, AUC and polyphenolic profiles of the six *Cyclopia* species was not unexpected as Joubert *et al.* (2003; 2008b) and De Beer and Joubert (2010) reported qualitative and quantitative differences between certain *Cyclopia* species. This large variation between the species could possibly be due to the different localities, climates, soil conditions, survival strategies (Schutte, 1997), the age of plant/regrowth (Joubert *et al.,* 2011), the presence of flowers/pods (Du Toit & Joubert, 1998) and the leaf-to-stem ratio (E. Joubert, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, 2010, personal communication). However, investigation into each of these factors would be necessary to confirm this. In addition to these factors the large variation in the chemical/instrumental parameters between and within each specie could also be due to the different processing conditions, in particular the fermentation temperature and time combinations used as it is well known that extensive heat treatments during fermentation have a detrimental effect on the levels of SS, TP and individual polyphenolic compounds (Du Toit & Joubert, 1999; Joubert *et al.,* 2008b). The long fermentation and or/high fermentation temperatures used by industry (70°C/60 h, 80-85°C/≥16 h) would have contributed to the much lower levels of SS, TP and the individual polyphenolic compound contents. For example, Int9, Int10 and Int11 (Addendum A) were fermented by industry and all three infusions had very low TP and specific polyphenolic compounds contents (Addendum E). Also, certain samples had a higher stem content resulting in a lower TP content. Normalisation of the AUC values (based on the SS content) indicated that the differences in the absorbance values reflect differences in the hue of the colour and not simply the differences in the SS content.

Infusions of *C. genistoides* not only had the highest concentration of the xanthones, mangiferin and isomangiferin, as previously reported (Joubert *et al.,* 2008b; De Beer & Joubert, 2010) but, together with *C. maculate,* also had the highest hesperidin content (De Beer & Joubert, 2010). Their investigation was limited to *C. genistoides, C. sessiliflora, C. intermedia* and *C. subternata. Cyclopia subternata* contained the lowest concentration of these three polyphenolic compounds, while being one of the two species containing compound A.

5.2. The relationship between the chemical composition and sensory attributes

Sour taste in food products is caused by small, soluble, inorganic cations and not by polyphenolic compounds (Jackson, 2009) which could explain the low correlation values between the polyphenolic compounds and sour taste. However, it has been reported that certain organic acids such phenolic acids have acidic or sour taste characteristics (Huang & Zayas, 1991; Peleg & Noble, 1995; Ramos Da Conceicao Neta *et al.*, 2007). According to Ramos Da Conceicao Neta *et al.* (2007) sour taste intensity is related to the total molar concentration of all organic acid species that have more than one protonated carboxyl groups plus the concentration of the free hydrogen ions. The phenolic acids *p*-coumaric and shikimic acid have been previously identified in *Cyclopia* species (Kamara*et al,* 2003; Ferreira *et al.,* 1998) but they have not been detected in the infusions. Previously the sour taste was significantly correlated to lemon and plantlike (green) sensory attributes (Chapter 3) indicating that a sour taste in honeybush tea may be related to the compounds responsible for the plantlike (green) flavour associated with under fermented honeybush tea.

Bitter taste is elicited by structurally diverse compounds and no clear definition of the molecular properties that confer bitterness has yet been proposed (Lesschaeve & Noble, 2005). According to Lesschaeve & Noble (2005) bitterness (and astringency) is elicited by flavonoid phenols such as flavanols and flavonols. Several of these types of compounds were present in varying concentration in the infusions. The chemical nature of the flavonoid phenols depends on structural class, degree of hydroxylation, other substituents and conjugations as well as the degree of polymerisation which in turn have an effect on its taste properties (Aherne & O'Brien, 2002; Ley, 2008). Some flavonoids are very bitter whereas others are not, depending on the type of glycoside chain (Drewnowski & Gomez-Carneros, 2000). Naringenin (flavanone neohesperidoside) and neohesperidin (hesperetin neohesperidoside) are very bitter whereas hesperidin (hesperetin rutinoside) is tasteless (Ley, 2008). Both eriocitrin and narirutin, similar to hesperidin, contain a rutinoside chain indicating that they might also be tasteless. Some of the flavanones in honeybush tea might thus very well be responsible for the bitter taste. It is quite interesting that hesperidin correlated significantly to bitter taste as this specific compound has been reported as tasteless by Ley (2008).

The moderate significant correlation between bitter taste and the xanthone, mangiferin (r = 0.740), indicates that this specific polyphenolic compound might be responsible for bitter taste in honeybush infusions. *Cyclopia genistoides,* the species with the highest mangiferin content, was shown to be significantly more bitter compared to the other *Cyclopia* species (Chapter 3). It is also possible that certain compounds, such as eriodictyol, might act as taste modulators. Hesperetin have previously been identified as a sweet enhancing flavanone whereas eriodictyol have been identified as a bitter masking compound (Reichelt *et al.,* 2010a, b).

It is also possible that the bitter taste could be due to the specific amino acids present as it is known that certain amino acids (in the L-form) such as leucine, phenylalanine, tryptophan and tyrosine have a bitter taste (Solms, 1969). As previously mentioned, a general trend could be identified; all those compositional parameters strongly correlating to bitter taste correlates negatively to sweet taste. It is known that slight changes to the structure of many sweet and bitter tasting compounds can cause a change in their taste quality from sweet to bitter and vice versa (Jackson, 2009). For example; the amino acids previously mentioned to have a biter taste have a sweet taste in its D-form (Solms, 1969).

There was no strong correlation between astringency and the compositional parameters. The weak, but significant correlations between astringency and the SS (r = 0.337), TP (r = 0.278), mangiferin (r = 0.392) and isomangiferin (r = 0.384) content respectively indicate that these compositional parameters might play a role in the mouthfeel of honeybush infusions. The weak correlations could perhaps be due to the fact that there was not a lot of variation between the astringency of the honeybush infusions analysed or it is possible that the panel could have confused astringency for bitterness which according to Lea & Arnold (1973) often happens. It might also be an indication that the chemical parameters considered in this study do not represent the compounds responsible for the subtle astringency of honeybush. Whether a specific compound exhibit astringency or not, depends on the structural characteristics of the compound. In general astringent polyphenols are intermediately sized with molecular weights of 500-3000 Da (Bakker, 1998) but smaller compounds, such as flavan-3-ol monomers, dimers and trimers, have also been shown to elicit astringency (Naish *et al.,* 1993; Peleg *et al.,* 1998). Scharbert *et al.* (2004) reported that astringency in black tea (*Camellia sinensis*) was due to the presence of a series of flavonol glycosides and not high molecular mass polyphenolic compounds. McManus *et al.* (1981) proposed that for a phenolic compound to elicit an astringent sensation it must possess two adjacent hydroxyl groups, a structural requirement fulfilled by the

xanthones. However, eriocitrin, which also possess two adjacent hydroxyl groups, did not significantly correlate with astringency, nor did compound A (tentatively as the flavone scolymoside) which also possess two adjacent hydroxyl groups. The presence of flavan-3-ol epigallocatechingallate (EGCG) which has previously identified in unfermented *C. subternata* (Kamara *et al.,* 2004) contributes to the astringency in black tea (*Camellia sinensis)* (Scharbert *et al.,* 2004; Scharbert & Hofmann,2005). However, no EGCG was detected in the infusions of fermented *C. subternata.*

It is known that honeybush tea has a low tannin content (Greenish, 1881; Marloth, 1925; Terblanche, 1982) and thus the very low average score obtained by descriptive analysis for astringency is not unexpected. It is possible that the quantification of the tannin content could reveal more insight into the astringency of honeybush tea but the levels were so low in the honeybush infusions used for this study that it was unquantifiable by the MCP tannin assay (unpublished results). This assay was developed for red wine analysis (Mercurio *et al.,* 2007). By using another method of tannin quantification it might be possible to precipitate and quantify those compounds associated with astringency. Koch (2011) found no correlation between the astringency of rooibos (*Asphalatuslinearis*) and the tannin content as determined using the MCP assay.

Based on the analysis used during this study the only conclusion in terms of taste which can be made is that bitterness might be caused by the xanthone, mangiferin, and that a relationship between the xanthones and astringency exist. The sour taste and more importantly the sweet taste of honeybush tea could not be explained by the phenolic composition of the infusions and further analysis of the chemical composition of honeybush infusions would be required in order to identify the compounds responsible.

6. CONCLUSION

Quantification of the levels of soluble solids, total polyphenol, absorbance as indication of colour and nine polyphenolic compounds in infusions of different batches of six *Cyclopia* species revealed that large variation exist within and between the different *Cyclopia* species. This large variation between the species could be due to many different factors, such as different localities, climates, soil conditions, survival strategies, the age of plant/regrowth, the presence of flowers/pods, the leaf-to-stem ratio and different fermentation temperature/time combinations used during processing. Mangiferin, isomangiferin, hesperidin and compound C were the only polyphenolic compound detected in all six *Cyclopia* species whereas eriocitrin, narirutin, compound A, B and C was only detected in some of the *Cyclopia* species. *Cyclopia genistoides* had the highest content of the xanthones whereas both *C. genistoides* and *C. maculata* had the highest hesperidin content. These two species would thus be ideal for the production of extracts containing high levels of mangiferin, isomangiferin and hesperidin.

Large variation in the composition of honeybush tea samples reflected large variation in the sensory quality of the herbal infusions, especially in terms of taste and mouthfeel. The correlation coefficients indicated that bitter taste was related to the TP content as well as the mangiferin, isomangiferin, compound C and hesperidin content. Unfortunately, it is these three polyphenols (mangiferin, isomangiferin and hesperidin) with their unique biological activities which are responsible for the many health benefits associated with honeybush tea. Not surprisingly, *C. genistoides*, a species identified as bitter (Chapter 3), had the highest mean SS, TP, mangiferin and isomangiferin contents as well as the highest mean AUC value. Additionally, *C. genistoides* also contained high concentrations of hesperidin and compound C.

Based on the high levels of mangiferin in *C. genistoides* and the strong significant correlation between mangiferin and bitter taste it appeared as if this compound might in fact be responsible for the bitter taste associated with this species. Plant improvement studies are currently focussing on selections based on phytochemical content. This could cause problems with regards to the sensory quality of the tea if the taste characteristics of the specific compounds are not taken into account.

No specific compound could be linked to the sweet taste of the infusions, however, sweetness was significantly negatively correlated to all components associated with bitterness. Astringency seemed to be caused by the xanthones, possibly due to the presence of two adjacent hydroxyl groups. It is possible that the tannin content may also play a role in astringency, however, the tannin content was unquantifiable by the MCP tannin assay. The effect certain taste modulating compounds, such as hesperetin and eriodictyol, could have on the taste of honeybush infusions should also be kept in mind. Additional research is needed to confirm the effect specific polyphenolic compounds have on the taste and mouthfeel of honeybush infusions. The impact of other components present in honeybush, such as amino acids, polysaccharides, volatile components and the interaction between these components, and their influence on the flavour should be considered in future research in order to obtain a more comprehensive picture of the effect the chemical composition has on the flavour of honeybush tea.

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Chapter 5

EFFECT OF FERMENTATION TEMPERATURE AND TIME ON THE SENSORY PROFILE, POLYPHENOLIC COMPOSITION AND COLOUR OF HONEYBUSH INFUSIONS

TABLE OF CONTENTS

1. ABSTRACT

The effect of fermentation temperature (80°C and 90°C) and time (8 h, 16 h, 24 h and 32 h) on the sensory characteristics of infusions of honeybush was investigated in order to establish the optimum fermentation conditions. The effect of fermentation conditions on the polyphenolic composition and the effect these changes have on the taste and mouthfeel properties of honeybush tea were determined. This was achieved by examining the changes in the soluble solids and total polyphenol content, instrumental colour and the concentration of specific polyphenolic compounds, such as mangiferin, isomangiferin and hesperidin of the infusions. Fermentation resulted in an increase (positive sensory attributes) and decrease (negative sensory attributes) of sensory attributes rather than the formation of new sensory attributes. In order to produce honeybush tea with an optimal sensory profile a fermentation period of 80°C/24 or 90°C/16 h is required for *C. genistoides, C. subternata* and *C. maculata*. Fermenting *C. genistoides* at 90°C would result in a honeybush infusion with slightly less rose geranium notes. *Cyclopia subternata* can be fermented at either 80°C or 90°C, depending on whether a floral or apricot jam tea is desired. *C. maculata* required a fermentation temperature of 80°C as fermentation at 90°C results in an increase of negative sensory attributes (hay/dried grass aroma and flavour and green grass aroma). A fermentation time period of 24 h are required for *C. maculata* in order to effectively reduce the intensity of the negative sensory attributes Fermentation reduced the soluble solids content, total polyphenol content and the concentration of the polyphenolic compounds. Absorbance, as a measure of colour, decreased with increasing fermentation temperature and time, reflecting the change in the polyphenolic composition. Changes in the taste and mouthfeel of honeybush tea could be attributed to changes in polyphenolic composition caused by high temperature oxidation. A significant correlation between the polyphenolic compounds, specifically the xanthones, mangiferin and isomangiferin, and bitter taste existed in *C. genistoides* whereas these compounds appeared to be correlated to astringency in *C. subternata*. The concentration of these compounds appear to be important as *C. subternata* contains a fraction of that present in *C. genistoides*. *Cyclopia maculata* on the other hand revealed a relationship between astringency and hesperetin.

2. INTRODUCTION

Chemical oxidation, more commonly referred to as “fermentation”, is responsible for the characteristic dark brown colour and the sweet, honeylike flavour of honeybush tea (Du Toit & Joubert, 1998a). Traditionally, fermentation heaps (Marloth, 1909; Marloth, 1925) or baking ovens (Hofmeyer & Phillips, 1922) were used for fermentation, followed by sun-drying (Du Toit *et al.,* 1998). These traditional processing methods did not allow for control of the processing parameters and problems with mould and bacterial growth, as well as under- and unfermented tea resulted in honeybush tea of poor quality (Du Toit & Joubert, 1998a). More than a decade ago these challenges were addressed by Du Toit and Joubert (1999) and elevated temperatures (> 60°C) were introduced in order to eliminate microbial contaminants (Du Toit *et al.,* 1999) and to produce tea of consistent high quality. Rotation drums for fermentation and drying is used in industry (Joubert *et al.,* 2011).

Du Toit and Joubert (1999) investigated the effect of different fermentation temperature and time combinations for *C. intermedia* and *C. buxifolia* (previously classified as *C. maculata*) (Schutte, 1997) showing that the development of the optimum characteristic sweet, honeylike flavour, with no grassy

undertones, depended on the fermentation-time combinations, with higher temperatures requiring shorter times. Honeybush fermented at 60°C, 70°C and 80°C was still under-fermented after 24-36 h and possessed an unpleasant grassy flavour whereas fermentation for longer than 36 h at 90°C resulted in a burnt flavour. The changes in sensory properties with fermentation were accompanied by a decrease in the levels of soluble solids, total polyphenol and individual polyphenolic compounds such as mangiferin, isomangiferin and hesperidin (Du Toit & Joubert, 1999; Joubert *et al.,* 2008).

Fermentation at 70°C for 60 h and 90°C for 36 h was selected as the optimum fermentation conditions for these two *Cyclopia* species. Currently industry employs fermentation conditions ranging from 70°C/60 h for *C. intermedia* and 80-85°C/18-24 h for other *Cyclopia* species such as *C. genistoides* and *C. subternata* (Joubert *et al.,* 2011), whereas the Agricultural Research Council (ARC, Infruitec-Nietvoorbij, Stellenbosch, South Africa) ferments honeybush, for research purposes, at 90°C/16 h (E. Joubert, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, 2010, personal communication).

Until recently, exports consisted mainly of *C. intermedia,* but as the demand for honeybush tea increased, both locally and internationally, the focus, due to necessity, shifted to other *Cyclopia* species (Joubert *et al.,* 2011). The fear of over-exploitation of the natural *Cyclopia* populations also played a role in the establishment of commercial plantations to lessen the pressure on the natural *Cyclopia* populations (De Lange & von Mollendorff, 2006). Currently, *C. intermedia, C. genistoides* and *C. subternata* are the three main species being utilized commercially (Joubert *et al.,* 2011). The potential to cultivate *C. longifolia, C. sessiliflora* and *C. maculata* is currently under investigation. It has become necessary to re-evaluate the fermentation temperature-time combination used in terms of sensory properties, chemical composition and extract colour of honeybush infusions in order to produce honeybush tea of optimum quality.

The objective of this study was thus to determine the effect of different fermentation temperature-time combinations on the sensory characteristics of three *Cyclopia* species (*C. genistoides, C. subternata* and *C. maculata*) and, thereby, to establish optimum fermentation conditions for each. Changes in phenolic composition were also determined in order to establish whether certain changes in the composition could be linked to changes in taste and mouthfeel attributes of the honeybush infusions.

3. MATERIALS AND METHODS

3.1. Preparation of fermented material

Three batches plant material of each of the *Cyclopia* species, *C. genistoides, C. subternata* and *C. maculata,* were harvested from different locations in the Western Cape Province of South Africa during 2010 and 2011 (Table 1). More than one plant per species was harvested and pooled to form a batch. The plants were harvested by removing two thirds of the shoot lengths. The shoots from each batch (±13 kg) were cut to 2-3 mm lengths using a mechanised fodder cutter, divided into eight parts (1.5 kg each, one for each temperature/time combination) and placed in stainless-steel containers. Deionised water (250 mL) was added to the shredded plant material and thoroughly mixed before sealing the containers with aluminium foil. The samples were placed in preheated CAL 3200 temperature controlled laboratory ovens (CAL Controls Ltd., UK) at 80 and 90°C. Every 8 h (8 h, 16 h, 24 h and 32 h) two containers (one from each oven) were removed and dried by spreading the contents onto four 30 mesh stainless steel drying racks and drying it in a temperature-controlled dehydration tunnel (Continental Fan Works, Parrow, South Africa) at 40°C for 6 h to

a moisture content of less than 10%. The dried tea was sieved (200 g/30 s at 90 rpm) using a mini-sifter (SMC, Cape Town) and the <12>40 mesh fraction collected. The fractions were stored in sealed glass jars at room temperature until needed. The experimental design can be viewed in Table 2 and consists of eight treatments and three blocks for each of the three *Cyclopia* species. Each block represents a temperature/time combination. Each sample was analysed in triplicate.

3. 2. Preparation of infusions

The infusions of the samples for descriptive analysis and chemical/instrumental analysis were prepared as described in Chapter 3.

