Development and validation of prediction models and rapid sensory methodologies to understand intrinsic bitterness of *Cyclopia genistoides*

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Declaration

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

This dissertation includes one original paper published in a peer-reviewed journal (Food Research International) and four unpublished publications. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and, for each of the cases where this is not the case, a declaration is included in the dissertation indicating the nature and extent of the contribution of co-authors.

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Abstract

Cyclopia species, endemic to South Africa, is used for the production of honeybush tea. This herbal tea has grown from a product consumed only locally to one currently marketed worldwide. *Cyclopia* species is high in polyphenols, well-known for its health related properties, but these compounds could contribute to bitterness, which may elicit negative consumer response. One of the species, *C. genistoides*, is high in polyphenols but also associated with bitterness, contrary to the characteristic sweet taste and honey-like flavour associated with honeybush.

The polyphenolic content of four *Cyclopia* species were quantified with high-performance liquid chromatography diode-array detection while descriptive sensory analysis (DSA) was used to determine the taste intensities of these samples. The link between phenolic content and bitterness was investigated. Based in the phenolic content of *C. genistoides* and *C. longifolia,* partial least squares (PLS) regression analysis identified four compounds, mangiferin, isomangiferin, iriflophenone-3-*C*-glucoside-4-*O*-glucoside and iriflophenone-3-*C*-glucoside, as candidate predictors of bitterness. This model will find application as screening tool in cultivar development research programs.

Production of honeybush lags behind demand, forcing the industry to use blends of *Cyclopia* species, including *C. genistoides*, to supply in the increased demand. The distinct differences in the sensory profiles associated with different *Cyclopia* species require that special care is given to blending to ensure a consistent, high quality product. DSA was used to evaluate the effect of blending of *C. genistoides* with other *Cyclopia* species on bitterness. Blending of *C. genistoides* with *C. subternata*, *C. intermedia* or *C. maculata* in a ratio of 2:3 were effective in reducing bitterness to below perceptible levels. The sensory profile of *C. genistoides*-*C. subternata* blends were further quantified using DSA and was described as “fynbos floral”, “apricot”, “woody”, “fruity sweet” and “fynbos sweet” aroma and a sweet taste. Based on these results, inclusion of *C. genistoides* at 40% when blending different *Cyclopia* species, is recommended. Blending at this standardised ratio will result in a well-rounded product with bitterness below perceptible levels.

The herbal tea industry expressed the need for time- and cost-effective methods for sensory screening of infusions to improve quality and product consistency. The validity of three rapid profiling methods {sorting, projective mapping and polarised sensory positioning (PSP)} for the sensory characterisation of honeybush infusions were investigated using a trained panel. The efficacy of partial (aroma or palate attributes) or global (all attributes) evaluation was compared within each rapid method. Product configurations similar to that of DSA demonstrated the validity of all three methods for broad sensory profiling of *Cyclopia* species. Sorting on palate attributes resulted in additional differentiation between samples with only subtle differences. Sorting demonstrated to be the most effective method for the broad sensory profiling of honeybush infusions and could find application in the honeybush industry as screening tool. PSP on the other hand could find application in quality control programs where poles that represent specific quality attributes, should be included. Implementation of valid, scientific methods, such as sorting and PSP, will aid the honeybush industry in their effort to supply a product with consistent quality and high consumer appeal.

Opsomming

Die eg Suid-Afrikaanse fynbosgenus, *Cyclopia,* word gebruik vir die produksie van heuningbostee, 'n unieke kruietee wat internasionaal bemark word. Die gebruik van heuningbostee hou verskeie gesondheidsvoordele in wat grootliks toegeskryf kan word aan die fenoliese samestelling, maar hierdie verbindings kan bydra tot 'n bitter smaak wat negatiewe verbruikersreaksie tot gevolg mag hê. Een van die spesies, *Cyclopia genistoides,* beskik oor 'n hoë fenoliese samestelling, maar word ook met 'n bitter smaak geassosieer.

Die fenoliese samestelling van vier *Cyclopia* spesies is gekwantifiseer deur middel van hoë-druk vloeistof chromatografie gekoppel aan ultraviolet-diode deteksie. Beskrywende sensoriese analise is gebruik om die sensoriese profiel te kwantifiseer. Die verband tussen fenoliese samestelling en bitterheid is ondersoek. Die fenoliese samestelling van *C. genistoides* en *C. longifolia* is gebruik vir die ontwikkeling van 'n model om bitterheid te voorspel. Gedeeltelike kleinste-kwadrate regressie analise (PLS) met seleksie van veranderlikes is op die data toegepas. Vier komponente, naamlik mangiferien, isomangiferien, iriflofenoon-3-*C*-glukosied-4-*O*-glukosied en iriflofenoon-3-*C*-glukosied, is geïdentifiseer as potensiële "kandidaat voorspellers" vir bitterheid. Hierdie model kan toepassing vind in die heuningbos kultivar ontwikkelingsprogram vir die seleksie van plantmateriaal.

Die vraag na heuningbos oorskry produksie, wat die bedryf noodsaak om *Cyclopia* spesies te vermeng. Beduidende verskille in die geurprofiel van die verskillende *Cyclopia* spesies vereis dat mengsels met omsigtigheid berei word om 'n konstante, hoë kwaliteit eindproduk te verseker. Beskrywende sensoriese analise is gebruik om die effek van vermenging van *C. genistoides* met ander *Cyclopia* spesies op bitterheid te bepaal. Vermenging van *C. genistoides* met enige van *C. subternata, C. intermedia* of *C. maculata* in 'n verhouding van 2:3 was effektief om bitterheid tot onder waarneembare vlakke te verlaag. Die sensoriese profiel van die *C. genistoides-C. subternata* mengsel kan beskryf word as 'n "fynbos-blomagtige", "appelkoos", "houtagtige", "vrugtige-soet" en "fynbos-soet" aroma en 'n soet smaak. Op grond van hierdie resultate kan aanbevelings aan die heuningbosteebedryf gemaak word. Insluiting van *C. genistoides* teen 40% in *Cyclopia* mengsels sal 'n produk met lae bitterheid en 'n karakteristieke heuningbostee profiel lewer.

Die heuningbosteebedryf is 'n klein, maar groeiende bedryf en die implementering van tyd- en koste-effektiewe metodes vir sensoriese evaluaring is belangrik. Hierdie studie het die geldigheid van drie vinnige profileringsmetodes {sortering, projektiewe kartering (PK) en gepolariseerde sensoriese posisionering (GSP)} vir die sensoriese beskrywing van heuningbostee, ondersoek. Die effektiwiteit van hierdie metodes, gefokus per modaliteit (aroma of geur) of met 'n holistiese benadering, is vergelyk. Al drie die genoemde vinnige profileringsmetodes is geldige metodes vir die oorsigtelike sensoriese kategorisering van *Cyclopia* spesies. Addisionele differensiasie tussen produkte met slegs subtiele verskille is verkry deur sortering, gefokus op geur-eienskappe. Sortering is die mees effektiewe metode vir die oorsigtelike beskrywing van heuningbostee en kan effektief in die heuningbosteebedryf toegepas word. Verder kan GSP toepassing vind in kwaliteitsbeheerprogramme waar produkte met spesifieke eienskappe ingesluit word as verwysingstandaarde. Implementering van geldige en betroubare sensoriese metodes, soos sortering en GSP, kan 'n waardevolle

bydrae lewer in die heuningbosteebedryf om ‘n kwaliteit produk met hoë verbruikersaanvaarbaarheid te voorsien aan ‘n groeiende mark.

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"The journey of a thousand miles begin with one step." *Lao Tzu*

Notes

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

Results from this dissertation that have been submitted for publication:

Moelich, E.I., Muller, M., Joubert, E., Næs, T., Kidd, M. (2017). Validation of projective mapping as potential screening tool for application by the honeybush herbal tea industry. *Food Research International*, 99, 275-286.

Results from this dissertation that have been presented at conferences:

Moelich, E.I. (2017). Exploring projective mapping for the rapid sensory profiling of *Cyclopia* species (honeybush). Oral presentation at the 22nd SAAFoST Biennial International Congress and Exhibition, 3-6 September 2017, Cape Town. South Africa

Moelich, E.I., Muller, M., Joubert, E. & Næs, T. (2016). Projective mapping as potential quality screening tool for Honeybush herbal tea. Poster presented at EuroSense 2016, 7th European Conference on Sensory and Consumer Research, 10-14 September 2016, Dijon, France

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Chapter 1

Introduction

The global demand for herbal teas has grown substantially over the past decades, primarily driven by consumer awareness of the health benefits associated with this type of product (Insight Survey, 2016). *Cyclopia* species, endemic to the fynbos biome of South Africa, is used for the production of honeybush tea (Joubert, Gelderblom, Louw, & De Beer, 2008). This herbal tea has grown from a product consumed only locally to one currently marketed worldwide. Researchers are working towards positioning honeybush as a unique herbal tea that can compete on local and global markets based on its quality, taste, flavour and health benefits (Joubert, Joubert, Bester, De Beer, & De Lange, 2011). The majority of the annual production comprises *C. intermedia,* mainly harvested from the wild, and to a lesser extent *C. subternata* and *C. genistoides*, both cultivated. Small quantities of *C. maculata, C. longifolia* and *C. sessiliflora* are also produced.

The distinguishing sensory profile of honeybush tea is the result of "fermentation", a high-temperature oxidation process (Du Toit & Joubert, 1999). All the *Cyclopia* species investigated to date are associated with the generic sensory profile, defined as "fynbos-floral", "woody" and "fynbos-sweet" aroma and flavour, with a slight sweet taste and slight astringent mouthfeel (Erasmus, Theron, Muller, Van Der Rijst, & Joubert, 2017). Furthermore, the respective species show higher intensities of specific sensory attributes, contributing to species-specific sensory profiles. *Cyclopia genistoides* is associated with a strong "rose-geranium" flavour and perceptible bitter taste, while *C. subternata* associate with "caramel" and "sweet-associated" aroma notes and a slight astringent mouthfeel. The distinct bitter taste of *C. genistoides* is contrary to the sweet taste and honey-like flavour that consumers associate with honeybush tea (Vermeulen, 2015).

Taste is one of the main drivers of consumers' food choice (Drewnowski, 2001). Bitterness, one of the basic tastes, is well-known for eliciting negative consumer responses when present at high intensities, resulting in lower consumption patterns (Drewnowski & Gomez-Carneros, 2000). In beverages such as black tea, cider, and red wine, the bitter taste and the astringent mouthfeel are primarily elicited by polyphenols (Lesschaeve & Noble, 2005). Theron (2012) investigated the link between the phenolic compounds in honeybush infusions and sensory data, and the results suggested that mangiferin might be responsible for the perceptible bitter taste. In a study on the polyphenol content of several *Cyclopia* species, Schulze et al. (2015) demonstrated that C. *genistoides* contained the highest content of the xanthone, mangiferin and its regio-isomer, isomangiferin, while *C. subternata* contained the lowest levels of these compounds.

Erasmus (2015) conducted stepwise-regression analysis to investigate the relationship between the phenolic compounds and sensory taste attributes (sweet, sour, bitter and astringent) associated with honeybush infusions. The results indicated that the bitterness could be associated with several phenolic compounds,

including mangiferin and isomangiferin. Several limitations were identified when performing step-wise regression analysis, with the main limitation being the inability to handle collinearity of variables. Collinearity of variables can result in different models. When two independent variables (predictors) are significantly and highly correlated to each other and to a dependent variable, the model selects only one of the predictors to be present in the model. A more targeted approach to prediction model development is thus necessary. A technique that could find application in the current context, is partial least square (PLS) regression analysis. The PLS regression method is a projection-based technique that can handle data with numerous and strongly collinear *X*-variables, while it is able to simultaneously model several *Y*-variables (Wold, Sjostrom, & Eriksson, 2001). A valid model that can predict sensory bitterness based on phenolic composition, would provide an effective screening tool within a research program to identify plant material of *Cyclopia* species producing herbal tea with unacceptable high levels of bitterness. In this way, screening of plant material could be conducted in a time- and cost-effective manner. Such a prediction model could find application in the Honeybush Cultivar Development Program of the Agricultural Research Council, South Africa to identify genotypes and plant selections with high levels of bitterness. Plant material with predicted high levels of bitterness, should not be included in cultivation programs for honeybush tea processors but rather utilised in the nutraceutical extract industry.

Prediction models are valuable tools in the research environment but in industry there is a need for effective methods to address bitterness associated with *C. genistoides*. The honeybush industry already blends different species to supply in the local and international demand. Blending of *C. genistoides* with sweet-tasting *C. subternata* may be a viable option to reduce bitterness, but to date, no targeted research to address this matter has been conducted. The effect of blending *C. genistoides* with *C. subternata* and other *Cyclopia* species on bitterness perception needs to be investigated to obtain actionable results that will aid the honeybush industry in effective blending practices to reduce bitterness associated with *C. genistoides*.

While it is important to reduce bitterness to below perceptible levels, blending of different *Cyclopia* species may have a significant effect on the species-specific sensory profiles. The effectiveness of blending in reducing bitterness without adversely affecting the complex aroma profile of honeybush, needs to be evaluated. Although descriptive sensory analysis delivers a detailed sensory profile of the products tested, this comprehensive method is considered time-consuming to conduct and can be regarded as too cumbersome for the honeybush industry to use in quality control programs. There is thus a need for flexible and rapid sensory profiling methods to determine the effect of blending on bitterness, as well as the species-specific sensory quality of honeybush.

Novel rapid methodologies for sensory characterisation of products have gained popularity, and have been the main focus of international sensory and consumer research programs in the last decade (Varela & Ares, 2014). Although numerous studies on the application of rapid sensory methods have been published, research to better understand the advantages, disadvantages and limitations of the various rapid profiling techniques, both with respect to efficiency, information obtained and simplicity in use, need to be conducted.

This opens up research opportunities for developing and identifying product-specific rapid profiling methods that will be suitable for application in the herbal tea industry.

The efficacy of several rapid sensory methods for the characterisation of different *Cyclopia* species needs to be evaluated. Preliminary research has demonstrated that *Cyclopia* species can be profiled using sorting (Erasmus, 2015); however, no information on the suitability of other rapid methods as applied to honeybush has been published. The main benefits of the development of rapid sensory profiling methods is that these methods can be used time- and cost-effectively within research and development (R&D) and quality control (QC) programs in industry.

In view of the above, the aim of the research is to 1) develop and validate a prediction model using polyphenol content to predict sensory bitterness in *Cyclopia* species*;* 2) to establish robust blending protocols for *C. genistoides* with other *Cyclopia* species to mask bitterness; 3) to validate and establish effective rapid profiling methods such as sorting, projective mapping and polarised sensory positioning, for application within R&D and QC programs in the honeybush industry.

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Chapter 2

Literature review

1 Introduction

Tea, prepared by infusing dried leaves of *Camilla sinensis* in hot water, is the second most consumed beverage in the world after water (Butt & Sultan, 2009). Tea can be classified into three major categories, mainly based on the extent to which fermentation, an enzymatic oxidation process, has taken place. Green tea is derived from unfermented leaves, oolong tea from partially fermented leaves and black tea from fermented leaves of plant species including *Camellia sinensis*. Fully fermented black tea accounts for approximately 80% of the total world tea production while green tea accounts for 20%, and oolong tea for 2% of production (Flaten, 2002). In the case of the endemic South African herbal teas, plant material from *Aspalathus linearis* is used for the preparation of rooibos tea while honeybush tea is prepared from several *Cyclopia* species (Joubert, Joubert, Bester, De Beer, & De Lange, 2011).

In 2017, the three main consumer trends driving consumer behavior in the beverage market were an interest in process-based beverages (including hot water infusions of dried plants), "earthy" flavours and a growing demand for more premium water (safe, clean and accessible water options) (Horwit & Zimmer, 2017). Health and wellness trends combined with changing consumer habits, contributed to the development of the fruit and herbal tea market. Growing consumer interest in the herbal tea market is confirmed by the SA Tea Industry Landscape Report, where a global annual growth rate in consumption of 10% in green tea and 4% in herbal and fruit teas, are reported (Insight Survey, 2016). Both honeybush and rooibos herbal teas are indigenous to South Africa, and as a result is marketed as unique, niche products.

South Africa has a well-established herbal tea industry, and is known worldwide for the production of several endemic herbal tea ranges. The most well-known of these herbal teas is rooibos tea, which is a product of the fynbos species *Aspalathus linearis,* endemic to the Western Cape region. Demand for rooibos tea has been increasing not only on a local scale, but globally; countries including the United States of America, United Kingdom, the Netherlands, Germany and Japan comprise the majority of the global market and total exports of rooibos tea have increased from 750 tons in 1993 to over 6000 tons in 2016 (SARC, 2016). Honeybush tea is another herbal tea endemic to the Western Cape regions of South Africa that is experiencing a similar surge in local and global consumer awareness.

2 Honeybush industry

2.1 Geographical distribution and botanical description

Honeybush (*Cyclopia* species) forms part of the Cape fynbos biome spread across the coastal and mountainous areas of the Eastern and Western Cape regions of South Africa (Du Toit & Joubert, 1998; Joubert, et al., 2011). The majority of *Cyclopia* species grow in mountainous areas, preferring the cool, shady and sandy southern slopes near the coast; the exception is *C. genistoides*, which prefers the flat, sandy regions right next to the Southern coast.

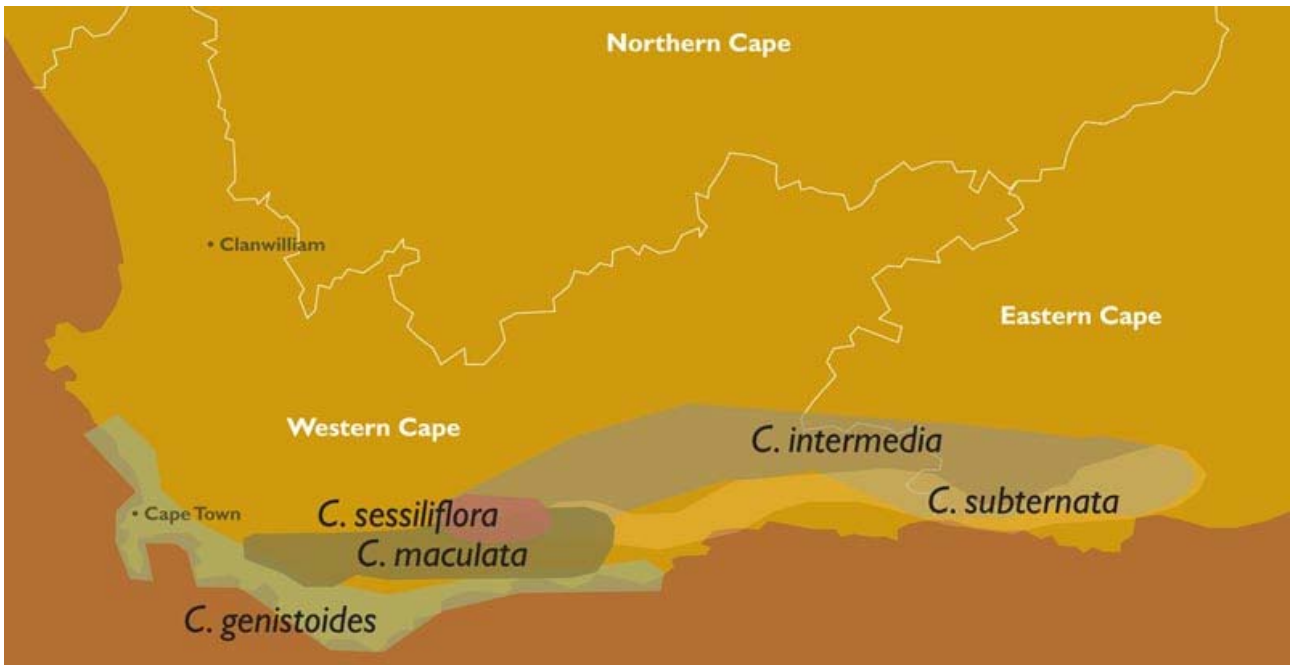

Fig. 1 Natural distribution of honeybush species in South Africa (SAHTA, 2017)

Traditionally harvesting occurred during the flowering periods in an attempt to maximise the sweet honey flavour in the tea; however, the global increase in demand led to changes in the harvesting methods, primarily to allow for earlier harvesting, starting before the plants have fully flowered. Sensory research conducted on this change of processing, suggested that the final product was regarded as satisfactory when compared to samples harvested after flowering (Du Toit & Joubert, 1998).

The physical plant is on average about 1.5 to 2 m in height, comprising woody yellow-to-brown stems, bright yellow flowers which give off a sweet, mellifluous odour during flowering months, and has a low leaf-to-stem ratio (Du Toit, Joubert, & Britz, 1998). The leaves are trifoliate, with variations in shape according to the different species. *Cyclopia genistoides* is characterised by pubescent, narrow leaves (14-20 mm long, 1-2 mm wide), whereas other commercial species, such as *C. intermedia* and *C. subternata*, both have large, flattened leaves (18-28 mm long, 2-5 mm wide) (Du Toit, et al., 1998). There are mainly two classifications of *Cyclopia* based on survival instincts: sprouters, which survive by forming coppice shoots from the woody roots; and non-sprouters, also known as re-seeders, which form seeds from which the new plant grow after scarification and germination (Du Toit et al., 1998). *Cyclopia genistoides* is classified as a sprouter and can

be harvested 2-3 years after planting, with annual harvesting after the first year of harvest. Non-sprouters tend to be preferred for cultivation as they can be harvested one year after planting. *Cyclopia subternata* is classified as a non-sprouter, it is regarded as a relative fast grower and can be harvested annually (Joubert et al., 2011). *C. intermedia* require at least two to three years of growth prior to the harvesting event and are therefore not always favoured for cultivation trials.

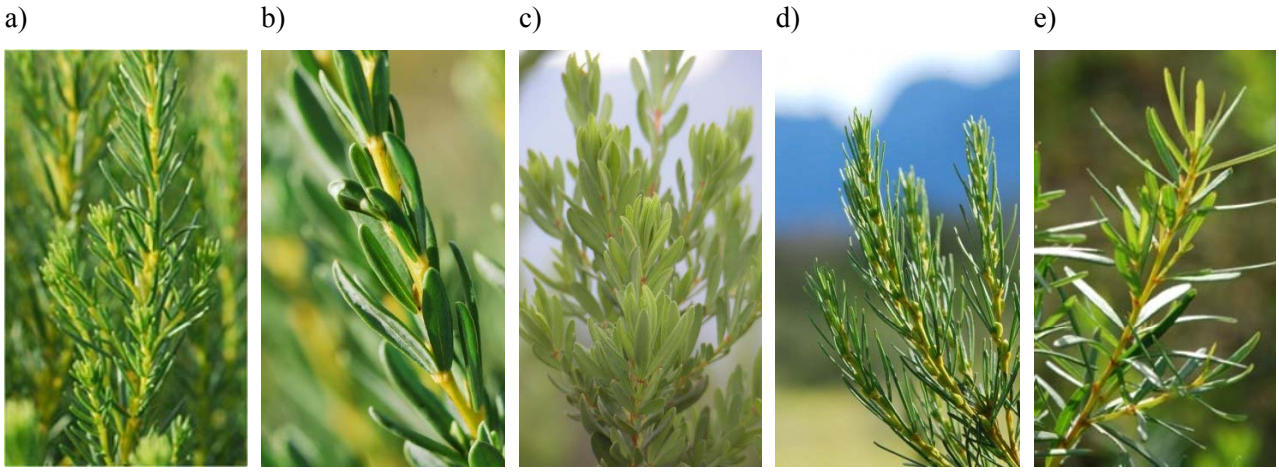

Fig. 2 Leaves of different *Cyclopia* species a) *C. genistoides* b) *C. subternata* c) *C. intermedia* d) *C. maculata* e) *C. longifolia*

2.2 Processing of honeybush

Unsustainable harvesting practices of honeybush plant-material from the wild have caused a significant decline in species populations (Joubert, et al., 2011). According to the South African Honeybush Tea Association (SAHTA, 2017), 70% of honeybush tea is harvested from the wild and only 30% cultivated.

The industry has established commercial plantations in an attempt to conserve the abundance of *Cyclopia* species and is working with local authorities in the Western and Eastern Cape, South Africa to implement measures to control wild harvesting. Species of commercial interest are *C. subternata, C. genistoides* and *C. intermedia (*Joubert et al., 2011). *Cyclopia subternata* and *C. genistoides* are the only species currently under cultivation, with about 200 ha designated for cultivation throughout the Overberg and Langkloof regions (Joubert et al., 2011). *Cyclopia intermedia* is almost exclusively harvested from the wild and currently, this species also contributes to the bulk of honeybush production. It is uneconomical to cultivate as it can only be harvested every second to third year. Small quantities of *C. sessiliflora* and *C. plicata* are also wild harvested (E. Joubert, personal communication, October 20, 2017).

Traditional fermentation of honeybush in curing heaps, resulted in tea with inconsistent and often poor quality (Du Toit & Joubert, 1999). In the last decade, research has focused on evaluating processing parameters for optimum product quality. Treating the tea with water prior to fermentation reduced the occurrence of white pieces of stem in the final dried product and enhanced colour development, resulting in an infusion with a dark red-brown colour (Du Toit, et al. 1998). Fermentation, a high temperature oxidation process of the plant

leaves, is universally proven to increase positive sensory attributes and decrease negative sensory attributes in the tea. In recent years, research has focused on evaluating fermentation conditions for optimum development of colour and flavour of different *Cyclopia* species (Bergh, Muller, Van der Rijst, & Joubert, 2017; Erasmus, Theron, Muller, Van der Rijst, & Joubert, 2017; Theron et al., 2014). Currently, the honeybush industry mostly apply fermentation conditions of 80-85°C/24 h or 90°C/16 h for the majority of *Cyclopia* species processed.

3 Characteristics of honeybush

Honeybush is a unique, indigenous crop, with several health benefits associated with this herbal tea, the latter contributing to its potential to be marketed as a niche product in global tea markets. The majority of the honeybush crop is exported to the Netherlands, Germany, the UK and the USA. This market has grown substantially over the last decade, with exports increasing from 50 to approximately 200 tons per annum (SAHTA, 2017).

3.1 Chemical composition

3.1.1 Volatile compounds

The volatile compounds add to the aroma profile of a product, whereas non-volatile components, i.e. polyphenols add to the basic taste and mouthfeel attributes (Bakker & Clarke, 2012). Honeybush herbal tea is associated with a wide range of volatile compounds and the identification of these compounds is important as aroma is regarded as one of the most important attributes for assessing tea quality (Yang, Baldermann, & Watanabe, 2013). Le Roux, Cronje, Joubert, & Burger (2008) used gas chromatography-mass spectrometry (GC-MS) to analyse the volatile organic compounds present in fermented and unfermented *C. genistoides*. A total of 79 compounds were identified in the volatile fraction of fermented *C. genistoides*, of which 46 were found to be terpenoids. A considerable difference in the quantity of volatile compounds in unfermented and fermented tea were revealed, indicating that the fermentation process increased the quantity of volatile compounds associated with sweet-woody and floral descriptors. The same methodology was applied to quantify the volatile organic compounds in fermented *C. subternata*. A total of 183 compounds, the majority of which were terpenoids, were identified. When employing gas chromatography−olfactometry (GC-O), 37 of these compounds were determined to be odor-active (Le Roux, Cronje, Burger, & Joubert, 2012).

Ntlhokwe, Tredoux et al. (2017) employed headspace solid phase micro-extraction (HS-SPME) in combination with two-dimensional gas chromatography (GC×GC) separation for the analysis of the organic volatile compounds in fermented *C. maculata*, *C. subternata* and *C. genistoides*. A total of 84 compounds were identified; most of the compounds were common to all three species, with observed differences mainly due to differences in relative amounts. Research by Ntlhokwe, Muller, Joubert, Tredoux, & de Villiers (2017) aimed to identify compounds potentially responsible for observed sensory differences between *Cyclopia* species. Two-dimensional gas chromatography (GC × GC) combined with time-of-flight mass spectrometry (TOF)-MS was used for the quantification of the volatile compounds associated with three *Cyclopia* species

(*C. genistoides*, *C. maculata* and *C. subternata*). A total of 287 compounds were identified, but only a limited number of these compounds seem to be species-specific. The results on the quantification of the volatile compounds associated with several *Cyclopia* species demonstrated the immense complexity of honeybush tea volatiles.

3.1.2 Non-volatile compounds

The taste (sweet, sour and bitter) and mouthfeel (astringency) of tea are determined by several classes of non-volatile compounds including polyphenolic compounds, amino acids, purine alkaloids, nucleotides, organic acids, carbohydrates and ions (Kaneko, Kumazawa, Masuda, Henze, & Hofmann, 2006). Pertaining to polyphenols, these compounds are abundant in plant food and beverages and exhibit a wide range of properties, depending on the structure of the compound (Lesschaeve & Noble, 2005). Honeybush is a herbal tea low in tannins and caffeine and rich in polyphenolic compounds (Joubert, Gelderblom, Louw, & De Beer, 2008; Kamara, Brandt, Ferreira, & Joubert, 2003).

Numerous studies have reported on the health promoting properties associated with polyphenols present in *Cyclopia* species. Van der Merwe et al. (2006) reported on the anti-mutagenic properties associated with *C. subternata*, *C. genistoides* and *C. sessiliflora.* Research further focused on the antioxidant capacity of polyphenols isolated from *C. genistoides* (Beelders, De Beer, & Joubert, 2015; Malherbe et al., 2014) and *C. genistoides*, *C. subternata*, *C. maculata* and *C. longifolia* (Schulze et al., 2015). Anticancer properties associated with *C. intermedia* were reported by Marnewick, Joubert, Swart, Van Der Westhuizen, & Gelderblom (2003) and Sissing et al. (2011). Polyphenols in honeybush tea are furthermore associated phyto-estrogenic properties (Verhoog, Joubert, & Louw, 2007) and anti-obesity properties were demonstrated in *C. maculata* and *C. subternata* (Dudhia et al. 2013) and *C. intermedia* (Jack et al., 2017).

Mangiferin is one of the major phenolic compounds associated with *Cyclopia* species and specifically with *C. genistoides.* Mangiferin is widely distributed in plants and has been identified in several flowering plants (De Nysschen, Van Wyk, Van Heerden, & Schutte, 1996). It is the major constituent of extracts of the mango plant, *Mangifera indica* L. (Anacardiaceae) (Barreto et al., 2008). A recent review on the biological and pharmacological effects of mangiferin on metabolism and metabolic disorders were published by Fomenko & Chi (2016). Mangiferin is a nutraceutical that demonstrates multiple beneficial effects, mainly ascribed to its antioxidant and anti-inflammation properties. Mangiferin modulates multiple biological processes involved with carbohydrate and lipid metabolism. The review article reported on the modulating effect of mangiferin on obesity, its ability to inhibit lipogenesis, to reduce hyperglycemia and therefore also reduce the risk for diabetes, its mitigating effect on nephropathy (one of the complications associated with diabetes) and its ability to reduce the risks associated with cardiac vascular diseases (Fomenko & Chi, 2016).

Non-volatile components, i.e. polyphenols not only contribute to the health promoting properties of honeybush tea as illustrated above, but also add to the colour and basic taste and mouthfeel attributes of food and beverages (Cheynier, 2005). In an effort to support the development of the honeybush tea industry, numerous studies have been conducted to investigate the phenolic composition of *Cyclopia* species. Several

of the first reported studies focused on the quantification of the polyphenol content of methanol extracts of selected *Cyclopia* species (Ferreira, Kamara, Brandt, & Joubert, 1998; Kamara, Brand, Brandt, & Joubert, 2004; Kamara et al., 2003), indicating that these species afforded a complex arrangement of polyphenolic derivatives, including xanthones, flavanones, flavones, iso-flavones and coumestans.

