# Characterisation of the sensory profile of *Cyclopia intermedia* and optimisation of fermentation parameters for improved product quality

By

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## DECLARATION

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## ABSTRACT

In light of the limited and inconsistent supply of good quality honeybush tea, a species-specific sensory profile and the physicochemical characteristics of *Cyclopia intermedia* (honeybush) tea were determined to ultimately establish the optimum fermentation parameters for this herbal tea on laboratory-scale and to validate these findings on commercial-scale. The characteristic sensory profile of *C. intermedia* can be described as sweet tasting and slightly astringent with a combination of "fynbos-floral", "fynbos-sweet", "fruity" (specifically "apricot jam", "cooked apple", "raisin" and "lemon/lemon grass"), "woody", "caramel/ vanilla" and "honey-like" aromas. The flavour can be described as distinctly "fynbos-floral", "fynbos-sweet" and "woody", including hints of "lemon/lemon grass" and "hay/dried grass". The results of the sensory study were used to create a *C. intermedia* sensory wheel and lexicon, and an elementary grading system that categorised samples into "good", "average" and "poor" sensory quality was proposed. Physicochemical parameters, i.e. soluble solids (SS) content, absorbance as a measure of colour, and turbidity, were evaluated as possible rapid predictors of sensory quality. High SS content, absorbance and turbidity correlated strongly with "poor" sensory quality. A linear relationship existed between the physicochemical parameters.

The effect of fermentation temperature (70, 80 and 90°C) and time (12, 16, 24, 36, 48 and 60 h) on the sensory and physicochemical characteristics of *C. intermedia* was determined on laboratory-scale. Increasing fermentation time increased the intensity of positive sensory attributes, while decreasing the intensity of negative sensory attributes. The SS content, colour and turbidity of infusions decreased with increasing fermentation time, while the SS content and turbidity of infusions increased with increasing fermentation temperature. Fermentation at 90°C for 36 h on laboratory-scale produced *C. intermedia* with the best sensory properties, while preserving the SS content and colour of infusions. Fermentation at 70°C and 80°C required longer fermentation times for development of positive sensory attributes. Fermentation at 90°C was subsequently validated on commercial-scale. Laboratory-scale fermentation of the same batches of plant material was also carried out concurrently to allow direct comparison of the scale of fermentation on tea quality. Commercial-scale fermentation, despite increased variability as a result of increased batch volumes and heating difficulties, produced *C. intermedia* of "good" sensory quality after 24 and 36 h of fermentation. Increasing fermentation time had little effect on the SS content and colour of infusions of tea produced on commercial-scale, but turbidity increased significantly after 36 h. Thus, to produce *C. intermedia* with consistently good quality on commercial-scale, fermentation at 90°C for 24 to 36 h is recommended. Increasing fermentation time past 48 h should be avoided to prevent turbidity and the development of sensory attributes characteristic of over-fermented tea. However, due to the large variability of commercial-scale honeybush tea production, it is recommended that each batch be monitored between 24 and 36 h to determine when optimum fermentation has been obtained.

## UITTREKSEL

Beperkte en wisselvallige beskikbaarheid van goeie gehalte heuningbostee noodsaak die optimisering van fermentasie parameters vir *Cyclopia intermedia.* Optimisering van fermentation parameters is op laboratorium skaal gedoen, gevolg deur validasie van die parameters op kommersiële skaal. Vooraf is die spesie-spesifieke sensoriese profiel en die fisies-chemiese eienskappe van *C. intermedia* tee bepaal. Die kenmerkende sensoriese profiel van *C. intermedia* kan beskryf word as soet en effens vrank met 'n kombinasie van "fynbos-blomagtige", "fynbos-soet", "vrugtige" (spesifiek "appelkooskonfyt", "gekookte appel", "rosyntjie" en "suurlemoen/sitroen gras"), "houtagtige", "karamel/vanilla" en "heuningagtige" aromas. Die smaak kan beskryf word as "fynbos-blomagtig", "fynbos-soet" en "houtagtig", met 'n tikkie "suurlemoen/sitroen gras" en "hooi/gedroogde gras". Die resultate van die sensoriese studie is gebruik om 'n *C. intermedia* sensoriese wiel en leksikon, asook 'n basiese graderingstelsel wat tee monsters in "goeie", "gemiddelde" en "swak" sensoriese kwaliteit klassifiseer, te ontwikkel. Fisies-chemiese parameters: oplosbare vastestof (SS) inhoud; absorbansie as 'n maatstaf van kleur; en troebelheid, is geëvalueer as moontlike indikasies van sensoriese kwaliteit. Hoë SS inhoud, absorbansie en troebelheid waardes het sterk met "swak" sensoriese kwaliteit gekorreleer. 'n Lineêre verwantskap bestaan tussen die fisies-chemiese parameters en kwaliteit.

Die effek van fermentasie temperatuur (70, 80 en 90°C) en -tyd (12, 16, 24, 36, 48 en 60 h) op die sensoriese en fisies-chemiese eienskappe van *C. intermedia* is op laboratorium skaal bepaal. Verlenging van fermentasie tyd het die intensiteit van die positiewe sensoriese eienskappe verhoog, terwyl dit die intensiteit van negatiewe sensoriese eienskappe verminder het. Die SS inhoud, kleur en troebelheid van die tee het met verlengde fermentasie tyd afgeneem, terwyl die SS inhoud en troebelheid met verhoging van fermentasie temperatuur toegeneem het. Fermentasie by 90°C vir 36 h op laboratorium skaal het tee met die beste sensoriese eienskappe geproduseer, met behoud van die SS inhoud en kleur. Fermentasie by 70°C en 80°C het 'n langer fermentasie tyd vir die ontwikkeling van positiewe sensoriese eienskappe vereis. Fermentasie by 90°C is daaropvolgens op kommersiële skaal uitgevoer, met gelyktydige laboratorium skaal fermentasie van dieselfde plantmateriaal lotte om die direkte effek van die skaal van fermentasie op tee kwaliteit te bepaal. Kommersiële fermentasie, ten spyte van verhoogde wisselvalligheid as gevolg van groot volumes tee en probleme met verhitting, het tee van "goeie" sensoriese kwaliteit na fermentasie periodes van 24 en 36 h geproduseer. Verlenging van fermentasie tyd het min uitwerking op die SS inhoud en kleur van kommersiel gefermenteerde tea gehad, maar troebelheid het beduidend na 36 h toegeneem. Fermentasie by 90°C vir 24 - 36 h word gevolglik aanbeveel om tee met goeie gehalte op kommersiële skaal te produseer. Fermentasie vir langer as 48 h moet vermy word om troebelheid te voorkom en die ontwikkeling van sensoriese eienskappe kenmerkend van oor-gefermenteerde tee te vermy. As gevolg van faktore wat groot variasie in kommersiële skaal heuningbostee produksie kan teweegbring, word aanbeveel dat elke produksielot tussen 24 en 36 h gemonitor word om die optimum fermentasie tyd te bepaal.

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# NOTES

This thesis is presented in the format prescribed by the Department of Food Science, Stellenbosch University. The structure is in the form of two research chapters and is prefaced by an introduction chapter with the study objectives, followed by a literature review chapter and culminating with a general discussion and conclusions. Language, style and referencing format used are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

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# CHAPTER 1

# INTRODUCTION

The foliage and stems of *Cyclopia* species, indigenous to the fynbos biome in the Western and Eastern Cape Provinces of South Africa, is used to prepare honeybush tea, a herbal drink also known as "South Africa's sweetest tea". The pleasant sweet aroma and flavour of "fermented" honeybush, its low tannin content and absence of caffeine led to widespread interest in the commercial cultivation and processing of honeybush tea during the mid-1990s. A major contributing factor to the increasing market share of honeybush is greater consumer awareness of its potential health-promoting properties, among others, its antioxidant activity (Joubert *et al*., 2011). However, poor and inconsistent quality, especially poor flavour and the presence of off-flavours, as well as turbid infusion characteristics, contribute to poor market share. Modern consumers have specific performance expectations for food and consistent quality is thus key to growing consumers' confidence in a product and creating a reliable market. The lack of good quality tea was identified as a major shortcoming in the successful commercialisation and advancement of the honeybush industry (Du Toit *et al*., 1998; SAHTA, 2014). Furthermore, in light of limited supply of plant material, losses due to poor quality should be limited.

Although more than 20 species of honeybush grow in the wild, only a few species are commercially exploited for the manufacturing of tea. Honeybush is exported to 55 countries, with the Netherlands, Germany, United Kingdom, and United States of America being the major importers since 2008, and traditional tea-drinking countries such as India, Sri-Lanka, Japan and China also contributing to the market (M. Joubert, SAHTA, Paarl, South Africa, 2013, personal communication). Until recently, exports consisted mainly of *C. intermedia*, *C. genistoides* and *C. subternata*, however, as the demand starting exceeding supply, other *Cyclopia* spp., such as *C. maculata*, *C. longifolia* and *C. sessiliflora* were included to cater for the larger demand for the product (Joubert *et al*., 2011). *Cyclopia intermedia* still contributes to the major portion of the export market and is thus the focus of this study.

Poor and inconsistent quality, a major factor at the initiation of the development of a formal honeybush tea industry, still remains a problem today. This is attributed to the lack of standardised processing conditions for the respective *Cyclopia* spp., as well as a lack of quality control systems. The food industry largely uses sensory tools such as sensory profiling, wheels and lexicons to standardise the sensory terminology used to define a product, which facilitates communication between role players in the industry and contributes to standardising the evaluation of a product (Drake & Civille, 2002). Theron *et al*. (2014) developed a generic honeybush sensory wheel and lexicon based on the sensory attributes of six *Cyclopia* species, including *C. intermedia*. The study highlighted that the species share many aroma and flavour attributes, yet distinct differences in their sensory profiles exist (Theron *et al*., 2014), which are not reflected in the generic sensory wheel. This merits the development of species-specific sensory wheels and lexicons for individual *Cyclopia* spp. The lack of information regarding defined differences between different *Cyclopia* spp., as well as the practice of mixing different species depending on availability, contributes to considerable variation in the sensory quality of honeybush tea on the market, which has detrimental consequences for the reputation of honeybush tea. Thus, a consistent supply of high quality tea with a consistent sensory profile cannot be ensured without this information. Variation between species, on the other hand also provides the honeybush industry with an opportunity to develop niche products, aimed at specific high-end markets. Evidence suggests that people tend to spend more money on high-end food products, even in recession times, due to an internal need to feel a reconnection with some values such as health and happier quality of life (Yeoman & McMahon-Beattie, 2010). Concepts such as "organic" (Wier *et al*., 2003), country of origin certified products (Van der Lans *et al*., 2001) and TSG (Traditional Specialty Guaranteed) (Tsakiridou *et al*., 2009) are food trends that have seen continuous growth. Examples of existing high-end products are French wines, Italian food products (Balabanis & Diamantopoulos, 2004), and Honeycrisp apples (West, 2010). From an economic perspective high-end food products have a high-income elasticity of demand, which means that a fluctuation in income will affect the expenditure on the same direction (Douglas & Isherwood, 1979). This behaviour is strongly related to exclusivity and scarcity, which are major indicators of high-end products. It is believed that honeybush tea has the potential to fit this food category.

The processing of honeybush tea entails high temperature chemical oxidation, also known as "fermentation", which is responsible for the characteristic colour and unique sweet-associated sensory attributes. Generally, the development of optimum infusion colour and sensory properties depends on the fermentation temperature x time combinations, but high fermentation temperature (>70°C) is required to inhibit growth of thermophillic contaminants (Du Toit *et al*., 1999). The optimisation of fermentation and drying conditions for *C. intermedia* has been addressed (Du Toit & Joubert, 1998, 1999). Du Toit and Joubert (1999) recommended fermentation at 70°C for 60 h or 90°C for 36 h to produce the best flavoured tea, however, only the sweet aroma and flavour of infusions of tea were assessed and could, thus, allegedly be inconclusive. Theron (2012), while investigating the optimum fermentation parameters for *C. subternata*, *C. genistoides* and *C. maculata,* also showed that differences exist between *Cyclopia* species in terms of their processing parameters. Therefore, a more in-depth study of the change in the sensory attributes of *C. intermedia* was necessary. Furthermore, although honeybush tea fermentation has been fairly well documented on laboratory-scale, these findings have not been given commercial application, taking the factors of large-scale production into account.

The objectives of this study were, therefore, to develop a sensory profile for *C. intermedia*, and present it graphically by means of a sensory wheel illustrating average intensity and occurrence of sensory attributes in an infusion of tea. An elementary quality grading system was also developed for honeybush tea, which is another tool used to standardise and commercialise food products. The sensory profile and elementary grading system were used to detect changes in sensory quality of infusions as a result of different fermentation temperature and time conditions in a controlled laboratory environment, which ultimately enabled the optimum fermentation conditions for *C. intermedia* on laboratory-scale to be established. These findings were then applied to commercial fermentation of honeybush tea to validate the optimum fermentation parameters for *C. intermedia* on commercial-scale. The change in physicochemical parameters closely correlated to the way in which consumers perceive product quality, i.e. "infusion strength" (soluble solids content and colour) and turbidity of infusions of tea were determined as additional measures of perceived quality and to determine whether they could be used as rapid quality monitoring tools. This MSc study forms part of the on-going honeybush research program conducted at the Agricultural Research Council (ARC) Infruitec-Nietvoorbij in Stellenbosch, South Africa, aimed at the development of a viable honeybush industry.

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# CHAPTER 2

# LITERATURE REVIEW

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## 1. INTRODUCTION

"Honeybush tea is one of the few indigenous South African plants that made the transition from the wild to a commercial product during the past 100 years" (Joubert *et al*., 2011). The increasing popularity of this herbal tea, both locally and internationally, can be ascribed to its pleasant characteristic flavour and aroma, its caffeine-free status, as well as to the fact that it is contains a wealth of polyphenolic compounds associated with health-promoting properties. The link between oxidative stress and ageing is fuelling global interest in natural antioxidants such as polyphenols, which augments the nutraceutical, and thus value-adding potential of honeybush tea as outlooks such as "food as medicine" drives modern society's perspective. This, in combination with its inherently sweet taste and distinctly fruity and floral aromas and flavours, make honeybush tea an extremely desirable and thus commercially viable product. This chapter provides a short background of honeybush tea, research undertaken and developments employed to encourage growth of the industry. Within the context of the present study, elements that require attention to support the growth of the industry will be discussed.

## 2. THE HONEYBUSH TEA INDUSTRY

### 2.1. *Cyclopia* species: Taxonomy, botanical description and geographical distribution

*Cyclopia* is a distinct genus that belongs to the leguminous family Fabaceae, which is a member of the tribe Podalyrieae (Schutte, 1995). Since the first identification of a *Cyclopia* species, namely *Cyclopia genistoides*, 23 species have been described (Kies, 1951; Bond & Goldblatt, 1984; Schutte, 1995, 1997) of which only a few are currently used or are under investigation for the commercial production of honeybush tea (Table 1).

*Cyclopia* spp. are distinguished by their trifoliate leaves (Levyns, 1920; Marloth, 1925), yellow flowers with indented calyx and characteristic sweet, honey-like aroma from which the name "honeybush" is derived (Kies, 1951; Schutte, 1997). The shape and size of the trifoliate leaves vary between species, from needle-like (*C. maculata*) (Fig. 1a), pubescent, narrow-leafed (*C. genistoides*) (Fig. 1b) to flat-leafed (*C. intermedia* and *C. subternata*) (Fig. 1c & d) (Kies, 1951; Bond & Goldblatt, 1984; Schutte, 1995, 1997). The distinctive yellow flowers allow the bushes to be easily recognised in the wild during the flowering period that occurs in spring (September and October) except for *C. sessiliflora*, which flowers during late autumn or early winter (May and June) (Du Toit *et al.,* 1998). *Cyclopia* bushes have woody stems with a low leaf-to-stem ratio and normally grow to 1.5 m high in nature, but may reach heights of up to 3 m depending on the area and climate (Bond & Goldblatt, 1984).

*Cyclopia* spp. are endemic to the Cape Floristic Region (CFR), the smallest yet richest of the world's six floral kingdoms (Turpie *et al*., 2003), where they grow on the coastal plains and mountainous regions of the Western and Eastern Cape Provinces of South Africa (Fig. 2a) (Joubert *et al*., 2011). Even within the fynbos region the natural habitat of individual species is localised (Kies, 1951). The species of current commercial interest, i.e. *Cyclopia intermedia*, *C. subternata*, *C. maculata*, *C. longifolia*, and *C. genistoides* (Joubert *et al*., 2011) grow in diverse conditions (Bond & Goldblatt, 1984). It varies from the shady and cooler southern slopes of the mountain ranges (*C. intermedia*), along riverbanks and streams (*C. subternata*) and the flat and sandy coastal regions (*C. genistoides*) (Du Toit *et al*., 1998). *Cyclopia* spp. have also developed survival strategies adapted to the frequent veld fires in their natural habitat, classifying them as sprouters (distinguished by the woody rootstock that survives fire and produces new shoots) or re-seeders (killed by fire and re-established from seeds). The seeds are hard-shelled that require scarification before germination, which is achieved by fire in nature (Schutte *et al*., 1995).

a)


c)

**Figure 1** Trifoliate leaves of a) *C. maculata*, b) *C. genistoides*, c) *C. intermedia* and d) *C. subternata* (SAHTA, 2014c).

**Table 1** General information on *Cyclopia* species of commercial and potential commercial value.

| Species | Descriptive name | Geographical distribution | Soil type | Survival strategy | Comments |
|---|---|---|---|---|---|
| ***C. intermedia*** | Bergtee/Kougabergtee | Witteberg, Anysberg, Swartberg, Touwsberg, Rooiberg, Kammanasie, Kouga, Baviaanskloof, Langeberg, Outeniqua, Tsitsikamma and Van Stadens mountains (most widespread) | Rocky, loamy, sandy soils | Sprouter | Very good quality tea, slow growing, harvest close to the ground, possibly drought resistant |
| ***C. genistoides*** | Kustee/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 eastward to Albertina in the southern region | Sandy soils | Sprouter | Good quality tea, excellent growth form, harvest close to the ground |
| ***C. maculata*** | Vleitee/Genadendaltee | Along riverbanks and streams in the south-western and southern region | Wet, peaty soils | Re-seeder | Good quality tea, vigorous growth, thick shoots, harvest knee-high |
| ***C. subternata*** | Vleitee | Widely distributed along the coastal mountain ranges (Tsitsikamma, Outeniqua and Langeberge) where it occurs on the southern slopes | Well-drained, stony, loamy soils | Re-seeder | Very good quality tea, rigorous grower, produces relatively thick shoots, harvest knee-high |
| ***C. longifolia*** | Unknown descriptive name | Van Stadens River mountains near Port Elizabeth | Moist, sandy soils along the banks of a river | Re-seeder | Tea quality relatively unknown, harvest close to the ground |

Compiled from Joubert *et al*. (2008b), Joubert *et al*. (2011), and ARC (2013).

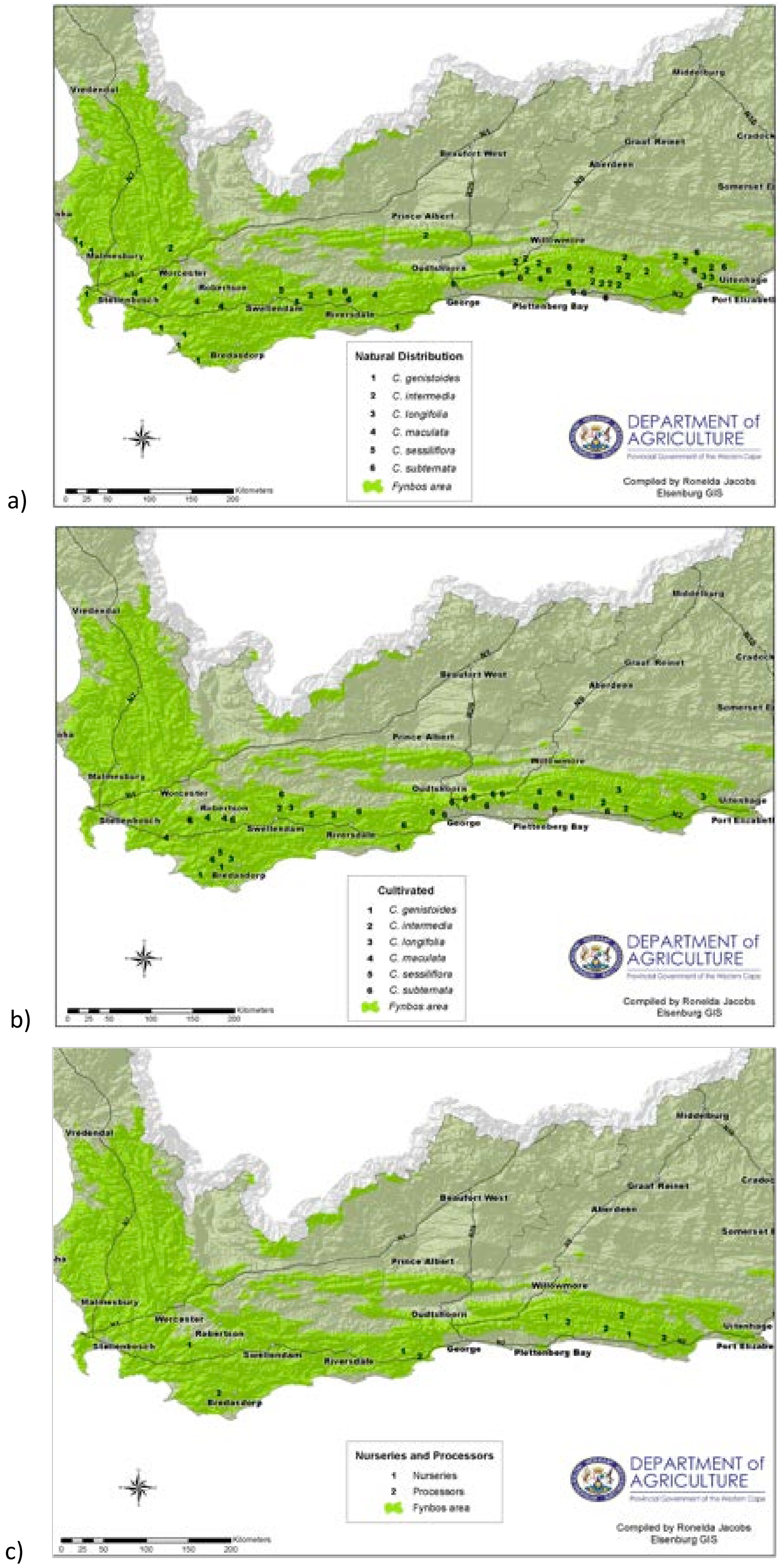



**Figure 2** Locations of (a) the natural distribution of *C. genistoides, C. intermedia, C. longifolia, C. maculata, C. sessiliflora* and *C. subternata*, (b) cultivated areas, and (c) nurseries and tea processors (Joubert *et al.,* 2011).

## 2.2. History

Honeybush tea, prepared from the foliage and stems of *Cyclopia* species, is a traditional South African herbal tea with a long history of regional use until the mid-1990s. A European taxonomic script of 1705 contains the earliest reference to honeybush (Kies, 1951). In the 1770's, C. Thunberg, a Swedish botanist, first recorded the use of the name "honigtee" (Dutch) to describe *Cyclopia* species during his travels in the Cape. This, along with other names such as honeybush and "heuningtee" or "heuningbostee" (Afrikaans), is derived from the sweet, honey-like scent of the plant in full bloom (Joubert *et al*., 2011). C. Latrobe was given 'tea-water' whilst travelling in the Langkloof area in 1815 (Latrobe, 1818). It is believed to be prepared from honeybush. Allegedly, the leafy shoots and flowers were traditionally "fermented" (oxidised) and dried to prepare tea as treatment for coughs and upper respiratory tract infections (Du Toit *et al*., 1998).

It was only in 1825 that the plant was classified and a specific species, *C. genistoides*, was named in an anatomical and chemical study of Cape tea, or "honig-thee," the most common plant used for tea in the Cape (Greenish, 1881). According to Marloth (1925) colonists praised honeybush as being wholesome, and valued it as a stomachic aiding weak digestion without stimulating the heart. Bowie (1830) also reported the use of a decoction of the plant as a restorative and as an expectorant in chronic catarrh and pulmonary tuberculosis. *Cyclopia vogelii* (renamed *C. subternata*) (Watt & Breyer-Brandwijk, 1962), *C. latifolia* and *C. longifolia* (Marloth, 1913, 1925) are other species with a history of use as tea by the colonists. In the 1920's all honeybush tea made in the Cape was from *C. genistoides*, while *C. subternata* was used in the Caledon (Overberg) and George areas (Marloth, 1925). This regional use of a specific species is probably owed to the prevalence of the plants in those areas and as a result honeybush remained relatively unknown outside its natural habitat localities.

Through the involvement of the pioneer of rooibos marketing, B. Ginsberg, the first branded product "Caspa *Cyclopia* Tea" was launched on the South African market in the 1960s. However, despite these early marketing efforts, the honeybush industry remained insignificant until the 1990s when the success of rooibos and global interest in herbal teas led to renewed interest in honeybush tea (Joubert *et al*., 2008b). Since then, research on honeybush focused on commercial cultivation, processing, chemistry, and health-promoting properties of honeybush tea (Joubert *et al*., 2011). The South African Honeybush Tea Association (SAHTA), originally known as the South African Honeybush Producers Association, was founded in 1999 to coordinate activities within the industry and deal with matters regarding conservation, production, processing, marketing and quality control (SAHTA, 2014a).

## 2.3. The industry

Less than 10 years after the initiation of honeybush research in response to the growing interest and demand for the product, commercial cultivation and factory-based production became a reality. This laid the foundation for the honeybush industry and, although no formal marketing campaign existed during this period, development of global markets and significant trade was seen. The export market grew from 50 to almost 200 tonnes during this time (Fig. 3) (Joubert *et al.*, 2011; ARC, 2013).

The dramatic growth in the honeybush tea industry has occurred concurrently with the growing interest in herbal teas both locally and internationally. Its popularity can be ascribed to its pleasant characteristic sweet, honey-like aroma and flavour. Other factors contributing to its popularity are its caffeine-free (Greenish, 1881) and low tannin content (Marloth, 1925; Terblanche, 1982) status, as well as the wealth of polyphenolic compounds associated with health-promoting properties. Extracts have been shown to possess strong antioxidant, immune-modulating, chemo-preventative and phytogenic actions in various models (as reviewed by McKay & Blumberg, 2007 and Joubert *et al.,* 2008b). The increasing awareness of consumers to the influence of diet on maintaining overall health and the trend toward self-medication have stimulated interest in natural products and functional foods and beverages. The search for products that can help prevent disease and aging, particularly those rich in antioxidants that combat oxidative stress and aging naturally, augments use of honeybush by the nutraceutical and cosmetics industries (Joubert *et al*., 2011).

The different *Cyclopia* species used to prepare honeybush tea have subtle differences in flavour that could offer a marketing advantage by catering for niche markets with a specific flavour profile. Its use in blends with rooibos tea, for example, also expands the range of naturally flavoured products, catering for a wider and more sophisticated taste (Du Toit *et al*., 1998).

Honeybush is currently being sold in bulk to 55 countries, with the Netherlands, Germany, United Kingdom, and United States of America being the major importers since 2008, and traditional tea-drinking countries such as India, Sri-Lanka, Japan and China also contributing to the market (Table 2) (M. Joubert, SAHTA, Paarl, South Africa, 2013, personal communication). However, issues regarding inconsistent quality (microbial and sensory) and availability still exist, which could harm the reputation of the relatively new and fast developing honeybush industry and restrict the expansion of product development (Joubert *et al*., 2011). SAHTA identified that a reliable supply of raw and processed product, as well as reliable standards and consistent product quality are some of the requirements for growth of the industry (SAHTA, 2014b).

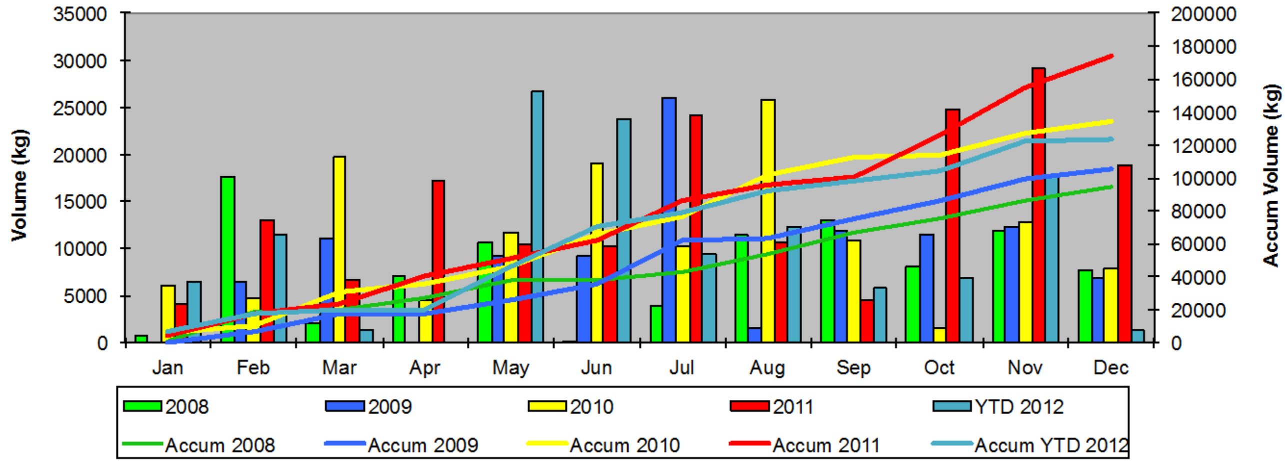



**Figure 3** Month to month comparison and total volume of honeybush tea exports from 1999 to 2012 (ARC, 2013; M. Joubert, SAHTA, Paarl, South Africa, 2013, personal communication).

**Table 2** Top importers of honeybush tea in 2012.

| | Country | Total (kg) | Percentage |
|---|---|---|---|
| 1 | Germany | 53 984 | 43.78% |
| 2 | USA | 31 703 | 25.71% |
| 3 | Netherlands | 16 680 | 13.53% |
| 4 | UK | 7 200 | 5.84% |
| 5 | Bulgaria | 6 005 | 4.87% |
| 6 | Belgium | 3 000 | 2.43% |
| 7 | Canada | 1 000 | 0.81% |
| 8 | Taiwan | 725 | 0.59% |
| 9 | Malaysia | 720 | 0.58% |
| 10 | China | 644 | 0.52% |
| 11 | Australia | 447 | 0.36% |
| 12 | Norway | 336 | 0.27% |
| 13 | Russia | 306 | 0.25% |
| | Other | 558 | 0.45% |
| | Total (kg) | 123 308 | 100% |

USA = United States of America, UK = United Kingdom. (M. Joubert, SAHTA, Paarl, South Africa, 2013, personal communication).

## 2.4. Commercial propagation and cultivation

With the growth in the demand for honeybush tea, fears of over-exploitation of the natural *Cyclopia* populations served as an incentive for the establishment of commercial plantations to ensure sustainable agriculture (Joubert *et al*., 2011). Harvesting activities in the past have led to the decline and even extinction of *Cyclopia* populations (Du Toit *et al.,* 1998). Currently, about 70% of the commercial crop is harvested in the wild and fewer than 10 farmers with honeybush plantations of more than 2 hectares produce the balance (SAHTA, 2014b) – a situation that is not sustainable. The industry aims to formalise cultivation practices by involving small and emerging farmers in order for the industry to remain sustainable, which would increase production of consistently good quality product and contribute to crucial economic development in poor communities in the Western and Eastern Cape (Den Hartigh, 2011).

Successful propagation and cultivation is dependent on the natural occurrence of *Cyclopia* species in specific areas, which aided in the identification and mapping of suitable honeybush cultivation areas (Jacobs, 2007, 2008). Figure 2a shows the natural distribution of *Cyclopia* spp., which mostly coincides with the locations of commercial plantations (Fig. 2b). The most important factors in determining areas apt for honeybush cultivation is soil type and rainfall (Jacobs, 2008). Currently approximately 200 ha of, mostly, *C. genistoides* and *C. subternata* shrubs are under cultivation to cater for demand. But wild harvesting, especially of *C. intermedia*, still contributes the major part of the annual production (Bester, 2013). *Cyclopia intermedia* is not favoured for cultivation as it can only be harvested every second or third year as frequent harvesting results in die-back due to insufficient build-up of energy reserves in the rootstock, deeming it uneconomical for cultivation (Joubert *et al*., 2011). About 30 ha of *C. intermedia* has been planted in the Langkloof and Southern Cape areas, but natural stands in the Kouga mountains and Langkloof are still the main source of tea from this species (Joubert *et al.,* 2011).

Either seed or cuttings can be used to propagate honeybush. Vegetative propagation is slower, requiring a more sophisticated nursery and is thus more expensive (De Lange, 2006) but higher plantation yields of a more uniform product is obtained by using selected propagation material and is the preferred propagation method for some producers (Malan, 2000; Erasmus, 2002; Gleason, 2004). The Agricultural Research Council has a formal breeding program to develop improved genetic material for the sustainable production of honeybush (Bester, 2013).

Honeybush production is thus challenging as farming practices vary depending on the species and area. Another concern regarding cultivation is the effect of the use of pesticides and fertilisers on the chemical composition of the herbal plant, distracting its "green status" as a naturally grown product (Du Toit *et al*., 1998). However, organic production is widely practiced while not many farmers are certified mainly due to the high cost of certification (Joubert *et al*., 2011). Furthermore, despite following strict organic practices, transportation of harvested tea through fruit growing areas to the processors, specifically in the Langkloof, causes contamination of "organic" tea material with chemicals sprayed on the fruit (J. Kritzinger, Honeybush Natural Products, Langkloof, South Africa, 2014, personal communication).

### 2.5. Conservation

Given the increasing popularity of honeybush tea as an herbal drink and interest in its health properties, it is anticipated that demand will continue to rise. Thus, conservation and regulatory control are essential to protect the natural resources in support of the long-term sustainability of the industry. Two species, *C. intermedia* and *C. subternata*, have been protected in the Eastern Cape since October 2011 (SAHTA, 2014b). Permits are required for harvesting and transport of plant material across provincial borders. The permit system also requires processors to keep meticulous records on the origin and volumes of each consignment of honeybush (SAHTA, 2014b). This also facilitates data collection to determine sustainable harvesting practices. *Cyclopia* is, however, unprotected in the Western Cape creating the need for alignment of regulatory control between the neighbouring honeybush-growing provinces to ensure the conservation of resources in support of the industry.

## 3. PROCESSING

### 3.1. Harvesting

Traditionally, plants were harvested during the flowering period (May or September, depending on the species) but this limited the harvesting period, which has been extended to meet the increasing demand (Du Toit & Joubert, 1999). The fragrant yellow flowers were thought to be essential in imparting the sweet, honey-like aroma and characteristic flavours of the product. However, contrary to popular belief, a study by Du Toit & Joubert (1999) showed that although the presence of flowers enhanced the aroma and flavour, it was not essential for the characteristic sensory properties of honeybush tea. Cronje (2010) also showed that the presence of flowers did not have a significant effect on the aroma attributes of *C. subternata* while studying the volatile fraction of a number of diverse honeybush teas, representing four species and variants thereof. Furthermore, flowers bulk up the plant material, yet disintegrate during fermentation and are lost as "dust" when the dried product is sieved (Joubert *et al*., 2011). Thus, plant material is currently harvested in summer to late autumn before the flowering period, as flowering also places the plants under unnecessary stress (Joubert *et al*., 2008b).

Different harvesting methods, using a sickle or pruning-shears, are practiced depending on the survival strategies of the species (sprouter on non-sprouter/re-seeder) (Viljoen, 1994). *Cyclopia intermedia* and *C. genistoides*, both sprouters, can be harvested 2 to 3 years after planting and are cut back to ground level to stimulate new shoot growth from the rootstock (Viljoen, 2001). *Cyclopia genistoides* can be harvested annually following the first harvest, whereas *C. intermedia* can only be harvested every second or third year depending on conditions to allow enough recovery time (Joubert *et al*., 2011). Non-sprouters, such as *C. subternata* and *C. maculata*, grow more vigorously and can be harvested within a year of planting. Following the first harvest it is harvested annually by cutting the shoots back leaving one third of active growth on the plant (30 to 50 cm above the ground) to prevent dieback due to severe pruning (Joubert *et al*., 2011). Regular harvesting yields better plant material for processing as the stems become softer and have higher leaf-to-stem ratios (Du Toit *et al*., 1998). The lifespan for non-sprouters is 7 to 8 years while sprouters live for at least 10 years (Joubert *et al*., 2011).

### 3.2. Cutting

Plant material is processed as soon as possible after harvesting (Du Toit *et al*., 1998). The first step in processing entails cutting the material into small pieces (2-3 mm lengths) to disrupt the cellular structure, which facilitates "fermentation". Small hand-held axes were originally used to achieve this, but advanced cutting tools such as mechanised fodder cutters, tobacco cutters, and Bovic cutters are now used that increase productivity, and give a smaller, more uniformly cut product (Du Toit *et al*., 1998). Poor and blunt cutting equipment produces course material that appears as unappealing white pieces of stem in the final product, which in turn affects the quality of the tea and causes unnecessary product loss during sieving (Du Toit & Joubert, 1998b).

### 3.3. Pre-treatment

The fresh plant material, especially old bushes or bushes not frequently harvested, with a high stem-to-leaf ratio has a relatively low moisture content (<50%) (Fig. 4a). Different treatments (bruising, hot water and cold water) were investigated by Du Toit and Joubert (1998b) for their effect on honeybush tea fermentation. Cut material treated with hot or cold water developed the desired dark-brown colour and honey-like flavour of honeybush faster during fermentation than bruising. The increased rate of colour development with the addition of water is considered to be the result of some of the oxidisable matter being extracted to the surface of the material where it is more accessible for oxidation, thus increasing the rate of fermentation. Treatment with water also decreased the presence of uncoloured bits of stems, giving a more uniformly coloured final product with better liquor characteristics. No significant differences were found between cold and hot water treatments, thus cold water is used in production, as it is more economical (Du Toit & Joubert, 1998b).

In practice, water is added to the cut honeybush plant material at a ratio of 1:4 to increase the moisture content to approximately 60% before fermentation begins. However, it is important to attain the correct moisture content as material that is too wet produces run-off during fermentation, causing possible loss of flavour and aroma. It also affects the drying process as wet tea tends to form balls and stick to the bed of the fluidised-bed driers causing uneven drying. It has been noted that these unevenly dried plant material sometimes has off-flavours (J. Kritzinger, Honeybush Natural Products, Langkloof, South Africa, 2013, personal communication), probably due to conditions favourable for microbial growth. On the other hand, material that is too dry tends to burn more readily at elevated fermentation temperatures producing undesirable sensory attributes. For some species, such as *C. genistoides* and young plant material, the cut plant material has an inherent moisture content of *ca*. 60% or more due to a low stem-to-leaf ratio (Fig. 4b). In such a case superficial wetting is required to aid the fermentation process (E. Joubert, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, 2014, personal communication).

Moisture content also seems to have an effect on the turbidity of the tea extract, as material that is fermented without the addition of water produces a clear, non-cloudy infusion (J. Kritzinger, Honeybush Natural Products, Langkloof, South Africa, 2013, personal communication). A likely explanation for infusion turbidity is the polymerisation of compounds extracted and deposited on the outside surface of the leaf and subsequent loss of solubility of the polymeric phenolic substances with increasing fermentation time (Du Toit & Joubert, 1998b), but the link to moisture content is still unknown.

Du Toit and Joubert (1998b) also studied the effect of pretreatments on polyphenol oxidase (PPO) and peroxidase (POD) activity as these enzymes play an important role in the development of colour in black tea (*Camellia sinensis*) (Mahanta *et al*., 1993). Interestingly, hot water treatment inactivated both the enzymes but did not impair fermentation, which indicates a chemical oxidation process rather than an enzymatic reaction is responsible for the "fermentation" of honeybush (Du Toit & Joubert, 1998b). Furthermore, the high temperatures used for fermentation do not sustain enzymatic oxidation due to denaturation of enzymes at high temperatures.

a)  b) 

**Figure 4** Old (a) vs. young (b) *C. intermedia* plant material illustrating low vs. high stem-to-leaf ratio, respectively.

### 3.4. Fermentation

"Fermentation" refers to the high-temperature chemical oxidation process integral for the development of the characteristic sweet, honey-like aroma and flavour and dark reddish brown colour of the leaves and infusion of honeybush tea (Du Toit & Joubert, 1998b). During this process, oxidative and other chemical reactions would be responsible for these changes. Research on black tea "fermentation" and high-temperature firing (drying process) showed that degradation of α-amino acids, carotenoids, fatty acids and hydrolysis of terpenoid glycoside precursors, lead to the formation of volatile organic compounds (VOCs), while oxidation of phenolic compounds results in formation of coloured compounds (Yamanishi, 1995; Drynan *et al*., 2010, 2012). A study by Le Roux *et al*. (2008) on the change in the VOCs of *C. genistoides* during fermentation demonstrated the presence of similar compounds in unfermented and fermented plant material and an increase in terpenoid concentration. The aroma fraction of *Cyclopia* will be further discussed in section 4.5. Early descriptions of the use of *Cyclopia* species as "bush tea" indicated that the leaves and flowers were sun-dried without first being "sweated" or fermented (Hofmeyer & Phillips, 1922). In such a case if not quickly dried, enzymatic oxidation would have taken place leading to browning, if not flavour development too (E. Joubert, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, 2014, personal communication).

Traditionally, small quantities were fermented for home use in the warm drawer of coal stoves (Joubert *et al*., 2011). Fermentation of larger quantities of honeybush tea was achieved using curing heaps (Fig. 5a) (Marloth, 1909, 1925) and fermentation at elevated temperatures in preheated "baking-ovens" (Fig. 5b) (Hofmeyer & Phillips, 1922; Snyman, 1990). A common method used to ferment large quantities of honeybush tea entailed piling and firmly packing the cut material into large heaps covered with Hessian bags allowing spontaneous heat generation and "fermentation" for 3-5 days (Du Toit *et al*., 1998). From the third day of fermentation onwards the heap was turned every 12 h to ensure that the cooler outer and bottom regions were mixed with the rest of the material, which also prevented oxygen depletion in the heap. Thereafter, once the material had developed a satisfactory brown to dark-brown colour and sweet aroma, the heap was spread open in a thin layer on canvas and allowed to dry in the sun (Du Toit *et al.*, 1998). This method did not require the input of additional energy and was thus favoured by the few processors in the Langkloof area who supplied the limited demand for honeybush tea during the twentieth century (Joubert *et al*., 2011). However, prolonged fermentation at temperatures ranging from ambient in the outer layers to *ca*. 60°C in the middle of the heap and moist conditions (50-60% moisture content) allowed the proliferation of thermotolerant mould and bacteria (Du Toit *et al*., 1998), with *Humicola grisea, H. lanuginose* and *Rhizomucor pusillus* found to be the predominant microbial contaminants (Du Toit *et al*., 1999). Additionally to giving a product of poor microbial quality, inadequate aeration and drying out of the fermentation heap resulted in under- and unfermented tea with a grassy flavour and poorly developed leaf colour (Du Toit & Joubert, 1999). The heap fermentation method subsequently became obsolete when faced with the demand for consistently high quality tea and export regulations requiring very low levels of microbial contaminants.

Du Toit *et al.* (1999) demonstrated that fermentation at elevated temperatures (>60°C) inhibits growth of thermophillic contaminants. The time required for the optimum development of sensory characteristics depends on the fermentation temperature, with increasing temperature requiring shorter time (Fig. 6). Sensory analysis of *C. intermedia* and *C. buxifolia* (previously identified as *C. maculata*) fermented at 60, 70 and 80°C were still "under-fermented" after 24-36 h and unacceptable due to the grassy flavour, whereas, fermentation longer than 36 h at 90°C produced a "burnt" flavour (Du Toit & Joubert, 1999). Fermentation is an energy intensive process, thus the minimum time required for satisfactory fermentation would be the choice in practice. However, fermentation at elevated temperatures is more critical and demands good control to prevent the development of negative sensory characteristics and to deliver a consistently high quality product.

With the rediscovery of the honeybush industry in the 1990s, crude "baking-ovens", consisting of steel drums buried in the ground (Fig. 5c), were the first attempt to elevate fermentation temperatures to shorten the fermentation time and to inhibit mould growth with a fair degree of success (Du Toit *et al*., 1998). Fires were made in the drums to heat the chamber and the coals were removed before Hessian bags filled with cut and wetted plant material were placed inside and covered. Although the initial temperature was high (>90°C), it rapidly declined and the only way to increase the temperature was to remove the bags and rekindle the fire, allowing little control (Du Toit *et al*., 1998). Given the historical use of "baking ovens" in the Riversdal area (Snyman, 1990), this crude concept of preheated steel drums was improved by building "baking-ovens" of bricks consisting of a baking chamber surrounded by a heating chamber (Fig. 5b). This design allowed reheating and maintenance of temperature throughout the fermentation process (Du Toit *et al*., 1998).

The commercialisation of honeybush subsequently required the modernisation of honeybush processing, which led to the adoption of batch rotary fermentation (Fig. 7a & b) (Joubert *et al*., 2008b), a concept developed for rooibos fermentation (Joubert & Muller, 1997). Different heating methods are currently employed with varying efficiency, such as heating of the walls of the rotary drum via short wavelength direct heating or by infrared lights. One processor aimed to further develop the fermentation system as the use of short wavelength infrared lights as source of heat results in slow temperature increases, which still allowed the opportunity for mould growth and extended the fermentation period, thus slowing productivity (J. Kritzinger, Honeybush Natural Products, Langkloof, South Africa, 2013, personal communication). Subsequently, the continuous screw drum (Fig. 8a) composed of a long fermentation chamber equipped with "screw and paddles" (Fig. 8b) that moves the tea along a continuous system of chambers was developed. In this system the chamber is heated to the desired temperature via direct steam injection into the tea while steam jackets surrounding the chambers aided in retaining the temperature of the tea. This system gave good insight into heating using a steam boiler, but insufficient knowledge of the fermentation process and poor management produced over-fermented and turbid tea (J. Kritzinger, Honeybush Natural Products, Langkloof, South Africa, 2013, personal communication).

This processor then reverted back to a batch system for better control over the fermentation process. The batch system consists of U-shaped stainless steel fermentation tanks (Fig. 9a) with rotary paddles (Fig. 9b & 10) that can hold up to 1.2 tons of tea each. The tanks were originally indirectly heated via electrical elements situated at the bottom of the tank (Fig. 10), however, the element caused the surface of the tank in contact with the tea to become extremely hot, causing the tea to burn and develop unacceptable “smokey” and “burnt” flavours as well as extremely turbid and dark-brown/black liquor colour. The heating methods used in the continuous screw drum were subsequently applied to the U-tank system with direct steam injection into the tea allowing for fast temperature increase while steam jackets surrounding the tank maintain the temperature. Additionally, electric heating elements situated inside the tanks (attached to the lid) heat the air surrounding the tea with the aim of simulating the functioning of a convection oven (Fig. 10). Rotating paddles (Fig. 9b & 10) thoroughly mix the tea at hourly intervals during the fermentation process ensuring even heat distribution throughout the tea. For better temperature control and reproducibility of the fermentation process, temperature probes connected to data loggers are positioned throughout the U-tank. This system has shaped advancements in honeybush fermentation methods, especially regarding temperature monitoring and control during the fermentation process, but still needs to be fine-tuned to achieve optimum fermentation and consistently reproduce a high quality product (J. Kritzinger, Honeybush Natural Products, Langkloof, South Africa, 2013, personal communication).

Fermentation conditions used by various processors range from 70°C/60 h for *C. intermedia*, as recommended by Du Toit & Joubert (1999) to 80-85°C/18-24 h for other *Cyclopia* species such as *C. genistoides*, *C. maculata* and *C. subternata* (Joubert *et al*., 2011). A recent study done on the optimum fermentation conditions of *C. genistoides, C. maculata* and *C. subternata* showed that no new sensory attributes develop during fermentation from 8 to 32 h, however, the average intensity of positive sensory attributes increased and the intensity of negative sensory attributes decreased (Theron, 2012). Fermentation for at least 16 h was required to increase the average intensity of positive attributes and decrease negative attributes to an acceptable level, whilst increasing the fermentation time to 24 h significantly reduced bitter taste. In general, a fermentation temperature of 80°C for 24 h is recommended for these species. Alternatively, fermentation at 90°C reduces the time for the development of an optimal sensory profile to 16 h, but different sensory attributes develop (Theron, 2012). *Cyclopia genistoides* fermented at 90°C has a slightly lower “rose geranium” note than that fermented at 80°C, while either “floral” or “apricot jam” notes are created when *C. subternata* is fermented at 80°C or 90°C, respectively. Fermentation of *C. maculata* at 90°C results in an increase of negative sensory attributes such as “hay/dried grass” aroma and flavour and “green grass” aroma. Each of these species has a slightly different sensory profile due to quantitative and qualitative differences in their volatile chemical composition (Cronje, 2010) and requires slightly different fermentation conditions for the development of an optimal sensory profile. From these results it is clear that fermentation conditions should be optimised for each *Cyclopia* species. Furthermore, optimised conditions determined on laboratory-scale should be verified on industrial scale to take into account scale and equipment employed in industry.

Despite knowledge gained on honeybush tea fermentation and advancements in processing methods, one of the major problems with the development of a reliable honeybush market is still the lack of standardised processing methods for all species and adaptation to commercial conditions necessary to ensure the production of tea with consistently good microbial and sensory quality.

Green ("unfermented") honeybush is also produced for the herbal tea market, as well as a source of extracts for the food, nutraceutical and cosmetic industries (Joubert *et al*., 2008b). During the production of green honeybush tea, slow greenish brown discolouration of the leaves occurs due to phenolic oxidation initiated when the cells are disrupted during shredding of the plant material (Martinez & Whitaker, 1995). Discolouration is thus assumed to indicate a change in the phenolic composition and a loss of bioactives, leading to a loss in perceived quality (Joubert *et al*., 2010). Browning of green honeybush is unacceptable (Anon., 2000) and could possibly be perceived as a poorly "fermented" honeybush, subsequently also negatively impacting on traditional "fermented" honeybush. It is thus also of great importance that processors employ processing and storage conditions that minimise colour and compositional changes of green honeybush (Joubert *et al*., 2010).

a)  b) 

c) 

**Figure 5** Curing heap (a) and baking ovens (b & c) traditionally used to ferment honeybush tea (De Lange, H; Joubert, E.).

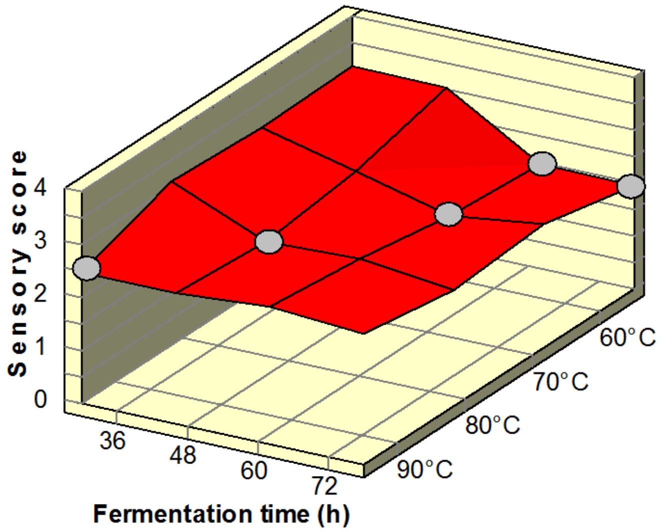



**Figure 6** Effect of fermentation temperature and time on overall quality of honeybush tea (average sensory score for *C. intermedia* and *C. maculata* (reclassified as *C. buxifolia*) with lowest score indicating best quality). Optimum temperature/time combinations are depicted as circles. Data from Du Toit & Joubert (1999) combined with unpublished data (Joubert *et al*., 2011).