3.3. Descriptive analysis

Descriptive analysis was conducted as described in Chapter 3. For this part of the study additional twelve one-hour training sessions were introduced (four per species) in order to select relevant sensory attributes (positive and negative) for each species (Table 3) from the sensory wheel. Nine judges rated the intensity of the selected aroma, flavour, taste and mouthfeel attributes for each of the samples. Each sample was analysed in triplicate on the same day and Compusense® *five* (Compusnse, Guelph, Canada) were used to capture the data

Table 1 Sample information for each *Cyclopia* species

Specie	Batch	Harvesting date	Area (Farm)	Source
C. genistoides	a	24.11.2010	Bredasdorp (Toekomst)	Cultivated
	b	01.12.2010	Pearly Beach (Koksrivier)	Cultivated
	c	29.11.2010	Pearly Beach (Koksrivier)	Cultivated
C. subternata	a	09.12.2010	Stellenbosch (Helderfontein)	Cultivated
	b	18.01.2011	Barrydale (Kanetberg)	Cultivated
	c	07.02.2011	Napier (Tolbos)	Cultivated
C. maculata	a	03.05.2010	Riviersonderend (Boskloof)	Wild
	b	28.06.2010	Riviersonderend (Boskloof)	Wild
	c	15.11.2010	Riversdale (Romansrivier)	Wild

Table 2 Illustration of the experimental design for fermentation-temperature combinations used for each of the *Cyclopia* species

Time	**8 h**		**16 h**		**24 h**		**32 h**	
Temperature	**80°C**	**90°C**	**80°C**	**90°C**	**80°C**	**90°C**	**80°C**	**90°C**
Batch a	1	2	3	4	5	6	7	8
Batch b	1	2	3	4	5	6	7	8
Batch c	1	2	3	4	5	6	7	8

3.4. Chemicals

The chemicals and reagents required for this study are identical to those listed in Chapter 4. Acetic acid (2 %) (Fluka, Sigma-Aldrich, Steinheim, Germany) was used as HPLC mobile phase B instead of formic acid (0.1%) to improve quantification of the xanthones in *C. subternata* (De Beer & Joubert, 2010).

3.5. Chemical/Instrumental analyses

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

3.6. Data analysis

The sensory and chemical/instrumental data were subjected to analysis of variance (ANOVA) using SAS® software (Version 9.2, SAS Institute Inc, Carry, USA). The Shapiro-Wilk test was used to test for non-normality of the residuals (Shapiro & Wilk, 1965) and in the event of significant non-normality ($p \leq 0.05$) outliers were identified and removed until the data were normally distributed. The student's t-least significant difference (LSD) was calculated at the 5% significance level to compare treatment means. Principal component analysis (PCA) was performed using XLStat (Version 7.5.2, Addinosoft, New York, USA) to visualise the relationship between the samples and their attributes.

Table 3 Selected sensory attributes for *C. genistoides, C. subternata* and *C. maculata*

Species	***Cyclopia genistoides***	***Cyclopia subternata***	***Cyclopia maculata***
Positive attributes	Fynbos floral, Rose geranium, Lemon, Apricot jam, Plant-like, Woody, Fruity sweet, Boiled syrup, Fynbos sweet, Spicy	Fynbos floral, Rose Geranium, Rose/Perfume, apricot jam, Cherry essence, Plant-like, Woody, Rooibos woody, Fruity sweet, Caramel, Fynbos sweet, Spicy, Walnut	Fynbos floral, Rose Geranium, Rose/Perfume, Plant-like, Woody, Pine, Fruity sweet, Boiled syrup, Fynbos sweet, Spicy, Walnut
Negative attributes	Dusty, Medicinal, Burnt caramel, Rotting plant water, Hay/Dried grass, Green grass, Cooked vegetables	Dusty, Burnt caramel, Rotting plant water, Hay/Dried grass, Green grass, Cooked vegetables, Seaweed	Dusty, Burnt caramel, Rotting plant water, Hay/Dried grass, Green grass, Cooked vegetables
Taste and mouthfeel	Sweet, Sour, Bitter, Astringent	Sweet, Sour, Bitter, Astringent	Sweet, Sour, Bitter, Astringent

*All attributes were analysed as aromas and flavours except for fruity sweet, boiled syrup, caramel and fynbos sweet which were only evaluated as aromas.

4. RESULTS

According to statistical terminology a main effect is the effect of one independent variable on the dependent variable (Rutherford, 2001). In this study it refers to the effect of temperature or time on a sensory attribute or a chemical/instrumental parameter. If there was significant statistical interaction ($p \leq 0.05$) between temperature and time the main effects could not be interpreted, and instead the interactions were interpreted. An interaction occurs when the effect of one independent variable on the dependent variable changes, depending on the level of another independent variable (Rutherford, 2001), for example, the effect of the fermentation temperature on a specific sensory attribute or chemical/instrumental parameter was influenced by the fermentation time. The F- and p-values for each temperature-time combination of each sensory attribute and chemical/instrumental parameter can be viewed in Addendum F and Addendum G, respectively. The main effects will be discussed first, followed by the interactions. Only sensory attributes with an average intensity of more than 10 (positive sensory attributes) and more than five (negative sensory attributes) are included in the discussion.

4.1. Effect of fermentation temperature and time on sensory attributes

Cyclopia genistoides

The main effects of four sensory attributes, rose geranium (Fig.1), fynbos floral, and fynbos sweet aroma, as well as sweet taste (Fig. 2), could be interpreted. Only fermentation temperature had a significant ($p \leq 0.05$) effect on the rose geranium aroma of *C. genistoides.* Tea fermented at 80°C had a significantly ($p \leq 0.05$) stronger rose geranium aroma compared to tea fermented at 90°C, but the difference in the average intensity is only five. Although the average intensity has doubled, at this low intensity the difference would hardly be noticeable. The rose geranium aroma was also influenced by the fermentation time (Fig. 2a). The average intensity of the rose geranium aroma increased significantly ($p \leq 0.05$) as the fermentation time increased from 8 h to 24 h. On the other hand, fynbos floral (Fig. 2b) and fynbos sweet aroma (Fig. 2c), as well as sweet taste (Fig. 2d) increased significantly ($p \leq 0.05$) as the fermentation time increased from 8 h to 16 h, but after 16 h of fermentation there was no significant ($p > 0.05$) difference in the average intensities of these attributes. Fynbos floral and fynbos sweet aroma are considered important aroma attributes, as both have average intensity attribute values higher than 30, even after 8 h. Furthermore, the difference between the lowest average intensity and highest average intensity for these two attributes are approximately 10 (out of 100).

The main effects for apricot jam, plantlike and fruity sweet aroma (Fig. 3), fynbos floral and plantlike flavour, bitter taste and astringency (Fig. 4) and hay/dried grass and cooked vegetables aroma (Fig. 5) could not be interpreted as there was significant ($p \leq 0.05$) interaction between fermentation temperature and time for these sensory attributes. The apricot jam aroma average intensity was not significantly ($p > 0.05$) affected during fermentation, however, the highest average intensity was obtained at 90°C/24 h and the lowest at 80°C/32 h. (Fig. 3a). The average intensity of plantlike aroma was significantly ($p \leq 0.05$) higher in *C. genistoides* fermented at 80°C/8 h compared to *C. genistoides* fermented at 90°C/8h (Fig. 3b). The average intensity of fruity sweet aroma remained relatively stable throughout fermentation, but similar to

apricot jam aroma, the highest average intensity was obtained at 90°C/24 h of fermentation (Fig. 3c). The average intensity for fynbos floral (Fig. 4a) and plantlike flavour (Fig. 4b) was significantly ($p \leq 0.05$) lower and higher, respectively, in *C. genistoides* fermented at 80°C/8 h compared to all other temperature-time combinations. The average intensity of bitter taste (Fig. 4c) decreased at 80°C as the fermentation time increased from 8 h to 24 h, whereas, at 90°C the average intensity only decreased significantly ($p \leq 0.05$) between 8 h and 16 h. The decrease in the average intensity of bitter taste at 80°C was substantial (approximately 15 (out of 100). Bitter taste was less intense at 90°C at 8 h and 16 h fermentation time. Special attention should be given to this specific attribute when determining the optimum fermentation conditions for *C. genistoides.* Astringency followed a similar pattern as bitter taste (Fig. 4d). The average intensity of astringency decreased significantly ($p \leq 0.05$) between 8 h and 16 h. Furthermore, the temperature appears to play a role if the fermentation time is 16 h or less as *C. genistoides* fermented at 80°C had significantly ($p \leq 0.05$) higher average intensity values at 8 h and 16 h than at 90°C. It appears as if most of the changes in terms of the flavour and taste attributes as well as astringency stabilised after 16 h.

Interestingly, the average attribute intensity of hay/dried grass aroma (Fig. 5a) decreased with fermentation time at 80°C, whereas at 90°C the average attribute intensity remained stable. However, a reduction of less than five (out of 100) in the average attribute intensity would hardly be noticeable and a fermentation temperature of 90°C should not be overlooked. A similar trend was observed for cooked vegetables aroma (Fig. 5b). The first 16 h of fermentation at 80°C resulted in a significant ($p \leq 0.05$) reduction of cooked vegetables aroma, similar to a level present in honeybush fermented at 90°C.

The effect of fermentation temperature and time combinations on the sensory properties of *C. genistoides* can be displayed by means of the PCA loadings plot and scores plot (Fig. 6) which reflect, respectively, the positioning of the sensory attributes and the positioning of the 24 honeybush samples analysed with respect to each other. The loadings plot shows that the negative sensory attributes, except for dusty aroma and green grass flavour, are situated on the right hand side of the plot whereas the positive attributes are situated on the left hand side (Fig. 6a). *Cyclopia* samples fermented at 80°C/8 h, 80°C/16 h and 90°C/8 h (Fig. 6b) are situated on the right side associating with the negative sensory attributes. Longer fermentation times, with the exception of two samples, are situated on the left side of the plot associating with the positive sensory attributes.

Cyclopia subternata

The main effects of four sensory attributes, rose geranium (Fig. 7), fruity sweet and rotting plant water aroma as well as astringency (Fig. 8), could be interpreted. Fermentation temperature had a significant ($p \leq 0.05$) effect on the rose geranium aroma of *C. subternata.* Tea fermented at 80°C had a significantly ($p \leq 0.05$) stronger rose geranium aroma compared to tea fermented at 90°C. The rose geranium aroma was also influenced by the fermentation time (Fig. 8a). The average intensity of the rose geranium aroma increased significantly ($p \leq 0.05$) as the fermentation time increased to 24 h. The average intensities of fruity sweet aroma (Fig. 8b) increased significantly ($p \leq 0.05$) as the fermentation time increased. The first 16 h of fermentation resulted in a significant ($p \leq 0.05$) reduction in rotting plant water aroma (Fig. 8c) and astringency (Fig. 8d). Rose geranium is considered one of the important aroma attributes associated with *C. subternata* (Chapter 3), although the highest average intensity was less than 15 (out of 100).

The main effects for fynbos floral, apricot jam, plantlike and fynbos sweet aroma (Fig. 9), fynbos floral and plantlike flavour, sweet taste (Fig. 10) and burnt caramel and cooked vegetables aroma (Fig. 11), could not be interpreted as there was significant ($p \leq 0.05$) interaction between temperature and time for these sensory attributes. The fynbos floral (Fig. 9a) and fynbos sweet aroma (Fig. 9b) increased significantly ($p \leq 0.05$) as the fermentation time increased to 24 h at 80°C whereas at 90°C the aroma intensities for these aroma attributes remained unaffected ($p > 0.05$). The reverse is true for apricot jam aroma (Fig. 9c). Apricot jam aroma increased significantly ($p \leq 0.05$) with fermentation time at 90°C, whereas at 80°C the aroma intensities remained unaffected ($p > 0.05$). Plantlike aroma (Fig. 9d) decreased substantially with fermentation time at 80°C, however, at 90°C plantlike aroma remained unaffected ($p > 0.05$). Fynbos floral and fynbos sweet aroma attributes, similar to *C. genistoides*, can be considered important for aroma as the maximum average intensity of these attributes was more than 40 (out of 100).

Fynbos floral flavour (Fig. 10a) followed the same trend as fynbos floral aroma. Fynbos floral flavour increased significantly ($p \leq 0.05$) with fermentation time at 80°C whereas at 90°C the flavour intensity remained unaffected ($p > 0.05$). Fermentation at 80°C for 24 h resulted in a significant ($p \leq 0.05$) reduction of the plantlike flavour aroma (Fig. 10b), to levels present in tea fermented at 90°C. In the latter case, the plantlike flavour intensity remained unaffected ($p > 0.05$) by fermentation time. The sweet taste (Fig. 10c) increased slightly, but significantly ($p \leq 0.05$) at 80°C as the fermentation time increased. At 90°C sweet taste fermentation time had no effect for the first 24 h ($p > 0.05$). Fynbos floral are the most important flavour attribute to consider as the maximum average intensity of this attribute was 30 (out of 100).

The first 8 h of fermentation resulted in a significant ($p \leq 0.05$) reduction, to the level of intensity at 90°C, of burnt caramel aroma (Fig. 11a) at 80°C. Fermentation at 90°C had no effect on the intensity of burnt caramel aroma ($p > 0.05$). In order to reduce the cooked vegetables aroma (Fig. 11b) a fermentation time of 24 h at 80°C was required. It appears as if fermentation time plays a very important role in reducing the average intensities of the negative sensory attributes if *C. subternata* is fermented at 80°C. On the other hand, a fermentation time had no effect at 90°C.

The effect of fermentation temperature and time has on the sensory properties of *C. subternata* can be viewed in Fig. 12 which depicts the positioning of the sensory attributes and the positioning of the 24 honeybush samples analysed with respect to each other. The loadings plot (Fig. 12a) shows that the negative sensory attributes, except for dusty aroma and flavour and green grass flavour, are situated on the right side whereas the positive sensory attributes are situated on the left side. The scores plot (Fig. 12b) reveals that *C. subternata* fermented for 8 h lies to the right side associating with the negative sensory attributes indicating that a fermentation of at least 16 h are required. Also, a distinction can be made between *C. subternata* fermented at 80°C and 90°C. *Cyclopia subternata* fermented at 90°C mostly lie at the bottom and those fermented at 80°C mostly lie at the top on the left side of the scores plot. This indicates that if the fermentation temperature is increased to 90°C *C. subternata* tends to become more fruity sweet with apricot jam notes, whereas, fynbos sweet with floral notes are associated with *C. subternata* fermented at 80°C.

Cyclopia maculata

The main effects of astringency, hay/dried grass aroma and flavour (Fig. 13) and fynbos floral and fynbos sweet aroma (Fig. 14) could be interpreted. Fermentation temperature had a significant ($p \leq 0.05$) effect on astringency (Fig. 13a), hay/dried grass aroma (Fig. 13b) and flavour (Fig. 13c). These three sensory attributes were significantly ($p \leq 0.05$) higher in tea fermented at 90°C. Fynbos floral (Fig. 14a) and fynbos sweet aroma (Fig. 14b) were influenced by the fermentation time. Fynbos floral aroma increased significantly ($p \leq 0.05$) as the fermentation time increased to 24 h. The highest average intensity value for fynbos sweet aroma was obtained after 24 h of fermentation, however, it did not differ significantly ($p > 0.05$) from *C. subternata* fermented for 16 h. Fynbos floral and fynbos sweet aroma are both important aroma attributes as their maximum intensities was more than 40 (out of 100).

The main effects for fynbos floral flavour, sweet taste (Fig. 15) and rotting plant water, cooked vegetables and green grass aroma (Fig. 16) could not be interpreted as there was significant ($p \leq 0.05$) interaction between temperature and time. Fynbos floral flavour (Fig. 15a) remained relatively stable as the fermentation time increased, however, at 80°C/24 h the fynbos floral flavour was significantly ($p \leq 0.05$) higher compared to 90°C/16h and 90°C/32 h. The sweet taste (Fig. 15b) increased significantly ($p \leq 0.05$) from 8 h to 24 h at 80°C whereas at 90°C the sweet taste decreased slightly from 8 h to 24 h.

Rotting plant water aroma (Fig. 16a) decreased significantly ($p \leq 0.05$) as the fermentation time increased from 8 to 24 h at 80°C, whereas at 90°C the aroma intensity remained unaffected ($p > 0.05$). The first 8 h of fermentation at 80°C resulted in a significant ($p \leq 0.05$) reduction in cooked vegetables aroma to the same level than at 90°C (Fig. 16b). At 90°C the average intensity of green grass aroma (Fig. 16c) increased significantly ($p \leq 0.05$) with fermentation time, however, at 80°C a fermentation time of 24 h effectively reduced the green grass aroma compared to 8 h.

The PCA loadings plot and scores plot (Fig. 17) which depict the positioning of the sensory attributes and the positioning of the *C. maculata* samples ($n = 24$) analysed with respect to each other, illustrate the relationship between the sensory attributes and fermentation temperature/time combinations. From the loadings plot (Fig. 17a) it is clear that all the negative sensory attributes, except for dusty aroma, are situated in the top two quadrants. It appears as if the different batches played larger role as all the samples produced from batch a are situated on the right side of the scores plot (Fig. 17b) and batch b and c are situated mostly on the left side of the plot. However, a general trend can still be observed as the majority of the *C. maculata* fermented at 90°C are situated at the top end of the plot while the majority of the *C. maculata* fermented at 80°C are located at the bottom of the plot. *Cyclopia maculata* fermented at 80°C thus appears to associate with a sweet taste and floral attributes whereas at 90°C it tend to associate with the negative sensory attributes and astringency.

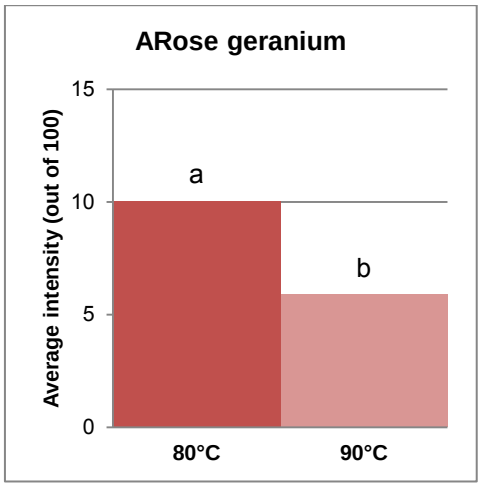

Figure 1 Effect of fermentation temperature (80 vs. 90°C) on the rose geranium aroma of *C. genistoides.* The letter "A" in front of the attribute name refers to aroma.

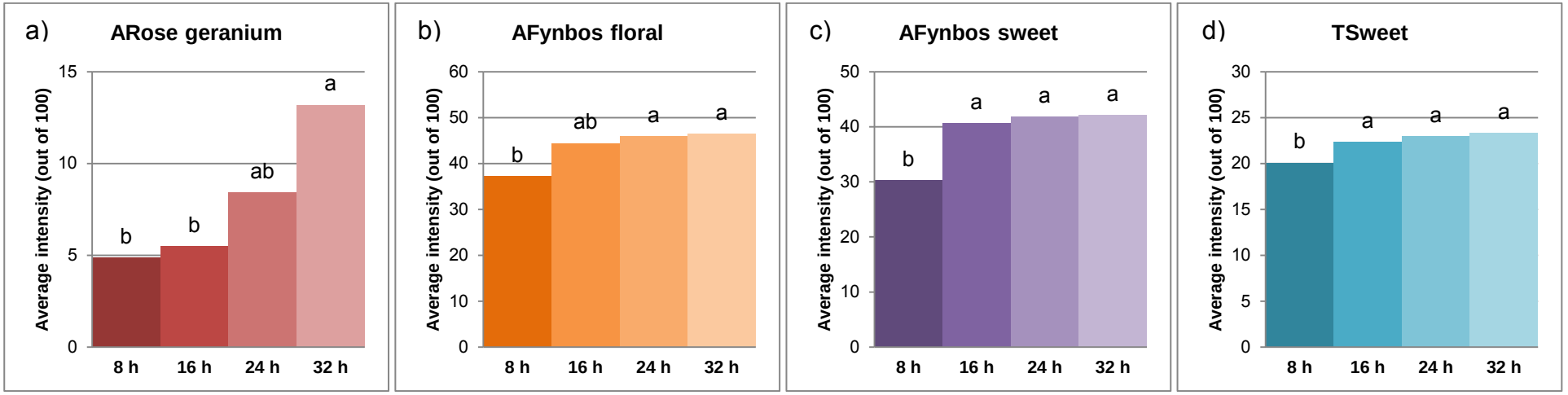

Figure 2 Effect of fermentation time (8, 16, 24 and 32 h) on the a) rose geranium aroma, b) fynbos floral aroma, c) fynbos sweet aroma and d) sweet taste of *C. genistoides*. The letters "A" and "T" in front of the attribute name refer to aroma and taste, respectively.