Mounting evidence on the health promoting properties associated with the polyphenols in honeybush, resulted in the need for effective, valid methods for the quantification of these compounds in hot water extracts of different *Cyclopia* species. A high-performance liquid chromatography –diode array detection (HPLC-DAD) method for the quantification of the major phenolic compounds in hot water extracts associated with *C. subternata*, were developed (De Beer et al., 2012). Extensive research on HPLC-DAD method development showed that a specie-specific method for phenolic profiling of the different *Cyclopia* specie is necessary. Using the method reported by De Beer et al. (2012) as starting point, species-specific HPLC-DAD methods were developed and validated for *C. maculata* (Schulze, De Beer, De Villiers, Manley, & Joubert, 2014) and *C. genistoides* (Beelders, De Beer, Stander, & Joubert, 2014). The phenolic content of hot water extracts of fermented and unfermented *Cyclopia genistoides* (L.) Vent. was determined by employing the specie-specific HPLC-DAD method. Forty phenolic compounds were identified, of which 31 were identified for the first time in *C. genistoides*. The xanthones, mangiferin and isomangiferin, were some of the major phenolic compounds identified (Beelders, et al., 2014).

Schulze et al. (2015) employed validated, species-specific HPLC-DAD detection methods to quantify the phenolic composition of infusions at 'cup-of-tea' strength of four *Cyclopia* species (*C. genistoides, C. subternata, C. maculata* and *C. longifolia).* 'Cup-of-tea' strength represent the strength of honeybush tea as normally consumed. Large variation in the qualitative and quantitative phenolic profiles of the different *Cyclopia* species were reported. Major phenolic compounds identified in one or more of the species were mangiferin, isomangiferin, vicenin-2, scolymoside, eriocitrin, hesperidin, iriflophenone-3-*C*-glucoside, iriflophenone-3-*C*-glucoside-4-*O*-glucoside, phloretin-3',5'-di-*C*-glucoside and 3-hydroxyphloretin-3',5'-di-*C*-hexoside. When comparing the xanthone content for the four *Cyclopia* species, *C. genistoides* demonstrated the highest mangiferin (102 mg/L) and isomangiferin (35 mg/L) content. *Cyclopia longifolia* demonstrated the second highest xanthone content while infusions of *C. maculata* and *C. subternata* contained a significantly lower amount of these compounds. Infusions of *C. genistoides* further contained the highest amount of the benzophenones, especially with regard to iriflophenone-3-*C*-glucoside. The benzophenone content of *C. subternata* were significantly lower, moreover, benzophenones were not present in quantifiable quantities in *C. maculata*. Regarding the sub-class of flavanones, infusions of *C. subternata* and *C. maculata* contained the highest concentration of eriocitrin (5.1 mg/L) and hesperidin (15.9 mg/L) respectively. Consumption of honeybush tea, particularly *C. genistoides* and *C. longifolia*, could therefore make a considerable contribution to the dietary intake of polyphenols, in particular the xanthone and benzophenone sub-classes (Schulze et al., 2015).

3.2 Sensory profile

Research on herbal teas endemic to South Africa commenced with work on Rooibos tea, *Aspalathus linearis.* Koch, Muller, Joubert, Van der Rijst & Næs (2012) reported on the sensory characterisation and development of a rooibos sensory wheel and lexicon. Prior to the work done by Koch et al. (2012), tools to ensure consistent sensory quality and control of the sensory properties of rooibos herbal tea did not exist. Sensory wheels and sensory lexicons provide a range of descriptive terms with which to describe the sensory perception of a specified food product (Drake & Civille, 2003). The research on the quantification and qualification of the sensory properties and development of a sensory flavour wheel was extended to honeybush.

In contrast to rooibos, a herbal tea which comprises only one species (*Aspalathus linearis)*, honeybush comprises of roughly 23 different species. Research on the cultivation and processing of *Cyclopia* species have focused on the following six species: *C. subternata*, *C. intermedia*, *C. genistoides*, *C. longifolia*, *C. maculata* and *C. sessiliflora.* Demand for honeybush has increased substantially over the last two decades, forcing the honeybush industry to use blends of the different species to supply in the demand for a commercial product that is consistent in quality (Joubert et al., 2011). There are considerable data to suggest variation in the morphological, genetic, chemical, and ecological characteristics of honeybush herbal tea, which can lead to a lack of consistency in the overall sensory quality of the final product; this complicates the national and global marketing process, which relies on the production of a consistent and uniform product.

The honeybush industry therefore expressed the need for similar quality control tools such as the sensory wheel and sensory lexicon developed for rooibos herbal tea. Theron et al. (2014) used six species of honeybush (*C. sessilliflora*, *C. longifolia*, *C. genistoides*, *C. intermedia*, *C. subternata* and *C. maculata*) to develop a "generic" honeybush sensory wheel which comprises the major positive and negative flavour, taste and mouthfeel attributes of this herbal tea. The characteristic sensory profile of honeybush was described as a combination of "sweet-associated", "floral", "fruity", "woody" and "plant-like" aromas with a sweet taste and a slight astringent mouthfeel. The different honeybush species have significantly different sensory profiles which can affect consumer preference and overall enjoyment of the tea. Erasmus (2015) used a large sample set of *C. genistoides*, *C. maculata*, *C. subternata* and *C. longifolia* to validate the "generic" honeybush sensory wheel and to develop specie-specific sensory wheels. All species were associated with the major positive aroma attributes "fynbos-floral" and "fynbos-sweet" aroma, while the "rose geranium" and "apricot/apricot jam" aromas was more prominent in *C. subternata* and *C. genistoides.* Sweet taste was equally high in all species while bitter taste was only perceptible in *C. genistoides* and under-fermented *C. longifolia* (Erasmus et al., 2017).

4 Bitter taste modality and role of polyphenols

The sense of taste is a specialised chemosensory system, with the main aim being to evaluate food and beverages (Yarmolinsky, Zuker, & Ryba, 2009). There are four basic taste sensations: sweet, bitter, sour, and

salty, while umami, a savory taste elicited by certain L-amino acids, may constitute a fifth "primary" taste (Yarmolinsky et al., 2009). The taste of food has a prominent impact on food choice and eating habits, and although bitter taste might not be the most prominent sensation in food, it contributes to the complexity and enjoyment (or lack of enjoyment) of food and beverages (Drewnowski, 2001). Although there is mounting evidence of the beneficial health related properties of polyphenols, these compounds are also associated with bitterness and astringency, which are well known for eliciting negative consumer response (Lesschaeve & Noble, 2005).

The perception of bitterness in animals is regarded as an evolutionary defense mechanism against the ingestion of potentially poisonous material (Chandrashekar et al., 2000). Animals are sensitised to bitter tasting compounds and therefore reject foods that are perceived to be overly bitter (Drewnowski & Gomez-Carneros, 2000). Compounds in food that elicit a bitter taste includes peptides, organic and inorganic salts, plant-derived phenols and polyphenols, flavonoids, catechins and caffeine (Drewnowski, 2001). The fact that bitter perception in food and beverages is caused by structurally diverse compounds, suggests that multiple mechanisms are responsible for perception and transduction of bitterness.

4.1 Biology of bitter perception

Humans perceive bitterness through bitter taste receptor cells (TRCs) present in the oral cavity. Taste receptor cells are clustered in multicellular structures, called taste buds. Taste buds are found on the tongue surface, embedded in fungiform, foliate and circumvallate papillae, as well as on the soft palate, epiglottis and pharynx (Drewnowski, 2001). TRCs regenerate during adult life; the average lifespan of taste cells is 10 days (Lindemann, 2001). Each bitter TRC comprises of a subset of 25 human bitter taste receptor genes (hTAS2Rs) (Behrens, Foerster, Staehler, Raguse, & Meyerhof, 2007). A vast array of different bitter compounds are therefore detected by a limited number of taste receptor genes. Meyerhof et al. (2010) reported on a study where 25 human taste receptors were challenged with 104 natural or bitter chemicals. Thirteen related bitter compounds for five orphan receptors and 64 new compounds for previously identified receptors were identified. The results revealed that some taste receptor genes are broadly tuned, therefore responding to a wide variety of bitter compounds whereas others are narrowly tuned, and therefore only a limited number of specific compounds would elicit the bitter taste. These researcher concluded that 3 of the hTAS2Rs together were able to detect approximately 50% of the bitter compounds used. Their results further suggested that the detection of numerous bitter chemicals is related to the molecular receptive ranges of the human taste receptor genes. Individuals differ in sensitivity to bitter perception (Bartoshuk, 1993).

In herbal teas, bitterness can be elicited by a number of chemical compounds present. The majority of phytonutrients, including polyphenolic acid derivatives, flavonoids, isoflavones, terpenes, and glucosinolates, which occur naturally in many herbal teas, can elicit a bitter perception (Bravo, 1998). Minor modifications to the polyphenol structure, within a class of compounds, could affect bitter intensity.

Soares et al. (2013) investigated the activation of the hTAS2Rs, by six polyphenol compounds present in wine, tea, beer and chocolate. The results indicated that different compounds activate different combinations

of the 25 hTAS2Rs. Hufnagel & Hofmann (2008) quantified 82 recognised taste-active compounds and mineral salts in red wine, and determined the sensory impact of these compounds using a dose-over-threshold (DoT) method. These researchers suggested that larger molecules were more bitter than smaller ones, and further demonstrated that the bitterness of red wine could be induced by phenolic acid ethyl esters and flavan-3-ols. Sáenz-Navajas et al. (2017) characterised the taste-active fractions in red wine by combining HPLC fractionation, sensory analysis and ultra-performance liquid chromatography coupled with mass spectrometry detection. These researcher concluded that the bitterness and astringency in wine cannot be simply related to the bitter and astringent character of the HPLC fractions, which might be explained by taste and physicochemical interactions. They concluded that the bitter taste of the bitterest fractions might be associated with some some flavonols (myricetin, quercetinandtheir glycosides). Researchers developing a polyphenol rich beverages found bitterness to be the key sensory attribute influencing consumer acceptability (Jaeger, Axten, Wohlers, & Sun-Waterhouse, 2009).

4.2 Incidence of bitterness in honeybush species

Sensory research on honeybush has noted bitterness as common descriptor, with one species in particular generating relatively high values on a consistent basis, namely *C. genistoides*. The bitter taste associated with this herbal tea, is largely viewed as being detrimental to the consumer's overall enjoyment of the tea, particularly in view of the fact that honeybush is regarded as a naturally slightly sweet-tasting beverage.

Theron et al. (2014) compared the sensory profiles of six *Cyclopia* species and found *C. genistoides* to be significantly higher in bitter taste compared to the bitterness in *C. sessiflora*, *C. intermedia*, *C. subternata, C. longifolia* and *C. maculata*. The effect of oxidation temperature and time on the sensory characteristics of *C. genistoides*, *C. subternata*, *C. maculata* and *C. longifolia* was investigated by Erasmus et al. (2017). The bitter taste was most pronounced in *C. genistoides* while bitter taste was not regarded as typical for the other three species (*C. subternata*, *C. longifolia* and *C. maculata*) and values for bitterness for these three species were extremely low. The mean bitterness values (measured on a 100 mm scale) for the respective *Cyclopia* species, as reported by Erasmus et al. (2017), are depicted in Fig. 3.

These *Cyclopia* species also differ in phenolic composition (Beelders et al., 2014; De Beer et al., 2012; Schulze et al., 2015), which could explain the perceived differences in bitterness intensities. Research is needed to delineate the contribution of specific phenolic compounds to the bitter taste associated with *C. genistoides* and *C. longifolia*.

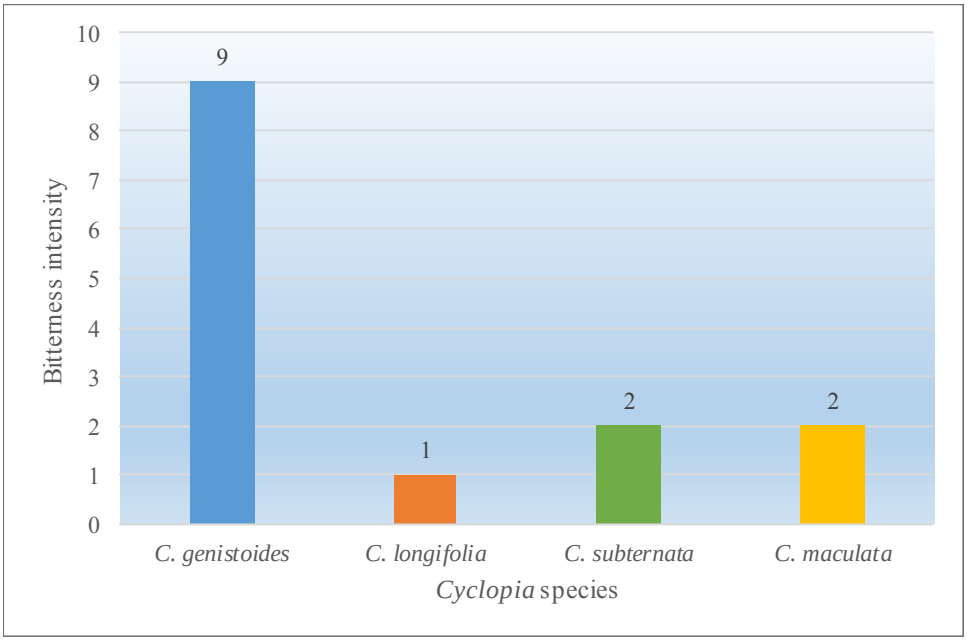

Fig. 3 Intensity for bitterness of *C. genistoides*, *C. longifolia*, *C. subternata* and *C. maculata* fermented at 90°C for 16h. Numbers above bars represent mean intensity.

4.3 Regression analysis of chemical and sensory data to determine chemical drivers of taste

Data obtained with descriptive sensory analysis is valuable in interpreting the sensory quality of food and beverages, but in many instances, relating sensory data to other types of measurements could aid in interpretation of results or used for prediction purposes (Naes, Brockhoff, & Tomic, 2010). Two parametric methods used to determine the relationship between variables are correlation and regression analysis. Correlation analysis is used as an exploratory technique to determine whether two variables are significantly correlated. Regression analysis, on the other hand, is used to describe the functional relationship between two variables, where the value of one variable (dependent variable) could be predicted by the independent variable (McKillup, 2005). In the context of food research, prediction models allows for an empirical understanding of food systems through regression analysis, for example by correlating the chemical and sensory properties of food and beverages. One application of regression analysis is that time-consuming and costly sensory analysis could be replaced by a more rapid instrumental method, given high linearity and good predictive power between sensory attributes and instrumental measurements were demonstrated (Bower, 2009). Many regression techniques for modeling and analyzing two or more sets of data are available and these include linear, multi-linear, probit- and logistic approaches (Granato, De Araújo Calado, & Jarvis, 2014). Linear models are often employed when investigating the relationship between sensory and chemical or physical measurements. Step-wise and partial least square (PLS) regression analysis are of interest for the current study and will be discussed.

4.3.1 Step-wise regression analysis

Step-wise regression analysis is a way to build a model by adding or removing predictor variables, usually by employing a series of F-tests (compares the ratio of two variances) or T-tests (Judd & McClelland, 1989). The t-test (also referred to as Student's T-test) compare the means of two variables, determines if means are different and indicates the significance of the difference (Bower, 2009). Variables to be added or removed are chosen based on the test statistics of the estimated coefficients. This technique has its benefits but require skill on the part of the researcher, specifically with regard to interpretation of the model. The forward step-wise regression test start with no predictor variables and variables are added, one at a time, as the regression model develops. The model calculates the F-test and at each step, the variable with the highest F-value will be added to the model (Snedecor & Cochran, 1989).

Stepwise regression was performed on honeybush infusions in order to build a model able to predict the sensory taste of an infusion, based on individual phenolic compounds. Theron (2012) found a moderate significant correlation between bitter taste and the xanthone, mangiferin, when employing step-wise regression analysis. Erasmus (2015) continued the research on investigating the correlation between chemical composition and sensory data. Validated HPLC-DAD methods for phenolic profiling of honeybush infusions and sensory data of the samples, spanning several harvest years, were employed. The development of a prediction model where the contribution of phenolic compounds to basic taste and astringency in four *Cyclopia* species were determined. When employing step-wise regression analysis, the prediction model could predict 50% for variation in sweetness, 81% for variation in bitterness and 69% of the variation in astringency (Erasmus, 2015). Collinearity of variables is a limitation when performing step-wise regression analysis and it was recommended that other regression analyses be employed to identify potential predictors of bitterness based on the compositional data of *Cyclopia* infusions.

Stepwise regression analysis is not often used for prediction of sensory quality, and only one recent published example could be found. The relationship of texture profile analysis (TPA) and Warner-Bratzler shear force (WBS) with sensory characteristics of beef rib steaks were investigated using step-wise regression analysis (Caine, Aalhus, Best, Dugan, & Jeremiah, 2003). A prediction model were developed that included the TPA parameters of hardness and adhesiveness, which accounted for 47, 36, 51 and 38% of the variation in initial tenderness, amount of connective tissue, tenderness and overall palatability, respectively. A prediction equations based on WBS accounted for 37, 24, 36 and 31% of the variation in initial tenderness, amount of connective tissue, overall tenderness and overall palatability, respectively. The researchers came to the conclusion that TPA values explained more of the variation in sensory tenderness of the rib steaks than WBS.

4.3.1.1 Advantages and disadvantages of step-wise regression

Step-wise regression has the ability to handle a large amount of potential predictor variables, and the model is adjusted to choose the best predictor variable (or combination of variables) from all the options available. Valuable information about the quality of the predictor variables could be obtained by observing the order in which variables are added. Although step-wise regression is a popular method of regression

analysis, many statisticians agree that it has numerous limitations and should be used with caution (Judd & McClelland, 1989). Some of the limitations of step-wise regression include that this method often has many potential predictor variables but too little data to estimate coefficients meaningful. Addition of more data do not make the model more reliable. Models based on stepwise regression frequently fail to replicate when applied on new sets of comparable data (Judd & McClelland, 1989). Collinearity of predictor variables further poses serious problems (Snedecor & Cochran, 1989). If predictor variables are highly correlated, only one will be chosen to be included in the model (Magidson, 2013). R^2 values, calculated by the model, are usually too high and predicted values and confidence intervals are too narrow.

4.3.2 Partial least square regression analysis

The partial least square (PLS) regression analysis method has gained interest in recent years since it is a projection based technique that handles data with numerous and strongly collinear *X*-variables, while it is able to simultaneously model several *Y*-variables (Wold, Sjostrom, & Eriksson, 2001). The use of Variable Importance in Projection (VIP) scores has been demonstrated to be useful for interpreting the more relevant variables in PLS models (Platikanov et al., 2017). VIP scores summarise the variance in the predictor variables that globally contributes most to the y variance explanation. A VIP value of >1 is used as criterion to indicate the most important variables in the PLS model for variable selection (Chong & Jun, 2005).

PLS regression analysis demonstrated good potential as method to predict sensory properties based on physicochemical parameters (Moskowitz, Beckley, Jacqueline, & Resurreccion, 2006), as illustrated by the following published research results. A prediction model, using PLS regression analysis, were successfully constructed to predict the quality of Longjing tea based on 10 volatile compounds (Lin, Dai, Guo, Xu, & Wang, 2012). A study by Platikanov et al. (2017) illustrated that it was possible to accurately predict consumers' preference for mineral content of bottled and tap water by PLS regression using physicochemical parameters.

Keenan, Brunton, Mitchell, Gormley, & Butler (2012) examined the volatile compounds and sensory attributes in fresh and processed fruit smoothies. These researchers used PLS regression to investigate the relationships between sensory attributes and volatile compounds. The correlations between sensory and instrumental data were lower than expected, possibly due to the high odour threshold values of the volatile compounds identified.

The fluorescence spectra of olive oils and the main quality parameters of olive oils (peroxide value, UV absorbance at 232 nm and 270 nm, and acidity) were used to develop a prediction model of quality, based on PLS regression analysis (Guzmán, Baeten, Pierna, & García-Mesa, 2015). Results indicated the potential use of fluorescence instruments for the overall evaluation of olive oil quality.

The physicochemical data, sensory data and consumer liking of sweetened mango nectar were correlated by employing partial least square (PLS) regression analysis (Cadena et al., 2013). The PLS method allows for the interpretation of the attributes that contributed positively and negatively to consumer acceptance of the mango nectar samples. Sucralose was shown to be the best substitute for sucrose when compared with the

other high intensity sweeteners. PLS regression identified sweet aftertaste and bitter aftertaste as undesirable attributes; these attributes contributes to lower acceptance according to the consumers.

The relationship between chemical flavour keys and consumer acceptability of ready-to-drink green tea beverages were investigated using PLS regression analysis (Yu, Low, & Zhou, 2018). The PLS model identified two flavour keys (floral and roasted) to be the main drivers of liking, while dislike were associated with the flavours described as green, bitter and astringent.

The relationship between sensory and chemical data of Norwegian dry fermented lamb sausages were studied by employing multivariate analytical techniques including principal component analysis and partial least square regression analysis (Helgesen & Næs, 1995). The PLS method extracts a few linear combinations (called PLS component or latent variables) from the chemical data, and this is used to predict the variation in the sensory data. Results from the PLS regression indicated a weak correlation between fat content and juiciness, while a good correlation between juiciness and water content and water activity were revealed.

The sensory properties of tea aromas were correlated to gas chromatography (GC)-profiles of the volatile components using stepwise regression, principal component regression (PCR) and partial least square regression (PLS) analysis. Seven aromas (fresh floral, sweet floral, citrus, sweet fruity, fresh green, resinous and roasted) were selected from 16 sensory terms, using the multivariate techniques indicated above, suggesting their importance for describing quality differences in aromas of black, Oolong and green tea samples (Togari, Kobayashi, & Aishima, 1995). The estimation accuracies obtained with PLS were better than those for PCR but mostly equivalent to that obtained with stepwise regression.

4.3.2.1 Advantages and disadvantages of PLS regression

The PLS regression method has the ability to model multiple dependent variables as well as multiple independents variables; furthermore, this technique has the ability to handle multi-collinear independent variables, and it is robust in handling data noise and missing data. Greater difficulty in interpreting the loadings of the independent latent variables is regarded as one of the main disadvantages associated with of PLS regression. PLS is favoured for use as a predictive technique, but not as interpretive technique.

5 Sensory analysis

Sensory analysis are defined as the scientific discipline that applies principles of experimental design and statistical analysis and the use of human senses (sight, smell, taste, touch and hearing) for the purpose of analysing food and beverages (Lawless & Heymann, 2010). The discipline employ panels of human assessors, by whom the products are tested, and the responses of the assessors are recorded. Statistical techniques are applied to the data, which gives insight in the sensory characteristics, and similarities and differences of the test products. The full range of test methodologies in sensory and consumer science can be divided into three main classes namely discrimination tests, descriptive tests and affective tests (Lawless & Heymann, 2010). Descriptive sensory analysis (DSA) aim to quantify the perceived intensities of the sensory characteristics of

a product as perceived by highly trained assessors (Lawless & Heymann, 2010). This technique uses humans as analytical instruments, and the outcome is a full sensory profile of the product/s in question. Consumer analysis measures consumers' degree of liking of a product. Statistical techniques such as regression and correlation analysis can be used to relate consumer liking to descriptive analysis.

5.1 Descriptive sensory analysis

Conventional sensory profiling is of major importance in describing differences between products (Risvik, McEwan, & Rødbotten, 1997) and the application of sensory analysis as tool in product development, quality control and research has grown substantially over the last decades (Lawless & Heymann, 2010; Varela & Ares, 2014). The selection, screening, training and maintaining of a well-trained sensory panel is of utmost importance for generating valid descriptive sensory data. This method could be time consuming and costly due to extensive training and testing phases (Chollet, Lelièvre, Abdi, & Valentin, 2011). Traditionally, only trained assessors were used for sensory characterisation while consumers were used to test acceptability of products. In recent years, the line between sensory analysis and consumer analysis has become less defined (Varela & Ares, 2012). The process of training the panel, testing products in replications, giving feedback to the panel, capturing and analysing data is time consuming and costly. A great advantage of DSA is that results can be correlated to instrumental, chemical, consumer and marketing related data of the same products (Murray, Delahunty, & Baxter, 2001).

Although DSA has been regarded as key to the detailed sensory descriptions of products, some questions about the analytical approach has been raised. Murray et al. (2001) reasons that descriptive sensory analysis is based on the assumption that attributes of a food matrix are independent and evaluated as such by trained assessors. Cartier et al. (2006) raised the same question: during analytical sensory evaluation, panelists are asked to evaluate different sensory attributes as independent dimensions. The approach of evaluating descriptors as independent dimensions might pose some problems as it does not reveal the complex interactions between sensory attributes as is revealed when following a holistic approach. Classic descriptive sensory analysis relies on the consensus method to test perception of assessors on defined attributes, but in some cases assessors differ in their perception and it is difficult to reach consensus (Delarue, 2014). This can pose a problem as assessors might find it difficult to articulate their perception and often information on this perception is then not captured (Barcenas, Elortondo, & Albisu, 2004;. Delarue & Sieffermann, 2004). Furthermore, in a very competitive environment such as the food industry, there is a need for valid sensory methods that is more cost-effective and less time consuming and which can incorporate consumers' perceptions.

6 Rapid sensory profiling methodologies

The development of novel rapid sensory profiling techniques started in the 1980's with the development of free choice profiling (FCP) and repertory grid (RG) methods and evolved to include a vast range of different

techniques that differ in level of difficulty to conduct, level of training of assessors and method of analysis (Varela & Ares, 2014). The most profound outcome of the development of rapid sensory techniques is most probably the use of non- or partially trained assessors in product characterisation. However, data analysis of rapid methods could be more cumbersome for the inexperienced sensory scientist.

Rapid methodologies for the sensory characterisation of products can be classified in different categories, based on the approach to product evaluation. These methodologies can be divided in three main groups: methods based on the verbal description of individual products (check-all-that-apply and flash profiling), methods with a holistic approach based on global similarities and dissimilarities between products (sorting and projective mapping) and methods based on comparison of products with references (polarised sensory positioning and pivot profile) (Valentin, Chollet, Lelièvre, & Abdi, 2012).

6.1 Sorting

The sorting task is a categorization method where products with perceived similarities are grouped together (Chollet et al., 2011). The method originated in psychology (Coxon, 1999). Sorting products into groups which share similar characteristics is a natural cognitive process, routinely applied in everyday life (Chollet et al., 2011; Qannari, Cariou, Teillet, & Schlich, 2010). The sorting task, also referred to as free sorting (Chollet, Valentin, & Abdi, 2014), was introduced to the sensory domain to determine the perceptual structure in odours (Lawless, 1989; Lawless & Glatter, 1990). The aim of the sorting task, as applied in sensory science, is to provide a sensory configuration of the products under investigation and to interpret the underlying dimensions (Chollet et al., 2011).

As with the other novel rapid sensory methods, no standardised procedure for the sorting task exists and the basic method can be adapted to meet the objectives of the study. Generally, the sorting task is conducted in one session; all samples are presented simultaneously and in a different order to the assessors participating. Assessors are asked to evaluate the samples and then sort them into groups according to perceived similarities (Chollet et al., 2011). Sorting can be done on specified modalities (aroma, flavour or mouthfeel) or on global attributes. Assessors use their own criteria to form groups of similar products; they can put as many products into a group and form as many groups as they want. The sorting task can end at this point or a next step can be added where assessors are asked to add descriptive terms to each group. Assessors can use their own set of descriptors but this pose some difficulty to the sensory scientist during data analysis where interpretation of descriptive terms would then be required (Chollet et al., 2014). The assessors could also be provided with a list of descriptors applicable to the set of samples evaluated. According to Chollet, et al. (2011) providing trained assessors with a list of descriptors did not increase the number of descriptors to describe groups of beer. This could be the result of using a list that is too long (44 attributes) or using a general list of attributes and not attributes used during the training of the panel. The importance of using a relative short list of attributes (Hughson and Boakes (2002) used 14 terms) that is relevant to the samples under investigation are discussed in the review by Chollet et al. (2011).

Application of the sorting task led to the development of different variations of this method. The first variation, directed sorting, entails that assessors are directed with regard to the number and/or type of groups. Assessors could be asked to sort products to specific modalities (aroma, flavour or global attributes) or even to form groups according to specified aromas (Lawless, 1989). Bécue-Bertaut & Lê (2011) conducted a multilingual study on wine using the sorting task. These researchers called the method of adding descriptors to the groups of sorted samples *labelled* sorting. *Hierarchical* sorting is another modification of the sorting task, aimed to encourage assessors to further discriminate products through sub-groupings of products within the groups they had already made (Santosa, Abdi, & Guinard, 2010).

Research further focused on variations on the level of training of assessors as well as number of assessors partaking in the sorting task. When applying the sorting task, the same sensory categorisation with trained and untrained assessors were obtained although trained assessors tend to use more groups (Cartier et al., 2006; Chollet et al., 2011). It is generally agreed that 20 assessors per sorting task would be sufficient to provide interpretable results (Chollet et al., 2011, 2014). The complexity of the product under investigation as well as familiarity with the product in the case with consumers might influence the sensory map obtained (Chollet et al., 2011; Lelièvre, Chollet, Abdi, & Valentin, 2008).

The number of products that can be successfully evaluated in one session is dependent on the complexity of the product as well as on the sensory ability of the assessors. Assessors have to remember the sensory characteristics of the set of samples presented to them, and sort these samples into groups according to perceived similarity of attributes. Assessors need to re-taste when they cannot remember the sensory character of a product, and re-tasting can cause even more confusion in forming groups of similar products. Re-tasting increases with increased number of samples. Assessors also tend to re-taste when products are very similar. Chollet et al. (2011) recommended between 9 and 20 samples for the sorting task, with the optimum number of samples being 12 while Cartier et al. (2006) recommended between 6 and 15 samples per sorting session.

There are different approaches to analysing sorting data. The first approach result in a map representation (Euclidian) and different techniques for analyzing the data such as multidimensional scaling (MDS), multiple correspondence analyses (MCA) and DISTATIS has been proposed (Chollet et al., 2011). The second approach would be to analyse data using clustering techniques which result in a tree-representation.

Cluster analysis may be used as an exploratory procedure to elucidate the structure in sorting data. When employing cluster analysis, objects are classified into groups or clusters where objects in a group are more similar to each other than to objects in another group. Cluster analysis finds the most significant solution possible; it is used as exploratory procedure when no *a priori* hypotheses are set. Therefore statistical significance testing in the traditional sense is not really appropriate. The tree clustering method uses dissimilarities or distance between objects to form clusters. These distances can be based on a single dimension or multiple dimensions.

The first sensory studies on sorting were analysed using multidimensional scaling, an approach to visualise the level of similarity of individual assessments in a dataset. When using the MDS approach, an

individual co-occurrence matrix is constructed for each assessor. Rows and columns represent products and a value of 1 at the intersection indicates that the assessor sorted these products into the same group. A global similarity matrix is obtained by the sum of the individual similarity matrices and MDS is performed on the global similarity matrix. Analysis of sorting data using MDS provides group data on similarity for the total group of assessors and representations for individual assessors are lost (Abdi, Valentin, Chollet, Chrea, 2007; Lawless, Sheng & Knoops, 1995; Nestrud & Lawless, 2008). The reliability and validity of the results of MDS is tested by computing R^2 values. R^2 values give an indication of what proportion of the variance of the dataset can be accounted for by the MDS procedure. An R^2 of 0.6 is considered as the minimum acceptable level while an R^2 of 0.8 is considered as a good representation for metric scaling. Lawless et al. (1995) are of the opinion that MDS in general and the sorting task in particular, might have the tendency to oversimplify product configurations as assessors choose a limited number of dimensions on which to base their sorting and important dimensions of differences between products might be disregarded.