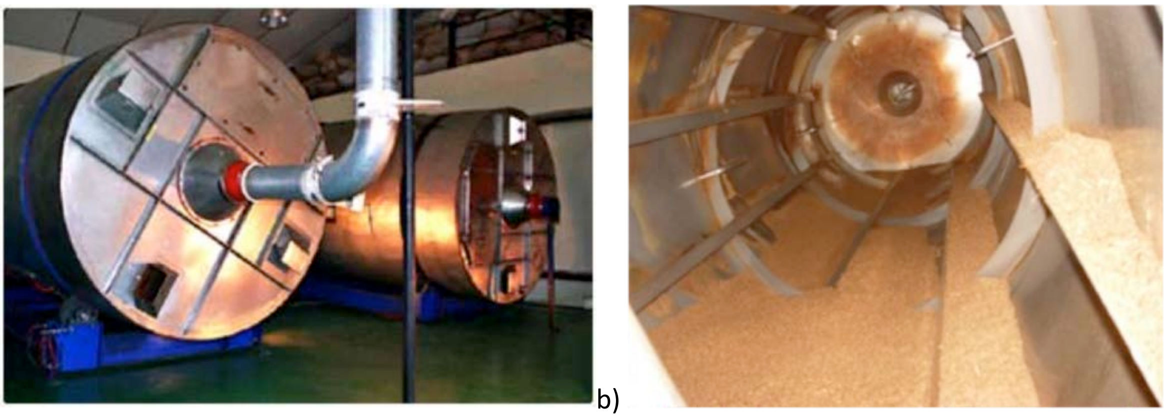
a)  b)

**Figure 7** Batch rotary fermentation tanks (a) and the interior of the tank (b) (Joubert, E.).

a)  b)


**Figure 8** Continuous screw drum fermentation tank (a) and the interior of the tank showing the rotating paddles (b).


**Figure 9** Modern batch fermentation tank (a) and interior of the tank with rotating paddles (b).

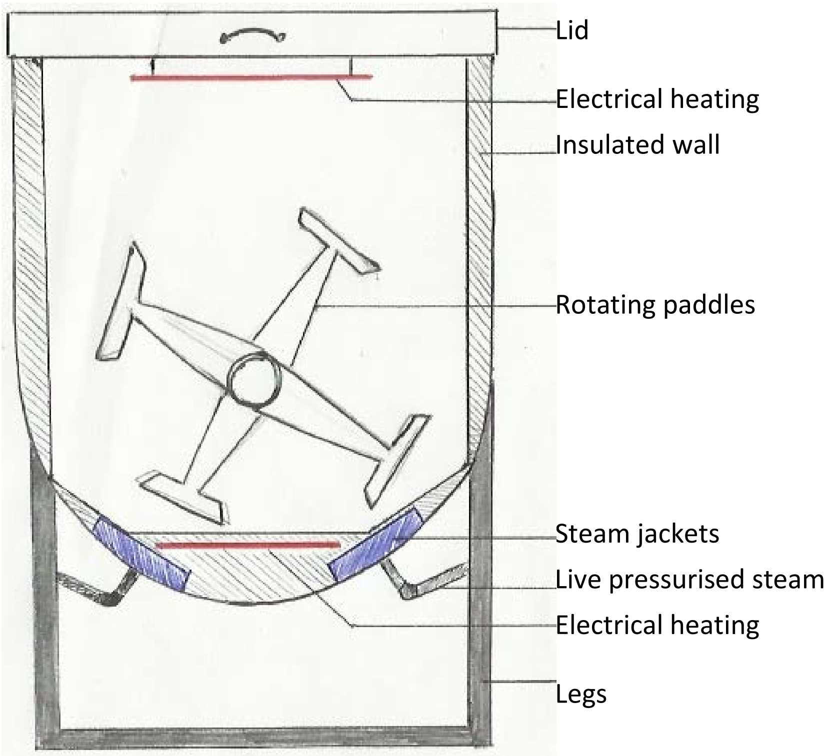



**Figure 10** Sketch of the U-tank fermentation system illustrating heating mechanisms and functioning.

### 3.5. Drying

Various methods are used to dry tea after fermentation. Traditional processors believe that the final product is improved by sun drying - it is thought to be essential for the development of the leaf colour and for the sensory characteristics of the infusion (Du Toit *et al*., 1998). However, Du Toit & Joubert (1998a) demonstrated that the drying method used (sun-drying vs. controlled drying) does not significantly affect the quality of honeybush tea. *Cyclopia intermedia* and *C. genistoides* dried at 40 - 70°C had no effect on taste or leaf colour, but loss of red colour was seen at high temperatures, and optimum aroma was obtained with drying at 50°C (Du Toit & Joubert, 1998a).

Sun drying has also been shown to not be essential for the development of the characteristic organoleptic properties of rooibos tea (Joubert & De Villiers, 1997). However, similar to honeybush tea, drying temperature has a significant effect on the aroma of rooibos tea with increasing temperatures (>40°C) causing a decrease in aroma (Joubert & De Villiers, 1997, Du Toit & Joubert, 1998a). The reason for this could be that high water activity of the plant material during slow drying promotes degradation reactions, which could result in the formation of beneficial or detrimental aroma compounds as reported for other plant material (Rockland & Nishi, 1980). For example, constituents of honeybush tea aroma such as linalool and α-terpineol increase during oven drying of raspberry leaves at 35°C (Kirsi *et al*., 1990); the different degradation products of β-carotene present in honeybush tea (i.e. β-ionone, β-damascenone, 5,6-epoxy-β-ionone and dihydroactinidiolide, identified by Cronje, 2010) are affected by temperature, and high temperature drying promotes thermal decarboxylation of some phenolic acids, forming phenolic volatiles (Fiddler *et al*., 1967; Pyysalo *et al*., 1977).

Furthermore, slow sun drying (1-2 days depending on the thickness of the layer of tea and the prevailing weather conditions) allows the growth of mould before the moisture content is sufficiently reduced. Some processors use drying racks raised off the ground, which allows shorter drying time due to better air circulation (Du Toit *et al*., 1998). However, drying is preferably done under controlled conditions as it prevents recontamination, ensuring the production of a hygienic product (Erasmus, 2002), and allowing tea processing during unfavourable weather conditions. Drying tunnels, rotary driers and fluidised-bed driers are currently employed by the honeybush industry.

### 3.6. Sieving

Honeybush tea is generally a course product and traditional belief is that the unrefined product has health giving properties (Du Toit *et al.,* 1998). Roadside stalls often sell it as "loose" tea: a mixture of short stems and leaves. Initially the tea was refined by sieving the dried product through an electrical cylindrical sieve with a 6.5 mm aperture screen that removed pieces thicker than a matchstick (Viljoen, 1994). The export market, however, demands a finer product, which dictated improvements to the sieving process (Du Toit *et al*., 1998). Today, more sophisticated sieving systems are employed that allow fractionation according to size. An example of such a system is a Locker Rotex Vibratory sieve, where the <10>60-mesh fraction is collected and sorted according to cut size. According to the Agricultural Product Standards Act (Act No. 119 of 1990; Anon., 2000) five classes of cuts exist, namely: extra course cut, regular course cut, regular fine cut, super fine cut, dust and other cut, which classify honeybush and green honeybush intended for export (Table 3). In the laboratory, dried tea is sieved (200 g/30 s) using a SMC mini-sifter and the <12>40-mesh fraction is collected for sensory analysis (Theron, 2012).

**Table 3** Specifications and applications for the five classes of tea cuts.

| Class name | Length | Mesh fraction | Application |
|---|---|---|---|
| **Extra course cut** | 7-10 mm | <95% shall pass through a 6-gauge mesh sieve and <50% shall pass through a 10-gauge mesh sieve. | Sold as loose tea. |
| **Regular course cut** | | >95% shall pass through a 6-gauge mesh sieve and <55% shall pass through a 10-gauge mesh sieve. | Used as filler for blends, or packaged in flat pillow tea bag. |
| **Regular fine cut** | 3-4 mm | >75% shall pass through a 10-gauge mesh sieve, >55% and <75% shall pass through a 12-gauge mesh sieve and >35% and <60% shall stay on a 40-gauge mesh sieve. | Used in all kinds of tea bags. |
| **Super fine cut** | 1-2 mm | >90% shall pass through a 10-gauge mesh sieve, >70% and <95% shall pass through a 12-gauge mesh sieve and <40% shall stay on a 40-gauge mesh sieve. | Used in fast packing machines and low volume dosage applications (e.g. double chamber string and tag tea bags). |
| **Dust** | | <40>60-mesh fraction | |
| **Other cut** | Classified according to buyer's specifications provided that the standards for honeybush as set out in item 6 of the Act, are complied with. | | |

(Anon., 2000; C. Cronje, Rooibos Ltd, Clanwilliam, South Africa, 2014, personal communication).

# 4. QUALITY ANALYSIS

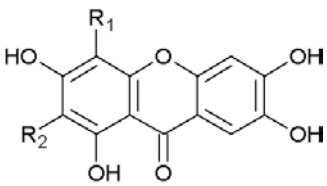
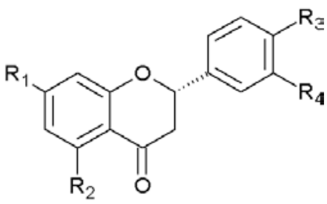
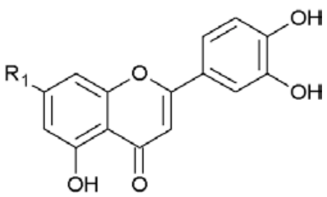
## 4.1. Chemical composition

Honeybush tea is a caffeine-free (Greenish, 1881), low tannin (Marloth, 1925; Terblanche, 1982) herbal infusion containing a wealth of polyphenolic compounds associated with health-promoting properties. The tannin, comprising approximately 30% of the total polyphenol content and 4.34% of the soluble solids of fermented *C. buxifolia* (previously *C. maculata*) (Du Toit & Joubert, 1998b), is of the proanthocyanidin type based on formation of a deep-red colour after reaction of the infusion with acidic butanol (Marnewick *et al*., 2005), a reaction that commonly used to indicate proanthocyanidins (Porter *et al*., 1985). Honeybush infusions contain 0.59 mg/mL fluoride (Touyz & Smith, 1982) and 20.5 mg/mL calcium (Malik *et al*., 2008), which play an important role in human health. The content of aluminium, a trace element that has been correlated with various human diseases, for example, Dementia, Parkinson and Alzheimer disease (Kröppl *et al*., 2012), is shown to be much less than that of *Camellia sinensis* teas (Malik *et al*., 2008).

To date only a few *Cyclopia* species have been subjected to in-depth analysis of their phenolic composition, represented by xanthones, benzophenones, dihydrochalcone, flavones and flavanones (Ferreira *et al*., 1998; Kamara *et al*., 2003, 2004; Kokotkiewicz *et al*., 2009, 2012, 2013; De Beer *et al*., 2012). Qualitative and quantitative differences exist between species. Major compounds, amongst others are the xanthones, mangiferin and isomangiferin, the benzophenone, iriflophenone-3-*C*-glucose, the dihydrochalcone, phloretin-3',5'-di-*C*-*β*-glucoside and the flavanone, hesperidin. Mangiferin is present in high concentrations in *C. genistoides* (Table 4). Scolymoside, a flavone glycoside, was present in notable quantities in *C. subternata*. Several compounds have been shown to possess strong antioxidant, immune-modulating, chemo-preventative and phytoestrogenic actions in various models (as reviewed by McKay & Blumberg, 2007; Joubert *et al*., 2008b and Louw *et al*., 2013), contributing to the health-promoting potential of *Cyclopia*. Mangiferin has been postulated to contribute to the anti-diabetic potential of *C. intermedia* (Muller *et al*., 2011). The presence of high levels of the less common and sought-after antioxidant compound, mangiferin, in honeybush tea merits the use of *Cyclopia* spp. as an alternative herbal tea and potential dietary supplement.

Apart from the dependence of the phenolic content of *Cyclopia* spp. on factors such as weather conditions, harvest date and frequency, and seed source as shown for *C. genistoides* (Joubert *et al.,* 2014), as well as stem-to-leaf ratio (De Beer et al., 2012; Du Preez, 2014), fermentation decreases total polyphenol and the content of individual compounds (Du Toit & Joubert, 1998b; Joubert *et al*., 2008a; De Beer & Joubert, 2010). Table 4 shows comparative changes in the content of some of the major polyphenols of hot water extracts prepared from unfermented and fermented *Cyclopia* spp. analysed to date. Apart from the decrease in content of these major compounds, the degradation of unknown compounds and polymerisation of compounds susceptible to oxidation, accompanied with lower solubility, could also contribute to the resulting lower total polyphenol content after fermentation (Joubert *et al*., 2005). Thus, the use of unfermented plant material is recommended for preparation of antioxidant extracts for the nutraceutical and cosmetics markets (Joubert *et al.,* 2003). Other processing practices such as steam treatment during production of green honeybush could also lead to quantitative changes (Joubert *et al.*, 2010).

**Table 4** Major phenolic compounds (g/100g extract) identified in unfermented honeybush aqueous extracts from four *Cyclopia* spp. and the effect of fermentation.

| Structure | Name | *C. genistoides* | *C. intermedia* | *C. sessiliflora* | *C. subternata* |
|---|---|---|---|---|---|
|   | **Xanthones** | | | | |
| | Mangiferin ($R_2$ = *C*-β-D-glucosyl; $R_1$ = H) | 9.55 (- 83%) | 4.35 (- 97%) | 4.67 (- 87%) | 2.73[a] (- 98%)[b] |
| | Isomangiferin ($R_2$ = H; $R_1$ = *C*-β-D-glucosyl) | 2.72 (- 59%) | 1.40 (- 81%) | 1.69 (- 54%) | 0.86 (- 83%) |
|   | **Flavanones** | | | | |
| | Hesperidin ($R_1$ = *O*-rutinosyl; $R_3$ = $OCH_3$; $R_2$, $R_4$ = OH) | 0.71 (- 56%) | 0.62 (- 47%) | 0.74 (- 49%) | 0.62 (- 61%) |
| | Eriocitrin ($R_1$ = *O*-rutinosyl; $R_2$, $R_3$, $R_4$= OH) | Traces | 0.13 (- 77%) | 0.32 (- 38%) | 0.32 (- 63%) |
| | Eriodictyol glucoside[c] (either $R_1$ or $R_2$ = *C*-β-D-glucosyl; $R_3$, $R_4$ = OH) | ND | 0.07 (- 100%) | ND | 0.35 (- 100%) |
|   | **Flavone** | | | | |
| | Scolymoside ($R_1$ = *O*-rutinosyl) | Traces | 0.04 (- 100%) | 0.06 (- 100%) | 0.68 (- 100%) |

(Adapted from Joubert *et al*., 2008b; De Beer & Joubert (2010)).

[a] Average compound content in aqueous extract from unfermented tea.

[b] Percentage decrease of compound content in extract due to fermentation of plant material.

[c] Position of sugar not yet determined.

### 4.2. Quality and regulatory control

Grading systems are used to standardise and commercialise food products to maintain consumer satisfaction (Feria-Morales, 2002). Such systems need to identify, define and measure the quality parameters of the product. Grading methods should also be quick, simple, reliable and scientifically validated and correlated to the consumers' perception of quality.

Honeybush tea (fermented and unfermented) that is exported is subject to the regulatory standards of the Department of Agriculture, as described in the Agricultural Product Standards Act 119 of 1990 (Regulation No. 1177 of 24 Nov 2000 and amended according to Notice No. R 1132 of 15 July 2005). The regulations specify classification according to cut size, moisture content, microbial content, pesticide levels and the presence of foreign matter. Furthermore, the plant material must be free of *Salmonella*, while limited counts for total bacteria, coliform bacteria, mould and yeast are allowed, with higher total bacterial counts and the presence of *Escherichia coli* allowed for unfermented honeybush. No provision is made for sensory quality, except that the regulation specifies that the "taste and aroma (both of unfermented and fermented honeybush) shall have the clean, characteristic taste and aroma and clear, distinctive colour of honeybush" and should be "free from any foreign flavours and odours which detrimentally affect the characteristic of the product". However, this vague description lacking sensory descriptors has no meaning in a quality control environment.

The sensory quality characteristics of black tea (*Camellia sinensis*) are assessed by tea tasters using sight, smell and taste to define quality (Obanda *et al*., 2001). The tasters take the appearance of the dry and infused tea leaves, and the liquor colour, aroma and flavour into consideration. Rooibos tea is similarly graded into seven grades (AA, A, B, C, D, E or F) according to the appearance of the dry and wet leaves and the appearance and flavour of the infusion (C. Cronje, Rooibos Ltd, Clanwilliam, South Africa, 2013, personal communication). Using only sensory attributes as determined by descriptive sensory analysis, Koch *et al*. (2012) showed that distinction between rooibos tea grades A to D were possible. Analysis of the phenolic composition of rooibos infusions of different grades also indicated quantitative differences, especially between grade A and D (Joubert *et al*., 2012; Stanimirova *et al*., 2013).

Quality assessment procedures such as for black tea and rooibos tea do not exist for honeybush, resulting in inconsistent product quality. Each processor exercises his/her own quality standards according to colour, aroma, flavour and cut size, which lead to large variation, including the sale of tea of inferior quality. As a step towards an uniform grading system for honeybush tea based on sensory quality, the ARC, in collaboration with the Sensory Laboratory of the Department of Food Science of Stellenbosch University, has developed a generic sensory wheel and lexicon with positive and negative aroma, flavour and taste descriptors for fermented honeybush tea, based on descriptive sensory analysis of *C. intermedia, C. sessiliflora, C. longifolia*, *C. genistoides, C. maculata,* and *C. subternata.* This quality control tool needs validation and refinement (Theron, 2012). Phenolic analysis of the infusions of *C. genistoides, C. maculata*, and *C. subternata* was also undertaken to investigate the association between individual compounds and taste modalities and mouthfeel attributes, i.e. astringency (Theron, 2012). The information generated during the latter study has not yet been translated into quality standards and further studies are in progress.

### 4.3. Flavour

Flavour is a vital quality of foodstuffs and a key driver of consumers' acceptance and appreciation or rejection of a product (Aparicio *et al*., 2006; Harker *et al*., 2008). Scientists have bestowed much effort in defining flavour. For example, in 1978, Beidler defined it as "the sensation realised when a food or beverage is placed into the oral cavity. It is primarily dependent upon the reactions of the taste and olfactory receptors to the chemical stimulus. However, some flavours also involve tactile, temperature and pain receptors." (Delwiche, 2004). According to the definition stated by the International Standards Organisation (ISO) flavour is the "complex combination of the olfactory, gustatory and trigeminal sensations perceived during tasting. The flavour may be influenced by tactile, thermal, painful and/or kinaesthetic effects." (ISO 5492, 1992).

It is challenging to capture the multi-faceted sensory experience when ingesting a foodstuff and the term is often inconsistently used. Flavour is the sensation arising from the complex combination of different non-volatile and volatile compounds that are released during eating, which are responsible for tastes by gustation (taste as perceived by taste buds containing specialised receptor cells that exist on the tongue, soft palate and extend partially down the throat), aroma by olfaction (sense of smell perceived indirectly by the olfactory organ, i.e. the nose) and trigeminal sensations (sense of irritation perceived by chemesthetic receptors present throughout the nose and oral cavity, which are also influenced by thermal, tactile, painful and/or kinaesthetic effects) (Fig. 11) (Delwiche, 2004; Deibler & Delwiche, 2004; Tournier *et al*., 2007).

The combination of taste and smell is known to have a unique impact on overall flavour perception (Delwiche, 2004; Lawless & Heymann, 2010). Chemesthetic sensations, named in an analogy to "somethesis" or the tactile and thermal sensations perceived over the body surface, are a separate class of tastes and smells that are perceived through the stimulation of the trigeminal nerves in the mouth, nose or eyes. They include the heat-related sensations from chilli, the nasal pungency of wasabi, tear-inducing stimuli from onions, cooling sensation from menthol, irritation from $CO_2$ and the tactile sensation known as astringency (Lawless, 1996; Lawless & Heymann, 2010). The importance of this sense in overall flavour perception is easily overlooked as the distinction between a chemical sense and tactile sense becomes blurred, particularly when sensing astringency (Lawless, 1996). For example, tannins in foods are chemical stimuli and yet the astringent sensations they produce, i.e. a rough and dry mouthfeel and drawing, puckering and tightening sensation in the cheeks and muscles of the face (Bate-Smith, 1954), are mostly tactile (Lawless, 1996). Although astringency is scientifically categorised as a group of chemically induced oral tactile sensations, most wine tasters regard it as a component of wine taste, which highlights the integrative nature of flavour in combining inputs from multiple modalities (Lawless, 1996).

The exact mechanism of flavour perception is not yet known due to the wide range of stimuli involved; the complex interaction of individual modalities (Fig. 12); and the fact that chemical compounds and food structures that activate the flavour sensors change due to temperature increases, salivation and mastication as the food is eaten (Tournier *et al*., 2007).  However, a striking characteristic of flavour perception is that information from all these modalities may be synthesised into a unitary experience with a single hedonic response – either appetitive or aversive (Lawless, 1996).

Theron (2012) conducted a study to define and describe the flavour of honeybush tea by characterising and analysing the sensory attributes associated with six commercially important *Cyclopia* species, i.e. *C. genistoides*, *C. subternata*, *C. sessiliflora, C. longifolia, C. intermedia* and *C. maculata.*  The study showed that the "characteristic" flavour of honeybush could be described as a combination of "floral, sweet, fruity and plant-like with a sweet taste and slight astringent mouthfeel" (Theron, 2012).

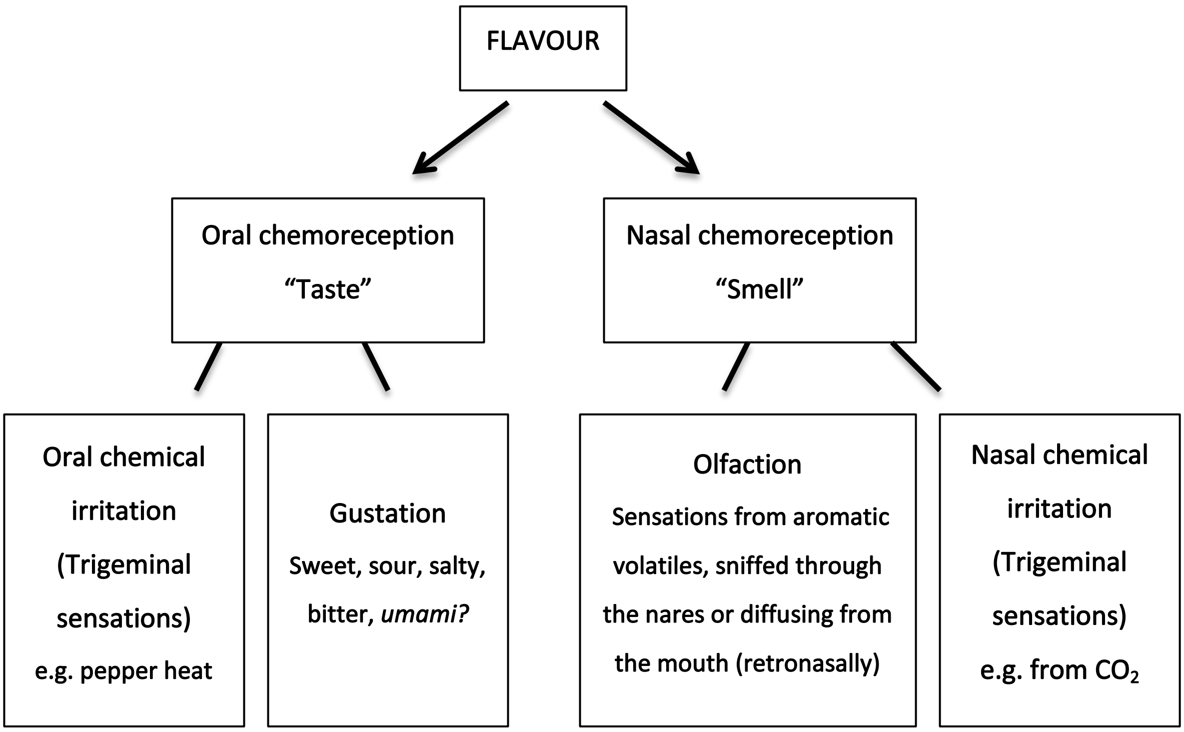



**Figure 11** Schematic of the anatomical systems mediating perception of flavours (Lawless, 1996).

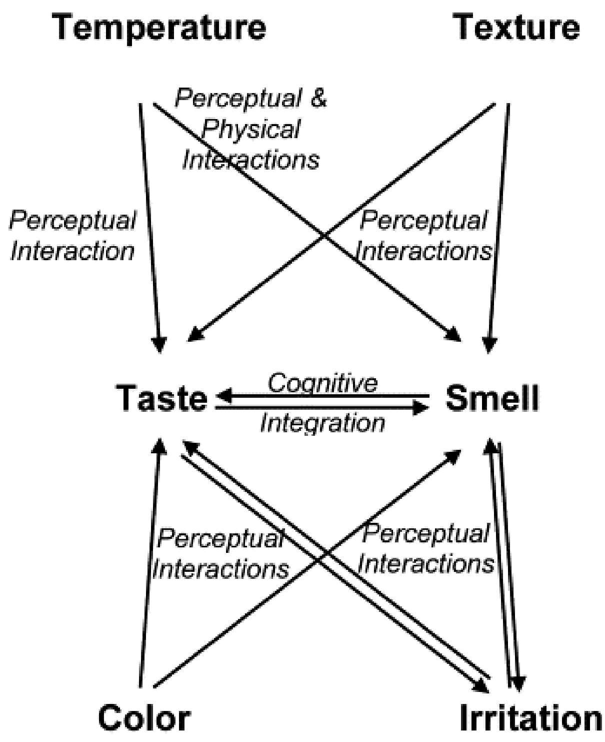



**Figure 12** Summary of perceptual interactions evoked while ingesting foodstuffs. Arrows indicate a modality that has been shown to interact with another modality (Delwiche, 2004).

### 4.4. Taste and mouthfeel

The four basic taste modalities (bitter, sweet, sour and salty) are perceived by taste receptors situated in the cell membranes of taste buds, which are located within a variety of projections known as papillae distributed on the tongue and within the oral cavity (Jackson & Linskens, 2002). Three types of papillae preferentially detect these modalities: foliate (appear as rows of ridges and valleys situated at the side of the tongue), fungiform (mushroom shaped papillae situated at the tip of the tongue) and circumvallate (button-shaped papillae situated at the back of the tongue) (Jackson & Linskens, 2002). Various other taste qualities such as umami and astringency have been proposed to be included in the group of basic tastes (Lawless & Heymann, 2010).

For sweet, bitter and umami tastes, the receptor protein families T1Rs and T2Rs (specific G-protein coupled receptors or GPCRs) are functional: the T1Rs for sweet and umami and the T2Rs for bitter tastes. Salt and sour tastes are detected by the activation of ion channels rather than via GPCRs (Lawless & Heymann, 2010).

Astringency can be defined as “a complex sensation combining three distinct aspects: drying of the mouth, roughing of oral tissues, and pucker or drawing sensations felt in the cheeks and muscles of the face” (Lee & Lawless, 1991). It has been shown that these qualities are distinctly separate from taste sensations such as sourness (Lawless & Corrigan, 1994) and the fact that they can be sensed from areas lacking in taste receptors such as the lips further substantiates their classification as tactile rather than gustatory sensations (Breslin *et al*., 1993). The mechanism for astringency involves the binding of polyphenols (the primary source of astringency in foods) to salivary proteins, particularly proline-rich proteins (PRPs), causing a change in configuration and subsequent loss of their lubricating properties (Bajec & Pickering, 2008).

The sense of taste has two important functional properties that are important to consider in sensory analysis: sensory adaptation and mixture interactions. Adaptation is a decrease in responsiveness under conditions of constant stimulation, which functions to alert an organism to change. For example, we are generally unaware of sodium in our saliva, but rinsing the tongue with deionised water and presenting the normal concentration of NaCl will produce a notable sensation. Interestingly, presentation of concentrations of NaCl or any other tastant below the adapting level takes on other taste qualities; for example, water can taste sour and/or bitter after salt adaptation, sweet after quinine or acid and bitter after sucrose (McBurney & Shick, 1971). Thus, water can elicit any of the four taste qualities depending on what preceded it. Taste adaptation should thus be taken into account during sensory evaluation as both the solvent and the taste molecules can elicit sensory responses. Secondly, mixtures of tastes have a tendency to show inhibitory or masking interactions known as mixture suppression, which are important in determining the overall balance of flavours in foods (McBurney & Bartoshuk, 1973). For example, the sourness from acids in wine and fruit juices are partially masked by sweetness from sugar (Lawless, 1977). Similarly, bitterness can be inhibited by sweetness (Lawless, 1979) or salt (Kroeze & Bartoshuk, 1985). However, hyperadditive relationships (also known as enhancement or synergism) also occur between tastes - the most common being the addition of sub-threshold amounts of monosodium glutamate in mixtures to produce strong taste sensations (Yamaguchi, 1967). The search for synergistic mixtures of sweeteners and salt flavours are currently of particular interest, due to the health-implications of sugar and salt, and restrictions placed on their use.

Additionally, the global trend to improve the healthiness of our diets has been met with taste and flavour challenges (Ley *et al*., 2011) as it is, in most cases, the potential beneficial phytonutrients such as phenolic acid derivatives, flavonoids, isoflavones, terpenes and glucosinolates that elicit unpleasant tastes, particularly bitter and astringent (as reviewed by Drewnowski & Gomez-Carneros, 2000). Consequently, there is a growing demand for ingredients with flavour-modifying capabilities that have a weak intrinsic taste but are able to enhance the positive hedonic experiences, while suppressing the negative, i.e. change the quality of the flavour profile indirectly, as the obvious solutions such as using high potency sweeteners are becoming less accepted by consumers (Ley *et al*., 2011). Thus, the molecular structures of taste stimuli have been investigated to better understand taste perception.

The taste of tea is linked to its complex chemical composition, i.e. sugars, polysaccharides, alcohols, acids, phenolics and nucleic acids (Jackson & Linskens, 2002; Chen *et al*., 2008). Small, soluble, inorganic cations and certain organic acids, such as phenolic acids, cause sour taste (Ramos Da Conceicao Neta *et al*., 2007), whereas flavonoids (low molecular weight), alkaloids and terpenoids elicit bitter taste and tannins are astringent (Lesschaeve & Noble, 2005; Ley, 2008). In attempts to correlate structural elements with bitter taste it has been found that a bitter molecule needs a polar group and a hydrophobic moiety (Belitz & Wieser, 1985) and the spatial arrangement of these structural features is critical as even a minor variation can alter the taste profile or strongly influence the threshold (Ley, 2008). For example, the hesperetin rutinoside (hesperidin) is tasteless, the isomer hesperetin neohesperidoside (neohesperedin) is intensely bitter (Ley, 2008) and neohesperedin dihydrochalcone is a sweet molecule used as a bitter-reducing compound (Cano *et al*., 2000; Kroeze, 2000). It is also accepted that the mechanism of sweet taste chemoreception is via hydrogen bonding (Mathlouthi & Portman, 1990).

Theron (2012) revealed that large variations exist between the levels of soluble solids, total polyphenols and individual polyphenolic compounds of six *Cyclopia* species, which reflected in the sensory quality of the infusions. Factors such as geographical area, climate, soil, survival strategies (Schutte, 1997; Joubert *et al*., 2014), the presence of flowers (Du Toit & Joubert, 1998b), the age of the plant/regrowth (Joubert *et al*., 2011; Joubert *et al*., 2014) and processing conditions (Du Toit & Joubert, 1999; Joubert *et al*., 2008a) contribute to this variation. The bitter taste perceived in some *Cyclopia* species, especially *C. genistoides*, appears to be caused by the xanthone content, particularly mangiferin, although isomangiferin, compound C (later defined as iriflophenone-3-*C*-glucoside) (De Beer *et al*., 2012) and tannins may also play a role as associations with these compounds have been indicated (Theron, 2012). Hesperidin, believed to be tasteless, also correlated significantly with bitter taste. The sweet and sour taste of honeybush tea has not yet been linked to specific compounds (Theron, 2012), however, hesperetin, a compound found in small quantities in honeybush extracts (Joubert *et al*., 2003), has been identified as a flavour-modulating compound with sweet enhancing properties (Ley *et al*., 2007).

Fermentation temperature and time has been shown to affect taste and astringency of teas such as rooibos (Joubert & De Villiers 1997) and black tea (Obanda *et al*., 2001) due to oxidative changes and polymerisation of phenolic compounds. Under-fermented rooibos tea was watery (without body) and astringent. Although honeybush tea relies on chemical oxidation to “ferment” at higher temperatures, it can be postulated that the polyphenols intrinsic to honeybush tea and subsequently its characteristic taste are similarly affected. It has been shown that astringency in honeybush tea decreases with increasing fermentation time (Theron, 2012).

### 4.5. Aroma

Odour is perceived in the upper part of the nasal cavity called the regio olfactoria. Odours can reach the regio olfactoria via a breath of air through the nasal cavity (orthonasal olfaction) or via the nasopharynx connecting the mouth with the nasal cavity during chewing and swallowing (retronasal olfaction) (Negoias *et al*., 2008). In contrast to the relatively limited taste sensations, a much larger number of distinct sensations arise via volatile chemicals stimulating smell, suggesting that olfaction provides the majority of the diversity in our flavour experiences (Lawless, 1996).

A foodstuff may contain hundreds of volatile compounds known as volatile organic compounds (VOCs), while only a few are of significance in determining the aroma, i.e. odour active (Delahunty *et al*., 2006). The latter compounds have three properties that affect their activity: absolute threshold, concentration-response (psychometric function), and quality. Thus, in a complex aroma, only the VOCs that exist in concentrations above their absolute threshold contribute to the aroma, with increased intensity perceived as the concentration of a compound increases (Delahunty *et al*., 2006). However, predicting the outcome of mixing odours is extremely difficult, as odours with different quality tend to suppress one another, whereas odours with similar quality tend to blend and produce a new aroma. Additionally, odours present at sub-threshold concentrations, or those with no odour activity when assessed individually, can contribute to the aroma when mixed (Delahunty *et al*., 2006).

A recent paper by Le Roux *et al*. (2012) gave a comprehensive list of VOCs identified in *C. subternata*. Of the 183 VOCs identified by gas chromatography–mass spectrometry (GC-MS), 37 were determined by gas chromatography–olfactrometry (GC-O) to be odour-active. (*E*)-β-Damascenone, (R/S)-linalool, (*E*)-β-damascone, geraniol, (*E*)-β-ionone, and (7*E*)-megastigma-5,7,9-trien-4-one were shown to be most odour-active (compounds with the highest flavour dilution (FD) factor), while the odours of (6*E*, 8Z)-megastigma-4,6,8-trien-3-one, (6*E*, 8*E*)-megastigma-4,6,8-trien-3-one, (7*E*)-megastigma-5,7,9-trien-4-one, 10-*epi*-*γ*-eudesmol, *epi*-*α*-muurolol, and *epi*-*α*-cadinol were perceived as typically honeybush-like (Le Roux *et al*., 2012).

A study of the volatile fraction of unfermented and fermented *C. genistoides* showed that the same volatile compounds existed in both unfermented and fermented honeybush (Le Roux *et al*., 2008). Cronje (2010) concluded that the VOCs present in the green (unfermented) honeybush material are thus formed via biogenesis and fermentation is only responsible for increasing or decreasing the relative concentrations of the VOCs already present. The change in the compositions of the VOCs with fermentation is characterised by an increase in terpenoid concentration and loss of saturated and unsaturated alcohols, aldehydes and methyl ketones, which contributed to the development of the typical honeybush aroma (Le Roux *et al*., 2008). The volatile compounds identified in unfermented *C. genistoides* mainly consisted of saturated and unsaturated alcohols, aldehydes and methyl ketones that are known to have distinct grassy odours. For example, the major constituent, 6-methyl-5-hepten-2-one is described as oily, green grass, and herbaceous. Mainly terpenoid compounds exist in the volatile fraction of fermented *C. genistoides*, which are known to have floral and sweet-associated odours. The volatile compounds identified in unfermented and fermented *C. genistoides* as well as the descriptors associated with these compounds are summarised in Table 5. Similarly, the odour active VOCs identified in unfermented and fermented *C. intermedia* (Table 6) (Cronje, 2010) indicate that VOCs present in higher concentrations in unfermented *C. intermedia* are described as citrus-like, herbaceous, camphoraceous and green, whereas those present in higher concentration in fermented *C. intermedia* are mostly described as sweet, floral, fruity, and woody (Cronje, 2010).

Cronje (2010) also compared the odour active volatile compounds of four *Cyclopia* species whilst taking the origin of two species into account [*C. genistoides* (Albertina), *C. genistoides* (Pearly beach), *C. subternata* (Bredasdorp), *C. subternata* (Genadendal), *C. intermedia*, *C. longifolia*]. It was found that the four species were qualitatively similar but quantitatively different (Fig. 13). This trend was also evident between species from different areas, however, since the number of samples was limited, no definitive conclusion can be made except that the volatile fraction of *Cyclopia* species show quantitative differences concentrations (Cronje, 2010).

As more *Cyclopia* species are introduced into the market and a greater demand for high quality honeybush tea is required, it has become imperative to increase our understanding of factors that differentiate species, but also contribute to the characteristic flavour of honeybush, irrespective of species. Descriptive analysis of the aroma profile of *Cyclopia* species showed that the primary/generic aroma attributes associated with all honeybush species are “floral”, “sweet-associated” and “plant-like”, while secondary attributes such as “rose geranium”, “fruity” and “boiled syrup”, amongst others, seem to differentiate between species (Theron, 2012). Furthermore, the subtle aroma notes need to be identified and the role of non-aroma active compounds and/or sub-threshold odorants in the modification of the sensory perception of aroma-active compounds of honeybush should be investigated to better understand compound interactions and improve our understanding of the association between aroma compounds and sensory attributes (Cronje, 2010).

**Table 5** The main volatile components in the aroma of unfermented and fermented *C. genistoides* and their descriptions compiled by Le Roux *et al*., 2008.

| 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.

**Table 6** Most intense odour active volatile organic compounds identified in unfermented and fermented *C. intermedia* compiled by Cronje (2010).

| Compound | Unfermented Area % | Fermented Area % | Aroma descriptors |
|---|---|---|---|
| **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.

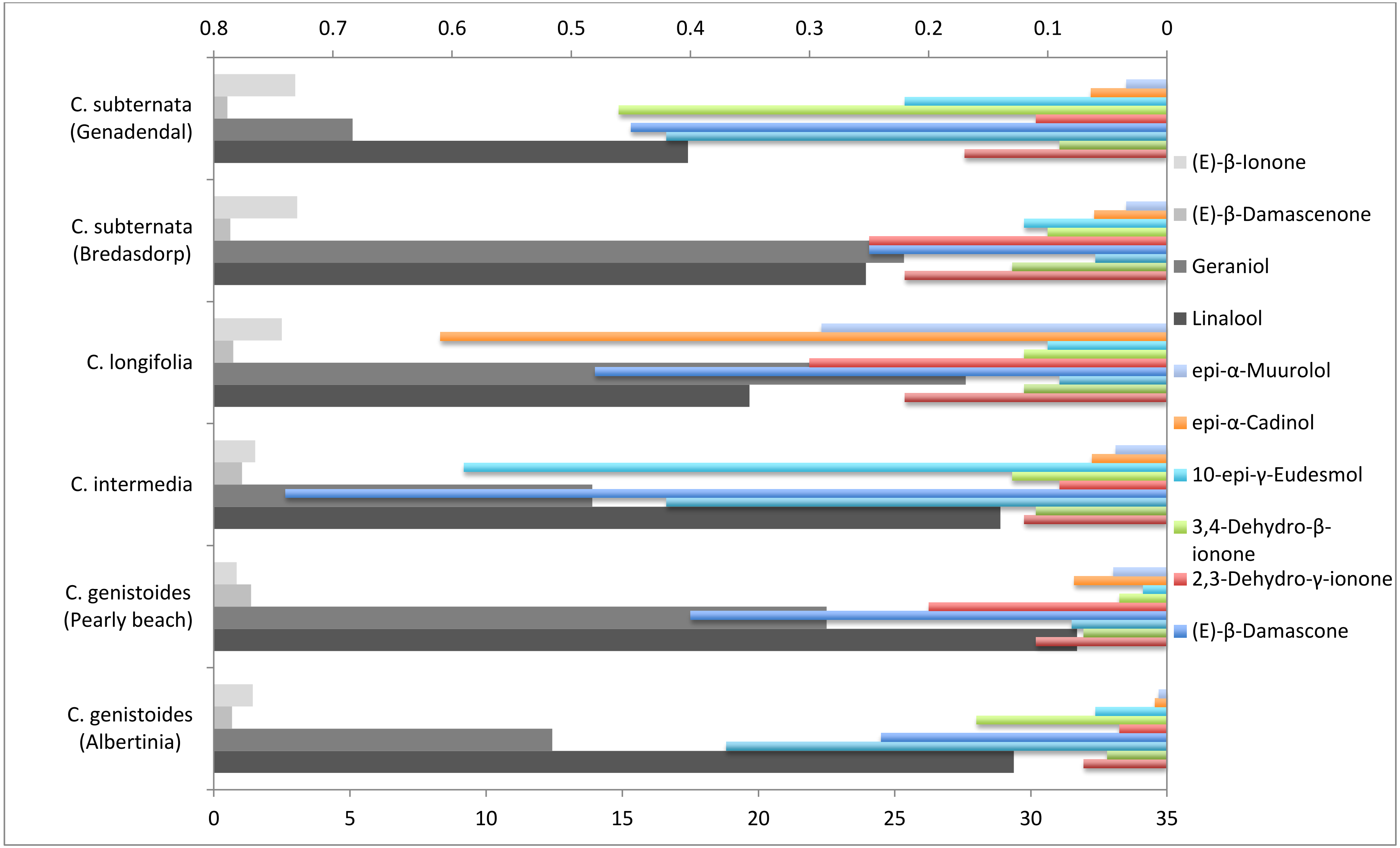



**Figure 13** Comparison of relative concentrations (area %) of the most intense odour active compounds identified in the headspace of infusions of four *Cyclopia* species (adapted from Cronje, 2010). Samples originating from different areas were analysed for both *C. genistoides* and *C. subternata.*

## 4.6. Colour

The integration of sensory inputs is not limited to chemical senses, but include factors such as appearance, visual texture and colour (Lawless, 1996). It has been demonstrated that individuals associate certain flavours with specific colours and when the colours are altered, the flavour identification is decreased. Also, learned colour-taste associations impact on perceived taste, even in complex stimuli such as wine (Delwiche, 2004).

Indian tea processors use colorimetric methods to indirectly detect the optimum fermentation time of *Camellia sinensis* (Bhattacharyya *et al*., 2007). The colour change of fermenting tea leaves is tracked using a digital colorimeter and optimum fermentation is obtained when optical absorbance attains the maximum value and the colour of the leaves are coppery brown (Liang *et al*., 2005; Bhattacharyya *et al*., 2007).

The colour of the infusion can also give an indication of quality. Colour measurement values, i.e. absorbance and tristimulus parameters, are affected by factors such as the soluble solids content and composition. The soluble solids content of an infusion also gives an indication of its "strength" and, therefore, influences the sensory quality of the tea infusion. Infusions of Grade A rooibos were found to have higher soluble solids content than infusions prepared from B, C and D grade rooibos (Joubert *et al*., 2012), where A grade samples were of the highest quality and D grade samples of the lowest quality. The association between objective colour measurements, using the CIELab scale, and the soluble solids content of rooibos infusions has been demonstrated by Joubert (1995). Another useful application of the link between soluble solids and colour of the infusion is that it can provide some insight into fermentation. Joubert (1984, 1988) found that increasing fermentation time of rooibos plant material results in a decrease in soluble solids due to polymerisation and subsequent loss of solubility of the polymeric phenolic substances. Similarly, it was postulated that a low level of soluble solids could be an indication of poor solubility due to over-fermentation of honeybush tea (Du Toit & Joubert, 1998b).

Du Toit and Joubert (1998b, 1999) applied the tristimulus reflectance and transmission measurements (CIELab scale) to determine the objective colour of honeybush plant material and infusions, respectively, as a measure to establish the effect of pretreatment on the fermentation of honeybush tea and the effect of fermentation temperatures and times on colour development in order to optimise the fermentation process. It was shown that browning and thus darkening of the fermenting leaves, measured as a decrease in the $L^*$ (lightness) and $b^*$ (yellowness) values and an increase in $a^*$ (redness) values, increased with increasing fermentation temperature and time. Colour development took place during the first 24 h of fermentation with the loss of green colour (- $a^*$) occurring simultaneously as the development of red pigments ($a^*$). No significant changes in $L^*$ values were observed (Du Toit & Joubert, 1999). The change in colour from green to red-brown is partially due to the breakdown of chlorophylls (Du Toit & Joubert, 1998b) but the major contribution is from the oxidation of polyphenols, for example, the oxidation of flavonoids to highly polymeric compounds results in the loss of yellow colour and contributes to browning. The fermentation rate, indicated by the rate of change in *L** value, was markedly higher in the water treated samples. Infusions of these samples also had lower *L** values, which corresponds with the higher amount of soluble solids extracted as well as the higher phenolic content of the soluble solids (Du Toit & Joubert, 1998b). Thus, treating the plant material with water could contribute to the partial diffusion of soluble solids to the surface of the leaves, resulting in more concentrated brown extracts. Theron (2012) also indicated that differences in absorbance values of honeybush tea infusions as a measure of colour correspond with the soluble solids content.

Thus, colour measurements of honeybush tea could be used to track fermentation and determine optimum time of fermentation indirectly. Additionally, colour can be used as an indication of quality.

### 4.7. Turbidity

As honeybush tea is a herbal drink, thus usually taken without milk (hot or as an iced beverage) the turbidity of the infusion also has a marked influence on the perception of its quality. In fact, buyers of honeybush tea have rejected turbid tea, which is of concern in view of the limited supply of the product (M. Bergh, Rooibos Ltd, Clanwilliam, South Africa, 2013, personal communication).

The cause of turbidity and formation of precipitate in honeybush infusions is unknown. In black tea (*Camellia sinensis*), tannins, accounting for 25% of the water-soluble components in tea leaves, interact with caffeine to form a complex known as “tea cream” which leads to the development of turbidity and precipitation in a strong infusion upon cooling (Rutter & Stainsby, 1975; Boadi & Neufeld, 2001). It has been shown that quantity of tea cream varies with temperature, pH, the concentration of the brew and the type of leaf used. Since the creaming properties of teas with similar caffeine contents differ greatly, these differences may be due to heterogeneity in the polyphenol constituents (Rutter & Stainsby, 1975). A few techniques are employed to remove or solubilise tea cream for producing tea extract concentrates or cold soluble tea powders, which should be clear and bright upon reconstitution for use as iced beverages.

Unlike black tea, honeybush tea does not contain caffeine, which is key to the formation of tea cream, yet turbidity is a factor that detracts the quality of some production batches. Turbidity in rooibos tea, also caffeine-free, is a characteristic associated with over-fermented tea that is of unacceptable quality (Joubert & De Villiers, 1997). Through observations in preliminary research for the present study, it can be postulated that turbidity of honeybush tea is associated with processing problems such as fermentation conditions (temperature and time, particle size and moisture content, moisture addition, heat distribution, equipment). Turbidity of honeybush infusions will be measured in this study to gain some insight into a possible link between processing parameters and this undesirable phenomenon.

### 4.8. Negative sensory attributes

Fermentation is a vital process in honeybush tea manufacturing and is primarily responsible for the characteristic flavour of the tea, thus deciding the final quality of the product. During this oxidation process, chemical reactions are dependent on temperature, humidity and aeration. Managing the reactions and chemical transformations during fermentation to an optimum limit via these factors is vital for producing good quality tea. It is thus crucial that the plant material is allowed to ferment only up to the desired limit as under-fermented and over-fermented teas suffer serious sensory and quality problems. Sensory attributes associated with over- and under-fermented teas are “dusty”, “medicinal”, “burnt caramel”, “rotting plant water”, “cooked vegetables”, “sour”, “green grass” and “hay/dried grass” (Theron, 2012).

Factory managers subjectively monitor the appearance and smell of the fermenting honeybush tea to estimate when optimum fermentation has been reached. This requires experience and an understanding of the factors that may affect the fermentation rate and ultimately quality. Apart from pre-harvest factors that may affect the quality of honeybush such as age of the plant material, leaf-to-stem ratio etc., good control over the processing conditions is vital to ensure good quality. The quality of the final product hinges to a large extent on termination of fermentation at the “right time”, which is achieved by drying. Poorly fermented tea, due to under- or over-fermentation, will not produce a final product of good quality. Poor control over the amount of water added can also lead to poor quality. If the plant material is too wet, drip loss during fermentation results in loss of valuable sensory components. On the other hand, plant material that is too dry may easily burn at elevated temperatures or result in inadequate fermentation at low temperatures. Experience also showed that it is vital to keep fermentation temperatures elevated (>70°C), but not too hot as this may accelerate the fermentation rate too much and cause development of unacceptable negative sensory attributes and burnt tea.

## 5. ANALYTICAL METHODS

### 5.1. Descriptive sensory analysis

Stone and Sidel (1993) defined sensory analysis as “a scientific discipline used to evoke, measure, analyse, and interpret reactions to those characteristics of products or materials as they are perceived by the senses of sight, smell, taste, touch and hearing.” Sensory analysis is a widely applied tool for quality inspection, product design and marketing (Zeng *et al*., 2008). It is considered to be the ultimate method of identifying, quantifying and qualifying information on the sensory aspects of food products as chemical and instrumental procedures lack the acuity of human senses and the ability to integrate perceptions (Aparicio *et al*., 1996; Murray *et al*., 2001).

Descriptive sensory analysis has been used for many years to study food and beverage products. Recent applications included products such as cheese (Drake *et al*., 2001), Malbec wines (Goldner &

Zamora, 2007), chocolate milks (Thompson *et al*., 2004), carbonated drinks (Kappes *et al*., 2006), fermented food products (Ghosh & Chattopadhyay, 2010), rooibos tea (Koch *et al*., 2012) and honeybush tea (Theron, 2012) to name a few.

Descriptive sensory analysis is a generic method carried out by a trained sensory panel; a group of 8 to 12 individuals that are familiar with the product and are able to discriminate between and describe different flavour and aroma sensations (Drake & Civille, 2002; Lawless & Heymann, 2010) when evaluating a number of samples. The panellists have a roundtable discussion to generate descriptive terms relating to the sensory attributes of the product and the intensity of each attribute. The overall amplitude, as well as a measure of the balance and blend of the sensory attributes, is evaluated (Zeng *et al*., 2008). The panel is then trained prior to evaluation in the terminology used to judge the product involving a 100-point descriptive intensity scale for describing major sensory attributes (Chawla *et al.,* 2011). Theron (2012) used the same procedure to analyse honeybush infusions, with each panellist scoring the intensity of the selected attributes on a linear scale anchored to 0 (no presence) and 100 (maximum intensity). Data acquisition was achieved through a computerised system using the software Compusense® *five*, which allows statistical analysis of the data.