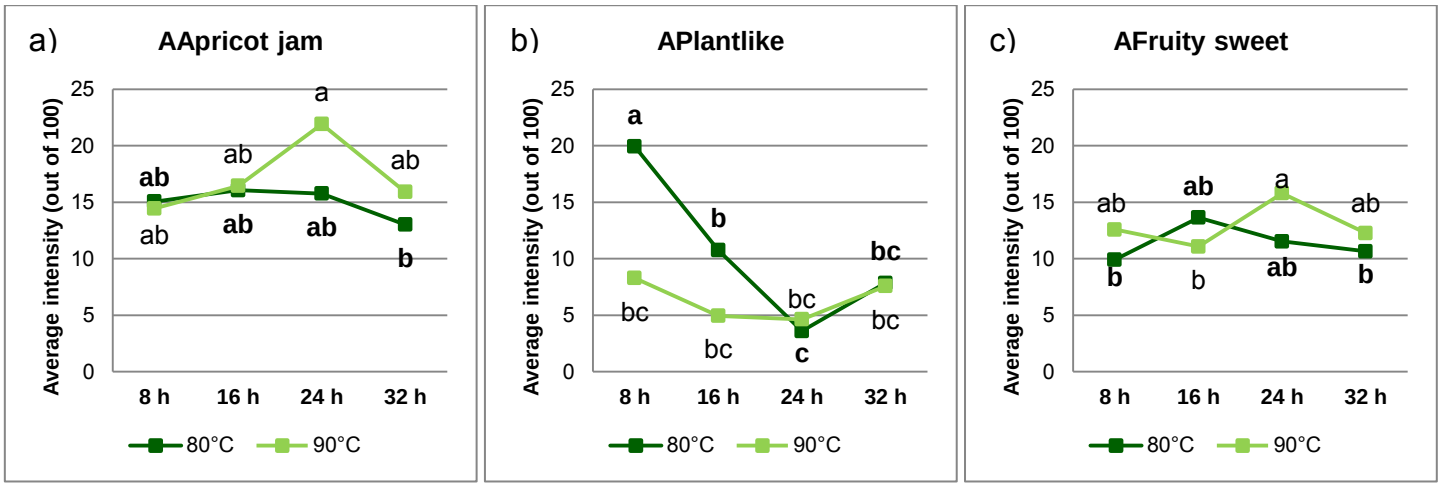

Figure 3 Average aroma intensity values of a) apricot jam aroma b) plantlike aroma and c) fruity sweet aroma for honeybush (*C. genistoides*) fermented at 80°C and 90°C for 8 h, 16 h, 24 h and 32 h. Values with different alphabetical letters differ significantly from each other ($p \leq 0.05$) and the alphabetical letters referring to honeybush fermented at 80°C are in bold. The letter "A" in front of the attribute name refers to aroma.

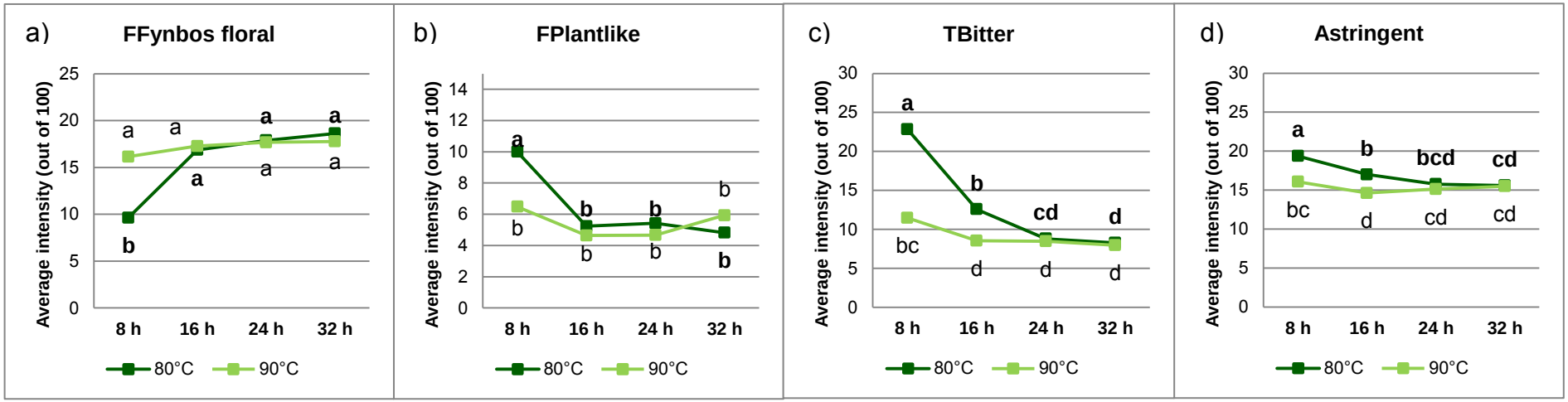

Figure 4 Average flavour and taste intensity values of a) fynbos floral flavour, b) plantlike flavour, c) bitter taste and d) astringency for honeybush (*C. genistoides*) fermented at 80°C and 90°C for 8 h, 16 h, 24 h and 32 h. Values with different alphabetical letters differ significantly from each other ($p \leq 0.05$) and the alphabetical letters referring to honeybush fermented at 80°C are in bold. The letters "F" and "T" in front of the attribute name refer to flavour and taste, respectively.

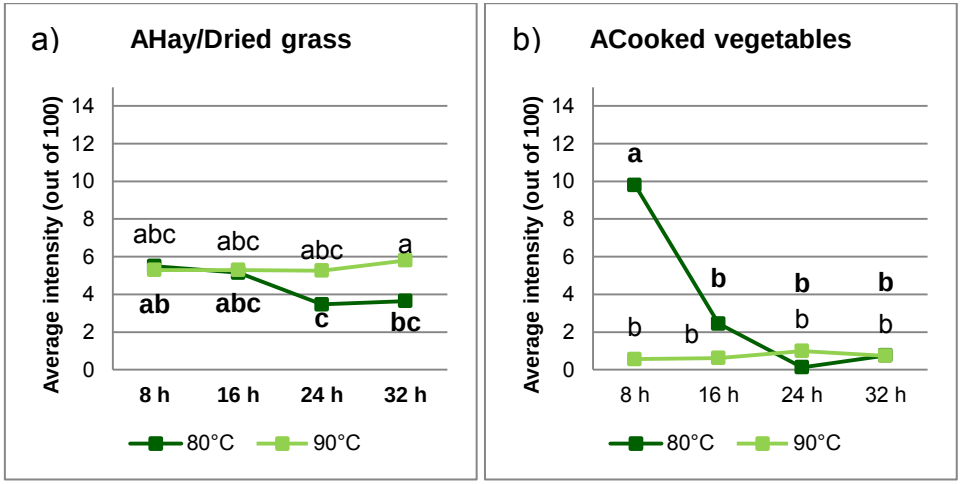

Figure 5 Average negative aroma intensity values of a) hay/dried grass aroma and b) cooked vegetables aroma for honeybush (*C. genistoides*) fermented at 80°C and 90°C for 8 h, 16 h, 24 h and 32 h. Values with different alphabetical letters differ significantly from each other ($p \leq 0.05$) and the alphabetical letters referring to honeybush fermented at 80°C are in bold. The letter "A" in front of the attribute name refers to aroma.

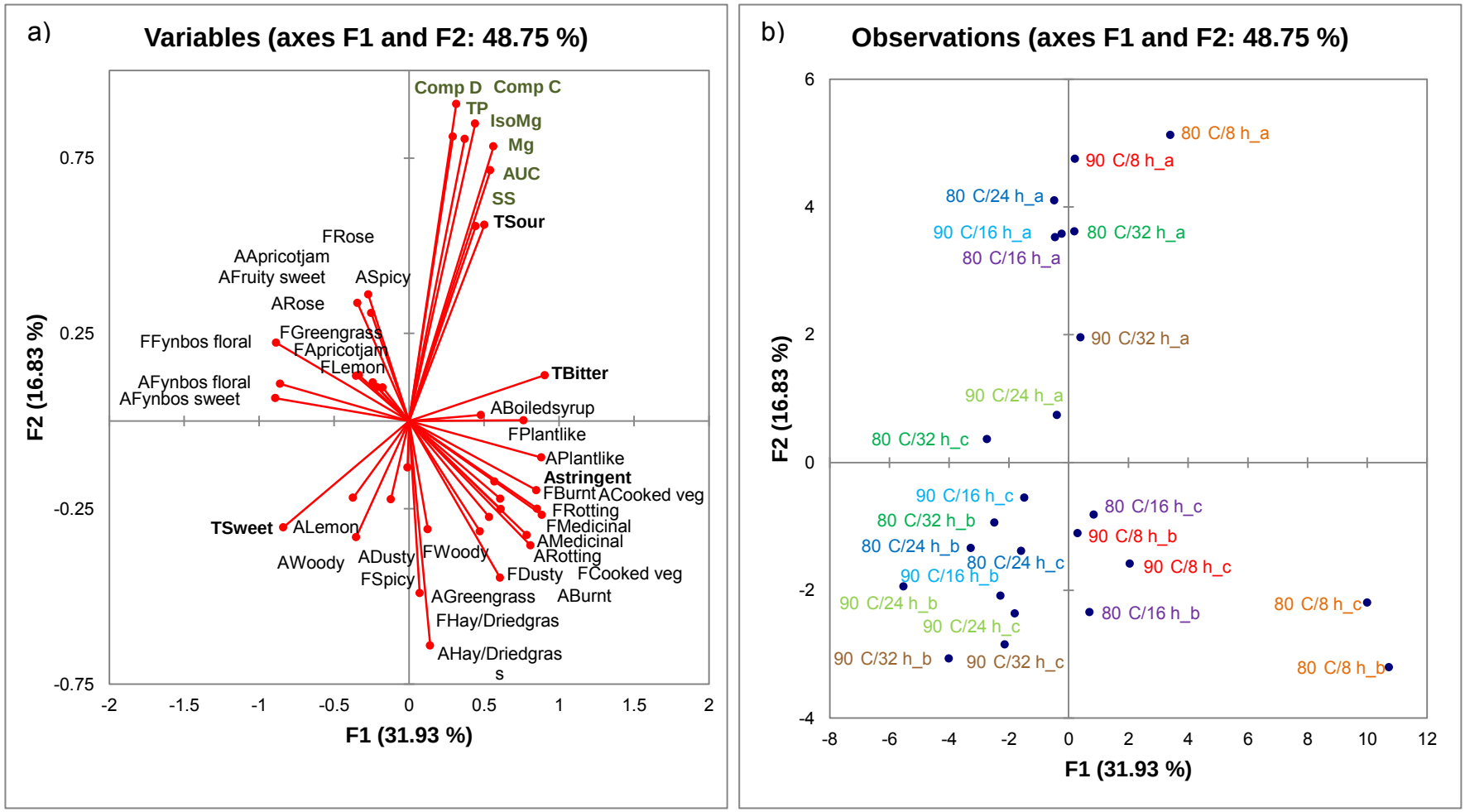

Figure 6 a) PCA loadings plot showing the positioning of both positive and negative sensory attributes for *C. genistoides*. The letters "A", "F" and "T" in front of the attributes refer to aroma, flavour and taste attributes, respectively. Rose = Rose geranium, Burnt = Burnt caramel, Cooked veg = Cooked vegetables, Rotting = Rotting plant water, SS = Soluble solids, TP = Total polyphenol, AUC (370 – 570 nm) = Area under curve, Mg = Mangiferin, IsoMg = Isomangiferin, Comp C = Component C, Comp D = Component D. b) PCA scores plot showing the positioning of the 24 honeybush (*C. genistoides*) samples. The letters "a", "b" and "c" refer to the specific batch.

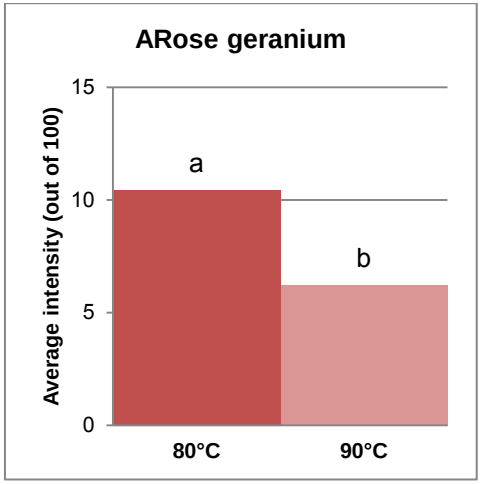

Figure 7 Effect of fermentation temperature (80 vs. 90°C) on the rose geranium aroma of *C. subternata.* The letter "A" in front of the attribute name refers to aroma.

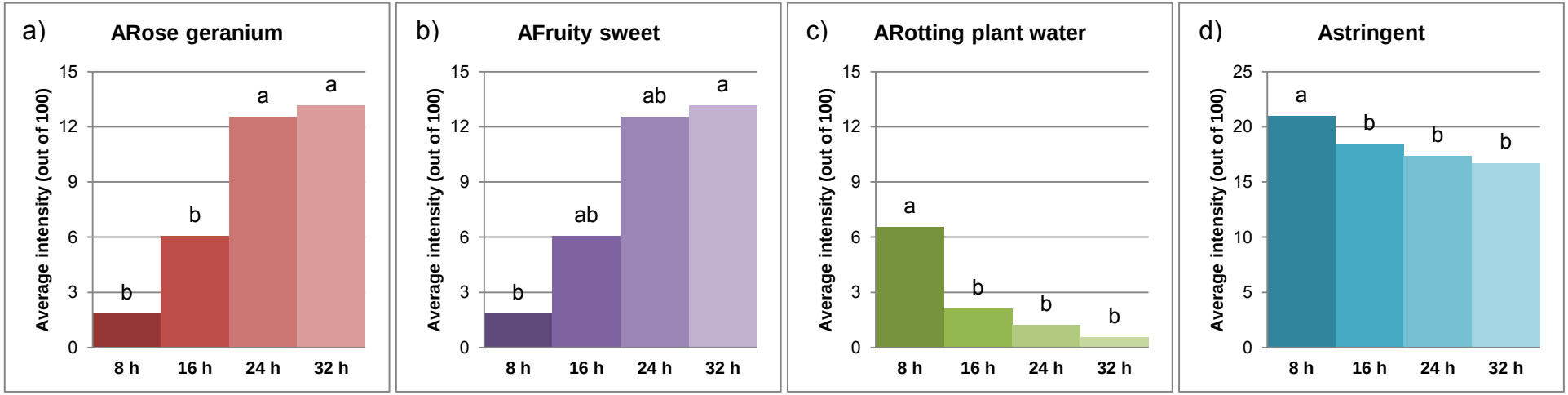

Figure 8 Effect of fermentation time (8, 16, 24 and 32 h) on the a) rose geranium aroma, b) fruity sweet aroma, c) rotting plant water aroma and d) astringency of *C. subternata*. The letter "A" in front of the attribute name refers to aroma.

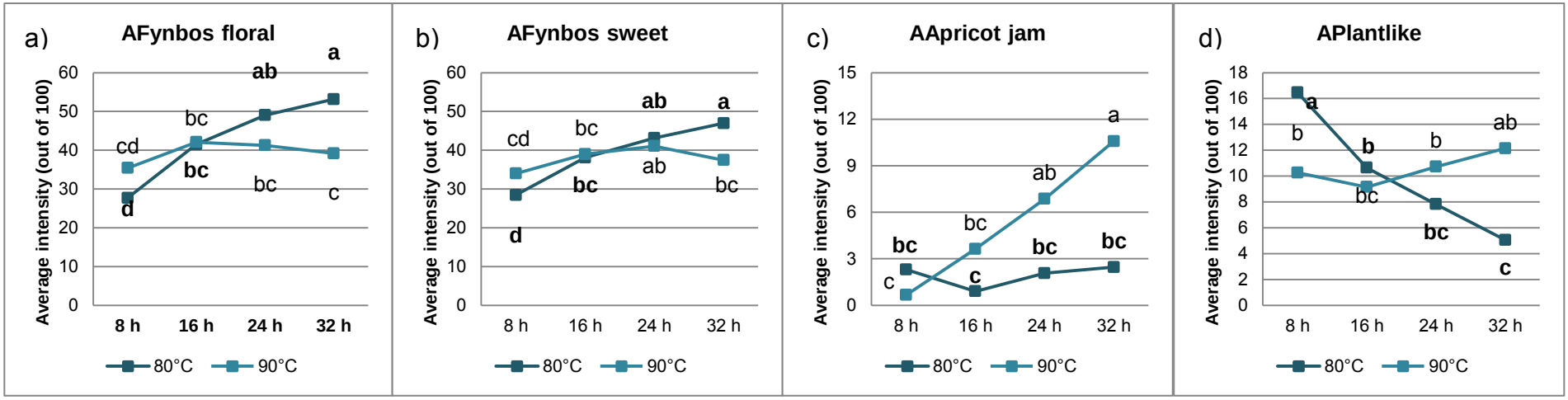

Figure 9 Average aroma intensity values of a) fynbos floral aroma, b) fynbos sweet aroma, c) apricot jam aroma and d) plantlike aroma for honeybush (*C. subternata*) fermented at 80°C and 90°C for 8 h, 16 h, 24 h and 32 h. Values with different alphabetical letters differ significantly from each other ($p \leq 0.05$) and the alphabetical letters referring to honeybush fermented at 80°C are in bold. The letter "A" in front of the attribute name refers to aroma.

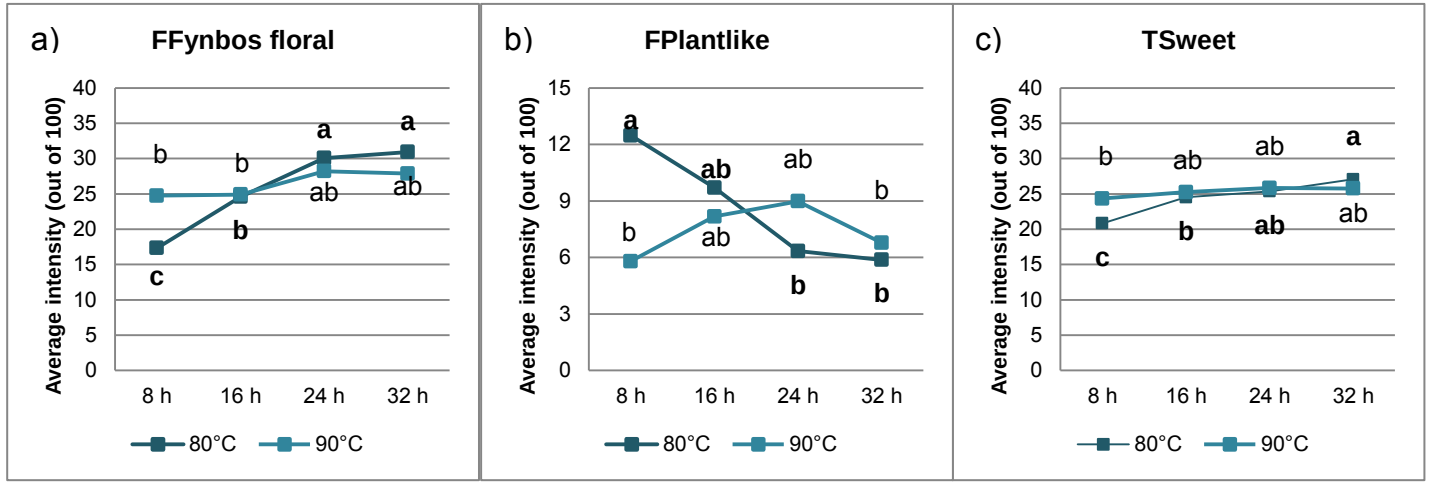

Figure 10 Average flavour and taste intensity values for a) fynbos floral flavour, b) plantlike flavour and c) sweet taste for honeybush (*C. subternata*) fermented at 80°C and 90°C for 8 h, 16 h, 24 h and 32 h. Values with different alphabetical letters differ significantly from each other ($p \leq 0.05$) and the alphabetical letters referring to honeybush fermented at 80°C are in bold. The letters "F" and "T" in front of the attribute name refer to flavour and taste, respectively.