Multi-block analysis, such as DISTATIS, take individual differences, therefore differences between assessors, into account (Chollet et al. 2011). When analysing sorting data using DISTATIS, the first step is to construct a co-occurrence matrix per assessor where the rows and the columns represent the products and a value of 1 between a row and a column indicates that these products have been sorted in the same group while a value of 0 would indicate that these products were not sorted together. Each co-occurrence matrix is transformed into a distance matrix where rows and columns are products and a value of 0 indicates that these products were grouped together whereas a value of 1 indicates that these products were not grouped together. The distance matrix of each assessor is transformed into a cross-product matrix which is then normalized. The individual normalized, cross-product matrices are combined and subjected to eigen-decomposition analysis to compute a compromise cross-product matrix (Qannari et al., 2010). The compromise cross-product matrix is a weighted average of the individual cross-product matrices where weighting is determined by the degree of agreement between assessors. Assessors that show more agreement with other assessors would therefore contribute more (larger weight) in defining the compromise map. The weighting of assessors are determined by computing RV coefficients between all pairs of assessors. The compromise matrix is a cross-product matrix and its eigen-decomposition is similar to that of a PCA (Abdi et al., 2007).

DISTATIS further provides a map of the each assessors' grouping relative to the compromise map (Abdi et al., 2007; Chollet et al., 2011). The results of the DISTATIS procedure is a two-dimensional product map which indicates the relative positions of products in the sensory space. The descriptive terms used to describe groups of products, are used to construct contingency tables. As quite a large number of descriptors can be used, descriptors with the same meaning can be grouped together while descriptors with a low frequency will be discarded. The frequency data can be projected on the product similarity map by calculating the correlation between the frequency of descriptors and the product factor scores (Faye et al., 2004). Alternatively, the contingency table could be subjected to correspondence analysis, which results in a map representing both products and associated descriptors.

6.1.1 Advantages and limitations of the sorting task

Classic descriptive sensory analysis relies on an analytical approach to product perception and product attributes which is difficult to explain or define, might go unnoticed. The sorting task is a more holistic approach and the sensory configuration of the products is determined by the perception of similarity or dissimilarity of the overall sensory perception. Descriptive words can be added, although this will only provide a broad association of descriptors for groups of products. The sorting task is easy to conduct and to understand, and can be performed by trained and untrained assessors (Chollet et al., 2011). The sensory product configuration obtained using the sorting task is similar to that of the classic descriptive analysis (Cartier et al., 2006; Lelièvre et al., 2008). However, Moussaoui & Varela (2010) reported that although sorting and projective mapping performed with naïve consumers could be used for broad classification of products, these methods performed poorly in terms of product discrimination and repeatability.

One of the disadvantages of the sorting task is the interpretation of descriptors used by consumers (Chollet et al., 2011). Consumers often use a variety of terms to describe the same concept or attribute or they use descriptors which are vague and difficult to interpret (Lelièvre et al., 2008). When a large number or vague descriptors have been used, the sensory scientist have to decide about grouping descriptors together or discarding descriptors which is difficult to interpret. When conducting the sorting task, all samples have to be presented simultaneously. This could pose a problem with samples where temperature control is necessary or with shelf life studies where all samples are not available at the same time. The sorting task is not suitable for hot products as all products have to be presented at the same time and controlling the temperature might pose problems (Cartier et al., 2006; Chollet et al., 2014).

6.1.2 Application of the sorting task

The sorting technique has been applied to a variety of food products. Cartier et al. (2006) used the sorting task on breakfast cereals and tested the effect of trained vs. untrained assessors. These researcher report similar product configurations when comparing sorting and DSA, furthermore products configurations for trained and untrained assessors were similar. Furthermore, replication of the sorting task may not be necessary as similar product configurations were obtained for repeated sessions (Cartier et al., 2006). The sorting task has been applied on food products including vanilla beans (Heymann, 1994), cheeses (Lawless et al., 1995), beer (Lelièvre et al., 2008), wine (Bécue-Bertaut & Lê, 2011), for evaluating textures (Picard, Dacremont, Valentin, & Giboreau, 2003) and astringent solutions (Fleming, Ziegler, & Hayes, 2015). The sorting task can be applied in the areas of R&D, quality control and marketing (Chollet et al., 2014).

6.2 Projective mapping

Projective mapping (PM) was first introduced to the field of sensory science by Risvik and co-workers in studies on chocolates (Risvik, Mcewan, Colwill, Rogersa, & Lyonb, 1994) and blueberry soups (Risvik et al., 1997). Projective mapping, originating from psychology, entails that all products are presented simultaneously and assessors are asked to place products in a two-dimensional space according to perceived

similarities and dissimilarities. Data analysis of the first PM studies was done using generalized Procrustes analysis (GPA) and principal component analysis (PCA). The product maps obtained with PM was compared to that of conventional profiling using RV coefficients. The bi-plots for conventional profiling and PM were very similar on the first dimension, suggesting that this method could be a useful technique in linking sensory analysis and consumer research (Risvik et al., 1994, 1997).

Projective mapping was re-introduced to the field of sensory science by Pagès and co-workers in the form of Napping® (Pagès, 2005; Perrin et al., 2008) where "nappe" is the French word for a tablecloth. In the study by Pagès (2005), assessors were asked to position ten white wines on a paper tablecloth of 40 cm x 60 cm in such a way that wines that are near seem to be identical and wines that are far apart, seems to be very different. Data were analysed using multiple factor analysis (MFA) (Pagès, 2005). The Napping® technique as proposed by Pagès evolved to include an attribute collection step (Perrin et al., 2008). The procedure therefore started with a Napping® task and was followed by ultra-flash profiling (UFP), a step where descriptors were added. The results suggested UFP to be a good compliment to Napping® (Perrin et al., 2008).

Some researchers regard PM and Napping® as essentially the same technique (Nestrud & Lawless, 2010) while others regard Napping® to be more restricted in the sense that Napping® is always carried out on a rectangular piece of paper of 40 cm x 60 cm, descriptor collection is done by a follow-up step using UFP and data analysis is done using multiple factor analysis (Dehlholm, Brockhoff, Meinert, Aaslyng, & Bredie, 2012).

Different variations of PM have been introduced since the initial work by Risvik and Pagès with one variation being global versus partial PM. Global PM has a more holistic approach where all attributes are taken into account while partial PM can direct the assessor to focus on a specified sensory modality.

Numerous studies on the validation of PM has been published over the past decade, reporting on the application of the method on a range of products: ewes milk cheeses (Barcenas et al., 2004), citrus juices (Nestrud & Lawless, 2008), white wine (Pagès, 2005; Perrin et al., 2008), cheddar cheese and apples (Nestrud & Lawless, 2010), liver pâté's (Dehlholm et al., 2012), red wines (Hopfer & Heymann, 2013), products with high alcohol content (Louw et al., 2014) and functional yoghurts (Cadena et al., 2014).

Pagès (2005) suggested a maximum of 12 samples per PM procedure in the study using white wine. Hopfer & Heymann (2013) used 18 samples in a PM task with red wines and found that individual judges showed high variability in their ability to position identical samples close to each other. This could be a result of using very similar samples in the sample set and/or a large number of samples. The optimum number of samples to include in a PM task is 12 (Pagès, 2005).

Research on the effect of level of training of assessors on the PM task have also been reported where researchers compared results for experts and consumers (Nestrud & Lawless, 2008; Torri et al., 2013) and the performance of consumers versus trained assessors (Barcenas et al., 2004; Moussaoui & Varela, 2010). The product configurations of citrus juices evaluated by experts and consumers using the PM technique were generally similar and showed a RV coefficient of 0.73 (Nestrud & Lawless, 2008). According to Moussaoui & Varela (2010), the PM task with consumers is suitable as quick profiling method when a broad description

is sufficient, although product description and discrimination is not as detailed as when using traditional profiling methods with trained assessors. These findings were confirmed by Ares, Deliza, Barreiro, Giménez, & Gámbaro (2010) who are of the opinion that PM conducted with consumers result in a valid product configuration. The repeatability of assessors' PM configurations of ewe's milk cheese were tested using naïve consumers and a trained panel. The trained assessors showed higher repeatability between replicates than the consumers although the product configurations were fairly similar (Barcenas et al., 2004). If naïve consumers are used for the PM task, more assessors need to participate to obtain sample separation (Dehlholm et al., 2012). The aim of the sensory project should guide the choice of assessor: if a more holistic and spontaneous approach is desirable, naïve consumers should be used (Dehlholm, 2014). Trained assessors on the other hand, would tend to evaluate products according to their learned constructs, therefore not as spontaneous as consumers but also with the advantage of using descriptors that is more defined.

Studies on the effect of the geometrical shape on the product configuration has also been done (Dehlholm, 2012; Hopfer & Heymann, 2013; Louw et al., 2015) as well as research on effect of replicate and individual panel performance (Hopfer & Heymann, 2013; Louw et al., 2013). Hopfer & Heymann (2013) and Dehlholm (2012) concluded that better results is obtained when using a rectangular space as assessors regard the horizontal axis is the main dimension to indicate dissimilarity between products with the vertical dimension being less important. Hopfer and Heynmann (2013) tested the repeatability of assessors by using the people performance index (PPI). The PPI is the ratio of the Euclidian distance between two replicated products and the maximum Euclidian distance between two different products on a PM plot. The PPI ranges between 0 and 1. A smaller value indicates that the assessor placed identical products together on the map. Louw et al. (2014) propose the use of a Relative Performance indicator (RPI) to measure assessors' repeatability. The RPI is based on the explained variance by generalized Procrustes analysis (GPA) after data transformation. It is used to measure the similarity between product maps as obtained through repeated sessions per assessor (Louw et al., 2014). Both RV coefficients and RPI measure assessor repeatability: RV coefficients measure the repeatability of the actual measurement per assessor (before statistical analysis) while RPI measure the repeatability of the resulting product configuration per assessor (after statistical analysis).

Data collection for PM is done by measuring the X and Y coordinates on the two-dimensional space for each product relative to the zero point (lower left corner of the paper), with X1 and Y1 representing coordinates for assessor 1 and X2, Y2 coordinates for assessor 2. Each row in the data table represents one sample. The assessor coordinates in the data table are followed by the sample descriptors, with descriptors listed in columns and frequency of descriptors as cited by assessors, indicated per product row (Delholm, 2014). Different methods for analysing PM data have been applied including variations of principal component analysis, generalised Procrustes analysis (GPA) or variations on the multidimensional scaling technique (Delholm, 2014). Pagès (2005) proposed using multiple factor analysis (MFA) for the analysis of PM data. When employing MFA, the x and y coordinates per panel member and product constitutes a group of two un-standardised variables per assessor. Multiple factor analysis collect the Euclidian configuration of each subject (assessor) and with simultaneous processing of all the maps, produces a configuration in which two products

are near each other if perceived to be similar by the whole panel, with each panel member using his own weighted set of criteria. When product descriptors are added using ultra-flash profiling, this qualitative data is added as another data table, therefore added as supplementary variables, and descriptors therefore do not interfere with the construction of the product map.

6.2.1 Advantages and limitations of the PM task

Projective mapping is a rapid method that can distinguish between groups of products in a broad sensory space but the major disadvantage of this method, is its poor precision (Dehlholm, 2014). Projective mapping is efficient in distinguishing between products on the first two model dimensions, but it is not possible to study differences that are only visible in the third and fourth model dimensions, making PM less suitable for products that are very similar. Another disadvantage of PM is the number of samples that can be evaluated in one session, with Varela & Ares (2012) proposing a maximum of 12 products, depending on the sensory characteristics of the products. Although PM is a categorisation method and should therefore be fairly easy to conduct, some assessors might experience difficulty in the spatial arrangements of products.

6.2.2 Application of the PM task

Nestrud & Lawless (2008) employed MFA for analysis of PM data and concluded that this method of analysis is robust and results in detailed information between individual (assessor) and product differences.

Louw et al. (2013) investigated PM as a tool for screening brandy samples by employing two variations of PM: global (conventional) PM, where the overall perception of the food product is considered; and partial PM, which focuses on each sensory modality individually. Results indicated a high degree of conformity between DSA and the PM methodology, suggesting that the use of PM is suitable for brandy and other high alcoholic beverages; overall, the partial PM method was more suitable in capturing the profile of the brandies, particularly where larger sample sets were employed. Results further indicated that training of assessors could improve assessors' ability to evaluate difficult sample sets.

The efficacy of PM and CATA, conducted by consumers when evaluating chocolate milk desserts were investigated (Ares, Deliza, et al., 2010). CATA and PM provided similar sensory maps and both methods were able to discriminate between products. Consumers found the CATA task easier to apply and less time-consuming compared to the PM task.

6.3 Polarised sensory positioning

Polarised sensory positioning (PSP) is a rapid sensory method developed by Teillet and Schlich to determine the taste of water (Teillet, 2014). The rationale behind development of the PSP method was the need for a comparative method that would not need inclusion of a large number of samples or the entire sample set, furthermore the method would need to be suitable to use with assessors with different levels of training and allow for aggregation of data from several sessions (Teillet, Schlich, Urbano, Cordelle, & Guichard, 2010).

Polarized sensory positioning was developed while Teillet et al. (2010) conducted a study on French bottled and tap water. Drinking water is a neutral product and the sensory characterisation is quite difficult as sensory stimuli are low and discrimination between products difficult. Classic sensory profiling, temporal dominance of sensation (TDS) and the free sorting task were used for the sensory analysis of water. When comparing results of the three methodologies, some disadvantages were encountered. Sensory profiling and TDS did not result in discrimination between samples. Although results for the free sorting task showed better discrimination between samples than the profile and TDS methods, the free sorting task does not allow data collection over different sessions (Teillet et al., 2010). All three methods revealed some limitations when applied for the sensory characterisation of water, thus the development of a new method named polarised sensory positioning (Teillet et al., 2010).

PSP is described as an approach for the rapid sensory characterisation of products where well-known products, which should be stable over time, are used as "poles" or references. During the evaluation phase, new products are positioned in a sensory space relative to the "sensory pole" (Teillet, 2014). The principle is to measure the similarity or dissimilarity of new products in relation to the reference or pole. Poles should be well chosen, representing the most important attributes associated with the sensory space for the food category or product range under question. PSP also enables data collection across multiple sessions, a great advantage not possible with the other rapid comparative methods.

Protocols and standard procedures for PSP are not established although the choice of poles is regarded as the most critical step when conducting PSP (De Saldamando, Antúnez, Giménez, Varela, & Ares, 2015). Three poles, representative of the total sensory space under question and which are stable over time, are recommended (Teillet, 2014). Two variations of PSP are proposed: PSP using a continuous scale and Triadic PSP (Telleit, 2014). PSP with continuous scale entails that assessors indicate the similarity (or dissimilarity) of each sample relative to each pole using an unstructured line scale where 0 would indicate that the sample were perceived to be the same as the pole and 100 indicates a perception of totally different from the pole (Teillet et al., 2010). Triadic PSP involves that three poles ae used and assessors asked to indicate to which pole the sample is most similar and to which least similar, therefore only similarity of dissimilarity without indicating the distance from the pole.

When using PSP with continuous scale, data are collected in the form of a dissimilarity matrix between samples and poles. Teillet (2014) propose two possible ways of analysing data of PSP with continuous scale. The first method is analysis by a multidimensional scaling (MDS) unfolding technique. When using the continuous scale with PSP, data can also be encoded to intensity values for different descriptors (if descriptors can be assigned to each pole) and factor analysis such as Principal Component Analysis (PCA), multiple factor analysis (MFA), STATIS or generalized Procrustes analysis (GPA) can then be employed for data analysis.

The data of triadic PSP is arranged in a co-occurrence matrix of product x variables, where occurrences are summed over assessors, and data can be analysed through correspondence analysis (CA).

The effect of pole selection on PSP results was investigated (De Saldamando, Delgado, Herencia, Giménez, & Ares, 2013). These researchers conducted two studies on widely different products namely on make-up foundation and orange flavoured powdered drinks using PSP and two sets of poles for each product. The results showed that the sensory space for the two sets of poles for both products were fairly similar suggesting that conclusions on the main similarities and differences between products were not influenced by the choice of poles. However, some differences in similarities and dissimilarities among products were observed when comparing the sensory space obtained with two sets of poles (De Saldamando et al., 2013). Therefore the selection of poles did not have an influence on the sensory space obtained but did have an influence on conclusions regarding similarities and dissimilarities among products, therefore poles should be selected with great care. Poles should be different, spanning the total sensory space of the products under question. Selection of poles using results of previous sensory characterisation is suggested and would allow researchers to select poles that are very different, covering the total sensory space of the product category. The complexity of the product under question could also influence the effect of the poles where changing poles of a more complex product would have a greater influence. The level of training of assessors should be taken into account when selecting a set of poles. The set of poles could have a more pronounced effect when working with consumers than with trained assessors who have previous knowledge of the sensory characteristics of the product.

Table 1 provide a summary of the published results on PSP, indicating that different numbers of consumers have participated in the PSP task ranging between 32 for the first PSP study to taste 10 types of water (Teillet et al., 2010) to up to 92, evaluating 8 orange-flavoured drinks (De Saldamando et al., 2013).

Although PSP has delivered promising results, application of the method on a limited range of products have been published, as summarised in Table 1. The application of polarised sensory positioning has tested on water (Teillet et al., 2010), make-up foundation and orange powdered drinks (De Saldamando et al., 2013) and functional yoghurts (Cadena et al., 2014), chocolate mild beverages (Antúnez et al., 2015) and astringent agents (Fleming et al., 2015).

Table 1 Summary of published studies on polarised sensory positioning comparing products evaluated, assessors, applied methods, number of poles, statistical techniques used and major findings

Publication	Products	Assessors	Applied methods	No of poles	Statistical analysis	Major findings
Teillet et al., (2010)	10 waters	32 consumers	TDS Sorting PSP	3 poles	MDS unfolding STATIS	Sensory profiling not suitable for products with low sensory stimuli PSP results in better discrimination in water than classic sensory profiling
De Saldamando et al., (2013)	8 make-up foundations	30 consumers	PSP	2 sets of 3 poles each	MFA	Sample configurations obtained with different sets of poles were similar Poles should be selected to span full sensory space of product category Different poles affected conclusions regarding similarities and dissimilarities of products to some extent
	8 orange-flavoured powdered drinks	92 consumers	PSP	2 sets of 3 poles each	MFA	
Ares et al. (2013)	9 orange-flavoured drinks (including blind duplicate)	45 consumers	PSP	3 poles	MFA	Three methodologies provided similar sensory spaces Methods differed in terms of discriminative ability, with t-PSP showing lower discriminative ability Conclusion regarding similarities between samples, differed for one of the products
		45 consumers	Triadic PSP (t-PSP)	3 poles	Multiple correspondence analysis (MCA)	
		45 consumers	Polarized PM	3 poles	MFA	

Publication	Products	Assessors	Applied methods	No of poles	Statistical analysis	Major findings
Cadena et al. (2014)	8 low-fat yoghurts	9 trained assessors	DSA		ANOVA and PCA	Three methods provided similar results on main differences between products
		81 consumers	CATA		Cochran's Q test Correspondence analysis	Product configuration of CATA most similar to DSA CATA and PSP showed lower discriminative ability compared to PM
		81 consumers	PM		MFA	Bootstrapping resampling method revealed sample configurations of PSP and CATA to be highly reliable
		81 consumers	PSP	3 poles	MFA	Sample configurations of PM least stable and showed lowest similarity to DSA
Antúnez et al. (2015)	7 orange-flavoured drinks	120 consumers split into three groups of 40 to evaluate subsets of samples	PSP with scales	3 poles	PCA	Good correlation between configurations for evaluation of whole vs. split samples sets by different groups of consumers
		120 consumers split into three groups of 40 to evaluate subsets of samples	Triadic PSP	3 poles	Correspondence analysis	Sample configurations similar for PSP with scales and t-PSP

Publication	Products	Assessors	Applied methods	No of poles	Statistical analysis	Major findings
	7 chocolate milk beverages	120 consumers split into three groups of 40 to evaluate subsets of samples	PSP with scales	3 poles	PCA	PSP with scales: difference in conclusions regarding similarities between products when evaluating whole set or subset of samples with different consumers
		120 consumers split into three groups of 40 to evaluate subsets of samples	Triadic PSP	3 poles	Correspondence analysis	Low agreement between configurations obtained PSP with scales and t-PSP Influence of data aggregation more evident with complex products and products with only small differences
Fleming et al., (2015)	10 astringent agents	41 consumers	CATA		Cochran's Q test Correspondence analysis	Similar visual product configurations when comparing three rapid methods
		30 consumers	Sorting		Multidimensional scaling	Sorting performed as 1:1 with experimenter, making it less time efficient
		41 consumers	PSP	3 poles	MFA	Recommends mixed approach of CATA with PSP where CATA results can be used to select poles for PSP

Publication	Products	Assessors	Applied methods	No of poles	Statistical analysis	Major findings
Ares, Antúnez, Oliveira, et al. (2015)	8 chocolate flavoured milks	40 consumers	PSP with different sets of poles	3 sets of poles: 3 poles vs. 2 sets with 2 poles each	MFA	Poles should be selected to represent the main sensory characteristics associated with products under question Two well-selected poles that represent the main sensory characteristics associated with the sensory space, are sufficient to obtain reliable product categorization Poles that are distinctly different in sensory characteristics, should be selected
	8 vanilla milk desserts	40 consumers				
	6 orange-flavoured drinks	40 consumers				

6.3.1 Advantages and limitations of the PSP task

Polarised sensory positioning is a promising method for the sensory characterisation of a given product with the great advantage of sample evaluation and data collection over multiple sessions. Data from different sessions and different panels have already been successfully aggregated by Teillet and co-workers (Teillet et al., 2010). A disadvantage of this method is that *a priori* information about the sensory space is necessary for selection of poles (Teillet, 2014). This would require that some sort of sensory technique be first applied to obtain a representation of the sensory space to aid in pole selection. A further limitation of the method is that descriptive information about the sensory characteristics of the samples is only obtained relative to the poles. The data obtained with PSP is not quantitative and therefore it is not possible to link PSP data to physical and chemical data. Furthermore, data analysis is more complex compared to that of DSA.

6.3.2 Future research

All the published studies on PSP reported on employing this method with consumers, research comparing the efficacy of PSP with trained vs. untrained assessors is therefore needed. Furthermore, work on statistical methods to test for repeatability and the discrimination power of the method by applying confidence ellipses is also recommended.

6.4 CATA (Check-all-that-apply)

Marketing research often applies a multiple choice question format to test consumers' perception on products. Consumers find these type of questions easy as there is no cognitive burden of comparing samples and rating intensities, furthermore consumers are allowed to select all terms that they regard applicable to the test product. The multiple-choice question format is increasingly applied in the field of sensory science. The sensory characterisation of products using *frequency of citation* was originally applied using trained assessors. McCloskey, Sylvan, & Arrhenius (1996) developed a method called *multi-wine descriptive analysis* (MWDA) where different wines were characterised by the frequency of use of terms as opposed to intensity rating, characteristic of DSA. A variation of this method, involving the sensory characterisation of Spanish mono-varietal white wines by trained assessors using a frequency of citation method, was introduced by Campo, Do, Ferreira, & Valentin (2008).

In recent years, Check-all-that-apply (CATA) questions, also known as choose-all-that-apply, has gained popularity for the sensory characterisation of products using consumers (Meyners & Castura, 2014). CATA questions entail that assessors are provided with products and CATA questions comprising of a list of descriptors and/or phrases. Participants are asked to evaluate the product and then select all the descriptors that they perceive as being relevant to the product under question. The list of descriptors might include sensory descriptors, hedonic responses, emotional responses, purchase intent, occasion of use or concepts on the ideal product (Ares, Barreiro, Deliza, Giménez, & Gámbaro, 2010; Meyners & Castura, 2014; Parente, Manzoni, & Ares, 2011). The list of descriptors need to be pre-determined, either using consumers and the focus group

technique or using previous elicited terms by trained panels evaluating the same product category (Dooley, Lee, & Meullenet, 2010).

Terms included in a CATA question should be selected with caution, and need to be clear and easily understood by consumers (Ares, Barreiro, et al., 2010). The number and presentation order of terms have been the focus of several studies, with major findings of these summarised in Table 2. The number of terms to include in a CATA question should be carefully considered. Primacy bias (selecting terms that appear first) and satisficing (not paying attention to all terms presented), fatigue associated with long lists of terms and the length of evaluation should be considered when deciding on the number of terms to include (Meyners & Castura, 2014). Research on CATA report on including between 10 and 113 terms (Table 2). A shorter list, with attributes grouped according to modality, seem to improve product characterisation (Ares, Jaeger, et al., 2013).

Studies on best practice when conducting CATA, have investigated the effect of attribute order on product characterisation. Consumers tend to mark the first term that they deem appropriate rather than to read through all terms and choose the best option. Furthermore, results could be skewed if descriptors are presented in a fixed order to all assessors. Ares & Jaeger (2013) investigated the influence of the order in which sensory terms are placed within a CATA question and secondly, the influence of the order of a CATA question within an extended product assessment questionnaire (Table 2). These researchers recommended to group sensory terms according to modality, present groups of terms in the order of normal perception (e.g. aroma followed by flavour attributes) and to randomise attributes within a group. Meyners & Castura (2016) recommended random presentation of attributes (within a group) per assessor rather than per product. By using this method, the attribute list are consistent per assessor, enabling them to "memorise" the questionnaire, thereby reducing the cognitive effort while completing the questionnaire. CATA questions can be successfully used in conjunction with the 9-point hedonic scale to determine overall liking (Ares & Jaeger, 2013). These researcher further reported no significant effect on hedonic scores as a result of the position of the hedonic question within a CATA questionnaire.

Research using CATA questions and consumers for the sensory characterisation of a wide range of products has been published in recent years: vanilla ice cream (Dooley et al., 2010), chocolate milk desserts (Ares, Barreiro, et al., 2010), functional yoghurts (Cadena et al., 2014), beer (Reinbach, Giacalone, Ribeiro, Bredie, & Frøst, 2014), hibiscus tea (Monteiro et al., 2017) and fish (Lazo, Claret, & Guerrero, 2016). Research comparing consumers' CATA response and trained panel sensory data resulted in similar sensory characterisation of products, indicating agreement between the different methods (Ares, Barreiro, et al., 2010; Dooley et al., 2010; Jaeger et al., 2013), deeming CATA questions a valid tool for the sensory characterisation of products by consumers.

Between 5 (Ares & Jaeger, 2013) and 23 products (Campo et al., 2008) have been included in the CATA task. The number of consumers necessary to obtain a stable configuration when using CATA questions depend on a number of factors, including the degree of difference between the products evaluated, the complexity of the products and the type of descriptors included (Ares, Tárrega, Izquierdo, & Jaeger, 2014). If widely different

samples are tested, 80 – 100 consumers would be sufficient to obtain a stable product configuration (Ares et al., 2014). Gacula & Rutenbeck (2005) also report that the number of consumers to participate in a hedonic test is determined by the size of the hedonic difference between products. Often CATA questions are combined with overall liking test using the 9-point hedonic scale, in which case 100 -120 consumers should be considered (Ares et al., 2014).

External preference mapping has been widely used to draw the link between consumers' preference and the sensory attributes (as determined by a trained panel) that are drivers of liking. The disadvantage of external preference mapping is that it assumes that trained assessors and consumers perceive products in the same way. The greatest advantage of CATA questions is that it is a valid method for determining both consumers' sensory impression and degree of liking of products (Ares, Barreiro, et al., 2010).

6.4.1 Advantages and limitations of CATA questions

CATA questions is regarded as an easy, straightforward task for consumers to complete (Ares, Deliza, et al., 2010). Moreover, it is easy to conduct from the sensory scientists' perspective, providing rapid results. CATA questions can be used to gather consumers' opinions on products without inducing an analytical approach that might interfere with consumers' hedonic assessment of products (Meyners & Castura, 2014). Research up to date demonstrated CATA to be a valid method for product characterisation by consumers.

One of the limitations of CATA questions is that it provide binary results with no indication of the intensity of the selected attribute. Furthermore, CATA questions with consumers are not recommended for the sensory characterisation of complex products or products with only subtle perceptual differences (Ares, Antúnez, Bruzzone, et al., 2015). When conducting descriptive sensory analysis, a well-defined vocabulary is used, panel members are trained in specific attributes and replicated sessions are conducted and therefore this classic technique show higher discriminative ability for complex products or products with only slight perceptual differences (Ares, Antúnez, Bruzzone, et al., 2015).

Table 2 Summary of published studies on CATA comparing products, assessors, number of terms, selection of terms, replications, statistical techniques used and major findings

Publication	Sensory methods	Products	Assessors	Number of terms	Analysis	Major findings
McCloskey et al., (1996)	Multi-wine descriptive analysis (MWDA)	16 Chardonnay wines	26 Wine experts	10 (select 2-5)	PCA of the sensory attribute scores; ANOVA; Polar plots	MWDA accounts for quality bias of wine professionals
Campo et al. (2008)	Sorting and CATA	23 Spanish mono-varietal white wines	36 novices	101	Sorting: MDS and hierarchical cluster analysis (HCA) CATA: Correspondence analysis (CA) and HCA	Sorting combined with CATA is a suitable tool to classify samples and describe wine aroma.
Campo, Ballester, Langlois, Dacremont, & Valentin (2010)	DSA Frequency of citation	12 Pinot Noir wines	DSA: 9 trained Frequency of citation (FC) = 33, consumers but trained	113 (select 5)	DSA: ANOVA and PCA FC: CA and HCA	Agreement of methods on main odor attributes Dimensionality of PCA and CA not the same FC better discrimination between products / large no of terms to describe DA in this study quicker than FC

Publication	Sensory methods	Products	Assessors	Number of terms	Analysis	Major findings
Ares, Deliza, et al., (2010)	CATA with hedonic Projective mapping (PM)	8 milk chocolate desserts	CATA: 50 consumers PM: 40 consumers	CATA 17 terms PM 4 terms	Hedonic: ANOVA CATA: MFA on frequency table with hedonics as supplementary data PM: MFA Comparison of methods: Hierarchical MFA	Two methods provided similar sensory profiles. Both methods could discriminate between products CATA questions easier and less time consuming
Parente et al. (2011)	CATA with hedonic	6 antiaging creams	69 consumers	42 terms, grouped in five categories	Hedonic: ANOVA Friedman's test Cochran's Q test MFA Cluster analysis External preference mapping	Largest no of terms used to describe products with highest liking scores Sensory characteristics influence consumers' emotional response to products. Sensory characteristics and positive emotional response highly correlated to overall liking. External preference mapping possible using only CATA results, therefore only consumers' perception

Publication	Sensory methods	Products	Assessors	Number of terms	Analysis	Major findings
Ares & Jaeger (2013)	CATA with hedonic	6 strawberry cultivars	116 consumers	21 terms (structure vs random)	Frequency of use of terms Fisher's exact test Cochran's Q test MFA on CATA counts Hedonic: ANOVA	Random presentation of all terms reduced frequency of use compared to grouping similar terms Order of terms influence frequency of use, first presented were more often used Researchers suggest group attributes according to modality, random order of terms within modality
	CATA with Hedonic	4 apple cultivars	109 consumers	14 terms (blocked vs interspersed)		
	CATA with Hedonic, purchase intent	2 apple cultivars	110 consumers	Four sections: sensory (18 terms), liking, emotions and purchase intent		

Publication	Sensory methods	Products	Assessors	Number of terms	Analysis	Major findings
Jaeger et al. (2013)	CATA	6 Vanilla milk dessert	50 consumers	21 terms	Within-assessor reproducibility Frequency of use of terms Fisher's exact test Cochran's Q test MFA for contingency tables RV coefficients	Consumer reproducibility was similar across four studies regardless of product, number of samples, or number of terms Trained assessors use higher number of terms than consumers Consumers provide reliable product characterisation, comparable to that of trained assessors
	CATA	1 milk chocolate on 2 occasions	100 consumers	40 terms		
	CATA	2 milk chocolates on 2 occasions	65 consumers	40 terms		
	CATA	5 flavoured waters on 2 occasions	48 consumers	46 terms, select 5		
	CATA	2 milk chocolates on 2 occasions	14 trained assessors	40 terms		
Reinbach et al., (2014)	CATA, RATA and partial PM	8 beers	135 consumers		ANOVA MFA A-PLSR D-PLSR	Agreement between three methods on perceived product differences Three methods had similar number of discriminating descriptors CATA easier and faster than PM.