The quality of data that the panel provides is vital for making proper research decisions, thus a good sensory panel should provide accurate, discriminating and precise results (Kermit & Lengard, 2005). An initial step in analysing sensory data is to identify individual assessors that perform abnormally or inconsistently in order to re-evaluate their data by further analysis and improve data quality in future sessions through increased training and targeting problematic areas (Kermit & Lengard, 2003; Tomic *et al*., 2010). PanelCheck software can be used to evaluate the repeatability, reproducibility and discriminatory ability of the panellists via univariate and multivariate statistical methods (Tomic *et al*., 2010). ANOVA (Analysis of variance) is performed for each panellist and attribute and the resulting p-value vs. Mean Square Error (MSE) value plot shows the panellist's ability to detect differences between samples against their repeatability (Næs *et al.*, 2010; Tomic *et al*., 2010). Profile and line plots visually demonstrate how each panellist rates the samples compared to other panellists and the panel consensus for a certain attribute (Tomic *et al*., 2010). The consonance of each assessor and the panel as a whole can also be visually detected and evaluated using correlation loadings plots based on the Tucker-1 method (Næs *et al*., 2010; Tomic *et al*., 2010).

More importantly, average sensory profiles of the product can be determined by univariate and multivariate approaches, allowing comparison of samples of different cultivars, region of origin, or that were exposed to different treatments, processes or storage conditions, for example (Corollaro *et al*., 2013). Principle component analysis (PCA) is a graphical tool used to visualise the sensory space of the samples and cluster them in homogenous groups via Hierarchical cluster analysis (Aprea *et al*., 2012). Such models can also be used to study the broad associations between sensory and chemical data to identify the most important compounds that contribute to odour and flavour perception (Aprea *et al*., 2012). It should, however, be stressed that this approach has limitations as an association may be indicated just because they show the same trend. Partial least squares (PLS) regression is an extension of the multiple linear regression model with features of PCA that is also a useful tool to predict or analyse a set of dependent variables from a set of independent variables or predictors, for example, correlating sensory and instrumental data. PLS regression is particularly useful in order to predict a set of dependent variables from a very large set of independent variables or predictors, which is achieved by extracting a set of orthogonal factors from the predictors called latent variables, which have the best predictive power (Næs *et al*., 2010).

While descriptive sensory analysis identifies and quantifies information on the sensory aspects of products, consumer tests provide information on consumer liking via acceptability and preference tests, which are critical for industry to identify the products and product attributes that are preferred by consumer market segments (Drake & Civille, 2002). Descriptive sensory properties can be related to consumer liking via simple and multivariate analysis to determine the possible direct relationships between sensory attributes and consumer liking or to relate the data sets and determine the combinations of attributes that affect liking, respectively (Drake & Civille, 2002).

### 5.2. Sensory lexicons and wheels

Sensory lexicons are commonly used tools to document and describe the sensory perception of foodstuffs, consisting of a set of words describing the flavour, taste, aroma and mouthfeel of a specific product (Drake & Civille, 2002). The development of a sensory lexicon entails several steps, including collecting a broad representative sample set, generating an appropriate descriptive language and descriptors using descriptive sensory analysis, and eliminating redundant terms before organising the final list of descriptors and designating definitions and reference standards (Aparicio *et al*., 1996; Drake & Civille, 2002; Corollaro *et al*., 2013). A good sensory lexicon should be discriminatory, descriptive and terms should be precise and non-redundant. The inclusion of descriptor definitions and reference standards (food- or chemical-based, as well as qualitative, quantitative, or both) provides language clarity and minimises confusion, which creates a platform from which research can be reproduced at different sites or times and compared (Meilgaard *et al*., 1999; Drake & Civille, 2002). Sensory lexicons are widely used for profiling new and competitive products, comparing and monitoring products within a category, quality control, as well as an interface relating to consumer acceptance and liking, and chemical and instrumental data (Drake & Civille, 2002).

The honeybush tea sensory lexicon developed by Theron (2012) comprises descriptive terms, generated by descriptive sensory analysis of infusions from six *Cyclopia* species, with definitions and reference standards for each attribute. The descriptive terms are grouped together as flavour (attributes determined orthonasally and retronasally) and taste terms (mouthfeel, i.e. astringency, and taste modalities, i.e. sweet, bitter and sour) (Table 7). The descriptors are also arranged into levels, where the first tier represents general characteristics of the product, and the second tier represents more specific descriptive characteristics. Reference standards chosen to represent the unique honeybush attributes are readily available foodstuffs (Noble *et al*., 1987). These reference standards serve to facilitate improved communication between different role players in the industry.

A sensory wheel is a simplified graphical representation of a sensory lexicon (Fig. 14) (Aparicio *et al*., 1996; Theron, 2012). The two classes of attributes (positive and negative) are located on the outer tier, generic terms used to describe general characteristics of the product in the middle tier, and more specific attributes in the inner tier. Since the honeybush sensory lexicon and wheel were developed using tea from six *Cyclopia* species, they are considered generic tools for honeybush and can be used in this study to develop a species-specific sensory profile for *C. intermedia*. Such tools will be valuable for monitoring and quantifying sensory changes during processing and subsequently distinguish the optimum fermentation conditions for *C. intermedia*. Additionally, it can be used for monitoring the consistency of quality of honeybush tea.

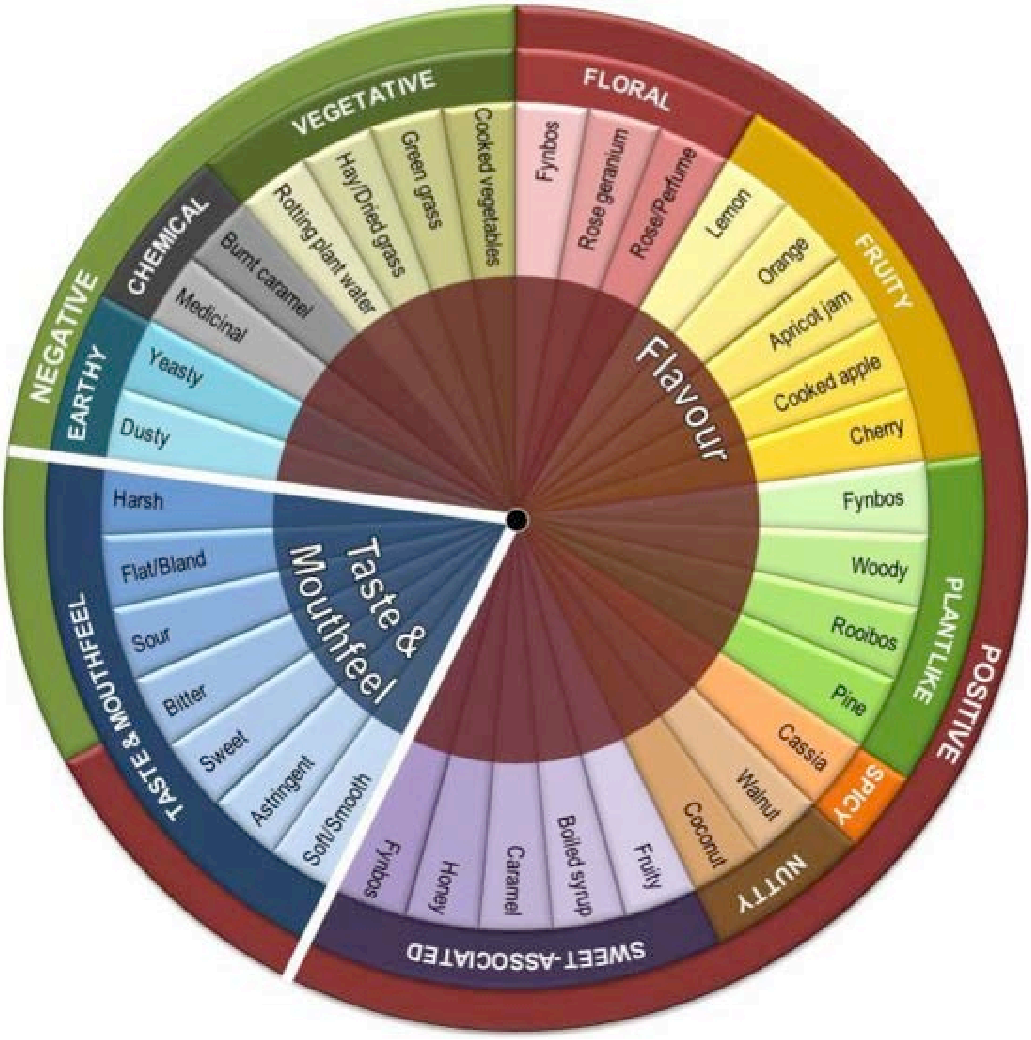



**Figure 14** Honeybush tea sensory wheel comprising 28 flavour and 7 taste and mouthfeel terms that describes the sensory attributes of infusions from six *Cyclopia* species (Theron, 2012).

**Table 7** Sensory lexicon describing flavour and mouthfeel characteristics of honeybush tea analysed by descriptive sensory analysis, compiled by Theron (2012).

| 1st Tier Attribute | 2nd Tier attribute | Definition | Reference Standard |
|---|---|---|---|
| **FLORAL** | 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** | 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%) |
| **PLANT-LIKE** | Plant-like* | 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-ASSOCIATED** | Fruity-sweet | Sweet aromatic reminiscent of non-specific fruity 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-sweet* | Aroma note reminiscent of the fynbos plant | Honeybush tea prepared from *C. intermedia* (3 g/100 mL) |
| **SPICY** | Cassia/Cinnamon | The sweet woody spicy aromatic of ground cinnamon/cassia bark | Soak cinnamon/cassia bark in water overnight[a] |
| **NUTTY** | Walnuts | Aroma note associated with fresh (not rancid) walnuts | Freshly chopped walnuts[c] |
| | Coconut | Aromatic associated with desiccated coconut | Desiccated coconut |
| **NEGATIVE** | 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 characteristics 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 & 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] |

* Adequate reference standards have not yet been found for some sensory attributes, i.e. fynbos-floral, plant-like, woody and fynbos-sweet, thus honeybush tea prepared from specific *Cyclopia* species are recommended for these attributes. All reference standards were prepared using distilled water. [a] Civille and Lyon (1996), [b] Koch (2011), [c] Heisserer and Chambers (1993), [d] Lee and Chambers (2007), [e] Galálan-Soldeville *et al*., (2005), [f] Preston *et al*., (2008), [g] ISO 5496:1992.

## 6. CONCLUSION

Honeybush tea is a caffeine-free, traditional South African herbal beverage that has low tannin content. It contains a host of polyphenolic compounds that are not only responsible for its supposed health-promoting properties, but contribute to its pleasant taste and mouthfeel properties. The dramatic growth in popularity of honeybush tea both locally and internationally is owed to these properties. The increasing international demand for honeybush tea requires a consistent supply of high quality product. In view of limited supply, it is thus of utmost importance that optimum quality of the available plant material is realised and loss due to poor quality limited.

The quality of honeybush tea available on the market varies widely due to lack of standardised and optimised processes. With the commercialisation of more *Cyclopia* species and the expansion of the number of processing facilities, even greater variation can be expected. The quantitative and qualitative differences in polyphenol composition and VOCs between *Cyclopia* species contribute to their different sensory profiles. These compositional differences underpin the need for different processing ("fermentation") conditions for each species to create species-specific optimum sensory profiles. The high temperature (bio)chemical oxidation process, known as fermentation, that is integral for the development of the sweet-associated, floral, honey-like characteristics of honeybush tea requires further investigation to arrive at optimum conditions for each species. Although these conditions were previously optimised for *C. intermedia,* the need to shorten fermentation time for more cost effective production, differences in factory management, experience of processors and type of processing equipment used, impact on quality. The current study will, therefore, revisit the fermentation parameters of *C. intermedia*, building on the sensory platform created by Theron (2012) to improve quality and product consistency.

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# CHAPTER 3

# CHARACTERISING THE SENSORY PROFILE OF *CYCLOPIA INTERMEDIA* AND DEVELOPMENT OF AN ELEMENTARY GRADING SYSTEM

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## ABSTRACT

Differences exist between the sensory profiles of *Cyclopia* species used for the production of honeybush tea, necessitating the need to descriptively analyse a large variety of *Cyclopia intermedia* samples to identify and quantify the sensory aspects of this species. The characteristic sensory profile of *C. intermedia* can be described as sweet tasting and slightly astringent with a combination of "fynbos-floral", "fynbos-sweet", "fruity" (specifically "apricot jam", "cooked apple", "raisin" and "lemon/lemon grass"), "woody", "caramel/vanilla" and "honey-like" aromas. The flavour can be described as distinctly "fynbos-floral", "fynbos-sweet" and "woody", including hints of "lemon/lemon grass" and "hay/dried grass". These sensory attributes were compiled into a sensory lexicon comprised of 11 flavour, 3 taste and 1 mouthfeel descriptors. Additionally, sensory wheels were created as simple graphical representations of the characteristic aroma, flavour, taste and mouthfeel of *C. intermedia*. A basic grading system was also proposed that categorised the samples into "good", "average" and "poor" sensory quality. "Good" quality samples associated with positive attributes such as those responsible for the characteristic *C. intermedia* profile, whereas the "poor" quality samples associated with negative attributes descriptive of under- and over-fermented *C. intermedia*, while "average" samples lay between these categories with a combination of positive and negative attributes. Physicochemical parameters (soluble solids (SS) content, absorbance as a measure of colour, and turbidity) were evaluated as a rapid means to indicate sensory quality. The SS content, absorbance and turbidity of *C. intermedia* infusions correlated strongly with "poor" sensory quality (representing both under- and over-fermented tea), signifying their usefulness as indicators to monitor the fermentation process. These quality control tools will find invaluable application in this study to optimise the processing conditions for *C. intermedia*.

## 1. INTRODUCTION

Globally consumers are increasingly health-conscious leading to greater appreciation for natural products, including herbal teas (Payne *et al*., 2014). As a result, the market for honeybush tea, produced from a number of *Cyclopia* species, has seen rapid growth over the past 20 years with demand currently exceeding supply (Joubert *et al.,* 2011). Additionally, it is unique to South Africa, is mostly organically grown and contains a wealth of polyphenolic compounds that can be used in value-added food products, medicinal products and cosmetics (Joubert *et al*., 2011), thus adding to the commercial viability of honeybush tea. The South African Honeybush Tea Association identified a reliable supply of raw and processed product, as well as reliable standards and consistent product quality, are some of the requirements for growth of the industry (SAHTA, 2014).

Despite the positive momentum of the honeybush industry, both locally and internationally, it has been plagued with inconsistent product quality and limited availability, which severely impacts on the reputation of this relatively new and fast developing industry on the international market, in particular (M. Bergh, Rooibos Ltd, Clanwilliam, South Africa, 2013, personal communication), and restricts the expansion of product development (Joubert *et al*., 2011). These quality inconsistencies are generally due to a lack of standardised processing conditions for the respective *Cyclopia* species used for honeybush production. Optimising processing conditions will thus ensure the production of tea with consistently good quality and limit losses due to poor quality. However, before processing conditions can be addressed and optimised, the distinctive sensory profiles of the species of interest need to be defined to establish a platform to determine the effect of different processing conditions on their sensory quality. The food industry has made extensive use of sensory lexicons and wheels to describe the sensory attributes of food and beverage products (Drake & Civille, 2002). These tools are employed to standardise the sensory terminology used to define and discuss a product and have been reported to facilitate communication between different role players in the industry, as well as aid in the evaluation of honeybush tea to compare and monitor the quality and consistency of the product.

Recently, progress has been made in this regard with the development of a generic honeybush sensory wheel based on the sensory attributes of six *Cyclopia* species (Theron *et al*., 2014). An important outcome of the latter study was that the species share many of the aroma and flavour attributes, yet distinct differences in their sensory profiles were evident, which merit species-specific sensory wheels.

Exports currently consist mainly of *C. intermedia*, underlying the commercial importance of this species. It is thus essential that the industry have the ability to sustain production of a high quality herbal tea of this species. For this reason the present study focused on *C. intermedia*, in particular the characterisation of its sensory profile, including sensory attributes foreign and detrimental to the profile, i.e. attributes that would impact negatively on the overall sensory quality. As there is currently no quality grading system in place for the evaluation of honeybush tea, this study also focused on developing an elementary grading system using the species-specific sensory profile as platform. Grading systems are used to enable standardisation and commercialisation of a food product by improving control over its overall quality and thereby increasing consumer satisfaction (Feria-Morales, 2002). The elementary grading system for *C. intermedia* was developed by identifying, defining and measuring quality parameters, i.e. the intensity of sensory attributes, of a wide range of *C. intermedia* samples representing varying quality. Descriptive sensory analysis was conducted as a scientifically validated method to identify and quantify the sensory aspects of *C. intermedia*, while parameters closely correlated to the way that consumers perceive product quality, i.e. "infusion strength" (soluble solids content), colour and turbidity of infusions, were measured to identify physicochemical parameters that could be used to predict product quality quickly and effectively.

## 2. MATERIALS AND METHODS

### 2.1. Commercial *C. intermedia* samples

A total of 54 fermented honeybush tea samples, specifically produced from the species *C. intermedia*, were sourced from various commercial honeybush tea processors, as well as from farm stalls and supermarkets throughout the Western and Eastern Cape provinces of South Africa. A complete list of the set of samples is provided in Addendum A. The samples differed in terms of the geographical growth area of the plant material, and whether it was cultivated or wild harvested, harvesting date, processing methods and conditions, and quality in order to capture potential sensory variation and a wide range of sensory attributes, including attributes associated with poor quality. The samples were stored in labelled and sealed glass jars (Consol, Stellenbosch, South Africa) at room temperature during the analysis period and moved into cold storage (4°C) thereafter.

### 2.2. Preparation of infusions

The infusions were prepared as described by Koch (2011) and Theron (2012) by pouring 1 L freshly boiled distilled water on 12.5 g tea leaves in a measuring jug to infuse for 5 min, thereafter it was decanted through a tea strainer into a 1 L stainless steel thermos flask (Woolworths, South Africa). Approximately 100 mL of each infusion was served in white porcelain mugs. The jug and mugs were covered with aluminium foil and plastic lids, respectively, to prevent evaporation and loss of volatile compounds. Steps taken to maintain the temperature of the infusions during preparation included preheating of the mugs in an industrial oven (Hobart CSD-UC 1012, France) set at 70°C and preheating the flasks with boiling water. Temperature was maintained at 65°C during sensory analysis by placing the mugs containing the infusions in scientific water baths (SMC, Cape Town, South Africa), regulated at 65°C. Photographs illustrating these procedures are provided in Addendum B.

### 2.3. Descriptive sensory analysis

#### *2.3.1. Sensory panel*

In this study, descriptive sensory analysis (Koch *et al*., 2012) was conducted by nine female panellists who were selected based on experience, availability and interest in the topic. They all had extensive experience in descriptive sensory analysis of a wide range of food products, including rooibos and honeybush teas. The panellists had previously been screened for their ability to discriminate between similar samples, rate products for intensity and identify tastes and aromas, as advised by Drake and Civille (2002).

### *2.3.2. Panellist training*

The panellists had previously undergone extensive training, using the consensus method (Lawless & Heymann, 2010) to identify and describe flavours, aromas and mouthfeel of honeybush infusions (Theron *et al*., 2014). Brief training was done to familiarise the panellists with the attributes and taints of the specific samples analysed and to calibrate them in terms of the range of intensities. Initially, the panel was briefed on the objectives of the study, as well as instructed on the analysis procedure. Training took place during ten half-hour sessions over a period of five days, with 7 samples presented per session. All the samples were presented to the panellists during training to familiarise them with the products, generate a list of terms describing the sensory characteristics of the infusions, and introduce a tentative score sheet to orientate the panellists to the test protocol.

The aroma of the infusions, defined as the fragrance perceived through orthonasal analysis (Ross, 2009), was analysed first by swirling the cup several times before removing the plastic lid and sniffing. Then, the panellists sucked a mouthful of infusion into the mouth from a round tablespoon to evaluate the flavour (retronasal perception), taste (basic taste modalities) and mouthfeel (sensation perceived in the oral cavity after drinking a sip of tea) (Ross, 2009). Distilled water and unsalted water biscuits (Carr, Carlisle, UK) were presented between samples as palate cleansers.

A total of 68 aroma and 51 flavour, taste and mouthfeel descriptors, generated by Theron *et al*. (2014) for honeybush tea, based on the sensory profiles of six *Cyclopia* species including *C. intermedia* (Addendum C), were used as a basis to generate a list of descriptors that best described the aroma, flavour, taste and mouthfeel of the *C. intermedia* samples in this study. The list was generated during an open discussion led by the panel leader. Honeybush tea samples produced from other *Cyclopia* species and exhibiting a high intensity of a specific attribute was used as reference standards during the training phase to familiarise the panellists with the sensory attributes in question. During further discussion redundant descriptors, as well as those present infrequently, were removed to simplify the list to 28 aroma and 27 flavour descriptors, 3 taste modalities and 1 mouthfeel descriptor that were relevant, unambiguous, and non-redundant (Table 1). Included in the table are the definitions of the attributes.

**Table 1** Complete list of descriptors and definitions (lexicon) used for descriptive analysis generated during the sensory training.

| Attribute descriptions | Definitions |
|---|---|
| **Floral** | |
| Fynbos-floral | Very sweet floral aroma note associated with the flowers of fynbos vegetation |
| Rose geranium | Floral aroma note associated with the rose geranium plant |
| Rose/Perfume | Floral aroma note associated with rose petals |
| **Fruity** | |
| Lemon | Aroma note associated with the general impression of fresh lemons |
| Apricot jam | Sweet, sour fruity aroma note reminiscent of apricot jam |
| Apple, cooked | The flat, slightly sour aroma note reminiscent of cooked apples |
| Raisin | Sweet aroma note reminiscent of hanepoot raisin |
| **Woody** | |
| Earthy | Aroma note associated with moist soil, mushrooms or a forest floor with pepper notes |
| Plant-like | Aroma note associated with green fynbos vegetation |
| Woody | Aroma note associated with dry bushes, stems and twigs of the fynbos vegetation |
| Pine | Aroma note reminiscent of pine needles |
| **Sweet-associated** | |
| Fruity-sweet | Sweet/sour aroma note reminiscent of non-specific fruit |
| Caramel/Vanilla | Sweet aroma note characteristic of molten sugar or caramel pudding with vanilla notes |
| Honey | Aroma note associated with the sweet fragrance of fynbos honey |
| Fynbos-sweet | Aroma note reminiscent of the fynbos plant |
| **Spice** | |
| Sweet spice | Sweet, woody and spicy aroma note |
| **Nutty** | |
| Walnuts | Aroma note associated with fresh walnuts |
| Almond/Marzipan | Aroma note associated with chopped almonds and marzipan confection |
| **Taints** | |
| Dusty | Earthy aroma note associated with wet hessian, wet cardboard, or a dusty road |
| Yeasty | Aroma note associated with active yeast culture |
| Medicinal | Aroma note characteristic of Band-Aid, disinfectant-like (phenolic) |
| Hay/Dried grass | Slightly sweet aroma note associated with dried grass or hay |
| Green grass | Aroma note associated with freshly cut green grass |
| Cooked vegetables | Aroma note associated with canned/cooked vegetables |
| Burnt caramel | Aroma note associated with blackened/acrid sugar solution |
| Sour | Sour aroma note characteristic of vinegar |
| Smokey | Smokey aroma note associated with burning hay/grass or tobacco |
| Wet fur/Farm animals | Musty and sour aroma note associated with wet dog fur, sheared sheep, or barnyards |
| **Taste & mouthfeel** | |
| Sweet | Basic taste sensation of which sucrose is typical |
| Sour | Basic taste sensation of which citric acid is typical |
| Bitter | Basic taste sensation of which caffeine is typical |
| Astringent | Dry, puckering sensation on the tongue and other mouth surfaces |

### *2.3.3. Analysis of C. intermedia infusions*

The quantitative aspect of the descriptive sensory analysis of *C. intermedia* infusions entailed expressing the intensity of the attributes or the degree to which attributes were present in each sample by assigning a value on a scale. Attribute intensities were digitally scored on an unstructured line scale (0 - 100) with verbal anchors on each end, using Compusense® *five* (Compusense, Guelph, Canada). The score sheet is presented in Addendum D. Six samples were evaluated per day with each sample analysed in triplicate during three consecutive sessions. The order of presentation was randomised and samples were labelled with different three-digit codes to ensure blind tasting. Panellists cleansed their palates with water and unsalted fat free biscuits (Carr, Carlisle, UK) between samples and were given a 10 min break between each session to reduce panel fatigue. All evaluations were conducted in a light- and temperature-controlled room (22°C).

### *2.3.4. Development of a sensory lexicon and wheel for C. intermedia*

A species-specific lexicon for *C. intermedia* was created by selecting relevant descriptors from the general honeybush tea lexicon, generated by Theron *et al*. (2014), as well as descriptors unique to *C. intermedia* identified during panel training. This lexicon comprised of 11 aroma and flavour descriptors, 3 basic taste modalities and 1 mouthfeel descriptor that were relevant, unambiguous, non-redundant and non-hedonic, along with a definition and reference standard for each term.

Additionally, sensory wheels were created to graphically represent the characteristic aroma and flavour attributes of *C. intermedia* infusions by weighting the descriptive terms according to their mean intensity scores from the descriptive sensory analyses conducted during this study.

## 2.4. Physicochemical parameters

### *2.4.1. Soluble solids (SS) content*

The hot water soluble solids content of the *C. intermedia* infusions, prepared for sensory analysis, was determined gravimetrically according to Joubert (1988). Briefly, the infusions were filtered through Whatman No. 4 filter paper and triplicate aliquots (20 mL) were evaporated to dryness in pre-weighed nickel moisture dishes on a steam bath (Merck Chemicals (Pty) Ltd, Belville, South Africa). Final drying took place at 100°C for 1 h in a laboratory oven. Thereafter the moisture dishes were allowed to cool to room temperature in a desiccator before re-weighing. The soluble solids content of the infusions was expressed in g/100 mL infusion.

### *2.4.2. Absorbance as a measure of colour*

Absorbance values of each infusion, collected from 370 to 510 nm at 10 nm intervals, were determined using a Biotek Synergy HT microplate reader (BioTek Instruments, Winooski, USA). The absorbance of a 200 uL aliquot of each infusion (undiluted) was measured in triplicate, using a clear 96-well flat-bottom microplate (Greiner Bio-one; Lasec (Pty) Ltd, Cape Town, South Africa). The integral of the absorbance values across the wavelength range, i.e. Area Under the Curve (AUC), reflecting the "total colour" of the sample, was obtained using Gen5 Secure software (Biotek Instruments, Winooski, USA).

### *2.4.3. Turbidity*

The turbidity of a 25 mL aliquot of each sample was measured in triplicate using a Thermo Scientific Orion AQUAfast AQ3010 Turbidity Meter (Labotec (Pty) Ltd, Cape Town, South Africa), autoranging from 0 to 1000 nephelometric turbidity units (NTU). The AQ3010 meter was calibrated with four EPA-approved SDVB (Styrene-divenylbenzene) primary standards (0.02, 20.0, 100 and 800 NTU) prior to analysing the infusions. The infrared LED light source eliminates temperature effects, is consistent, and provides better results for coloured samples compared to tungsten meters (Thermo Fisher Scientific, 2009).

## 2.5. Statistical procedures

### *2.5.1. Univariate analysis of data*

A randomised complete block design was used for descriptive analysis. The data collected, representing scores for individual sensory attributes of each sample by all the assessors, were considered continuous interval scale data, meaning that the differences between values were considered meaningful.

PanelCheck® software (Version 4.1.0; Nofima, Norway) was used to preliminarily monitor the performance of the panel during the descriptive sensory analysis period, i.e. to check the reliability of the panel. It also serves as a tool to continuously check the quality of the sensory data set.

All the data were subjected to test-retest analysis of variance (ANOVA), using SAS® software (Version 9.2; SAS® Institute, Cary, NC, USA) to test the reliability of the panel, i.e. temporal stability (Judge*Replication interaction) and internal consistency (Judge*Level interaction). The Shapiro-Wilk test was used to test for normality of the residuals (Shapiro & Wilk, 1965), i.e. deviation between the scores for the three replicates of a sample by a particular judge. The outliers were identified and removed from non-normal data ($p \leq 0.05$) until the data were normally distributed (Glass *et al*., 1972). Thereafter, the data were subjected to analysis of variance (ANOVA) to determine significant differences between the mean attribute scores. The student's t-tests were performed to calculate the least significant difference (LSD) at the 95% confidence/5% significance level where the F-test indicated significant differences.

### *2.5.2. Multivariate analysis of data*

Multivariate statistical analysis was performed using XLSTAT software (Version 2014.01.02, Addinsoft, Paris, France). Principal Component Analysis (PCA), using the correlation matrix, was conducted to illustrate the positioning of the samples with respect to each other and their characteristic attributes and also to investigate sample patterns (Mirarefi *et al*., 2004). The same multivariate analysis procedure was followed to evaluate the relationship between descriptive and physicochemical analyses.

# 3. RESULTS AND DISCUSSION

## 3.1. Profiling *C. intermedia* and development of a sensory lexicon and wheel

Sensory wheels and lexicons have seen extensive use in the food industry for quality control, research and development and marketing. Application during processing operations is useful to compare product quality between different production sites, as well as to monitor production processes. These tools also aid accurate and clear communication between role players in industry, such as the research and development, and production and marketing departments, to produce consistently good quality products. For the development of a sensory lexicon and wheel for a specific product, the sensory attributes of the product need to be profiled. Following this procedure, Theron *et al*. (2014) profiled the sensory characteristics of the infusions of several *Cyclopia* species, demonstrating three distinct groups according to their sensory properties. According to the groupings, *C. intermedia* had similar sensory properties to *C. sessiliflora* and *C. genistoides,* however, subtle differences existed between the species within a group. Prominent to these species were "fynbos-floral", "fynbos-sweet" and "plant-like" attributes, but many other aroma and flavour notes were also observed and incorporated into a generic sensory wheel and lexicon for honeybush. Given the generic nature of the honeybush sensory wheel, subtle differences between species could not be accommodated. Another shortcoming of this wheel was that all sensory attributes are afforded the same prominence, thus giving the user no indication of the importance of a specific sensory attribute.

In order to efficiently generate a sensory profile it was necessary to reduce the number of sensory descriptors identified by panellists during training in order to eliminate redundancies and disregard attributes that were perceived in only a limited number of samples. Using the aroma, flavour, taste and mouthfeel descriptors generated by Theron *et al*. (2014) (Addendum C) to describe honeybush tea as a basis, a list of descriptors was generated to describe *C. intermedia* specifically. Box plots, illustrating the mean, minimum, maximum and range of intensity scores of attributes, graphically summarise the distribution and variation of the data generated from descriptive sensory analysis of *C. intermedia* infusions (Figs. 1, 2 & 3). Certain attributes, particularly "fynbos-floral", "woody" and "fynbos-sweet", generally had much higher intensities (mean > 30) than the more subtle, specific fruity and floral attributes such as "rose geranium", "rose perfume", "lemon/lemon grass" and "cooked apple" (intensities ranged between 0 and 15 on a 100-point scale). Additionally, "fynbos-floral", "woody" and "fynbos-sweet" had minimum intensities of > 25, which highlight their prominence in an infusion.

The intensity scores for the aroma and flavour attributes followed similar trends, however, flavour attributes were perceived at lower intensities. Interestingly, our diverse flavour perception comes from volatile airborne molecules, released from foods in the mouth that pass up into the nasal cavity retronasally and are sensed by the olfactory receptors. Thus, the vast diversity of food flavours, whether sniffed through the nose or arising from odours present in the mouth, is mediated by smell (Lawless & Heymann, 2010). This explains the correlation between the aroma (orthonosal) and flavour (retronasal) perception. Additionally, Aubrey *et al*. (1999) demonstrated that some attributes (specifically fruity notes) are evaluated more effectively by the nose, which explains the reduced retronasal perception of attributes present in *C. intermedia* (Figs. 1 & 2). In terms of negative attributes, only “hay/dried grass” had relatively high intensity scores (mean ≤ 10), however, the abundance of outliers (dots above the whiskers) for “dusty”, “medicinal”, “green grass”, “cooked vegetables”, “smokey” and “wet fur/farm animals” indicate that they are important to describe tainted *C. intermedia* samples and were prominent with relative high intensities in some samples. The intensities of “woody” and “hay/dried grass” would be decisive in their classification as negative or positive. Looking at the distribution of intensity ratings for “woody” across the sample set (Fig. 4a), it can be postulated that an intensity rating of >40 would be considered negative due to the overpowering nature of this attribute. On the other hand, the distribution of “hay/dried grass” intensity ratings across the sample set is more random (Fig. 4b), thus insight into its positive/negative contribution is not clear.

Graphs that gave an indication of the perceived intensity of the sensory attributes in an infusion, as well as their prevalence amongst the sample set, were also used to scrutinise the relative importance of the sensory attributes of *C. intermedia* infusions (Figs. 5 & 6). The positive attributes “woody”, “fynbos-floral”, “fynbos-sweet” and “fruity-sweet” were present at the highest intensities and perceived in all the samples, thus clearly indicating them as the most prominent positive aroma attributes (Fig. 5). “Apricot jam” and “caramel/vanilla” aromas were perceived in more the 50% of the samples at intensities > 5 (Fig. 5), and can thus also be considered important. Other minor positive aroma attributes were “honey”, “raisin”, “cooked apple”, “lemon/lemon grass” and “rose geranium”. These attributes occurred in more than 20% of samples at low intensities (Fig. 5). Attributes that occurred in less than 20% of samples were excluded. These attributes also had low average intensities (< 5 out of 100). In terms of negative aroma attributes, “hay/dried grass” is the only prevalent negative aroma perceived in more the 90% of the samples, although its average intensity is relatively low (< 10 out of 100) (Fig. 5).

Similarly to the aromas, “fynbos-floral”, “woody” and “fynbos-sweet” flavours obtained the highest intensity scores, as well as being most prevalent (Fig. 6). “Lemon/lemon grass” flavour, at mean intensity < 5, occurred in 20% of samples. Interestingly, this was the only fruity attribute that was perceived in both aroma and flavour. Of the negative flavours, only “hay/dried grass” demonstrated to be prevalent in all the *C. intermedia* infusions (Fig. 6). Sweet taste and astringency were perceived in all the samples (Fig. 6) with minimal difference between the minimum and maximum intensity ratings (Fig. 2). This indicates that all the samples were considered slightly sweet and astringent, although some less than others. Sour taste was detected in less than 50% of the samples, however, at low intensity. Bitter taste was negligible in *C. intermedia* infusions (Fig. 6).

These attributes were considered important due to their prevalence in *C. intermedia* infusions, however, the less prevalent attributes at lower intensities should not necessarily be disregarded completely as they may contribute to the overall aroma and flavour of the tea. Conversely, attributes such as “green grass”, “cooked vegetables”, “smokey”, “sour” and “wet fur/farm animals” with low prevalence but high maximum intensity ratings were not “characteristic” of *C. intermedia* infusions, but were rather extreme cases. The “characteristic” sensory profile of the honeybush tea species, *C. intermedia*, can thus be described as sweet tasting and slightly astringent with a combination of “fynbos-floral”, “fynbos-sweet”, “fruity” (specifically “apricot jam”, “cooked apple”, “raisin” and “lemon/lemon grass”), “woody”, “caramel/vanilla” and “honey-like” aromas with a distinctly “fynbos-floral”, “fynbos-sweet” and “woody” flavour, including hints of “lemon/lemon grass” and “hay/dried grass”.

These attributes were compiled into a *C. intermedia* sensory lexicon comprising 11 flavour, 3 taste and 1 mouthfeel descriptors, along with definitions and reference standards to clarify the exact meaning of each descriptor (Table 2). Reference standards, created by Theron *et al*. (2014), were retained to maintain the integrity of the standardised terminology. This lexicon is an important quality control tool, primarily used to clarify sensory attribute terminology. It also aims to facilitate improved communication among the different role players in the industry. The original list of attributes, generated by Theron *et al*. (2014), was expanded with descriptors unique to *C. intermedia*, thus contributing to the existing honeybush lexicon.

Sensory wheels were created that graphically represent the characteristic aroma (Fig. 7a) and flavour (Fig. 8a) of *C. intermedia* infusions. The descriptive terms were assembled to form a three-tiered wheel with attributes separated into two classes (positive and negative) on the outer tier and grouped according to generic terms in the middle tier, while the specific attributes that are weighted according to their average intensity in the infusion are located in the inner tier. Thus, wider slices represent higher average perceived intensities of the attributes in the infusion and vice versa. The bar graphs accompanying the sensory wheels (Figs. 7b & 8b) represent the percentage occurrence of each of the attributes in the sample set. These sensory wheels, indicating average intensity and occurrence, incorporate the large variation within a large set of *C. intermedia* samples and could thus be considered to comprehensively describe the sensory attributes of *C. intermedia*. They may thus be useful in developing a formal grading system for *C. intermedia*, which will further facilitate precise communication between all members of the honeybush industry when applying quality control measures. Extension of the concept of species-specific sensory wheels to other *Cyclopia* species may also assist in identifying niche markets, profiling new and competitive products within the tea industry, as well as marketing endeavours. These tools will also find invaluable application in the present study to optimise processing conditions for *C. intermedia* (Chapter 4) as it will serve as a measurement tool to determine the effect of different fermentation regimes on sensory quality.

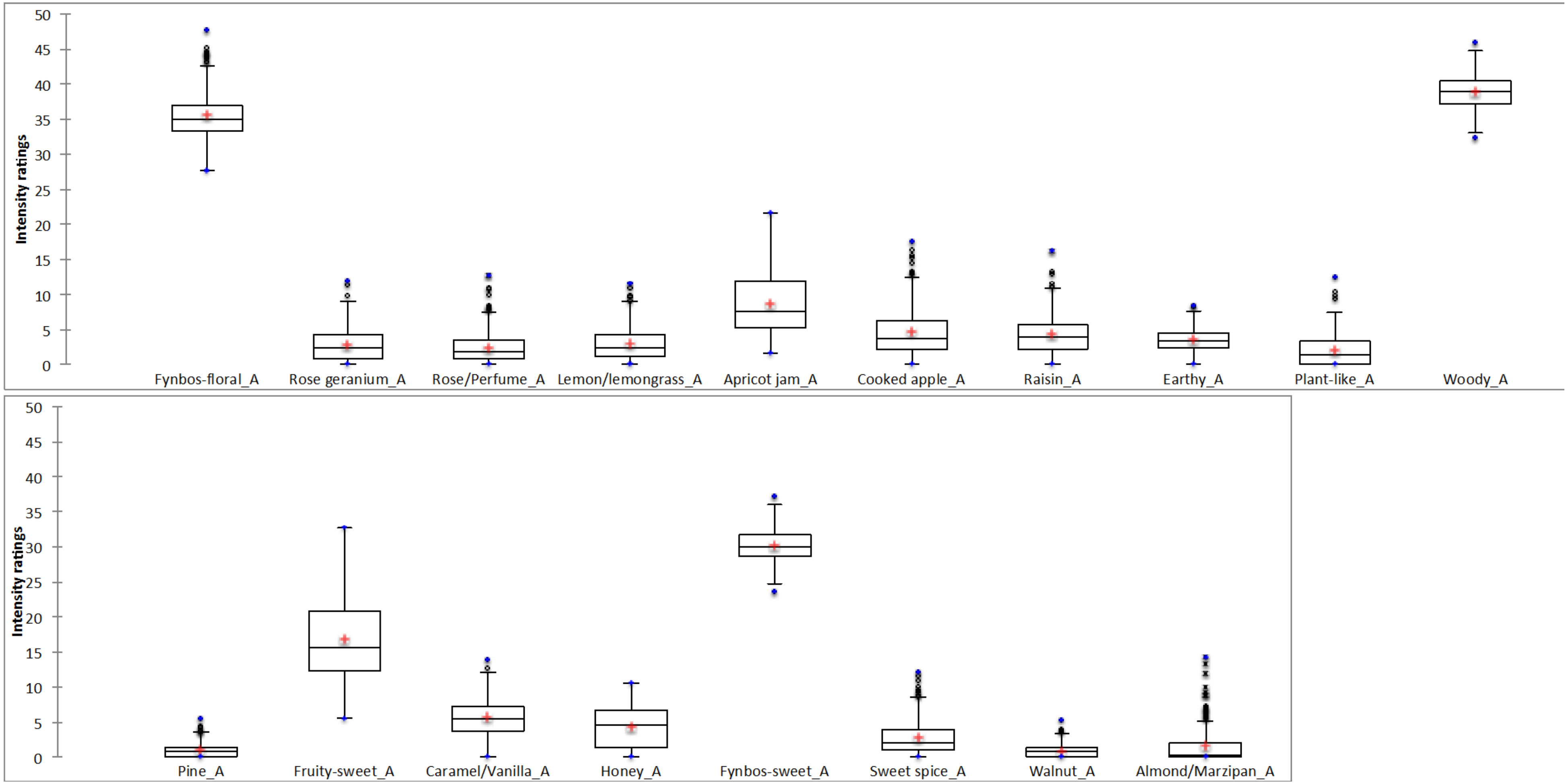



**Figure 1** Box plots illustrating the mean, minimum, maximum and range of intensity scores for the positive aroma attributes of *C. intermedia* infusions. The letters "A" after the attributes refers to aroma attributes.

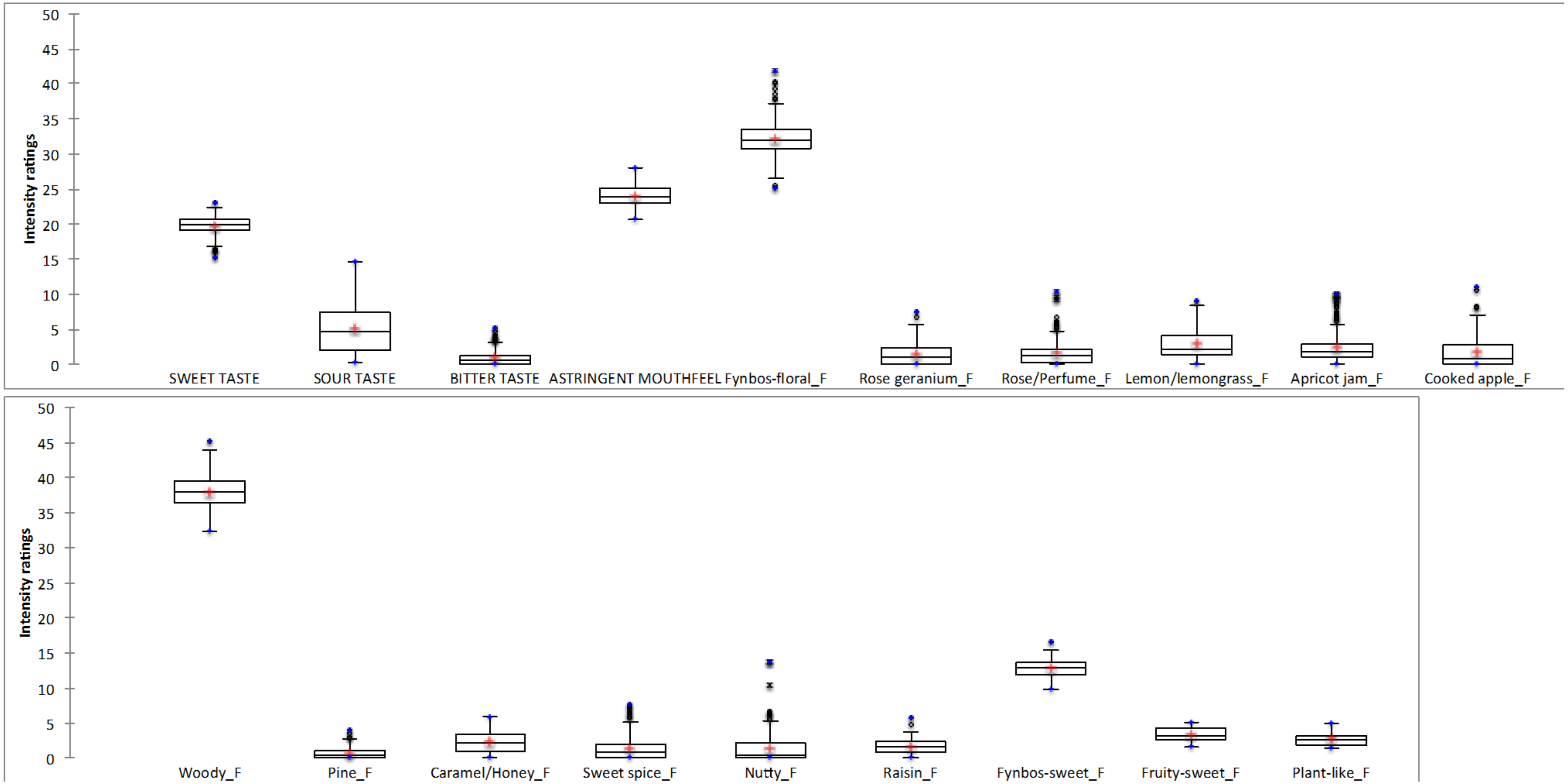



**Figure 2** Box plots illustrating the mean, minimum, maximum and range of intensity scores for the taste, mouthfeel and positive flavour attributes of *C. intermedia* infusions. The letter "F" after the attributes refers to flavour attributes.

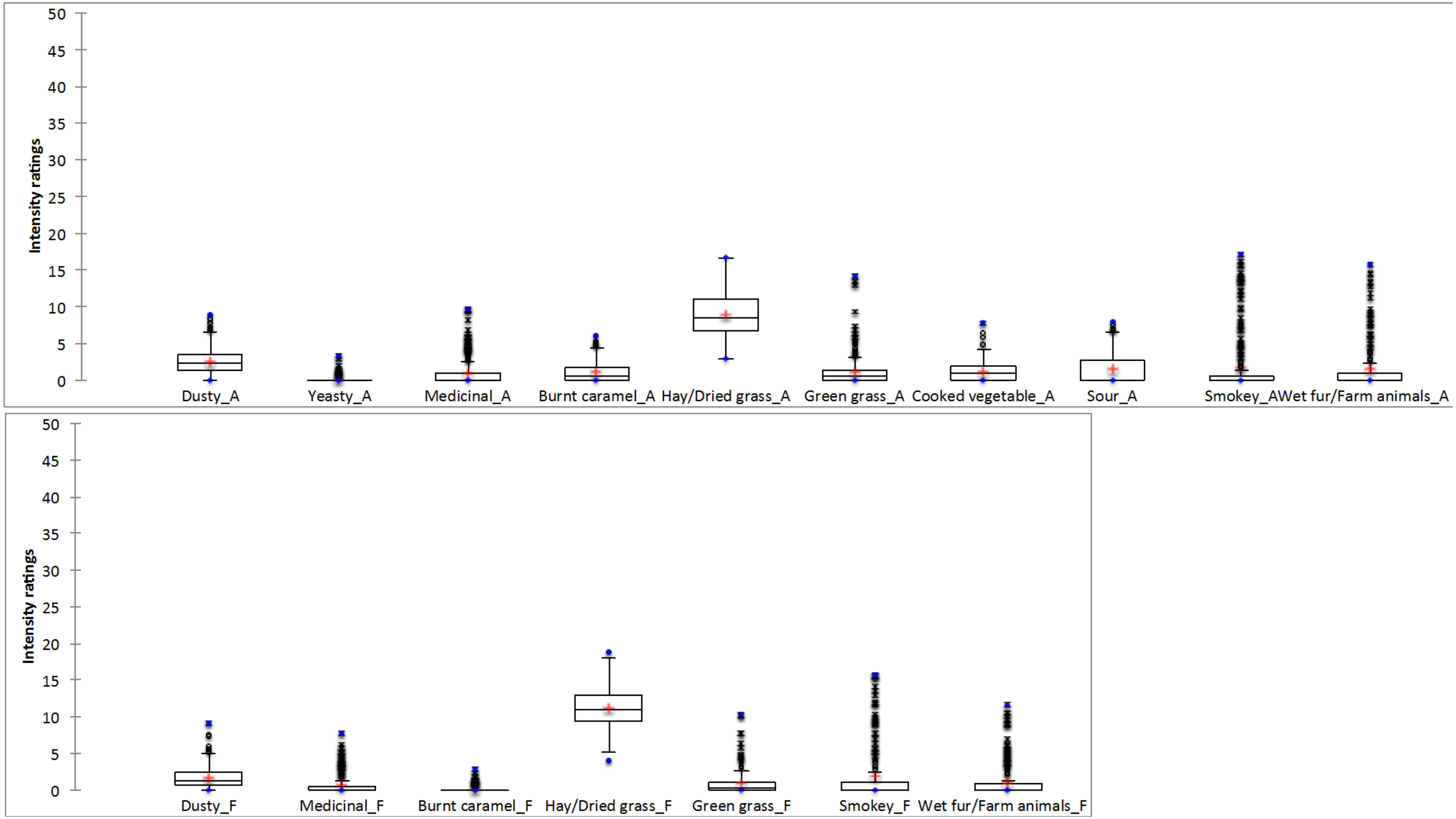



**Figure 3** Box plots illustrating the mean, minimum, maximum and range of intensity scores for the negative aroma and flavour attributes of *C. intermedia* infusions. The letters "A" and "F" after the attributes refer to aroma and flavour attributes, respectively.

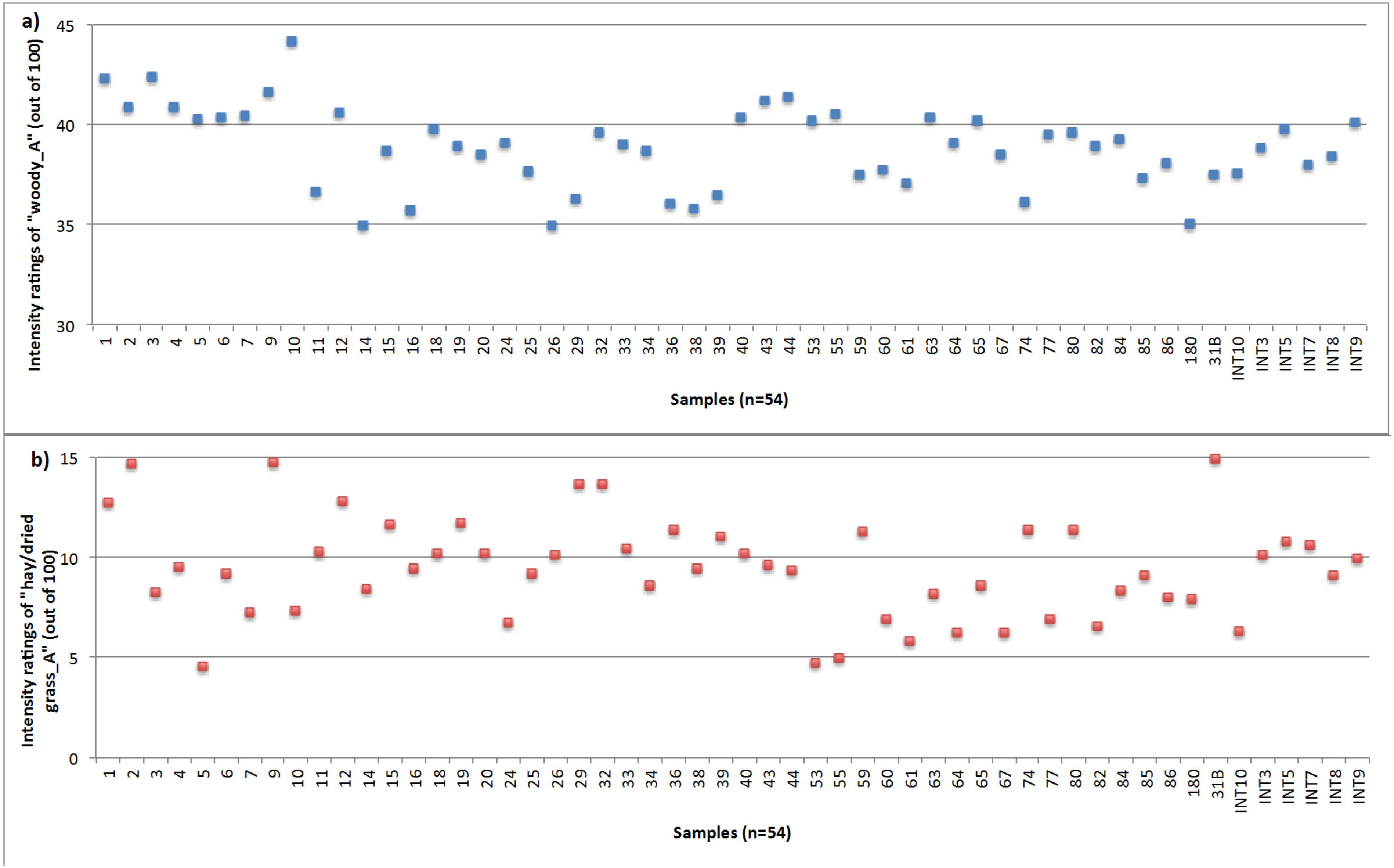



**Figure 4** Scatter plots illustrating the distribution of intensity ratings for a) “woody” and b) “hay/dried grass” aroma of individual samples.