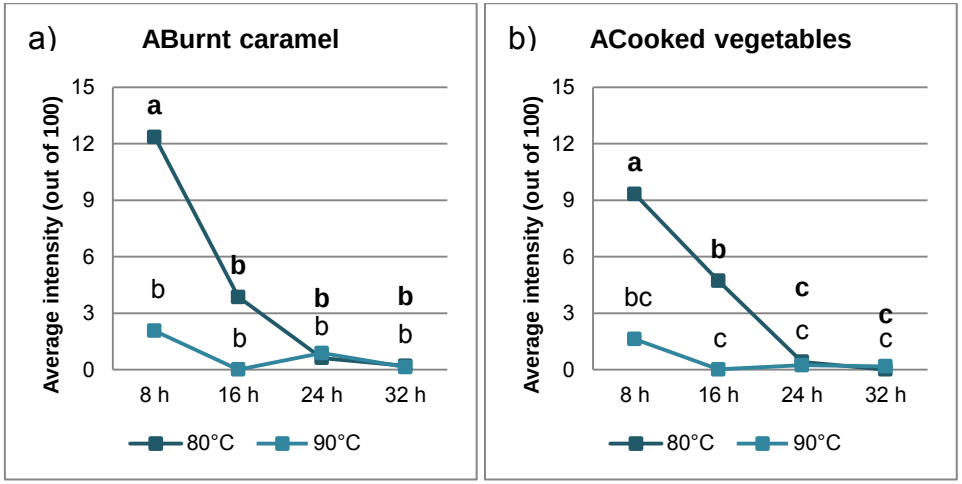

Figure 11 Average negative aroma intensity values of a) burnt caramel and b) cooked vegetables for honeybush (*C. subternata*) fermented at 80°C and 90°C for 8 h, 16 h, 24 h and 32 h. Values with different alphabetical letters differ significantly from each other ($p \leq 0.05$) and the alphabetical letters referring to honeybush fermented at 80°C are in bold. The letter "A" in front of the attribute name refers to aroma.

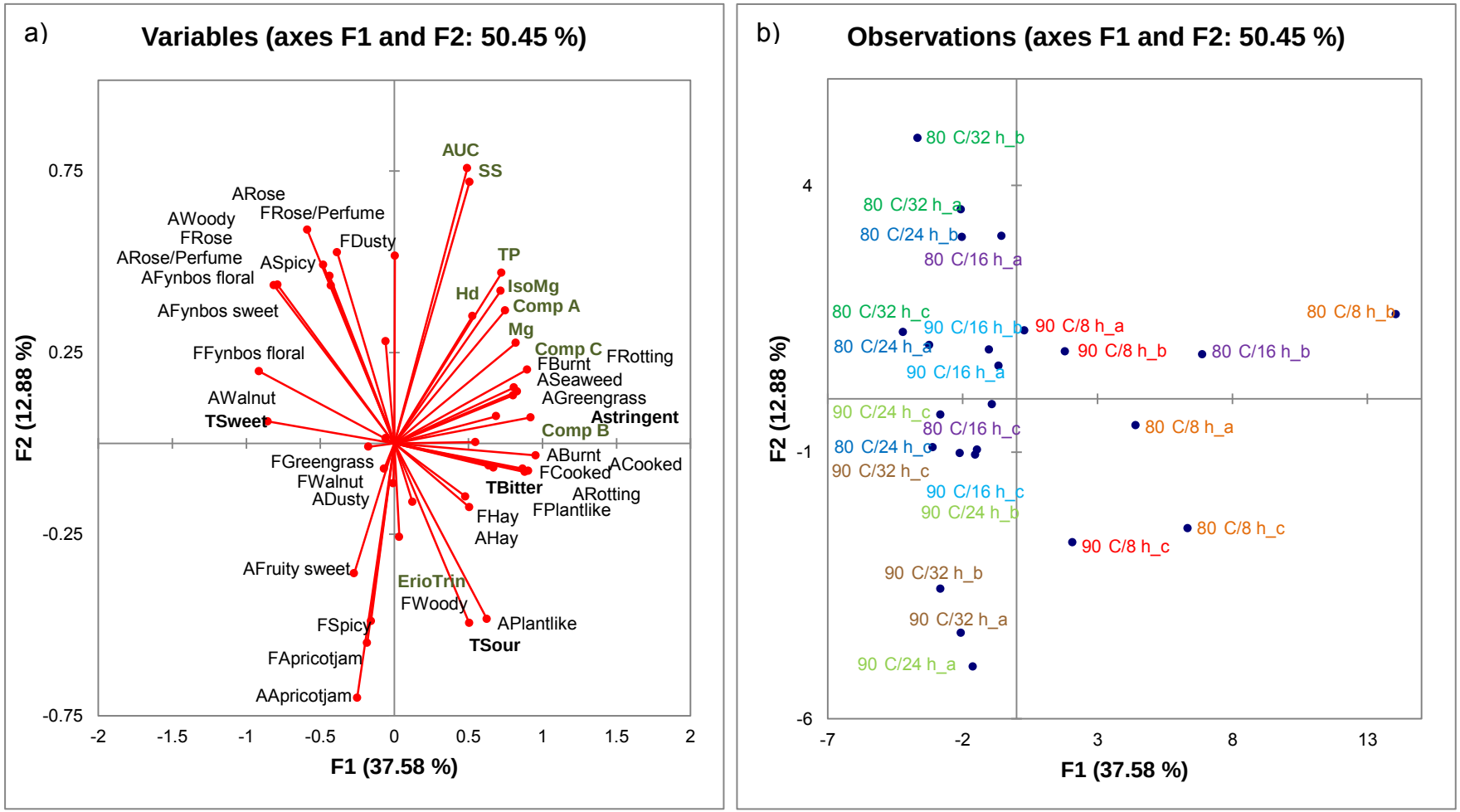

Figure 12 a) PCA loadings plot showing the positioning of both positive and negative sensory attributes of *C. subternata*. The letters "A", "F" and "T" in front of the attributes refer to aroma, flavour and taste attributes, respectively. Rose = Rose geranium, Burnt = Burnt caramel, Hay = Hay/Dried grass, Cooked veg = Cooked vegetables, Rotting = Rotting plant water, SS = Soluble solids, TP = Total polyphenol, AUC (370 – 570 nm) = Area under curve, Mg = Mangiferin, IsoMg = Isomangiferin, ErioTrin = Eriocitrin, Hd = Hesperidin, Comp A = Component A, Comp B = Component B, Comp C = Component C. b) PCA scores plot showing the positioning of the 24 honeybush (*C. subternata*) samples. The letters "a", "b" and "c" refer to the specific batch.

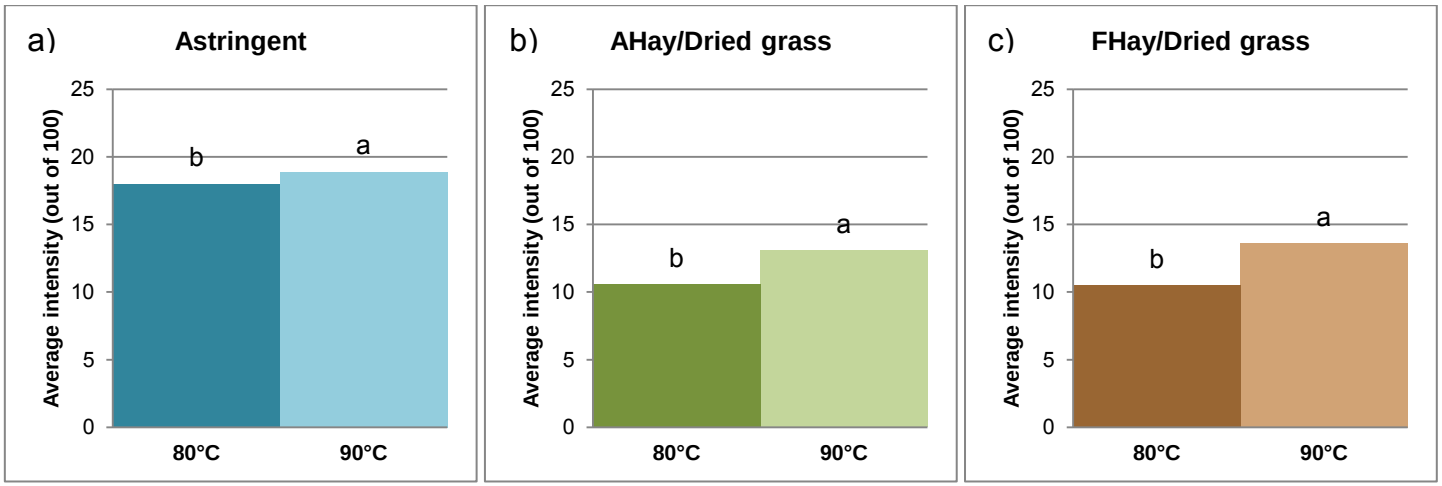

Figure 13 Effect of fermentation temperature (80 vs. 90°C) on the a) astringency, b) hay/dried grass aroma and c) hay/dried grass flavour of *C. maculata.* The letters "A" and "F" in front of the attribute name refer to aroma and flavour, respectively.

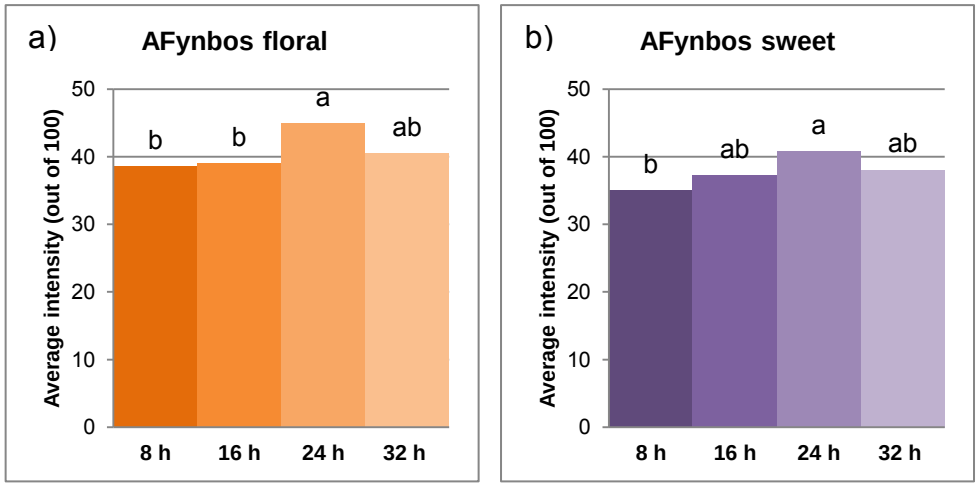

Figure 14 Effect of fermentation time (8, 16, 24 and 32 h) on the a) fynbos floral aroma and b) fynbos sweet aroma of *C. maculata*. The letter "A" in front of the attribute name refers to aroma.

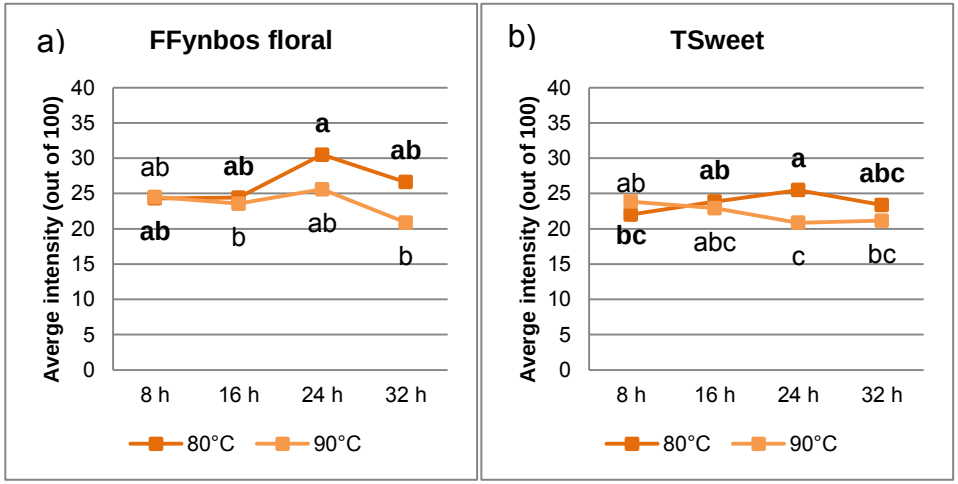

Figure 15 Average attribute intensity values of a) fynbos floral flavour and b) sweet taste for honeybush (*C. maculata*) fermented at 80°C and 90°C for 8 h, 16 h, 24 h and 32 h. Values with different alphabetical letters differ significantly from each other ($p \leq 0.05$) and the alphabetical letters referring to honeybush fermented at 80°C are in bold. The letters "F" and "T" in front of the attribute name refer to flavour and taste, respectively.

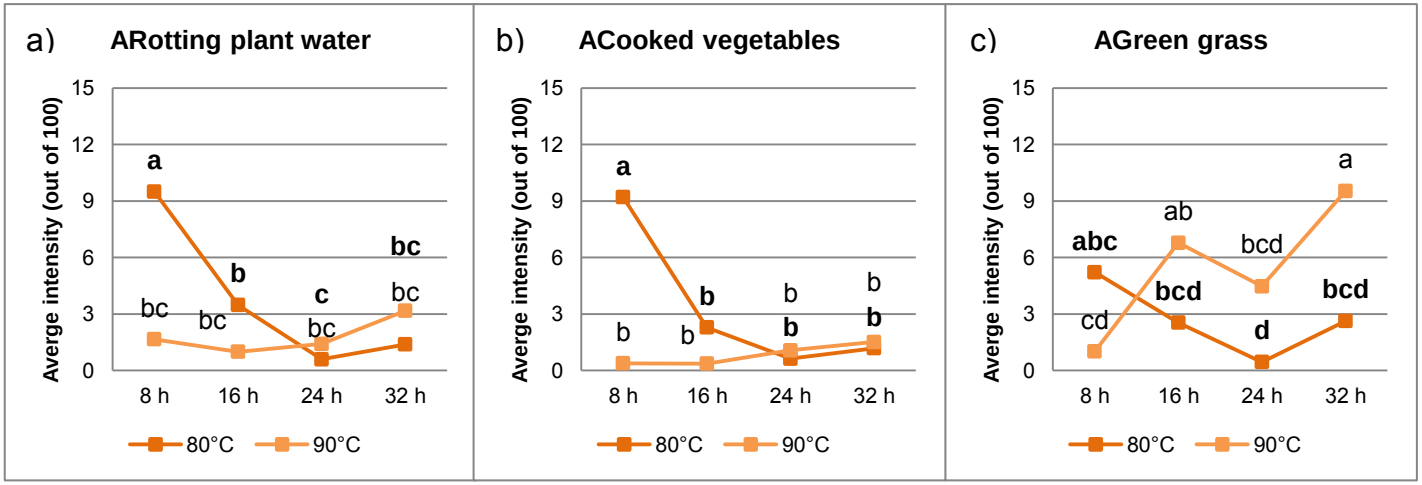

Figure 16 Average negative attribute intensity values of a) rotting plant water aroma, b) cooked vegetables aroma and c) green grass aroma for honeybush (*C. maculata*) fermented at 80°C and 90°C for 8 h, 16 h, 24 h and 32 h. Values with different alphabetical letters differ significantly from each other ($p \leq 0.05$) and the alphabetical letters referring to honeybush fermented at 80°C are in bold. The letter "A" and in front of the attribute name refers to aroma.

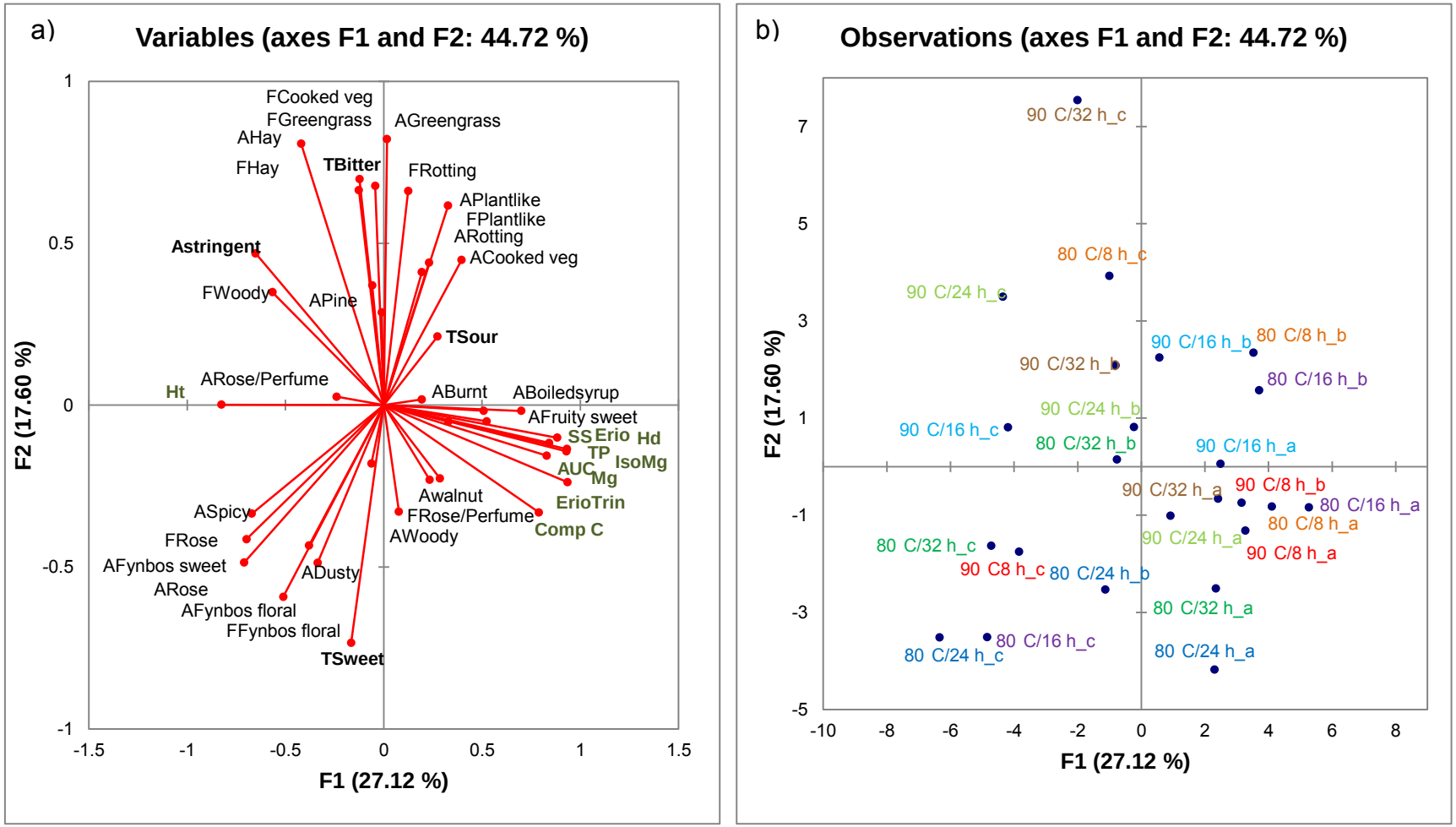

Figure 17 a) PCA loadings plot showing the positioning of both positive and negative sensory attributes for *C. maculata*. The letters "A", "F" and "T" in front of the attributes refer to aroma, flavour and taste attributes, respectively. Rose = Rose geranium, Rotting = Rotting plant water, Hay = Hay/Dried grass, Cooked veg = Cooked vegetables, Burnt = Burnt caramel, SS = Soluble solids, TP = Total polyphenol, Mg = Mangiferin, IsoMg = Isomangiferin, Comp C = Component C, Hd = Hesperidin, Erio = Eriodictyol, ErioTrin = Eriocitrin, Ht = Hesperetin , AUC (370 – 570 nm) = Area under curve, AUCnorm = Area under curve normalized. b) PCA scores plot showing the positioning of the 24 honeybush (*C. maculata*) samples. The letters "a", "b" and "c" refer to the specific batch.