Publication	Sensory methods	Products	Assessors	Number of terms	Analysis	Major findings
Monteiro et al. (2017)	DSA CATA	22 Hibiscus teas	DSA: Trained assessors from Senegal and Portugal CATA: 490 consumers from four countries	CATA: 28 terms	ANOVA and PCA MFA	CATA is a valuable tool in development of sensory lexicons, interpretable across cultures Consumers' sensory profiling of hibiscus teas using CATA question are comparable to that of DSA by trained assessors

7 Conclusions

Honeybush is a traditional South African herbal tea produced from the endemic *Cyclopia species*. It grows along the coastal and mountainous regions of the Eastern and Western Cape provinces of South Africa. The increasing interest in honeybush coincided with the increasing global demand for health-promoting food and beverages. Honeybush tea is naturally caffeine free and has a low tannin content; it is associated with a complex array of polyphenolic compounds, contributing to the antioxidant properties of this herbal tea. Different species differ in chemical composition, which may result in differences in sensory characteristics, which in turn influences the marketing ability of the product.

The honeybush industry has the potential to become as successful as the rooibos industry, based on its unique flavour profile and health-related properties. Marketing strategies, underpinned by research on cultivation, production, processing and the sensory and chemical characteristics of honeybush, is needed to compete in local and international herbal tea markets. Six *Cyclopia* species, namely *C. subternata*, *C. intermedia*, *C. genistoides*, *C. longifolia*, *C. maculata* and *C. sessiliflora,* are of commercial interest, and research on production, agri-processing and sensory and chemical composition have focused on these species. Demand for honeybush has increased substantially over the last two decades, forcing the honeybush industry to use blends of the different species to supply in the demand for a commercial product that is consistent in quality.

The volatile compounds of the different species have been quantified, revealing a diverse array of volatile compounds. Qualitative and quantitative profiling of the phenolic composition of the major species have revealed differences between species. Mangiferin is one of the major phenolic compounds associated with *Cyclopia* species and specifically with *C. genistoides.* When comparing the xanthone content of four *Cyclopia* species, *C. genistoides* demonstrated the highest mangiferin and isomangiferin content while *Cyclopia longifolia* demonstrated the second highest xanthone content. Consumption of honeybush tea, particularly *C. genistoides* and *C. longifolia*, could make a considerable contribution to the dietary intake of polyphenols, in particular the xanthone and benzophenone sub-classes. Qualitative and quantitative sensory profiling of the major *Cyclopia* species revealed that all the species are associated with “fynbos-floral” and “fynbos-sweet” aromas while species-specific sensory profiles were confirmed, mainly driven by higher intensities of specific attributes. Sweet taste was equally high in all species while bitter taste was only perceptible in *C. genistoides* and under-fermented *C. longifolia*

The abundance of polyphenols identified in honeybush tea, not only contribute to the health promoting properties, but are also associated with bitter taste and astringent mouthfeel. The bitter taste is largely viewed as being detrimental to the consumer’s overall enjoyment of the tea, which opens up research opportunities to find ways of reducing the bitterness to below detection levels without detracting from the quality and health benefits of the tea. Different statistical techniques could be employed to understand the relationship between polyphenolic content and bitterness associated with *Cyclopia* species.

Although classic descriptive sensory analysis is regarded as the cornerstone of sensory methods, the analytical approach of this method, as well as the cost and time involved in maintaining highly trained panels, lead to the development of rapid sensory methods. Sensory scientists have used classic descriptive analysis for many decades and best practice for this method has been established. This is however not the case for the new rapid sensory methods. Research is necessary to determine the applicability, reliability and reproducibility of the new rapid sensory characterisation methods.

Sorting, a characterisation method with a holistic approach, entails that samples are sorted into groups based on perceived similarity or dissimilarity. Projective mapping has the advantage that additional differentiation between products might be possible as products are placed on a two-dimensional map, according to perceived similarities or dissimilarities. Polarised sensory positioning is a reference based method where the holistic sensory properties of products are evaluated in relation to a reference or sensory pole. Check-all-that apply is a verbal based method, where descriptors applicable to the product under question, are checked. All these methods can conducted by assessors with different levels of training. The number of product to be presented and the number of assessors to obtain stable results, differ for the various rapid methods. Rapid methods further differ in method of data analysis.

The development of new sensory methodologies are characterised by specific phases, which also functions as the basis for future application of the method. Phases of new method development in the sensory field, are: initial application of the proposed method; application of the method using different products; validation of the method, usually with results obtained through DSA; application of the method with different scenarios to determine best practice including number of products, number of assessors and statistical analysis of data. This process was apparent in the development of the rapid methods discussed in this chapter.

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Chapter 3

Chemical composition of *Cyclopia* species as potential predictors of bitter taste

Abstract

The aim was to identify phenolic compounds that could contribute to the potentially bitter taste associated with selected *Cyclopia* species. The sample set (n = 90) included samples of *C. genistoides* and *C. longifolia,* spanning different production seasons and different fermentation conditions. Infusions of these *Cyclopia* species were analysed using high-performance liquid chromatography–diode array detection (HPLC-DAD) for quantification of the phenolic compounds. Descriptive sensory analysis (DSA) was used to evaluate the bitter taste of the same infusions using an extended scale. Data obtained with HPLC-DAD and DSA were subjected to univariate (Pearson's linear correlation) and multivariate (partial least squares (PLS) regression) analyses to investigate the association between the bitter intensity and the phenolic composition of the honeybush infusions. Differences in the phenolic composition between species were demonstrated. PLS regression analysis with variable selection was effective in identifying candidate predictors for sensory bitterness. The xanthones, mangiferin and isomangiferin, were highly correlated to sensory bitterness and were identified as the major predictors of sensory bitterness. Two of the benzophenones, iriflophenone-3-*C*-glucoside-4-*O*-glucoside and iriflophenone-3-*C*-glucoside, also contributed to the bitter taste, but illustrated lower predictive ability. External validation of the proposed model demonstrated that 76% of observed bitterness values were within the 95% confidence interval of predicted bitterness. This prediction model could find application as screening tool in the Honeybush Cultivar Development Programme to identify plant material with high bitterness. Furthermore, batches of plant material with unacceptable high bitterness could be identified prior to blending to ensure an end-product with acceptable taste and high consumer appeal.

Keywords: *Cyclopia* species; Polyphenol composition; Bitter taste; Prediction model; Regression analysis, descriptive sensory analysis

1. Introduction

Following the growing global consumption of herbal teas, the South African honeybush herbal tea, prepared from *Cyclopia* species, has gained popularity worldwide. The rise in consumption of this beverage can be ascribed to numerous factors, including the perception of tea as a natural and healthy product, the wide range of speciality teas offered globally, as well as the affordability and availability of tea (Insight Survey, 2016). The taste (sweet, sour and bitter) and mouthfeel (astringency) of tea are determined by several classes

of non-volatile compounds including polyphenolic compounds, amino acids, purine alkaloids, nucleotides, organic acids, carbohydrates and ions (Kaneko, Kumazawa, Masuda, Henze, & Hofmann, 2006). Polyphenols are abundant in plant foods and beverages, and are associated with the major organoleptic properties of these products (Cheynier, 2005). In recent years, numerous studies have focused on the biological properties of polyphenols and their importance in human health (Jack et al., 2017; Mortimer, Visser, De Beer, Joubert, & Louw, 2015; Murakami et al., 2017).

The qualitative and quantitative differences in the polyphenol composition of *Cyclopia* species have been extensively researched (De Beer et al., 2012; Beelders, De Beer, Stander, & Joubert, 2014; Schulze, De Beer, De Villiers, Manley, & Joubert, 2014). Various studies on the antioxidant activity (Beelders, De Beer, & Joubert, 2015; Malherbe et al., 2014; Schulze et al., 2015) and health related effect (Beelders, Brand, et al., 2014; Jack et al., 2017; Mortimer et al., 2015; Murakami et al., 2017) contributed to the knowledge of the phenolic composition of various species, and their role as health-promoting constituents. Mangiferin, present in all *Cyclopia* species, is increasingly valued for its wide range of bioactivity, in particular its effect on metabolic homeostasis (Fomenko & Chi, 2016). Comparison of the xanthone content of infusions of four *Cyclopia* species demonstrated *C. genistoides* to have the highest content of the xanthones, mangiferin and isomangiferin. Infusions of *C. longifolia* had the second highest content of these xanthones while infusions of *C. maculata* and *C. subternata* contained significantly lower amounts (Schulze et al., 2015). When comparing the sensory profiles of the same *Cyclopia* species, bitterness was only perceived in *C. genistoides* and to lesser extent in *C. longifolia* (Erasmus, Theron, Muller, Van der Rijst, & Joubert, 2017).

Polyphenolic compounds contribute to the bitterness and astringency associated with tea, wine and some types of fruits and berries (Lesschaeve & Noble, 2005). High levels of bitterness in these foodstuffs may lead to negative consumer responses (Drewnowski & Gomez-Carneros, 2000). Bitterness perception is elicited by thousands of structurally diverse compounds through the activation of dedicated bitter taste receptors located in the oral cavity (Behrens et al., 2009). Taste receptor cells are organised in multicellular structures called taste buds (Adler et al., 2000). Each bitter taste receptor cell consists of a subset of the 25 human bitter taste receptor genes (hTAS2Rs) (Behrens et al., 2009). Several transduction mechanism for bitter perception have been identified, and these mechanisms appear to be compound specific. However, the mechanism for bitterness perception is not fully understood, with ongoing research focussing on bitter transduction methods and compounds responsible for bitter perception (Behrens et al., 2009; Roland et al., 2011, 2013; Soares et al., 2013).

In the current study, the association between bitter perception and the phenolic composition of honeybush infusions is of special interest. Stepwise-regression analysis was used to investigate the predictive value of the phenolic compounds for intensity of sensory taste attributes (sweet, sour, bitter and astringent) associated with honeybush infusions (Erasmus, 2015; Theron, 2012). Limitations to the step-wise regression method were identified, including that an assortment of compounds were used to build the model. In some instances, the independent variables (compositional parameters) had negative parameter estimates and in other instances positive parameter estimates. Collinearity of variables is a further problem when performing step-wise

regression analysis. When two independent variables (predictors) are significantly and highly correlated to each other and to a dependent variable, the model selects only one of the predictors to be present in the model.

An added limitation of the proposed model was that although considerable variation in the phenolic content of the different species were detected, intensities of individual sensory attributes demonstrated limited variation (Erasmus, 2015). To address the latter limitation, a so-called *extended* scale was proposed. The rationale for application of the extended scale was that when analysing the full profile of the *Cyclopia* species, the aroma are more prominent and when evaluating intensity of taste attributes relative to that of aroma, only a small part of the scale is used to indicate perceived differences. The idea was to evaluate only taste attributes and astringency, thereby forcing assessors to use a wider part of the scale to indicate perceived differences.

In the present study, the model proposed by Erasmus (2015) using step-wise regression analysis will be re-evaluated. Other selection criteria will be used, primarily to improve the prediction ability of the model. As discussed, traditional statistical techniques such as multiple regression analysis have limitations in handling co-linearity, therefore might not be appropriate because of co-linearity among compositional parameters (Magidson, 2013). One method that could find application in the current study, is partial least squares (PLS) regression analysis. The PLS regression method has gained interest in recent years, since it is a projection based technique that handles data with numerous and strongly collinear *X*-variables, while it is able to simultaneously model several *Y*-variables (Wold, Sjostrom, & Eriksson, 2001). The use of Variable Importance in Projection (VIP) scores has been demonstrated to be useful for interpreting the more relevant variables in PLS models (Platikanov et al., 2017). VIP scores summarise the variance in the predictor variables that globally contribute most to the y variance explanation. A VIP value >1 is used as criterion to indicate the most important variables in the PLS model for variable selection (Chong & Jun, 2005).

PLS regression analysis demonstrated good potential as method to predict sensory properties based on physicochemical parameters. A prediction model, using PLS regression analysis, were successfully constructed to predict the quality of Longjing tea based on 10 volatile compounds (Lin, Dai, Guo, Xu, & Wang, 2012). A study by Platikanov et al. (2017) illustrated that it was possible to accurately predict consumers' preference for mineral content of bottled and tap water by PLS regression using physicochemical parameters. The objective of the present study was to employ PLS regression analysis to predict the bitter taste of infusions of *C. genistoides* and *C. longifolia* based on the phenolic composition.

2. Materials and methods

An outline of the sample sets and the different analyses conducted are provided in Fig. 1.

2.1 Samples

Two sample sets were used i.e., one set for the development of the prediction model (training set) and a second set of independent samples for validation of the model. All plant material, sourced either from commercial plantations or the wild (in the case of *C. maculata*) was processed according to a standard protocol,

described by Theron et al. (2014). Fermentation time and temperature of sub-batches was varied to introduce additional variation in terms of sensory taste intensities and phenolic composition.

For prediction model development, batches of plant material of *C. genistoides* (n = 36), *C. maculata* (n = 44) and *C. subternata* (n = 44), harvested over a four year period (2010, 2012 and 2013), were sourced and processed, i.e. fermented at optimum conditions (80°C for 24h or 90°C for 16h). Batches of plant material of *C. longifolia* (n = 54), harvested in 2013, were fermented at different temperature/time regimes, i.e. 80°C and 90°C for 8, 16 and 24 h (Erasmus et al., 2017). Plant material representing different batches and different fermentations conditions were included as this address the variation necessary for the development of prediction models.

An independent set of samples were sourced for external validation of the model. Batches representing *C. genistoides* (n = 24), *C. subternata* (n = 24) and *C. maculata* (n = 24), fermented at eight different temperature/time regimes, i.e. 80°C and 90°C for 8, 16, 24 h and 32h (Theron, 2012), served as validation set.

After fermentation, all samples were mechanically sieved to obtain the "tea bag" fraction (<12 mesh and > 40 mesh) as described by Theron et al. (2014). The sieved plant material was stored at ambient temperature (21ºC) in sealed glass jars, until analysed.

2.2 Preparation of infusions

Infusions were prepared as described by Theron et al. (2014). Briefly, freshly boiled distilled water (1000 g) was poured onto 12.5 g of the plant material and allowed to infuse for 5 min before straining through a fine-mesh strainer directly into a 1 L pre-heated stainless steel thermos flask (Woolworths, Bellville, South Africa). The mugs used for tasting were pre-heated in an industrial oven (Hobart, France) at 70°C before aliquots of each infusion (ca. 100 mL) were poured into the mugs and covered with plastic lids to prevent loss of volatiles. Coded samples were served in a random order per assessor as generated by the Compusense® five software program (Compusense version 5.6, Guelph, Canada) where all assessors evaluated all the samples while controlling for first order carry-over effects. The samples were served in temperature controlled (65°C) water baths (Scientific Manufacturing Company, Cape Town, South Africa). Preparation and evaluation of the infusions are depicted in Addendum A.

2.3 Descriptive sensory analysis (DSA)

Trained panellists (n = 9, female between the ages of 40 and 65) with several years of experience in the sensory analysis of infusions prepared from rooibos (Jolley, Van der Rijst, Joubert, & Muller, 2017; Koch, Muller, Joubert, Van der Rijst, & Næs, 2012) and honeybush (Erasmus et al., 2017; Theron et al., 2014) were selected. Panel members completed an official consent form before commencing with DSA. The panel was further trained on the samples and the generic DSA technique as described by Lawless and Heymann (2010). Assessors were screened for bitterness sensitivity using solutions with three concentrations of caffeine (0.07%, 0.14% and 0.035%). All panel members correctly completed the ranking test and were included in the final panel.

Intensities for three taste attributes (sweet, sour and bitter) and astringency as mouthfeel attribute were rated on the extended, unstructured line scale (0 – 100), using the Compusense® five software program (Compusense, Guelph, Canada). Only taste attributes were scored since the focus of the study was to determine sensory bitterness of honeybush infusions. The experimental design was a completely random design. The different batches of plant material per species served as replicates. Six samples were presented in a random order to each assessor per session. The hot water infusions were evaluated during three consecutive sessions per day with a rest period of 15 min between each test session to avoid panel fatigue. Unsalted water biscuits (Woolworths, Stellenbosch, South Africa) and still natural spring water (Woolworths, Stellenbosch, South Africa) were used as palate cleansers between each sample. Assessors were seated at individual booths in a temperature (21ºC) and light-controlled room.

2.4 Phenolic content and soluble solids content

The same infusions prepared for the DSA were used for compositional analysis. A 100 mL aliquot of each of the latter infusions was filtered through Whatman No. 4 filter paper and allowed to cool. The filtrate was transferred to 2 mL microfuge tubes and stored at -18°C until required for high-performance liquid chromatography (HPLC) analyses.

2.4.1 Soluble solids content

The soluble solids content of the each infusion was determined directly after filtration. The procedure described by (De Beer et al., 2012) was employed.

2.4.2 HPLC analysis

All analyses were conducted on an Agilent 1200 series HPLC instrument, consisting of a quaternary pump, auto-sampler, column thermostat, in-line degasser and diode array detector (DAD), controlled by Chemstation software (Agilent Technologies Inc., Santa Clara, CA). Authentic standards (purity ≥95%) were sourced from Extrasynthèse, Genay, France (mangiferin, eriocitrin, luteolin), Sigma-Aldrich (hesperidin, iriflophenone-3-*C*-glucoside) and Chemos, Regenstauf, Germany (isomangiferin). Aspalathin (3-hydroxyphloretin-3'-*C*-glucoside) and nothofagin (phloretin-3'-*C*-glucoside) were obtained from PROMEC (Medical Research Council of South Africa, Tygerberg, South Africa). Prior to PHLC analysis, an aqueous ascorbic acid solution was added (final ascorbic acid concentration of 5mg/L and 9mg/L for standards and samples, respectively) to prevent oxidative degradation during analysis. Infusions of *C. genistoides*, *C. longifolia*, *C. maculata* and *C. subternata* were analysed using species-specific HPLC-DAD methods described by Beelders, De Beer, et al. (2014), Schulze et al. (2015), Schulze, De Beer, De Villiers, Manley, & Joubert (2014) and De Beer et al. (2012). The xanthones and flavones were quantified at 320 nm, while the flavanones and the benzophenones were quantified at 288 nm. The phenolic compounds were calibrated using calibration curves for authentic reference standards, whereas compounds for which no standards could be acquired, quantification were performed with equivalents of related compounds (i.e. the eriodictypol-*O*-deoxyhexoside-*O*-hexocide isomere was given as eriocitirn equivalents) (Jack et al., 2017).

2.5 Statistical procedures

2.5.1 DSA

The performance of the DSA panel was monitored using PanelCheck Software (Version 1.3.2, http://www.panelcheck.com/). Descriptive sensory analysis data were pre-processed to test for panel reliability by means of a model that includes assessor, replication and sample effects and interactions (Naes, Brockhoff, & Tomic, 2010). The Shapiro-Wilk test was performed to test for non-normality of residuals (Shapiro & Wilk, 1965). In the event of significant non-normality ($P \leq 0.05$), outliers were removed when the standardised residuals for an observation deviated more than three standard deviations from the model value. Following confirmation of panel reliability and normality of data, subsequent statistical analyses were performed on data representing means over assessors. All univariate analyses were performed using SAS® software (Statistical Analysis System 2006, Version 9.4, SAS Institute Inc., Cary, NC, USA).

2.5.2 Descriptive statistics

Preliminary exploratory data analysis was performed using univariate descriptive statistics. Sensory and phenolic compositional data for all four *Cyclopia* species were included in exploratory data analysis.

2.5.3 Correlation and regression analyses

Pearson's correlations analysis was performed to determine the closeness of the linear relationships between the compositional parameters and sensory attributes (Snedecor & Cochran, 1989). Pearson's correlations analysis was performed on the training data set, including bitterness and phenolic compositional parameters for all four *Cyclopia* species, as well as on a reduced set comprising the data of only *C. genistoides* and *C. longifolia*.

Partial least squares regression (PLS) analysis was performed to determine the association between bitterness and phenolic composition (Abdi, 2013; Jolliffe, 2002), enabling the development of a model for predicting bitterness based on phenolic composition of selected *Cyclopia* species. The sensory and chemical data were standardised to equal variance prior to PLS. The number of components for the PLS model was optimized on the training data by assessing the quality of the prediction models. By eliminating predictors that did not contribute to the model, a final model that fitted the data well, were obtained. The most important variables for inclusion in the PLS regression model were identified, based on a VIP value >1 as criterion for variable selection (Chong & Jun, 2005). The robustness of the prediction model was tested by performing external validation using the second set of independent samples not included in the model. The quality of the prediction model was evaluated by calculating the percentage of observed bitterness values of the validation set falling within the 95% confidence bounds for prediction.

Correlation and PLS regression analyses were performed using XLStat (Version 7.5.2, Addinsoft, New York, USA).

3. Results and discussion

The typical aroma, flavour and taste associated with honeybush tea contributes to consumers' enjoyment of this herbal tea. Recent studies have focused on identifying the volatile and aroma-active compounds associated with *C. genistoides* (Le Roux, Cronje, Joubert, & Burger, 2008) and *C. subternata* (Le Roux, Cronje, Burger, & Joubert, 2012). Following this research, 84 volatile compounds were identified in infusions of fermented *C. subternata*, *C. maculata* and *C. genistoides* (Ntlhokwe et al., 2017). However, limited research on the effect of non-volatile compounds on taste attributes of honeybush infusions have been published. To date, two studies (Erasmus, 2015; Theron, 2012) aimed to address the effect of phenolic compounds on the taste and mouthfeel of honeybush tea. Theron (2012) indicated that mangiferin might contribute to the bitter taste of honeybush infusions but only a limited number of phenolic compounds have been identified for this study. Advances were made in the identification and quantification of phenolic compounds associated with several *Cyclopia* species, including compounds belonging to the benzophenone and dihydrochalcone sub-classes (Beelders, De Beer, et al., 2014; Schulze et al., 2014). These advances merited re-investigation of the link between taste and mouthfeel and chemical composition of a large sample set consisting of four *Cyclopia* species.

3.1 Phenolic content and sensory intensities

The notations used to indicate individual phenolic compounds in Tables and Figures are presented in Table 1. The minimum, maximum, mean and standard deviation values of the sensory attributes (taste and mouthfeel) and content of individual phenolic compounds of *C. genistoides*, *C. longifolia*, *C. maculata* and *C. subternata* are summarised in Table 2. Large qualitative and quantitative differences were observed when comparing the content of individual phenolic compounds between these species. This was expected as previous research by Schulze et al. (2015) demonstrated large intra- and inter-species variation when comparing phenolic content of infusions of the same *Cyclopia* species. Fifteen phenolic compounds were quantified, however, all compounds could not be quantified in all species, either due to their absence or presence in trace quantities. The concentration of compounds in the infusions varied considerably between species, in particular when comparing the mangiferin (X1) and isomangiferin (X2) content. The highest mangiferin content was observed in infusions of *C. genistoides* (118.20 mg/L) followed by *C. longifolia* infusions (71.33 mg/L). The mean mangiferin content for *C. maculata* infusions was 16.65 mg/L while the mean mangiferin content of *C. subternata* infusions was as low as 2.34 mg/L. *C. genistoides* infusions presented the highest mean values for the following phenolic compounds: iriflophenone-3-*C*-glucoside-4-*O*-glucoside (B1), maclurin-di-*O*,*C*-hexocide (B2), maclurin-3-*C*-glucoside (B3), iriflophenone-3-*C*-glucoside (B4), naringenin-*O*-hexose-*O*-deoxyhexose A (Fl1), naringenin-*O*-hexose-*O*-deoxyhexose B (Fl2) and isomangiferin (X2). *C. longifolia* illustrated the highest mean values for vicenin-2 (Fv1) and tetrahydroxyxanthone-C-hexoside isomer A (X3), *C. subternata* the highest mean values for phloretin-3'-5'-di-*C*-glucoside (D2), eriocitrin (Fl4) and scolymoside (Fv2) and *C. maculata* the highest mean value for hesperidin (Fl3).

When comparing interspecies variation in phenolic composition, *C. genistoides* presented a more complex composition than the other species. Two of the benzophenone compounds {maclurin-di-*O*, *C*-hexocide (B2) and maclurin-3-*C*-glucoside (B3)}, the flavanone, naringenin-*O*-hexose-*O*-deoxyhexose A (Fl1), and the xanthone, tetrahydroxyxanthone-*C*-hexoside isomer B (X4), were only present in *C. genistoides* infusions. Only four compounds of those quantified were present in all four *Cyclopia* species, namely hesperidin (Fl3), vicenin-2 (Fv1), mangiferin (X1) and isomangiferin (X2).

Limited variation was observed for astringency and sour taste when comparing mean values across species, but considerable variation in the mean values for bitterness was observed. The normal distribution of bitterness per species is illustrated in Fig. 2. The highest mean value for bitterness was observed for *C. genistoides* (27.19), moreover, the bitterness for this species varied considerable (minimum = 17.81 and maximum = 37.11). The mean bitterness for C. *longifolia* was 19.17, with an even larger difference between minimum (7.28) and maximum (41.94) bitterness. The variation in bitterness associated with *C. subternata* was limited; the mean value for bitterness was 8.85, while the minimum and maximum values were 6.28 and 14.17 respectively. *Cyclopia maculata* illustrated more variation in bitterness (mean value = 10.85, minimum = 5.67 and maximum = 21.72). Both *C. genistoides* and *C. longilfolia* were perceived to be lower in sweetness (mean values 26.01 and 26.53, respectively) compared to the sweetness of *C. subternata* and *C. maculata* (mean values 31.77 and 32.44, respectively).

3.2 Correlation between bitterness and compositional parameters

Pearson's correlation coefficients for bitterness and phenolic composition of the four *Cyclopia* species are summarised in Table 3. *Cyclopia genistoides* illustrated significant positive correlations ($p \leq 0.05$) between bitter taste and mangiferin (X1) ($r = 0.659$), isomangiferin (X2) ($r = 0.444$), iriflophenone-3-*C*-glucoside-4-*O*-glucoside (B1) ($r = 0.371$) and vicenin-2 (Fv1) ($r = 0.332$). A significant positive correlations between bitterness and mangiferin (X1) ($r = 0.814$), vicenin-2 (Fv1) ($r = 0.779$), isomangiferin (X2) ($r = 0.763$), iriflophenone-3-*C*-glucoside-4-*O*-glucoside (B1) ($r = 0.705$) and iriflophenone-3-*C*-glucoside (B4) ($r = 0.658$), were observed for *C. longifolia*. This species further illustrated significant positive correlations between bitterness and tetrahydroxyxanthone-*C*-hexoside isomer A (X3) ($r = 0.586$), eriocitrin (Fl4) ($r = 0.569$), naringenin-*O*-hexose-*O*-deoxyhexose B (Fl2) ($r = 0.482$) and hesperidin (Fl3) ($r = 0.436$). Of the five compounds quantified in *C. maculata*, only mangiferin (X1) illustrated a significant positive correlation with bitterness ($r = 0.357$, $p \leq 0.05$). No correlations between bitter taste and the phenolic compounds quantified in *C. subternata,* were observed.

3.2.1 Combined data set for *C. genistoides* and *C. longifolia*

Given the range of bitter intensities, and the positive correlation obtained for *C. genistoides* and *C. longifolia* for several phenolic compounds, Pearson correlation analysis were subsequently performed on a combined data set including only these two species. Furthermore, only corresponding compounds of *C. genistoides* and *C. longifolia* were included in this analysis. These compounds were iriflophenone-3-*C*-glucoside-4-*O*-glucoside (B1), iriflophenone-3-*C*-glucoside (B4), naringenin-*O*-hexose-*O*-deoxyhexose B

(Fl2), hesperidin (Fl3), vicenin-2 (Fv1), mangiferin (X1), isomangiferin (X2) and tetrahydroxyxanthone-*C*-hexoside isomer A (X3).

Pearson's correlation coefficients for bitterness and the selected polyphenolic compounds of the combined data set for *C. genistoides* and *C. longifolia* are summarised in Table 4. Scatter plots, illustrating the relationship between bitterness and the individual phenolic compounds, are presented in Fig. 3. Significant positive correlations ($p \leq 0.05$) were obtained between bitter taste and all the polyphenolic compounds included in the analysis except tetrahydroxyxanthone-*C*-hexoside isomer A (X3).

3.3 PLS regression analysis

Given that the aim of the current study was to predict sensory bitterness based on phenolic composition, only data for *C. genistoides* and *C. longifolia* were included during PLS regression analysis as these species were associated with a wide range of bitterness intensities (Fig. 2). Furthermore, only data of phenolic compounds quantified in both these species were included in the regression analysis. Results reported further are therefore based on bitterness and selected compositional parameters of *C. genistoides* and *C. longifolia* only. The individual phenolic compounds for the infusions of the two *Cyclopia* species were imported to the *X*-matrix while the sensory intensity scores for bitterness of the same infusions were imported to the *Y*-matrix. This data set is referred to as the training set. PLS regression analysis was employed to determine the association between the two matrices of the training set.

When including all eight compositional parameters in the PLS regression, the first two dimensions explained 63% of the variance of *X* and 66% of *Y*. Model quality did not improve by including more dimensions. The plot for the PLS regression analysis performed on the training set is provided in Fig. 4. Four of the phenolic compounds, mangiferin (X1), isomangiferin (X2) and iriflophenone-3-*C*-glucoside-4-*O*-glucoside (B1) and iriflophenone-3-*C*-glucoside (B4) were positioned in close proximity to bitterness towards the right of the plot, indicating high correlation. Vicenin-2 (Fv1), hesperidin (Fl3) and naringenin-*O*-hexose-*O*-deoxyhexose B (Fl2) were also situated towards the right of the PLS plot but further apart from bitterness, indicating a lower degree of correlation. Tetrahydroxyxanthone-*C*-hexoside isomer A (X3) was situated in the upper left quadrant of the plot, opposite to bitterness and therefore not correlated to bitter taste.

The importance of an *X*-variable for both *Y* and *X* is given by a VIP value (variable importance for the projection) (Wold et al., 2001). A VIP value >1 is used as criterion for variable selection (Chong & Jun, 2005). VIP values for the respective variables are presented in Fig. 5. Mangiferin (X1), isomangiferin (X2), iriflophenone-3-*C*-glucoside-4-*O*-glucoside (B1) and iriflophenone-3-*C*-glucoside (B4) illustrated VIP values >1 and were selected for inclusion in the final model. Components with VIP values <1 were hesperidin (Fl3), naringenin-*O*-hexose-*O*-deoxyhexose B (Fl2), vicenin-2 (Fv1) and tetrahydroxyxanthone-*C*-hexoside isomer A (X3).

A subsequent PLS regression was performed with inclusion of the four selected variables with VIP values >1. In this case, the first two dimensions explained 92% of the variance of *X* and 66% of Y. The plot for PLS regression analysis with variable selection is provided in Fig. 6. In this second PLS plot, the two

xanthones, mangiferin (X1) and isomangiferin (X2), were situated in close proximity to bitterness in the upper right quadrant of the plot, indicating high correlation to sensory bitterness. Two of the benzophenones, iriflophenone-3-*C*-glucoside-4-*O*-glucoside (B1) and iriflophenone-3-*C*-glucoside (B4) were also situated towards the right of the plot, therefore contributing to the bitter taste, but these compounds have a smaller contribution to prediction of bitterness compared to the two xanthones (X1 and X2).

Based on this PLS regression analysis, the equation of the prediction model for bitterness was: Bitter = 5.6409 + 0.1052*X1 + 0.1844*X2 + 9.0989*B1 - 0.1259*B4

Model quality indexes are used to evaluate the quality of the prediction model. The quality is related to the cumulative contribution of the components to the indexes. The first two components were included in the current model. Addition of components did not improve the model quality. The Q^2cum index measures the overall contribution of the first two components to the prediction ability of the model. The Q^2cum index for the current model was 0.614. The R^2Ycum index measures the explanatory power of the first two components for the dependent variables included in the current model and was 0.663. Sixty six percent of the variance in Y (bitterness) is therefore explained by dimension one and two of the current model. The R^2Xcum index measures the explanatory power of the first two components for the explanatory variables of the model and was 0.918 for the current model. This indicates that 92% of the variance in X (phenolic compounds) were explained by the first two components included in the current model. The model quality indexes, based on the cumulative contribution of the first two components, is presented in Fig. 7.