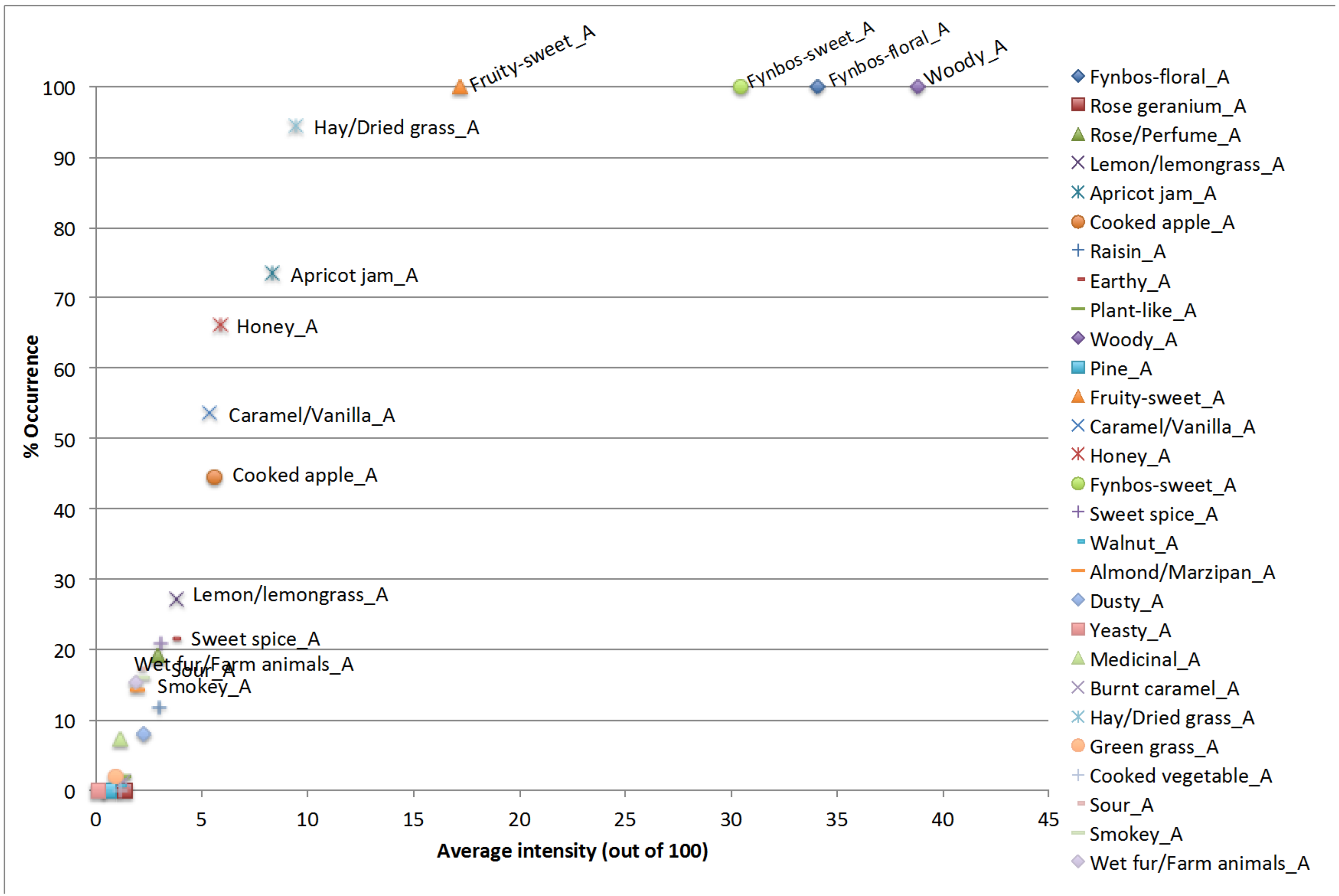



**Figure 5** Scatter plot illustrating the mean intensities of the full set of aroma attributes analysed, as well as the percentage of samples exhibiting a specific attribute. The letter "A" after the attributes refers to aroma attributes.

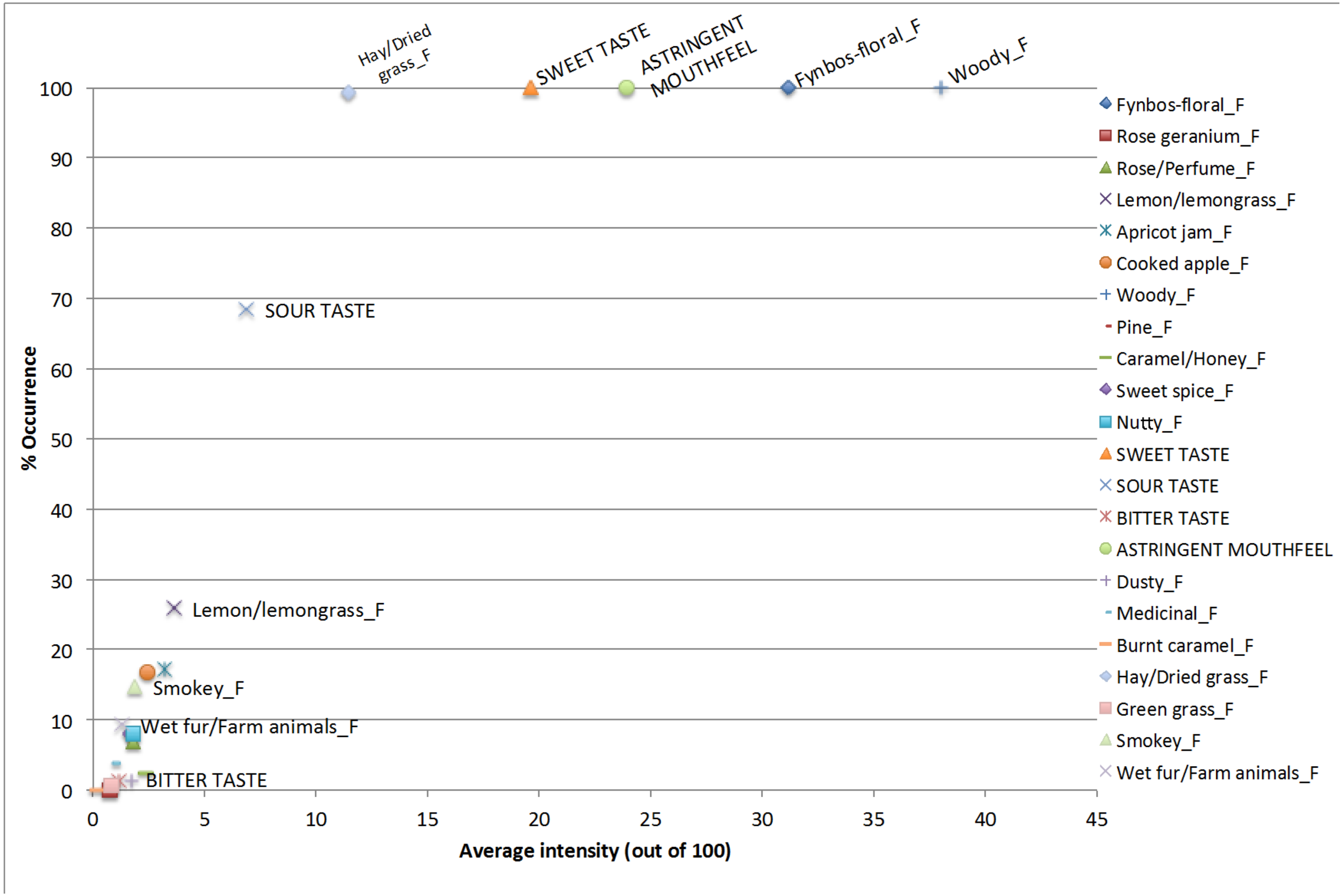


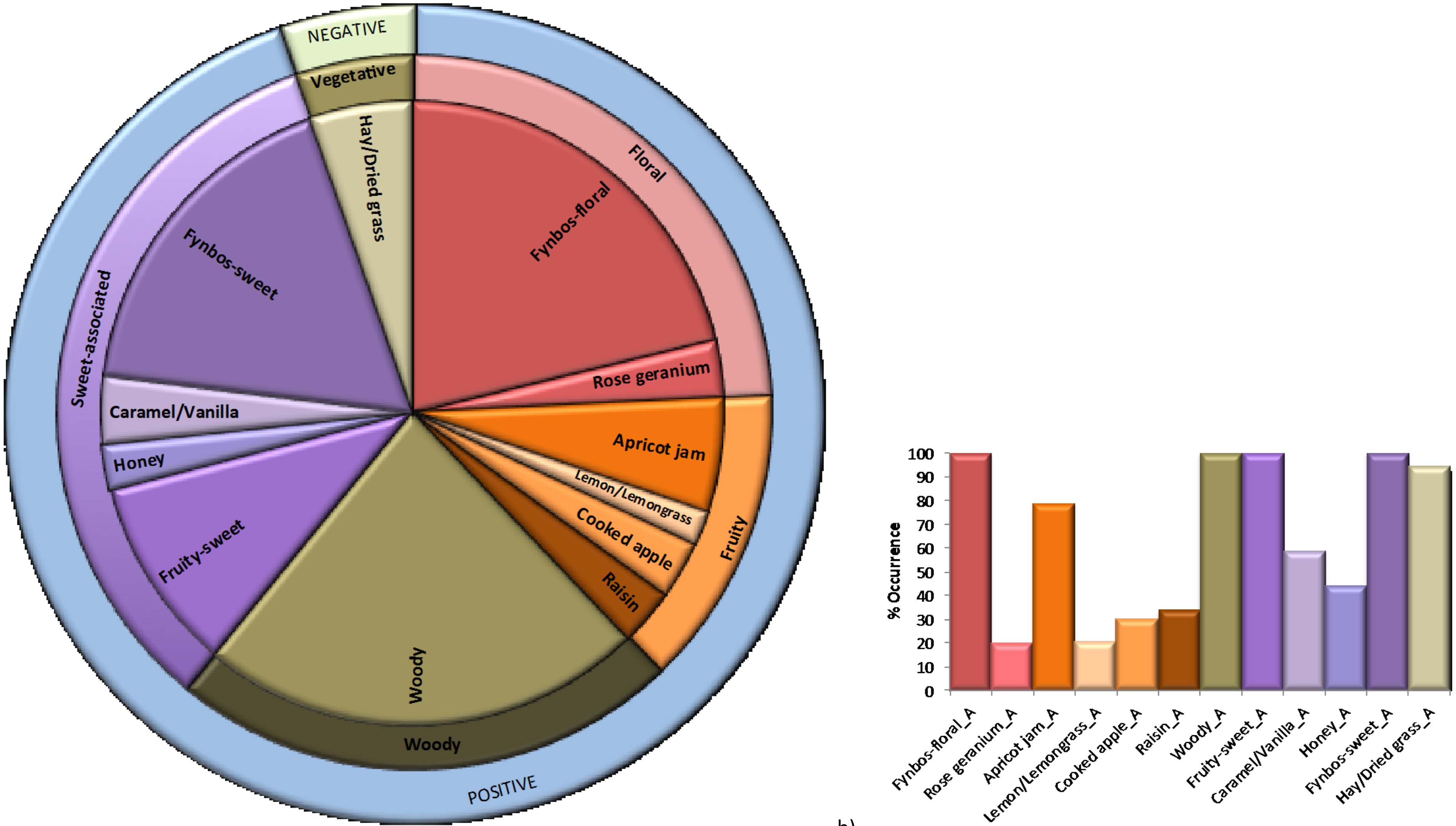

**Figure 6** Scatter plot illustrating the mean intensities of the full set of taste, mouthfeel and flavour attributes, as well as the percentage of samples exhibiting a specific attribute. The letter "F" after the attributes refers to flavour attributes.

**Table 2** Sensory lexicon describing aroma, taste and mouthfeel attributes and appropriate reference standards of *C. intermedia* infusions.

| 1st Tier Attributes | 2nd Tier Attributes | Definitions | Reference standards |
|---|---|---|---|
| **FLORAL** | Fynbos-floral* | Very sweet 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%) |
| **FRUITY** | Apricot jam | Sweet, sour fruity aroma reminiscent of apricot jam | Superfine apricot jam (15 g/100 mL hot water) |
| | Lemon/lemon grass | Aromatic associated with general impression of fresh lemons or lemon grass | Lemon juice (5%); crushed lemon grass |
| | Apple, cooked | The flat, slightly sour aroma of cooked apples | Apple puree (2.5 g/100 mL) |
| | Raisin | Sweet aroma reminiscent of hanepoot raisin | Hanepoot raisins (10 raisins in capped wine tasting glass) |
| **WOODY** | Woody* | Aromatic associated with dry bushes, stems and twigs of the fynbos vegetation | Honeybush tea prepared from *C. maculata* (3 g/100 mL) |
| **SWEET-ASSOCIATED** | Fruity-sweet | Sweet/sour aromatic reminiscent of non-specific fruit | Superfine apricot jam and strawberry jam (5 g each/100 mL hot water)[b] |
| | Caramel/Vanilla | Sweet aromatic characteristic of molten sugar or caramel pudding with occasional vanilla notes | Caramel, natural flavour (0.4%)[b] |
| | Honey | Aromatics associated with the sweet fragrance of fynbos honey | Wild flower honey, Hillcrest[b] |
| | Fynbos-sweet* | Aroma note reminiscent of the fynbos plant | Honeybush tea prepared from *C. intermedia* (3 g/100 mL) |
| **NEGATIVE** | Dusty | Earthy aromatic associated with wet hessian, wet cardboard, or a dusty road | Old/dry tree bark (*Jacaranda mimosifolia*) (1 piece/100 mL hot water, infuse for 5 min, filter)[a] |
| **TASTE & MOUTHFEEL** | Sweet taste | Fundamental taste sensation of which sucrose is typical | Sucrose (0.1%)[d] |
| | Sour taste | Fundamental taste sensation of which citric acid is typical | Citric acid (0.035%)[d] |
| | Astringent mouthfeel | The drying, puckering sensation on the tongue and other mouth surfaces | Alum solution (0.05%)[c] |

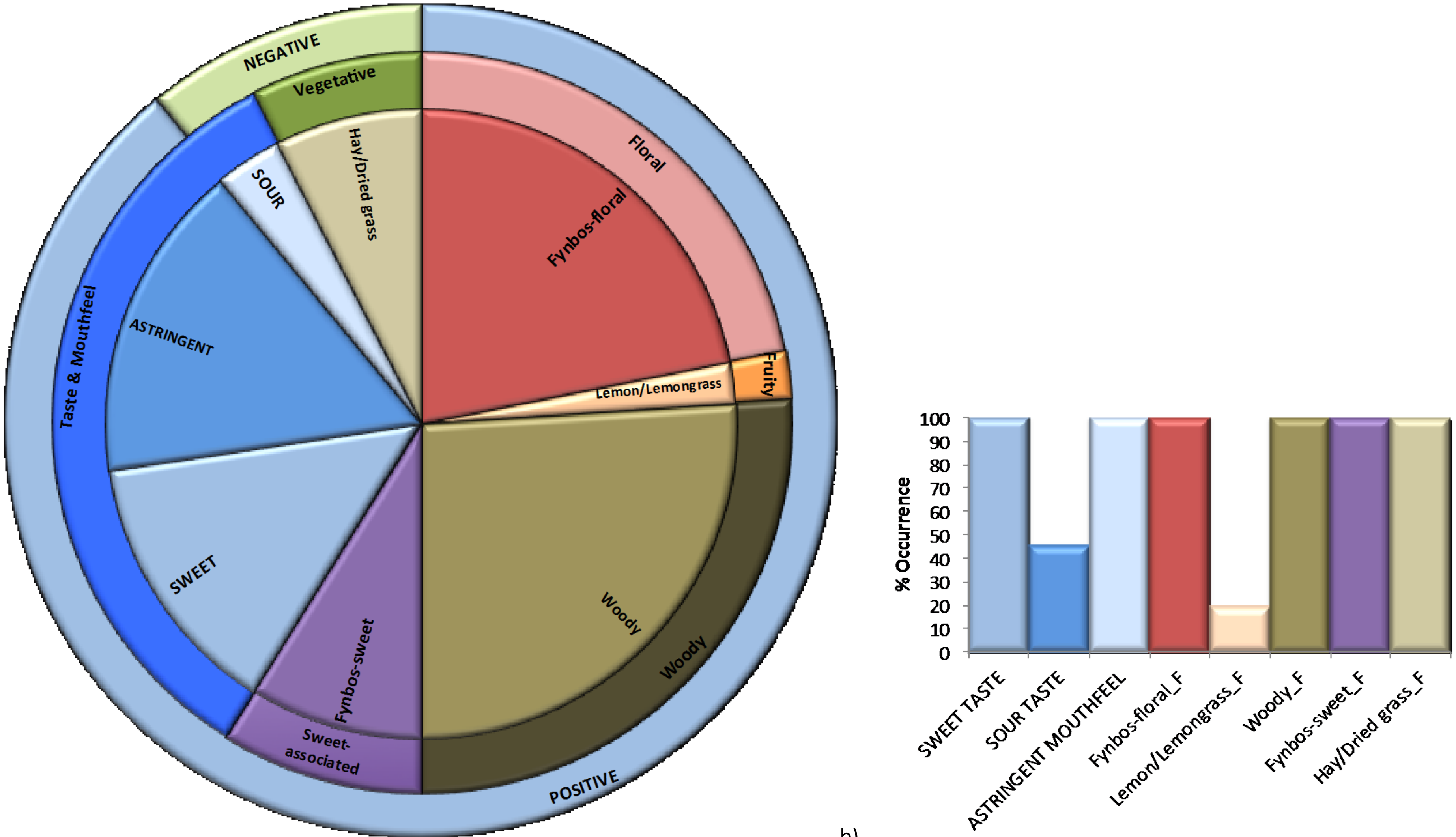
*For certain sensory attributes ("fynbos-floral", "plant-like", "woody" and "fynbos-sweet") adequate reference standards have yet to be found and honeybush tea prepared from specific *Cyclopia* species are recommended for these attributes. All reference standards were prepared using distilled water.

[a] Civille & Lyon (1996), [b] Koch (2011), [c] Galálan-Soldeville *et al.*, (2005), [d] ISO 5496:1992.




a) b)

**Figure 7**(a) *C. intermedia* sensory wheel depicting the mean intensity of the aroma attributes. Graph (b) illustrates the percentage occurrence of attributes in *C. intermedia* infusions. Attributes that occurred in less than 20% of samples were not included.




a) b)

**Figure 8**(a) *C. intermedia* sensory wheel depicting the mean intensity of the flavour, taste and mouthfeel attributes. Graph (b) illustrates the percentage occurrence of attributes in *C. intermedia* infusions.

### 3.2. Relationship between sensory attributes of *C. intermedia* infusions

Principle component analysis (PCA) is a commonly used multivariate data analysis method that is useful to visualise the relationship between the variables in a 2-dimensional space and to establish whether associations exist between attributes, i.e. aroma, flavour, taste and mouthfeel attributes.

The length of the vector from the origin of the unit circle indicates how well the variation in that attribute is being explained – the longer the vector the better the representation (Hasted, 2014). F1 and F2 explain most of the attributes adequately (63.54% variance explained), although "fynbos-floral", "fynbos-sweet" and "honey" aromas are not well represented by these components (Fig. 9a), i.e. the variation in these attributes are not well explained by the first and second principle components. At least 70% recovery of variance is recommended to explain the correlations with confidence, thus the third component (F3) was included to improve the interpretation (Fig. 9b). Although F3 only contributes 9.49% of the variance, the variation in "fynbos-floral" and "fynbos-sweet" aromas are represented better by this dimension. "Honey" aroma is not well explained by this factor either, indicating that it may not be a characteristic attribute of *C. intermedia* infusions.

The cosine of the angle between vectors joining attributes to the origin on a PCA loadings plot is approximately the correlation between the attributes. Thus, the smaller the angle between attributes the greater the correlation between them. Vectors in the same direction indicate the attributes are positively correlated, while vectors in opposite directions indicate the attributes are negatively correlated (Hasted, 2014). However, visually interpreting the correlations between the variables and between the variables and factors should be done with certain precaution because some sensory attributes may seem inaccurately highly correlated on a PCA loadings plot where the variance is not fully explained. These representations are only reliable if the sum of the variability percentages associated with the axes of the representation space is sufficiently high, i.e. 70% (XLSTAT, 2013; Hasted, 2014). Thus, it is also useful to study the correlation coefficients. The correlation coefficient is a measure of the similarity of the trends of attribute intensities, thus similar trends in attribute intensities will have high correlation coefficients. It is a measure of the relationship between the attribute intensities, thus the coefficient r-value has a magnitude and a direction (either positive or negative) (Taylor, 1990).

According to the PCA loadings plot (Fig. 9a) the corresponding aroma and flavour attributes lie closely together, which indicates that these attributes are perceived similarly on the nose and the mouth. This observation is confirmed by Pearson's correlation coefficients (Table 3) that show that there are significant ($p \leq 0.05$) and strong positive correlations ($r > 0.7$) between all of the corresponding aroma and flavour attributes. This is due to the perception of volatile airborne molecules passing into the nasal cavity from the mouth retronasally and sensed by the olfactory receptors, as explained in the previous section (Lawless & Heymann, 2010).

Most of the variance was explained by the first component (F1 = 44.91%), showing a clear separation of positive and negative attributes (Figs. 9a & b). The positioning of positive and negative attributes on opposites sides of the PCA loadings plot, specifically vectors in opposite directions, indicate that positive and negative attributes are negatively correlated (Hasted, 2014). In this case it indicated that a decrease in the intensity of the negative attributes was accompanied by an increase in the intensity of positive attributes. It is vital to establish the point where the intensities of negative attributes are at a minimum to produce a product of good sensory quality.

On the PCA loadings plot (Fig. 9a), several of the positive attributes were placed closely together, indicating a strong positive correlation. The correlation coefficients confirm that significant positive correlations exist between "fynbos-sweet" and "fynbos-floral" aromas (r = 0.828) (Table 4), "fruity-sweet" aroma and "fynbos-floral" flavour (r = 0.385) (Table 3), and "apricot jam" and "fruity-sweet" aromas (r = 0.723) (Table 3). Using the classification of Taylor (1990), a correlation coefficient of 0.36 - 0.67 shows a moderate correlation, while r > 0.68 indicates a strong correlation. "Caramel/vanilla" only correlated with one of the closely positioned attributes, i.e. "fruity-sweet" (Table 3). The inaccurate grouping of "caramel/vanilla" aroma may have arisen from a general tendency of this attribute to change in a similar way to the others over a large group of samples (Wolters & Allchurch, 1994; Talavera-Bianchi *et al*., 2010). Strong positive correlations indicate associations between different attributes, i.e. the intensities change in a similar pattern, the presence of one attribute may highlight another, and perhaps similarities exist between these attributes (Chambers *et al*., 2004).

The negative attributes also showed strong correlations. "Smokey" and "wet fur/farm animals" correlated strongly across aromas and flavours (r > 0.8) (Tables 3 – 5). The attribute "hay/dried grass" also correlated significantly with the negative attributes mentioned, although the correlations were not very strong (r < 0.4) and thus it is slightly isolated on the PCA loadings plot (Fig. 9a). "Sour" aroma significantly correlated with "smokey", "wet fur/farm animals" and, to a lesser extent, with "hay/dried grass" aroma and flavour (Table 7). Sour taste also correlates significantly with bitter taste and astringent mouthfeel, while bitter taste and astringent mouthfeel have a very strong positive correlation (r = 0.812) (Table 8). It should be noted that the correlation analysis measures the proximity of a variable to a straight line, however, does not validly measure the strength of a nonlinear relationship and thus false or accidental associations between variables may also exist (Taylor, 1990). Correlation of taste modalities with aroma and flavour attributes will fall into this category (Tables 6 & 7). Evidently, most of the negative attributes are closely correlated due to the intensely overpowering nature of these attributes. Tainted samples are mostly very obvious, either due to the absence of positive attributes (under-developed in under-fermented tea or transformed into negative attributes in over-fermented tea), while good honeybush tea has a finer balance of various positive attributes. It is, however, clear from these PCA plots and correlation coefficients for negative attributes that they are closely linked and that some root cause, such as poor processing conditions/practices may be responsible.

Interestingly, the seemingly positive attribute "woody" correlates significantly with all negative attributes, as well as with sour and bitter taste and astringent mouthfeel, and negatively correlates with most positive attributes and sweet taste as depicted in the PCA loadings plot (Fig. 9a) and qualified by the correlation coefficients (Tables 3 - 7). At low intensities, "woody" aroma and flavour may not be considered negative but at higher intensities it overpowers positive attributes, thus masking the usual floral profile of *C. intermedia*. Similarly, mild astringent mouthfeel is considered a positive attribute, while harsh astringency is negative. These attributes may possibly be used to monitor the fermentation process and indicate once optimal fermentation has been reached, however, a fine balance of intensities is key to good sensory quality.

The correlations between sensory attributes provide insight into the variation of sensory profiles of *C. intermedia* within the sample set, probably due to different fermentation conditions, and to a lesser extent, natural variation in the raw plant material. For example, *C. intermedia* infusions with a high intensity of "fynbos-floral" aroma also had high "fynbos-sweet" aroma and "fynbos-floral" flavour intensities (highly correlated with "fynbos-floral" aroma) with subtle "cooked apple", "caramel/vanilla" and "fruity-sweet" aroma and flavour intensities (weaker positive correlations), while "smokey" or "wet fur/farm animal" aromas and flavours would be unexpected (strong negative correlations). *Cyclopia intermedia* infusions with a strong "apricot jam" aroma would be expected to have a "fruity-sweet" aroma, sour taste and "apricot jam" flavour (highly correlated) with subtle "caramel/vanilla" and "sour" aromas (weaker positive correlations), while "cooked apple", "hay/dried grass", "smokey" and "wet fur/farm animals" aromas and flavours would be unexpected (negative correlations). On the other hand, "poor" sensory quality *C. intermedia* infusions would be expected to have high "woody", "hay/dried grass", "sour", "smokey", and "wet fur/farm animals" aroma and flavour intensities with bitter and sour tastes and astringent mouthfeel.

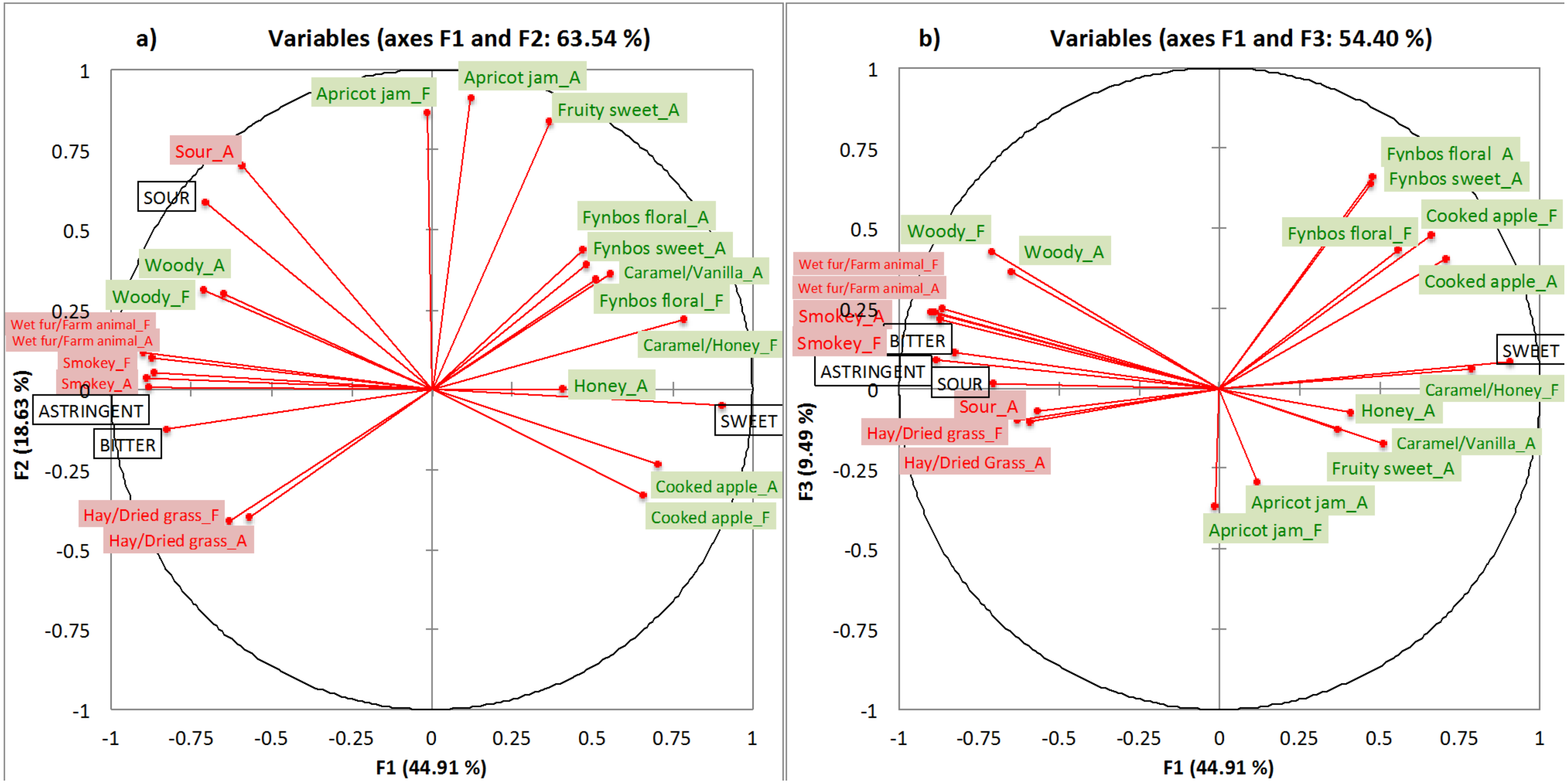



**Figure 9** PCA loadings plots a) (F1; F2) and b) (F1; F3) showing the positioning of the most prevalent positive (green) and negative (red) aroma (A) and flavour (F) attributes, as well as taste and mouthfeel (black) attributes of *C. intermedia* infusions.

**Table 3** Pearson's correlation coefficients (r) illustrating the relationship between aroma and flavour attributes of *C. intermedia* infusions.

| Variables | Fynbos-floral_F | Apricot jam_F | Cooked apple_F | Woody_F | Caramel/ Honey_F | Hay/Dried grass_F | Smokey_F | Wet fur/Farm animals_F |
|---|---|---|---|---|---|---|---|---|
| **Fynbos-floral_A** | **0.820** | 0.173 | **0.368** | 0.033 | **0.430** | **-0.393** | **-0.276** | **-0.281** |
| **Apricot jam_A** | 0.265 | **0.901** | **-0.315** | 0.093 | 0.253 | **-0.372** | -0.140 | -0.092 |
| **Cooked apple_A** | **0.306** | **-0.355** | **0.920** | **-0.352** | **0.560** | **-0.292** | **-0.504** | **-0.511** |
| **Woody_A** | -0.173 | 0.156 | **-0.319** | **0.919** | **-0.341** | **0.352** | **0.555** | **0.557** |
| **Fruity-sweet_A** | **0.385** | **0.723** | 0.002 | -0.085 | **0.442** | **-0.505** | -0.264 | -0.231 |
| **Caramel/Vanilla_A** | 0.172 | **0.290** | 0.210 | **-0.290** | **0.652** | **-0.367** | **-0.391** | **-0.417** |
| **Honey_A** | 0.264 | 0.059 | 0.110 | -0.203 | **0.322** | -0.237 | **-0.477** | **-0.445** |
| **Fynbos-sweet_A** | **0.705** | 0.112 | **0.425** | 0.024 | **0.453** | **-0.435** | -0.255 | -0.241 |
| **Hay/Dried grass_A** | **-0.458** | -0.232 | **-0.297** | **0.409** | **-0.630** | **0.839** | **0.328** | **0.368** |
| **Sour_A** | -0.176 | **0.654** | **-0.575** | **0.572** | **-0.346** | 0.101 | **0.504** | **0.606** |
| **Smokey_A** | **-0.436** | -0.086 | **-0.457** | **0.655** | **-0.631** | **0.339** | **0.986** | **0.881** |
| **Wet fur/Farm animals_A** | **-0.433** | -0.009 | **-0.483** | **0.683** | **-0.650** | **0.348** | **0.937** | **0.978** |

Significant correlations ($p \leq 0.05$) are indicated in bold. Correlations > ± 0.7 are indicated in red. The letters "A" and "F" after the attributes refer to aroma and flavour attributes, respectively.

**Table 4** Pearson's correlation coefficients (r) illustrating the relationship between aroma attributes of *C. intermedia* infusions.

| Variables | Fynbos-floral_A | Apricot jam_A | Cooked apple_A | Woody_A | Fruity-sweet_A | Caramel/ Vanilla_A | Honey_A | Fynbos-sweet_A | Hay/ Dried grass_A | Sour_A | Smokey_A | Wet fur/Farm animals_A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Fynbos-floral_A** | **1** | | | | | | | | | | | |
| **Apricot jam_A** | 0.255 | **1** | | | | | | | | | | |
| **Cooked apple_A** | **0.371** | -0.231 | **1** | | | | | | | | | |
| **Woody_A** | -0.012 | 0.098 | **-0.297** | **1** | | | | | | | | |
| **Fruity-sweet_A** | **0.385** | **0.863** | 0.128 | -0.031 | **1** | | | | | | | |
| **Caramel/Vanilla_A** | 0.206 | **0.369** | **0.397** | -0.158 | **0.573** | **1** | | | | | | |
| **Honey_A** | 0.170 | 0.063 | 0.152 | -0.191 | 0.040 | -0.065 | **1** | | | | | |
| **Fynbos-sweet_A** | **0.828** | 0.232 | **0.415** | -0.007 | **0.386** | 0.154 | 0.226 | **1** | | | | |
| **Hay/Dried grass_A** | **-0.476** | **-0.375** | **-0.359** | **0.450** | **-0.559** | **-0.502** | -0.165 | **-0.504** | **1** | | | |
| **Sour_A** | -0.055 | **0.595** | **-0.532** | **0.545** | **0.405** | -0.075 | -0.232 | -0.103 | 0.140 | **1** | | |
| **Smokey_A** | **-0.306** | -0.155 | **-0.514** | **0.575** | **-0.290** | **-0.411** | **-0.463** | **-0.301** | **0.360** | **0.505** | **1** | |
| **Wet fur/Farm animals_A** | -0.265 | -0.086 | **-0.542** | **0.588** | -0.225 | **-0.429** | **-0.467** | -0.239 | **0.376** | **0.615** | **0.937** | **1** |

Significant correlations ($p \leq 0.05$) are indicated in bold. Correlations > ± 0.7 are indicated in red. The letter "A" after the attributes refers to aroma attributes.

**Table 5** Pearson's correlation coefficients (r) illustrating the relationship between flavour attributes of *C. intermedia* infusions.

| Variables | Fynbos-floral_F | Apricot jam_F | Cooked apple_F | Woody_F | Caramel/ Honey_F | Hay/Dried grass_F | Smokey_F | Wet fur/Farm animals_F |
|---|---|---|---|---|---|---|---|---|
| **Fynbos-floral_F** | **1** | | | | | | | |
| **Apricot jam_F** | 0.171 | **1** | | | | | | |
| **Cooked apple_F** | **0.286** | **-0.461** | **1** | | | | | |
| **Woody_F** | -0.130 | 0.159 | **-0.347** | **1** | | | | |
| **Caramel/Honey_F** | **0.426** | 0.100 | **0.491** | **-0.425** | **1** | | | |
| **Hay/Dried grass_F** | **-0.395** | -0.217 | **-0.277** | **0.367** | **-0.575** | **1** | | |
| **Smokey_F** | **-0.427** | -0.071 | **-0.442** | **0.634** | **-0.609** | **0.339** | **1** | |
| **Wet fur/Farm animals_F** | **-0.470** | -0.013 | **-0.456** | **0.652** | **-0.609** | **0.342** | **0.880** | **1** |

**Table 6** Pearson's correlation coefficients (r) illustrating the relationship between positive aroma and flavour attributes and taste and mouthfeel modalities of *C. intermedia* infusions.

| Variables | Fynbos-floral_A | Apricot jam_A | Cooked apple_A | Woody_A | Fruity-sweet_A | Caramel/ Vanilla_A | Honey_A | Fynbos-sweet_A |
|---|---|---|---|---|---|---|---|---|
| **SWEET TASTE** | **0.467** | 0.035 | **0.678** | **-0.538** | **0.296** | **0.381** | **0.358** | **0.465** |
| **SOUR TASTE** | -0.050 | **0.438** | **-0.618** | **0.656** | 0.204 | -0.236 | -0.228 | -0.110 |
| **BITTER TASTE** | **-0.367** | -0.246 | **-0.543** | **0.558** | **-0.449** | **-0.416** | -0.251 | **-0.370** |
| **ASTRINGENT MOUTHFEEL** | **-0.316** | -0.150 | **-0.614** | **0.606** | **-0.386** | **-0.443** | **-0.330** | **-0.365** |
| **Variables** | **Fynbos-floral_F** | **Apricot jam_F** | **Cooked apple_F** | **Woody_F** | **Caramel/ Honey_F** | | | |
| **SWEET TASTE** | **0.500** | -0.026 | **0.692** | **-0.604** | **0.687** | | | |
| **SOUR TASTE** | -0.152 | **0.514** | **-0.633** | **0.709** | **-0.425** | | | |
| **BITTER TASTE** | **-0.442** | -0.144 | **-0.493** | **0.582** | **-0.638** | | | |
| **ASTRINGENT MOUTHFEEL** | **-0.364** | -0.024 | **-0.595** | **0.662** | **-0.630** | | | |

Significant correlations ($p \leq 0.05$) are indicated in bold. Correlations > ± 0.7 are indicated in red. The letters "A" and "F" after the attributes refer to aroma and flavour attributes, respectively.

**Table 7** Pearson's correlation coefficients (r) illustrating the relationship between negative aroma and flavour attributes and taste and mouthfeel modalities of *C. intermedia* infusions.

| **Variables** | **Hay/Dried grass_A** | **Sour_A** | **Smokey_A** | **Wet fur/Farm animals_A** | **Hay/Dried grass_F** | **Smokey_F** | **Wet fur/Farm animals_F** |
|---|---|---|---|---|---|---|---|
| **SWEET TASTE** | **-0.475** | **-0.529** | -0.798 | -0.802 | **-0.435** | -0.786 | -0.775 |
| **SOUR TASTE** | **0.299** | 0.822 | **0.595** | **0.659** | 0.196 | **0.590** | **0.633** |
| **BITTER TASTE** | **0.473** | **0.347** | **0.747** | **0.721** | **0.406** | **0.704** | **0.722** |
| **ASTRINGENT MOUTHFEEL** | **0.510** | **0.483** | **0.750** | **0.740** | **0.476** | **0.721** | **0.707** |

Significant correlations (p ≤ 0.05) are indicated in bold. Correlations > ± 0.7 are indicated in red. The letters "A" and "F" after the attributes refer to aroma and flavour attributes, respectively.

**Table 8** Pearson's correlation coefficients (r) illustrating the relationship between taste and mouthfeel modalities of *C. intermedia* infusions.

| **Variables** | **SWEET TASTE** | **SOUR TASTE** | **BITTER TASTE** | **ASTRINGENT MOUTHFEEL** |
|---|---|---|---|---|
| **SWEET TASTE** | **1** | | | |
| **SOUR TASTE** | **-0.656** | **1** | | |
| **BITTER TASTE** | **-0.731** | **0.446** | **1** | |
| **ASTRINGENT MOUTHFEEL** | **-0.822** | **0.678** | **0.812** | **1** |

Significant correlations (p ≤ 0.05) are indicated in bold. Correlations > ± 0.7 are indicated in red.

### 3.3. Categorising *C. intermedia* samples to develop an elementary quality grading system

Grading systems are essential for the standardisation and commercialisation of food products by improving the regulation of their overall quality and ultimately improving consumer satisfaction (Feria-Morales, 2002). In order to develop such systems, quality parameters need to be identified, defined and measured. Descriptive sensory analysis is a widely applied tool for quality inspection, product design and marketing (Zeng *et al*., 2008). It is considered to be the ultimate method of identifying, quantifying and qualifying information on the sensory aspects of food products (Aparicio *et al*., 1996; Murray *et al*., 2001) and was therefore used in the present study to generate a baseline sensory description, as well as quality parameters for *C. intermedia* infusions. A wide range of samples were analysed to determine both the positive and negative attributes of the product, which prompted the categorisation of the individual samples in an initial step to developing an elementary quality grading system.

The simple categorisation was achieved by calculating the sum total of the positive and negative attribute intensity scores, respectively, as a percentage of the total intensity score for each infusion. Thus, the percentage of positive and negative attribute intensities that constitute each infusion was obtained. An example of the calculations to achieve this is presented in Addendum E. The box plots (Fig. 10) illustrate the distribution of the infusions according to the composition of positive and negative attribute intensities. Positive attributes contributed more to the overall aroma and flavour profile than the negative attributes (Fig. 10), because they had much larger mean intensity scores (Figs. 1-3). On average, the total intensities of positive attributes constitute 76% of the infusion and that of the negative attributes constitute 15% (indicated by red crosses on Fig. 10). This provided a “yardstick” to categorise samples according to their constitution of positive and negative sensory attribute intensities above or below average: “good” quality samples constituted a higher percentage of positive and lower percentage of negative attributes than the average, “average” quality samples comprised of a combination of positive and negative attributes at moderate intensities and samples of “poor” quality were composed of a higher percentage of negative attributes and lower percentage of positive attributes than the average. These sensory quality grades are defined in Table 9.

The PCA plot, drawn to illustrate the association between the most prevalent sensory attributes and the graded samples (Fig. 11), only gives 57.84% recovery of the correlation matrix, i.e. it portrays 57.84% of the correlation between the samples, and is thus not a reliable tool to determine definite associations between attributes. However, it is useful to visualise the patterns that exist between the sensory attributes and samples that were graded according to their sensory quality. Most of the variance is explained by the first component (F1 = 38.42%), with most of the positive attributes positioned on the right of the plot and the negative attributes located on the left (Fig. 11a). Attributes with short vector lengths may not be characteristic of the infusions in this sample set, and thus not well represented on this plot. However, a few positive attributes (“apricot jam” flavour, and “lemon/lemon grass” and “woody” aroma and flavour) were positioned on the left of the PCA plot associating with the negative attributes, and could thus perhaps be indicators of a transition between positive and negative quality tea (over- or under-fermented) and could be useful indicators to monitor the fermentation process.

The positioning of the graded *C. intermedia* samples on the corresponding PCA scores plot (Fig. 11b) gives an indication of the sensory characteristics associated with each sample when compared with the PCA loadings plot (Fig. 11a). The association between “good” quality samples and positive sensory attributes on the right of the plots confirms that “good” quality samples have a large constituent of positive sensory attributes with high intensity scores, while those on the left are of “poor” quality (over- or under-fermented). Under-fermented tea would have green notes at high intensity, whilst “wet fur/farm animals” and “smokey” clearly indicate poor control over fermentation conditions. The samples positioned in the centre of the map were of “average” quality, thus having moderate intensities of positive and negative sensory attributes, often termed “flat”. Interestingly, some of the “average” quality samples associate with fruity attributes. Evolvement of aroma and flavour attributes during fermentation may play a role here. As negative attributes associated with under-fermented tea such as “green grass” and “cooked vegetables” decrease during fermentation, fruity attributes may become dominant while positive attributes such as “fynbos-floral”, “caramel/honey” and “cooked apple” are still developing and thus not perceived at high intensities. Theron *et al*. (2014) showed that the average positive attribute intensities increased while the negative attribute intensities decreased during fermentation of *C. genistoides*, *C. maculata* and *C. subternata* at 80 and 90°C from 8 to 32 h. It can thus be postulated that the different sensory quality grades in this sample set are to a large extent as a result of different fermentation conditions. The fermentation conditions need to be optimised for *C. intermedia* to standardise the quality of the product on the market and aid its commercial viability.

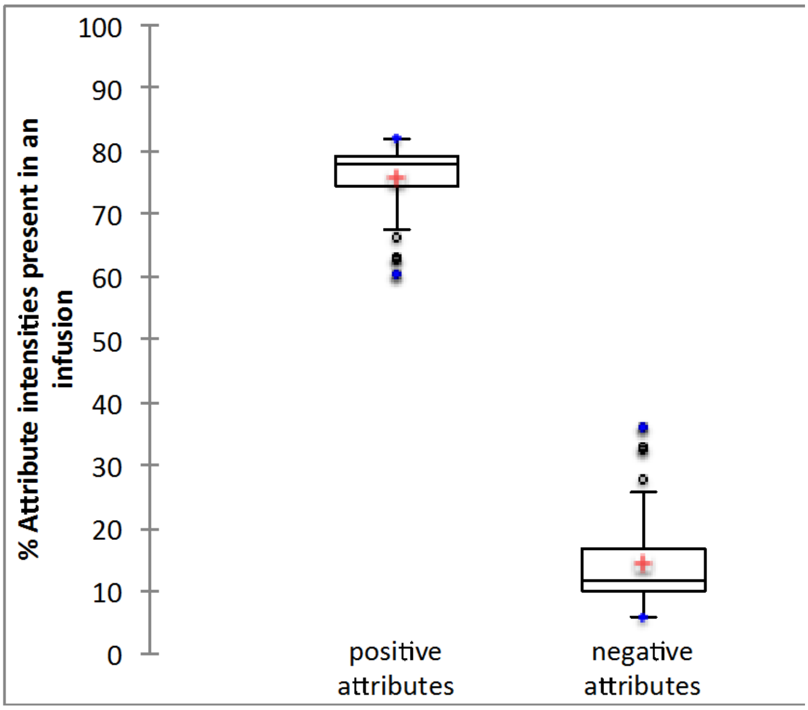



**Figure 10** Box plots illustrating the distribution of the percentage positive and negative attribute intensities in the infusions analysed.

**Table 9** Parameters used to categorise *C. intermedia* infusions into quality grades.

| | **% Attribute intensities present in an infusion** | |
|---|---|---|
| **Quality category** | **Positive** | **Negative** |
| Good | > 80% | < 10% |
| Average | > 75 < 80% | > 10 < 15% |
| Poor | < 75% | > 15% |

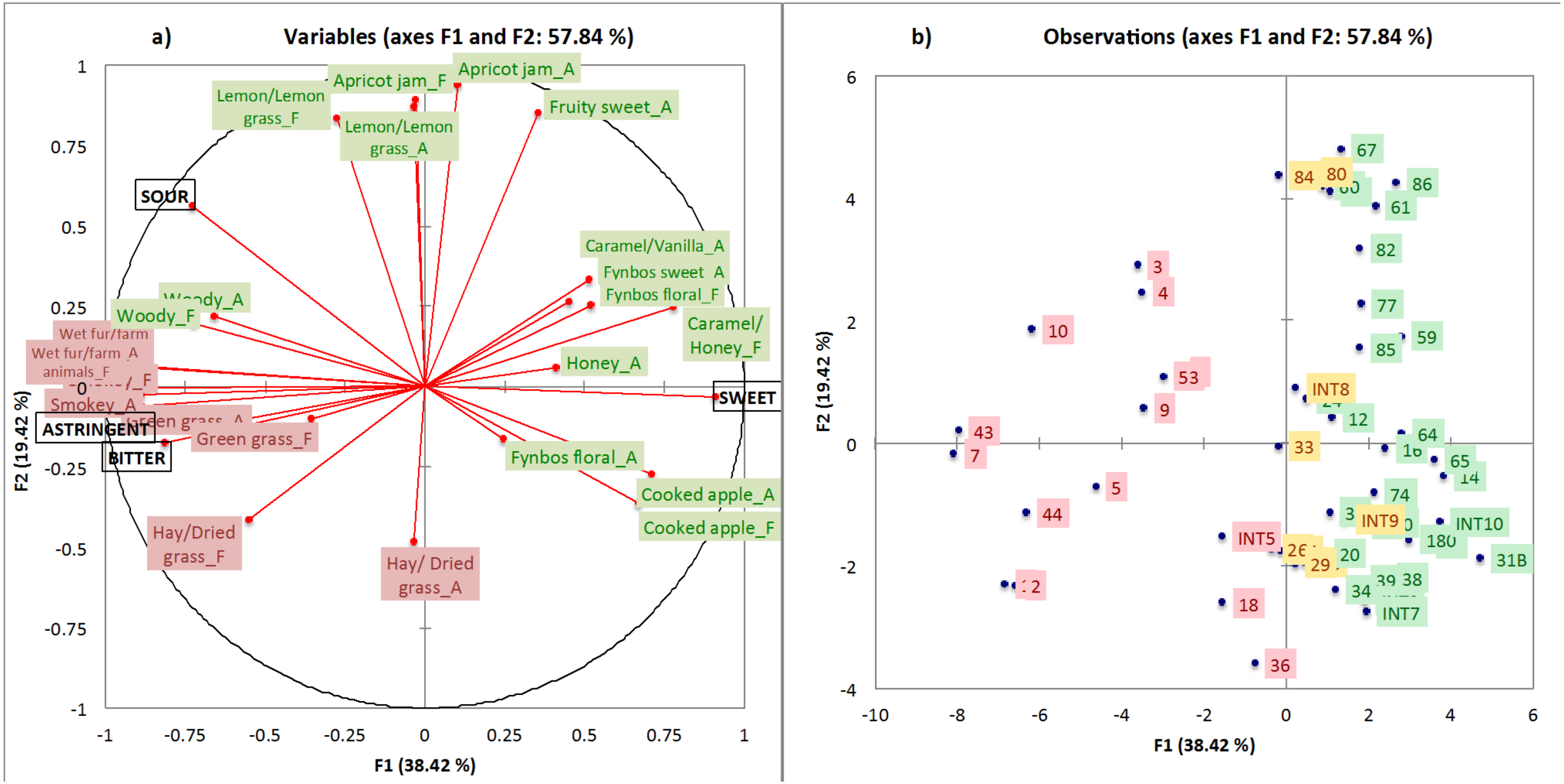



**Figure 11** a) PCA loadings plot showing the positioning of sensory attributes of *C. intermedia* infusions. Aroma (A) and flavour (F) attributes are indicated as positive (green) and negative (red). Taste and mouthfeel attributes are indicated in black. b) PCA scores plot showing the positioning of *C. intermedia* samples (n = 54) used in the analysis, graded as "good" (green), "average" (yellow) and poor (red) quality categories.