4.2. Effect of fermentation temperature and time on composition and colour

Cyclopia genistoides

The effect of fermentation temperature (80°C vs. 90°C) on the composition of *C. genistoides* are summarised in Table 4 whereas the effect of fermentation time (8 h, 16 h, 24 h and 32 h) are summarised in Table 5. The two fermentation temperatures used to ferment *C. genistoides* differed significantly ($p \leq 0.05$) with regard to its effect on the SS, mangiferin and isomangiferin contents and instrumental colour with 80°C being less detrimental than 90°C. The TP, compound C and compound D contents were not influenced by fermentation temperature. On the other hand, the TP and individual polyphenolic compounds contents, as well as instrumental colour decreased as the fermentation time of *C. genistoides* increased. The SS content was the lowest at 24 h. The curves for absorbance and normalised absorbance can be viewed in Addendum H.

Cyclopia subternata

The effect of fermentation temperature (80°C vs. 90°C) on the composition of *C. subternata* are summarised in Table 6 whereas the effect of fermentation time (8 h, 16 h, 24 h and 32 h) are summarised in Table 7. The SS, TP, isomangiferin, compound C and compound B contents, as well as instrumental colour of *C. subternata* infusions, were significantly ($p \leq 0.05$) higher when the tea was fermented at 80°C compared to 90°C. There was no difference between the two fermentation temperatures with regards to the concentration of mangiferin, compound A, eriocitrin and hesperidin. The content of TP and individual polyphenolic compounds decreased significantly ($p \leq 0.05$) as the fermentation period of *C. subternata* increased. The same effect was also observed for instrumental colour.

Cyclopia maculata

The effect of fermentation temperature (80°C vs. 90°C) on the composition of *C. maculata* are summarised in Table 8 whereas the effect of fermentation time (8 h, 16 h, 24 h and 32 h) are summarised in Table 9. Fermentation temperature had no significant effect on any of the parameters. Fermentation time, however, played a significant role ($p \leq 0.05$), with the content of several of the individual phenolic compounds, i.e. mangiferin, isomangiferin, hesperidin and eriocitrin content decreasing. Eriodictyol increased significantly ($p \leq 0.05$) as the fermentation time increased from 8 h to 16 h. Increased fermentation time significantly ($p \leq 0.05$) reduced the TP content. SS content showed no clear pattern, whilst instrumental colour were not significantly affected ($p > 0.05$).

4.3. Relationship between composition and taste and mouthfeel

PCA plots are commonly used to demonstrate whether correlations exist between certain sensory attributes and the chemical composition of a product. It is often more useful to examine the correlation coefficients as certain groupings may arise from a general tendency of certain attributes to change in a similar way over a large group of samples (Wolters & Alchurch, 1994; Talavera-Bianchi *et al.,* 2010). For this reason, the PCA plots and the pearson correlations coefficients will be considered. The focus will fall on the taste and

mouthfeel attributes as these are the sensory attributes influenced by the polyphenolic composition. Only significant ($p \leq 0.05$) correlations, albeit positive or negative, will be discussed.

Cyclopia genistoides

Examining the PCA loadings plot (Fig. 6) reveals that all of the chemical/instrumental parameters are situated in the same quadrant (top) of the loadings plot as the negative taste and mouthfeel attributes, i.e. sour and bitter taste and astringency. Sweet taste, on the other hand, is situated directly opposite all of the chemical/instrumental parameters. In order to determine whether certain compositional parameters, specifically the polyphenolic compounds, influenced the taste and mouthfeel of *C. genistoides* the correlation coefficients between the basic tastes and mouthfeel and chemical/instrumental parameters were compared (Table 10). This revealed that sweet taste negatively correlated with all chemical/instrumental parameters whereas bitterness positively correlated with all the chemical/instrumental parameters ($p \leq 0.05$). Sour taste correlated significantly ($p \leq 0.05$) with all the parameters, except for SS content. Interestingly, there was no significant ($p > 0.05$) correlation between astringency and any of the chemical/instrumental parameters.

Cyclopia subternata

As for *C. genistoides,* the PCA loadings plot for *C. subternata* (Fig. 12) reveals that all the chemical/instrumental parameters are situated in the top right quadrant except eriocitrin. Eriocitrin is situated in the bottom right quadrant along with sour and bitter taste, as well as astringency. The correlation coefficients of the basic tastes and mouthfeel with the chemical/instrumental parameters of *C. subternata* are summarised in Table 11. Sweet taste negatively correlated with all parameters (r varies between -0.461 to -0.820), except eriocitrin and compound B ($p \leq 0.05$). Sour taste significantly correlated only with compound C ($r = 0.478$), whereas bitter taste significantly correlated with mangiferin ($r = 0.456$), compound A ($r = 0.560$), compound C ($r = 0.616$) and hesperidin ($r = 0.495$). Astringency significantly ($p \leq 0.05$) correlated with all parameters, except for eriocitrin.

Cyclopia maculata

All the chemical/instrumental parameters, except for hesperetin, are situated in the bottom, right quadrant of the PCA loadings plot (Fig. 17). Hesperetin is located on the left hand side of the PCA loadings plot. Only sour taste is located on the right side, whereas bitter and sweet taste as well as astringency are located on the left side of the PCA loadings plot. The correlation coefficients of the basic taste and mouthfeel attributes with the chemical/instrumental parameters of *C. maculata* are shown in Table 12. Only the SS content correlated significantly with sour taste ($r = 0.447$), whereas, all of the chemical/instrumental parameters, except for hesperetin, negatively correlated with astringency ($p \leq 0.05$). On the other hand, hesperetin positively correlated with astringency ($r = 0.555$).

Table 4 Effect of fermentation temperature (80 vs. 90°C) on the composition of *C. genistoides* infusions

Temp	SS (mg/L)		TP (mg GAE/L)		Mg (mg/L)		IsoMg (mg/L)		Comp C (mg/L)		Comp D (mg/L)		AUC	
80	2471.2	a	469.7	a	130.29	a	54.21	a	18.1	a	5.63	a	93.95	a
90	2303.2	b	433.83	a	106.62	b	46.8	b	15.7	a	4.07	a	84.17	b
LSD	117.22		36.25		11.42		4.45		2.46		2.14		6.27	

Values in the same column with the same letters are significantly different ($p \leq 0.05$). SS = Soluble solids, TP = Total polyphenol, Mg = Mangiferin, IsoMg = Isomangiferin, Comp C = Component C, Comp D = Component D, AUC = Area under the curve (370-570 nm), LSD = Least significant difference.

Table 5 Effect of fermentation time (8, 16, 24 and 32 h) on the composition of *C. genistoides* infusions

Time	SS (mg/L)		TP (mg GAE/L)		Mg (mg/L)		IsoMg (mg/L)		Comp C (mg/L)		Comp D (mg/L)		AUC	
8	2497.45	a	490.36	a	153.71	a	61.53	a	22.32	a	7.45	a	97.291	a
16	2373.1	ab	465.89	ab	121.12	b	51.55	b	16.56	b	5.75	ab	90.434	ab
24	2207.71	b	429.1	b	99.51	c	42.97	c	14.64	b	3.23	b	82.625	b
32	2470.46	a	421.72	b	99.47	c	45.97	bc	14.09	b	2.99	b	85.877	b
LSD	165.77		51.27		16.15		6.3		3.48		3.03		8.8642	

Values in the same column with the same letters are significantly different ($p \leq 0.05$). SS = Soluble solids, TP = Total polyphenol, Mg = Mangiferin, IsoMg = Isomangiferin, Comp C = Component C, Comp D = Component D, AUC = Area under the curve (370-570 nm), LSD = Least significant difference.

Table 6 Effect of fermentation temperature (80 vs 90°C) on the composition and colour of *C. subternata* infusions

Temp	SS (mg/L)		TP (mg GAE/L)		Mg (mg/L)		IsoMg (mg/L)		Comp A (mg/L)		Comp C (mg/L)		ErioTrin (mg/L)		Comp B (mg/L)		Hd (mg/L)		AUC	
80	1664.61	a	245.09	a	4.41	a	5.16	a	3.76	a	2.92	a	6.54	a	4.24	a	3.17	a	34.57	a
90	1527.73	b	205.14	b	2.01	a	3.47	b	3.51	a	2.00	b	5.89	a	2.79	b	3.01	a	28.03	b
LSD	105.23		30.55		3.1	a	1.3		0.4		0.8		0.8		1.2		0.3		3.02	

Values in the same column with the same letters are significantly different ($p \leq 0.05$). SS = Soluble solids, TP = Total polyphenol, Mg = Mangiferin, IsoMg = Isomangiferin, Comp A = Component A, Comp C = Component C, ErioTrin = Eriocitrin, Comp B = Component B, Hd = Hesperidin, AUC = Area under the curve (370-570 nm), LSD = Least significant difference.

Table 7 Effect of fermentation time (8, 16, 24 and 32 h) on the composition and colour of *C. subternata* infusions

Time	SS (mg/L)		TP (mg GAE/L)		Mg (mg/L)		IsoMg (mg/L)		Comp A (mg/L)		Comp C (mg/L)		ErioTrin (mg/L)		Comp B (mg/L)		Hd (mg/L)		AUC	
8	1672.91	a	266.05	a	6.48	a	6.14	a	4.36	a	4.10	a	7.18	a	4.99	a	3.77	a	34.79	a
16	1622.41	ab	242.97	ab	3.20	ab	4.96	ab	3.81	b	2.65	b	6.52	ab	4.10	ab	3.24	b	32.90	ab
24	1522.04	b	203.97	bc	1.47	b	3.26	bc	3.20	c	1.46	c	5.60	b	2.48	b	2.74	c	27.65	c
32	1567.31	ab	187.47	c	1.15	b	2.91	c	3.19	c	1.62	bc	5.55	b	2.51	b	2.60	c	29.85	bc
LSD	148.82		43.21		4.43		1.88		0.54		1.18		1.06		1.70		0.47		4.28	

Values in the same column with the same letters are significantly different ($p \leq 0.05$). SS = Soluble solids, TP = Total polyphenol, Mg = Mangiferin, IsoMg = Isomangiferin, Comp A = Component A, Comp C = Component C, ErioTrin = Eriocitrin, Comp B = Component B, Hd = Hesperidin, AUC = Area under the curve(370-570 nm), LSD = Least significant difference.

Table 8 Effect of fermentation temperature (80 vs 90°C) on the composition and colour of *C. maculata* infusions

Temp	SS (mg/L)		TP (mg GAE/L)		Mg (mg/L)		IsoMg (mg/L)		Comp C (mg/L)		Hd (mg/L)		Erio (mg/L)		ErioTrin (mg/L)		Ht (mg/L)		AUC	
80	1875.06	a	274.12	a	12.62	a	12.41	a	2.5	a	11.81	a	0.25	a	10.99	a	0.14	a	39.581	a
90	1948.01	a	283.74	a	10.66	a	11.82	a	1.82	a	10.95	a	0.19	a	10.01	a	0.09	a	40.232	a
LSD	118.99		35.25		3.59		2.38		0.93		1.44		0.07		2.88		0.06		3.8511	

Values in the same row with the same letters are significantly different ($p \leq 0.05$). SS = Soluble solids, TP = Total polyphenol, Mg = Mangiferin, IsoMg = Isomangiferin, Comp C = Component C, Hd = Hesperidin, Erio = Eriodictyol, ErioTrin = Eriocitrin, Ht = Hesperetin, AUC = Area under the curve (370-570 nm), LSD = Least significant difference.

Table 9 Effect of fermentation time (8, 16, 24 and 32 h) on the composition and colour of *C. maculata* infusions

Time	SS (mg/L)		TP (mg GAE/L)		Mg (mg/L)		IsoMg (mg/L)		Comp C (mg/L)		Hd (mg/L)		Erio (mg/L)		ErioTrin (mg/L)		Ht (mg/L)		AUC	
8	1884.10	ab	295.78	a	18.59	a	15.68	a	2.59	a	13.21	a	0.15	b	13.44	a	0.11	a	41.93	a
16	1955.88	ab	309.36	a	13.57	a	14.02	a	2.76	a	12.73	a	0.26	a	11.48	ab	0.15	a	40.68	a
24	1808.66	b	242.22	b	7.43	b	9.39	b	1.69	a	9.89	b	0.24	ab	8.57	b	0.13	a	37.76	a
32	1997.50	a	268.35	b	6.98	b	9.36	b	1.58	a	9.69	b	0.24	ab	8.50	b	0.09	a	39.26	a
LSD	168.27		49.85		5.07		3.37		1.32		2.03		0.10		4.07		0.09		5.45	

Values in the same row with the same letters are significantly different ($p \leq 0.05$). SS = Soluble solids, TP = Total polyphenol, Mg = Mangiferin, IsoMg = Isomangiferin, Comp C = Component C, Hd = Hesperidin, Erio = Eriodictyol, ErioTrin = Eriocitrin, Ht = Hesperetin, AUC = Area under the curve (370-570 nm), LSD = Least significant difference.

Table 10 Correlation coefficients for the taste and mouthfeel attributes and the chemical/instrumental variables of *C. genistoides* infusions

Variables	SS	TP	Mg	IsoMg	Comp C	Comp D	AUC
TSweet	**-0.520***	**-0.668***	**-0.698***	**-0.639***	**-0.594***	-0.495*	**-0.599***
TSour	0.291	**0.582***	**0.673***	**0.594***	**0.712***	**0.575***	**0.638***
TBitter	0.482*	0.416*	**0.605***	**0.543***	0.430*	0.488*	**0.514***
Astringent	0.288	0.153	0.299	0.228	0.040	0.174	0.258

*Values marked with an asterix are significantly different from 0 with a significance level of p=0.05. Values in bold are higher than ± 0.5. SS = Soluble solids, TP = Total polyphenol, Mg = Mangiferin, IsoMg = Isomangiferin, Comp C = Component C, Comp D = Component D, AUC = Area under the curve (370-570 nm).

Table 11 Correlation coefficients for the taste and mouthfeel attributes and the chemical/instrumental variables of *C. subternata* infusions

Variables	SS	TP	Mg	IsoMg	Comp A	Comp C	ErioTrin	Comp B	Hd	AUC
TSweet	**-0.518***	-0.461*	**-0.727***	**-0.641***	**-0.591***	**-0.820***	0.164	-0.216	**-0.614***	-0.416*
TSour	0.034	0.084	0.302	0.188	0.156	0.478*	-0.075	0.089	0.069	-0.041
TBitter	0.353	0.351	0.456*	0.387	**0.560***	**0.616***	0.076	0.301	0.495*	0.344
Astringent	**0.530***	**0.742***	**0.702***	**0.663***	**0.723***	**0.786***	0.195	**0.552***	0.459*	**0.543***

*Values marked with an asterix are significantly different from 0 with a significance level of p=0.05. Values in bold are higher than ± 0.5. SS = Soluble solids, TP = Total polyphenol, Mg = Mangiferin, IsoMg = Isomangiferin, Comp A = Component A, Comp C = Component C, ErioTrin = Eriocitrin, Comp B = Component B, Hd = Hesperidin, AUC = Area under the curve (370-570 nm).

Table 12 Correlation coefficients for the taste and mouthfeel attributes and the chemical/instrumental variables for *C. maculata* infusions

Variables	SS	TP	Mg	IsoMg	Comp C	Hd	Erio	ErioTrin	Ht	AUC
TSweet	-0.156	-0.104	0.018	-0.041	0.134	-0.124	-0.187	-0.047	0.293	-0.145
TSour	0.447*	0.337	0.002	0.131	0.147	-0.029	0.317	0.183	-0.392	0.364
TBitter	-0.007	0.048	0.009	0.051	-0.065	-0.080	-0.048	-0.091	0.070	0.141
Astringent	**-0.551***	**-0.582***	**-0.527***	**-0.616***	**-0.588***	**-0.574***	**-0.530***	**-0.659***	**0.555***	**-0.567***

*Values marked with an asterix are significantly different from 0 with a significance level of p=0.05. Values in bold are higher than ± 0.5. SS = Soluble solids, TP = Total polyphenol, Mg = Mangiferin, IsoMg = Isomangiferin, Comp C = Component C, Hd = Hesperidin, Erio = Eriodictyol, ErioTrin = Eriocitrin, Ht = Hesperetin , AUC = Area under the curve (370-570 nm).

5. DISCUSSION

High temperature chemical oxidation, more commonly referred to as “fermentation”, is responsible for the characteristic dark brown colour and the sweet, honeylike flavour of honeybush tea (Du Toit & Joubert, 1998a). In the present study it was demonstrated that no new sensory attributes developed during fermentation from 8 h to 32 h, but the average attribute intensity of the sensory attributes increased or decreased. In general, the positive sensory attributes increased with fermentation time whereas the negative sensory attributes decreased. It is possible that some of the sensory attributes intensities did not increase/decrease by fermentation, but that the perceived intensity changed due to the increase/decrease in the intensity of another attribute. Furthermore, differences also existed between the different species.