3.4 Model validation

When developing a model, it is important to ascertain the prediction ability of the model by determining how well the *X*-data can predict the *Y*-data (Naes et al., 2010). In the current study, this involves determining how well the individual phenolic compounds (*X*-matrix) can predict sensory intensity scores for bitterness (*Y*-matrix). A prediction model can be regarded as valid when it consistently and precisely predicts the *Y*-values of observations when new *X*-values are fitted on the model (Wold et al., 2001). The best procedure for model validation is by subjecting new *X*-values of an independent data set to the model and evaluate how consistently and precisely *Y*-values are predicted. According to Wold et al. (2001), an independent and representative validation set is rare and most often cross-validation is used, which entails that a subset of samples is removed and a "parallel" model developed based on the reduced data set. The "removed data set" is projected on the model to determine how precise and consistent the model predicts the *Y*-values.

For the current study, external validation of the prediction model was possible using an independent data set not included in the model. The validation data was fitted on the model to determine the accuracy of the model for predicting bitterness. The observed values for bitterness were therefore compared to the predicted values for bitterness. A scatter plot of the predicted bitter values from the PLS regression model against the observed values is provided in Fig. 8. A 95% confidence interval around the predicted bitterness was calculated for each observation. The percentage of observed bitterness values that fall within the 95% confidence interval of the predicted bitterness was calculated. Of the training set, 96% of observed values fall

within the 95% confidence interval, while 76% of the observed values for the validation set falls within the 95% confidence interval.

3.5 Limitations and recommendations

The so-called "extended scale" was employed in the current study in an attempt to improve variation in the intensities of sensory taste attributes. This scale demonstrated to aid in capturing variation in bitterness however, extensive training of the panel in the application of the scale is essential. Some inconsistencies relating to the sensory analysis of bitterness, merits further discussion. External validation indicated that the model could accurately predict 76% of the observed bitterness values, but the model seems to under predict infusions with high bitterness. This current research indicated that samples with low or moderate bitterness were accurately predicted, however, the bitterness of infusions that the panel perceived as extremely bitter, were under predicted. A possible explanation for this problem was the time lapse between the sensory analysis of the training set and the validation set (almost 12 months) and therefore inconsistent use of the scale by the panel. This concern could be addressed by including a range of reference standards with known and defined bitter intensities.

Although good results were obtained with PLS regression analysis, some limitations were identified. When comparing the phenolic composition for *C. genistoides* and *C. longifolia*, only eight compounds were common to both these *Cyclopia* species and therefore included in model development. When developing prediction models, it is important to include data that encompass as much variation as possible. Apart from external factors such as climate, soil type, slope and cultivation area, the phenolic content of honeybush is also influenced by processing conditions such as fermentation time and temperature (Du Toit & Joubert, 1999). Considerable variation in the phenolic content and sensory bitterness was observed for *C. genistoides*, although all samples representing this species were processed at optimum fermentation conditions (80°C/24h or 90°C/16h). By including a larger sample set of only *C. genistoides*, that spans different processing conditions, model development to address bitterness could be based on this species only. In this way more variation in phenolic content will be included, opening up more opportunities for model development. Future research to address the latter, is recommended.

The results of the current study demonstrated that the polyphenolic composition provided valuable qualitative and quantitative information for modelling bitter taste in *Cyclopia* species, and PLS regression analysis identified four candidate predictors for sensory bitterness. Further research to determine the contribution of the individual compounds, identified as candidate predictors, as well as the phenolic sub-classes to bitter taste, is recommended. Recent studies investigated the sensory contribution of identified phenolic compounds by employing sensory-guided fractionation (Liu et al., 2015; Sáenz-Navajas et al., 2017). To investigate the contribution of fractions, and individual phenolic compounds isolated within specific fractions, to sensory bitterness in *Cyclopia* species, this research approach could be followed. The contribution of individual isolated compounds to taste and mouthfeel could furthermore be investigated using dose-over-threshold (Dot) values (i.e. the ratio of the concentration and the threshold of a compound). The taste impact

of the compounds with the largest Dot values could be confirmed by means of reconstitution and omission experiments (Scharbert & Hofmann, 2005).

4. Conclusions

The current study investigated the phenolic composition of four *Cyclopia* species and the relationship with bitter taste evaluated on the extended scale. Results from the present work demonstrated the extended scale to be an effective tool to capture the differences in perceived bitter taste of different *Cyclopia* species. High intensities and considerable variation in bitterness were observed for *C. genistoides* and *C. longifolia*. These species further illustrated substantial variation in phenolic content. Although some variation in the phenolic content of *C. subternata* was observed, the variation in bitterness was limited. *C. maculata* illustrated some variation in bitterness but a limited number of phenolic compounds were identified in this species.

Pearson's correlation analysis and PLS regression analysis were used to investigate the relationship between polyphenolic compounds and sensory bitterness. Partial least squares regression analysis with variable selection provided a valid model for predicting bitterness based on the polyphenolic composition of selected *Cyclopia* species. The validity of the model was confirmed by the high percentage of observed bitterness values accurately predicted when using external validation. Candidate predictors for sensory bitterness associated with *Cyclopia* species are mangiferin and isomangiferin while iriflophenone-3-*C*-glucoside and iriflophenone-3-*C*-glucoside-4-*O*-glucoside were also identified as candidate predictors, but with a slightly lower predictive ability. Future research, exploring the contribution of phenolic sub-classes (xanthones and benzophenones) and individual phenolic compounds to bitter taste, is recommended.

The prediction model demonstrated to be a valid tool to screen a large number of batches of *Cyclopia* samples to identify batches with potential high degree of bitterness. Such a model will find application in research programs such as the Honeybush Cultivar Development Program of the Agricultural Research Council, South Africa where the aim is to identify genotypes and plant selections with high levels of bitterness.

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Table 1 Notations used to indicate individual phenolic compounds in Tables and Figures.

Notations used in Tables and Figures	Class	Compositional parameters
B1	Benzophenone	Iriflophenone-3-*C*-glucoside-4-*O*-glucoside
B2	Benzophenone	Maclurin-di-*O*,*C*-hexocide[a]
B3	Benzophenone	Maclurin-3-*C*-glucoside
B4	Benzophenone	Iriflophenone-3-*C*-glucoside
D2	Dihydrochalcone	Phloretin-3'-5'-di-*C*-glucoside[b]
Fl1	Flavanone	Naringenin-*O*-hexose-*O*-deoxyhexose A[c]
Fl2	Flavanone	Naringenin-*O*-hexose-*O*-deoxyhexose B[c]
Fl3	Flavanone	Hesperidin
F14	Flavanone	Eriocitrin
Fv1	Flavone	Vicenin-2[d] (apigenin-6,8-di-*C*-glucoside)
Fv2	Flavone	Scolymoside (luteolin-7-*O*-rutinoside)
X1	Xanthone	Mangiferin
X2	Xanthone	Isomangiferin
X3	Xanthone	Tetrahydroxyxanthone-*C*-hexoside isomer A[e]
X4	Xanthone	Tetrahydroxyxanthone-*C*-hexoside isomer B[e]

[a] Quantified as maclurin-3-C-glucoside equivalents.

[b] Quantified as nothofagin equivalents.

[c] Quantified as narirutin equivalents.

[d] Quantified as luteolin equivalents.

[e] Quantified as mangiferin equivalents.

Table 2 Minimum, maximum**,** mean and standard deviation for the intensities of the respective sensory attributes (scored on the extended scale) and compositional parameters [a] (mg/L in water) for *C. genistoides*, *C. longifolia*, *C. maculata* and *C. subternata*.

Species	Variable	Minimum	Maximum	Mean	Standard deviation
C. genistoides (n = 36)	Bitter	17.81	37.11	27.19	5.35
	Sweet	19.72	32.39	26.01	2.65
	Sour	12.78	25.33	18.31	3.16
	Astringent	39.63	56.33	48.44	3.24
	B1	11.86	49.87	33.28	10.92
	B2	0.25	2.40	1.29	0.54
	B3	0.58	9.05	3.73	2.23
	B4	4.88	41.03	17.01	8.62
	D2	0.00	0.00	0.00	0.00
	Fl1	0.96	18.62	6.48	4.50
	Fl2	1.02	17.30	7.55	4.95
	Fl3	2.50	13.13	8.91	2.55
	F14	0.00	0.00	0.00	0.00
	Fv1	2.41	8.34	5.48	1.38
	Fv2	0.00	0.00	0.00	0.00
	X1	61.23	198.49	118.20	36.56
	X2	26.51	62.99	38.58	8.36
	X3	0.14	0.77	0.42	0.16
	X4	0.32	1.21	0.69	0.23
C. longifolia (n = 54)	Bitter	7.28	41.94	19.17	10.31
	Sweet	15.78	34.75	26.53	4.73
	Sour	8.81	33.72	18.32	6.06
	Astringent	37.33	55.61	45.49	4.59
	B1	10.33	50.45	22.76	10.94
	B2	0.00	0.00	0.00	0.00
	B3	0.00	0.00	0.00	0.00
	B4	0.78	26.15	5.44	7.12
	D2	0.00	0.00	0.00	0.00
	Fl1	0.00	0.00	0.00	0.00
	Fl2	-0.02	2.23	0.87	0.48
	Fl3	5.18	12.63	8.37	1.87
	F14	1.56	6.35	3.30	1.21
	Fv1	4.81	10.36	6.85	1.23
	Fv2	3.02	10.99	5.67	1.72
	X1	17.35	254.76	71.33	55.85
	X2	12.01	67.12	29.71	13.25
	X3	0.89	2.79	1.66	0.43
	X4	0.00	0.00	0.00	0.00

Species	Variable	Minimum	Maximum	Mean	Standard deviation
C. maculata (n = 44)	Bitter	5.67	21.72	10.85	3.08
	Sweet	22.56	42.00	32.44	4.17
	Sour	9.22	21.11	13.75	2.75
	Astringent	35.78	47.89	42.55	2.33
	B1	0.00	0.00	0.00	0.00
	B2	0.00	0.00	0.00	0.00
	B3	0.00	0.00	0.00	0.00
	B4	0.00	0.00	0.00	0.00
	D2	0.00	0.00	0.00	0.00
	Fl1	0.00	0.00	0.00	0.00
	Fl2	0.00	0.00	0.00	0.00
	Fl3	9.49	21.32	15.36	2.87
	F14	0.90	10.98	5.02	2.14
	Fv1	2.37	5.60	3.73	0.78
	Fv2	0.00	0.00	0.00	0.00
	X1	3.02	38.67	16.65	8.67
	X2	2.47	20.74	11.52	4.52
	X3	0.00	0.00	0.00	0.00
	X4	0.00	0.00	0.00	0.00
C. subternata (n = 44)	Bitter	6.28	14.17	8.85	2.10
	Sweet	25.78	39.89	31.77	3.22
	Sour	9.11	21.50	14.17	2.93
	Astringent	34.67	48.61	41.33	2.77
	B1	3.71	55.72	22.75	12.78
	B2	0.00	0.00	0.00	0.00
	B3	0.00	0.00	0.00	0.00
	B4	0.00	0.00	0.00	0.00
	D2	0.87	14.51	4.32	2.96
	Fl1	0.00	0.00	0.00	0.00
	Fl2	0.00	0.00	0.00	0.00
	Fl3	2.73	11.99	5.91	2.45
	F14	1.71	12.29	5.36	2.58
	Fv1	1.02	4.70	2.55	0.92
	Fv2	3.20	32.64	13.64	7.55
	X1	0.44	6.95	2.34	1.87
	X2	0.46	7.50	2.49	1.86
	X3	0.00	0.00	0.00	0.00
	X4	0.00	0.00	0.00	0.00

[a] The notations used for the compositional parameters are explained in Table 1.

Table 3 Pearson's correlation table for bitter taste and compositional parameters [a] (mg/L in water) for four *Cyclopia* species.

Variables	*C. genistoides*		*C. longifolia*		*C. maculata*		*C. subternata*	
	Correla-tion	p-value	Correla-tion	p-value	Correla-tion	p-value	Correla-tion	p-value
B1	**0.371**	**0.026**	**0.705**	**0.0001**	-	-	-0.005	0.975
B2	0.281	0.097	-	-	-	-	-	-
B3	0.206	0.228	-	-	-	-	-	-
B4	0.293	0.083	**0.658**	**0.0001**	-	-	-	-
D2	-	-	-	-	-	-	0.047	0.763
Fl1	0.281	0.097	-	-	-	-	-	-
Fl2	0.281	0.096	**0.482**	**0.0001**	-	-	-	-
Fl3	-0.144	0.400	**0.436**	**0.001**	0.083	0.594	0.110	0.478
F14	-	-	**0.569**	**0.0001**	0.209	0.173	0.132	0.392
Fv1	**0.332**	**0.048**	**0.779**	**0.0001**	0.173	0.261	-0.077	0.618
Fv2	-	-	-0.159	0.249	-	-	-0.125	0.417
X1	**0.659**	**0.000**	**0.814**	**0.0001**	**0.357**	**0.017**	-0.158	0.307
X2	**0.444**	**0.007**	**0.763**	**0.0001**	0.274	0.072	-0.122	0.430
X3	-0.010	0.956	**0.586**	**0.0001**	-		-	-
X4	0.198	0.247	-	-	-	-	-	-

Values in bold are significantly different from 0 ($p<0.05$)

[a]The notations used for the compositional parameters are presented in Table 1.

Table 4 Pearson's correlation coefficients for bitter taste and selected polyphenolic compounds [a] (mg/L in water) for *C. genistoides* and *C. longifolia.*

Variables	Bitter	
	Correlation	*p*-value
B1	**0.661**	**0.000**
B4	**0.622**	**0.000**
Fl2	**0.403**	**0.000**
Fl3	**0.256**	**0.015**
Fv1	**0.304**	**0.004**
X1	**0.821**	**0.000**
X2	**0.732**	**0.000**
X3	-0.130	0.223

Values in bold are significantly different from 0 ($p<0.05$)

[a] The notations used for the polyphenolic compounds are presented in Table 1.

Table 5 Model quality indexes for the prediction model based on PLS regression analysis with variable selection.

Statistic	Component 1	Component 2
B1	**0.661**	**0.000**
B4	**0.622**	**0.000**
Fl2	**0.403**	**0.000**
Fl3	**0.256**	**0.015**
Fv1	**0.304**	**0.004**
X1	**0.821**	**0.000**
X2	**0.732**	**0.000**
X3	-0.130	0.223

Values in bold are significantly different from 0 ($p<0.05$)

[a] The notations used for the polyphenolic compounds are presented in Table 1.

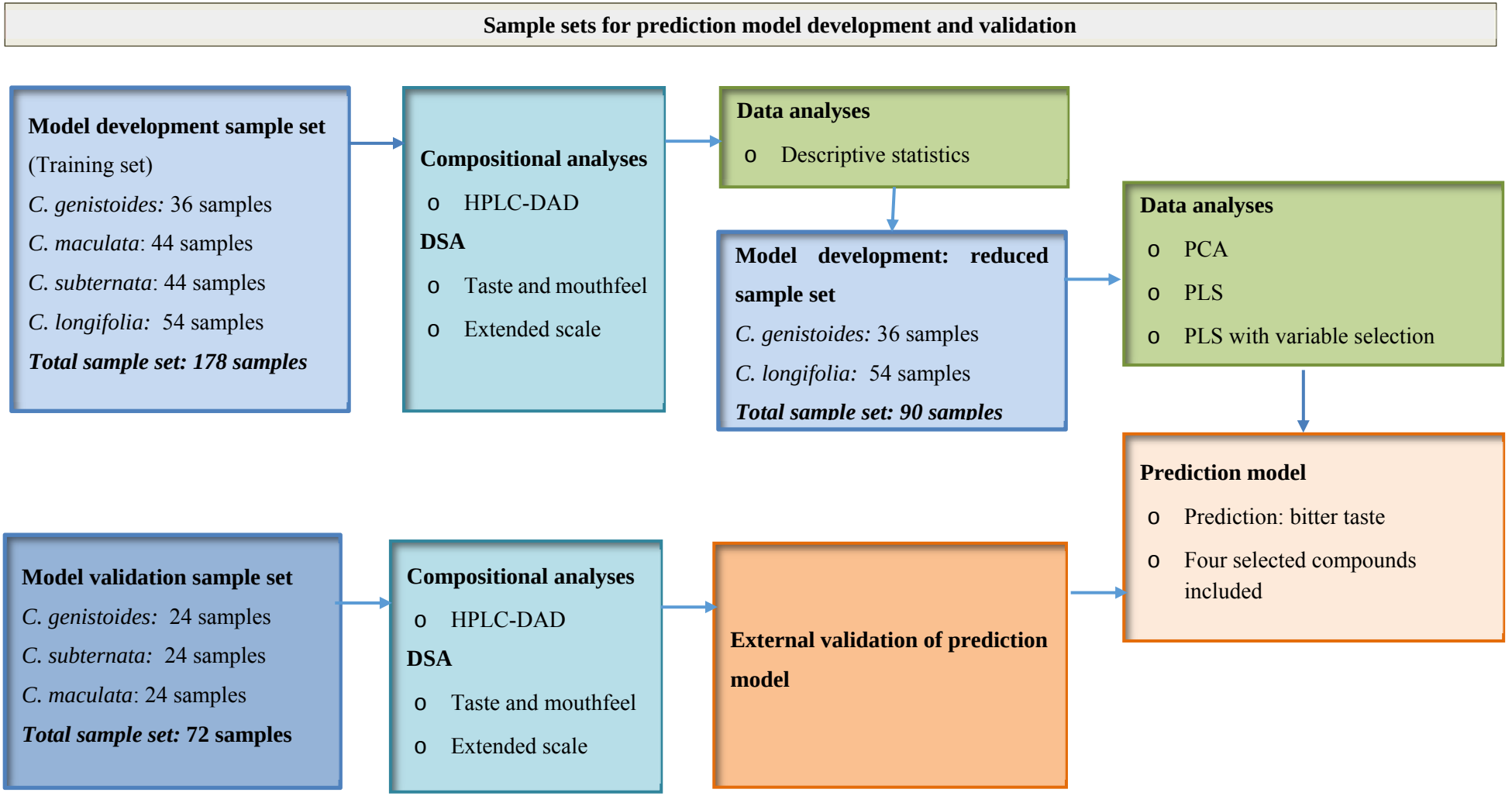


Fig. 1. Summary of sample sets, compositional and sensory data, data analyses and outcome of model development and validation for *Cyclopia* species. DSA refer to descriptive sensory analysis, HPLC-DAD to high-performance liquid chromatography–diode array detection, PCA to principal component analysis and PLS to partial least squares regression analysis.

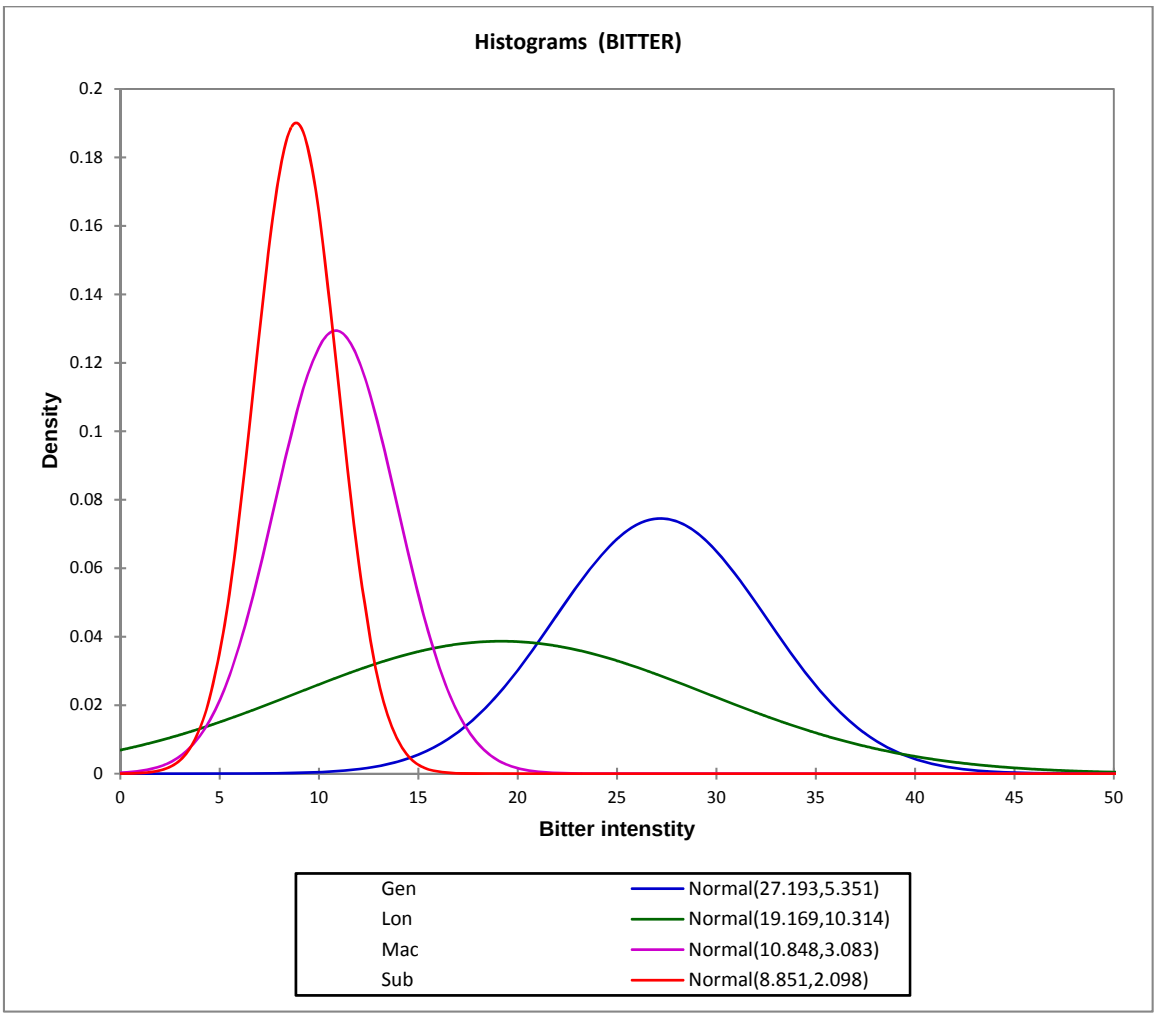


Fig. 2. Normal distribution of bitterness for four *Cyclopia* species. Gen, Lon, Mac and Sub refer to *C. genistoides*, *C. longifolia*, *C. maculata* and *C. subternata* respectively.

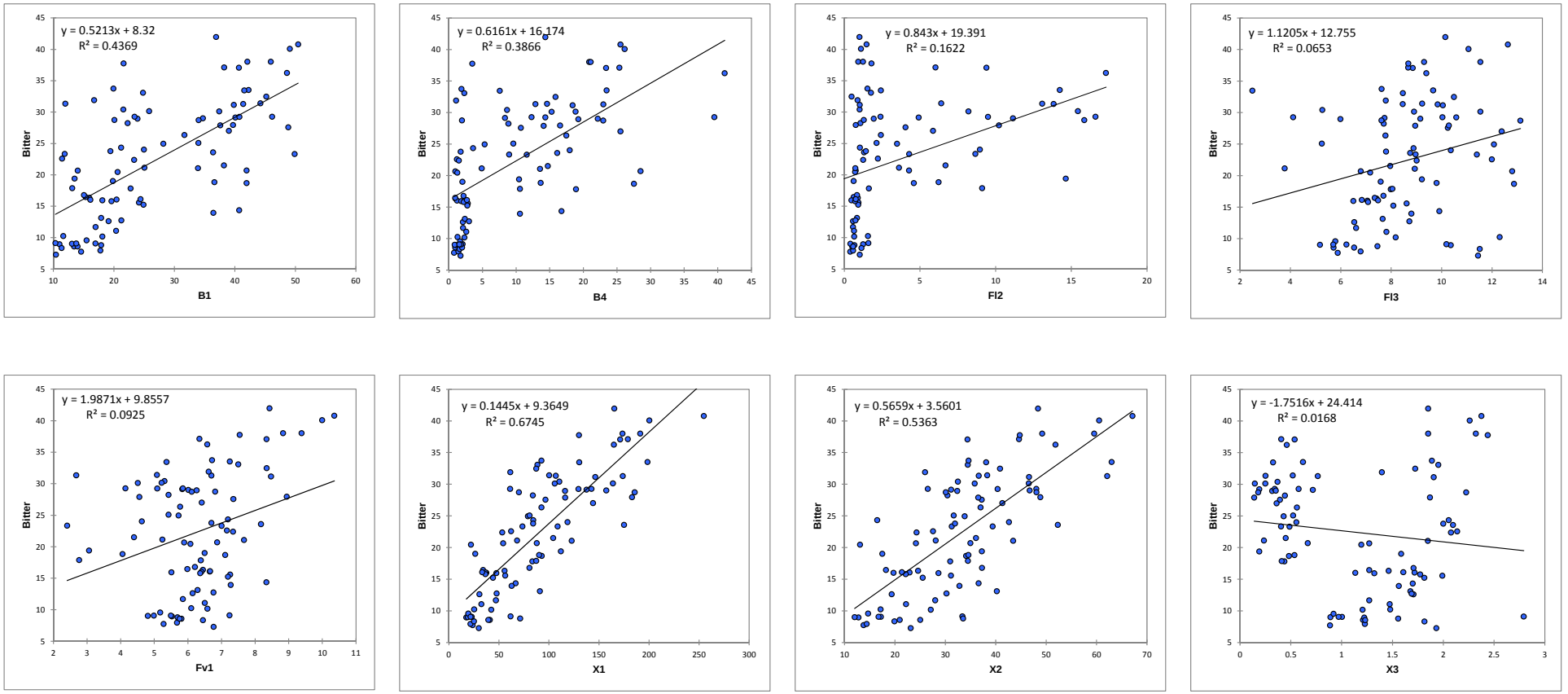


Fig. 3. Scatter plots displaying the relationship between bitterness associated with *C. genistoides* and *C. longifolia* and individual phenolic compounds. The notations for the phenolic compounds are explained in Table 1.

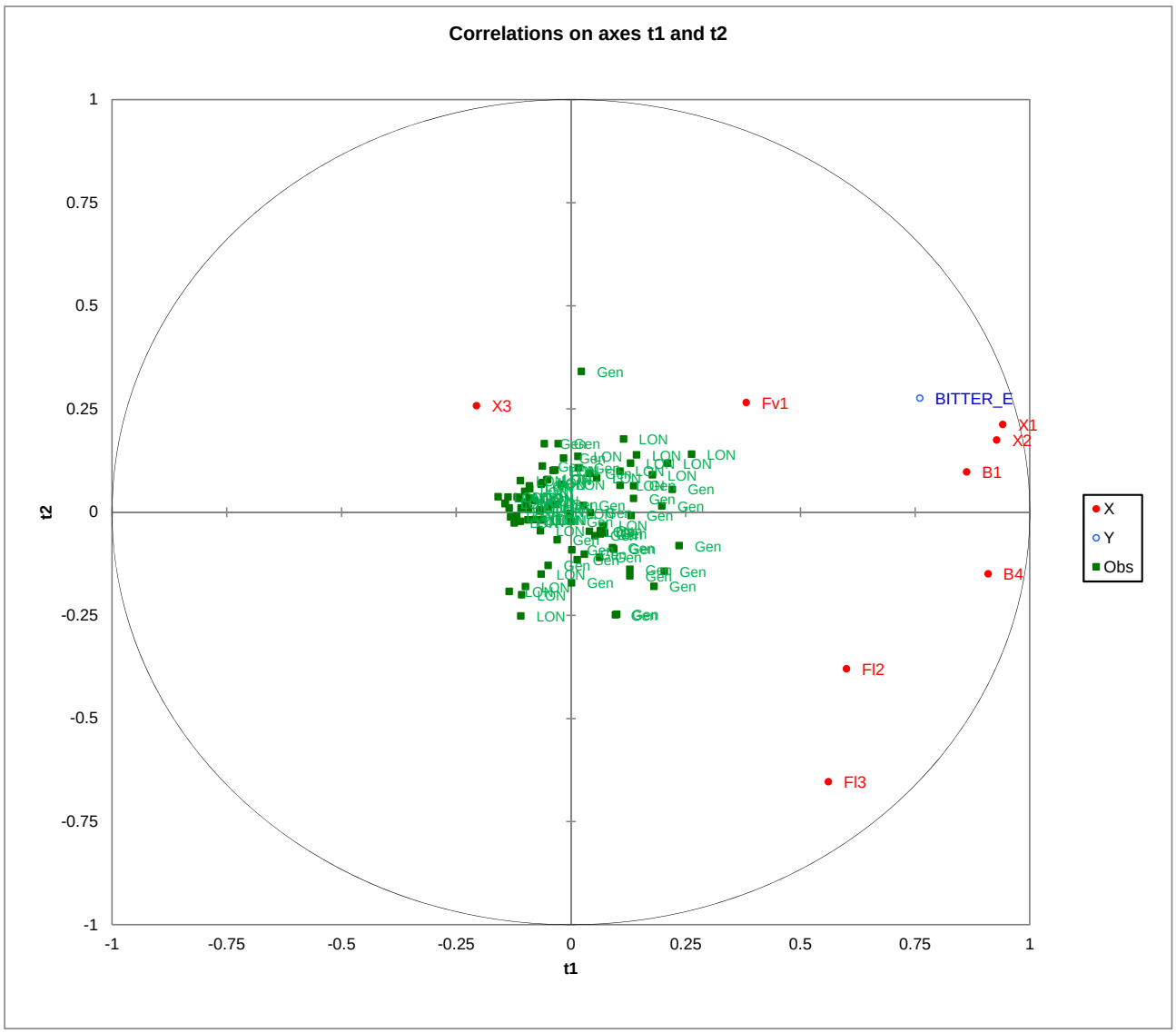


Fig. 4. PLS regression analysis plot on training data for *C. genistoides* and *C. longifolia* displaying the relationship between compositional parameters and bitter taste. The notations for the phenolic compounds are explained in Table 1. Gen and Lon refer to *C. genistoides and C. longifolia* respectively.

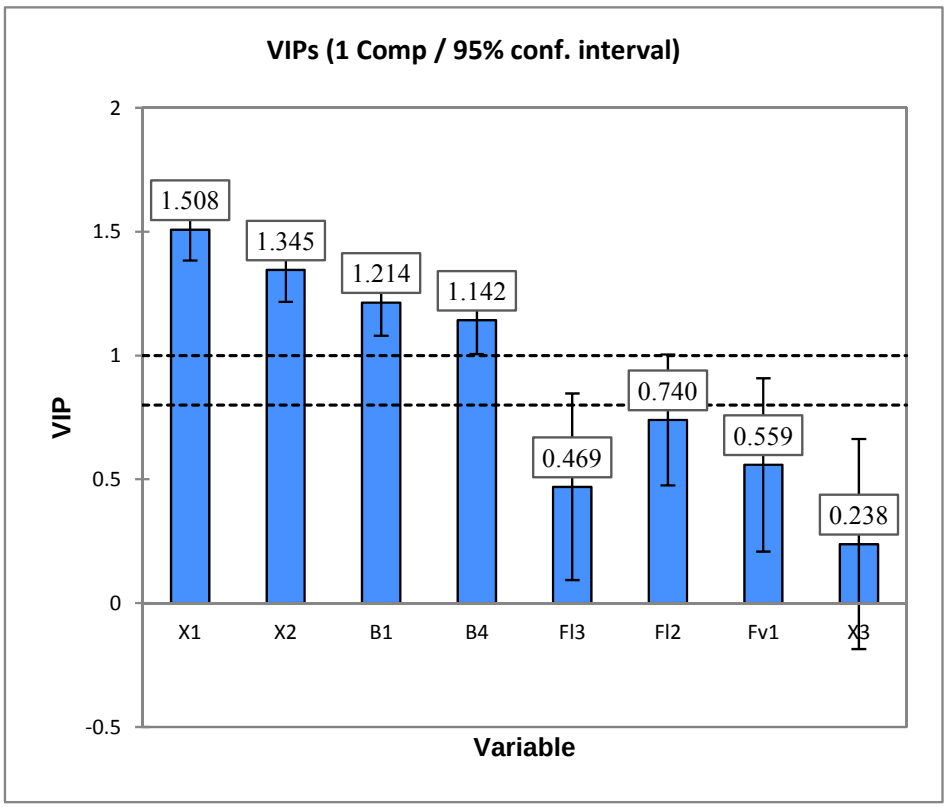


Fig. 5. Bar chart representing the VIP values on component 1 for the compositional variables (phenolic compounds) included in PLS regression analysis. VIP denotes variable importance in the projection, PLS denotes partial least squares regression regression. The notations for the phenolic compounds are explained in Table 1.