### 3.4. Relationship between the soluble solids content, absorbance as a measure of colour, turbidity and sensory quality of *C. intermedia* infusions

The descriptive statistics (minimum, maximum, range, mean and standard deviation) for the soluble solids (SS), absorbance as a measure of colour (AUC) and turbidity (NTU) parameters of *C. intermedia* infusions, categorised according to sensory quality grade, are summarised in Table 10 and graphically presented by box plots (Fig. 12) to show the distribution of the data across quality grades. This categorisation of physicochemical measurements could be useful to serve as indicators of sensory quality in the future. The absorbance vs. wavelength (nm) plot (Fig. 13) illustrates the extent to which *C. intermedia* infusions (categorised according to sensory quality) absorb light at different wavelengths and provides insight into the differences in AUC ("total colour") values between the different sensory quality grades. Figure 14 illustrates the variation in colour and turbidity of a few of the *C. intermedia* infusions analysed. The association between the physicochemical parameters, as well as their association with the sensory attributes and quality-graded samples is displayed on the principal component analysis (PCA) plot (Fig. 15), followed by the correlation coefficients for the association between these variables presented in Table 11, 12 and 13. Graphical presentation of the correlations between the physicochemical parameters is presented in Figures 16a, b and c and Figure 17.

The soluble solids content of a tea sample gives an indication of the strength of the infusion and, therefore, is an indication of the sensory quality of the tea infusion. Joubert (1984) found that increasing fermentation time of rooibos plant material resulted in a decrease in soluble solids due to polymerisation and subsequent loss of solubility of the polymeric phenolic substances. Both Du Toit and Joubert (1999) and Theron (2012) showed a decrease in soluble solids content of honeybush infusions with increasing fermentation time, although the effect was not significant, while Joubert *et al*. (2008) demonstrated that fermentation reduced the yield of SS of all *Cyclopia* spp. significantly, while also reducing the phenolic content of the SS. Thus, a low level of soluble solids could be an indication of poor solubility due to over-fermentation of honeybush tea (Du Toit & Joubert, 1998).

The SS content of the *C. intermedia* infusions analysed differed considerably between the quality grades. The average SS content of samples of "good" quality was 0.096 g/100 mL, 0.145 g/100 mL for "average" quality samples, and 0.171 g/100 mL for "poor" quality samples (Table 10), thus decreased solubility was observed in infusions of better quality (Fig. 13). This relationship is not as simple as both under-fermented and over-fermented samples (grades of "poor" quality) could have high SS contents. Under-fermented samples would yield high SS contents as the solubility of compounds are unaffected by polymerisation at this stage. On the other hand, extensive degradation of the plant matrix as a result of over-fermentation due to excessive fermentation temperature and time exposure or extensive microbial activity (at low temperature fermentation) would lead to higher than expected SS content. Theron (2012) demonstrated that the SS content of *C. genistoides* and *C. subternata* infusions decreased with increasing fermentation time until 24 h, after which an increase was observed (32 h). It can be postulated that polymerisation occurred within 24 h of fermentation and extending the fermentation time had a detrimental effect on the compounds produced, qualifying the benefit of SS content determination to monitor the fermentation process and determine optimum fermentation conditions. The use of microbial enzymes to increase the release of soluble solids from plant material, including *Camellia sinensis* tea and rooibos tea, has been demonstrated (Pengilly *et al*., 2008; Coetzee *et al*., 2013; Murugesh & Subramanian, 2014).

The colour of a tea infusion is an important parameter that has a marked influence on the perception of its "strength" and thus quality and may be useful to physically monitor the fermentation process. The average AUC value for "good" quality samples was 42.86, 77.97 for "average" quality samples, and 89.35 for "poor" quality samples (Table 11). The higher AUC values for samples of "poor" and "average" quality are qualified by the higher absorbance values of these samples (Fig. 13). When white light passes through a coloured substance, certain colours of light are absorbed while other colours pass through or are reflected by the substance. The colour seen by the eye is the visible light that is not absorbed by the sample. The *C. intermedia* infusions have greatest absorbance in the violet range (370-400 nm), thus appear yellow/orange. Paradoxically, "good" quality *C. intermedia* appears lighter in colour to those of "poor" and "average" quality. Monomeric compounds generally have higher absorbance capabilities than polymers (a phenomenon known as hypochromism) (Rhodes, 1961). It can thus be postulated that the decreased absorbance observed for "good" quality samples is as a result of polymerisation during fermentation. Over-fermented tea would then be expected to have the least colour, however, degradation of the plant matrix due to excessive fermentation temperature and time exposure has been observed to produce a dark red/black colour and excessively turbid infusion (Fig. 14). Theron (2012) also observed the same effect of fermentation time on the colour (AUC) of *C. genistoides* and *C. subternata* infusions as for the SS content, illustrating that the colour of infusions reflected the change in the SS content due to polymerisation.

Turbidity is the optical property that describes the scattering and absorption of light as it travels through a liquid due to particles in suspension, making the liquid look cloudy or smokey (Bhuyan, 2007). This property of the particles in suspension, i.e. they scatter a light beam focused on them, is considered a meaningful measure of turbidity in water (Ankcorn, 2003) and beverages such as beer (Steiner *et al*., 2010). Turbidity measured in this manner uses an instrument called a nephelometer with the detector setup to the side of the light beam. More light reaches the detector if there are many small particles scattering the source beam than if there are few. The units of turbidity from a calibrated nephelometer are called Nephelometric Turbidity Units (NTU). Little is known about the turbidity of honeybush tea infusions, although it also has a marked influence on the perception of quality and has negatively influenced international sales (M. Bergh, Rooibos Ltd, Clanwilliam, South Africa, 2013, personal communication).

There was also a large variation between the infusions in terms of their turbidity, which ranged from a mean of 39.83 NTU for “good” quality infusions, 72.07 NTU for “average” quality infusions, to 313.9 NTU for “poor” quality infusions (Table 10). Turbidity, measured as a quality parameter in beer, is descriptively categorised as “brilliant” (0-2 NTU), “almost brilliant” (2-4 NTU), “very slightly turbid” (4-8 NTU), “slightly turbid” (8-16 NTU), “turbid” (16-32 NTU), and “very turbid” (>32 NTU) (Norman, 2012). Using the classification for beer turbidity, a few “good” quality *C. intermedia* infusions were “very slightly turbid”, however, “average” and “poor” quality infusions were “very turbid”, indicating that turbidity is important in terms of quality. *Cyclopia intermedia* infusions that were particularly turbid could be described as analogous to unfiltered coffee (Fig. 14). It should be noted that the different methods and technologies used to measure turbidity indicate that turbidity is not an absolute value, but a relative value representing a qualitative measurement that can yield different readings based on the method used (Ankcorn, 2003). Further work on this aspect is required to define the clarity/turbidity levels for honeybush specifically.

It can be postulated that the large variation in the physicochemical parameters within the sample set, representing different qualities of tea made from one species (*C. intermedia*), could largely be due to different processing conditions, in particular the fermentation temperature and time combination used. It is well known that extensive heat treatments during fermentation have a detrimental effect on SS content (Joubert *et al*., 2008), which in turn affects the colour and perhaps the turbidity of infusions. This is confirmed by the positioning of most of the samples of “good” quality (representing optimally fermented *C. intermedia*) on the right of the PCA loadings plot (Fig. 15a), illustrating a negative correlation with the physicochemical parameters, while mostly samples of “poor” quality (representing under- and over-fermented *C. intermedia*) closely associated with these parameters.

The SS content correlated highly significantly with the colour of the infusions (AUC) ($r = 0.921$) and less so with turbidity (NTU) ($r = 0.601$) (Table 11). The colour and turbidity measurements also correlated significantly, but to a lesser degree ($r = 0.517$). These correlations are reflected by the PCA loadings plot (Fig. 15a) where the physicochemical parameters are situated on the left of the plot. The relationships between the individual physicochemical parameters are illustrated by the scatter plots (Figs. 16 a, b & c). All the parameters, specifically SS content and colour (AUC) measurements (Fig. 16a) are positively linearly correlated (i.e. one parameter increases as the other increases or vice versa), indicating that the SS content of an infusion of tea largely governs the colour (AUC). Both Du Toit and Joubert (1999) and Theron (2012) demonstrated a relationship between the SS content and colour of honeybush infusions. Polymerisation decreases the solubility of compounds and thus the SS content of an infusion, resulting in a lighter coloured infusion. The mechanism of turbidity formation in honeybush tea, however, is not known. In black tea (*Camellia sinensis*), tannins interact with caffeine to form a complex that causes turbidity and precipitation in a strong infusion upon cooling (Rutter & Stainsby, 1975; Boadi & Neufeld, 2001). Although honeybush has a low tannin content and does not contain caffeine, it can be postulated that polyphenolic compounds in honeybush infusions complex with polymeric compounds such as proteins and cause turbidity (Siebert *et al*., 1996). The relationship between the SS content and turbidity could then be elucidated as the loss of solubility of polyphenolic compounds due to polymerisation, which would prevent their extraction into the infusions and subsequently decrease complex formation, resulting in less turbidity as fermentation time is increased. Decreased turbidity would also lower the absorbance (AUC) values. The highly turbid samples are thus under-fermented or, on the other hand, over-fermented due to fermentation at excessive temperatures for extended time. The breakdown of plant material would be the cause of turbidity in over-fermented infusions of tea, rather than phenolic compounds forming complexes.

The interrelated relationship between physicochemical parameters is illustrated by the bubble plot (Fig. 17), which presents the linear relationship between the SS content and colour (AUC), as well as turbidity represented by the size of the bubbles. Both the colour and turbidity of infusions increased with increasing SS content. It can thus be postulated that increased fermentation time would decrease the SS content due to polymerisation of phenolic compounds and subsequently decrease the colour and turbidity of infusions, rendering these physicochemical parameters as possible indicators to monitor the fermentation process.

The link between certain sensory attributes, particularly taste and mouthfeel modalities, and the physicochemical composition, as well as the impact on the sensory quality of *C. intermedia* infusions is evident in the PCA plot (Fig. 15). The physicochemical parameters correlate negatively with the positive flavour attributes and have significant positive correlations with the negative flavour attributes (descriptive of under- and over-fermented tea) (Table 12), confirming that these physicochemical parameters could be indicators of sensory quality. Additionally, the physicochemical parameters have significantly negative correlations with sweet taste and positive correlations with sour and bitter taste and astringent mouthfeel (Table 13). Sour taste in food products is caused by small, insoluble, inorganic cations (Jackson, 2009), while certain organic acids such a phenolic acids have been also reported to elicit acidic or sour taste (Ramos Da Conceicao Neta *et a*l., 2007). The phenolic acids *p*-coumaric and shikimic acid, have previously been identified in *C. intermedia* (Ferreira *et al*., 1998; Kamara *et al*., 2003), which could contribute to the sour taste. However, fermentation records provided for some of the tainted samples analysed in this study show that the tea had been fermented between 40°C and 60°C for up to 5 days, indicating that microbial products were most likely contributing to these taints. According to Lesschaeve and Noble (2005) bitterness and astringency are elicited by flavonoids such as flavanols and flavonols. The chemical nature of the flavonoids, which determines their taste properties, depends on the structural class, degree of hydroxylation, other substituents and conjugations as well as the degree of polymerisation (Ley, 2008, Roland *et al*., 2013). Theron (2012), investigating the relationship between the combined data for composition and astringency of infusions prepared from *C. genistoides, C. maculata* and *C. subternata*, demonstrated weak, but significant, correlations for 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 = 0.46). The same study indicated that mangiferin, specifically, could be responsible for the bitter taste of honeybush infusions. Furthermore, Hongsoongnern and Chambers (2008), as well as Theron (2012), reported that astringent and bitter attributes were intrinsically associated with the green character of many products. Thus, changes in flavonoid content and chemical structure during fermentation would affect taste. It is known that slight structural changes to many sweet and bitter tasting compounds results in a change of their taste quality from sweet to bitter and vice versa (Jackson, 2009). However, it is not clear why teas fermented for up to 5 days (over-fermented) were also then strongly associated with these attributes. The release of peptides and some amino acid constituents (i.e. leucine, phenylalanine, tryptophan and tyrosine) due to breakdown of proteins and other polymers could be contributing to the bitter taste associated with over-fermented tea (Solms, 1969; Guigoz & Solms, 1976). Taking observations from this study into account, it can be postulated that astringent mouthfeel and bitter taste are associated with non-optimal fermentation (either under- or over-fermented) honeybush tea.

**Table 10** Descriptive statistics for the SS content, colour (AUC) and turbidity (NTU) of *C. intermedia* infusions (n = 54) categorised according to sensory quality grade.

| | **SS (g/100 mL)** | | | **AUC (370 -510 nm)** | | | **NTU** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Sensory quality grade** | **Good** | **Average** | **Poor** | **Good** | **Average** | **Poor** | **Good** | **Average** | **Poor** |
| **Minimum** | 0.076 | 0.091 | 0.106 | 22.497 | 45.960 | 42.967 | 5.697 | 21.600 | 67.323 |
| **Maximum** | 0.139 | 0.207 | 0.250 | 83.437 | 106.913 | 177.977 | 107.833 | 138.777 | 802.887 |
| **Range** | 0.063 | 0.116 | 0.144 | 60.940 | 60.953 | 135.010 | 102.137 | 117.177 | 735.563 |
| **Mean** | 0.096 | 0.145 | 0.171 | 42.859 | 77.967 | 89.352 | 39.826 | 72.071 | 313.897 |
| **Standard deviation** | 0.016 | 0.045 | 0.036 | 13.217 | 27.965 | 38.196 | 31.500 | 34.938 | 210.881 |

SS = soluble solids, AUC = area under the curve (370 – 510 nm), NTU = nephelometric turbidity units.

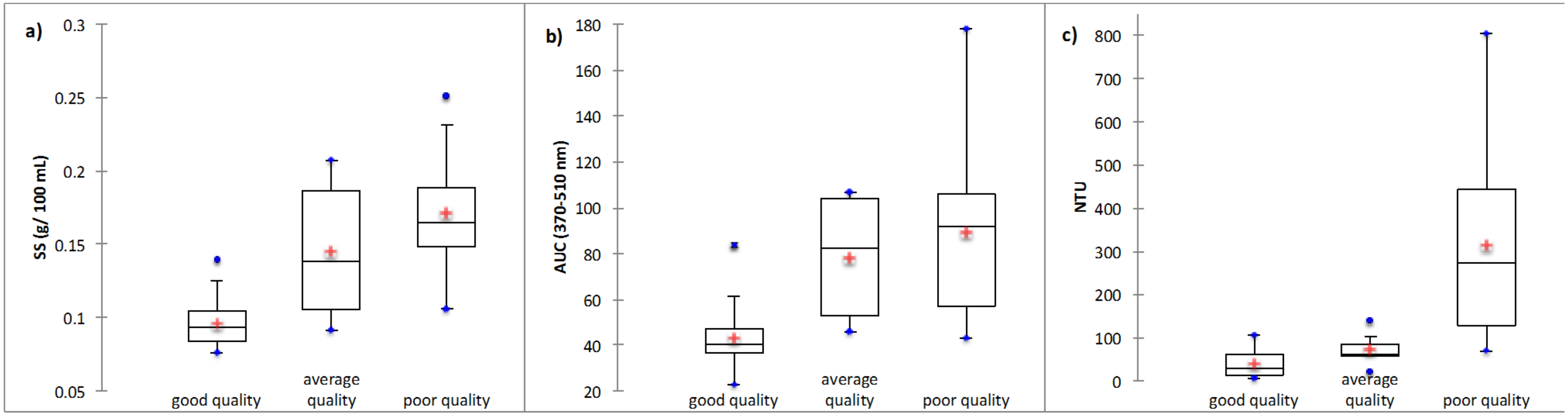



**Figure 12** Box plots of SS content, colour (AUC) and turbidity (NTU) of *C. intermedia* infusions (n = 54) categorised according to sensory quality grades, indicating the symmetry and distribution of the data. SS = soluble solids, AUC = area under the curve (370 – 510 nm), NTU = nephelometric turbidity units.

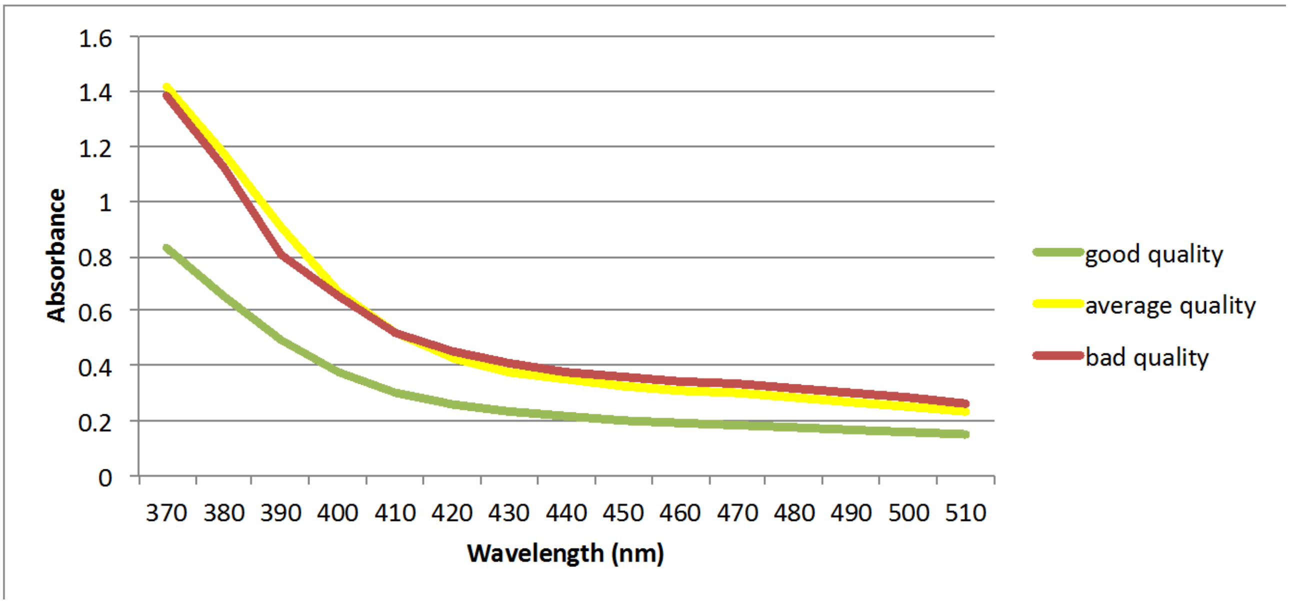



**Figure 13** Average absorbance values for the *C. intermedia* samples categorised according to "good", "average" and "poor" sensory quality.

**Figure 14** *Cyclopia intermedia* infusions varying in colour and turbidity.

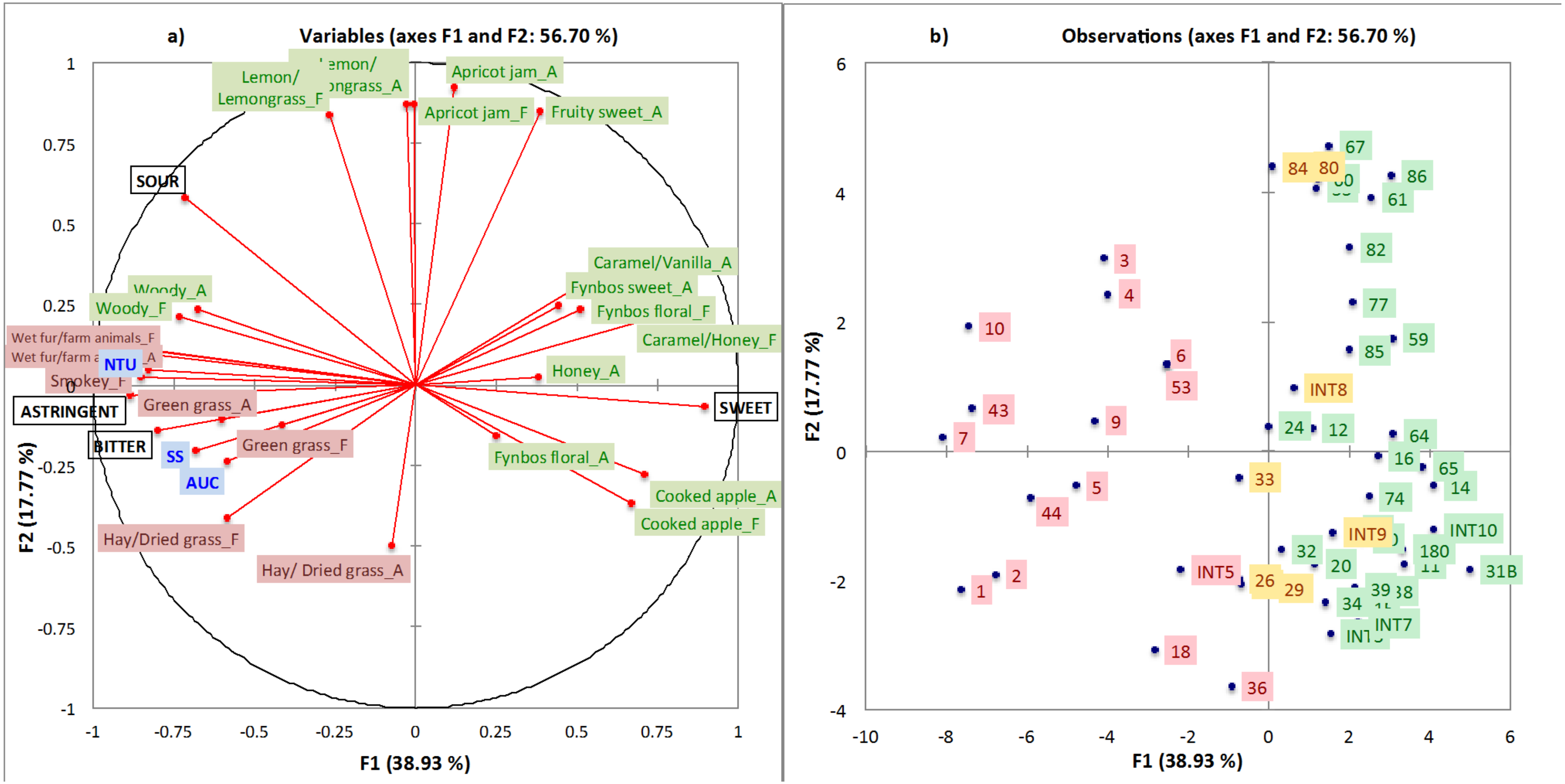



**Figure 15** a) PCA loadings plot showing the positioning of sensory attributes and physicochemical parameters of *C. intermedia* infusions. Aroma (A) and flavour (F) attributes are indicated as positive (green) and negative (red). Taste and mouthfeel attributes are indicated in black and physicochemical parameters in blue. SS = Soluble solids (g/100 mL), AUC = Area under the curve (370-510 nm), NTU = Nephelometric turbidity units. b) PCA scores plot showing the positioning of *C. intermedia* samples (n = 54) used in the analysis, graded as “good” (green), “average” (yellow) and “poor” (red) quality categories.

**Table 11** Pearson's correlation coefficients (r) illustrating the relationship between physicochemical parameters of *C. intermedia* infusions.

| Variables | SS (g/100 mL) | AUC (370-510 nm) | NTU |
|---|---|---|---|
| **SS (g/100 mL)** | **1** | **0.921** | **0.601** |
| **AUC (370-510 nm)** | **0.921** | **1** | **0.517** |
| **NTU** | **0.601** | **0.517** | **1** |

Significant correlations ($p \leq 0.05$) are indicated in bold. Correlations > ± 0.7 are indicated in red.

**Table 12** Pearson's correlation coefficients (r) illustrating the relationship between physicochemical parameters and certain flavour attributes of *C. intermedia* infusions.

| Variables | Fynbos-floral_F | Cooked apple_F | Woody_F | Caramel/Honey_F | Hay/Dried grass_F | Green grass_F | Smokey_F | Wet fur/farm animals_F |
|---|---|---|---|---|---|---|---|---|
| **SS (g/100 mL)** | **-0.284** | **-0.467** | **0.472** | **-0.578** | **0.557** | **0.567** | **0.311** | **0.354** |
| **AUC (370-510 nm)** | **-0.318** | **-0.415** | **0.430** | **-0.471** | **0.496** | **0.466** | 0.218 | 0.260 |
| **NTU** | **-0.300** | **-0.509** | **0.693** | **-0.602** | **0.470** | **0.424** | **0.536** | **0.624** |

Significant correlations ($p \leq 0.05$) are indicated in bold. The letters "A" and "F" after the attributes refer to aroma and flavour attributes, respectively.

**Table 13** Pearson's correlation coefficients (r) illustrating the relationship between physicochemical parameters and the taste and mouthfeel attributes of *C. intermedia* infusions.

| Variables | SWEET TASTE | SOUR TASTE | BITTER TASTE | ASTRINGENT MOUTHFEEL |
|---|---|---|---|---|
| **SS (g/100 mL)** | **-0.532** | **0.352** | **0.430** | **0.563** |
| **AUC (370-510 nm)** | **-0.422** | 0.260 | **0.374** | **0.450** |
| **NTU** | **-0.640** | **0.591** | **0.573** | **0.645** |

Significant correlations ($p \leq 0.05$) are indicated in bold.

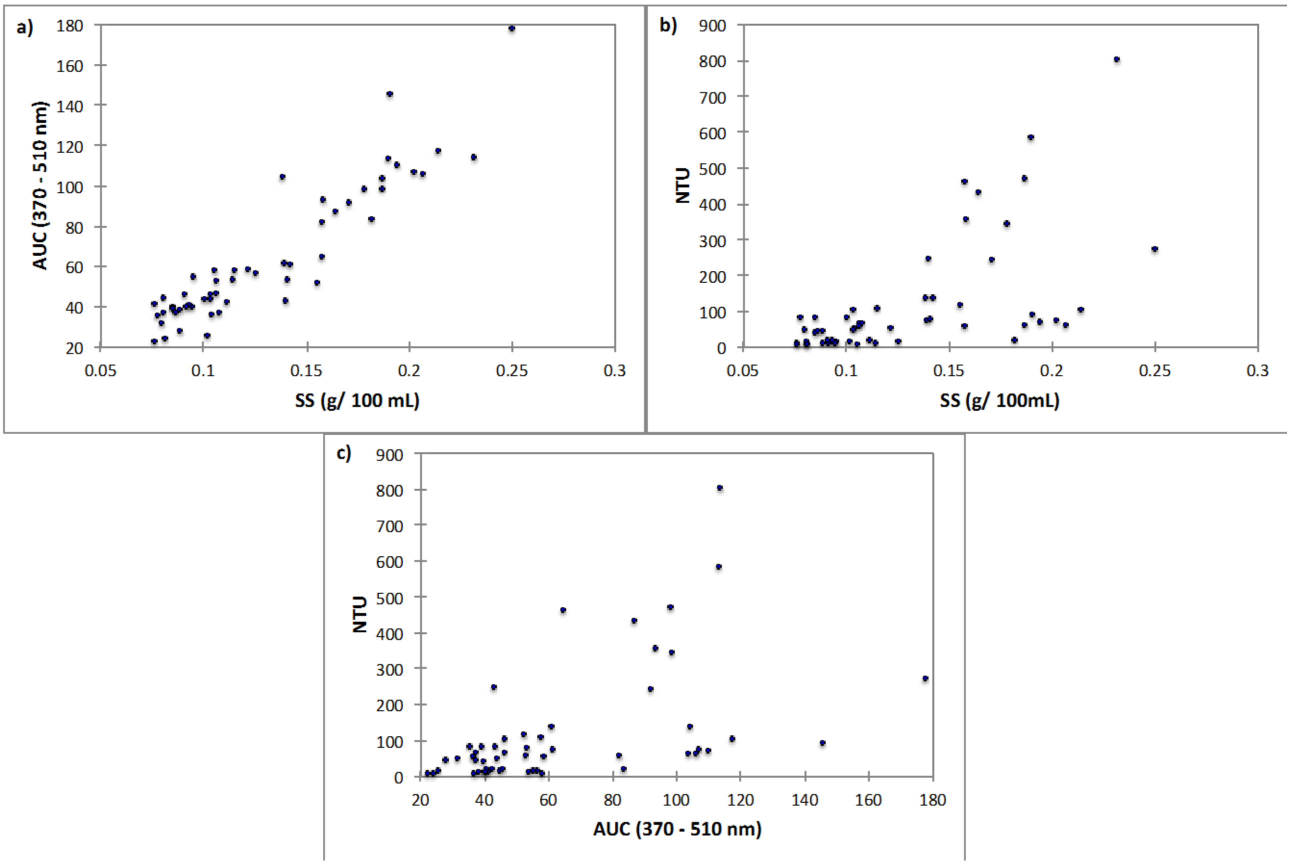



**Figure 16** Scatter plots illustrating the relationship between a) SS content and colour (AUC), b) SS content and turbidity (NTU), and c) colour (AUC) and turbidity (NTU) of *C. intermedia* infusions. SS = soluble solids, AUC = area under the curve (370 – 510 nm), NTU = nephelometric turbidity units.

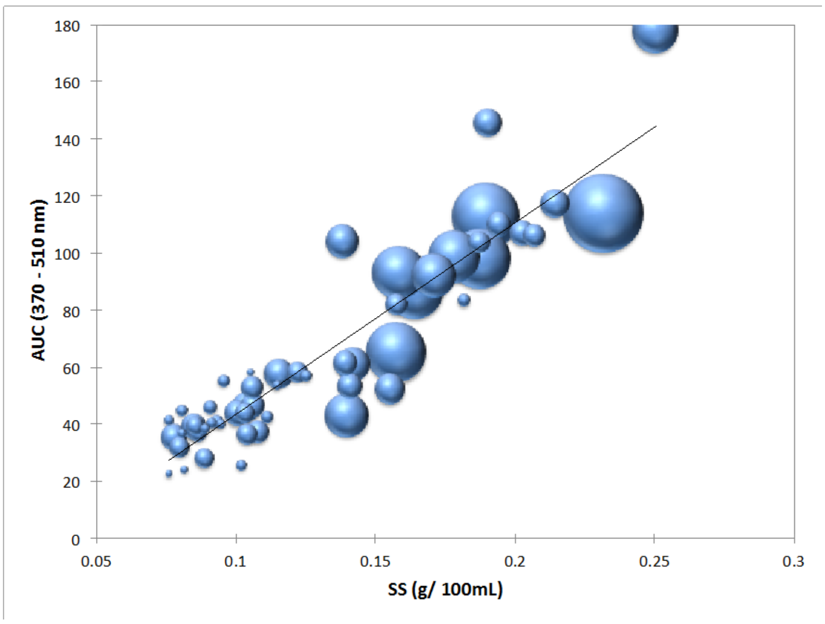



**Figure 17** Bubble plot showing the relationship between the SS content, colour (AUC) and turbidity (NTU) of *C. intermedia* infusions. Turbidity is represented by the size of the bubbles. SS = soluble solids, AUC = Area under the curve (370-510 nm).

## 4. CONCLUSIONS

Data from descriptive sensory analysis of a wide range of *C. intermedia* infusions were consolidated to generate a characteristic sensory profile, which can be described as sweet tasting and slightly astringent with a combination of “fynbos-floral”, “fynbos-sweet”, “fruity” (specifically “apricot jam”, “cooked apple”, “raisin” and “lemon/lemon grass”), “woody”, “caramel/vanilla” and “honey-like” aromas. The flavour is distinctly “fynbos-floral”, “fynbos-sweet” and “woody”, including hints of “lemon/lemon grass” and “hay/dried grass”. These sensory attributes were compiled into a sensory lexicon comprised of 11 flavour, 3 taste and 1 mouthfeel descriptors. Additionally, sensory wheels were created as simple graphical representations of the characteristic aroma, flavour, taste and mouthfeel of *C. intermedia*.

Additionally, the large number of samples analysed during descriptive sensory analysis contributed to the development of an elementary categorisation of *C. intermedia* samples into “good”, “average” and “poor” quality grades according to the composition of sensory attributes. The variation in sensory attributes within this specific species seems to be due to different processing conditions. The sensory attributes associated with each of these quality grades indicate that the grades were representative of well-, under- and over-fermented samples, respectively. The associations between the physicochemical parameters and graded samples showed that these physicochemical parameters could be indicative of the extent of fermentation: the SS content, colour and turbidity of infusions decreased as the sensory quality of improved, thus qualifying their use as quick physicochemical measurements to monitor the fermentation process. More research is needed to accurately define ranges and confirm their link to quality.

The definitive *C. intermedia* sensory profile, as well as the elementary sensory quality grading system and physicochemical parameters will find invaluable application in this study to optimise the processing conditions for *C. intermedia*. Furthermore, the sensory lexicon, wheels and grading system may be used in industry as standardised communication tools to develop a formal grading system, with the intention to produce consistently good quality product and aid its commercial viability.

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# CHAPTER 4

# OPTIMISATION OF FERMENTATION PARAMETERS FOR *CYCLOPIA INTERMEDIA* AND VALIDATION ON COMMERCIAL SCALE

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## ABSTRACT

The effects of fermentation temperature (70°C, 80°C and 90°C) and time (12, 16, 24, 36, 48, and 60 h) on the sensory and physicochemical (SS, colour and turbidity) characteristics of *C. intermedia* infusions were investigated on laboratory-scale to establish species-specific optimum fermentation parameters. Process conditions were tested thereafter on commercial-scale for validation for commercial use. Fermentation at 90°C on laboratory-scale proved more efficient at increasing the intensity of positive sensory attributes, eliminating the negative sensory attributes and producing *C. intermedia* with a characteristic sensory profile. Fermentation for 36 h at 90°C and 48 h at 70°C and 80°C was required to produce tea with "good" sensory quality. Increasing the fermentation time did not improve the sensory quality but rather caused a further decrease in SS content and colour of the infusions of tea, which are indicators of strength and thus also important for the overall quality of the product. Fermentation for 36 h at 90°C on laboratory-scale is, therefore, recommended to produce *C. intermedia* with optimal sensory characteristics, while preserving the SS content and colour of infusions. Fermentation of plant material from the same batch concurrently on laboratory- and commercial-scale proved that the commercial-scale fermentation process was more efficient than laboratory-scale fermentation, producing samples of "good" sensory quality after 24 and 36 h of fermentation, compared to 48 h for the laboratory-scale counterparts. Increasing fermentation time to 48 h on commercial-scale caused the sensory quality of infusions to decrease, while the turbidity increased significantly. However, no effects on the SS content and colour of infusions (particularly between 24 and 36 h) were observed. Thus, fermentation at 90°C on commercial-scale should be terminated between 24 and 36 h, especially taking optimum sensory quality and turbidity into account. However, due to the large variability between commercial-scale batches due to various other factors, it would be recommended that each batch be monitored between 24 and 36 h to determine optimum fermentation.


## 1. INTRODUCTION

It is well known that chemical oxidation, more commonly referred to as "fermentation", is responsible for the development of the characteristic sweet-associated, fruity and floral aroma and flavour and reddish colour of honeybush tea infusions (Du Toit & Joubert, 1998). Traditionally, small quantities were fermented for home use in the warm drawer of coal stoves (Joubert *et al*., 2011). Fermentation of larger quantities of honeybush tea was achieved using curing heaps (Marloth, 1909; 1925) and preheated "baking-ovens" (Hofmeyer & Phillips, 1922; Snyman, 1990), which was suitable for the few small processors that supplied the limited demand for honeybush during the twentieth century (Du Toit *et al*., 1998). These methods did not allow for control of the processing parameters and delivered a product lacking uniformity and, in many cases, was of poor sensory and microbial quality (Du Toit & Joubert, 1998). The renewed interest in honeybush tea in the 1990's, due to the growing interest in herbal teas both locally and internationally, necessitated the need for a consistent supply of high quality product to build consumer confidence and continue to expand the market.

As literature suggested higher fermentation temperatures to reduce fermentation time and inhibit growth of thermophillic contaminants (Du Toit & Joubert, 1999; Du Toit *et al*., 1999), processors adapted their methods accordingly to reduce processing time and reduce energy outputs. Furthermore, the commercialisation of honeybush subsequently required the modernisation of processing equipment, which led to the adoption of batch rotary fermentation, a concept developed for rooibos fermentation (Joubert & Muller, 1997). However, the use of short wavelength infrared lights as source of heat in the batch rotary system resulted in slow temperature increases, which allowed the opportunity for mould growth and extended the fermentation period and slowed productivity, led one processor to further develop the fermentation system (J. Kritzinger, Honeybush Natural Products, Langkloof, South Africa, 2013, personal communication). The movement towards increasing production volumes with modernised equipment at elevated temperatures has been accompanied by dramatic decline in sensory and physicochemical quality. This can be attributed to the critical nature of fermentation at elevated temperatures, which demands good control, and lack of standardised processing conditions to produce tea of optimum sensory and physicochemical characteristics.

Theron (2012), while investigating the optimum fermentation conditions for *C. subternata*, *C. genistoides* and *C. maculata*, demonstrated that optimum fermentation conditions were species-specific. Fermentation at 80°C/24 h is recommended for *C. genistoides*, *C. subternata* can be fermented at 80°C or 90°C depending on what sensory characteristics are desired, while *C. maculata* requires fermentation for 24 h at 80°C. Du Toit and Joubert (1999) also demonstrated that optimum fermentation conditions for *C. intermedia* and *C. buxifolia* (previously classified as *C. maculata*) (Schutte, 1997) are 70°C/60 h or 90°C/36 h. However, only the sweet aroma and flavour of infusions was considered in determining the sensory quality of *C. intermedia* and *C. buxifolia* infusions and can be considered inconclusive. Furthermore, these findings have not been tested on commercial-scale where many other variables may affect the fermentation process.

*Cyclopia intermedia* currently contributes to the major part of the annual honeybush production (Joubert *et al*., 2011). In view of its commercial importance, this study aims to optimise the fermentation parameters for *C. intermedia* on laboratory-scale and validate these conditions for commercial use in order to produce consistently good quality product. In view of limited supply, it is thus of utmost importance that optimum quality is realized and loss due to poor quality is limited as it is believed that the honeybush industry has the potential to emulate the success of the rooibos industry.

# 2. MATERIALS AND METHODS

## 2.1. Preparation of fermented material

### *2.1.1. Laboratory-scale fermentation to optimise the fermentation temperature x time regime for C. intermedia*

For the first part of this study three batches of *Cyclopia intermedia* plant material were harvested from different locations in the Western and Eastern Cape Provinces of South Africa (Table 1) and fermented on laboratory-scale at the research facilities of the Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Stellenbosch, to determine the optimum fermentation temperature x time combination. For a representative sample, shoots of several plants were harvested and pooled to form a batch of *ca*. 30 kg. Thick stems, largely devoid of thin side branches with leaves, were removed from the shoots, which were then cut to *ca*. 3 mm lengths using a mechanised fodder cutter. The cut plant material (*ca*. 25 kg) was moistened by adding distilled water (4 L), followed by thorough mixing. The moistened cut material at *ca.* 60% moisture content was then divided into 18 samples of 1.5 kg each (one for each temperature x time treatment). The samples were placed in stainless steel containers and covered with a double layer of heavy weight aluminium foil to prevent excessive moisture loss during fermentation. Fermentation took place at 70°C, 80°C and 90°C in preheated CAL 3200 temperature controlled laboratory ovens, equipped with CAL 3200 temperature controls (CAL Controls Ltd., UK). One sample from each oven was removed after predetermined time intervals (12, 16, 24, 36, 48, and 60 h) and dried. For drying to a moisture content of less than 10%, the fermented plant material of each sample was spread out on four 395 mm x 565 mm Polymon 30 mesh (Swiss Silk Bolting Cloth Mfg. Co. Ltd., Zurich) trays and placed in a cross-flow temperature-controlled dehydration tunnel (Continental Fan Works, Parow, South Africa) at 40°C for 6 h. The dried tea was mechanically sieved (200 g/1.5 min at 90 rpm) (SMC, Cape Town, South Africa) and the <12>40-mesh fraction collected and stored in sealed glass jars (Consol, Stellenbosch, South Africa) until analysis. Table 2 summarises the experimental design, which consists of three blocks (with a block represented by a batch), each with 18 treatments (representing different fermentation temperature x time regimes).

**Table 1** Information regarding the plant material used for laboratory-scale fermentation.

| Batch | Source | Farm, area | Harvest date | Processing date | Moisture content (before processing) |
|---|---|---|---|---|---|
| **1** | 3 yr-old plantation | Boplaas, Barrydale[a] | 03/06/2013 | 04/06/2013 | 51.48% |
| **2** | Natural population | Louterwater, Langkloof[b] | 04/07/2013 | 08/07/2013 | 51.59% |
| **3** | 4 yr-old plantation | Boplaas, Barrydale[a] | 16/07/2013 | 16/07/2013 | 53.62% |

[a] Western Cape Province, South Africa
[b] Eastern Cape Province, South Africa

**Table 2** Experimental design for the 18 controlled laboratory-scale fermentation temperature x time treatments.

| Time | 12 h | | | 16 h | | | 24 h | | | 36 h | | | 48 h | | | 60 h | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tempe-rature (°C) | 70 | 80 | 90 | 70 | 80 | 90 | 70 | 80 | 90 | 70 | 80 | 90 | 70 | 80 | 90 | 70 | 80 | 90 |
| Batch 1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| Batch 2 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| Batch 3 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |

### *2.1.2. Validation of optimum fermentation for C. intermedia on commercial-scale*

For the second part of the study, four batches of young *Cyclopia intermedia* plant material (long green stems with an abundance of leaves) were harvested from natural stands at locations in the Langkloof in the Eastern Cape Province of South Africa. The plant material was delivered to Honeybush Natural Products Ltd (Misgund, Langkloof, South Africa) (Fig. 1) for processing to validate the optimum fermentation regime obtained in the first part of the study on commercial-scale. More information regarding the plant material is provided in Table 3. The plant material was then mechanically cut up to < 5 mm lengths using a Bovic cutter (Sandveld Ingineurswerke, Graafwater, South Africa) into clean double-walled stainless steel fermentation tanks (Fig. 2) equipped with rotating paddles to ensure adequate mixing during fermentation. The fermentation tanks were preheated using steam to 90°C. The young plant material had a high inherent moisture content of *ca.* 60% or more due to a low stem-to-leaf ratio, thus only superficial wetting was required to aid the fermentation process. Following addition of water the cut material was thoroughly mixed to spread the water throughout and to "homogenise" the batch. Laboratory-scale fermentation was executed concurrently to commercial fermentation. Four samples of *ca.* 1.5 kg each (one for each treatment) were taken from the homogenous commercial batch and fermented at 90°C as described in section 2.1.1.

Samples were removed (one each from the laboratory oven and the commercial fermentation tank) at different time intervals (16, 24, 36, and 48 h) and spread onto four 50-mesh stainless steel drying racks (370 mm x 310 mm). The drying racks were placed in a cross-flow dehydration tunnel, controlled at 40°C, and dried for 6 h to less than 10% moisture content. The dried tea was mechanically sieved (*ca*. 200 g/1.5 min at 90 rpm) and the <12>40-mesh fraction was collected and stored in labelled and sealed glass jars.

Table 4 summarises the experimental design, which consists of four blocks (batches) with 8 treatments, representing different fermentation scale x time regimes.

**Table 3** Information regarding the plant material used for the laboratory- and commercial-scale fermentation treatments.

| Batch | Plant material | Area | Harvest date | Production date | Commercial batch size |
|---|---|---|---|---|---|
| **1** | Young (low stem-to-leaf ratio) | Langkloof area | 14/02/2014 | 17/02/2014 | 330 kg |
| **2** | Young (low stem-to-leaf ratio) | Langkloof area | 18/02/2014 | 19/02/2014 | 666 kg |
| **3** | Young (low stem-to-leaf ratio) | Langkloof area | 21/02/2014 | 24/02/2014 | 653 kg |
| **4** | Young (low stem-to-leaf ratio) | Langkloof area | 24/02/2014 | 24/02/2014 | 485 kg |

**Figure 1** Honeybush plant material being delivered and weighed at Honeybush Natural Products Ltd.

**Figure 2** Plant material being cut and deposited into fermentation tanks (Honeybush Natural Products Ltd).

**Table 4** Experimental design used for concurrent fermentation on laboratory- and commercial-scale.

| | Fermentation temperature (90°C) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Commercial-scale | | | | Laboratory-scale | | | |
| **Time** | **16 h** | **24 h** | **36 h** | **48 h** | **16 h** | **24 h** | **36 h** | **48 h** |
| **Batch 1** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| **Batch 2** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| **Batch 3** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| **Batch 4** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |

### 2.2. Preparation of infusions

Infusions of the fermented tea samples used for descriptive sensory analysis and physicochemical analyses were prepared as described in Chapter 3.

### 2.3. Descriptive sensory analysis

Descriptive sensory analysis was conducted as described in Chapter 3. In order to determine the development of sensory attributes during fermentation, all samples collected at the different time intervals were included in the sample set even though some were under- or over-fermented. Attributes such as "green grass", "plant-like", "cooked vegetables" and "dusty" aroma and flavour, identified during the training session, were thus included to accurately describe the development/loss of attributes during fermentation.

### 2.4. Quality grading of infusions

The sensory quality of the infusions was determined as described in Chapter 3, Section 3.3.

### 2.5. Physicochemical analyses

Determination of soluble solids content, absorbance as a measure of colour and turbidity of infusions were conducted as described in Chapter 3. Additionally, CIELab colour parameters of infusions of the dried fermented tea leaves and infusions of tea, produced during the validation phase of the study, were determined as described in the next section.

#### *2.5.1. CIELab colour measurements*

CIELab colour parameters of both the leaves and infusions of each sample were determined using a Konica Minolta spectrophotometer CM-5 (Konica Minolta, Inc. Tokyo, Japan) set at the standard/default protocol for petri dish (30 mm) and liquid measurements, respectively. Following zero and white calibration, 10 measurements of each leaf sample (15-20 g), presented in a quartz petri dish, were taken. The leaf sample was removed from the petri dish and mixed before the next measurement. Following 0% and 100% calibration of the spectrophotometer set on the default protocol for liquids, triplicate measurements of the undiluted infusions were taken using disposable plastic cuvettes (10 mm path length; Greiner Bio-one International GmbH).

### 2.6. Statistical procedures

Statistical procedures were performed as described in Chapter 3. The experimental designs were also randomised block with batches as block repetitions. Fermentation temperature and time were considered factors effecting sensory variation in the first part of the study, while fermentation scale (laboratory- and commercial-scale) and time were factors in the second party of the study.

## 3. RESULTS

### 3.1. Laboratory-scale fermentation to optimise the fermentation temperature x time regime for *C. intermedia*

In this study the main effects (the effect of one independent variable on the dependent variable) are the effect of temperature or time on a sensory attribute or physicochemical parameter. According to statistical methods, if a significant statistical interaction ($p \leq 0.05$) exists between temperature and time, i.e. an interaction occurs when the effect of one independent variable (temperature) on the dependent variable changes as a result of changes in another independent variable (time), then the main effects cannot be interpreted and interactions are interpreted instead.

#### *3.1.1. Effect of fermentation temperature x time on the sensory attributes of C. intermedia infusions*

The p-values for the effect of temperature (70, 80, and 90°C), time (12, 16, 24, 36, 48 and 60 h), and their interaction on the sensory attributes characteristic of *C. intermedia* are presented in Tables 5, 6 and 7. The main effects will be discussed first, then the interactions.

Fermentation temperature had a significant ($p \leq 0.05$) effect on both “woody” aroma and flavour (Tables 5 & 6). *Cyclopia intermedia* infusions fermented at 90°C had a significantly stronger “woody” flavour than tea fermented at 80°C, while “woody” aroma was significantly stronger in tea fermented at 90°C than at both 70°C and 80°C (Fig. 3a). Fermentation temperature had the same effect on “lemon/lemon grass” flavour as “woody” aroma (Fig. 3). “Caramel/vanilla” aroma was significantly stronger in *C. intermedia* infusions fermented at 80°C and 90°C compared to 70°C, while “cooked apple” aroma was significantly stronger in infusions of tea fermented at 70°C and 90°C than at 80°C (Fig. 3a). Conversely, the average intensity of “honey” aroma was significantly lower in infusions of tea fermented at 90°C compared to 70°C and 80°C (Fig. 3a). A significant increase in attribute intensities with increasing fermentation time was demonstrated (Fig. 3b). The average intensity of “raisin” aroma increased significantly as the fermentation time increased from 12 to 36 h, thereafter no significant change in intensity occurred (Fig. 3b). Similarly, “cooked apple”, “caramel/vanilla”, “woody” and “dusty” aromas, and “fynbos-floral” flavour increased significantly over time and reached their highest intensities after 48 h (Fig. 3b).

The main effects on “fynbos-floral”, “fruity-sweet”, “fynbos-sweet”, “hay/dried grass”, “cooked vegetables” and “green grass” aroma attributes, “hay/dried grass” and “green grass” flavours, and sweet and sour taste could not be interpreted, because a significant ($p \leq 0.05$) interaction existed between fermentation temperature and time for these sensory attributes (Tables 5, 6 & 7). The average intensities of “fynbos-floral”, “fruity-sweet” and “fynbos-sweet” aromas were significantly higher in *C. intermedia* infusions fermented at 90°C for 36 h, after which no significant differences were demonstrated (Figs. 4a, b

& c). Fermentation at 70°C also caused a significant increase of these positive aroma intensities over time, although at lower levels than in infusions of tea fermented at 90°C. Additionally, the highest intensities were only established after 48 h when fermenting at 70°C (Figs. 4a, b & c). The development of these attribute intensities over time in infusions of tea fermented at 80°C showed no trend, although the highest intensities were only reached after 60 h (Figs. 4a, b & c). This indicates that fermentation at 90°C allows positive sensory attributes to develop faster (in less time) than the lower temperatures. "Fynbos-floral" and "fynbos-sweet" aroma attributes are prominent attributes with average intensities of > 30, even after only 12 h of fermentation at 90°C, and can thus be considered good indicators of the improvement of sensory quality over fermentation time. Sweet taste was relatively unaffected by fermentation temperature and time but *C. intermedia* infusions fermented at 70°C was significantly less sweet after 12 h and 16 h than when fermented at 80°C and 90°C (Fig. 4d).

On the contrary, negative attribute intensities decreased with increasing fermentation temperature and time (Fig. 5). The average intensities of "hay/dried grass", "cooked vegetables" and "green grass" aromas were significantly stronger in infusions of tea fermented at 70°C for 12 to 24 h than those fermented at 80°C and 90°C (Figs. 5a, b & c). The negative flavour attribute intensities followed a similar trend to that of the aromas (Figs. 5d & e), although at slightly lower intensities. Interestingly, "hay/dried grass" aroma increased between 16 and 48 h of fermentation at 80°C, whereas at 70°C and 90°C the average intensity decreased (Fig. 5a). Apart from "hay/dried grass", the average intensity of negative attributes were negligible after 36 h of fermentation at all the temperatures investigated. However, it should be noted that the average intensity of negative attributes are significantly lower in infusions of tea fermented at 90°C, even after 12 h.