The characteristic sensory profile of honeybush tea was described in Chapter 3 as a combination of floral, sweet, fruity and plantlike flavours with a sweet taste and a slightly astringent mouthfeel. The most detrimental negative sensory attributes were shown to be hay/dried grass and burnt caramel aroma and flavour. *Cyclopia genistoides* also had a problem with a bitter taste. These attributes mentioned above should thus mainly be taken into account when determining the optimum fermentation conditions for the production of honeybush tea. In order to produce honeybush tea (*C. genistoides*) with an optimal sensory profile a fermentation period of at least 16 h was required in order to increase the average attribute intensities of the positive sensory attributes and lower the average intensities of the negative sensory attributes to an acceptable level. However, at 80°C/16 h bitter taste may still pose a problem and increasing the fermentation time to 24 h will result in a significant ($p \leq 0.05$) reduction. Although the hay/dried grass aroma remained stable at 90°C the highest average intensity value (six) was very low and would hardly be noticeable. A fermentation temperature of 80°C and a fermentation time of 24 h would thus be recommended for *C. genistoides*. Alternatively, a fermentation temperature of 90°C and a fermentation time of 16 h may be used, but will result in a honeybush infusion with less rose geranium notes as the rose geranium aroma was significantly ($p \leq 0.05$) higher in *C. genistoides* fermented at 80°C.

In terms of *C. subternata* a fermentation temperature of either 80°C or 90°C can be used, depending on whether a floral (fynbos floral and rose geranium) or apricot jam honeybush tea is desired. It is possible that the volatile components responsible for the floral notes are more sensitive to higher temperatures compared to those responsible for the apricot jam aroma. Another possibility is that the significantly ($p \leq 0.05$) lower fynbos floral and rose geranium notes in *C. subternata* fermented at 90°C might cause the perceived intensity of the apricot jam aroma to be higher. A fermentation time of at least 16 h is required to effectively reduce the rotting plant water aroma and astringency of *C. subternata.* Similar to *C. genistoides* a fermentation period of 24 h would be recommended to effectively reduce/eliminate the negative sensory attributes and to increase the intensities of the positive sensory attributes.

Cyclopia maculata requires a fermentation temperature of 80°C/24 h in order to effectively reduce the rotting plant water and green grass aroma. Although both rotting plant water and cooked vegetables aroma can be reduced to an acceptable level after only 8 h of fermentation at 90°C, green grass aroma increases significantly ($p \leq 0.05$) at this temperature. This is quite interesting as Du Toit and Joubert (1998b) reported that a grassy aroma is associated with insufficiently fermented honeybush tea (*C. buxifolia*). Furthermore, Le Roux *et al.* (2008) and Cronje (2010) reported that the volatile compounds present in the highest concentrations in unfermented honeybush tea (*C. genistoides*) were associated with green aroma

attributes. One would thus expect the grassy aroma to decrease as the fermentation temperature and time increased. It is possible that another aroma may mask or suppress the green grass aroma at 80°C. Additionally, astringency and hay/dried grass aroma and flavour were significantly ($p \leq 0.05$) higher in *C. maculata* fermented at 90°C. The highest fynbos floral flavour and sweet taste average intensities were also obtained at 80°C/24 h. A fermentation temperature of 80°C and a fermentation time of 24 h would thus be recommended for this particular species.

These differences between the species could be attributed to the quantitative and qualitative differences in the chemical composition, i.e. the volatile fraction (Cronje, 2010) and the polyphenols (De Beer & Joubert, 2010). It is known that neither new volatile compounds (Le Roux *et al.*, 2008; Cronje, 2010) nor new major monomeric polyphenolic compounds form during fermentation (De Beer & Joubert, 2010). However, the concentration of certain compounds increase whereas others decrease, i.e. hexanal, 6-methyl-5-hepten-2-one, limonene, 3,5-octadien-2-one, 6-methyl-3,5-heptadien-2-one, β-cyclocitral, geranyl acetone and dihydroactinidiolide decrease during fermentation in *C. genistoides*, whereas *trans*-furanoid linalool oxide, *cis*-furanoid linalool oxide, linalool, α-terpineol, nerol and geraniol increase (Le Roux *et al.,* 2008; Cronje, 2010). Most of the volatile compounds which decrease are associated with aroma attributes such as fatty, green and grassy while the volatile compounds which increased associated with sweet, woody, fruity and floral aroma attributes. It is thus not surprising that during fermentation only the aroma attribute intensities increased or decreased and no new aroma attributes developed. The impact of other components and specifically the interaction between these components and the volatile compounds could also help to explain the differences in the sensory profiles between the species, batches and samples.

Fermentation reduced the soluble solids content, total polyphenol content and the concentration of the individual polyphenolic compounds quantified. Also, absorbance as a measure of colour decreased with increasing fermentation temperature and time reflecting the change in the soluble solids content. This is similar to the results obtained in Chapter 4. The decrease in the bitter taste associated with *C. genistoides* with fermentation can be attributed to the decrease in the mangiferin and isomangiferin content as there was a relatively strong significant correlation between bitter taste and each of these two compounds. They were present at very high levels compared to the other species. Additionally, bitter taste might also be influenced by compound C and D. In *C. subternata* mangiferin, compound A and C also appear to play a role in bitter taste. The fact that hesperidin, which is tasteless (Ley, 2008), correlated significantly ($p \leq 0.05$) to bitter taste could be explained by the fact that there was very little variation in the bitter taste of the *C. subternata* samples. Similarly, the lack of correlations of bitter taste with any of the polyphenolic compounds in *C. maculata* could be explained by the lack of variation in bitter taste between the samples. The absence of a bitter taste in *C. maculata* could also be due to the presence of eriodictyol, which possess bitter masking properties (Ley *et al.,* 2005; Ley, 2008), and hesperetin, a compound with sweet enhancing properties (Reichelt *et al.,* 2010a; 201b),

The sweet taste in *C. genistoides* and *C. subternata* correlated negatively with most of the chemical/instrumental parameters whereas in *C. maculata* there existed no significant ($p > 0.05$) correlation between sweet taste and the chemical/instrumental parameters. No specific compound could yet been linked to sweet taste, however, it is clear from these results that sweet taste is not associated with any of the major polyphenolic compounds quantified in this study. Other types of compounds may play a role. For example, certain amino acids (mostly in the D-form) such as leucine, phenylalanine, tryptophan and tyrosine

have a sweet taste (Solms, 1969). Further studies are needed to determine which compounds and to what extent play a role.

It is surprising that the xanthones appear to be related to the bitter taste, and not astringency, as McManus *et al.* (1981) proposed that a phenolic compound with two adjacent hydroxyl groups would elicit an astringent sensation. This structural requirement is fulfilled by the xanthones and one would expect a correlation to exist between astringency and the xanthones. This is not the case for *C. genistoides* nor *C. maculata,* but in *C. subternata* mangiferin and isomangiferin correlate relatively strongly to astringency. It is possible that the concentration of these compounds may play a role as a fraction of that present in *C. genistoides* was present in *C. subternata.* Interestingly, hesperetin (sweet enhancing flavanone) correlated significantly with astringency (r = 0.555). This could possibly be explained by the fact that hesperetin was only present in one of the *C. maculata* batches (batch c). As mentioned previously (Chapter 4), It is possible that the quantification of the tannin content could reveal more insight into the astringency of honeybush tea but the levels were so low in the honeybush infusions used for this study that it was unquantifiable by the MCP tannin assay (unpublished results). Furthermore, the impact of other compounds, as well as the interaction between the different compounds and their influence on the taste and mouthfeel should be considered in future research in order to obtain a more comprehensive picture of the effect the chemical composition has on the taste and mouthfeel properties of honeybush tea.

6. CONCLUSION

Chemical oxidation or “fermentation” resulted in an increase (positive sensory attributes) and decrease (negative sensory attributes) of sensory attributes rather than the formation of new sensory attributes. Fermentation at 80°C/24 h is recommended for *C. genistoides* (stronger rose geranium aroma) but an acceptable product can be obtained by fermenting at 90°C/16 h. *Cyclopia subternata* can be fermented at either 80°C or 90°C, depending on whether a floral or apricot jam notes is desired. *Cyclopia maculata* requires a fermentation temperature of 80°C as fermentation at 90°C resulted in an increase of negative sensory attributes (hay/dried grass aroma and flavour and green grass aroma). A fermentation time period of 24 h is required for *C. maculata* in order to effectively reduce the intensity of the negative sensory attributes.

No specific compounds could be linked to the sweet taste of honeybush tea, however, sweet taste correlated negatively to most compounds correlating to bitterness. The decrease in bitter taste and astringency can be attributed to the decrease in the concentration of the polyphenolic compounds with fermentation. The xanthones, mangiferin and isomangiferin, appear to be important polyphenolic compounds with regards to bitter taste and astringency. Furthermore, the concentration of the xanthones seems to be important. At low concentrations they appear to be perceived as bitter whereas at very high concentrations they elicit astringency. *Cyclopia maculata* on the other hand revealed a relationship between astringency and hesperetin. The impact of a number of other components present in honeybush, such as amino acids, polysaccharides, volatile compounds and the interaction between these components could additionally influence the changes occurring in the sensory profiles of these species during fermentation and need to be considered in future studies. Attention should also be given to taste modulating compounds such as eriodictyol and hesperetin.

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Chapter 6

GENERAL DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS

The success of any food product depends mainly on its sensory quality and consistency. Quality control and sensory assessment are used to analyse quality and to ensure a reliable supply of a standardised product with unchanging sensory characteristics. Currently, the sensory quality of honeybush infusions is not monitored by a standard set of parameters. Until recently, honeybush infusions were produced using *C. intermedia, C. genistoides* and *C. subternata,* however, as the demand began exceeding the supply, the focus shifted to include other *Cyclopia* species, such as *C. sessiliflora, C. longifolia* and *C. maculata* (Joubert *et al.,* 2011). In industry one species is often substituted for another or blended with one another without considering the different sensory profiles of each species. Variation in sensory quality due to inherent species differences, as well as differences in localities, environmental conditions and processing parameters are not taken into account, resulting in considerable variation in the sensory profile of honeybush infusions currently being sold in the market place. This could lead to detrimental consequences as a consistent supply of high quality honeybush infusions with unchanging flavour profiles cannot be ensured. Furthermore, in a market where new herbal tea products are appearing daily, it is becoming increasingly important to successfully differentiate honeybush infusions from its competitors to identify niche markets. In order to do this an accurate definition and description of the sensory attributes associated with the product is required. To date, no research has been published on the sensory profile of *Cyclopia* species as previous research mainly focused on the chemical and medicinal properties of honeybush infusions. Moreover, no standard set of tools are available to the industry to monitor the sensory quality of honeybush infusions.

In terms of processing, fermentation conditions ranging from 70°C/60 h for *C. intermedia* (Du Toit & Joubert, 1999) to 80-85°C/18-24 h for other *Cyclopia* species currently employed by the industry, is based on research done on *C. intermedia* and *C. buxifolia* (previously classified as *C. maculata*) (Joubert *et al.,* 2011). It is quite possible that these conditions are not necessarily optimum for producing honeybush infusions from *Cyclopia* species, other than *C. intermedia* and *C. buxifolia*. In addition, previous research only considered one sensory attribute, namely the characteristic sweet aroma (Du Toit & Joubert, 1999). The effect of fermentation temperature and time on species beside *C. intermedia* thus needs to be investigated in order to determine the optimum fermentation temperature-time regime for each *Cyclopia* species to prevent the production of honeybush infusions of inferior sensory quality. A set of standardised terms to describe the characteristic honeybush aroma and flavour, as well as specific negative attributes associated with honeybush infusions is needed to achieve this. Tools such as sensory lexicons and sensory wheels are often used in the industry or research-related environments to describe the sensory attributes associated with a certain food and beverage product (Drake & Civille, 2002). The successful application of these sensory tools by a number of industries, i.e. wine (Noble *et al*., 1987; Gawel *et al*., 2000), beer (Meilgaard *et al*., 1979) and tea (*Camellia sinensis*) (Bhuyan & Borah, 2001) industries, suggest that the development of a sensory lexicon and wheel would make a valuable contribution to the honeybush industry.

The objectives of this study were, therefore, to develop a suitable set of sensory descriptors that can be used to describe the sensory characteristics of honeybush infusions, to determine whether there exist differences in the sensory profiles of six *Cyclopia* species, and to determine whether some of these

descriptive attributes, i.e. taste and mouthfeel, are associated with specific phenolic compounds, with the aim of identifying some of the major sensory impact compounds of honeybush infusions. Changes in the sensory characteristics and phenolic composition of honeybush infusions prepared from three *Cyclopia* species (*C. genistoides, C. subternata* and *C. maculata*) during different temperature-time fermentation combinations were determined. This enabled establishment of optimum fermentation conditions. This also gave insight into the change in the phenolic honeybush composition and the role of the phenolic composition in the perceived taste and mouthfeel characteristics of honeybush infusions. Furthermore, the aroma of *C. maculata* was investigated by means of gas chromatograpgy-olfactometry (GC-O) to identify aroma-impact volatiles.

The sensory properties of 58 honeybush samples prepared from six fermented *Cyclopia* species (*C. sessiliflora, C. longifolia, C. genistoides, C. intermedia. C. subternata* and *C. maculata*) were evaluated by characterising the aroma, flavour, taste and mouthfeel attributes of their infusions. In order to capture as much variation as possible, samples from different localities, commercial producers, harvesting dates, processing conditions and size fractions were used. Experimental samples prepared for research purposes were also included. Descriptive analysis was used to establish sensory profiles for all the samples and by comparing these profiles the variation in their sensory attributes was established. In addition, a honeybush sensory wheel and a sensory lexicon consisting of descriptors, definitions and reference standards were developed by selecting the most frequently occurring positive and negative sensory descriptors.

Analysis of the sensory profiles of the honeybush samples revealed that the “characteristic” sensory profile of honeybush infusions can be described as a combination of floral, sweet, fruity and plantlike flavours with a sweet taste and a slight astringent mouthfeel. Similarities and differences in the sensory characteristics between the different *Cyclopia* species were also established and, based on discriminant analysis, the species could be divided into three distinct groups; group A (*C. sessiliflora, C. intermedia* and *C. genistoides)*, group B (*C. longifolia* and *C. subternata)* and group C (*C. maculata*). Group A associated with fynbos floral, fynbos sweet and plantlike attributes, group B with rose geranium and fruity sweet attributes and group C with woody, boiled syrup and cassia/cinnamon attributes. The most detrimental negative sensory attributes appeared to be hay/dried grass and burnt caramel aroma and flavour. It appeared as if most of the variation in the sensory attributes within a specific species, especially in terms of the negative sensory attributes, was due to different processing conditions rather than being species-specific. Most of the samples associated with negative sensory attributes were commercial samples and not necessarily optimally processed and this could have resulted in the emergence of the negative sensory attributes. The fermentation conditions used during processing was not available for most of the commercial samples and those that were, indicated that the infusions was fermented at 70°C for 60 hours or 80-85°C for 16-24 hours. These fermentation conditions are based on a study done on *C. intermedia* and *C. buxifolia,* however, the same conditions are not necessarily optimum fermentation conditions for all the *Cyclopia* species. It is thus very likely that some of the latter samples might have been over- or even under-fermented. Besides, these conditions are based on a study which only considered the sweet aroma of honeybush infusions (Du Toit & Joubert, 1999). In order to effectively determine the optimum fermentation conditions, a number of sensory attributes should be considered and not just the sweet aroma of honeybush infusions.

The spicy cassia/cinnamon note present in *C. maculata* was unexpected as honeybush infusions have not previously been described as “spicy”. However, cinnamon-like or spicy volatile compounds (6-

methyl-3,5-heptadien-2-one, 4-acetyl-1-methyl-cyclohexene, (+)-*p*-menth-1-en-9-al, eugenol and (7*E*)-megastigma-5,7,9-trien-4-one) have been identified in a number of *Cyclopia* species (Le Roux *et al.,* 2008; Cronje, 2010) and it was postulated that one or more of these volatiles may be responsible for the cassia/cinnamon-spicy note. In order to identify which compounds are responsible for this characteristic aroma note, a representative cassia/cinnamon-spicy *C. maculata* sample was selected and analysed using GC-O. Although eugenol is known to have a warm-spicy, dry aroma reminiscent of cloves rather than a cassia/cinnamon spicy aroma it was the only compound associated with a spicy aroma identified in *C. maculata*. The spicy aroma could thus possibly partly be due to the high concentration of eugenol although this compound is not usually associated with a cinnamon aroma. In general aromas of different qualities can mask or suppress one another, compounds with similar qualities can blend and produce a new aroma, and certain compounds present in concentrations below their odour threshold or which has no odour activity when assessed individually can contribute to the aroma when they are in a mixture (Delahunty *et al.,* 2006).

Large variation existed in the levels of soluble solids, total polyphenol and individual monomeric polyphenolic compounds, as well as the absorbance of the infusions between the different *Cyclopia* species and the different samples for an individual species. Factors such as locality, climate, soil conditions, survival strategies (Schutte, 1997), the age of plant/regrowth (Joubert *et al.,* 2011), the presence of flowers/pods (Du Toit & Joubert, 1998) and the leaf-to-stem ratio (Joubert, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, 2010, personal communication) could contribute to this variation. Only mangiferin, isomangiferin, hesperidin and compound C (unidentified phenolic compound) were detected in all six *Cyclopia* species. Infusions of *C. genistoides, C. longifolia* and *C. sessiliflora* in order of prominence had the highest concentration of both mangiferin and isomangiferin whereas *C. genistoides* and *C. maculata* had the highest hesperidin content. These species would thus be ideal for production of extracts containing high levels of these phytochemicals.

Having developed a set of descriptors to describe the flavour of honeybush infusions, and having established that there is considerable variation in the sensory and polyphenolic profiles of the different species and between the different samples from individual species, it was determined whether correlations could be found between certain sensory attributes, specifically taste and mouthfeel, and specific compounds in a honeybush infusion, with the focus being on non-volatile phenolic components. The bitter taste present in *C. genistoides* appeared to be due to the high mangiferin content, however, compounds such as isomangiferin and compound C (unidentified phenolic compound) might also play a role. Hesperidin, considered to be tasteless (Ley, 2008), also correlated significantly with bitter taste. The xanthones also appeared to be linked to astringency.

With the understanding gained from having analysed the relationship between composition and sensory quality of honeybush infusions, the effect of fermentation on the sensory characteristics and phenolic composition was investigated in order to determine the optimum fermentation temperature/time combinations for *C. genistoides, C. subternata* and *C. maculata* and to validate the relationship between specific polyphenolic compounds and bitter taste and mouthfeel. Fermentation resulted in an increase (positive sensory attributes) and decrease (negative sensory attributes) of sensory attributes rather than the formation of new sensory attributes. This is not surprising as the sensory profile is influenced by the chemical composition and it is known that no new volatile compounds (Le Roux *et al.,* 2008; Cronje, 2010) or detectable monomeric phenolic compounds (De Beer & Joubert, 2010) form during fermentation. However, it has been reported that the concentration of certain volatile compounds increase whereas others decrease

which would results in changes in the sensory profile of honeybush infusions. In order to produce honeybush infusions with an optimal sensory profile a fermentation period of 80°C/24 hours or 90°C/16 h is required for *C. genistoides* and *C. subternata.* Fermenting *C. genistoides* at 90°C would result in a honeybush infusion with slightly less rose geranium notes, but an acceptable product can be obtained. *Cyclopia subternata* can be fermented at either 80°C or 90°C, depending on whether floral or apricot jam notes are desired, whereas *C. maculata* requires a fermentation temperature of 80°C. Fermentation of *C. maculata* at 90°C results in an increase of negative sensory attributes (hay/dried grass aroma and flavour and green grass aroma). A fermentation time period of at least 24 h is required for *C. maculata* in order to effectively reduce the intensity of the negative sensory attributes. In the future, different fermentation temperature/time combinations may be used as a means of manipulating the flavour characteristics of certain *Cyclopia* species.