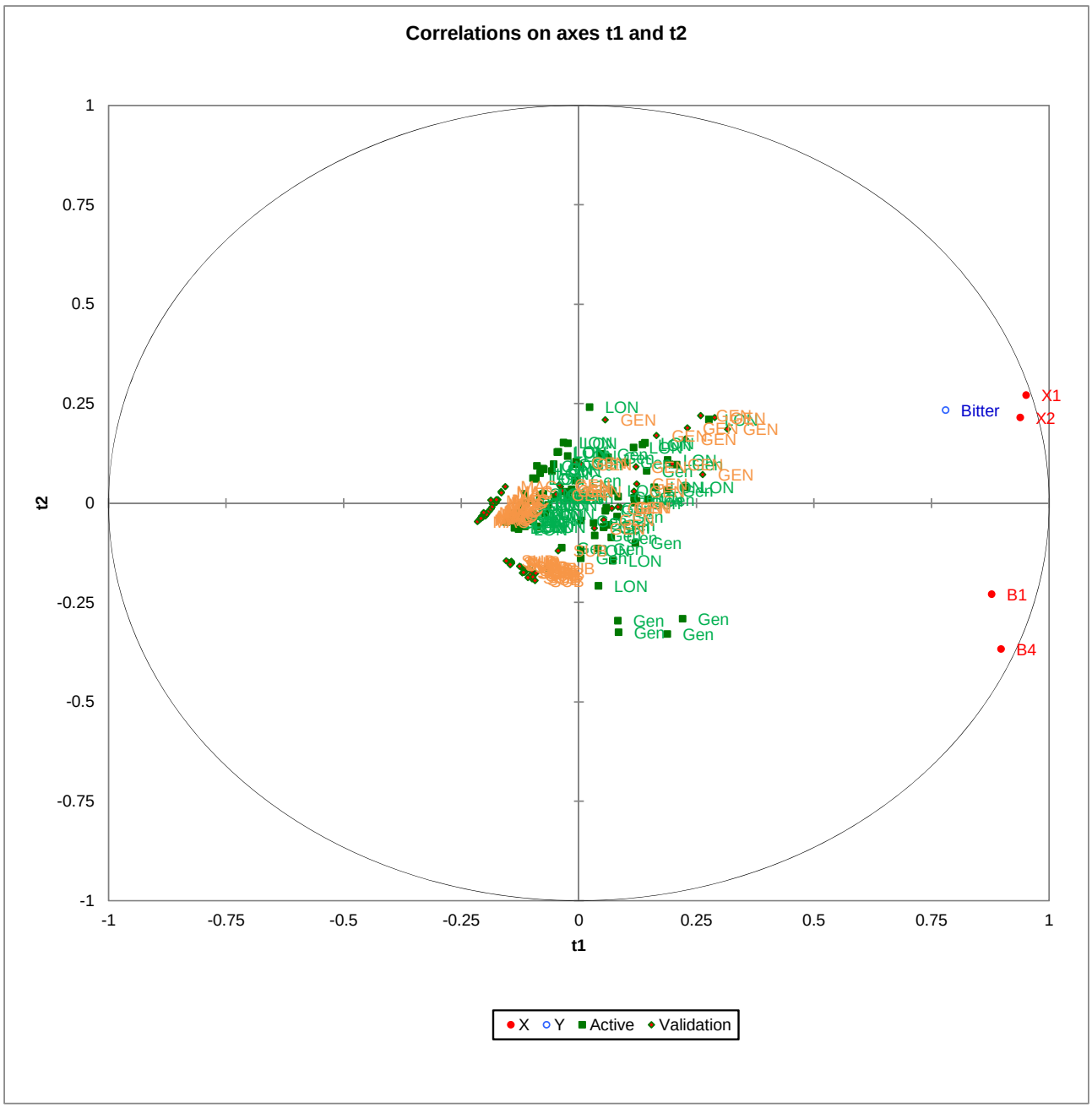


Fig. 6. PLS regression analysis for the final model on training data for *C. genistoides* and *C. longifolia* displaying the relation between selected compositional parameters (VIP >1) and bitter taste. The validation data are projected on the plot. The notations for the phenolic compounds are explained in Table 1. Gen and Lon refer *C. genistoides and C. longifolia* respectively.

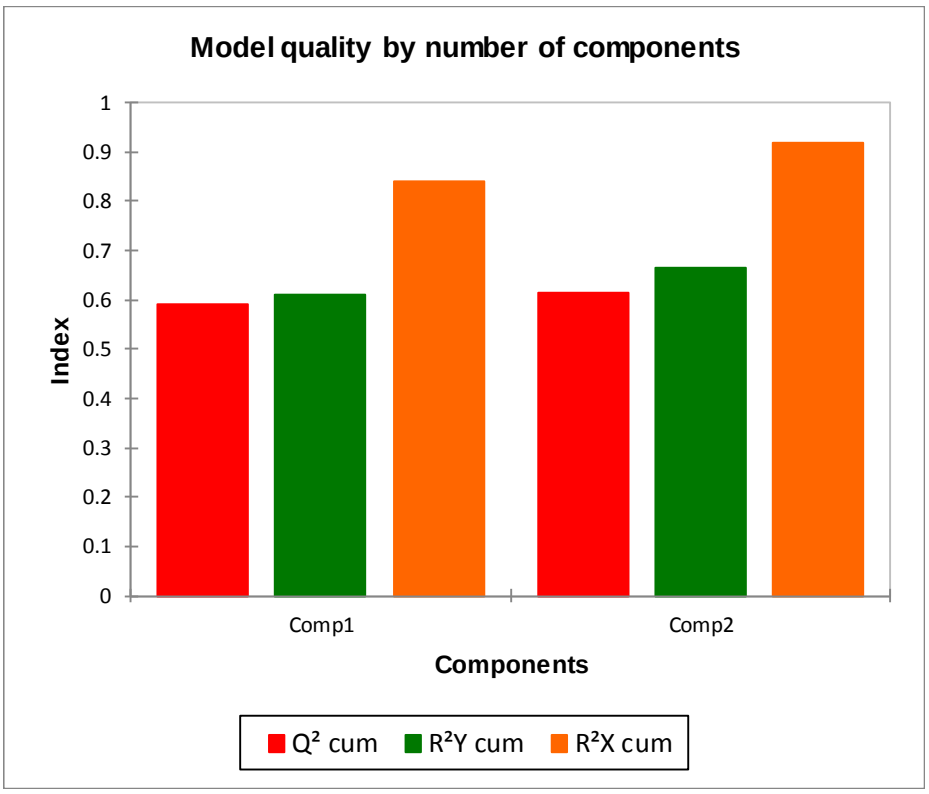


Fig. 7. Bar chart representing the model quality indexes based on the contribution of the first two components to the predictive quality of the model. Based on the first two components, the Q^2cum index denotes the global contribution, the R^2Ycum index the contribution of the dependent variables and the R^2Xcum the contribution of the independent variables on the predictive quality of the model.

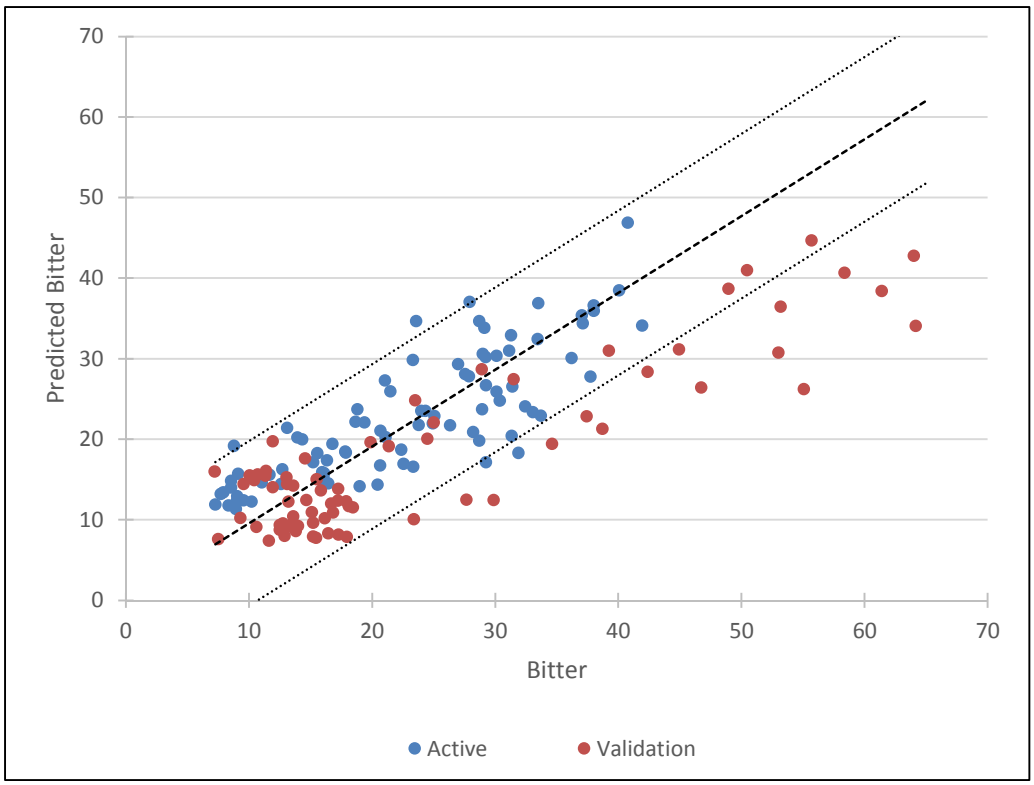


Fig. 8. Scatter plot of the predicted values for bitterness with 95% confidence intervals from PLS regression analysis against observed values for the training set and the validation set.

ADDENDUM A

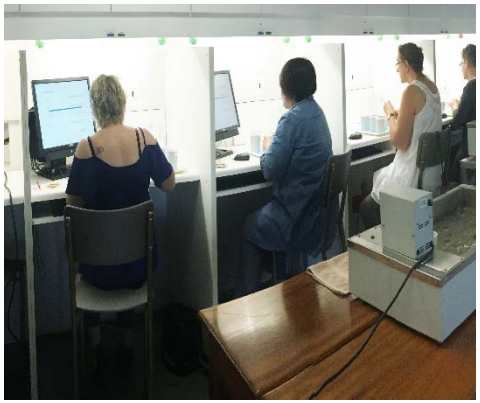
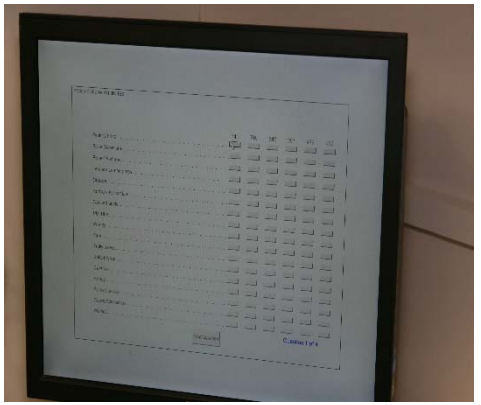
Flow diagram illustrating the preparation of honeybush infusions for descriptive sensory analysis

a) b) c) d)



e) f) g) h)



a) Tea (12.5 g), infused in 1 L freshly boiled distilled water for 5 min before straining into preheated stainless steel flasks b) Infusions poured into pre-heated porcelain mugs c) Mugs coded with three-digit random code d) Mugs covered with plastic lids to prevent loss of volatiles e) Mugs presented in random order per assessor in scientific water bath at 65°C to maintain temperature f) Descriptive sensory analysis of infusions using Compusense 5 software g) Samples served in waterbath per assessor h) Score sheet for evaluating full sensory profile of honeybush infusions

Chapter 4

Addressing intrinsic bitterness of *Cyclopia genistoides* by blending with *C. subternata* and other *Cyclopia* species

Abstract

Production of honeybush lags behind demand, forcing processors and tea merchants to use blends of *Cyclopia* species to supply in the increasing demand. One of the species, *C. genistoides*, is favoured for cultivation, but some batches are associated with a distinct bitter taste contrary to the characteristic sweet-like taste associated with honeybush tea. Descriptive sensory analysis was used to evaluate hot water infusions of blends of *C. genistoides* with *C. subternata*, *C. maculata* and *C. intermedia* respectively, focussing only on taste modalities and astringency using an extended scale. The results indicated that bitterness could be reduced to below perceptible levels by blending *C. genistoides* with any of *C. subternata, C. maculata* or *C. intermedia* in a ratio of 2:3. In addition to assessing the effect of blending on taste modalities, the full sensory profile of infusions of *C. genistoides*-*C. subternata* blends were quantified. The sensory profile of the *C. genistoides*-*C. subternata* infusion blended in a ratio of 2:3 was described by the prominent aroma attributes, "fynbos floral", "apricot", "woody", "fruity sweet" and "fynbos sweet", and a sweet taste. Consumers' sensory description of infusions of *C. genistoides*-*C. subternata* blends were determined using check-all-that-apply (CATA) questions. A group of 105 consumers completed CATA questions and a hedonic rating of five *C. genistoides*-*C. subternata* blends, varying from 100% *C. genistoides* to 100% *C. subternata*. Consumers showed an equal degree of liking for the infusions of the different *C. genistoides*-*C. subternata* blends, indicating that consumers did not find the levels of bitterness associated with the current sample set unacceptable. Furthermore, consumers were unable to differentiate between infusions with only subtle perceptual differences when applying CATA questions.

Keywords: Descriptive sensory analysis, Check-all-that-apply, *Cyclopia* species, Blending, Consumer preference, Bitterness

1. Introduction

The demand for herbal tea has grown substantially over the past two decades, primarily driven by a more concerted marketing effort, but also by consumer awareness of the health benefits associated with this beverage. The global increase in consumer demand for herbal tea resulted in an annual growth rate in consumption; a 10% growth rate for green tea and 4.4% for herbal and fruit tea has been reported (Insight Survey, 2016). Several *Cyclopia* species, endemic to the fynbos biome of South Africa, are used for the production of honeybush tea. Honeybush made the transition from a product being consumed regionally to a

product currently marketed worldwide. Three honeybush species provide the bulk of the production: *C. genistoides* and *C. subternata* are both produced commercially, while *C. intermedia* is mainly harvested from the wild (Joubert, Joubert, Bester, De Beer, & De Lange, 2011). Two *Cyclopia* species, *C. maculata* and *C. longifolia* are under development, whereas small quantities of *C. sessiliflora* and *C. plicata* are currently also wild harvested (E. Joubert, personal communication, October 20, 2017). As production lags behind demand, tea processors are forced to blend the different species to supply a well-rounded commercial product for the growing local and international markets.

Blending of wines to create a product with a characteristic sensory profile is common practice. Blending of wines of different cultivars may result in an altered aroma description, furthermore, the variation in phenolic composition of different cultivars may also influence the taste and mouthfeel character of blended wines (Cáceres-Mella et al., 2014). In the tea industry, blending of plant material of different origins, grades and quality to obtain a product of consistent quality, is standard practice (Joliffe, 2003; Liang, Lu, Zhang, Wu, & Wu, 2003). Expert buyers or tea blenders judge the appearance and aroma of the dried plant material, as well as the colour, aroma and flavour characteristics of the infusion of the final blend.

One of the *Cyclopia* species, *C. genistoides,* is favoured for cultivation and often used in blending, however, this *Cyclopia* species is often associated with a bitter taste that could have a negative impact on consumer acceptability. The chemical composition of several *Cyclopia* species have been quantified using high performance liquid chromatography (HPLC). In a study on the polyphenol content of several *Cyclopia* species, Schulze et al. (2015) demonstrated that C. *genistoides* contained the highest content of the xanthones, mangiferin and isomangiferin, while *C. subternata* contained the lowest levels of these compounds. These results were confirmed by our research (Chapter 3). As reported in Chapter 3, the prediction model revealed that the xanthones, mangiferin and isomangiferin, were highly correlated to sensory bitterness and were thus identified as the major predictors of sensory bitterness. Mangiferin is regarded as a major bioactive constituent of honeybush and high levels of this compound is deemed desirable in terms of the nutraceutical value of this herbal tea. Optimum levels of inclusion of *C. genistoides* in blends of different *Cyclopia* species is therefore obvious. The challenge is thus to maximise mangiferin content by optimum levels of inclusion of *C. genistoides*, whilst reducing bitterness below perceptible levels.

Detailed sensory profiling of the different *Cyclopia* species has been undertaken (Bergh, Muller, Van der Rijst, & Joubert, 2017; Erasmus, Theron, Muller, Van der Rijst, & Joubert, 2017; Theron et al., 2014). As limited research on consumers' perception of honeybush has been conducted, consumers' opinion on the acceptability and sensory profile of honeybush tea would afford valuable information for this growing agro-processing industry. In recent years, check-all-that-apply (CATA) questions have gained popularity as sensory technique to gain insight on consumers' perception of products (Meyners & Castura, 2014). CATA questions entail that assessors are provided with products and CATA questions comprising of a list of descriptors and/or phrases. Participants taste each product and then select all the descriptors perceived to be applicable to the product in question. The list of descriptors could include sensory descriptors, hedonic responses, emotional responses, purchase intent, occasion of use or ideas on the ideal product (Ares, Barreiro, Deliza, Giménez, &

Gámbaro, 2010; Meyners & Castura, 2014; Parente, Manzoni, & Ares, 2011). The list of descriptors need to be pre-determined, either by using consumers and the focus group technique or by using previous elicited terms by trained panels when evaluating the same product category (Dooley, Lee, & Meullenet, 2010). CATA questions can also be used successfully in conjunction with the 9-point hedonic scale to determine overall liking (Ares & Jaeger, 2013), in which case 100 - 120 consumers would be sufficient to provide stable results (Ares, Tárrega, Izquierdo, & Jaeger, 2014). CATA questions are regarded as an easy, straightforward task for consumers to complete (Ares, Deliza, Barreiro, Giménez, & Gámbaro, 2010) and provide rapid results. Consumers' CATA responses and trained panel sensory data resulted in similar sensory characterisations of products, indicating agreement between these two methods (Ares, Barreiro, et al., 2010; Dooley et al., 2010; Jaeger et al., 2013).

The aim of the current study was to determine the effect of blending *C. genistoides* with *C. subternata*, *C. maculata* and *C. intermedia* respectively, on bitterness perception of prepared infusions. Also of interest was to determine the effect of blending on the sensory profile of infusions of *C. genistoides*-*C. subternata* blends. As limited research on consumers' perception of honeybush infusions is available, consumers' degree of liking of honeybush infusions of different *C. genistoides*-*C. subternata* blends were assessed. Lastly, the efficacy of consumers' CATA response for the sensory characterisation of *C. genistoides*-*C. subternata* blends were evaluated.

2. Materials and methods

The effect of blending different *Cyclopia* species on taste perception was addressed in a series of experiments. Firstly, the taste perception of blends was determined of *C. genistoides* with *C. subternata*, *C. maculata* and *C. intermedia,* respectively (Experiment 1), with the focus on various blend ratios. Secondly, the taste perception of an independent sample set comprising of six *C. genistoides*-*C. subternata* blends were determined (Experiment 2), employing blend ratios established in experiment 1. The aim of the third experiment was to determine the full sensory profile (aroma, flavour and taste) of *C. genistoides*-*C. subternata* blends (Experiment 3). These tests were all conducted using an existing, trained sensory panel. Lastly, consumers' perception of *C. genistoides*-*C. subternata* blends were determined using CATA questions and the hedonic scale (Experiment 4).

2.1 Descriptive sensory analysis

For the development of aroma and flavour wheels, illustrating the sensory profiles of different *Cyclopia* species, a trained panel rated the intensities of aroma, flavour and taste attributes on a standard unstructured 100-point line scale (Erasmus, 2015). As the aroma attributes were perceived to be more prominent than the flavour and taste attributes, a condensed part of the scale was used to indicate perceived intensities of the taste and mouthfeel attributes. This led to the use of the *extended scale,* as discussed in Chapter 3, where three taste modalities (sweet, sour and bitter) and astringency (mouthfeel attribute) were measured using the entire "space" of the scale. The main aim of the current research was to test for the basic taste modality, bitterness.

When using an extended scale for testing a limited number of attributes of reasonably similar attribute intensity, better differentiation between samples on these attributes, including bitterness, would be possible. The *extended scale* was therefore used when the aim was to determine bitterness perception of blending ratios, while the *standard scale* was used for the full sensory profiling of the blends.

2.1.1 Sample selection

2.1.1.1 Blending C. genistoides with selected Cyclopia species

As described in Chapter 5, descriptive sensory analysis (DSA) was conducted on a randomly selected set of samples (n = 36) representing different production batches of five *Cyclopia* species namely *C. genistoides*, (n = 7), *C. subternata* (n = 9), *C. maculata* (n = 6), *C. intermedia* (n = 8) and *C. longifolia* (n = 6) of harvest year 2014/2015. Based on the principal component analysis (PCA) bi-plot of the DSA results (presented in Chapter 5), three samples of each of the following species were selected for inclusion in the blending experiment: *C. genistoides*, *C. subternata*, *C. maculata* and *C. intermedia.* A composite sample was prepared by blending three samples per species for *C. subternata*, *C. intermedia* and *C. maculata.* The respective *C. genistoides* batches were treated as separate samples. Six blend ratios were prepared, representing 100% *C. genistoides* to 100% of the respective *Cyclopia* species. Each of the three *C. genistoides* samples were therefore blended at 6 blend ratios with the composite samples of *C. subternata*, *C. intermedia* and *C. maculata* respectively, resulting in a total of 54 blends. Three replications of the 54 samples were subjected to DSA, testing three taste modalities (sweet, sour, bitter) and astringency on the extended scale. In Chapter 3 we illustrated that *C. longifolia* also contributes to bitter taste, hence the exclusion of this species in the blending experiment.

2.1.1.2 Blending C. genistoides and C. subternata

The taste profile of 198 independent samples representing five *Cyclopia* species, namely *C. genistoides* (n = 44), *C. subternata* (n = 44), *C. maculata* (n = 44), *C. longifolia* (n = 54) and *C. intermedia* (n = 12) was determined using DSA and the extended scale. For the study on *C. genistoides*-*C. subternata* blend ratios, the latter DSA results were used to classify *C. genistoides* samples as high (mean bitterness ≥25) or low (mean bitterness ≤20) in bitterness. Based on this classification, ten *C. genistoides* samples were selected, 5 for high bitterness and 5 for low bitterness. A composite sample of *C. subternata* was prepared by selecting and blending sixteen *C. subternata* samples with minimal sensory taints. Plant material of the selected *C. genistoides* and composite *C. subternata* sample were blended in six blend ratios of *C. genistoides*-*C. subternata*, varying from 100% *C. genistoides* to 100% *C. subternata*, resulting in a total of 60 samples. Three replications of the 60 samples were subjected to DSA using a trained panel and testing three taste modalities (sweet, sour, bitter) and astringency as mouthfeel attribute on the extended scale. The *Cyclopia* species, and representing sample codes are presented in Table 1.

2.1.1.3 Sensory profile of C. genistoides-C. subternata blends

Five independent batches of *C. genistoides*, representing different levels of bitterness, were selected from the sample set described in 3.1.1.1 and 3.1.1.2, to blend with a composite sample of *C. subternata*. Six blend

ratios were again prepared, representing 100% *C. genistoides* to 100% *C. subternata,* with varying levels of inclusion of *C. genistoides,* resulting in a total of 30 samples. The attributes and references generated during the development of the revised generic honeybush sensory wheel were used as the basis for training of the panel (Erasmus, 2015). Three replications of the 30 samples were subjected to DSA, testing 22 aroma, 17 flavour and 4 taste and mouthfeel attributes (Addendum A) using the standard unstructured line scale to determine the full sensory profile of the samples.

2.1.2 Sample preparation

Samples were prepared by blending plant material of *C. genistoides* with the respective composite samples of *Cyclopia* species to obtain inclusion levels of *C. genistoides* at 100%, 80%, 60%, 40%, 20% and 0% respectively. The total leaf mass per blended sample was 12.5g. Infusions were prepared by pouring 1000 g freshly boiled distilled water onto 12.5 g of the blended plant material and infused for 5 min. Each infusion was strained through a fine-mesh strainer directly into a 1 L pre-heated stainless steel thermos flask (Woolworths, Bellville, South Africa). The infusions were served in white porcelain mugs, each mug was coded with a 3-digit random code. The mugs were pre-heated in an industrial oven (Hobart, France) at 70°C before aliquots of each infusion (ca. 100 mL) were poured into the mugs and covered with plastic lids. The mugs were arranged in a complete random order per assessor as generated by the Compusense® five software program (Compusense version 5.6, Guelph, Canada) where all assessors evaluated all the samples. The samples were served in temperature-controlled (65°C) water baths (Scientific Manufacturing Company, Cape Town, South Africa). Refer to Addendum A in Chapter 3 for a visual representation of the process employed for sample preparation and sensory analysis of honeybush infusions.

2.1.3 Sensory panel

An existing sensory panel (n = 9, all female between the ages of 40 and 65) participated in the DSA to determine the taste profile of the respective *Cyclopia* samples. The panellists have several years of experience in the sensory analysis of infusions prepared from rooibos (Jolley, Van der Rijst, Joubert, & Muller, 2016; Koch, Muller, Joubert, Van der Rijst, & Næs, 2012) and honeybush herbal tea (Erasmus et al., 2017; Theron et al., 2014). Panel members completed an official consent form before commencing with the DSA. The generic DSA technique, as described by Lawless and Heymann (2010), was used as basis for training. Assessors were screened for bitterness sensitivity using solutions with three concentrations of caffeine (0.07%, 0.14% and 0.035%). All panel members correctly completed the ranking test and were included in the final panel.

The effect of blending *C. genistoides* with *C. subternata*, *C. maculata* and *C. intermedia* on bitterness perception were determined by evaluating three taste attributes (sweet, sour and bitter) and astringency on the extended scale. For the full sensory profiling of *C. genistoides*-*C. subternata* blends, the standard scale was used for evaluating all attributes. For both the extended scale and standard scale, attribute intensities were rated on an unstructured line scale (0 – 100), using the Compusense® five software program (Compusense version 5.6, Guelph, Canada) with six samples presented per session. The experimental design for the blending

experiments were a completely random design. Assessors completed three sessions per day with a rest period of 15 min between each test session. Unsalted water biscuits (Woolworths, Stellenbosch, South Africa) and still natural spring water (Woolworths, Stellenbosch, South Africa) were used as palate cleansers between each sample. Assessors were seated at individual booths in a temperature- (21ºC) and light-controlled room.

2.2 Consumer perception

2.2.1 Sample selection and preparation

Based on the results of the DSA as described in section 3.1.1, one sample representing *C. genistoides* and three samples representing *C. subternata* were selected for inclusion in the consumer test. The three selected *C. subternata* samples were blended to obtain one composite sample. Five blend ratios of *C. genistoides*-*C. subternata* were prepared, including *C. genistoides* at levels of 100%, 60%, 40%, 20% and 0%, resulting in 5 samples presented to each consumer. Only five *C. genistoides*-*C. subternata* blends were included in the consumer test to reduce consumers' sensory fatigue. Blend ratios for *C. genistoides*-*C. subternata* and corresponding sample codes are presented in Table 1. The total leaf mass per blended sample was 12.5g. The infusions were prepared in the same manner as for DSA, described in section 2.1.2.

The infusions, five per consumer, were served in white porcelain mugs, each coded with a 3-digit random code. The mugs were pre-heated in an industrial oven (Hobart, France) at 70°C before aliquots of each infusion (ca. 100 mL) were poured into the mugs and covered with plastic lids. The mugs were arranged in a random order per assessor as generated by Compusense-at-hand® (Compusense, Guelph, Canada). Samples were presented in a sequential monadic order, therefore a full crossover test where all consumers evaluated all the products. The experimental design was a completely random design. The serving temperature was maintained by placing the samples in temperature controlled (65°C) water baths (Scientific Manufacturing Company, Cape Town, South Africa).

2.2.2 Consumers

Consumers, 105 in total, were recruited based on their consumption of herbal tea, as well as their interest and availability to participate. Qualification criteria included adults over 18 years of age and regular users of herbal tea (at least once per week). Participants gave informed consent and received a small gift after completion of the test. Nine sessions were scheduled for conducting the CATA questions as the sensory facility can accommodate 14 consumers at a time and to allow for preparation and temperature control of the infusions. A short explanation of the method was presented at the start of each session. Unsalted water biscuits (Woolworths, Stellenbosch, South Africa) and still natural spring water (Woolworths, Stellenbosch, South Africa) were available to use as palate cleansers. All the consumers completed the CATA questions within 30 min.

2.2.3 Hedonic test and CATA questions

Consumers were asked to taste each sample and rate their overall degree of liking using a nine-point hedonic scale where 1 = "dislike extremely" and 9 = "like extremely". Thereafter consumers had to answer

the CATA questions which consisted of 25 terms (Table 2). Consumers were asked to check all the terms that they considered appropriate to describe each of the samples. The list was compiled based on results of a focus group session, previous research on honeybush infusions (Erasmus et al., 2017; Theron et al., 2014) and consumer research conducted by the Bureau for Food and Agricultural policy (Vermeulen, 2015). The terms included in the CATA questions were grouped into four categories: aroma attributes (8), taste attributes (5), consumer descriptors (5) and tea strength descriptors (7). The categories of terms were presented to consumers in a fixed order while terms within a category were presented in a randomised order, as suggested by Ares and Jaeger (2013). It is further suggested that sensory attributes be listed in the order that they would be perceived. Consumers were asked to first complete the hedonic test and thereafter the CATA questions. Data were collected using Compusense-at-hand® (Compusense, Guelph, Canada) at a central location (Sensory research laboratory, Department of Food Science, Stellenbosch University) where consumers were seated at individual booths in a temperature- (21ºC) and light-controlled room.

2.3 Statistical procedures

2.3.1 Descriptive sensory analysis

DSA data were subjected to PanelCheck Software (Version 1.3.2, http://www.panelcheck.com/) to monitor panel performance. Pre-processing of DSA data were performed to test for panel reliability by means of a model that includes assessor, replication and sample effects and interactions (Naes, Brockhoff, & Tomic, 2010). The Shapiro-Wilk test was performed to test for non-normality of residuals (Shapiro & Wilk, 1965). If significant non-normality was detected ($p \leq 0.05$), outliers were removed when the standardised residuals for an observation deviated more than three standard deviations from the model value. Following confirmation of panel reliability and normality of data, statistical analyses were performed on means over triplicate infusions and assessors of DSA data. The data were subjected to analysis of variance (ANOVA) according to the experimental design to test for treatment differences. Treatments means were compared by calculating Fisher's LSD where a probability level of 5% was considered significant. Univariate analyses were performed using SAS® software (Statistical Analysis System 2006, Version 9.4, SAS Institute Inc., Cary, NC, USA). Principal component analysis (PCA), using the correlation matrix, was conducted using XLStat (Version 7.5.2, Addinsoft, New York, USA) to visualise and elucidate the relationships between the samples and the attributes (Næs, Brockhoff, & Tomic, 2010).