The overall effect of fermentation temperature and time on the development of sensory attributes of *C. intermedia* infusions is illustrated by the PCA loadings and scores plots (Fig. 6). These plots present the positioning of the samples (representing different temperature x time fermentation regimes) with respect to each other and their characterising attributes. The sensory quality of the samples is also presented, which was determined by the constitution of positive and negative attribute intensities of each infusion. This classification according to "quality" classes was described in Chapter 3, Section 3.3, and serves as an indication of the progress of fermentation and when optimum fermentation was achieved. The PCA loadings plot shows a clear spatial separation between positive and negative (except "dusty" aroma and flavour) sensory attributes on the right and left quadrant of the plot, respectively (Fig. 6a). *Cyclopia intermedia* infusions fermented at 70°C and 80°C for 12 h, 16 h and 24 h were situated on the left side of the plot, associating with the negative sensory attributes. These samples were of "poor" and "average" sensory quality (Fig. 6b), indicating that they were under-fermented, as the intensities of negative sensory attributes constituting these samples were still prominent. Interestingly, fermentation at 80°C demonstrated a decrease in sensory quality from 24 to 36 h. Samples positioned in the centre of the

PCA scores plot (Fig. 6b), i.e. samples fermented at 90°C for 12 to 24 h and 80°C for 48 h, were of "average" sensory quality indicating that these fermentation regimes produced tea with both positive and negative attributes, indicative of tea that is still maybe under-fermented as the development of the desired attributes has not been completed. The positioning of "lemon/lemon grass" aroma and flavour and "dusty" flavour in the centre of the PCA loadings plot (Fig. 5a) suggests that these attributes are characteristic of slightly under-fermented *C. intermedia* infusions. Longer fermentation times (> 48 h) at 70°C and 80°C and fermentation at 90°C for > 36 h were positioned on the right side of the plot associating with positive sensory attributes and "good" sensory quality. Thus, to produce *C. intermedia* infusions with good sensory quality fermentation for at least 36 h at 90°C and 48 h at 70°C and 80°C was required to allow the development of positive sensory attributes at high intensities and, more importantly, to reduce the intensity of negative sensory attributes to an acceptable level. Infusions of tea fermented at 90°C had a good constituent of positive sensory attributes at relatively high intensity and significantly low intensity of negative sensory attributes across the different time regimes analysed, making fermentation at this temperature most flexible and leaving less room for error.

A distinction between samples fermented at 70°C, 80°C and 90°C can also be made. *Cyclopia intermedia* fermented at 70°C for 48 h associated with sweet taste and "cooked apple", "rose geranium" and "fynbos-floral" sensory attributes (Fig. 6). Increasing the fermentation time to 60 h at 70°C caused a greater association with "dusty" notes. Conversely to the other negative attributes, "dusty" aroma and flavour associated with positive sensory attributes on the right side of the PCA loadings plot (Fig. 6a), being an indication of over-fermented *C. intermedia* infusions. Increasing the fermentation temperature to 80°C for 60 h tend to produce tea with fruity attributes, i.e. "apricot jam" and "fruity-sweet", whereas "fynbos-sweet", "caramel/vanilla", "honey", "raisin" and "woody" notes were associated with *C. intermedia* fermented at 90°C for 36, 48 and 60 h.

**Table 5** p-Values for ANOVA table showing the effect of fermentation temperature and/or time or the interaction of temperature and time on positive aroma attributes characteristic of *C. intermedia* infusions (n = 54).

| | | Fynbos-floral_A | Rose geranium_A | Apricot jam_A | Cooked apple_A | Lemon/ Lemon grass_A | Raisin_A | Woody_A | Fruity-sweet_A | Caramel/ Vanilla_A | Honey_A | Fynbos-sweet_A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Main factors | **Temperature** | **0.027** | 0.088 | 0.388 | **0.010** | 0.704 | 0.063 | **0.005** | **0.024** | **0.017** | **0.003** | **0.002** |
| | **Time** | **0.001** | 0.153 | 0.366 | **<0.0001** | 0.724 | **<0.0001** | **0.0002** | **<0.0001** | **<0.0001** | 0.658 | **<0.0001** |
| Interaction | **Temperature x Time** | **0.014** | 0.220 | 0.164 | 0.244 | 0.658 | 0.664 | 0.479 | **0.016** | 0.454 | 0.214 | **0.005** |

**Table 6** p-Values for ANOVA table showing the effect of fermentation temperature and/or time or the interaction of temperature and time on positive flavour and taste attributes characteristic of *C. intermedia* infusions (n = 54).

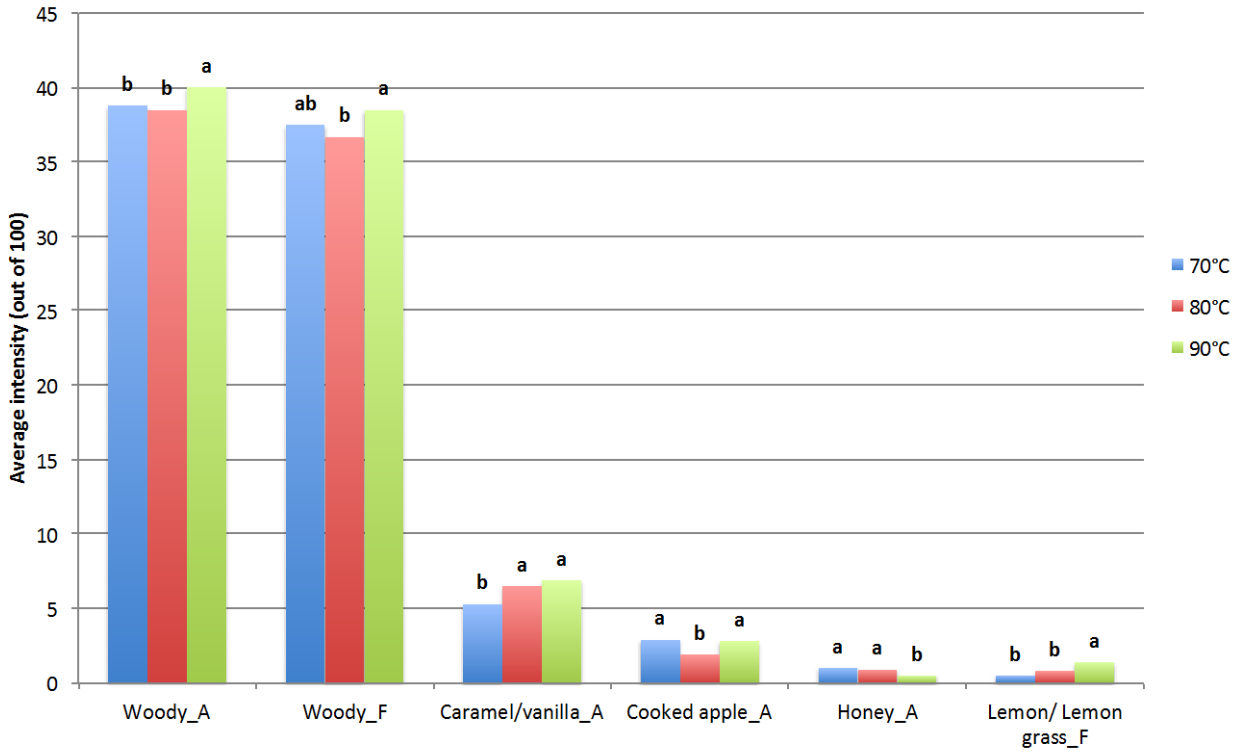
| | | Fynbos-floral_F | Lemon/Lemon grass_F | Woody_F | SWEET TASTE | SOUR TASTE | ASTRINGENT MOUTHFEEL |
|---|---|---|---|---|---|---|---|
| Main factors | **Temperature** | 0.319 | **<0.0001** | **0.004** | 0.077 | **0.004** | 0.424 |
| | **Time** | **0.0001** | 0.303 | 0.089 | **<0.0001** | **<0.0001** | 0.169 |
| Interaction | **Temperature x Time** | 0.256 | 0.973 | 0.694 | **0.003** | **<0.0001** | 0.487 |

**Table 7** p-Values for ANOVA table showing the effect of fermentation temperature and/or time or the interaction of temperature and time on negative sensory attributes characteristic of *C. intermedia* infusions, as well as attributes that describe under- and over-fermented *C. intermedia* (n = 54).

| | | Hay/Dried grass_A | Plant-like_A | Green grass_A | Cooked vegetable_A | Dusty_A | Hay/Dried grass_F | Green grass_F | Dusty_F |
|---|---|---|---|---|---|---|---|---|---|
| Main factors | **Temperature** | **0.036** | **<0.0001** | **<0.0001** | **0.001** | 0.203 | **<0.0001** | **<0.0001** | 0.680 |
| | **Time** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **0.0003** | **<0.0001** | **<0.0001** | 0.979 |
| Interaction | **Temperature x Time** | **<0.0001** | **<0.0001** | **<0.0001** | **0.002** | 0.492 | **0.024** | **<0.0001** | 0.791 |

Significant interactions ($p \leq 0.05$) are indicated in bold. The letters “A” and “F” after the attribute names refer to aroma and flavour, respectively.

a)




b)

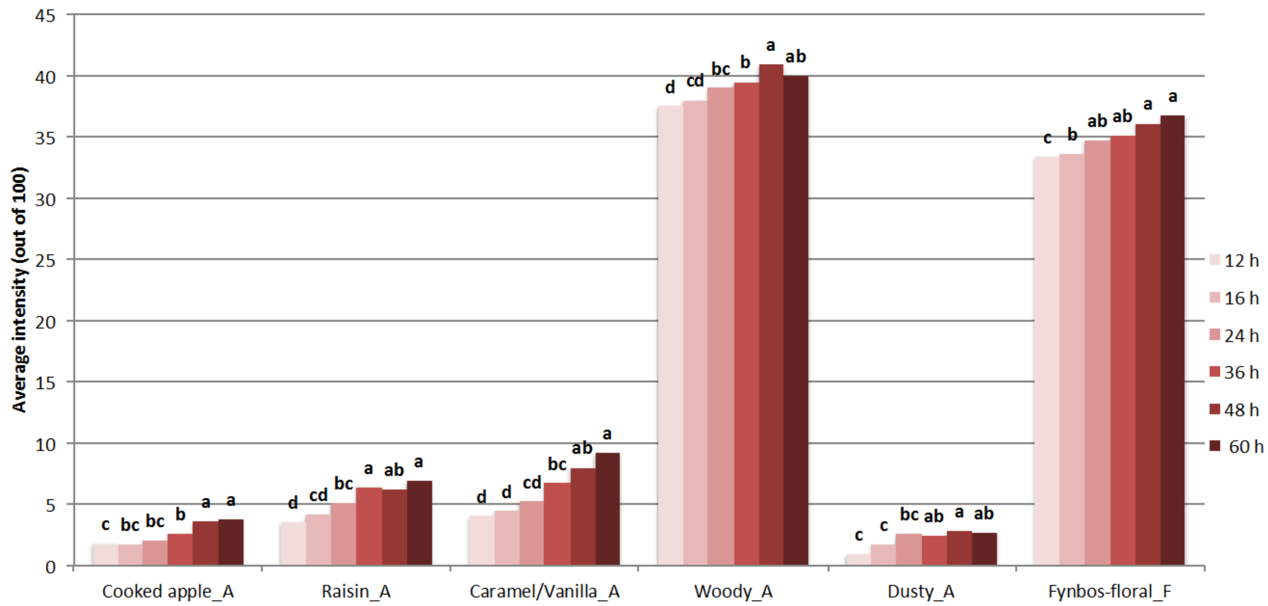



**Figure 3** Effect of fermentation a) temperature (70, 80 and 90°C) and b) time (12, 16, 24, 36, 48 and 60 h) on the average intensity ratings of aroma and flavour attributes of *C. intermedia* infusions. Bars with different letters differ significantly from each other ($p \leq 0.05$). The letters "A" and "F" after the attribute names refer to aroma and flavour, respectively.

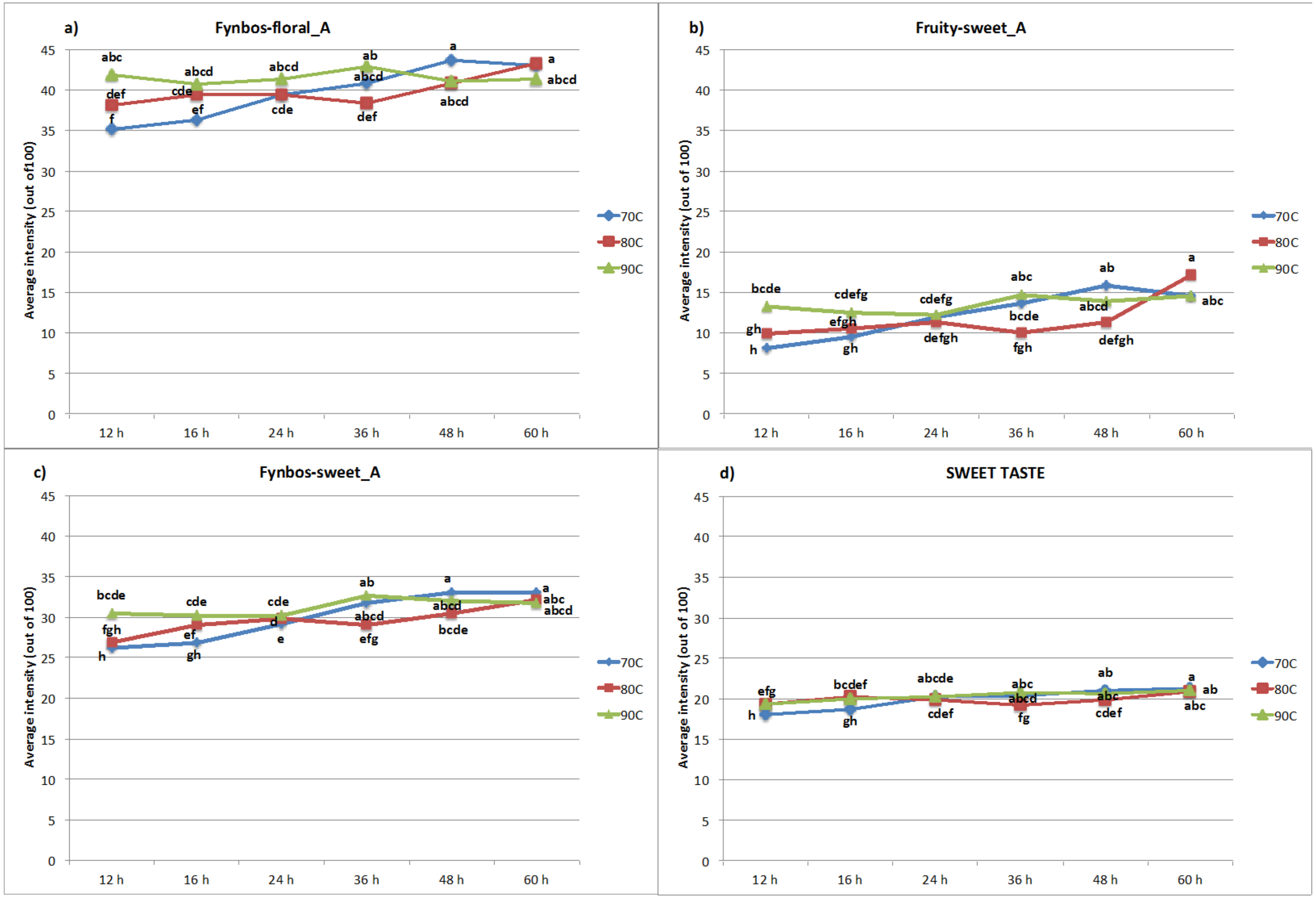



**Figure 4** Effect of fermentation temperature x time on the average intensity ratings of a) "fynbos-floral", b) "fruity-sweet" and c) "fynbos-sweet" aroma, and d) sweet taste attributes of *C. intermedia* fermented at 70, 80 and 90°C for 12, 16, 24, 36, 48 and 60 h. Values with different letters differ significantly from each other ($p \leq 0.05$). The letter "A" after the attribute name refers to aroma.

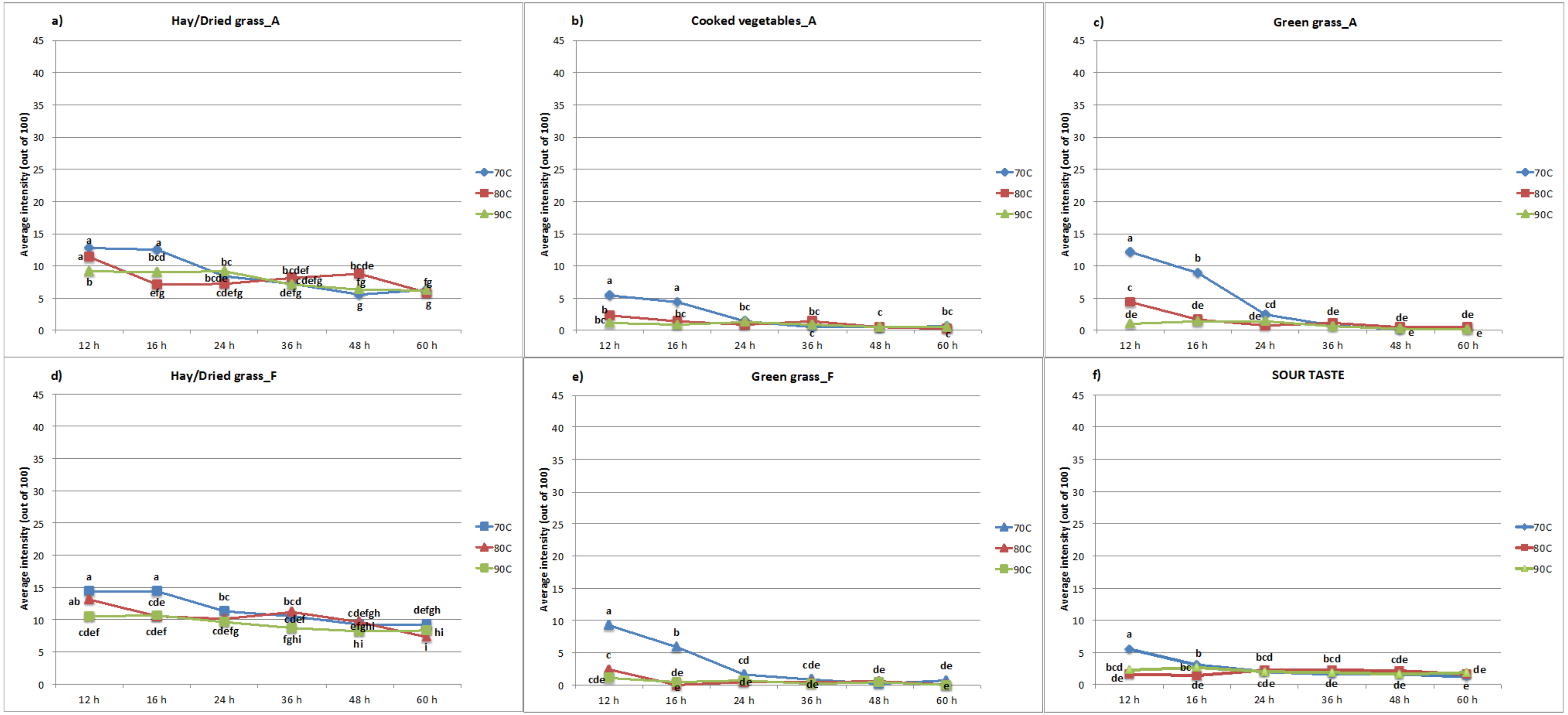



**Figure 5** Effect of fermentation temperature x time on the average intensity ratings of a) "hay/dried grass", b) "cooked vegetables" and c) "green grass" aroma, d) "hay/dried grass" and e) "green grass" flavour, and f) sour taste attributes of *C. intermedia* fermented at 70, 80 and 90°C for 12, 16, 24, 36, 48 and 60 h. Values with different letters differ significantly from each other ($p \leq 0.05$). The letter "F" after the attribute name refers to flavour.

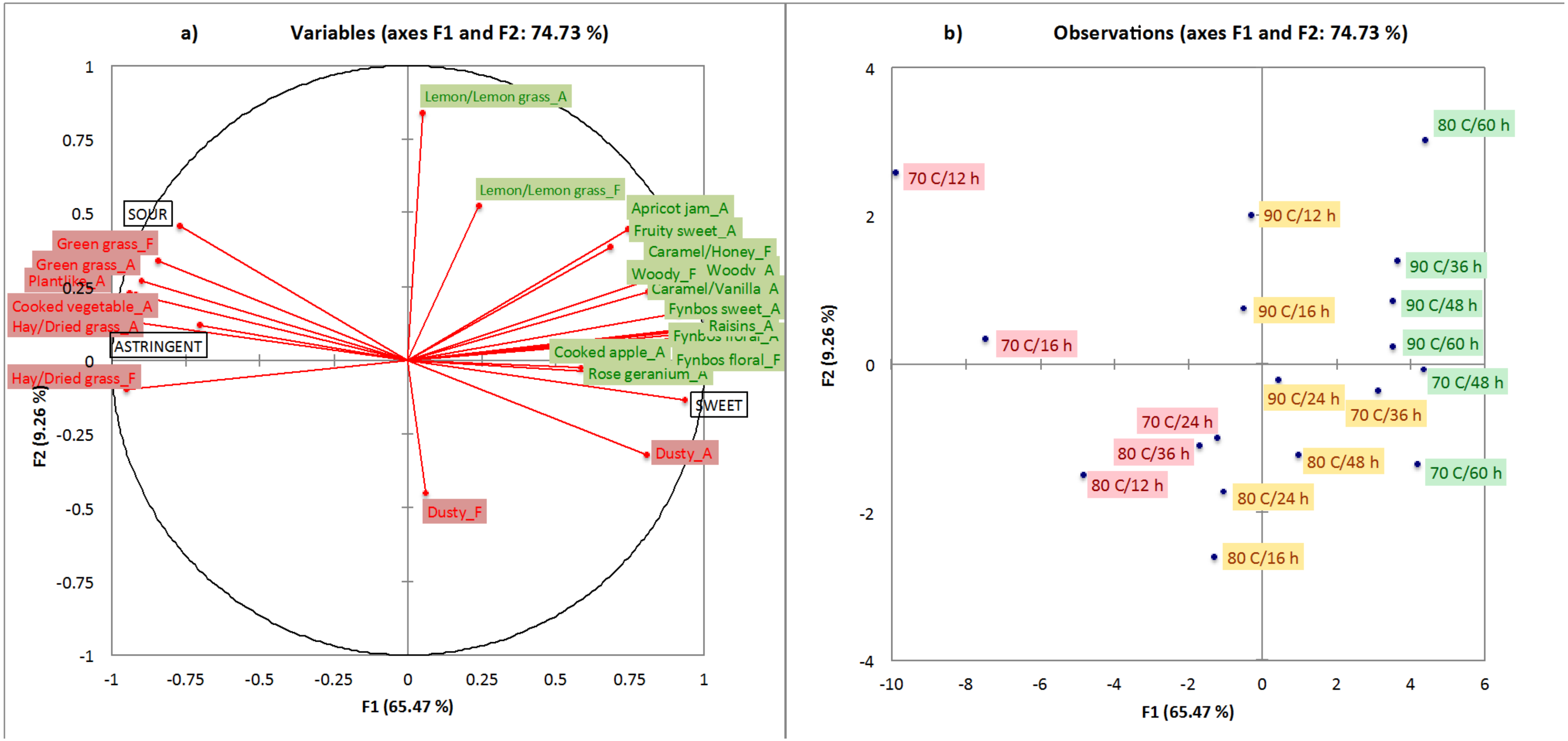



**Figure 6** a) PCA loadings plot showing the positioning of sensory attributes of *C. intermedia* infusions. Aroma (A) and flavour (F) attributes are indicated as positive (green) and negative (red). Taste and mouthfeel attributes are indicated in black. b) PCA scores plot showing the positioning of *C. intermedia* samples fermented with different temperature x time regimes on laboratory-scale, graded as “good” (green), “average” (yellow) and “poor” (red) sensory quality.

### *3.1.2. Effect of fermentation temperature x time on the physicochemical parameters of C. intermedia infusions*

The interaction of temperature and time did not have a significant effect on any of the physicochemical parameters of *C. intermedia* infusions, thus the main effects will be discussed (Table 8). The effect of fermentation temperature (70, 80 and 90°C) on the physicochemical parameters are summarised in Table 9, while the effect of fermentation time (12, 16, 24, 36, 48 and 60 h) is summarised in Table 10. The different temperatures differed significantly ($p \leq 0.05$) with regard to their effect on the soluble solids (SS) content and turbidity of infusions (Table 8). Infusions fermented at 90°C had significantly higher SS contents and more were significantly more turbid than those fermented at 70°C and 80°C (Table 9). The colour (AUC) of infusions was not influenced by fermentation temperature (Table 8). All the physicochemical parameters measured were affected significantly by fermentation time (Table 8). Both SS content and colour (AUC) of infusions showed a sequential decrease as fermentation time increased, particularly after 36 h. The turbidity of infusions remained relatively stable from 12 to 36 h; thereafter a significant decrease was demonstrated (Table 10).

The relationship between physicochemical parameters, and between physicochemical parameters and sensory quality of infusions of tea was investigated. Strong positive correlations (>0.7) existed between all physicochemical parameters (Table 11) and sensory quality (Fig. 7). According to the scatter plots (Fig. 7), which also indicate the fermentation temperature and time regimes of a specific data point, increasing fermentation time had the most detrimental effect on the physicochemical parameters, in particular SS content and colour. Low turbidity is preferred. Thus, infusions fermented for 60 h, regardless of the temperature, had the least SS, colour and turbidity (Fig. 7). This also indicates that samples of "good" sensory quality had lower SS, colour and turbidity (Fig. 7). With regard to turbidity, increasing fermentation time would thus be a solution to decreasing the turbidity of infusions fermented on laboratory-scale, however, the detrimental effect on SS and colour is not desirable. Interestingly, fermentation at 90°C for 12 to 36 h effected the physicochemical parameters of infusions the least. Fermentation at 90°C for 36 h would thus be optimal to produce *C. intermedia* of good sensory quality with high SS content and good colour. Although the turbidity of infusions fermented at 90°C for 36 h was relatively high, in comparison to the extremely turbid commercial samples described in Chapter 3, these NTU values seem to be acceptable (Fig. 8c).

Comparing the physicochemical parameters of the commercial *C. intermedia* infusions described in Chapter 3 with those in this study (Table 12; Fig. 8) illustrated that the means of the SS content and colour of infusions fermented on laboratory-scale were higher, while the turbidity was lower, and the range of all measurements was lower.

**Table 8** p-Values for ANOVA table showing the effect of fermentation temperature and/or time or the interaction of temperature and time on the physicochemical parameters of *C. intermedia* infusions fermented on laboratory-scale (n = 54).

| | | SS (g/100 mL) | AUC (370-510 nm) | NTU |
|---|---|---|---|---|
| Main factors | **Batch** | **0.0003** | **<0.0001** | **<0.0001** |
| | **Temperature** | **<0.0001** | 0.136 | **0.004** |
| | **Time** | **<0.0001** | **<0.0001** | **0.002** |
| Interaction | **Temperature x Time** | 0.585 | 0.408 | 0.241 |

Significant interactions ($p \leq 0.05$) are indicated in bold. SS = soluble solids, AUC = area under the curve, NTU = nephelometric turbidity units.

**Table 9** Effect of fermentation temperature (70, 80 and 90°C) on the physicochemical parameters of *C. intermedia* infusions fermented on laboratory-scale (n = 54).

| Temperature | SS (g/100 mL) | AUC (370-510 nm) | NTU |
|---|---|---|---|
| 70°C | 0.168[b] | 72.14[ab] | 96.65[b] |
| 80°C | 0.176[b] | 69.74[b] | 88.13[b] |
| 90°C | 0.198[a] | 75.36[a] | 134.01[a] |
| LSD | 0.01 | 5.58 | 27.69 |

Values in the same column with different superscript letters are significantly different ($p \leq 0.05$). SS = soluble solids, AUC = area under the curve, NTU = nephelometric turbidity units.

**Table 10** Effect of fermentation time (12, 16, 24, 36, 48 and 60 h) on the physicochemical parameters of *C. intermedia* infusions fermented on laboratory-scale (n = 54).

| Time | SS (g/100 mL) | AUC (370-510 nm) | NTU |
|---|---|---|---|
| 12 h | 0.196[a] | 82.71[a] | 124.52[a] |
| 16 h | 0.189[ab] | 80.56[a] | 118.48[ab] |
| 24 h | 0.187[ab] | 79.38[ab] | 135.73[a] |
| 36 h | 0.179[bc] | 71.70[bc] | 118.11[ab] |
| 48 h | 0.172[cd] | 66.32[c] | 83.68[bc] |
| 60 h | 0.160[d] | 53.81[d] | 57.05[c] |
| LSD | 0.014 | 5.58 | 39.16 |

Values in the same column with different superscript letters are significantly different ($p \leq 0.05$). SS = soluble solids, AUC = area under the curve, NTU = nephelometric turbidity units.

**Table 11** Pearson's correlation coefficients (r) illustrating the relationship between physicochemical parameters of *C. intermedia* infusions.

| Variables | SS (g/100 mL) | AUC (370-510 nm) | NTU |
|---|---|---|---|
| **SS (g/100 mL)** | **1** | **0.791** | **0.865** |
| **AUC (370-510 nm)** | **0.791** | **1** | **0.843** |
| **NTU** | **0.865** | **0.843** | **1** |

Significant correlations ($p \leq 0.05$) are indicated in bold. Correlations > ± 0.7 are indicated in red. SS = soluble solids, AUC = area under the curve (370 – 550 nm), NTU = nephelometric turbidity units.

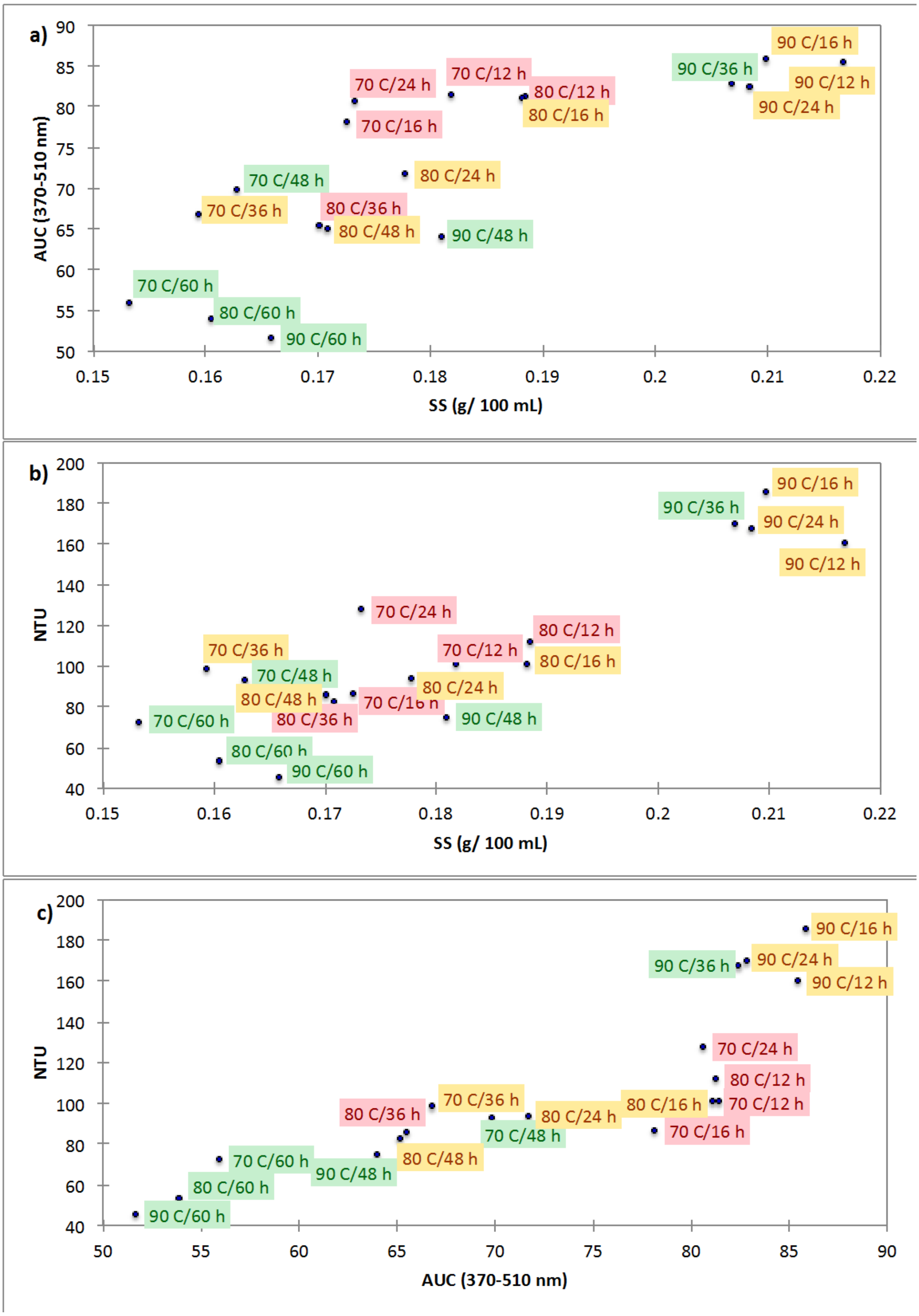



**Figure 7** Scatter plots illustrating the relationship between a) SS content and colour (AUC), b) SS content and turbidity (NTU), and c) colour (AUC) and turbidity (NTU) of *C. intermedia* infusions. The fermentation temperature x time treatments as well as the sensory quality of the samples is also presented as labels. Samples of "good" sensory quality are illustrated in green, "average" quality in yellow and "poor" quality in red. SS = soluble solids, AUC = area under the curve, NTU = nephelometric turbidity units.

**Table 12** Descriptive statistics comparing the SS content, colour (AUC) and turbidity (NTU) of commercial *C. intermedia* infusions from Chapter 3 and *C. intermedia* infusions fermented on laboratory-scale.

| | SS (g/100 mL) | | AUC (370-510 nm) | | NTU | |
|---|---|---|---|---|---|---|
| | **Commercial *C. intermedia*** | **Laboratory fermented *C. intermedia*** | **Commercial *C. intermedia*** | **Laboratory fermented *C. intermedia*** | **Commercial *C. intermedia*** | **Laboratory fermented *C. intermedia*** |
| **Minimum** | 0.076 | 0.153 | 22.497 | 51.633 | 5.697 | 45.330 |
| **Maximum** | 0.250 | 0.217 | 145.567 | 85.839 | 802.887 | 185.956 |
| **Range** | 0.174 | 0.064 | 123.070 | 34.206 | 797.190 | 140.626 |
| **Mean** | 0.131 | 0.180 | 62.103 | 72.414 | 120.174 | 106.263 |
| **Standard deviation** | 0.046 | 0.019 | 30.095 | 11.250 | 165.247 | 40.568 |

SS = soluble solids, AUC = area under the curve, NTU = nephelometric turbidity units.

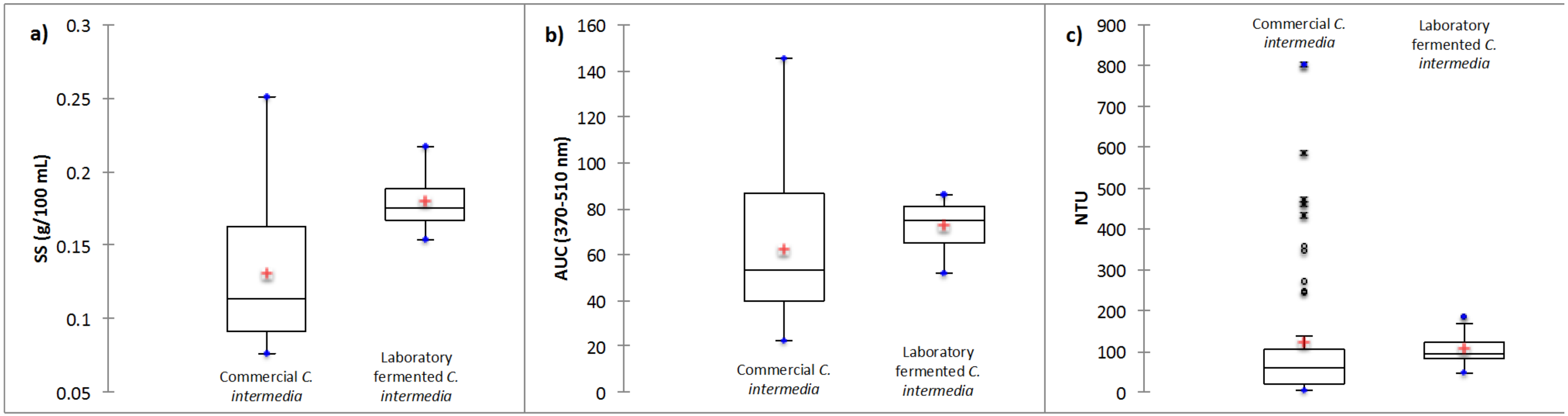



**Figure 8** Box plots comparing the a) SS content, b) colour (AUC) and c) turbidity (NTU) of commercial *C. intermedia* infusions from Chapter 3 and *C. intermedia* infusions fermented on laboratory-scale. SS = soluble solids, AUC = area under the curve, NTU = nephelometric turbidity units.

### 3.2. Comparison of laboratory- and commercial-scale fermentation of *C. intermedia* to validate the optimum fermentation temperature x time regime for commercial application

In this part of the study the main effects (the effect of one independent variable on the dependent variable) are the different fermentation scales (laboratory- and commercial-scale) and/or fermentation time on the sensory attributes and physicochemical parameters of *C. intermedia* infusions.

#### *3.2.1. Effect of fermentation scale x time on the sensory attributes of C. intermedia infusions*

The p-values for the effect of the different fermentation scales, time or their interaction on the sensory attributes of *C. intermedia* infusions are presented in Tables 13-15. The main effects will be discussed first, then the interactions.

Fermentation scale (laboratory- and commercial-scale) had a significant ($p \leq 0.05$) effect on "dusty" aroma and flavour, "raisin", "caramel/vanilla" and "honey" aroma and sour taste (Tables 13-15). These aroma and flavour attributes were significantly stronger in infusions of tea fermented on a laboratory-scale, except "honey" aroma was stronger in infusions of tea fermented on commercial-scale. Those fermented on commercial-scale were also significantly more sour (Fig. 9). The average intensity of <2 (out of 100) for sour taste would, however, be undetectable in an infusion.

Fermentation time had a significant effect on the attributes mentioned above, as well as "cooked apple" aroma and "green grass" aroma and flavour (Tables 13-15). No significant effects on the intensity of "raisin" and "caramel/vanilla" aromas and "dusty" aroma and flavour were demonstrated from 16 to 36 h of fermentation, however, the intensities increased significantly after 48 h of fermentation (Fig. 10). Sour taste increased significantly after 36 h of fermentation. Conversely, the intensity of "honey" and "green grass" aroma decreased significantly after 24 h of fermentation. Although the effect of fermentation time on "green grass" flavour does not show a clear trend, the intensity decreased significantly after 24 and 48 h of fermentation. The average intensity of "green grass" aroma and flavour were, however, very low. "Cooked apple" aroma showed no clear pattern (Fig. 10).

Fermentation scale and time had an interactive effect on the intensities of "rose geranium", "woody" and "hay/dried grass" aromas, "fynbos-floral" and "woody" flavours and astringent mouthfeel (Tables 13-15). The development of "woody" aroma and flavour showed similar trends over time when fermenting on laboratory- or commercial-scale, i.e. the intensities increased significantly as fermentation time increased to 48 h (Figs. 11b & f). No significant change in astringent mouthfeel was demonstrated during fermentation on laboratory-scale, while astringency increased significantly after 48 h of fermentation on commercial-scale (Fig. 11d). The intensity of "fynbos-floral" flavour remained more or less constant during fermentation, while the scale of fermentation only had an effect at 16 h (Fig. 11e). "Rose geranium" aroma demonstrated a similar trend to "fynbos-floral" flavour, however the intensity of "rose geranium" aroma decreased significantly after 24 h of fermentation on commercial-scale, thereafter no significant difference in intensity between laboratory- and commercial-scale fermentation was observed (Fig. 11a). Fermentation scale x time also had opposite effects on “hay/dried grass” aroma where the intensity decreased significantly during fermentation on laboratory-scale and increased on commercial-scale (Fig. 11c). The change in intensity was, however, very small and was not significant for tea fermented on commercial-scale. The laboratory-scale samples only differed significantly between 16 and 48 h with regard to “hay/dried grass” aroma.

PCA loadings and scores plots were used to assist in analysing the relationship between the observations and variables (Fig. 12) as the plots illustrate the positioning of samples (representing different fermentation scales and times) with respect to each other and their characterising attributes. The means of the individual batch scores, which were graded according to their sensory quality, were presented to illustrate the variation between batches (Fig. 12b). The positive and negative sensory attributes were separated across F2 of the PCA loadings plot (Fig. 12a). The negative attributes were further separated across F1 into attributes characteristic of under-fermented *C. intermedia*, i.e. “green grass”, “plant-like” and “hay/dried grass”, and over-fermented tea, i.e. “dusty”. The positive attributes were separated into floral (i.e. “fynbos-floral”, “rose geranium”, “fynbos-sweet”), “honey” and “cooked apple” attributes in the top left corner of the PCA loadings plot and fruity (i.e. “fruity-sweet”, “apricot jam” and “raisin”), “woody” and “caramel/vanilla” attributes in the top right of the plot (Fig. 12a). This separation of attributes is indicative of the development of attributes over time on different fermentation scales as the majority of commercial-scale fermented samples were positioned on the left of the PCA scores plot (Fig. 12b) associating with floral attributes, whereas the majority of laboratory-scale samples were positioned on the right (Fig. 12b) associating with fruity attributes. The effect of fermentation scale on the development of sensory attributes is highlighted by the incoherent positioning of corresponding laboratory- and commercial-scale samples (Fig. 12b). Additionally, the large variation between the laboratory- and commercial- scale batches, respectively, made distinctions challenging. It would be expected of the laboratory-scale batches to display less variation, i.e. lie closely together on the PCA scores plot (Fig. 12b) and be of similar sensory quality, due to the controlled and consistent fermentation conditions on small-scale, indicating that the plant material used may contribute to the sensory variation between batches. However, the only samples of “poor” sensory quality were those from batch 1, indicating that fermentation at 90°C generally produces tea of acceptable sensory quality despite the fermentation time, but also highlights other fermentation conditions that effect quality, which will be addressed at a later stage.

In order to extract more information, PCA bi-plots were generated displaying the relationship between *C. intermedia* samples and negative and positive sensory attributes, separately (Figs. 13a & b). It is evident that the presence and intensity of negative attributes in an infusion was the driver in determining sensory quality (Fig. 13a). The negative sensory attributes are separated into under-fermented and over-fermented attributes on the right and left hand side of the PCA bi-plot, respectively (Fig. 13a), while the samples were spread across this factor space between these attributes from 16 h to 48 h. Samples fermented for shorter fermentation times (16 h) associated with “green grass” and “plant-like” on the right of the PCA bi-plot (Fig. 13a). Most of the laboratory-scale samples fermented for 16 h were of “poor” sensory quality while the commercial-scale counterparts were all of “average” sensory quality (Fig. 13a). This is qualified by the positioning of these commercial-scale samples close to positive sensory attributes such as “honey” and “rose geranium” on the left of the PCA bi-plot (Fig. 13b). Samples fermented for 24 h were positioned predominantly in the centre of the plot associating with “hay/dried grass”. Most of the laboratory-scale samples fermented for 24 h were of “average” sensory quality due to the high intensity of “hay/dried grass” in those infusions, while half of the commercial-scale samples fermented for 24 h were of “good” sensory quality (Fig. 13a). Samples fermented for 36 and 48 h were typically positioned furthest away from the negative sensory attributes and were mostly of “average” and “good” sensory quality (Fig. 13a), indicating a transition towards increased intensity of positive sensory attributes positioned on the right of the PCA bi-plot at this point (Fig. 13b). Most of the laboratory-scale samples were still of “average” sensory quality after 36 h of fermentation and still associated slightly with “hay/dried grass”, while most of the commercial-scale counterparts were of “good” sensory quality (Fig. 13a). After 48 h of fermentation, most of the laboratory-scale samples were of “good” sensory quality associating with “apricot jam”, “caramel/vanilla” and “raisin” (Fig. 13b), while the commercial-scale counterparts were of “average” sensory quality and associated with sensory attributes characteristic of over-fermented tea, i.e. “dusty”.

Batch 1 displayed uncharacteristic conduct, which will be discussed at a later stage (Fig. 13a). A log of temperature during fermentation on commercial- and laboratory-scale (Figs. 14 & 15) was investigated to provide insight into the differences that exist between scales of fermentation. Fermentation temperature increased to > 85°C in *ca*. 1 h on commercial-scale (Fig. 14), while it took *ca*. 6 h to reach this point on laboratory-scale (Fig. 15). Despite a slow “come-up” time, fermentation temperature remained relatively constant between 88 and 90°C on laboratory-scale (Fig. 15), whereas temperature fluctuations up to 10°C occurred during commercial-scale fermentation (Fig. 14). Additionally, the temperature did not reach 90°C during fermentation on commercial-scale and a major decrease in temperature occurred between 17 and 23 h of fermentation of batch 1 (Fig. 14).

**Table 13** p-Values for ANOVA table showing the effect of fermentation scale (laboratory- and commercial-scale) and/or time or the interaction of fermentation scale and time on positive aroma attributes characteristic of *C. intermedia* infusions (n = 32).

| | | Fynbos-floral_A | Rose geranium_A | Apricot jam_A | Cooked apple_A | Raisin_A | Woody_A | Fruity-sweet_A | Caramel/ Vanilla_A | Honey_A | Fynbos-sweet_A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Main factors | **Batch** | **0.042** | **0.0004** | **0.001** | 0.386 | 0.449 | **<0.0001** | **0.028** | 0.102 | **0.019** | **0.025** |
| | **Scale** | 0.864 | **0.023** | 0.096 | 0.593 | **0.024** | **<0.0001** | 0.120 | **0.0001** | **0.031** | 0.990 |
| | **Time** | 0.793 | 0.313 | 0.092 | **0.018** | **0.0004** | **<0.0001** | 0.140 | **<0.0001** | **0.007** | 0.150 |
| Interaction | **Scale x Time** | 0.096 | **0.027** | 0.477 | 0.139 | 0.564 | **0.008** | 0.281 | 0.448 | 0.141 | 0.357 |

**Table 14** p-Values for ANOVA table showing the effect of fermentation scale (laboratory- and commercial-scale) and/or time or the interaction of fermentation scale and time on positive flavour and taste attributes characteristic of *C. intermedia* infusions (n = 32).

| | | Fynbos-floral_F | Woody_F | Fynbos-sweet_F | SWEET TASTE | SOUR TASTE | ASTRINGENT MOUTHFEEL |
|---|---|---|---|---|---|---|---|
| | **Batch** | **0.003** | **<0.0001** | **0.017** | **<0.0001** | 0.277 | **0.037** |
| Main factors | **Scale** | 0.305 | **<0.0001** | 0.569 | 0.312 | **0.003** | 0.253 |
| | **Time** | 0.720 | **<0.0001** | 0.093 | 0.628 | **0.0002** | **0.001** |
| Interactions | **Scale x Time** | **0.038** | **0.034** | 0.408 | 0.109 | 0.052 | **0.023** |

**Table 15** p-Values for ANOVA table showing the effect of fermentation scale (laboratory- and commercial-scale) and/or time or the interaction of fermentation scale and time on negative aroma and flavour attributes characteristic of *C. intermedia* infusions (n = 32).

| | | Hay/Dried grass_A | Plant-like_A | Green grass_A | Dusty_A | Hay/Dried grass_F | Green grass_F | Dusty_F |
|---|---|---|---|---|---|---|---|---|
| | **Batch** | **0.003** | 0.849 | 0.054 | 0.095 | **0.0002** | 0.633 | **<0.0001** |
| Main factors | **Scale** | 0.740 | 0.062 | 0.060 | **0.031** | 0.062 | 0.065 | **0.002** |
| | **Time** | 0683 | 0.096 | **0.013** | **0.0003** | 0.979 | **0.018** | **0.001** |
| Interaction | **Temperature x Time** | **0.039** | 0.687 | 0.370 | 0.746 | 0.398 | 0.458 | 0.966 |

Significant interactions (p ≤ 0.05) are indicated in bold. The letters "A" and "F" after the attribute names refer to aroma and flavour, respectively.

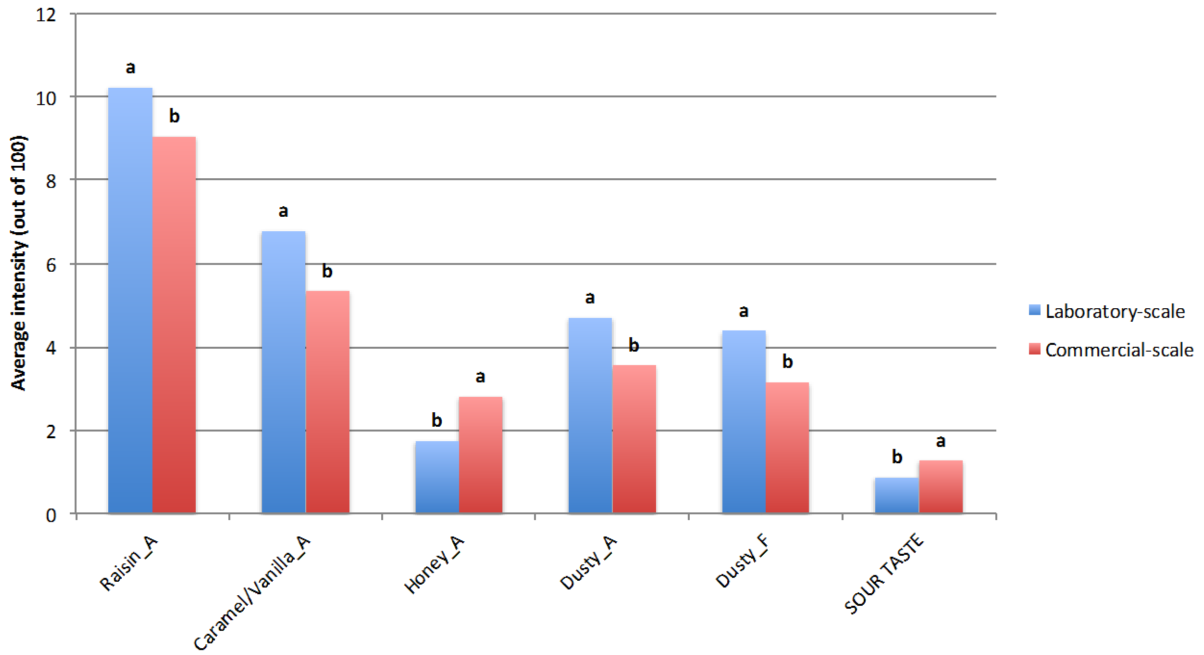



**Figure 9** Effect of fermentation scale (laboratory- and commercial-scale) on the average intensity ratings of "raisin", "caramel/vanilla" and "dusty" aromas, "dusty" flavour and sour taste of *C. intermedia* infusions. Columns with different letters differ significantly from each other ($p \leq 0.05$). The letters "A" and "F" after the attribute name refer to aroma and flavour, respectively.