Fermentation reduced the soluble solids content, total polyphenol content and the concentration of the individual polyphenolic compounds quantified. Absorbance as a measure of colour decreased with increasing fermentation temperature and time, reflecting the change in the soluble solids content and polyphenolic composition. The decrease in bitter taste and astringency could be attributed to the decrease in the concentration of the polyphenolic compounds with fermentation. In *C. genistoides* the bitter taste seemed to be caused by a high concentration of xanthones whereas these compounds appeared to cause astringency in *C. subternata,* confirming the previous results indicating that these compounds might be linked to bitter taste and astringency. The concentration of the xanthones seems to be important as *C. subternata* contains a fraction of that present in *C. genistoides.* These compounds were negatively correlated with astringency in *C. maculata* but no correlation with bitterness was observed. Infusions of this species confirm higher levels of mangiferin and isomangiferin than *C. subternata,* but not to the extent present in *C. genistoides.* Another reason could possibly be the very small variation which exists between the different *C. maculata* samples in terms of astringency. Also, the presence of measurable quantities of eriodictyol, a compound possessing bitter masking properties and hesperetin (Ley *et al.* 2005; Ley 2008), a compound with sweet enhancing properties (Reichelt *et al.,* 2010), in *C. maculata* could possibly explain the low average intensity score for bitter taste in this particular *Cyclopia* species.

This study scientifically demonstrated for the first time the variation which exists in the sensory profiles of a number of *Cyclopia* species. These differences, coupled with blending and the lack of quality control, indicate that a number of products on the market place most likely have inconsistent flavour profiles. Blending also leads to a loss of the unique flavour associated with individual species which could possibly, in the future, be used to establish niche markets. On the other hand, blending could also result in a standardised honeybush infusion as the sensory profile of a particular species can be adjusted by blending it with one or more species. Consumer sensory analysis using the standard nine point hedonic scale and the target consumer (Lawless & Heymann, 2010) can be used in future to measure degree of liking of the different *Cyclopia* species. Combining these results with results obtained by descriptive analysis could indicate sensory drivers of liking (McEwan *et al.,* 1998). This would enable the identification of species with the most commercial potential.

The effect of both fermentation time and temperature on the sensory profile and chemical composition of the infusions of *C. genistoides, C. subternata* and *C. maculata* was also shown. The optimum fermentation time and temperature conditions were determined for each species. From the results it was evident that the same processing conditions can not be used for all *Cyclopia* species. It was shown

that the polyphenolic profile of the species differs and that the levels of some of these compounds may partly explain the variation in the taste and mouthfeel characteristics of the species. In future, different fermentation temperature and time combinations may be employed to manipulate the flavour characteristics of certain species. Consumer sensory analysis may additionally facilitate determination of optimum fermentation temperature and time regimes for specific species by pinpointing the most acceptable (and unacceptable) sensory characteristics associated with honeybush infusions.

Although certain compounds, such as the xanthones, were linked to bitter taste and mouthfeel (astringency) the impact of other constituents present in honeybush, such as amino acids, polysaccharides, volatile components, their interaction and their influence on the flavour should be considered in future research in order to gain more insight into the relationship between the sensory characteristics and the chemical composition of honeybush infusions. Only with the findings obtained from such studies it may then be possible to formulate a prediction model based on the chemical composition of honeybush infusions, which could be used to predict sensory quality. This is important with regards to plant improvement as selections are currently based on phenolic compounds such as the xanthones (Joubert *et al.,* 2011) which could cause problems with regards to the sensory quality of the infusions if the taste characteristics of the specific compounds are not taken into account. In order to effectively reduce the levels of these compounds to obtain an acceptable taste and mouthfeel profile increased fermentation temperatures and times might be needed, which has cost implications due to increased energy demand.

A sensory wheel and lexicon were developed to facilitate the characterisation and description of honeybush sensory characteristics in a way that can be understood by anyone. These tools lend itself to the training of sensory panels and quality control personnel in the industry, and may be useful for improving the communication of product characteristics between different role players in the industry. It would also be beneficial to eventually develop flavour wheels tailored for each *Cyclopia* species as not all the descriptive terms currently included on the sensory wheel necessarily apply to all *Cyclopia* species. Analysing the sensory profiles of a number of samples prepared from one *Cyclopia* species from different localities and harvesting seasons would add great value to these tools since this would provide an indication of the seasonal variability in the sensory profile of honeybush. This would allow for refinement of the sensory terminology as redundant attributes can be removed while new attributes may be added. Some of the attributes may be rephrased or broken down into more suitable descriptors. The term astringency, for instance, is a multifaceted concept which may be divided into a number of sub-qualities. Gawel *et al.* (2000) developed several groupings for astringent mouthfeel characteristics associated with red wine such as drying, unripe, harsh and surface smoothness. The term astringency is likely too general to describe all mouthfeel characteristics associated with a honeybush infusion. These tools will not only be able to improve understanding and communication of different honeybush attributes, but may also form the foundation of many future studies involving sensory related aspects of honeybush.

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ADDENDUM A

Sample information for *C. sessiliflora, C. longifolia, C. genistoides, C. intermedia, C. subternata* and *C. maculata*

Specie	Geographic area (Producer)	Harvesting date	Fermentation	Drying	Fraction
Ses 1	Helderfontein (ARC)	1/3/2010_Batch a	90°C/16 h	40°C/6 h	<12>40 mesh
Ses 2	Helderfontein (ARC)	1/3/2010_Batch a	80°C/24 h	40°C/6 h	<12>40 mesh
Ses 3	Helderfontein (ARC)	1/3/2010_Batch b	90°C/16 h	40°C/6 h	<12>40 mesh
Ses 4	Helderfontein (ARC)	1/3/2010_Batch b	80°C/24 h	40°C/6 h	<12>40 mesh
Ses 5	Riversdale	3/2/2010	80°C/24 h	40°C/6 h	<12>40 mesh
Ses 6	Riversdale	3/2/2010	90°C/16 h	40°C/6 h	<12>40 mesh
Ses 7	Bredasdorp	27/1/2010	90°C/16 h	40°C/6 h	<12>40 mesh
Lon 1	Bredasdorp (ARC)	22/2/2010_Batch a	90°C/16 h	40°C/6 h	<12>40 mesh
Lon 2	Bredasdorp (ARC)	22/2/2010_Batch a	80°C/24 h	40°C/6 h	<12>40 mesh
Lon 3	Bredasdorp (ARC)	22/2/2010_Batch b	90°C/16 h	40°C/6 h	<12>40 mesh
Lon 4	Bredasdorp (ARC)	22/2/2010_Batch b	80°C/24 h	40°C/6 h	<12>40 mesh
Lon 5	Bredasdorp	2009	Unavailable	Unavailable	<12>40 mesh
Lon 6	(De Lange)	2009	70°C/60 h	Unavailable	<12>40 mesh
Lon 7	Loerie (De Lange)	Augustus 2008	70°C/60 h	Unavailable	<12>40 mesh
Gen 1	Koksrivier (ARC)	24/03/2010	90°C/16 h	40°C/6 h	<12>40 mesh
Gen 2	Koksrivier (ARC)	24/03/2010	80°C/24 h	40°C/6 h	<12>40 mesh
Gen 3	Koksrivier Overberg	19/01/2010	90°C/16 h	40°C/6 h	<12>40 mesh
Gen 4	Koksrivier Overberg	19/01/2010	80°C/24 h	40°C/6 h	<12>40 mesh
Gen 5	Koksrivier West Coast	19/01/2010	90°C/16 h	40°C/6 h	<12>40 mesh
Gen 6	Koksrivier West Coast	19/01/2010	80°C/24 h	40°C/6 h	<12>40 mesh
Gen 7	KoksrivierKirstenbosch	19/01/2010	90°C/16 h	40°C/6 h	<12>40 mesh
Gen 8	KoksrivierKirstenbosch	19/01/2010	80°C/24 h	40°C/6 h	<12>40 mesh
Gen 9	Bredasdorp	Nov-09	Unavailable	Unavailable	<12>40 mesh
Gen 10	Koksrivier	18/01/2010; 16/01/2010; 21/01/2010	Unavailable	Unavailable	<12>40 mesh
Gen 11	Bredasdorp	2/04/2009; 18/11/2009; 3/12/2009	Unavailable	Unavailable	<!12>40 mesh
Int 1	Helderfontein (ARC)	15/03/2010_Batch a	90°C/16 h	40°C/6 h	<12>40 mesh
Int 2	Helderfontein (ARC)	15/03/2010_Batch a	80°C/24 h	40°C/6 h	<12>40 mesh
Int 3	Helderfontein (ARC)	15/03/2010_Batch b	90°C/16 h	40°C/6 h	<12>40 mesh
Int 4	Helderfontein (ARC)	15/03/2010_Batch b	80°C/24 h	40°C/6 h	<12>40 mesh
Int 5	Kritzinger	2009	Unavailable	Unavailable	<12>40 mesh
Int 6	Helderfontein	17/11/2009	90°C/16 h	40°C/6 h	<12>40 mesh
Int 7	Ackerman	24/11/2009	Unavailable	Unavailable	Unavailable
Int 8	Nortje	1999 & 2000	70°C/60 h	Unavailable	Unavailable
Int 9	Nortje	5/6/2009	70°C/60 h	Unavailable	<12>40 mesh
Int 10	CHT Co	1/12/2009	80-85°C/≥16 h	Unavailable	Coarse
Int 11	Haarlem	10/3/2000	70°C/60 h	40°C/24 h	<2.00
Sub 1	Barrydale (ARC)	23/03/2010_Batch a	90°C/16 h	40°C/6 h	<12>40 mesh
Sub 2	Barrydale (ARC)	23/03/2010_Batch a	80°C/24 h	40°C/6 h	<12>40 mesh
Sub 3	Barrydale (ARC)	23/03/2010_Batch b	90°C/16 h	40°C/6 h	<12>40 mesh
Sub 4	Barrydale (ARC)	23/03/2010_Batch b	80°C/24 h	40°C/6 h	<12>40 mesh
Sub 5	Helderfontein (ARC)	3/3/2010_Batch a	90°C/16 h	40°C/6 h	<12>40 mesh
Sub 6	Helderfontein (ARC)	3/3/2010_Batch a	80°C/24 h	40°C/6 h	<12>40 mesh
Sub 7	Helderfontein (ARC)	3/3/2010_Batch b	90°C/16 h	40°C/6 h	<12>40 mesh

Sub 8	Helderfontein (ARC)	3/3/2010_Batch b	80°C/24 h	40°C/6 h	<12>40 mesh
Sub 9	Helderfontein (ARC)	17/11/2009	90°C/16 h	40°C/6 h	<12>40 mesh
Sub 10	Behr (CHT Co)	7/10/2009	85°C/24 h	Unavailable	<12>40 mesh
Sub 11	Du Toitskloof	Unavailable	70°C/72 h (Pods present)	40°C/6 h	<12>40 mesh
Mac 1	Riversdale	3/2/2010_Batch a	90°C/16 h	40°C/6 h	<12>40 mesh
Mac 2	Riversdale	3/2/2010_Batch a	80°C/24 h	40°C/6 h	<12>40 mesh
Mac 3	Riversdale	3/2/2010_Batch b	90°C/16 h	40°C/6 h	<12>40 mesh
Mac 4	Riversdale	3/2/2010_Batch b	80°C/24 h	40°C/6 h	<12>40 mesh
Mac 5	Riversdale (CHT Co)	Feb-10	80-85°C/≥16 h	Unavailable	<12>40 mesh
Mac 6	Riversdale (CHT Co)	Feb-10	80-85°C/≥16 h	Unavailable	Superfine
Mac 7	Riversdale (CHT Co)	Feb-10	80-85°C/≥16 h	40°C/6 h	<12>40 mesh
Mac 8	Welgedacht	2/12/2009	90°C/16 h	40°C	Sweepings
Mac 9	Welgedacht	2/12/2009	90°C/16 h (Flowers present)	40°C/6 h	<8>12 mesh
Mac 10	Genadendal museum (Flowers present before sieving)	2009	Overnight in baking oven	Unavailable	<12>40 mesh
Mac 11	Riversdale (CHT Co)	Feb-10	80-85°C/≥16 h	Unavailable	<12>40 mesh

Ses = *C. sessiliflora,* Lon = *C. longifolia,* Gen = *C. genistoides,* Int = *C. intermedia,* Sub = *C. subternata,* Mac = *C. maculata.* Batch a and batch b refer to plant material harvested at the same location on the same day but from different areas.

ADDENDUM B

A complete list of the descriptors generated during the training period

Aroma attributes	Descriptors
Floral	Fynbos floral, Rose geranium, Rose petals (dry), Rose petals (fresh), Perfume
Fruity	Fruity, Lemon, Orange, Apple, Cooked apple, Apricot jam, Banana, Bananna bread, Berry, Guava, Cherry essence, Dried fruit mix, Raisins, Lemon essence, Lemongrass
Plantlike	Fynbosplantlike, Geranium plantlike, Herbaceous
Woody	Woody, Rooibos woody, Pine, Smokey, Plankey, Burnt
Sweet	Fruity sweet, Boiled syrup, Caramel, Honey, Fynbos sweet
Spicy	Cinnamon, Cassia, Nutmeg, Mixed spices
Nutty	Walnut, Coconut, Almond
Negative	Dusty, Musty, Mouldy, Rotting plant water, Seaweed, Hay/Dried grass, Green grass, Cooked vegetables, Soapy, Medicinal, Yeasty, Green beans, Sour, Sweaty, Compost, Wet carpet, Fishy, Burnt caramel, Burnt vegetables, Penicillin, Antiseptic
Other	Minty, Cheesy, Earl Grey, Whiskey, Oily, Metallic

Flavour, taste and mouthfeel	Descriptors
Taste	Sweet, Sour, Salty, Bitter
Mouthfeel	Astringent, Flat, Bland
Floral	Fynbos floral, Rose geranium, Rose petals (dry), Rose petals (fresh), Perfume
Fruity	Fruity, Lemon, Orange, Cooked apple, Apricot jam, Banana, Berry, Guava, Cherry essence
Plantlike	Fynbosplantlike, Geranium plantlike, Herbaceous
Woody	Woody, Rooibos woody, Pine
Spicy	Cinnamon, Cassia, Nutmeg, Mixed spices
Nutty	Walnut, Coconut, Almond
Negative	Dusty, Musty, Mouldy, Rotting plant water, Seaweed, Hay/Dried grass, Green grass, Cooked vegetables, Soapy, Medicinal, Yeasty, Green beans
Other	Minty, Earl Grey, Whiskey, Oily, Metallic

ADDENDUM C

Questionnaire for trained panel – honeybush tea profiling

Date: _____ Session: ___ Judge no: ___ Name: _____ Sample:_____

BASIC	Min – Max: 0-40 Default: 0	SOUR	0	100
	Min –Max: 0-60 Default: 0	BITTER	0	100
	Min – Max: 5-30 Default: 5	ASTRINGENT	0 R	100
	Min - Max: 5-50 Default: 20	SWEET	0 R	100
FLAVOUR	Max			
FLORAL	30	Fynbos floral	0	100
	60	Rose Geranium	0 R	100
	60	Rose/Perfume	0	100
FRUITY	25	Lemon	0	100
	10	Orange	0	100
	0	Apricot jam	0	100
	0	Cooked Apple	0	100
	0	Cherry essence	0	100
PLANTLIKE	20	Plantlike	0	100
	40	Woody	0 R	100
	10	Rooibos	0	100
	40	Pine	0	100
SPICY	60	Cassia/Cinnamon	0	100
NUTTY	10	Walnut	0	100
	10	Coconut	0	100
NEGATIVE	30	Dusty	0	100
	0	Yeasty	0	100
	50	Medicinal	0	100
	40	Burnt caramel	0	100
	0	Rotting plant water	0	100
	50	Hay/Dried grass	0	100
	0	Green grass	0	100
	0	Cooked vegetables	0	100

AROMA	Max		
FLORAL	40	Fynbos floral	0 R 100
	70	Rose Geranium	0 R 100
	60	Rose/Perfume	0 100
FRUITY	30	Lemon	0 100
	40	Orange	0 100
	50	Apricot jam	0 100
	25	Cooked Apple	0 100
	20	Cherry essence	0 100
PLANTLIKE	40	Plantlike	0 100
	50	Woody	0 R 100
	0	Rooibos	0 100
	50	Pine	0 100
SWEET	60	Fruity sweet	0 R 100
	50	Boiled syrup	0 100
	50	Caramel	0 100
	0	Honey	0 100
	50	Fynbos sweet	0 R 100
SPICY	75	Cassia/Cinnamon	0 100
NUTTY	40	Walnut	0 100
	30	Coconut	0 100
NEGATIVE	40	Dusty	0 100
	60	Yeasty	0 100
	50	Medicinal	0 100
	70	Burnt caramel	0 100
	60	Rotting plant water	0 100
	50	Hay/Dried grass	0 100
	60	Green grass	0 100
	20	Cooked vegetables	0 100

R = Control sample attribute intensities, Min = Minimum attribute intensity, Max = Maximum attribute intensity.

ADDENDUM D

Suppliers of compounds used as reference standards during descriptive analysis training and compilation of the honeybush sensory lexicon

Compound/Product	Supplier
Honeybush tea	Agricultural Research Council, Stellenbosch, South Africa
Rose geranium oil	La Motte, Franschoek, South Africa
Lemon juice	Tru-Lem, Brookes', South Africa
Orange flavour (ukrma010055a002)	Sensient Flavours, South Africa
Apple Puree	Purity, South Africa
Superfine Apricot jam & Strawberry jam	All Gold, South Africa
Cherry essence	Moir's, South Africa
FTNF Rooibos extract	Rooibos Ltd., Clanwilliam, South Africa
Golden syrup	Illovo, South Africa
Caramel flavour	Wild®, Comhan products, South Africa
Wild flower honey	Hillcrest, Franschoek, South Africa
Cassia/Cinnamon bark	Robertson, South Africa
Band-aid	Elastoplast, Hamburg, Germany
Cis-3-hexen-1-ol	Fluka, Sigma-Aldrich, Steinheim, Gemany
Canned green beans	KOO, Pioneer Foods, South Africa
Sucrose	Huletts, South Africa
Citric acid	Sigma, Sigma-Aldrich, Steinheim, Germany
Caffeine	Fluka, Sigma-Aldrich, Steinheim, Gemany
Alum (aluminium potassium sulfate)	Sigma-Aldrich, Steinheim, Germany

ADDENDUM E

Variation, mean, SD, minimum, maximum and range of *C. sessiliflora, C. longifolia, C. genistoides, C. intermedia, C. subternata* and *C. maculata*

Table 1 The variation between the *C. sessiliflora* samples analysed as well as the mean, SD, minimum, maximum and range

Sample	SS	TP	AUC	AUCnorm	Mg	IsoMg	ErioTrin	Hd	Comp B	Comp C
Ses1	1650.00	299.87	50.93	55.12	15.51	17.04	4.51	3.83	5.63	17.06
Ses2	1878.33	367.96	62.42	59.29	20.79	22.04	5.19	5.00	8.76	26.57
Ses3	1653.33	347.54	51.81	56.07	18.14	18.61	3.87	4.08	5.75	16.02
Ses4	1806.67	343.95	64.16	63.30	20.16	21.01	4.25	4.86	8.14	19.17
Ses5	2453.33	616.98	104.71	76.32	104.27	53.48	6.17	9.16	5.49	35.94
Ses6	2415.00	429.03	95.03	70.13	88.41	48.83	5.80	8.73	4.01	31.69
Ses7	1843.33	576.54	69.00	66.96	29.75	25.82	5.70	5.27	10.24	21.67
Mean	1957.14	425.98	71.15	63.88	42.43	29.55	5.07	5.85	6.86	24.02
SD	337.71	123.36	20.85	7.77	37.37	15.08	0.87	2.18	2.22	7.63
Minimum	1650.00	299.87	50.93	55.12	15.51	17.04	3.87	3.83	4.01	17.06
Maximum	2453.33	616.98	104.71	76.32	104.27	53.48	6.17	9.16	10.24	21.68
Range	803.33	317.10	53.78	21.20	88.75	36.44	2.30	5.33	6.23	4.62

Compounds not detected: narirutin, compound A and compound F. Ses = *C. sessiliflora;* SD = Standard deviation; SS = Soluble solids; TP = Total polyphenol; AUC = Area under the curve (370 – 570 nm); AUCnorm = AUC normalised; Mg = Mangiferin; IsoMg = Isomangiferin; ErioTrin = Eriocitrin; Hd = Hesperidin; Comp B = Compound B; Comp C = Compound C. All chemical/instrumental analysis provided in mg/L except for TP (mg GAE/L), AUC and AUCnorm.