2.3.2 Hedonic test

The overall liking data were subjected to ANOVA where the samples were considered as fixed source of variation and the consumer as a random effect. Significant differences between samples were calculated using Tukey's test at a 95% confidence level.

2.3.3 CATA questions

Occurrence of descriptors were indicated per sample x assessor in rows. A value of 1 was allocated to a sample if the descriptor was used for that sample and a value of 0 if the descriptor was not used. The number

of descriptors selected by each consumer to describe each product were determined. The CATA counts were totalled for each product and descriptor and the resulting frequency table was used in subsequent analyses.

Data obtained with CATA were analysed using Cochran's Q test (Manoukian, 1986) to test for significant differences between samples for each of the descriptors included in the CATA question. Cochran's Q test is a non-parametric statistical test which is used in the analysis of two-way block designs to determine whether a number of treatments have identical effects when the response variable is binary. The test is applied to one attribute at a time. The frequency tables were further analysed using Correspondence Analysis (CA). A two-dimensional map representing samples and descriptors were obtained. Data analyses for the hedonic test and CATA questions were performed using XLStat (Version 7.5.2, Addinsoft, New York, USA) and Statistica, (Dell Inc. 2016, version 13).

3. Results and discussion

3.1 Descriptive sensory analysis

3.1.1 Blending *C. genistoides* with selected *Cyclopia* species

Three independent batches of *C. genistoides* samples were blended with composite samples of *C. subternata*, *C. maculata* and *C. intermedia,* respectively using six blend ratios. The ANOVA results, presented in Table 3, revealed no significant effect of *C. genistoides* batch. However, a significant effect ($p \leq 0.05$) of *Cyclopia* species and blend ratio on the three taste attributes, as well as astringency, was demonstrated.

The effect of *Cyclopia* species on the basic taste modalities and astringency when blending at different ratios with *C. genistoides*, is provided in Fig. 1. Bitterness increased with increased levels of *C. genistoides*, as illustrated in Fig. 1a. Considering the effect of species on bitterness, *C. subternata* demonstrated the highest ability to reduce the bitterness associated with *C. genistoides* (Fig. 1a), especially in the case of blends that included 20%, 40% and 60% *C. genistoides*. The results further indicated that blend ratios of 40:60 of *C. genistoides* with *C. subternata*, *C. maculata* or *C. intermedia,* respectively, resulted in bitter perception of between 20 and 25, as measured on the extended scale (Fig. 1a).

A significant effect ($p \leq 0.05$) of *Cyclopia* species and blend ratio on astringency was observed, evident when comparing blends of 100% *C. genistoides* to blends that included 60% or less *C. genistoides* (Table 4). Astringency increased with increasing levels of *C. genistoides* (Fig. 1b). Considering the effect of blending on sweetness, inclusion of *C. maculata* in blends with *C. genistoides* did not affect sweetness. However, sweetness decreased with increased levels of *C. genistoides* in blends with *C. intermedia* and *C. subternata* (Fig. 1c). When evaluating the effect of blending on sourness, blends that included *C. subternata* and *C. intermedia* were perceived to be less sour compared to blends with *C. maculata*, as illustrated in Fig. 1d.

Mean intensity values for the sensory attributes (scored on the extended scale) of six blend ratios of *C. genistoides* with three *Cyclopia* species (*C. subternata*, *C. maculata* and *C. intermedia*) are presented in Table

5. The data were pooled for *C. subternata*, *C. maculata* and *C. intermedia* to test the effect of blend ratio. Bitterness, sourness and astringency increased with increased levels of *C. genistoides* while sweetness decreased, as illustrated in Fig. 2. Increasing levels of *C. genistoides* had a more pronounced effect on bitterness than on sweetness, sourness and astringency. Intensities for sweetness ranged between 26.80 (0% *C. genistoides*) and 21.42 (100% *C. genistoides*), sourness between 29.86 and 33.43 and astringency between 37.09 and 41.21 for corresponding ratios. The range for bitterness, however, was 19.34 for samples with 0% *C genistoides* to 30.17 for samples with 100% *C. genistoides* (Table 5). To obtain maximum inclusion of *C. genistoides* without compromising on bitterness perception, an inclusion level of 40% *C. genistoides* in blends with *C. subternata*, *C. maculata* or *C. intermedia* is recommended.

In Chapter 3, we reported on the intensities for taste modalities of a large number of *C. genistoides* samples. According to the latter results, the bitterness for this species illustrated considerable variation and the question arose as to how bitterness level of *C. genistoides* samples (low or high) would influence taste perception of blends. The current study has shown that *C. subternata* has a distinct ability to reduce the bitterness associated with *C. genistoides*, more so than *C. maculata* and *C. intermedia*. Furthermore, the current study has demonstrated that a blend ratio of 40:60 of *C. genistoides* with selected *Cyclopia* species result in bitter perception just above the threshold level. A new set of samples were therefore selected to determine the effect of different bitterness levels of *C. genistoides* on taste perception of *C. genistoides*-*C. subternata* blends, and to substantiate the blend ratios employed in experiment 1.

3.1.2 Blending *C. genistoides* and *C. subternata*

The results of the DSA of 198 samples were used to classify *C. genistoides* samples according to bitterness perception as high (mean bitterness ≥25) or low (mean bitterness≤20). Based on this classification, ten *C. genistoides* samples were selected, 5 high and 5 low in bitterness (data not included). The ten *C. genistoides* samples were blended with a composite *C. subternata* sample according to the same blend ratios used in experiment 1. The ANOVA model, presented in Table 6, includes the classification of *C. genistoides* as high or low in bitterness as factor. A significant effect ($p \leq 0.05$) of *C. genistoides* class (low or high bitter) and blend ratio was observed for all four sensory attributes (Table 6). Furthermore, a significant interaction ($p \leq 0.05$) of *C. genistoides* class (low or high bitter) and blend ratio was revealed for all attributes except sweetness.

Table 7 summarises the ANOVA results for the three taste modalities (sweet, sour and bitter) and astringency with data pooled for *C. genistoides* class. The effect of blend ratio is illustrated in Fig. 3, indicating that bitterness, sourness and astringency increased with increased levels of *C. genistoides* while sweetness decreased. Significant differences in bitterness perception for all six *C. genistoides*-*C. subternata* blends were observed (Table 7). It is clear from the results presented in Fig. 4 and Table 7, that blend ratios of *C. genistoides:C. subternata* of 60:40 resulted in a bitterness perception of below 20. This blend ratio is, however, only applicable when blending *C. genistoides* with the sweet-associated *C. subternata*.

The effect of *C. genistoides* class (low or high bitterness) on the four sensory attributes tested for six blend ratios are presented in Figure 4 (a-d). Inclusion of *C. genistoides* at 60% resulted in bitter perception of >20 when blending with high bitter *C. genistoides* samples, in contrast, <20 bitter perception was obtained when blending with low bitter *C. genistoides* samples. *C. genistoides* samples with high bitter taste, had a significant effect on astringency when including 60% in *C. genistoides*-*C. subternata* blends, but the effect was not significant when including 40% *C. genistoides* in these blends. No significant effect of *C. genistoides* class (low or high bitterness) on sweetness was observed (Fig. 4c). Sourness were only effected by *C. genistoides* class (low or high bitter) when including 60% *C. genistoides* in blends.

No significant difference between the classes of *C. genistoides* (high and low bitter) was found at 40% inclusion of *C. genistoides* for all attributes tested (Fig. 4, a-d). These results indicate that the honeybush industry could use *C. genistoides*-*C. subternata* blend ratios of 40:60 in blending practices if no prior information on bitterness level of batches of plant material is available.

3.1.3 Full sensory profile of *C. genistoides*-*C. subternata* blends

The different *Cyclopia* species are characterised by species-specific sensory profiles (Erasmus, et al., 2017). One of the objectives of the current study was to quantify the full sensory profile of the *C. genistoides*-*C. subternata* blends, primarily to determine how blending affected the species-specific sensory profiles. Note that the standard profile scale was used to evaluate the full profile of the *C. genistoides*-*C. subternata* blends.

Sensory attributes with a mean intensity of ca. 5 and more on the 100-point scale were included as mean intensity scores in the principal component analysis (PCA), however, mean attribute intensities of lower than 5 were regarded as being barely perceptible and not included in the PCA. The PCA scores and loading plot (Fig. 5) displays the association between sensory attributes and samples (*C. genistoides*-*C. subternata* blends), as well as the positioning of the samples relative to each other. The first two principal components explained 55.36% of the variability in the data with 47.51% explained by principal component (PC) 1 and 7.84% by PC2. The main differentiation between samples is clear on PC1, differentiating samples on *C. genistoides*-*C. subternata* blend ratio. Samples of *C. genistoides*-*C. subternata* blends including 60%, 80% and 100% *C. genistoides*, were situated towards the left of the PCA bi-plot and were associated with the positive attributes of “apricot / apricot jam” and “rose geranium” and negative attributes of “burnt caramel” and “hay / dried grass”. These samples further associated with “fruity sweet” aroma, “woody” flavour and a bitter and sour taste. The *C. genistoides*-*C. subternata* blends that included a higher percentage of *C. subternata*, were scattered towards the positive side of PC1, associating with the positive aroma and flavour attributes “fynbos-floral”, “sweet spice / cassia”, “rose perfume” and “cooked apple” and the negative aroma and flavour described as “dusty”. These samples also associated with the positive aroma attributes described as “woody”, “fynbos-sweet”, “pine”, “walnut” and “caramel” and sweet taste.

In the current research, a *C. genistoides*:*C. subternata* blend ratio of 2:3 demonstrated to be effective in reducing bitterness to below perceptible levels (Fig. 1a). As previous research has indicated that the flavour attributes are usually perceived at lower intensities than the corresponding aroma attributes (Bergh et al., 2017), the average intensity values of the only aroma attributes and taste modalities and astringency for each *C.*

genistoides-C. subternata blend ratio will be displayed (Table 8) and discussed. The sensory profiles of four of the *C. genistoides:C. subternata* blends are illustrated in Fig. 6 (a-d). The *C. genistoides:C. subternata* sample with blend ratio of 2:3 (Fig. 6 a) was associated with a aromas of "fynbos-floral", "apricot / apricot jam", "woody", "fruity sweet" and "fynbos-sweet" and medium to low intensities of the taste modalities sweet, sour and astringent. The bitterness of this blend was <10 (as measured on the standard scale), indicating that bitterness was barely perceptible. The blend ratio representing 60:40 of *C. genistoides:C. subternata*, shows a similar sensory profile, only with higher "rose geranium" aroma intensities and a more bitter taste (Fig. 6 b).

The blend with 100% inclusion of *C. genistoides* (Fig. 6 c), was associated with high intensities of "fynbos-floral", "rose geranium", "apricot / apricot jam", "woody", "fruity sweet" and "fynbos-sweet" aroma, low sweetness and a sour and astringent taste. The bitterness of this blend was just above perceptible levels (bitterness >10 as measured on the standard scale). The sensory profile of samples representing a *C. genistoides:C. subternata* blend ratio of 0:100, is illustrated in Fig. 6 d. These samples were associated with high intensities of "fynbos-floral", "woody", "fruity sweet" and fynbos-sweet" aroma and a lower intensity of "apricot / apricot jam" aroma, a sweet taste and lower intensity of sourness and astringency. Bitterness in this sample was below perceptible levels (bitterness <10 as measured on the standard scale). Our results have thus shown that the recommended 40:60 blend ratio of *C. genistoides:C. subternata* result in a product with a sensory profile that is a combination of the complex sensory profile associated with *C. genistoides*, enhanced by the positive aroma attributes typical to *C. subternata*.

The sensory profiles of five *Cyclopia* species, including *C. genistoides* and *C. subternata,* were described in detail by Erasmus et al. (2017). The positive aroma attributes "fynbos-floral" and "fynbos-sweet" are common to all *Cyclopia* species, whereas species-specific attributes distinguish one species from another, primarily driven by differences in attribute intensities. The most prominent positive aroma attributes associated with *C. genistoides* were "fynbos-floral" and "fynbos-sweet", followed by "apricot / apricot jam" and "rose geranium" while the negative aroma attributes associated with this species, were "plant-like", "cooked vegetables" and "hay / dried grass". *Cyclopia subternata* were associated with an overall sweet and floral aroma including "apricot / apricot jam", "rose geranium" and "fruity-sweet" while this species also associated with the negative aroma attribute, "plant-like". Considering taste and mouthfeel attributes, the *Cyclopia* species were perceived to be equally sweet while a perceptible bitter taste was only identified in *C. genistoides* and in under-fermented *C. longifolia*. Considering the sensory profiles of the *C. genistoides-C. subternata* blends in the current study, it is clear that infusions of the blended products were also associated with "fynbos-floral" and "fynbos-sweet" aromas, identified as common to all *Cyclopia* species by Erasmus et al. (2017). In the set of blended samples, the positive aroma attributes of "fynbos-floral", "apricot / apricot jam", "woody", "fruity sweet" and "fynbos-sweet" aroma were enhanced by blending *C. genistoides* and *C. subternata*, while bitterness was reduced to below perceptible levels.

3.2 Consumer perception

Before making recommendations on optimum levels of *C. genistoides* inclusion in blends of *Cyclopia* species, it was necessary to determine consumers' perception of infusions prepared from blended samples.

Consumers' degree of liking of *C. genistoides*-*C. subternata* blends were determined using the nine-point hedonic scale. Check-all-that-apply (CATA) questions were further applied to determine consumers' perception on the sensory characteristics of the *C. genistoides*-*C. subternata* blends. Results of DSA of blends of *C. genistoides* with different *Cyclopia* species revealed that inclusion of *C. genistoides* at 40% or less, resulted in bitter perception between 20 and 25 as measured on the extended scale. Five *C. genistoides*-*C. subternata* blend ratios were therefore selected for the consumer tests namely 0%, 20%, 40%, 60% and 100% inclusion of *C. genistoides*.

3.2.1 Hedonic test

Consumers, 105 in total, completed the hedonic test and CATA questions. Participants were between 19 and 65 years old, 81% female and 19% male. Ninety three percent of consumers indicated that they consume herbal tea once a week or more.

According to the results as presented in Table 9, there were no significant differences ($p>0.05$) in the overall degree of liking of the five blends of honeybush infusions. The mean overall liking scores for all five blends were above 6, indicating that consumers liked the products. According to Muñoz, Civille, and Carr (1992), an overall liking score of above 6 when using the nine-point hedonic scale, indicates that the product has a reasonable chance of commercial success when launched.

Since the trained panel found significant differences in bitterness as a result of *C. genistoides* level (Table 5 and 7), it was expected that consumers would indicate a higher degree of liking for samples with a lower ratio of *C. genistoides* and therefore a less bitter taste. Hedonic assessment is done on the holistic sensory perception of the product and degree of liking are not tested per attribute. This group of consumers found the five blends equally acceptable when evaluating the holistic sensory perception. Consumers were recruited on the basis that they are regular users of herbal tea, including green tea. This group of consumers might not have found these samples unacceptably bitter, especially given the fact that they were regular consumers of green tea, a product which is usually associated with a bitter taste.

3.2.2 CATA questions

Consumers checked between 4 and 16 terms to describe each infusion of the *C. genistoides*-*C. subternata* blends. The average number of CATA terms checked were 8, with no significant difference ($p>0.05$) in the average number of terms checked per product (Table 9). These results corresponds to that of the hedonic test where no significant preference for one of the blends was found. Consumers showed an equal degree of liking for the five products and used the same number of terms to describe the five products.

The frequency in which each of the CATA terms were used to describe each of the blended infusions are presented in Table 10. No significant difference ($p>0.05$) was found in the frequency of terms used to describe the infusions of blended samples except for the term "not sweet". Consumers checked the term "not sweet" significantly more for the blend with 40% *C. genistoides* compared to the blend with 0% *C. genistoides*. These results indicate that consumers perceived the products to be similar and that they could not differentiate between the samples by applying CATA questions using this set of terms.

Considering the different categories of terms used in the CATA questions, significant differences ($p<0.0001$) between the average number of terms used per category were found (Table 11). Consumers used the terms related to the aroma of the infusions significantly more than those in the other categories of the CATA questions. Terms in the taste category were used less frequently, suggesting that consumers found these terms to be less relevant when describing the samples. Bitter was one of the terms in the taste category and considering that this category was used infrequently, one could conclude that consumers did not regard the bitterness of the samples as important. As results for the Cochran Q test indicated no significant difference in the frequency of use of terms to describe the products, no further analyses are reported.

Significant differences in bitterness of different blend ratios of *C. genistoides*-*C. subternata* were demonstrated using DSA and a trained panel (Table 4). Consumers, however, indicated an equal degree of liking for the different blend ratios. One could postulate that this group of consumers did not perceive differences in bitterness, nor did they find the level of bitterness in this herbal tea too high and therefore unacceptable. Furthermore, consumers used the same terms to describe the different blend ratios, indicating that they could not differentiate between the samples using CATA questions and perceived the samples to be fairly similar in sensory profiles. Differences in the sensory profiles of different blend ratios of *C. genistoides*-*C. subternata* were demonstrated using DSA and a trained panel (Table 8), however, these differences were subtle.

Various studies have indicated similar product characterisation when comparing consumer-generated CATA results and trained assessor results, indicating CATA to be a valid tool for consumer-based product characterisation (Ares, Deliza, et al., 2010; Dooley et al., 2010; Jaeger et al., 2013), however, in the latter studies it was clear that analysed products differed considerably in terms of sensory profiles. The validity of CATA questions for the profiling of complex (as opposed to non-complex) products and products with only subtle differences were also addressed by Ares et al. (2015). CATA questions with consumers might not be the ideal technique to use when the aim of the study is to detect small differences between products. These researchers found the discriminative ability of DSA with trained panels to be higher for complex products and products with only subtle sensory differences compared to the discriminative ability of CATA questions with consumers for the same samples. When conducting DSA, assessors are trained on discriminating between attributes, as well as on scoring intensities whereas consumers are usually not trained on attributes neither on detecting differences by scoring intensities. In the current study, there were an overlap of sensory attributes, shared by all the samples as blends of two products were presented and only subtle differences between the different blend ratios were demonstrated using DSA and a trained panel. Furthermore, the samples can be regarded as quite complex. Although terms included in the CATA questions were based on the results of focus groups, attributes used for DSA of honeybush infusions were also included. The group of consumers participating in the consumer perception test might have had difficulty in understanding some of the terms, they were not trained in describing product using these terms, and samples included were very similar in sensory profiles. All of these factors may have contributed to the low degree of differentiation when applying CATA question with consumers on this sample set. A study by Antúnez, Vidal, De Saldamando, Giménez, &

Ares (2017) also reported a higher degree of discrimination between samples with only subtle differences with trained assessors and descriptive analysis than for CATA questions with consumers. Oppermann, De Graaf, Scholten, Stieger, and Piqueras-Fiszman (2017) applied Rate-all-that-apply (RATA) questions, i.e. rating intensities of selected attributes of a set of samples that differed subtly in terms of sensory attributes. These researchers found RATA questions to be more powerful in discriminating between samples with small differences than CATA. Based on the results of the current study, it can be concluded that when working with samples with only subtle differences and/or complex products, consumers using CATA questions might not be an appropriate method for sensory characterisation.

4. Conclusions

The aim of the study was to address intrinsic bitterness associated with *C. genistoides* by blending this species with sweet-associated *C. subternata* and other *Cyclopia* species to reduce bitterness to below perceptible levels. A blend ratio of 2:3 of *C. genistoides* with *C. subternata, C. maculata* or *C. intermedia* demonstrated to be effective to reduce bitterness to below perceptible levels. The sensory profile of *C. genistoides-C. subternata* blends were quantified using DSA and a trained panel. The optimum *C. genistoides*:*C. subternata* blend associated with several aroma notes, "fynbos-floral", "apricot", "woody", "fruity-sweet" and "fynbos-sweet", and a sweet taste, while bitterness was below perceptible levels. Blending demonstrated to be effective in reducing bitterness by combining the complex sensory profile of *C. genistoides* with the more floral and sweet-associated notes associated with *C. subternata*, resulting in a well-rounded product with a complex array of sensory attributes.

It is important to determine consumers' perception on the sensory properties of the blended infusions of herbal tea before making recommendations to the industry. Consumers found the different *C. genistoides-C. subternata* blend ratios equally acceptable. Bitterness, associated with a high *C. genistoides-C. subternata* blend ratios, were therefore not driving consumer acceptability. The efficacy of CATA for the sensory characterisation blended infusions of herbal tea was also evaluated. Check-all-that-apply questions, applied by consumers, is not recommended for the sensory characterisation of samples with only subtle perceptual differences.

Based on the results of the current study, a recommendation can be made to the honeybush industry that inclusion of *C. genistoides* at 40% when blending different *Cyclopia* species will result in a well-rounded product with bitterness below perceptible levels. This ratio could be regarded as the optimum level of inclusion for successfully reducing bitterness while maintaining maximum levels of inclusion of *C. genistoides.*

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Table 1 *Cyclopia genistoides-C. subternata* blend ratios and corresponding sample codes for samples used in descriptive sensory analysis (Experiment 1) and CATA questions (Experiment 4). Gen refers to *C. genistoides,* 100, 80, 60, 40, 20 and 0 refer to the percentage *C. genistoides* included in the blended sample, A and D refer to experiment 1 and 4, respectively.

Descriptive sensory analysis (Exp. 1)			Consumer perception (Exp. 4)		
Sample code	Percentage plant material		Sample code	Percentage plant material	
	C. genistoides	*C. subternata*		*C. genistoides*	*C. subternata*
A_Gen_100	100	0	D_Gen_100	100	0
A_Gen_80	80	20			
A_Gen_60	60	40	D_Gen_60	60	40
A_Gen_40	40	60	D_Gen_40	40	60
A_Gen_20	20	80	D_Gen_20	20	80
A_Gen_0	0	100	D_Gen_0	0	100

Table 2 List of terms considered in the check-all-that-apply (CATA) question for evaluating infusions of *C. genistoides-C. subternata* blends.

Category	Terms
Aroma	Sweet-associated
	Apricot
	Floral
	Woody
	Plant-like
	Green grass
	Hay / dried grass
	Fruity
Taste	Sweet
	Not sweet
	Bitter
	Not bitter
	Astringent
Consumer terms	Honey
	Healthy
	Herbal
	Green-tea like
	Earthy
Tea strength	Lingering
	Strong
	Sharp / harsh
	Soft / smooth
	Soothing
	Watery / diluted
	Bland

Table 3 Analysis of variance for descriptive sensory analysis of blends of *C. genistoides* with *C. subternata*, *C. maculata* and *C. intermedia* (*p*-values of the effects that were significant (p<0.05) are highlighted in red).

		Sweet				Sour				Bitter				Astringent			
Source	DF[a]	SS[b]	MS[c]	F[d]	*p*-value	SS[b]	MS[c]	F[d]	*p*-value	SS[b]	MS[c]	F[d]	*p*-value	SS[b]	MS[c]	F[d]	*p*-value
Species	2	121.306	60.653	26.58	<.0001	39.964	19.982	10.41	0.0004	48.820	24.410	5.68	0.0081	17.036	8.518	5.88	0.0070
Species (Gen Batch)	6	24.352	4.059	1.78	0.137	10.703	1.784	0.93	0.4882	15.131	2.523	0.59	0.7382	18.160	3.027	2.09	0.844
Blend ratio	5	237.639	47.528	20.83	<.0001	102.557	20.511	10.69	<.0001	739.286	147.857	34.40	<0.0001	112.464	22.493	15.51	<.0001
Species x Blend ratio	10	61.211	6.121	2.68	0.0178	21.181	2.118	1.10	0.3909	119.073	11.907	2.77	0.0150	20.838	2.084	1.44	0.2121
Error	30	68.445	2.281			57.566	1.919			128.963	4.299			43.494			
Corrected Total	53	512.952				231.971				1051.273				211.992			

[a] Degrees of Freedom
[b] Sum of Squares
[c] Mean Square
[d] *F* test

Table 4 Mean values and standard deviations (SD) for the sensory attributes (scored on the extended scale) of six blends of *C. genistoides* with *C. intermedia*, *C. maculata* and *C. subternata.* Gen refers to *C. genistoides,* 100, 80, 60, 40, 20 and 0 refer to the percentage *C. genistoides* included in the blended sample, A refer to the sample set selected for experiment 1.

Species	Blend ratio	N	Sweet		Sour		Bitter		Astringent	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
C. intermedia	A_Gen_100	3	21.29^{f}	2.28	32.71^{abc}	1.78	29.44^{ab}	3.54	41.07^{ab}	1.40
	A_Gen_80	3	23.14^{def}	0.65	31.91^{bc}	0.71	27.70^{bcd}	1.80	40.10^{abcde}	2.23
	A_Gen_60	3	26.35^{bc}	1.77	30.58^{cdefg}	0.27	21.76^{fgh}	1.47	38.55^{defghi}	1.15
	A_Gen_40	3	27.84^{ab}	2.21	29.59^{defg}	0.60	20.99^{ghij}	1.88	37.12^{hijk}	0.33
	A_Gen_20	3	29.53^{a}	0.92	28.54^{g}	1.01	21.63^{fghi}	1.32	36.39^{ijk}	1.59
	A_Gen_0	3	28.28^{ab}	1.10	29.11^{efg}	1.12	19.30^{hij}	2.05	36.05^{k}	0.50
C. maculata	A_Gen_100	3	21.60^{def}	0.64	33.25^{ab}	1.30	28.49^{bc}	1.10	40.88^{abc}	1.05
	A_Gen_80	3	21.94^{def}	0.97	33.23^{ab}	1.24	26.65^{bcd}	1.59	39.27^{bcdef}	1.12
	A_Gen_60	3	22.30^{def}	0.59	32.31^{abc}	1.15	25.48^{cde}	1.08	40.45^{abcd}	0.67
	A_Gen_40	3	22.88^{def}	1.08	32.63^{abc}	2.52	24.57^{def}	3.32	39.16^{bcdefg}	2.02
	A_Gen_20	3	23.05^{def}	0.81	31.37^{bcde}	1.70	23.05^{efg}	2.19	38.12^{efghij}	0.69
	A_Gen_0	3	23.84^{cde}	1.12	31.26^{bcde}	2.52	21.01^{ghij}	1.42	38.60^{defghi}	1.17
C. subternata	A_Gen_100	3	21.37^{ef}	0.78	34.41^{a}	0.47	32.57^{a}	2.64	41.68^{a}	0.46
	A_Gen_80	3	22.90^{def}	2.74	31.76^{bcd}	2.13	25.33^{cde}	2.25	38.95^{cdefgh}	2.47
	A_Gen_60	3	24.07^{cd}	2.33	30.86^{cdef}	0.92	23.15^{efg}	2.68	37.94^{fghijk}	1.37
	A_Gen_40	3	27.26^{ab}	0.85	28.84^{fg}	0.81	18.36^{hij}	0.29	36.90^{ijk}	0.51
	A_Gen_20	3	28.36^{ab}	1.19	28.84^{fg}	0.49	18.25^{ij}	1.07	37.26^{ghijk}	1.12
	A_Gen_0	3	28.27^{ab}	3.20	29.21^{efg}	1.09	17.72^{j}	1.19	36.63^{ijk}	1.11

[a-k]Values in the same column with different superscripts differ significantly at $p<0.05$

Table 5 Mean values and standard deviations (SD) for the sensory attributes (scored on the extended scale) of six blends of *C. genistoides* with *C. subternata*, *C. maculata* and *C. intermedia*. Data pooled for *C. subternata*, *C. maculata* and *C. intermedia.* Gen refers to *C. genistoides,* 100, 80, 60, 40, 20 and 0 refer to the percentage *C. genistoides* included in the blended sample, A refer to the sample set selected for experiment 1.

Sample code	N	Sweet	SD	Sour	SD	Bitter	SD	Astrin-gent	SD
A_Gen_100	9	21.42^{c}	1.25	33.46^{a}	1.35	30.17^{a}	2.93	41.21^{a}	0.97
A_Gen_80	9	22.66^{c}	1.59	32.30^{ab}	1.46	26.56^{b}	1.94	39.44^{b}	1.83
A_Gen_60	9	24.24^{b}	2.31	31.25^{bc}	1.10	23.46^{c}	2.30	38.98^{b}	1.48
A_Gen_40	9	25.99^{a}	2.69	30.35^{cd}	2.20	21.30^{d}	3.31	37.73^{c}	1.51
A_Gen_20	9	26.98^{a}	3.11	29.58^{d}	1.69	20.98^{d}	2.55	37.26^{c}	1.28
A_Gen_0	9	26.80^{a}	2.84	29.86^{d}	1.82	19.34^{d}	1.99	37.09^{c}	1.43

$^{a-d}$Values in the same column with different superscripts differ significantly at $p<0.05$

Table 6 Analysis of variance for descriptive sensory analysis of *C. genistoides-C. subternata* blends (*p*-values of the effects that were significant (p<0.05) are highlighted in red).

		Sweet				Sour				Bitter				Astringent			
Source	DF[a]	SS[b]	MS[c]	F[d]	*p*-value	SS[b]	MS[c]	F[d]	*p*-value	SS[b]	MS[c]	F[d]	*p*-value	SS[b]	MS[c]	F[d]	*p*-value
Gen class (high or low)	1	10.688	10.688	5.95	0.0195	10.860	10.860	8.21	0.0066	280.203	280.203	45.52	<0.0001	39.039	39.039	27.47	<0.0001
Gen (sample)	8	10.154	1.270	0.71	0.6837	42.307	5.288	4.00	0.0015	144.212	18.026	2.93	0.0113	38.102	4.763	3.35	0.0050
Blend ratio	5	673.130	134.632	74.99	<0.0001	923.155	184.631	139.53	<0.0001	4599.635	919.927	149.46	<0.0001	684.895	136.979	96.38	<.0001
Gen class x Blend ratio	5	16.091	3.218	1.79	0.1365	22.657	4.531	3.42	0.0114	173.950	34.790	5.65	0.0005	18.573	3.715	2.61	0.0389
Error	40	71.817	1.795			52.930	1.323			246.207	6.155			56.849			
Corrected Total	59	781.910				1051.909				5444.207				837.459			

[a] Degree of Freedom
[b] Sum of Squares
[c] Mean Square
[d] *F* test

Table 7 Mean values and standard deviations (SD) for the sensory attributes (scored on the extended scale) of six *C. genistoides*-*C. subternata* blends. Data pooled for *C. genistoides* class (low and high bitter). Gen refers to *C. genistoides,* 100, 80, 60, 40, 20 and 0 refer to the percentage *C. genistoides* included in the blended sample, B refer to samples selected for experiment 2.

Sample	N	Sweet	SD	Sour	SD	Bitter	SD	Astringent	SD
B_Gen_100	10	22.81^{f}	1.49	24.36^{a}	1.80	30.43^{a}	6.27	41.39^{a}	2.25
B_Gen_80	10	24.02^{e}	1.18	22.52^{b}	1.58	25.00^{b}	4.66	39.50^{b}	1.29
B_Gen_60	10	25.59^{d}	1.50	20.82^{c}	2.08	17.71^{c}	4.56	37.41^{c}	2.47
B_Gen_40	10	27.89^{c}	1.04	17.66^{d}	1.24	12.85^{d}	2.17	35.18^{d}	1.17
B_Gen_20	10	29.80^{b}	1.06	15.21^{e}	0.89	8.51^{e}	1.98	33.14^{e}	0.99
B_Gen_0	10	32.50^{a}	2.00	13.36^{f}	1.39	5.77^{f}	1.86	31.84^{f}	1.32

$^{a\text{-}f}$Values in the same column with different superscripts differ significantly at $p<0.05$

Table 8 Mean values and standard deviations (SD) for aroma attributes (scored on the full profile scale) of six *C. genistoides-C. subternata* blends. Gen refers to *C. genistoides,* 100, 80, 60, 40, 20 and 0 refer to the percentage *C. genistoides* included in the blended sample, C refer to the sample set selected for experiment 3.