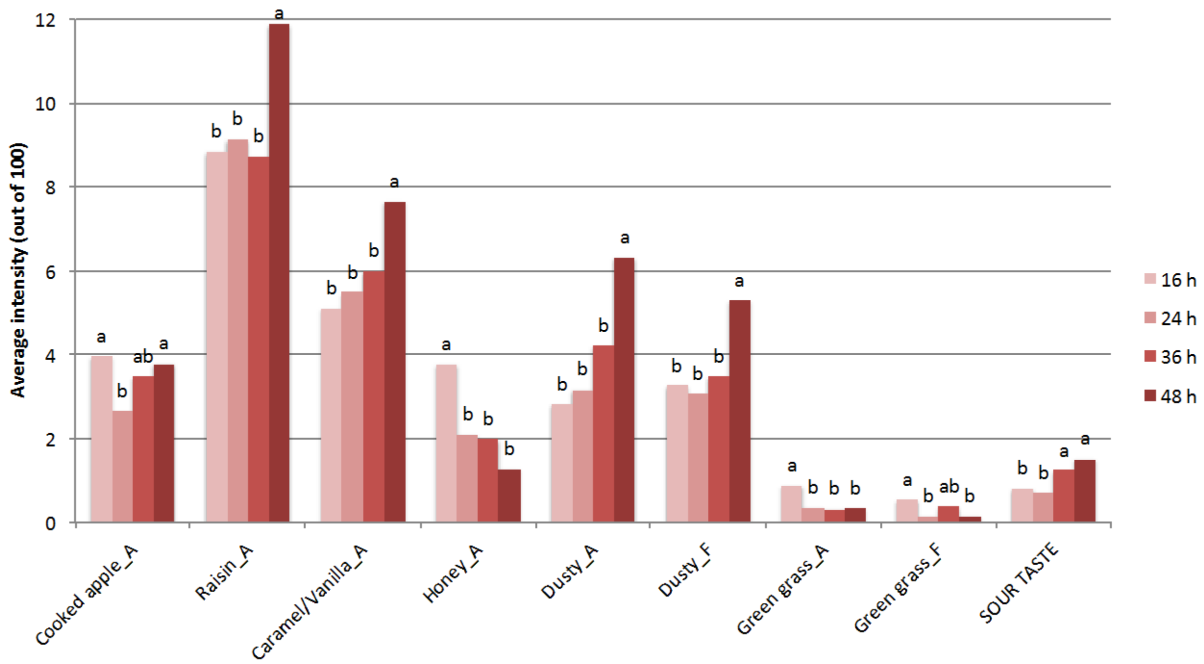



**Figure 10** Effect of fermentation time on the average intensity ratings of "cooked apple", "raisin", "caramel/vanilla", "dusty" and "green grass" aromas, "dusty" and "green grass" flavours and sour taste of *C. intermedia* infusions fermented on laboratory- and commercial-scale. Columns with different letters differ significantly from each other ($p \leq 0.05$). The letters "A" and "F" after the attribute name refer to aroma and flavour, respectively.

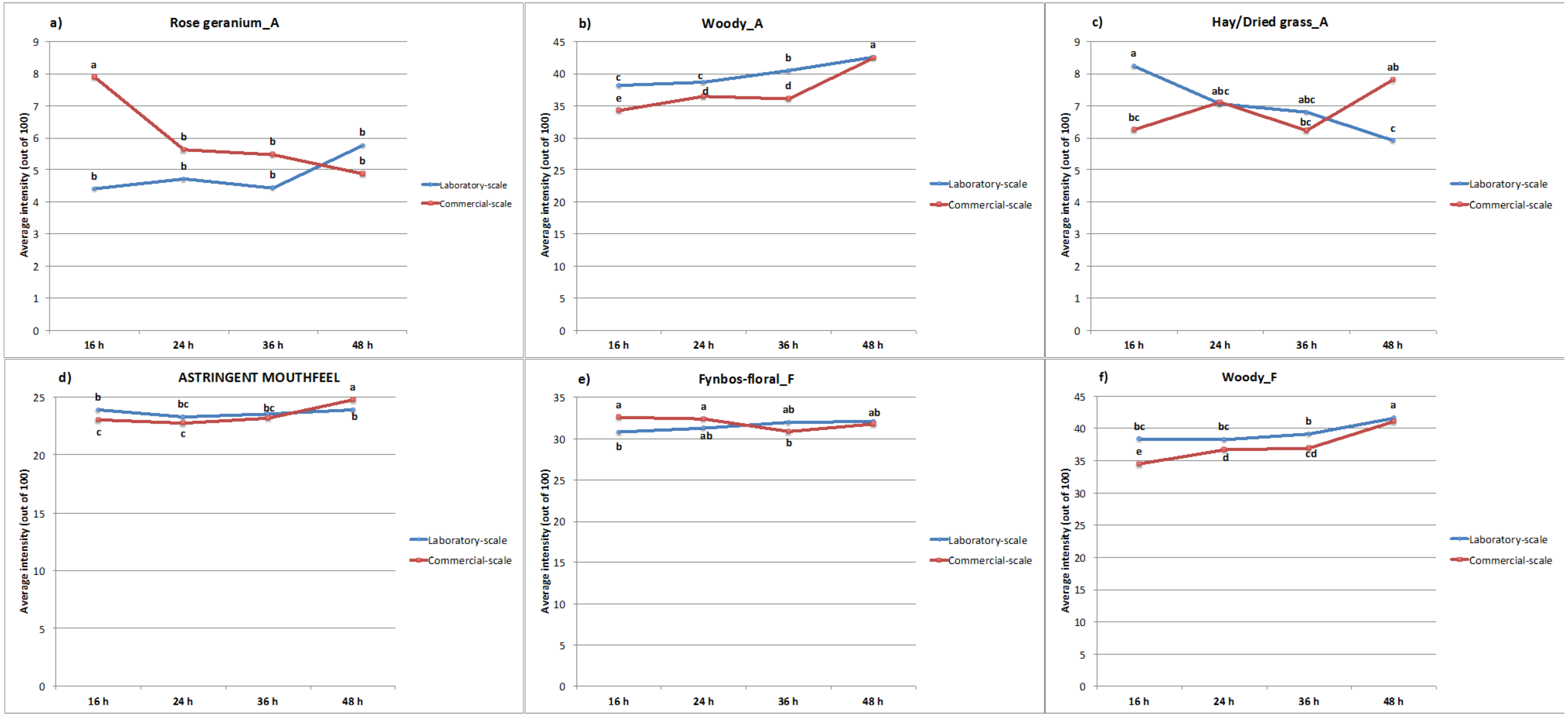



**Figure 11** Effect of fermentation scale x time on the average intensity ratings of a) “rose geranium”, b) “woody” and c) “hay/dried grass” aromas, d) astringent mouthfeel, and e) “fynbos-floral” and f) “woody” flavours of *C. intermedia* infusions fermented at laboratory- and commercial-scale for 16, 24, 36 and 48 h. Values with different letters differ significantly from each other ($p \leq 0.05$). The letters “A” and “F” after the attribute name refer to aroma and flavour, respectively.

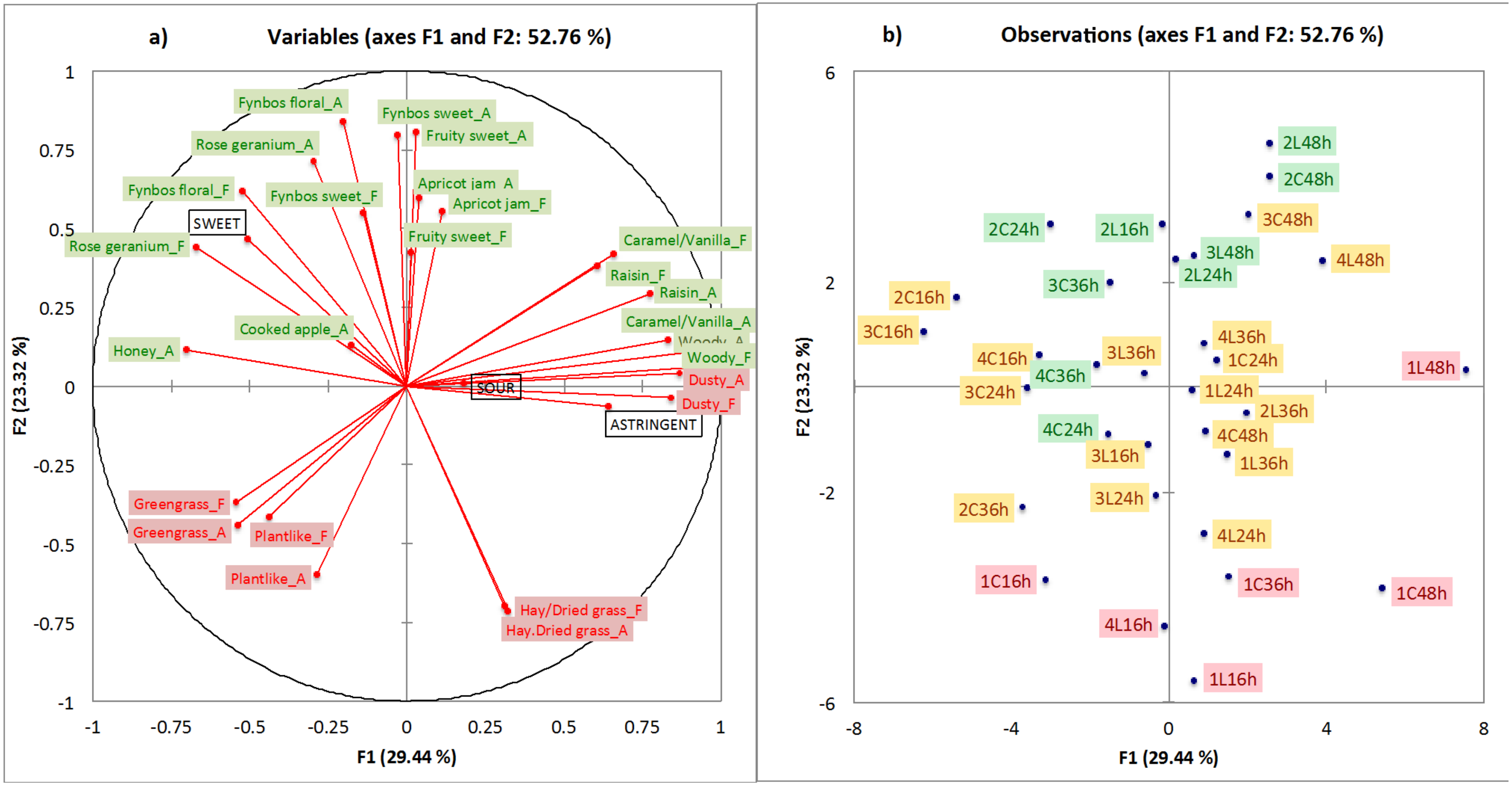



**Figure 12** a) PCA loadings plot showing the positioning of sensory attributes of *C. intermedia* infusions. Aroma (A) and flavour (F) attributes are indicated as positive (green) and negative (red). Taste and mouthfeel attributes are indicated in black. b) PCA scores plot showing the positioning of *C. intermedia* samples fermented on laboratory (L)- and commercial (C)-scale for 16, 24, 36 and 48 h, graded as “poor” (red), “average” (yellow) and “good” (green) sensory quality. Numbers 1, 2, 3 and 4 denote individual batches.

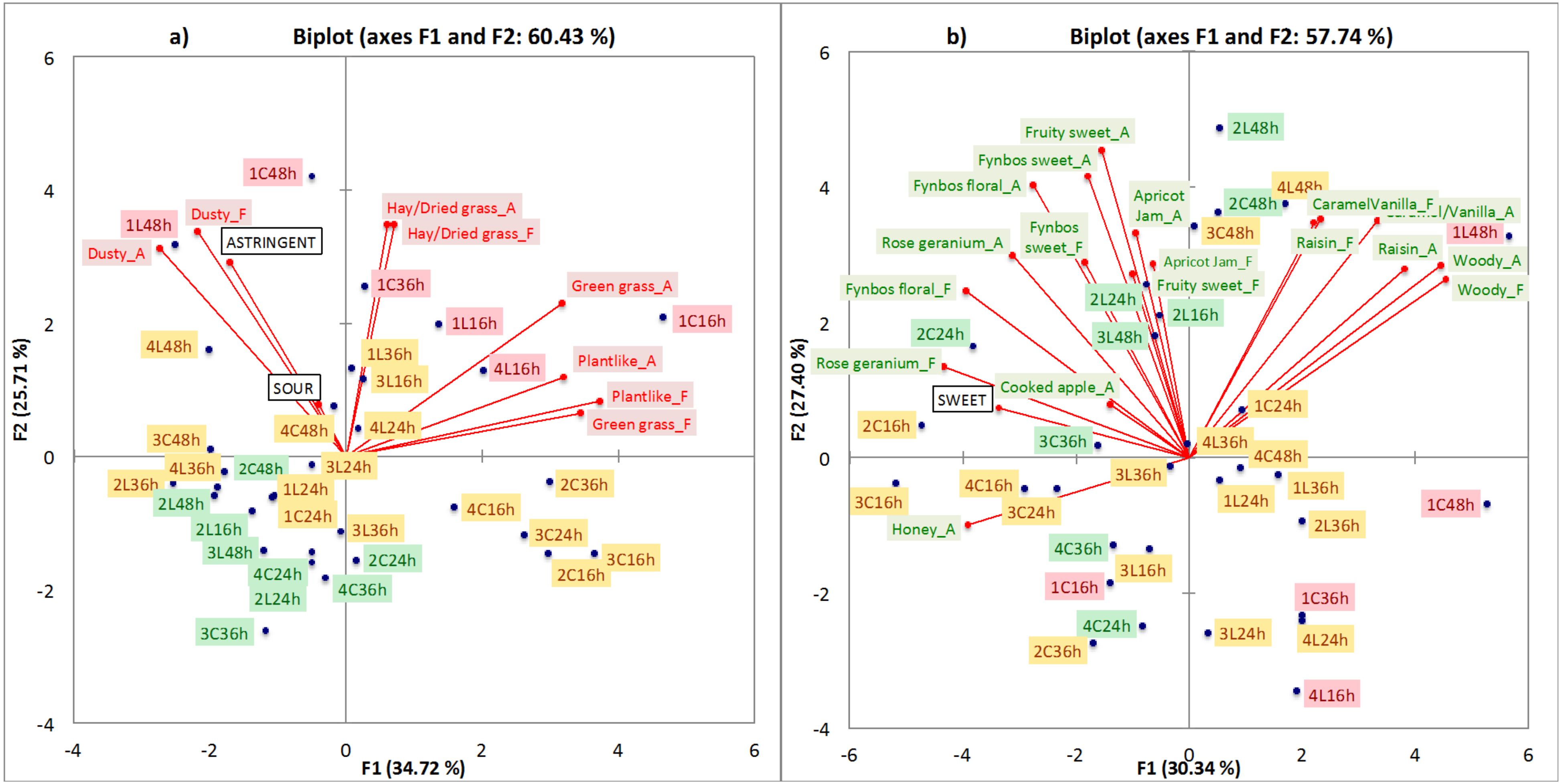



**Figure 13** PCA bi-plots illustrating the relationship between a) negative and b) positive sensory attributes of *C. intermedia* samples fermented on laboratory (L)- and commercial (C)-scale for 16, 24, 36 and 48 h, graded as "good" (green), "average" (yellow) and "poor" (red) sensory quality. Numbers 1, 2, 3 and 4 denote individual batches.

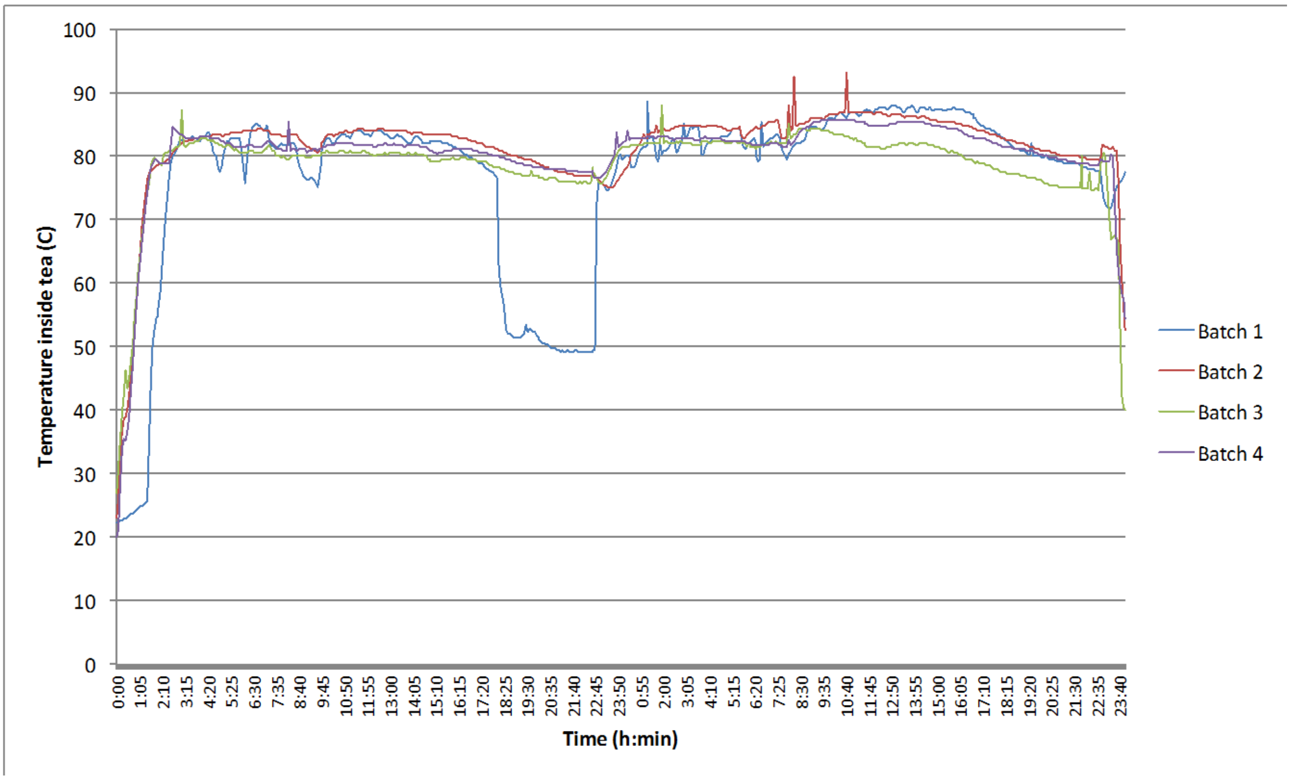



**Figure 14** Variation of fermentation temperature over time during commercial-scale fermentation. The temperature was monitored by a probe situated in the centre of the tea, near the centre of the fermentation tank.

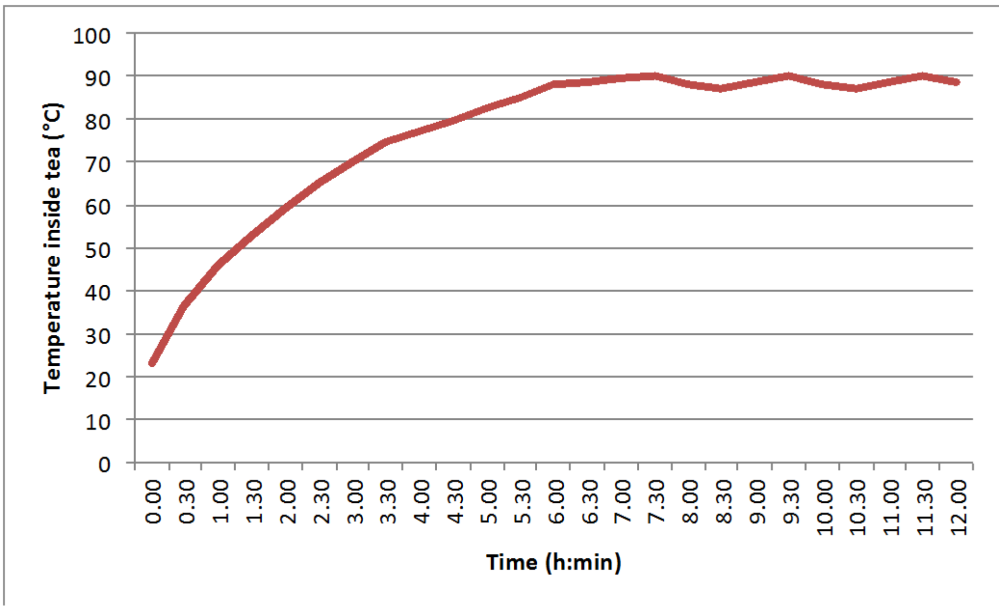



**Figure 15** Log of the temperature in the centre of 1.5 kg tea fermented on laboratory-scale demonstrating the come-up time and variation of temperature over time.

### *3.2.2. Effect of fermentation scale x time on the physicochemical parameters of C. intermedia infusions*

The p-values for the effect of different fermentation scales, time and their interaction on the physicochemical parameters of *C. intermedia* infusions and dried fermented leaves are presented in Table 16. The correlation coefficients, which illustrate the relationship between physicochemical parameters, are presented in Table 17. The three coordinates of CIELab represent the lightness of the colour ($L^*$ = 0 indicates black and $L^*$ = 100 indicates white), its position between red and green ($a^*$, negative values indicate green while positive values indicate red) and its position between yellow and blue ($b^*$, negative values indicate blue and positive values indicate yellow). Values of $a^*$ and $b^*$ of *C. intermedia* infusions were on the positive scales suggesting that the infusions were varying degrees of red and yellow in colour. The interaction of fermentation scale x time had a significant ($p \leq 0.05$) effect on all the physicochemical parameters, except $a^*$ (redness) of dried fermented *C. intermedia* leaves (Table 16). The main effects will be discussed first, then the interactions.

Dried *C. intermedia* leaves fermented on commercial-scale were significantly redder than those fermented on laboratory-scale (Fig. 16a). The redness of dried fermented *C. intermedia* leaves were highest at 16 h of fermentation and decreased significantly with increasing fermentation time (Fig. 16b). Fermentation scale x time had an interactive effect on the lightness ($L^*$) and yellowness ($b^*$) of dried fermented *C. intermedia* leaves (Table 16). The dried leaves fermented on laboratory-scale became significantly darker (less light) as fermentation time increased from 16 to 48 h, while those fermented on commercial-scale only became darker between 24 and 36 h and remained constant thereafter (Fig. 17a). Similarly, the $b^*$-value of dried leaves decreased with increasing fermentation time, however, the yellowness of leaves fermented on commercial- and laboratory-scale did not change after 24 h and 36 h, respectively (Fig. 17b). The relationship between the lightness and yellowness of leaves is confirmed by the strong positive correlation between them (Table 17).

The SS content of *C. intermedia* infusions fermented on laboratory-scale decreased significantly between 16 and 36 h of fermentation, thereafter no change was observed, while the SS content of those on commercial-scale did not change with increasing fermentation time. However, no significant difference between the SS content of infusions of tea fermented on laboratory- and commercial-scale was observed after 24 h (Fig. 18a). The colour (AUC) of infusions of tea fermented on laboratory-scale decreased significantly between 16 and 48 h of fermentation, while the colour (AUC) of those fermented on commercial-scale remained relatively constant over fermentation time (Fig. 18b). The effect of fermentation scale x time on the colour (AUC) of infusions was also observed for the CIELab colour parameters of infusions. The lightness ($L^*$), redness ($a^*$) and yellowness ($b^*$) of infusions of tea fermented on commercial-scale remained constant over time, while the infusions fermented of laboratory-scale were significantly lighter, less red and less yellow as fermentation time increased (Figs. 18d, e & f). Interestingly, no significant changes in physicochemical parameters occurred between 24 and 36 h of fermentation on laboratory-scale (Fig. 18a, b, c, d, e & f). The correlation coefficients confirm that strong positive correlations exist between the SS content, colour (AUC) and CIELab colour parameters *a** and *b**, i.e. the redness and yellowness, of *C. intermedia* infusions, while the *L** parameter (lightness) of infusions shows strong negative correlations with these parameters (Table 17). In other words, *C. intermedia* infusions became lighter as the SS content, colour (AUC), redness (*a**) and yellowness (*b**) decreased over fermentation time. Conversely, the turbidity of infusions fermented on laboratory-scale remained constant over fermentation time, while the turbidity of those fermented on commercial-scale increased significantly after 48 h of fermentation (Fig. 18c). However, the turbidity of infusions presented a strong positive correlation with all the other parameters, except the lightness of infusions (Table 17), indicating that the turbidity of infusions also decreased as the SS content, colour (AUC), redness and yellowness decreased over fermentation time. It should be noted that the effect of fermentation scale and time on the physicochemical parameters of infusions were based on the means of all the batches.

The PCA loadings and scores plots (Figs. 19a & b) show the associations between physicochemical parameters and *C. intermedia* samples from individual batches. These plots demonstrate that samples of batch 1, except those fermented for 16 h, were overly turbid as they were the only samples that associated with high turbidity readings on the left of the corresponding loadings plot. The samples fermented on laboratory-scale for 16 h associated with high SS content, total colour (AUC) and red and yellow infusion colour on the top left of the PCA plot (Fig. 19a & b), while those fermented on commercial-scale for 16 h were situated on the opposite side of the plot indicating that commercial-scale samples appear more fermented than their laboratory-scale counterparts. Samples fermented for 24 h associated with red leaf colour. Both laboratory- and commercial-scale samples fermented for 36 and 48 h were positioned on the bottom right of the PCA scores plot (Fig. 19b) associating with high *L**-values, i.e. light infusion colour (Fig. 19a). All of the samples fermented on commercial-scale, except those of batch 1, were situated on the right hand side of the plot (Fig. 19b) indicating that increasing fermentation time had little effect on the physicochemical parameters of these infusions, whereas those fermented on laboratory-scale were more spread out across the plot with varying physicochemical characteristics as a result of increasing fermentation time (Figs. 19a & b). Furthermore, most of the samples of “good” and “average” quality were positioned on the right of the PCA scores plot (Fig. 19b) associating with light infusion colour and red leaves (Fig. 19a).

A closer look at the relationship between the physicochemical parameters of infusions and their sensory quality provided more insight. Due to the large variation between batches, particularly on commercial-scale, it was not advisable to look at batch means, but rather at individual batches to possibly extract more information regarding commercial-scale fermentation of honeybush tea. The physicochemical parameters of samples fermented on commercial-scale differed considerably between batches, with batch 3 and 4 being the most similar, however, little variation was demonstrated due to increasing fermentation time (Figs. 20a, b & c). Commercial-scale samples fermented for longer times (36 and 48 h) had slightly higher SS content than those fermented for 16 and 24 h, however, the increase in SS content with increased fermentation time within each batch did not affect the colour of the infusions (Fig. 20a). Furthermore, no relationship was demonstrated between SS content, colour (AUC) and turbidity. For example, although batch 2 samples fermented on commercial-scale had relatively high SS contents and colour (AUC), these parameters did not affect the turbidity of these infusions (Figs. 20a & b). Samples fermented for 16 to 36 h on commercial-scale showed little difference in turbidity (except batch 1), while the turbidity of those fermented for 48 h were higher, however, it did not necessarily result in increased colour (AUC) (Figs. 20b & c). On the other hand, the physicochemical parameters of samples fermented on laboratory-scale showed less variation between batches, and more variation due to increasing fermentation time (Figs. 20d, e & f). The SS contents and colour of laboratory-scale samples were slightly higher than the commercial-scale samples, while the turbidity was lower (Fig. 20d). The SS content of samples fermented on laboratory-scale decreased with increasing fermentation time, as did the colour and turbidity (Figs. 20d, e & f). Thus, samples fermented on laboratory-scale demonstrated a far more linear relationship between physicochemical parameters than their commercial-scale counterparts.

Investigating the relationship between the SS content, colour (AUC) and turbidity (NTU) parameters with CIELab colour parameters indicated that the lightness ($L^*$) of infusions decreased, while redness ($a^*$) and yellowness ($b^*$) increased as the SS content, colour (AUC) and turbidity (NTU) of infusions increased when fermenting on laboratory- and commercial-scale (Fig. 21). Batch 1 samples fermented on commercial-scale had the highest SS content, colour (AUC) and turbidity, as well as the darkest infusion with the most red and yellow colour, while little difference was demonstrated between the samples of the other commercial-scale batches (Figs. 21a, b & c). These properties were also shown for samples of batch 1 fermented on laboratory-scale, however, less variation was displayed between batches and variation was rather due to increased fermentation time (Figs. 21d, e & f). Infusions of samples fermented on laboratory-scale became lighter, and less red and yellow in colour, while the SS content, colour (AUC) and turbidity decreased with increasing fermentation time (Figs. 21d, e & f).

Regarding the relationship between physicochemical parameters and the sensory quality of infusions, the sensory quality of infusions naturally also differed considerably between the batches (Fig. 20). Furthermore, as increasing fermentation time did not seem to have much of an effect on the physicochemical properties of samples fermented on commercial-scale, using these parameters to measure sensory quality would not be accurate. However, samples fermented on commercial-scale that were of "good" sensory quality were generally fermented for 24 to 36 h, thus had relatively high SS content and colour, and little turbidity. Increasing fermentation time to 48 h on commercial-scale caused an increase in turbidity, which would thus be undesirable for the overall quality of the product.

Comparing the physicochemical parameters of the wide range of commercial *C. intermedia* samples described in Chapter 3 with those in this study (Fig. 22), illustrated that differences exist between all of the sample sets, however, there is less variation between the commercial samples produced during this study regarding their physicochemical parameters than the commercial samples collected in Chapter 3. More importantly, the means of the SS content and colour of infusions fermented on commercial-scale in this study were higher than the wide range of commercial samples described in Chapter 3, while the turbidity was less.

**Table 16** p-Values for ANOVA table showing the effect of fermentation scale (laboratory- and commercial-scale) and/or time or the interaction of fermentation scale x time on the SS content, absorbance as a measure of colour (AUC), turbidity (NTU) and CIELab colour parameters of *C. intermedia* infusions and dried fermented leaves (n = 32).

| | | | | | CIELab (infusions) | | | CIELab (leaves) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **SS (g/100 mL)** | **AUC (370-510 nm)** | **NTU** | ***L**** | ***a**** | ***b**** | ***L**** | ***a**** | ***b**** |
| Main factors | **Batch** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **0.002** | **<0.0001** | **<0.0001** |
| | **Scale** | 0.170 | **<0.0001** | 0.673 | **<0.0001** | **<0.0001** | **<0.0001** | 0.753 | **<0.0001** | **0.033** |
| | **Time** | 0.743 | **<0.0001** | 0.115 | **0.027** | 0.053 | **<0.0001** | **<0.0001** | **0.003** | **<0.0001** |
| Interaction | **Scale x Time** | **0.006** | **<0.0001** | **0.029** | **0.001** | **0.001** | **0.005** | **0.001** | 0.358 | **0.034** |

**Table 17** Pearson's correlation coefficients (r) illustrating the relationships between the SS content, absorbance as a measure of colour (AUC), turbidity (NTU) and CIELab colour parameters of *C. intermedia* infusions and leaves.

| | **SS (g/100 mL)** | **AUC (370-510 nm)** | **NTU** | ***L*_Infusions** | ***a*_Infusions** | ***b*_Infusions** | ***L*_Leaves** | ***a*_Leaves** | ***b*_Leaves** |
|---|---|---|---|---|---|---|---|---|---|
| **SS (g/100 mL)** | **1** | | | | | | | | |
| **AUC (370-510 nm)** | **0.866** | **1** | | | | | | | |
| **NTU** | **0.784** | **0.738** | **1** | | | | | | |
| ***L*_Infusions** | **-0.864** | **-0.981** | **-0.776** | **1** | | | | | |
| ***a*_Infusions** | **0.882** | **0.957** | **0.753** | **-0.989** | **1** | | | | |
| ***b*_Infusions** | **0.849** | **0.947** | **0.651** | **-0.961** | **0.972** | **1** | | | |
| **L*_Leaves** | -0.174 | 0.010 | -0.269 | 0.025 | -0.017 | 0.141 | **1** | | |
| **a*_Leaves** | **-0.393** | **-0.637** | **-0.551** | **0.653** | **-0.584** | **-0.472** | 0.344 | **1** | |
| **b*_Leaves** | -0.195 | -0.126 | **-0.445** | 0.136 | -0.077 | 0.084 | 0.860 | **0.543** | **1** |

Significant correlations (p ≤ 0.05) are indicated in bold. Correlations > ± 0.7 are indicated in red. SS = soluble solids, AUC = area under the curve (370 – 510 nm), NTU = nephelometric turbidity units, *L** = lightness, *a** and *b** = colour-opponent dimensions.

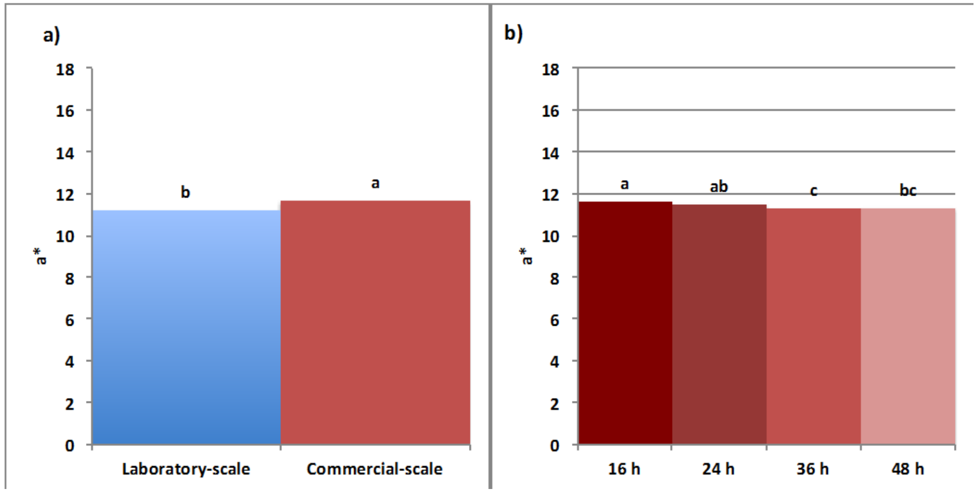



**Figure 16** Effect of a) fermentation scale and b) fermentation time on *a** (redness) of dried fermented *C. intermedia* leaves. Columns with different letters differ significantly from each other ($p \leq 0.05$).

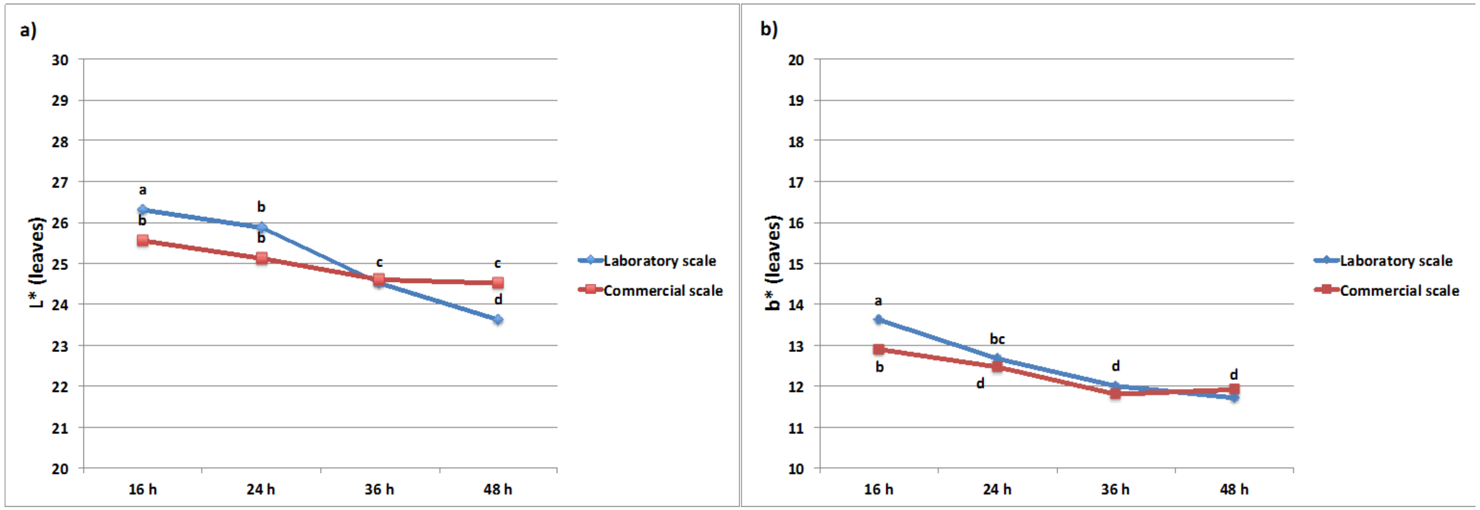



**Figure 17** Effect of fermentation scale x time on the a) *L** and b) *b** values of dried fermented *C. intermedia* leaves. *L** = lightness, *a** and *b** = colour-opponent dimensions. Values with different letters differ significantly from each other ($p \leq 0.05$).

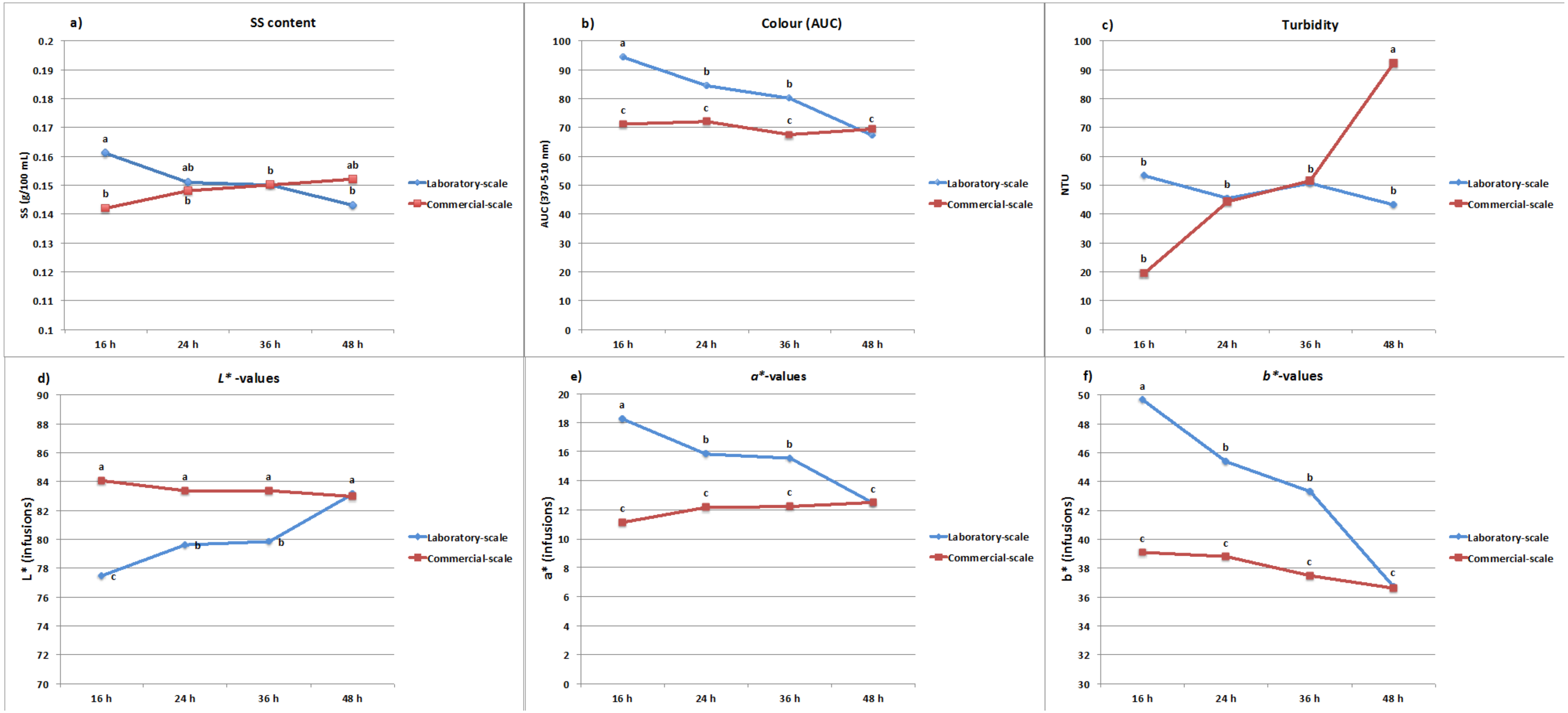



**Figure 18** Effect of fermentation scale x time on the a) SS content, b) colour (AUC), c) turbidity, d) *L**, e) *a**, and f) *b**-values of *C. intermedia* infusions. Values with different letters differ significantly from each other ($p \leq 0.05$). SS = soluble solids, AUC = area under the curve, NTU = nephelometric turbidity units, *L** = lightness, *a** and *b** = colour-opponent dimensions.

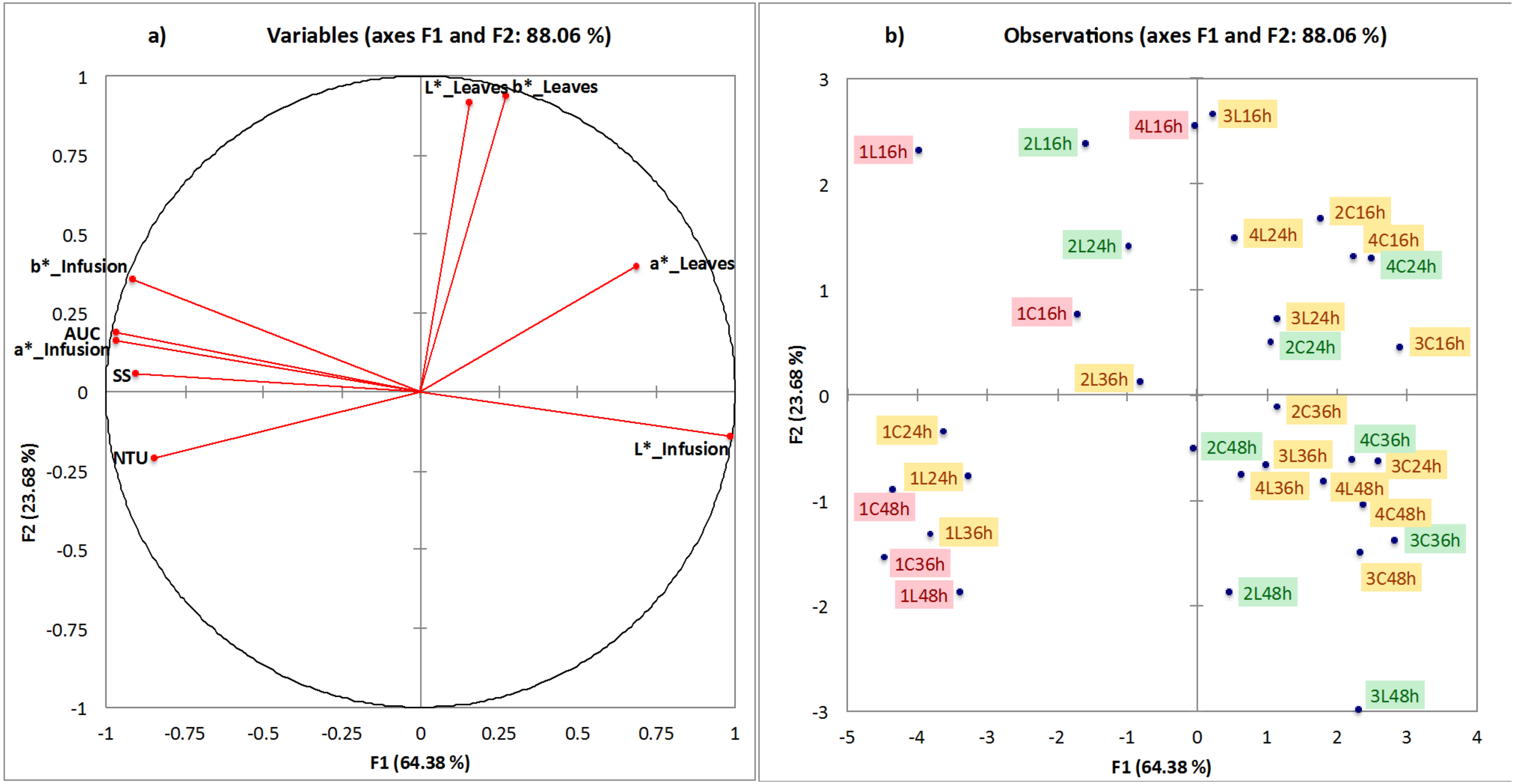



**Figure 19** a) PCA loadings plot showing the positioning of physicochemical parameters of *C. intermedia* infusions. SS = soluble solids, AUC = area under the curve (370 – 510 nm), NTU = nephelometric turbidity units, *L** = lightness, *a** and *b** = colour-opponent dimensions. b) PCA scores plot showing the positioning of *C. intermedia* samples fermented on laboratory (L)- and commercial (C)-scale for 16, 24, 36 and 48 h, graded as “poor” (red), “average” (yellow) and “good” (green) sensory quality. Numbers 1, 2, 3 and 4 denotes individual batches.

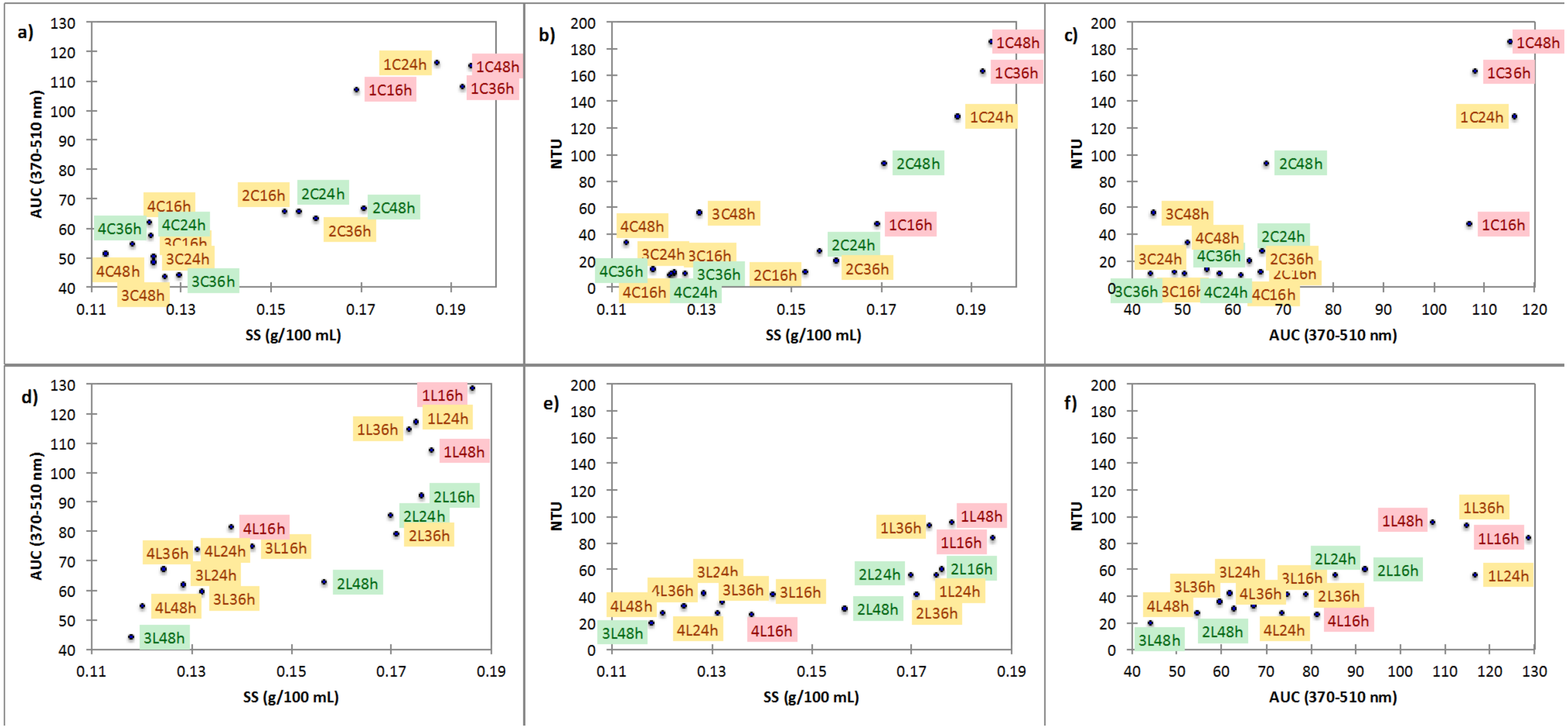



**Figure 20** Scatter plots illustrating the relationship between a) SS content and colour (AUC), b) SS content and turbidity (NTU) and, c) colour (AUC) and turbidity (NTU) of *C. intermedia* infusions fermented on commercial-scale for 16, 24, 36 and 48 h. Scatter plots illustrating the relationship between d) SS content and colour (AUC), e) SS content and turbidity (NTU) and, f) colour (AUC) and turbidity (NTU) of *C. intermedia* infusions fermented on laboratory-scale for 16, 24, 36 and 48 h. SS = soluble solids, AUC = area under the curve (370 – 510 nm), NTU = nephelometric turbidity units.

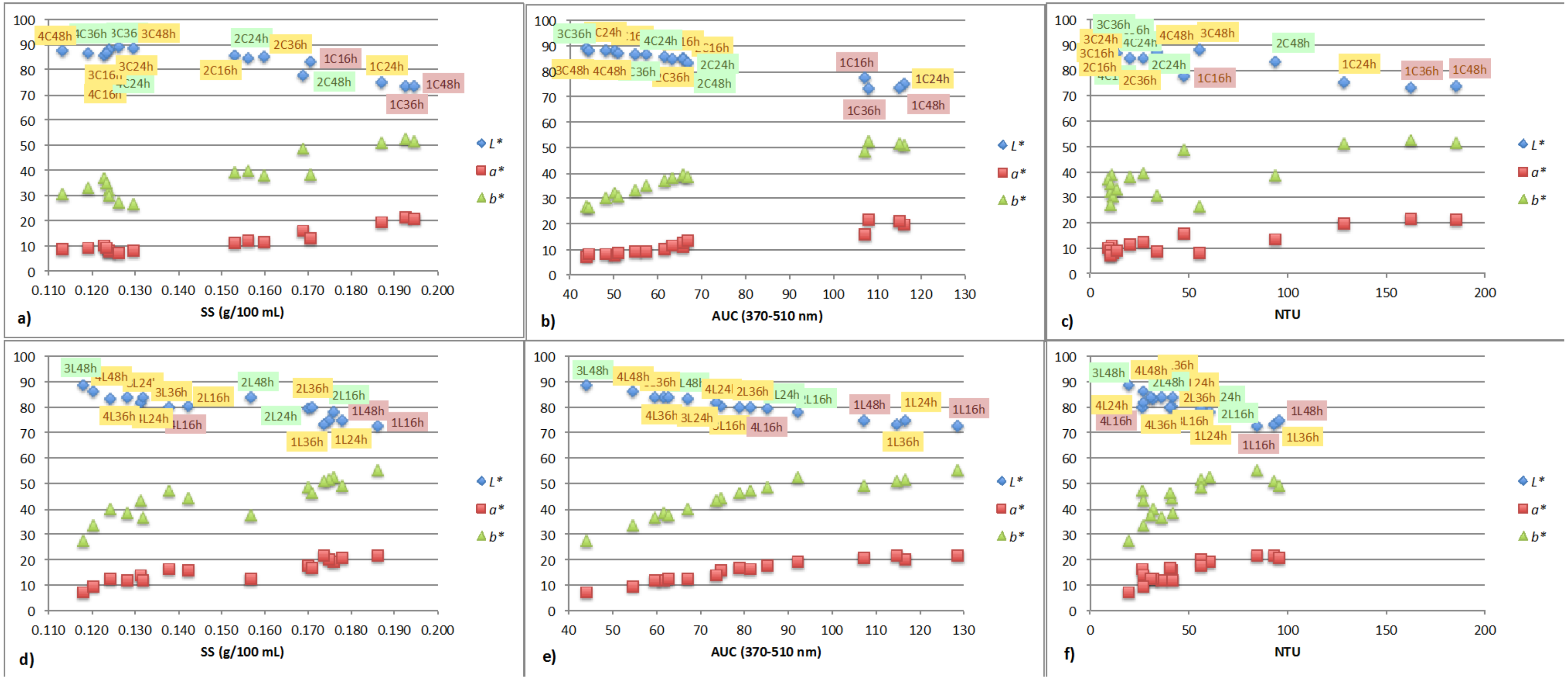



**Figure 21** Scatter plots illustrating the relationship between a) SS content, b) colour (AUC), and c) turbidity (NTU) and CIELab colour parameters of *C. intermedia* infusions fermented on commercial-scale for 16, 24, 36 and 48 h. Scatter plots illustrating the relationship between d) SS content, e) colour (AUC), and f) turbidity (NTU) and CIELab colour parameters of *C. intermedia* infusions fermented on laboratory-scale for 16, 24, 36 and 48 h. SS = soluble solids, AUC = area under the curve (370 – 510 nm), NTU = nephelometric turbidity units, *L** = lightness, *a** and *b** = colour-opponent dimensions.