Table 2 The variation between the *C. longifolia* samples analysed as well as the mean, SD, minimum, maximum and range

Sample	SS	TP	AUC	AUCnorm	Mg	IsoMg	ErioTrin	NarRut	Hd	Comp A	Comp B	Comp C
Lon1	2525.00	476.85	82.52	58.48	81.63	47.13	4.76	1.51	6.33	5.47	2.02	8.64
Lon2	2665.00	465.60	82.42	55.33	100.69	52.04	5.01	1.63	7.58	5.58	2.39	9.63
Lon3	2618.33	454.91	79.34	54.08	71.06	44.37	4.05	1.68	8.00	3.57	1.46	9.33
Lon4	2633.33	535.38	86.96	59.04	102.32	51.42	4.35	1.80	8.50	4.94	1.94	11.66
Lon5	2638.33	506.68	105.62	71.45	69.34	32.77	1.89	BD	14.09	1.64	3.26	2.95
Lon6	1548.33	172.65	46.24	53.28	15.44	9.54	BD	BD	4.19	BD	BD	BD
Lon7	1478.33	85.25	34.86	42.06	9.68	9.83	BD	BD	7.11	BD	BD	BD
Mean	2300.95	385.33	74.00	53.71	64.31	35.30	2.87	0.95	7.97	3.03	1.58	6.03
SD	540.20	178.97	24.64	6.16	37.67	18.62	2.21	0.89	3.05	2.47	1.21	4.91
Minimum	1478.33	85.25	34.86	42.06	9.68	9.54	0.00	0.00	4.19	0.00	0.00	0.00
Maximum	2665.00	535.38	105.62	71.45	102.32	52.04	5.01	1.80	14.09	5.58	3.26	11.66
Range	1186.67	450.13	70.77	29.39	92.64	42.50	5.01	1.80	9.90	5.58	3.26	11.66

Compounds not detected: compound F. Lon = *C. longifolia;* SD = Standard deviation; BD = Below detection; SS = Soluble solids; TP = Total polyphenol; AUC = Area under the curve (370 – 570 nm); AUCnorm = AUC normalised; Mg = Mangiferin; IsoMg = Isomangiferin; ErioTrin = Eriocitrin; NarRut = Narirutin; Hd = Hesperidin; Comp A = Compound A; Comp B = Compound B; Comp C = Compound C. All chemical/instrumental analysis provided in mg/L except for TP (mg GAE/L), AUC and AUCnorm.

Table 3 The variation between the *C. genistoides* samples analysed as well as the mean, SD, minimum, maximum and range

Sample	SS	TP	AUC	AUCnorm	Mg	IsoMg	Hd	Comp C
Gen1	1970.00	369.37	81.49	73.86	136.24	35.65	7.46	9.33
Gen2	2091.67	408.20	78.30	66.90	165.12	39.74	8.65	10.32
Gen3	2378.33	606.85	80.98	60.76	153.12	42.18	9.08	28.25
Gen4	2600.00	512.87	94.88	65.16	199.05	47.61	11.78	30.86
Gen5	2426.67	380.06	70.98	52.16	100.56	45.92	13.02	34.19
Gen6	2441.67	357.36	80.54	58.94	116.15	48.87	13.45	37.18
Gen7	2536.67	421.64	87.81	61.73	164.34	60.74	BD	49.08
Gen8	2558.33	434.72	92.76	64.70	194.74	66.25	14.17	51.91
Gen9	2891.67	578.08	137.56	85.00	201.03	55.89	13.62	5.02
Gen10	2370.00	324.36	92.27	69.52	144.72	51.55	13.26	3.47
Gen11	1950.00	315.54	86.45	79.17	81.91	33.03	5.43	4.63
Mean	2383.18	428.10	89.45	65.29	150.63	47.95	9.99	24.02
SD	284.37	98.14	17.48	7.69	39.87	10.27	4.42	18.19
Minimum	1950.00	315.54	70.98	52.16	81.91	33.03	0.00	3.47
Maximum	2891.67	606.85	137.56	85.00	201.03	66.25	14.17	51.91
Range	941.67	291.30	66.58	32.84	119.12	33.22	14.17	48.44

Compound not detected: eriocitrin, narirutin, compound A, compound B and compound F. Gen = *C. genistoides;* SD = Standard deviation; BD = Below detection; SS = Soluble solids; TP = Total polyphenol; AUC = Area under the curve (370 – 570 nm); AUCnorm = AUC normalised; Mg = Mangiferin; IsoMg = Isomangiferin; Hd = Hesperidin; Comp C = Compound C. All chemical/instrumental analysis provided in mg/L except for TP (mg GAE/L), AUC and AUCnorm.

Table 4 The variation between the *C. intermedia* samples analysed as well as the mean, SD, minimum, maximum and range

Sample	SS	TP	AUC	AUCnorm	Mg	IsoMg	Hd	Comp C	Comp F
Int1	1828.33	270.85	57.12	55.73	26.11	19.72	5.21	6.57	1.71
Int2	2003.33	377.25	68.09	60.79	37.81	25.72	7.19	8.49	1.90
Int3	1725.00	369.76	53.43	55.47	21.94	17.78	5.39	5.23	1.33
Int4	1781.67	339.29	63.36	63.57	25.50	20.36	6.13	5.41	1.51
Int5	1930.00	324.60	100.54	93.03	27.51	18.79	8.16	6.17	1.23
Int6	2040.00	325.50	67.73	59.29	32.76	25.48	1.93	7.47	3.88
Int7	953.33	77.08	30.20	56.77	4.77	5.54	7.68	BD	BD
Int8	803.33	105.28	27.92	62.33	6.43	5.48	3.65	1.76	BD
Int9	968.33	89.88	28.48	52.43	6.34	5.48	BD	1.93	BD
Int10	658.33	45.16	15.64	42.33	2.19	2.29	2.58	BD	BD
Int11	708.33	82.52	18.56	46.68	BD	2.99	BD	BD	0.93
Mean	1400.00	218.83	48.28	55.54	17.40	13.60	4.42	3.91	1.17
SD	570.93	136.39	26.30	6.80	13.61	9.24	2.82	3.23	1.14
Minimum	658.33	45.16	15.64	42.33	0.00	2.29	0.00	0.00	0.10
Maximum	2040.00	377.25	100.54	93.03	37.81	25.72	8.16	8.49	3.88
Range	1381.67	332.08	84.90	50.70	37.81	23.43	8.16	8.49	3.78

Compounds not detected: eriocitrin, narirutin, compound A and compound B. Int = *C. intermedia;* SD = Standard deviation; BD = Below detection; SS = Soluble solids; TP = Total polyphenol; AUC = Area under the curve (370 – 570 nm); AUCnorm = AUC normalised; Mg = Mangiferin; IsoMg = Isomangiferin; Hd = Hesperidin; Comp C = Compound C, Comp F = Compound F. All chemical/instrumental analysis provided in mg/L except for TP (mg GAE/L), AUC and AUCnorm.

Table 5 The variation between the *C. subternata* samples analysed as well as the mean, SD, minimum, maximum and range

Sample	SS	AUC	AUCnorm	TP	Mg	IsoMg	ErioTrin	Hd	Comp A	Comp B	Comp C
Sub1	1783.33	39.56	39.69	306.32	1.50	3.72	6.14	2.33	15.99	5.02	2.91
Sub2	1826.67	44.13	43.07	345.03	1.80	4.93	7.38	3.09	18.80	9.90	4.08
Sub3	2155.00	48.59	40.35	349.67	2.01	4.61	7.15	3.06	17.03	6.51	3.31
Sub4	1661.67	42.21	45.41	264.39	2.13	4.87	6.56	2.99	14.50	9.21	3.80
Sub5	1388.33	31.54	40.52	201.32	2.87	5.41	4.08	2.63	8.36	1.65	1.71
Sub6	1638.33	42.06	45.79	235.73	3.91	7.14	5.41	3.05	10.67	4.11	2.26
Sub7	1775.00	44.06	44.45	251.96	4.33	7.83	6.21	3.67	10.86	2.48	2.35
Sub8	1536.67	39.36	45.64	234.72	4.02	7.25	5.38	2.90	9.56	3.28	2.22
Sub9	1481.67	26.33	31.77	129.39	4.04	6.04	2.02	2.14	3.79	BD	1.82
Sub10	1058.33	17.02	28.67	37.25	0.27	1.85	BD	4.70	3.19	2.26	BD
Sub11	1423.33	40.12	50.45	180.21	BD	3.95	2.71	2.68	5.21	BD	1.57
Mean	1611.67	230.55	2.44	5.24	4.82	3.02	10.72	4.04	2.37	37.73	41.44
SD	285.45	92.56	1.52	1.77	2.35	0.69	5.38	3.36	1.15	9.20	6.38
Minimum	1058.33	37.25	0.00	1.85	0.00	2.14	3.19	0.00	0.00	17.02	28.67
Maximum	2155.00	349.67	4.33	7.83	7.38	4.70	18.80	9.90	4.08	48.59	50.45
Range	1096.67	312.42	4.33	5.98	7.38	2.56	4.33	9.90	4.08	31.57	21.78

Compounds not detected: narirutin and compound F. Sub = *C. subternata;* SD = Standard deviation; BD = Below detection; SS = Soluble solids; TP = Total polyphenol; AUC = Area under the curve (370 – 570 nm); AUCnorm = AUC normalised; Mg = Mangiferin; IsoMg = Isomangiferin; ErioTrin = Eriocitrin; Hd = Hesperidin; Comp A = Compound A, Comp B = Compound B; Comp C = Compound C. All chemical/instrumental analysis provided in mg/L except for TP (mg GAE/L), AUC and AUCnorm.

Table 6 The variation between the *C. maculata* samples analysed as well as the mean, SD, minimum, maximum and range

Sample	SS	TP	AUC	AUCnorm	Mg	IsoMg	ErioTrin	Hd	Comp C
Mac1	1716.67	271.56	58.20	60.43	22.27	20.25	1.87	10.73	2.60
Mac2	2051.67	349.11	71.67	62.43	40.72	31.96	2.46	12.41	4.03
Mac3	1588.33	241.46	54.03	60.68	23.97	18.70	1.49	12.35	2.65
Mac4	2058.33	364.87	77.10	66.83	51.30	32.93	2.51	17.54	4.95
Mac5	948.33	98.17	30.75	57.81	4.38	4.42	BD	9.37	BD
Mac6	1255.00	155.09	39.38	56.25	7.08	6.44	BD	16.07	BD
Mac7	1188.33	70.44	35.94	53.93	6.27	5.92	BD	12.22	BD
Mac8	1611.67	209.05	41.71	46.26	20.02	14.46	1.87	8.98	1.65
Mac9	885.00	51.95	19.40	38.93	4.11	3.90	BD	4.64	BD
Mac10	1073.33	104.81	23.73	39.60	19.31	10.00	1.48	4.35	BD
Mac11	850.00	73.05	25.38	53.32	3.57	3.77	BD	3.09	BD
Mean	1384.24	180.87	433.90	54.22	18.45	13.89	1.06	10.16	1.44
SD	444.99	113.39	19.47	9.14	15.89	10.86	1.07	4.69	1.85
Minimum	850.00	51.95	19.40	38.93	3.57	3.77	0.00	3.09	0.00
Maximum	2058.33	364.87	77.10	66.83	51.30	32.93	2.51	17.54	4.95
Range	1208.33	312.92	57.70	27.91	47.73	29.17	2.51	14.46	4.95

Compounds not detected: narirutin, compound A, compound B and compound F. Mac = *C. maculata;* SD = Standard deviation; BD = Below detection; SS = Soluble solids; TP = Total polyphenol; AUC = Area under the curve (370 – 570 nm); AUCnorm = AUC normalised; Mg = Mangiferin; IsoMg = Isomangiferin; ErioTrin = EriocitrinHd = Hesperidin; Comp C = Compound C. All chemical/instrumental analysis provided in mg/L except for TP (mg GAE/L), AUC and AUCnorm.

ADDENDUM F

F- and p-values for each sensory attribute for the interaction between temperature and time

Cyclopia genistoides			*Cyclopia subternata*			*Cyclopia maculata*		
	Temp x Time			Temp x Time			Temp x Time	
Attribute	**F value**	**Pr > F**	**Attribute**	**F value**	**Pr > F**	**Attribute**	**F value**	**Pr > F**
AFynbos floral	0.290	0.831	AFynbos floral	4.650	**0.019**	AFynbos floral	3.340	**0.050**
ARose geranium	3.320	0.051	ARose geranium	1.970	0.165	ARose geranium	0.740	0.548
ALemon	0.950	0.444	ARose/Perfume	2.330	0.119	ARose/Pefume	1.390	0.287
AApricot jam	0.680	0.577	AApricot jam	2.280	0.124	APlantlike	1.320	0.307
APlantlike	3.820	**0.034**	APlantlike	6.320	**0.006**	AWoody	0.190	0.902
AWoody	0.100	0.956	AWoody	2.220	0.131	APine	0.320	0.812
AFruity sweet	1.860	0.182	AFruity sweet	1.640	0.226	AFruity sweet	1.810	0.192
ABoiled syrup	0.370	0.778	AFynbos sweet	4.110	**0.028**	ABoiled syrup	0.410	0.746
AFynbos sweet	1.260	0.326	ASpicy	0.550	0.655	AFynbos sweet	1.540	0.248
ASpicy	0.690	0.571	AWalnut	0.310	0.818	ASpicy	3.320	0.051
ADusty	1.710	0.211	ADusty	7.530	**0.003**	AWalnut	0.450	0.718
AMedicinal	0.970	0.433	ABurnt caramel	6.840	**0.005**	ADusty	0.520	0.676
ABurnt caramel	2.630	0.091	ARotting	1.490	0.261	ABurnt caramel	1.380	0.290
ARotting	1.450	0.270	AHay	0.070	0.973	ARotting	10.660	**0.001**
AHay	1.620	0.230	AGreen grass	4.260	**0.025**	AHay	1.280	0.319
AGreen grass	0.380	0.768	ACooked veg	3.630	**0.040**	AGreen grass	5.100	**0.014**
ACooked	5.020	**0.014**	Aseaweed	1.140	0.368	ACooked veg	11.980	**0.000**
TSweet	2.000	0.160	TSweet	4.190	**0.026**	TSweet	4.170	**0.026**
TSour	0.800	0.517	TSour	4.880	**0.016**	TSour	0.700	0.568
TBitter	15.930	**<0.0001**	TBitter	1.210	0.344	TBitter	1.130	0.371
Astringent	5.460	**0.011**	Astringent	2.340	0.117	Astringent	1.460	0.268
FFynbos floral	4.650	**0.019**	FFynbos floral	4.140	**0.027**	FFynbos floral	1.030	0.409
FRose geranium	4.660	**0.019**	FRose geranium	1.640	0.225	FRose geranium	1.230	0.336
FLemon	2.780	0.080	FRose/Perfume	2.410	0.111	FRose/Perfume	0.800	0.517
FApricot jam	1.670	0.220	FApricot jam	0.520	0.678	FPlantlike	1.490	0.259
FPlantlike	1.820	0.189	FPlantlike	2.560	0.097	FWoody	0.980	0.429
FWoody	0.930	0.452	FWoody	1.820	0.190	FPine	0.770	0.531
FSpicy	0.870	0.481	FSpicy	1.490	0.260	FSpicy	1.100	0.381
FDusty	0.030	0.994	FWalnut	0.720	0.555	FWalnut	1.050	0.401
FMedicinal	0.820	0.506	FDusty	1.700	0.214	FDusty	0.820	0.505
FBurnt caramel	0.970	0.433	FBurnt caramel	1.410	0.281	FBurnt caramel	0.600	0.628
FRotting	5.230	0.013	FRotting	1.330	0.304	FRotting	2.530	0.100
FHay	0.830	0.499	FHay	0.310	0.818	FHay	2.360	0.115
FGreen grass	1.330	0.303	FGreen grass	0.750	0.540	FGreen grass	1.530	0.249
FCooked veg	3.440	**0.046**	FCooked veg	6.030	0.007	FCooked veg	3.520	**0.043**

Rotting = Rotting plant water, Cooked veg = Cooked vegetables, Hay= Hay/Dried grass. Significant interactions are indicated in bold.

ADDENDUM G

F- and p-values for each chemical/instrumental variable for the interaction between temperature and time

Cyclopia genistoides			*Cyclopia subternata*			*Cyclopia maculata*		
	Temp x Time			**Temp x Time**			**Temp x Time**	
Variable	**F value**	**Pr> F**	**Variable**	**F value**	**Pr> F**	**Variable**	**F value**	**Pr> F**
SS	0.410	0.744	SS	0.330	0.802	SS	0.290	0.829
TP	0.900	0.465	TP	0.810	0.507	TP	0.410	0.750
Mg	1.730	0.206	Mg	0.640	0.603	Mg	0.440	0.728
IsoMg	1.490	0.259	IsoMg	0.230	0.875	IsoMg	0.490	0.694
Comp C	0.560	0.652	Comp A	0.600	0.628	Comp C	0.960	0.440
Comp D	0.870	0.481	Comp C	0.740	0.546	Hd	0.460	0.717
AUC	0.900	0.465	ErioTrin	0.850	0.489	Erio	0.200	0.896
			Comp B	0.300	0.828	ErioTrin	0.600	0.628
			Hd	1.110	0.378	Ht	0.600	0.628
			AUC	1.930	0.171	AUC	0.720	0.555

SS = Soluble solids, TP = Total polyphenol, Mg = Mangiferin, IsoMg = Isomangiferin, Comp C = Component C, Comp D = Component D, AUC = Area under the curve (370 - 570 nm), Comp A = Component A, Comp B = Component B, Hd = Hesperidin, Ht = Hesperetin, Erio = Eriodictyol, ErioTrin = Eriocitrin.

ADDENDUM H

Absorbance and normalised absorbance values for *Cyclopia* species (*C. genistoides, C. subternata* and *C. maculata*)