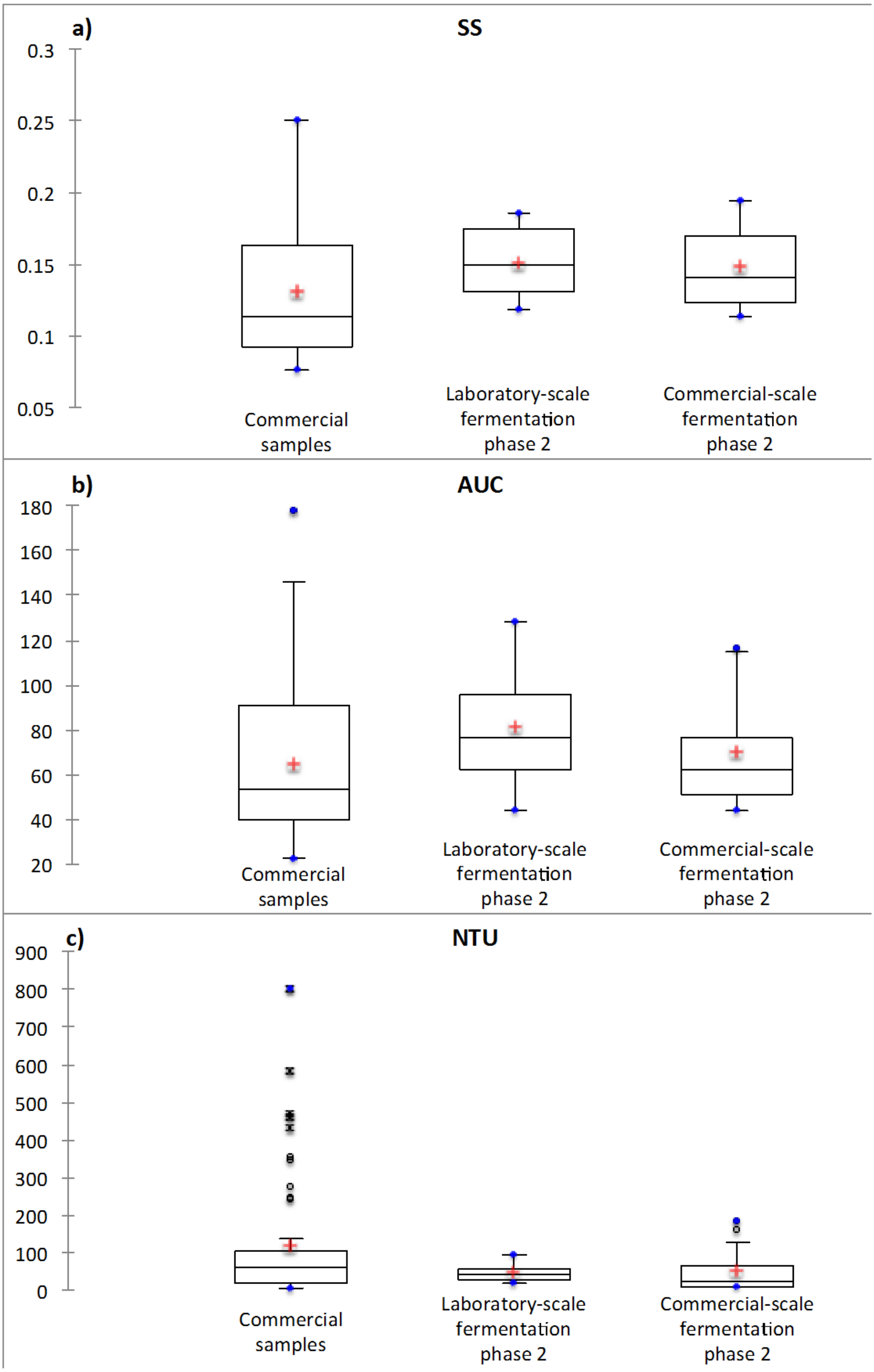



**Figure 22** Scatter plots illustrating the variation in a) SS content, b) colour (AUC) and c) turbidity (NTU) between the commercial *C. intermedia* samples analysed in Chapter 3 and the laboratory- and commercial-scale samples analysed in the second part of this study. SS = soluble solids, AUC = area under the curve (370-510 nm), NTU = nephelometric turbidity units.

## 4. DISCUSSION

During the high temperature fermentation of honeybush tea, oxidation of polyphenols takes place leading to the formation of compounds that are responsible for the sensory and physicochemical characteristics of honeybush tea infusions (Du Toit & Joubert, 1998). Prices of various teas are quite variable and are dependent on the quality (Liang *et al*., 2003; 2005). While assessing the cash valuation of black (*Camellia sinensis*) and rooibos (*Aspalathus linearis*) teas, "tea tasters" mainly take the infusion characteristics into consideration (Liang *et al*., 2005; Joubert *et al*., 2012; C. Cronje, Rooibos Ltd, Clanwilliam, South Africa, 2013, personal communication). Although the quality of tea largely depends on the components in the plant material (Liang *et al*., 2005), the fermentation conditions play a major role in the development of the sensory and physicochemical characteristics of honeybush infusions (Du Toit & Joubert, 1998; 1999; Theron, 2012). Theron (2012) demonstrated that different *Cyclopia* spp. required different fermentation temperature and time conditions to produce honeybush tea of good sensory quality. As Du Toit and Joubert (1999) only assessed infusions of *C. intermedia* for their sweet aroma and flavour, a more in-depth study of the change in its sensory attributes was necessary. Thus, the effect of fermentation temperature and time on the sensory quality and physicochemical parameters of *C. intermedia* infusions were investigated to optimise fermentation conditions to produce consistently good quality tea. Moreover, selected conditions were then applied on commercial-scale to optimise the fermentation conditions for *C. intermedia* in a commercial processing environment. Physicochemical characteristics of infusions, i.e. the SS content, colour and turbidity, were also used to investigate the relationship between physicochemical parameters and sensory quality in order to assess their usefulness for on-site quality monitoring during honeybush tea manufacture.

The sensory profile of *C. intermedia* was described in Chapter 3 as sweet tasting and slightly astringent with a combination of "fynbos-floral", "fynbos-sweet", "fruity" (specifically "apricot jam", "cooked apple", "raisin" and "lemon/lemon grass"), "woody", "caramel/vanilla" and "honey-like" aromas. The flavour is distinctly "fynbos-floral", "fynbos-sweet" and "woody", including hints of "lemon/lemon grass" and "hay/dried grass". Hence, the focus was on these attributes when determining the optimum fermentation parameters for the production of good quality honeybush tea (*C. intermedia*). In order to produce *C. intermedia* infusions with optimal sensory profiles on laboratory-scale a fermentation period of 36 h at 90°C or 48 h at 70°C and 80°C demonstrated to be necessary for development of positive attributes to their maximum intensities and to eliminate/reduce the negative attribute intensities to acceptable levels. Furthermore, although infusions fermented at 70°C were sweeter and had higher "fynbos-floral" intensities, those fermented at 90°C contained the entire sensory profile characteristic of *C. intermedia.* Infusions fermented at 70°C were predominantly floral in nature whereas those fermented at 80°C were fruity. Thus, fermentation at 90°C was more efficient at increasing and decreasing the intensity of positive and negative attributes, respectively, and produced a product with a good constituent of sensory attributes, characteristic of *C. intermedia* infusions.

The SS content, absorbance as a measure of colour and turbidity of infusions of tea decreased with the degree of fermentation on laboratory-scale, particularly after 36 h of fermentation. The positive linear correlations between the physicochemical parameters indicate that the reduction of these physicochemical parameters with increasing fermentation time could be attributed to the formation of insoluble polymerisation products from monomeric polyphenols during fermentation that reduces the amount of soluble polyphenols and thus the SS extracted during seeping (Joubert, 1994). The colour (AUC) of *C. intermedia* infusions was unaffected by fermentation temperature, however, the SS content and turbidity of infusions fermented at 90°C were higher than those fermented at 70°C and 80°C. It can be postulated that the harsher treatment of plant material during fermentation at 90°C on laboratory-scale caused the breakdown of compounds in leaves allowing these compounds to diffuse easily, therefore, increasing the turbidity of the infusion and leading to higher than expected SS content (Heong *et al*., 2011). Turbidity would be caused by complexation between polymers such as polysaccharides or proteins with tannin-type polyphenols (Rutter & Stainsby, 1975; Boadi & Neufeld, 2001). Additionally, the physicochemical parameters of infusions fermented at 90°C on laboratory-scale were most stable over fermentation time, rendering fermentation at 90°C paramount to produce *C. intermedia* infusions with the most consistent sensory and physicochemical properties. Fermentation for 36 h at 90°C on laboratory-scale is recommended to produce *C. intermedia* with optimal sensory characteristics, while preserving the SS content and colour of infusions. Fermentation time may be extended to improve the turbidity of infusions, however sensory quality as well as SS content and colour may be forfeited.

To investigate the validity of these findings for application on commercial-scale, *C. intermedia* plant material was fermented at 90°C on laboratory- and commercial-scale simultaneously in order to study the effect of the scale of fermentation on the sensory and physicochemical properties of infusions. As with the different temperature and time regimes, fermentation on laboratory- and commercial-scale had different effects on the sensory profile. Infusions of tea fermented on laboratory-scale had higher attribute intensities and were predominantly fruity in nature, whereas those fermented on commercial-scale had floral characteristics. It can be postulated that the sensory differences between infusions of tea fermented on laboratory- and commercial-scale are due to temperature variation during fermentation. There were large fluctuations in temperature (±5-10°C) during fermentation on commercial-scale (Fig. 14), particularly overnight, compared to the small fluctuation of temperature (88-90°C) during fermentation on laboratory-scale (Fig. 15). This was to be expected as temperature-control due to batch size presents a challenge on commercial-scale. The plant material in commercial fermentation tanks is heated via steam jackets with the steam generated by a boiler that is manually kindled. The fire in the boiler invariably burns out overnight, particularly during cold weather conditions, causing the fermentation tanks to cool down during the fermentation process. Additionally, it was difficult for the tea to reach 90°C in the fermentation tanks (Fig. 14) and temperature measurements taken simultaneously throughout the tea in the fermentation tank showed large temperature variation (±5°C) within the tank, highlighting the challenges of heating large volumes of *C. intermedia* plant material. Despite temperature fluctuations, fermentation on commercial-scale proved more efficient as samples fermented on commercial-scale were of "good" sensory quality after 24 and 36 h of fermentation, whereas those fermented on laboratory-scale were only of "good" sensory quality after 48 h. Increasing the fermentation time to 48 h on commercial-scale caused the sensory quality of infusions to decrease as attributes associated with over-fermented tea, such as "dusty", became more prominent. It can be postulated that laboratory-scale fermentation was "slower" than commercial-scale fermentation due to the slow increase of fermentation temperature initially ("come-up" time) - the temperature of the tea took *ca*. 5 h to reach 90°C on laboratory-scale (Fig. 15) compared to 1 h on commercial-scale (Fig. 14). Additionally, the fact that tea fermented on laboratory-scale was not mixed during the fermentation process resulted in less oxygen available for the oxidation reaction as the oxygen supply would be by slow diffusion from the atmosphere and may even have led to oxygen depletion within the laboratory oven, resulting in slow oxidation rates. The law of mass action that states that increased activities (usually equated to concentration) of reactants lead to increased reaction rates could also have contributed to the increased fermentation rates during commercial-scale fermentation.

With regard to the effect of fermentation scale and time on physicochemical parameters of *C. intermedia* infusions, the decrease in SS content, colour (AUC), turbidity and CIELab colour parameters ($a^*$ and $b^*$) of infusions of tea fermented on laboratory-scale with increasing fermentation time were consistent with the previous laboratory-scale fermentation experiment. Thus, the SS content, colour (AUC) and turbidity of infusions of tea fermented on laboratory-scale decreased, became lighter in colour, and less red and yellow with increasing fermentation time. Joubert (1994) found that a decrease is SS content resulted in an increase of $L^*$ values of rooibos extracts, while $a^*$ and $b^*$ values decreased, which was attributed to the polymerisation and subsequent loss of solubility of polyphenols. Degradation of unknown compounds and polymerisation of compounds susceptible to oxidation, accompanied by lower solubility, are possible contributors to the decrease in SS, however, no studies have been undertaken to explain these changes in the aqueous extract of honeybush with fermentation in terms of the chemical reactions involved (Joubert *et al*., 2008). On the other hand, increasing fermentation time had little effect on the physicochemical parameters (except turbidity) of infusions fermented on commercial-scale. Du Toit and Joubert (1999) demonstrated that the fermentation period from 24 to 60 h did not significantly affect colour values for tea fermented at 60, 70 and 80°C, but prolonging fermentation from 60 to 72 h resulted in an increase of $L^*$, while $a^*$ and $b^*$ decreased. However, at 90°C, fermentation time had a marked effect on infusion colour with optimum development occurring at 36 h (Du Toit & Joubert, 1999). Thus, the fluctuation of temperature below 90°C during commercial-scale fermentation may have affected the physicochemical parameters of infusions. Furthermore, infusions fermented on commercial-scale had slightly higher SS content, less colour (AUC) and more turbidity than those fermented on laboratory-scale (Fig. 22), highlighting the non-linear relationship between physicochemical parameters of infusions fermented on commercial-scale. This also indicates that the turbidity of infusions fermented on commercial-scale may not be linked to the SS content, but rather is a result of other conditions during fermentation. Preliminary trials indicated that minimising the amount of water added and rather superficially wetting, particularly of young plant material, to aid the fermentation process, as well as reducing the use of the rotating paddles to mix the plant material during fermentation reduced the turbidity of infusions dramatically. Although mixing the plant material is essential to ensure even heat distribution throughout the mass of fermenting tea and incorporates oxygen, leading to a higher oxygen supply and thus increased oxidation rate, mixing should be minimised to reduce turbidity. Adding too much water and thorough mixing during fermentation created a mush of plant material, which also caused drip loss and possible loss of compounds valuable for sensory quality. Although the turbidity of infusions of tea fermented on commercial-scale were more turbid than their laboratory-scale counterparts, they were far less turbid than the wide range of commercial *C. intermedia* samples analysed in Chapter 3 (Fig. 22), indicating that this fermentation regime, as well as monitoring of the moisture content prior to fermentation and minimising the use of the rotating paddles, improved the turbidity of infusions of tea produced on commercial-scale. Furthermore, the infusions of tea produced on commercial-scale during the second part of this study also had improved colour and SS content (Fig. 22).

The turbidity and colour of tea are important sensory qualities, as these are the first parameters of quality evaluated by consumers and are thus critical factors in the acceptance of the product before it enters the mouth. The $L^*a^*b^*$ colour space is the most used for measuring the colour of foodstuffs due to the uniform distribution of colours, and because it is very close to human perception of colour (León *et al*., 2006). $L^*$ is the luminance or lightness component, which ranges from 0 to 100, and parameters $a^*$ (from green to red) and $b^*$ (from blue to yellow) are the two chromatic components, which range from 120 to 120 (Yam & Papadakis, 2004). As with honeybush tea, the $L^*$ value decreased (from 99.42 to 98.38) with the degree of fermentation in *Centella asiatica* tea. A similar trend was found in *Camellia sinensis* (96.22±0.11) teas (Heong *et al*., 2011), however, honeybush tea showed lower $L^*$ values ranging from 72.30 to 88.42. The $-a^*$ values of black (-1.91±0.01) and *Centella asiatica* (-0.07±0.04) teas indicate that the infusions were greenish in colour (Heong *et al*., 2011), compared to the $+a^*$ values of honeybush infusions ranging from 21.58 to 7.10. The $b^*$ values of honeybush infusions (ranging from 55.29 to 26.40) were also much greater than those of *Camellia sinensis* (19.9±0.54) and *Centella asiatica* (2.27±0.46) teas. All these values indicate that honeybush infusions were darker, more yellow and red in colour than the other teas. Liang *et al*. (2005) showed that the $\Delta L^*$ was negatively correlated to various quality attributes of black tea but positively correlated to quality attributes of green and oolong tea, and vice versa for the $\Delta a^*$ and $\Delta b^*$.

It suggests that, the deeper the infusion colour and more red and yellow, the better for black tea quality but the worse for green and oolong tea quality.

Browning and thus darkening of the fermenting leaves, measured as a decrease in *L** values (Du Toit & Joubert, 1999), took place within 36 h of fermentation on commercial-scale, thereafter no significant changes in the *L** values were observed. The change in colour from green to red-brown can partially be attributed to the breakdown of chlorophylls (Du Toit & Joubert, 1998), but oxidation of polyphenols is the major contributor to the colour of the leaves and infusions. Decreasing *b** values is indicative of oxidation of flavonoids to highly polymeric compounds that results in the loss of yellow colour and contributes to browning (Harborne, 1965). Dry leaf colour is not a reliable indication of the infusion colour since the leaves became darker in colour and the infusions lighter with increasing fermentation time.

Since differences between physicochemical properties of *C. intermedia* infusions fermented on commercial-scale at 90°C were indistinguishable, particularly between 24 and 36 h, fermentation could thus be terminated during this period, taking optimum sensory quality, colour and turbidity development into account. Fermentation for longer than 36 h should be avoided to reduce "dusty" aroma and flavour, sour taste, astringent mouthfeel, and turbid infusion characteristics. A high level of turbidity in beverages such as herbal teas is known to decrease their aesthetic value, as mentioned by Hutchings (1999), Harbourne *et al*. (2009) and M. Bergh (Rooibos Ltd, Clanwilliam, South Africa, 2013, personal communication). However, taking the large variation between batches into account, it is recommended that each batch be monitored between 24 and 36 h to determine when optimum fermentation has been reached. Furthermore, as fermentation time did not have a significant effect on the physicochemical properties of samples fermented on commercial-scale, using these parameters to measure the sensory quality of *C. intermedia* infusions would not be helpful.

The large variation between batches of *C. intermedia* samples regarding their sensory and physicochemical characteristics is a good indication of the poor reproducibility of honeybush tea fermentation, which highlights the significance of this study to improve the quality and consistency of the product. It also provides further insight into other sources of variation that exist during fermentation of honeybush tea. The variation between samples fermented in a controlled laboratory-scale environment with little temperature fluctuation, consistent batch size and consistent drying conditions, highlights the effect of variation within the plant material on sensory quality. *Cyclopia intermedia* plant material may differ with regard to their polyphenol (and other constituent compounds) due to many factors such as growing conditions (e.g. soil, altitude, and environmental conditions), seasonal differences and plant age (Owuor *et al*., 1990; Du Toit & Joubert, 1999; Joubert *et al.*, 2008; Owuor *et al*., 2008; Joubert *et al*., 2011; Joubert *et al*., 2012; Stanimirova *et al*., 2013; Jayasekera *et al*., 2014; Joubert *et al.,* 2014). However, there is more variability during fermentation on commercial-scale. The effect of batch size is highlighted by batch 1 being particularly different from the other batches produced on commercial-scale, which can be attributed to the fact that it was half the size of the other batches. The small amount of plant material fermented in batch 1 (330 kg) negatively affected its ability to retain heat, particularly overnight while the functioning of the boiler was impaired, causing more extreme temperature fluctuation during fermentation (Fig. 14). However, it would then be expected that the characteristics of samples fermented in batch 2 and 3 be most alike, which was not the case. Other causes of variation of the fermentation product may be attributed to differences in moisture content of the plant material and temperature fluctuation during fermentation.

## 5. CONCLUSIONS

Chemical oxidation of *C. intermedia*, known as "fermentation", resulted in an increase of positive sensory attributes and a decrease of negative sensory attributes. Fermentation at 90°C for 36 h, or 48 h at 70°C and 80°C, was required to effectively increase positive sensory attributes, while fermentation for 12 h at 90°C and 24 h at 70°C and 80°C was required to eliminate/reduce the negative attributes to negligible levels on laboratory-scale. Different fermentation temperatures produced teas with slightly different sensory profiles. Those fermented at 90°C comprised most of the sensory attributes characteristic of *C. intermedia,* while infusions of tea fermented at 70°C were predominantly floral and those fermented at 80°C were fruity. The SS content of infusions of tea fermented on laboratory-scale decreased significantly between 16 and 36 h of fermentation, after which no change was observed. The colour (AUC) of infusions decreased with increasing fermentation time on laboratory-scale, although was unaffected by fermentation temperature, while the turbidity remained constant. The sensory and physicochemical properties of infusions fermented at 90°C on laboratory-scale showed less variation with increasing fermentation time, indicating that fermentation at 90°C is paramount to produce *C. intermedia* infusions with the most consistent sensory and physicochemical properties. Fermentation for 36 h at 90°C on laboratory-scale is, therefore, recommended to produce *C. intermedia* with optimal sensory characteristics, while preserving the colour of infusions.

Fermentation on commercial-scale showed markedly more variability between batches due to various other processing factors, highlighting the relevance of this study. Fermentation scale (laboratory- and commercial-scale) affected the sensory profiles of infusions differently, which can be attributed to temperature fluctuations during fermentation. Infusions of tea fermented on laboratory-scale had higher attribute intensities and were predominantly fruity in nature, while those fermented on commercial-scale had floral characteristics. However, fermentation on commercial-scale proved more efficient, producing samples of "good" sensory quality after 24 and 36 h of fermentation, compared to 48 h on laboratory-scale. The effect of fermentation time on the physicochemical parameters (except turbidity) of infusions fermented on commercial-scale was indistinguishable, particularly between 24 and 36 h. Increasing fermentation time to 48 h on commercial-scale caused the sensory quality of infusions to decrease and turbidity to increase significantly. Thus, fermentation on commercial-scale at approximately 90°C should be terminated between 24 and 36 h to produce *C. intermedia* with optimum sensory quality and good physicochemical characteristics. However, for want of reproducibility, it is recommended that each batch be monitored during this period to determine optimum fermentation. Furthermore, since no relationship between physicochemical properties of samples fermented on commercial-scale was demonstrated, these parameters cannot be used to measure the sensory quality of infusions.

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# CHAPTER 5

## GENERAL DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS

Definitions of quality include, "The distinctive trait, characteristic, capacity or virtue of a product that sets it apart from all others." (Ferree, 1973). Honeybush tea, with its characteristic sweet-associated, floral, fruity and woody aroma and flavour (Theron *et al*., 2014), low tannin content and the absence of caffeine (Joubert *et al*., 2011) certainly fulfills this definition of quality. However, another perspective of quality is the standard the consumer will accept (Ferree, 1973), which ultimately determines the success of the product. Consumers rely on external factors such as size, shape, colour, gloss, and consistency to establish the quality of the product before using the judgment of flavour and taste. Furthermore, consumers have specific performance expectations for food, thus consistent quality is key to growing consumers' confidence in a product and creating a reliable market. The lack of a consistent supply of good quality honeybush tea was identified as a major stumbling block in successful commercialisation and advancement of the industry (Du Toit *et al*., 1998; SAHTA, 2014).

Quality control and sensory assessment are common practices used to analyse food product quality to ensure a standardised product with consistent sensory and physicochemical properties is supplied. Currently, the quality of honeybush tea infusions is not monitored by a standard set of parameters (C. Cronje, Rooibos Ltd, Clanwilliam, South Africa, personal communication). Furthermore, honeybush tea is produced using various *Cyclopia* spp. with inherent species differences, as well as differences caused by varying growth localities, environmental conditions and processing parameters, which are not taken into account and contribute to considerable variation in the sensory and physicochemical profile of honeybush sold on the market (Joubert *et al*., 2011). In order to differentiate honeybush tea in an increasing competitive herbal tea market and to identify possible high-end niche markets, species-specific sensory profiles need to be generated and processing conditions need to be optimised for each *Cyclopia* spp. to ensure consistent quality tea is supplied. Exports currently consist mainly of *C. intermedia* and its commercial importance thus motivated the present study.

The sensory attributes and descriptions, defined by Theron *et al*. (2014) to describe honeybush tea in general, were used to characterise the aroma, flavour, taste and mouthfeel of *C. intermedia*. In order to capture as much variation as possible, samples from different localities, commercial producers, farm stalls, harvesting dates, processing conditions and size fractions were used. Experimental samples prepared for research purposes were also included. The characteristic sensory profile of *C. intermedia* was determined based on occurrence and intensities of sensory attributes. *Cyclopia intermedia* was described as typically sweet tasting and slightly astringent with a combination of "fynbos-floral", "fynbos-sweet", "fruity" (specifically "apricot jam", "cooked apple", "raisin" and "lemon/lemon grass"), "woody", "caramel/vanilla" and "honey-like" aromas. The flavour can be described as distinctly "fynbos-floral", "fynbos-sweet" and "woody", including hints of "lemon/lemon grass" and "hay/dried grass". The most prominent negative sensory attribute appeared to be "hay/dried grass", while the intensity of "woody" was decisive in its classification as negative or positive. A species-specific *C. intermedia* sensory wheel consisting of descriptors, weighted according to their average intensity in an infusion, was developed to visually represent the sensory attributes of "a cup of *C. intermedia* (honeybush tea)". A lexicon including the definitions and reference standards of the descriptors was also constructed. Moreover, as there is no quality grading system in place for the evaluation of honeybush tea, an elementary grading system was developed by identifying, defining and measuring the sensory characteristics of *C. intermedia* infusions. Large variation existed between this range of commercial *C. intermedia* samples in terms of their sensory characteristics, as well as soluble solids content, colour and turbidity of the infusions. Factors such as locality, climate, soil conditions, the age of the plant/regrowth (Joubert *et al*., 2011; Joubert *et al*., 2014), the presence of flowers/pods (Du Toit & Joubert, 1999) and the leaf-to-stem ratio could contribute to this variation. However, most of the variation in the sensory attributes, especially in terms of negative sensory attributes, and physicochemical properties appeared to be due to different processing conditions. Most of the samples having "poor" sensory quality that associated with negative sensory attributes such as "wet fur/farm animals" and "smokey" and extremely turbid infusions were not necessarily optimally processed (i.e. over-fermented).

The effect of fermentation temperature, time and scale on the sensory characteristics and physicochemical properties of *C. intermedia* infusions was investigated using the newly developed sensory profile and grading system and ultimately the optimum fermentation conditions for *C. intermedia* on laboratory- and commercial-scale were determined. Fermentation on laboratory-scale resulted in an increase of positive sensory attributes and reduction of negative sensory attributes to a certain extent, after which the quality did not improve. Increasing fermentation temperature increased the rate of sensory change/development, thus fermentation at 90°C/36 h was required to produce *C. intermedia* of optimum sensory quality, compared to 48 h at 70°C and 80°C. The reduction/elimination of negative sensory attributes was the driver in determining sensory quality. Fermentation temperature also seemed to affect the composition of positive sensory attributes, with fermentation at 90°C producing infusions with the characteristic *C. intermedia* sensory profile as defined during this study, whereas those fermented at 70°C were predominantly floral in nature, while those fermented at 80°C were fruity. This is not surprising as the sensory profile is influenced by the chemical composition. It is known that no new volatile compounds form during fermentation (Le Roux *et al*., 2008; Le Roux *et al*., 2012). However, it has been reported that the concentration of some volatile compounds increase while others decrease, which result in the change/development of the sensory profile of honeybush infusions. Increasing fermentation time resulted in a decrease of SS content, absorbance as a measure of colour and turbidity of infusions, particularly after 36 h. Fermentation temperature did not affect the colour of infusions, however, infusions fermented at 90°C had higher SS content and were more turbid but showed less variation over time. It can be postulated that the colour and turbidity of infusions reflect the change in the SS content due to the formation of insoluble polymerisation products during fermentation. Turbidity would be caused by complexation between polymers such as polysaccharides or proteins with tannin-type polyphenols (Rutter & Stainsby, 1975; Boadi & Neufeld, 2001). Moreover, the harsher treatment during fermentation at 90°C may have caused the breakdown of compounds in leaves allowing these compounds to diffuse easily, therefore, increasing the turbidity of the infusion and leading to higher than expected SS content (Heong *et al*., 2011). Thus, fermentation at 90°C for 36 h is recommended to consistently produce *C. intermedia* of optimum sensory quality, while preserving the SS content and colour of infusions on laboratory-scale.

Fermentation on commercial-scale had a slightly different effect compared to fermentation on laboratory-scale in terms of the sensory and physicochemical properties of *C. intermedia* infusions produced and, as expected, displayed more variability. Although fermentation on commercial-scale resulted in an increase and decrease in intensity of positive and negative sensory attributes, respectively, the rate of change was quicker during commercial-scale fermentation and extending fermentation time for more than 36 h resulted in a decrease of sensory quality. Infusions of tea fermented concurrently on laboratory-scale had higher attribute intensities and were predominantly fruity in nature, where as those fermented on commercial-scale had floral characteristics. The predominant floral notes would suggest that temperatures lower than 90°C was attained during fermentation on commercial-scale. It can be postulated that differences in sensory characteristics can primarily be attributed to temperature fluctuations and maintenance issues due to batch size and heat source, while variation in plant material may also play a role. The degree of fermentation had little effect on the SS content and colour of infusions of tea fermented on commercial-scale, however, their turbidity increased significantly after 36 h of fermentation. The cause of turbidity of infusions produced on commercial-scale may partly be attributed to mechanical breakdown of plant material during to mixing. Mixing the plant material is necessary to evenly spread the heat throughout the volume of tea to reduce temperature differences within the batch and aerates the tea to aid fermentation, however, it should be minimised to reduce turbidity. Additionally, wetting of the plant material should be carefully monitored and dosed based on the moisture content of the plant material to prevent over-wetting, which is also believed to contribute to turbidity. However, no studies have been undertaken to explain changes in the aqueous extract with fermentation in terms of the chemical reactions involved (Joubert *et al*., 2008) that could contribute to turbidity. Thus, fermentation for approximately 24-36 h at 90°C is required to produce *C. intermedia* of good sensory and physicochemical quality on commercial-scale with batch size ranging between 500 – 700 kg. Fermentation for longer than 36 h should be avoided to reduce “dusty” aroma and flavour, sour taste, astringent mouthfeel, and turbid infusion characteristics. However, for want of reproducibility and consistency between batches, it is recommended that each batch be monitored between 24 and 36 h to determine when fermentation should be terminated. Commercial-scale fermentation was performed on batches ranging between 300 – 700 kg in the present study, which demonstrated to have a marked effect on the overall quality of the product. A more in-depth investigation on the effect of batch size on the sensory and physicochemical parameters of honeybush tea would be recommended to further define optimum fermentation parameters of honeybush tea on commercial-scale. It should also be noted that, due to the presence of many commercial honeybush fermentation systems, the optimum parameters stipulated in this study may need to be adapted to produce *C. intermedia* of optimum sensory and physicochemical quality in other commercial fermentation systems.

To further eliminate sensory variation of commercial *C. intermedia* various attributes may be compensated by blending. For example, a good blended tea may be obtained by blending a tea with a strong flavour and weak aroma with a tea with weak flavour and strong aroma. Blending may also be used to introduce certain sensory attributes that one tea is lacking or to dilute negative sensory attributes to produce tea with the characteristics sensory profile. On the other hand, the sensory profile of a particular *Cyclopia* species can be adjusted by blending with one or more species to produce a standard honeybush infusion. There is thus a need to define blending ratios for honeybush tea. This, however, may lead to a loss of the unique sensory profiles of individual *Cyclopia* spp. and subsequent economic loss by disregarding possible high-end niche markets. Consumer analysis can be used to measure the degree of liking of the different *Cyclopia* spp., while combining these findings with results from descriptive sensory analysis could give an indication of sensory drivers of liking (McEwan *et al*., 1998; Lawless & Heymann, 2010). This will allow the identification of the most desired sensory characteristics and species with the most commercial potential.

As the sensory characteristics of honeybush tea is only as good as the inherent flavour potential of the plant, a longer-term solution to reduce quality variation in honeybush tea would be improvement of plant material through screening of available plant material for selection, breeding and propagation. This provides researchers with further opportunities to eliminate variation in plant material and improve product quality, but will also help commercial production management. Currently, plant material is brought in sporadically from external sources (wild and cultivated), which limits the ability to manage and plan batch size and production volumes per week. Furthermore, reduction in variation of plant material and improved control of fermentation conditions could allow for the manipulation of the desired flavour characteristics (determined via consumer analysis) by different fermentation temperature and time regimes.

The sensory profile of *Cyclopia intermedia* was defined and quantified by determining the occurrence and intensity of sensory attributes. This led to the development of the first species-specific sensory wheel and lexicon for *C. intermedia,* which expanded the generic honeybush profile. Descriptive sensory analysis of a wide range of *C. intermedia* samples for profiling further prompted the development of the first elementary grading system for *C. intermedia*, which may by adapted for other *Cyclopia* spp. and formalised by use in industry. These quality-monitoring tools are invaluable to improve product quality and consistency. Furthermore, this study was a principle investigation into the effect of fermentation parameters on the sensory and physicochemical quality of *C. intermedia* infusions on commercial-scale, which provided further insight into the development of sensory attributes and change of physicochemical properties of infusions of tea due to fermentation scale, temperature and time regimes and can be applied for commercial use.

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# ADDENDUM A

*C. intermedia sample information*

| N | Sample code | Producer/Geographic area | Harvesting date | Batch | Fermentation conditions |
|---|---|---|---|---|---|
| **1** | **INT1** | Helderfontein (ARC laboratory-scale fermentation) | 15/03/2010 | Batch a | 90°C/16 h |
| **2** | **INT2** | Helderfontein (ARC laboratory-scale fermentation) | 15/03/2010 | Batch a | 80°C/24 h |
| **3** | **INT3** | Helderfontein (ARC laboratory-scale fermentation) | 15/03/2010 | Batch b | 90°C/16 h |
| **4** | **INT4** | Helderfontein (ARC laboratory-scale fermentation) | 15/03/2010 | Batch b | 80°C/24 h |
| **5** | **INT5** | J. Kritzinger, Joubertina | 2009 | Unavailable | Unavailable |
| **6** | **INT6A** | Helderfontein (ARC laboratory-scale fermentation) | 17/11/2009 | Unavailable | 90°C/16 h |
| **7** | **INT6B** | Helderfontein (ARC laboratory-scale fermentation) | 17/11/2009 | Unavailable | 90°C/16 h |
| **8** | **INT7** | Ackerman | 24/11/2009 | Unavailable | Unavailable |
| **9** | **INT8** | Nortje, Kouga (ARC laboratory-scale fermentation) | 1999/2000 | Unavailable | 70°C/60 h |
| **10** | **INT9** | Nortje, Kouga (ARC laboratory-scale fermentation) | 06/05/2009 | Unavailable | 70°C/60 h |
| **11** | **INT10** | CHT Co (ARC laboratory-scale fermentation) | 12/01/2009 | Unavailable | 80-85°C/16 h |
| **12** | **INT11** | Haarlem (ARC laboratory-scale fermentation) | 03/10/2000 | Unavailable | 70°C/60 h |
| **13** | **4** | J. Kritzinger, Joubertina | Unavailable | L2127/2013 | 22-52°C/70 h |
| **14** | **5** | J. Kritzinger, Joubertina | Unavailable | L2129/2013 | Unavailable |
| **15** | **6** | J. Kritzinger, Joubertina | Unavailable | ORG409/2013 | 28-52°C/90 h |
| **16** | **9** | J. Kritzinger, Joubertina | Unavailable | L2124/2013 | 23-52°C/90 h |
| **17** | **10** | J. Kritzinger, Joubertina | Unavailable | ORG418/2013 | 32-73°C/82 h |
| **18** | **11** | Melmont Organic Mountain Honeybush Tea | Unavailable | Unavailable | Unavailable |
| **19** | **12** | George Ferreira | Unavailable | Unavailable | Unavailable |
| **20** | **14** | Helgard | Unavailable | Unavailable | Unavailable |
| **21** | **15** | H. Ackerman, Honeyblossom tea traders | 24/11/2009 | Unavailable | Unavailable |
| **22** | **16** | H. Ackerman, Honeybush Agroprocessing | 2011 | Unavailable | Unavailable |
| **23** | **1** | J. Kritzinger, Joubertina | Unavailable | L2145 | Unavailable |
| **24** | **2** | J. Kritzinger, Joubertina | Unavailable | L2138 | Unavailable |
| **25** | **3** | J. Kritzinger, Joubertina | Unavailable | L2124 | Unavailable |
| **26** | **7** | J. Kritzinger, Joubertina | Unavailable | L2132 | Unavailable |
| **27** | **8** | J. Kritzinger, Joubertina | Unavailable | ORG 418 | Unavailable |
| **28** | **18** | J. Kritzinger, Joubertina | 2009 | Unavailable | Unavailable |
| **29** | **19** | J. Kritzinger, Joubertina | 2009 | Unavailable | Unavailable |
| **30** | **20** | Melmont Organic Mountain Honeybush Tea | Unavailable | Unavailable | Unavailable |
| **31** | **26** | Nortje, Kouga (ARC laboratory-scale fermentation) | 26/02/2013 | Unavailable | 80°C/16 h |
| **32** | **27** | Nortje, Kouga (ARC laboratory-scale fermentation) | 27/02/2013 | Unavailable | 80°C/24 h |
| **33** | **24** | Nortje, Kouga (ARC laboratory-scale fermentation) | 28/02/2013 | Unavailable | 80°C/32 h |

| | | | | | |
|---|---|---|---|---|---|
| **34** | **29** | Nortje, Kouga (ARC laboratory-scale fermentation) | 01/03/2013 | Unavailable | 80°C/48 h |
| **35** | **30** | Nortje, Kouga (ARC laboratory-scale fermentation) | 02/03/2013 | Unavailable | 90°C/16 h |
| **36** | **25** | Nortje, Kouga (ARC laboratory-scale fermentation) | 03/03/2013 | Unavailable | 90°C/24 h |
| **37** | **32** | Nortje, Kouga (ARC laboratory-scale fermentation) | 04/03/2013 | Unavailable | 90°C/32 h |
| **38** | **33** | Nortje, Kouga (ARC laboratory-scale fermentation) | 05/03/2013 | Unavailable | 90°C/48 h |
| **39** | **28** | Mountain Honeybush Tea | Unavailable | Unavailable | Unavailable |
| **40** | **31 B** | Unavailable | Unavailable | Unavailable | Unavailable |
| **41** | **34** | H. Ackerman, Elandsrivier | Unavailable | Unavailable | Unavailable |
| **42** | **35** | H. Ackerman, Dwarsfontein | Unavailable | Unavailable | Unavailable |
| **43** | **36** | David Mills | Unavailable | Unavailable | Unavailable |
| **44** | **38** | Nortje, Nooitgedacht (Melmont) | May 2012 | Unavailable | Unavailable |
| **45** | **39** | M. van Dyk, Tsitsikamma | Unavailable | Unavailable | Unavailable |
| **46** | **40** | M. van Dyk, Kareedouw | Unavailable | Unavailable | Unavailable |
| **47** | **43** | J. Kritzinger, 0377 Brandhoek | Unavailable | Unavailable | Unavailable |
| **48** | **44** | J. Kritzinger, 0376 Brandhoek | Unavailable | Unavailable | Unavailable |
| **49** | **53** | J. Kritzinger, Joubertina | 15/08/2012 | L1806 | Unavailable |
| **50** | **55** | J. Kritzinger, Joubertina | 16/08/2012 | L1833 | Unavailable |
| **51** | **57** | J. Kritzinger, Joubertina | 17/08/2012 | L1905 | Unavailable |
| **52** | **58** | J. Kritzinger, Joubertina | 18/08/2012 | L1906 | Unavailable |
| **53** | **59** | J. Kritzinger, Joubertina | 19/08/2012 | L1908 | Unavailable |
| **54** | **60** | J. Kritzinger, Joubertina | 20/08/2012 | L1908 | Unavailable |
| **55** | **61** | J. Kritzinger, Joubertina | 21/08/2012 | L1909 | Unavailable |
| **56** | **63** | J. Kritzinger, Joubertina | 22/08/2012 | L1912 | Unavailable |
| **57** | **64** | J. Kritzinger, Joubertina | 23/08/2012 | L1913 | Unavailable |
| **58** | **65** | J. Kritzinger, Joubertina | 24/08/2012 | L1913 | Unavailable |
| **59** | **66** | J. Kritzinger, Joubertina | 25/08/2012 | L1914 | Unavailable |
| **60** | **67** | J. Kritzinger, Joubertina | 26/08/2012 | L1915 | Unavailable |
| **61** | **72** | J. Kritzinger, Joubertina | 27/08/2012 | L1921 | Unavailable |
| **62** | **74** | J. Kritzinger, Joubertina | 28/08/2012 | L1923 | Unavailable |
| **63** | **77** | J. Kritzinger, Joubertina | 29/08/2012 | L1928 | Unavailable |
| **64** | **80** | J. Kritzinger, Joubertina | 30/08/2012 | L1932 | Unavailable |
| **65** | **82** | J. Kritzinger, Joubertina | Unavailable | L1936 | Unavailable |
| **66** | **83** | J. Kritzinger, Joubertina | Unavailable | L1937 | Unavailable |
| **67** | **84** | J. Kritzinger, Joubertina | 20/03/2012 | L1938 | Unavailable |
| **68** | **85** | J. Kritzinger, Joubertina | Unavailable | L1939 | Unavailable |
| **69** | **86** | J. Kritzinger, Joubertina | 15/03/2012 | L1942 | Unavailable |
| **70** | **180** | Cape Honeybush Tea Company | 10/10/2012 | Unavailable | Unavailable |

**ADDENDUM B**

*Photographs illustrating the preparation of C. intermedia infusions for sensory evaluation*

**Figure 1** 12.5 g tea was infused in 1 L freshly boiled distilled water for 5 min before being decanted through a tea strainer into preheated stainless steel flasks for serving. *Ca*. 100 mL tea was filtered for soluble solids determination and absorbance measurements, and *ca*. 20 mL unfiltered tea was retained for turbidity measurements.

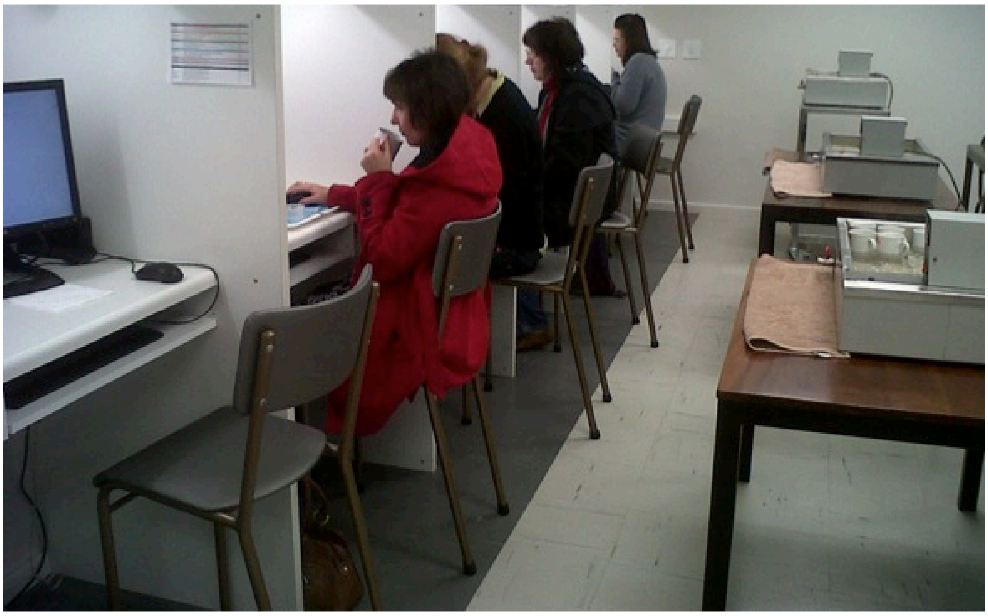

**Figure 2** *Cyclopia intermedia* infusions were served in preheated white porcelain mugs and placed in water baths regulated at 65°C to maintain the temperature of the infusions during descriptive sensory analysis. Panellists performed sensory analysis of the infusions in individual booths in a light- and temperature-controlled room (22°C).

**ADDENDUM C**

*List of descriptors generated for honeybush tea by Theron (2012)*

| 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, Banana bread, Berry, Guava, Cherry essence, Dried fruity mix, Raisins, Lemon essence, Lemongrass |
| **Plant-like** | Fynbos plant-like, Geranium plant-like, Herbaceous |
| **Woody** | Woody, Rooibos woody, Pine, Smokey, Plankey, Burnt |
| **Sweet-associated** | Fruity-sweet, Boiled syrup, Caramel, Honey, Fynbos-sweet |
| **Spicy** | Cinnamon, Cassia, Nutmeg, Mixed spice |
| **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 & mouthfeel attributes** | **Descriptors** |
| **Taste** | Sweet, Sour, Bitter, Salty |
| **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 |
| **Plant-like** | Fynbos plant-like, Geranium plant-like, Herbaceous |
| **Woody** | Woody, Rooibos woody, Pine |
| **Spicy** | Cinnamon, Cassia, Nutmeg, Mixed spice |
| **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, Cheesy, Earl Grey, Whiskey, Oily, Metallic |

# ADDENDUM D

*Descriptive sensory analysis questionnaire – C. intermedia profiling*

**Good morning!**
**There will be THREE tasting sessions today –**
**The same SIX samples will be presented in each session.**
**Before evaluating the tea sample, take off the lid and swirl the cup.**
**Please FIRST evaluate the AROMA of the tea, and then the PALATE attributes.**

**Question # 1**
**POSITIVE AROMA ATTRIBUTES**

**Sample <<Sample1>>**

Fynbos-floral
0 =none 100 =Prominent

Rose geranium
0 100

Rose/Perfume
0 100

Lemon/lemon grass
0 100

Apricot jam
0 100

Cooked apple
0 100

Raisin
0 100

Earthy
0 100

Plant-like
0 100

Woody
0 100

Pine
0 100

Fruity-sweet
0 100

Caramel/Vanilla
0 100

Honey
0 100

Fynbos-sweet
0 100

Sweet spice
0 100

Walnut
0 100

Almond/Marzipan
0 100

**Question # 2**
**NEGATIVE AROMA ATTRIBUTES**

**Sample <<Sample1>>**

Dusty
0 = none 100 = prominent

Yeasty
0 100

Medicinal
0 100

Burnt caramel
0 100

Hay/Dried Grass
0 100

Green grass
0 100

Cooked vegetables
0 100

Sour
0 100

Smokey
0 100

Wet fur/farm animals
0 100

**Question # 3**
**TASTE AND MOUTHFEEL ATTRIBUTES**

**Sample <<Sample1>>**

SWEET TASTE
0 = none 100 = prominent

SOUR TASTE
0 100

BITTER TASTE
0 100

ASTRINGENT MOUTHFEEL
0 100

**Question # 4**
**FLAVOUR ATTRIBUTES**

**Sample <<Sample1>>**

Fynbos-floral
0 = none 100 = prominent

Rose geranium
0 100

Rose/Perfume
0 100

Lemon/lemon grass
0 100

Apricot jam
0 100

Cooked apple
0 100

Raisin
0 100

Woody
0 100

Pine
0 100

Caramel/Honey
0 100

Sweet spice
0 100

Nutty
0 100

Dusty

0 100

Medicinal

0 100

Burnt caramel

0 100

Hay/Dried grass

0 100

Green grass

0 100

Wet fur/farm animals

0 100

CONTINUE TO THE NEXT REPETITION

THANK YOU

Have a lovely day!

**ADDENDUM E**

*Example of calculations done on the attribute intensities of one sample for the categorisation of samples in to sensory "quality" grades*

**Table 1** Calculations to determine "quality" grade of samples according to % intensities of positive and negative attributes.

<table>
<tr><th></th><th></th><th>Attributes</th><th>Attribute intensities</th><th>Total intensities</th><th>% Attribute intensities</th><th>Attribute count</th><th>Weighted % attribute intensities</th></tr>
<tr><td rowspan="23">POSITIVE ATTRIBUTE INTENSITIES</td><td rowspan="13">AROMAS</td><td>Fynbos-floral</td><td>31</td><td rowspan="23">227</td><td rowspan="23">(227/326) *100= 70%</td><td rowspan="23">22</td><td rowspan="23">(227/326)/ (22/39)*0.5 *100 = 62%</td></tr>
<tr><td>Rose geranium</td><td>3</td></tr>
<tr><td>Lemon/lemon grass</td><td>2</td></tr>
<tr><td>Apricot jam</td><td>3</td></tr>
<tr><td>Cooked apple</td><td>2</td></tr>
<tr><td>Raisin</td><td>2</td></tr>
<tr><td>Woody</td><td>42</td></tr>
<tr><td>Earthy</td><td>6</td></tr>
<tr><td>Pine</td><td>0</td></tr>
<tr><td>Fruity-sweet</td><td>6</td></tr>
<tr><td>Caramel/vanilla</td><td>2</td></tr>
<tr><td>Honey</td><td>5</td></tr>
<tr><td>Fynbos-sweet</td><td>26</td></tr>
<tr><td rowspan="9">FLAVOURS</td><td>Fynbos-floral</td><td>29</td></tr>
<tr><td>Rose geranium</td><td>1</td></tr>
<tr><td>Lemon/lemon grass</td><td>3</td></tr>
<tr><td>Apricot jam</td><td>2</td></tr>
<tr><td>Cooked apple</td><td>0</td></tr>
<tr><td>Pine</td><td>0</td></tr>
<tr><td>Woody</td><td>42</td></tr>
<tr><td>Caramel/Honey</td><td>1</td></tr>
<tr><td>TASTE</td><td>SWEET</td><td>17</td></tr>
<tr><td rowspan="18">NEGATIVE ATTRIBUTE INTENSITIES</td><td rowspan="8">AROMAS</td><td>Dusty</td><td>5</td><td rowspan="18">99</td><td rowspan="18">(99/326) *100= 30%</td><td rowspan="18">17</td><td rowspan="18">(99/326)/ (17/39)*0.5 *100 = 35%</td></tr>
<tr><td>Medicinal</td><td>4</td></tr>
<tr><td>Burnt Caramel</td><td>4</td></tr>
<tr><td>Hay/dried grass</td><td>13</td></tr>
<tr><td>Green grass</td><td>4</td></tr>
<tr><td>Cooked vegetables</td><td>3</td></tr>
<tr><td>Smokey</td><td>11</td></tr>
<tr><td>Wet fur/farm animals</td><td>5</td></tr>
<tr><td rowspan="8">FLAVOURS</td><td>Dusty</td><td>4</td></tr>
<tr><td>Medicinal</td><td>4</td></tr>
<tr><td>Burnt Caramel</td><td>0</td></tr>
<tr><td>Hay/Dried grass</td><td>15</td></tr>
<tr><td>Green grass</td><td>2</td></tr>
<tr><td>Smokey</td><td>9</td></tr>
<tr><td>Wet fur/farm animals</td><td>4</td></tr>
<tr><td>(blank)</td><td></td></tr>
<tr><td rowspan="2">TASTE</td><td>SOUR</td><td>10</td></tr>
<tr><td>BITTER</td><td>3</td></tr>
<tr><td colspan="3">TOTAL (positive + negative attributes)</td><td></td><td>326</td><td></td><td>39</td><td></td></tr>
</table>

* Astringent mouthfeel was not included in calculation, as its perception was not noteworthy in the overall infusion characteristic.

**Table 2** Parameters used to categorise *C. intermedia* infusions into quality grades.

| | % Attribute intensities present in an infusion | |
|---|---|---|
| **Quality category** | **Positive** | **Negative** |
| Good | > 80% | < 10% |
| Average | > 75 < 80% | > 10 < 15% |
| Poor | < 75% | > 15% |