Sample	N	Fynbos Floral	SD	Rose Gera-nium	SD	Rose Perfume	SD	Apricot / Apricot jam	SD	Cooked Apple	SD	Woody	SD	Fruity sweet	SD
C_Gen_100	5	42.90^{c}	2.75	18.78^{a}	4.05	4.95^{d}	2.44	24.54^{a}	2.65	7.50^{c}	3.94	45.74^{bc}	1.68	37.82^{a}	2.48
C_Gen_80	5	43.70^{bc}	1.21	16.08^{ab}	2.82	5.64^{cd}	2.51	22.65^{ab}	2.17	9.23^{bc}	2.96	45.38^{c}	0.79	37.85^{a}	1.81
C_Gen_60	5	43.54^{bc}	1.76	15.25^{bc}	1.70	6.78^{bcd}	1.82	21.58^{ab}	2.89	11.16^{bc}	1.97	45.06^{c}	0.73	36.55^{a}	0.58
C_Gen_40	5	45.19^{ab}	1.31	13.62^{bcd}	2.00	8.00^{bc}	2.34	20.41^{abc}	3.36	12.02^{ab}	2.06	46.10^{abc}	1.30	37.23^{a}	1.53
C_Gen_20	5	44.17^{ab}	1.61	12.50^{cd}	1.85	9.15^{ab}	1.96	18.27^{bc}	2.51	13.08^{ab}	4.29	46.80^{ab}	0.77	36.84^{a}	1.23
C_Gen_0	5	45.88^{a}	0.52	10.88^{d}	1.27	10.82^{a}	1.10	16.56^{c}	4.51	15.92^{a}	2.83	47.23^{a}	0.70	36.45^{a}	1.59

Caramel	SD	Fynbos sweet	SD	Sweet spice / Cassia	SD	Dusty	SD	Sweet	SD	Sour	SD	Astrin-gent	SD	Bitter	SD
4.52^{ab}	1.26	36.34^{c}	2.74	3.00^{cd}	0.71	5.03^{ab}	1.38	19.53^{d}	1.79	24.67^{a}	1.63	30.79^{a}	1.36	13.13^{c}	5.24
3.70^{b}	1.31	38.14^{bc}	0.71	2.54^{d}	2.52	3.59^{b}	1.77	21.16^{c}	0.99	23.54^{ab}	0.71	28.30^{b}	1.35	9.40^{b}	3.54
4.66^{ab}	1.39	39.33^{b}	1.82	4.13^{bcd}	1.27	5.52^{a}	0.56	21.45^{bc}	1.38	22.45^{bc}	1.25	28.39^{b}	1.18	9.26^{b}	2.85
5.09^{ab}	0.80	40.13^{ab}	1.13	5.39^{b}	1.97	5.38^{ab}	1.79	22.40^{bc}	0.66	22.32^{bcd}	0.80	26.99^{c}	1.36	5.72^{c}	1.59
4.82^{ab}	1.38	39.30^{b}	1.38	4.95^{bc}	1.34	5.66^{a}	1.15	22.71^{b}	0.71	21.79^{dc}	0.87	26.73^{c}	0.34	6.32^{bc}	1.86
5.25^{a}	1.91	41.93^{a}	1.36	7.55^{a}	1.29	5.81^{a}	1.17	24.39^{a}	0.89	20.82^{d}	1.60	26.01^{c}	0.85	2.89^{c}	1.43

$^{a-d}$Values in the same column with different superscripts differ significantly at $p<0.05$

Table 9 Mean overall liking scores and average number of terms used to describe infusions of five *C. genistoides-C. subternata* blends. Gen refers to *C. genistoides,* 100, 60, 40, 20 and 0 refer to the percentage *C. genistoides* included in the blended sample, D refers to the sample set selected for experiment 4.

Sample	Mean overall liking*	Average number of terms used in CATA question [a]
D_Gen_100	6.19[a]	8.2[a]
D_Gen_60	6.24[a]	8.4[a]
D_Gen_40	6.16[a]	8.2[a]
D_Gen_20	6.18[a]	8.3[a]
D_Gen_0	6.28[a]	8.3[a]

* Evaluated on a nine-point hedonic scale

[a]Different superscripts within a column indicates significant differences ($p<0.05$)

Table 10 Frequency of mention of terms of the Check-all-that-apply questions when evaluating infusions of *C. genistoides*-*C. subternata* blends. Gen refers to *C. genistoides,* 100, 60, 40, 20 and 0 refer to the percentage *C. genistoides* in the blended sample, D refers to the sample set selected for experiment 4.

Category	Terms	Frequency of mention (%)					
		Sample					
		D_Gen_100	D_Gen_60	D_Gen_40	D_Gen_20	D_Gen_0	*p*-value
Aromas	Sweet associated	40.95	37.14	34.29	31.43	34.29	0.596
	Apricot	29.52	23.81	22.86	20.95	20.95	0.476
	Floral	35.24	35.24	35.24	29.52	35.24	0.842
	Woody	29.52	36.19	28.57	37.14	33.33	0.505
	Plant-like	26.67	36.19	36.19	33.33	39.05	0.316
	Green grass	19.05	14.29	17.14	14.29	12.38	0.655
	Hay / dried grass	31.43	36.19	36.19	40.00	35.24	0.756
	Fruity	34.29	29.52	31.43	37.14	35.24	0.758
Tastes	Sweet	33.33	32.38	30.48	33.33	32.38	0.988
	Not sweet	50.48^{ab}	45.71^{ab}	57.14^{b}	47.62^{ab}	37.14^{a}	0.029
	Bitter	14.29	23.81	18.10	18.10	25.71	0.155
	Not bitter	42.86	40.00	34.29	47.62	41.90	0.269
	Astringent	22.86	24.76	33.33	24.76	31.43	0.192
Consumer terms	Honey	33.33	28.57	37.50	26.67	32.38	0.417
	Healthy	42.86	46.67	48.08	49.52	46.67	0.893
	Herbal	61.90	69.52	59.62	52.38	57.14	0.070
	Green tea-like	24.76	32.38	28.85	26.67	24.76	0.587
	Earthy	39.05	44.76	38.46	49.52	51.43	0.157
Tea strength	Lingering	35.24	38.10	35.58	40.00	39.05	0.911
	Strong	21.90	25.71	29.81	27.62	34.29	0.370
	Sharp/harsh	15.24	17.14	16.35	21.90	19.05	0.713
	Soft/smooth	47.62	44.76	42.31	41.90	40.00	0.881
	Soothing	34.29	38.10	38.46	37.14	43.81	0.626
	Watery/diluted	31.43	25.71	18.27	25.71	16.19	0.074
	Bland	22.86	18.10	20.19	20.00	15.24	0.701

Table 11 Average number of mentions of terms within each category considered in the Check-all-that-apply questions

Category	Average number of terms
Aroma	12.2[a]
Tastes	8.3[c]
Consumer terms	10.4[b]
Tea strength	10.3[b]

[a-c] Different superscripts within a column indicates significant differences ($p<0.05$)

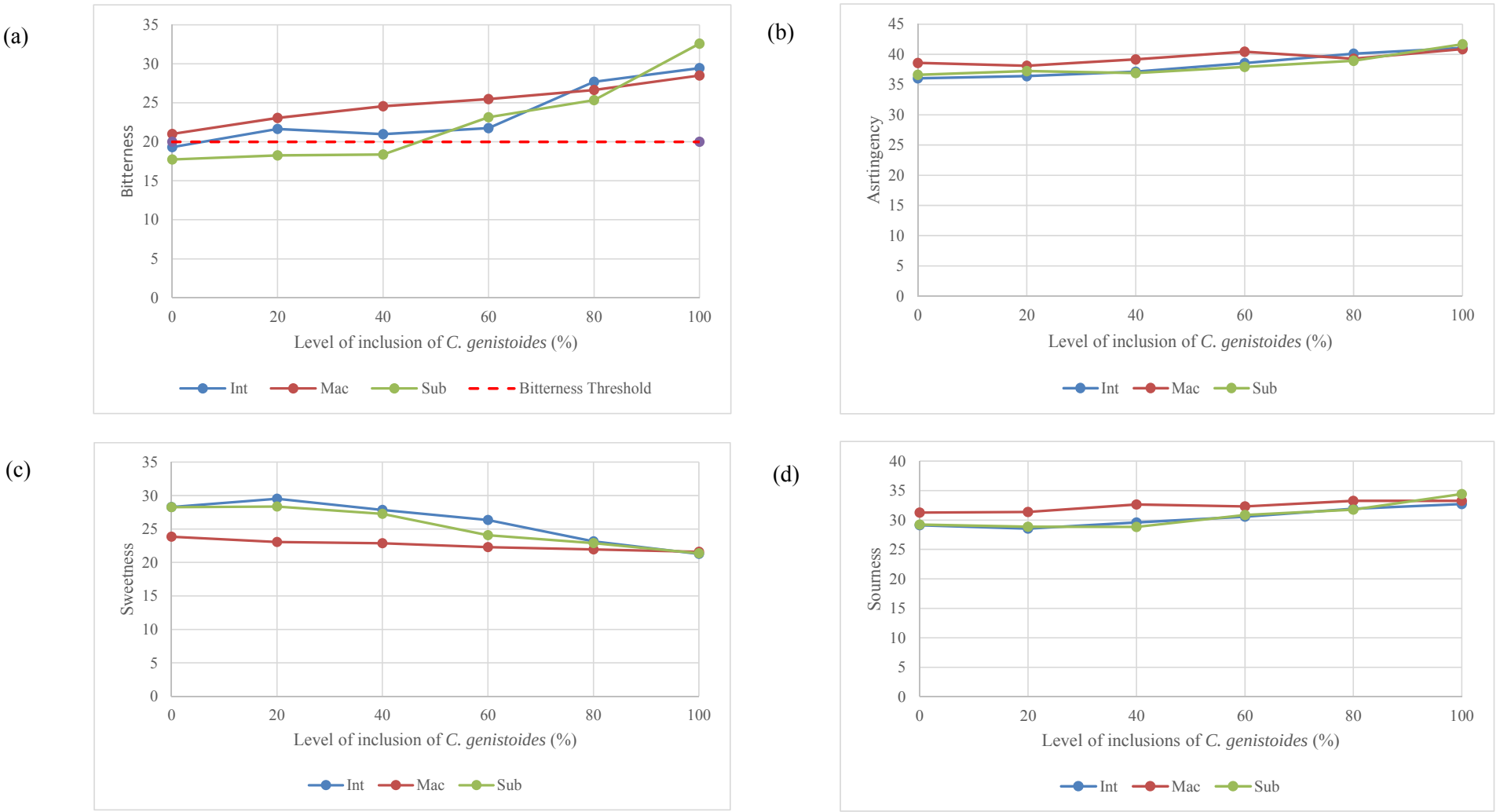

Fig. 1. Intensities of sensory attributes of blends of *C. genistoides* with *C. subternata*, *C. maculata* and *C. intermedia* respectively. *Cyclopia genistoides* included at 6 levels (0 – 100%) Int, Mac and Sub refers to *C. intermedia*, *C. maculata* and *C. subternata* respectively. Graphs a-d represent intensities for bitterness, astringency, sweetness and sourness respectively.

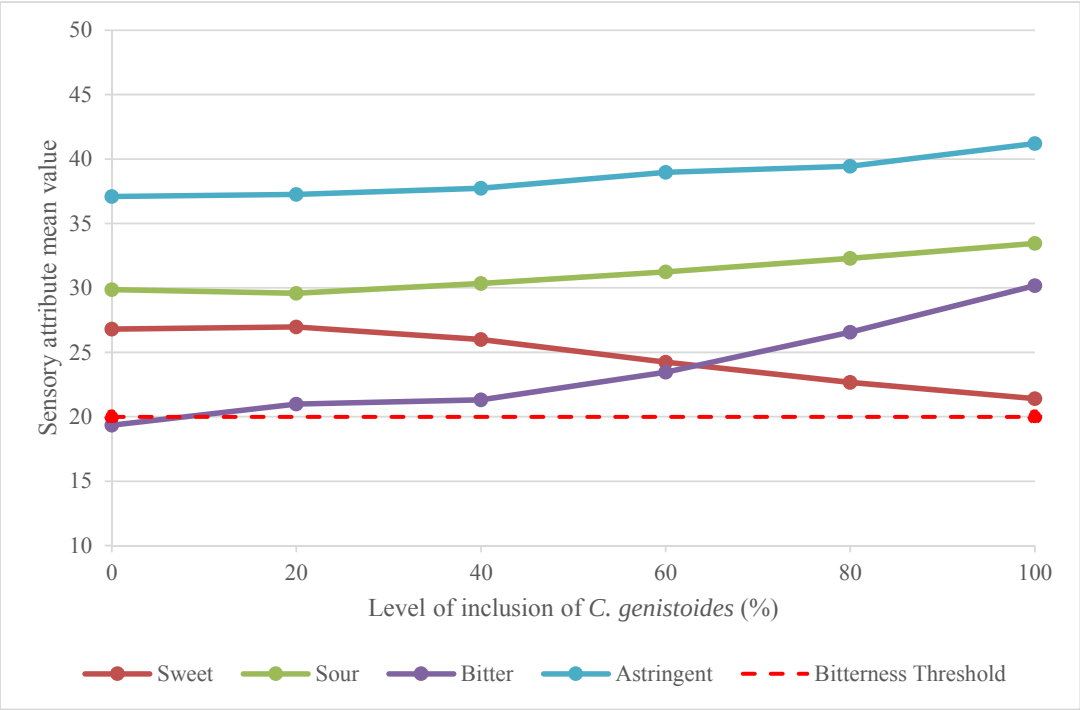

Fig. 2. Intensities of sensory attributes (extended scale) when blending *Cyclopia genistoides* with three *Cyclopia* species (*C. subternata*, *C. maculate* and *C. intermedia*) at six levels of inclusion. Data pooled for the three *Cyclopia* species.

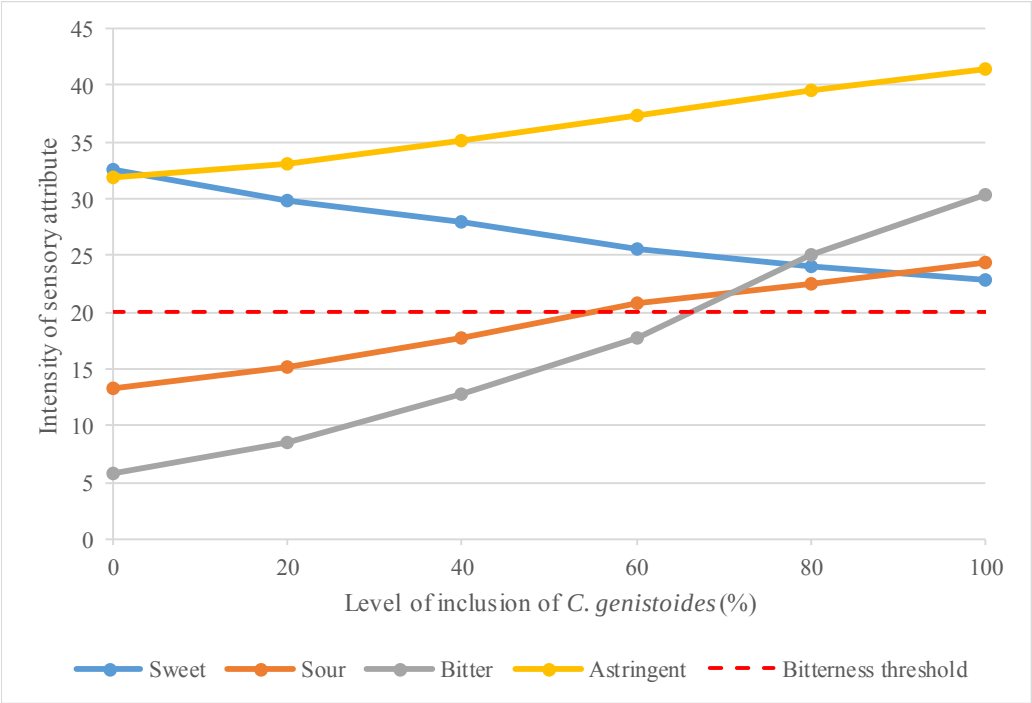

Fig. 3. Intensities of sensory attributes of infusions of *C. genistoides*-*C. subternata* blends. *Cyclopia genistoides* included at six levels. Different levels of inclusion were significantly different for all attributes ($p<0.05$).

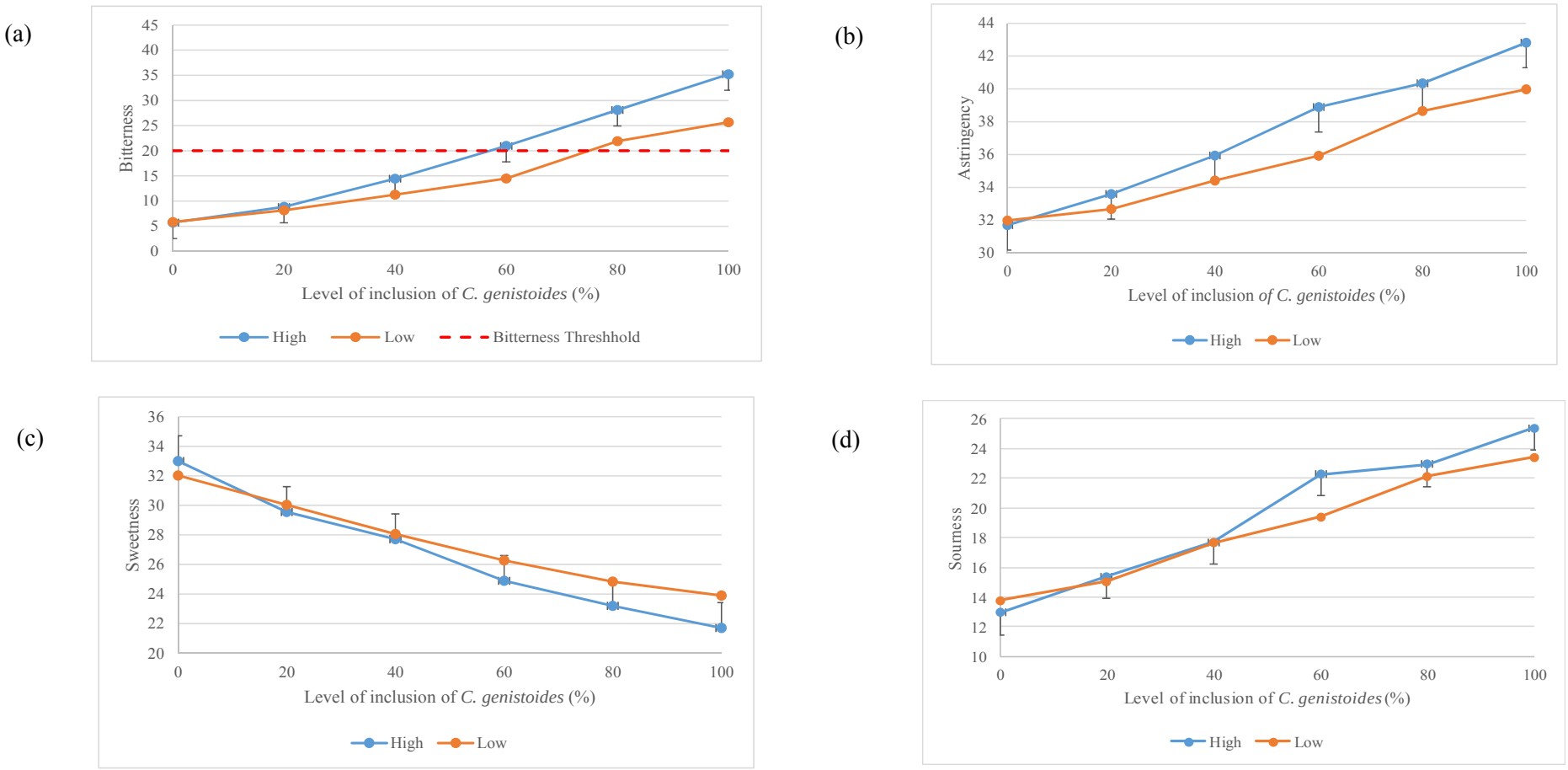

Fig. 4. Intensities of sensory attributes of blends of *C. genistoides* (high and low bitter) and *C. subternata*. *Cyclopia genistoides* included at 6 levels (0 – 100%). The vertical bars denote the least significant difference per attribute. Graphs a-d represent intensities for bitterness, astringency, sweetness and sourness, respectively.

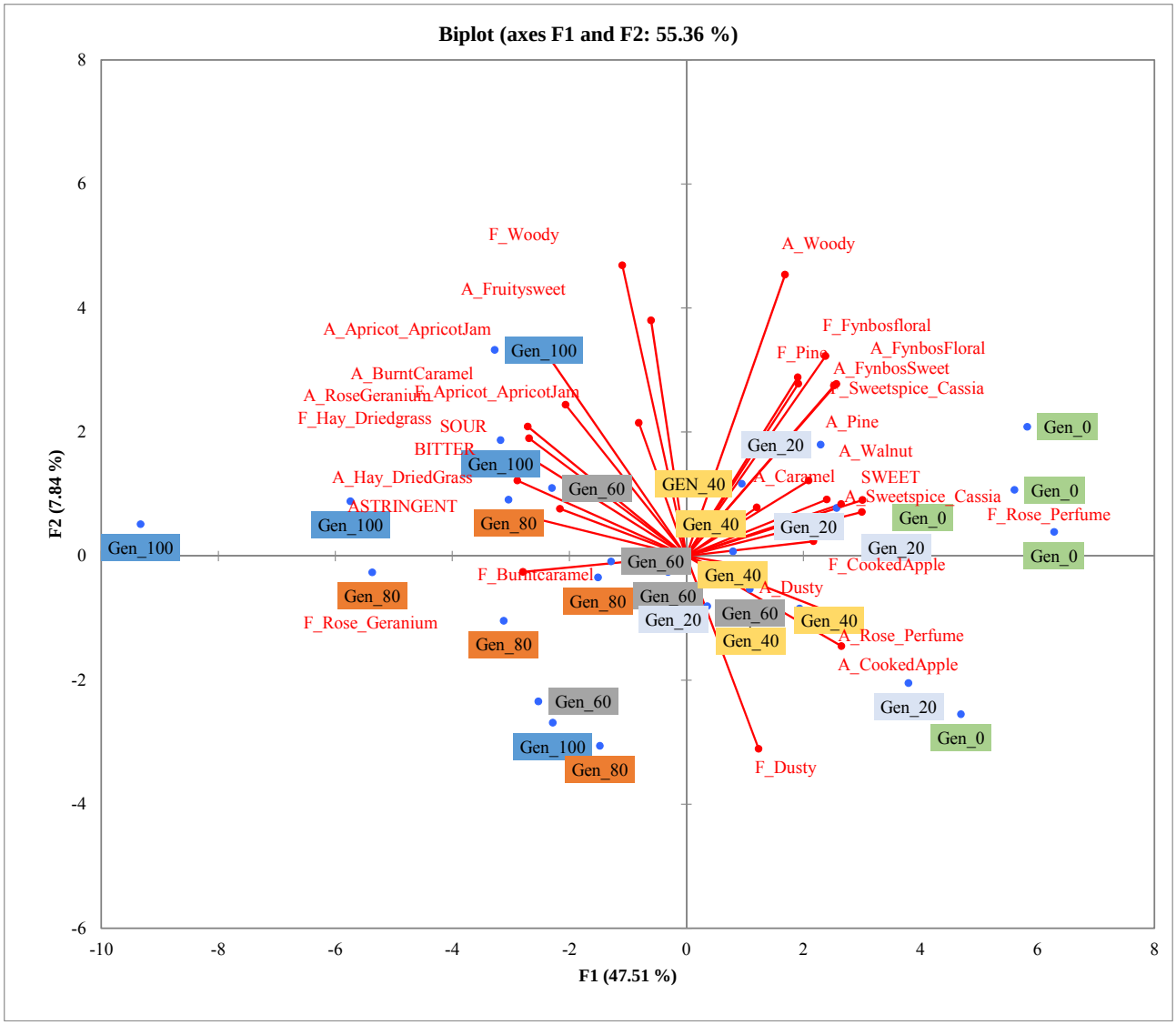

Fig. 5. PCA bi-plot representing the association between *C. genistoides-C. subternata* blends and sensory attributes. Capital letters added to attributes indicate A: aroma (orthonasal) and F: flavour (retronasal), Gen refers to *C. genistoides*, 100 – 0 refer to the percentage *C. genistoides* included in the blend.

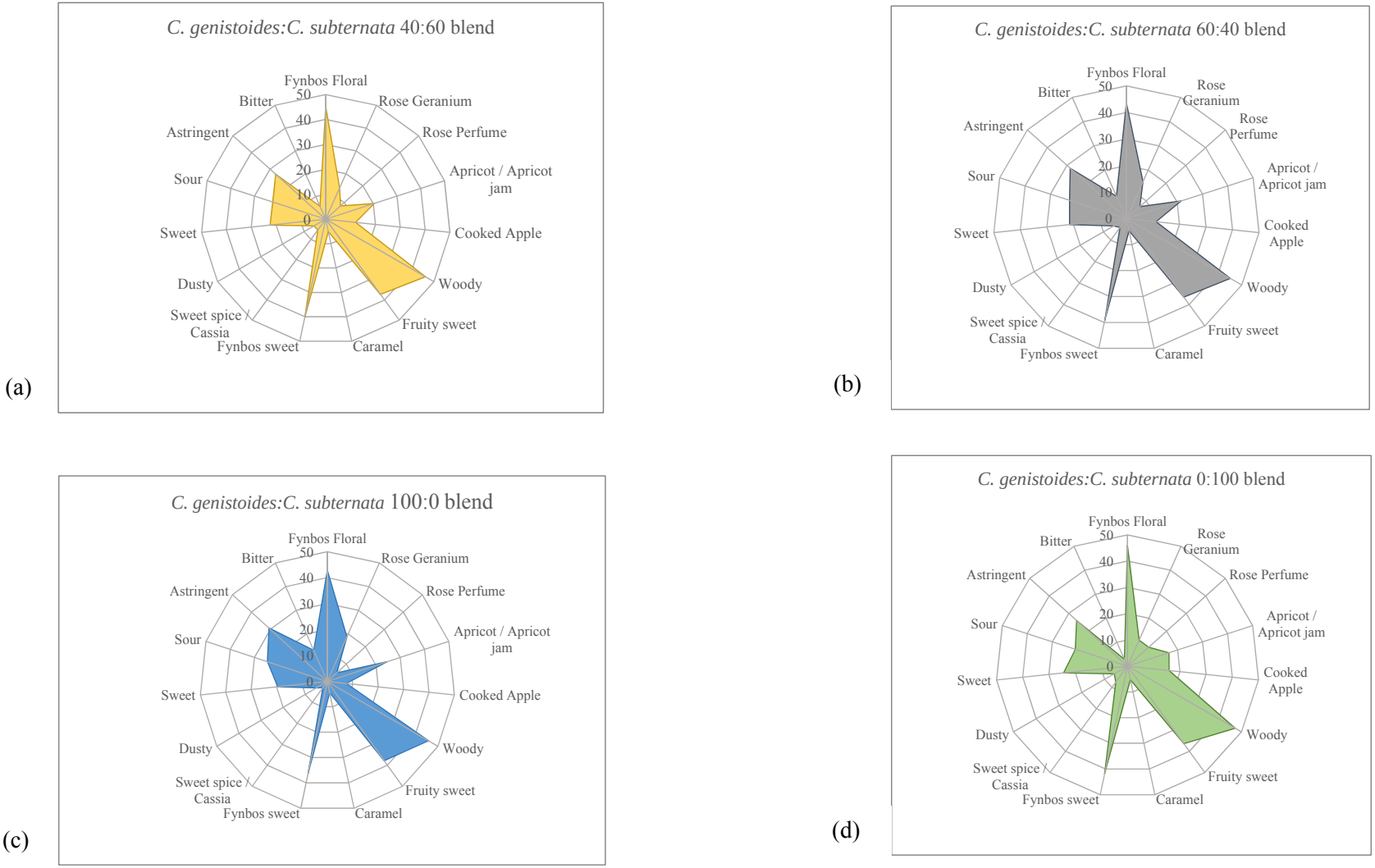

Fig. 6. Spider plots representing the mean aroma, taste and mouthfeel intensities for *C. genistoides-C. subternata* blends. Blend ratios of *C. genistoides:C. subternata* in the respective plots are (a) 40:60, (b) 60:40, (c) 100:0 and (d) 0:100.

ADDENDUM A

Sensory lexicon describing aroma, flavour, taste and mouthfeel characteristics of infusions of Cyclopia species

Aroma attributes	Descriptors	Flavour, taste and mouthfeel attributes	Descriptors
Floral	Fynbos floral[a]	*Floral*	Fynbos floral[a]
	Rose geranium		Rose geranium
	Rose / Perfume		Rose / Perfume
Fruity	Lemon / Lemongrass	*Fruity*	Lemon / Lemongrass
	Apricot jam / Apricot		Apricot jam / Apricot
	Apple cooked		Apple cooked
Woody	Woody	*Woody*	Woody
	Pine		Pine
Sweet	Fruity sweet	*Spice*	Sweet spice / Cassia
	Caramel		Coconut
	Honey	*Taints*	Dusty
	Fynbos sweet		Medicinal
Spice	Sweet spice / Cassia		Burnt caramel
Nutty	Walnut		Rotting plant water
	Coconut		Hay/dried grass
Taints	Dusty		Green grass
	Medicinal		Cooked vegetables
	Burnt caramel		
	Rotting plant water	*Taste*	Sweet
	Hay/dried grass		Sour
	Green grass		Bitter
	Cooked vegetables	*Mouthfeel*	Astringent

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

Chapter 5

Validation of projective mapping as potential sensory screening tool for application by the honeybush herbal tea industry

Abstract

Honeybush herbal tea is produced from the endemic South African *Cyclopia* species. Plant material subjected to a high-temperature oxidation step ("fermentation") forms the bulk of production. Production lags behind demand forcing tea merchants to use blends of available material to supply local and international markets. The distinct differences in the sensory profiles of the herbal tea produced from the different *Cyclopia* species require that special care is given to blending to ensure a consistent, high quality product. Although conventional descriptive sensory analysis (DSA) is highly effective in providing a detailed sensory profile of herbal tea infusions, industry requires a method that is more time- and cost-effective. Recent advances in sensory science have led to the development of rapid profiling methodologies. The question is whether projective mapping can be used successfully for the sensory characterisation of herbal tea infusions. Trained assessors performed global and partial projective mapping to determine the validity of this technique for the sensory characterisation of infusions of five *Cyclopia* species. Similar product configurations were obtained when comparing results of DSA and global and partial projective mapping. Comparison of replicate sessions showed RV coefficients >0.8. A similarity index, based on multifactor analysis, was calculated to determine assessor repeatability. Global projective mapping demonstrated to be a valid method for providing a broad sensory characterisation of *Cyclopia* species, thus suitable as a rapid quality control method of honeybush infusions. Its application by the honeybush industry could improve the consistency of the sensory profile of blended products.

Keywords: Projective mapping; Descriptive sensory analysis; Panel performance; *Cyclopia* species; Multiple factor analysis; Similarity index

1. Introduction

1.1 Honeybush industry

Honeybush herbal tea is produced from the endemic South African *Cyclopia* species (Joubert, Joubert, Bester, de Beer, & De Lange, 2011). Of the 23 *Cyclopia* species identified, only a few are currently of commercial interest (Joubert et al., 2011). The majority of the production comprises *C. intermedia,* mainly harvested from the wild, and to a lesser extent *C. subternata* and *C. genistoides*, both cultivated. Species under

evaluation are *C. maculata* and *C. longifolia* (Joubert et al., 2011). The distinguishing sensory profile of this herbal tea is the result of "fermentation", a high temperature oxidation process (Du Toit & Joubert, 1999). The generic sensory profile of honeybush has been defined as "fynbos-floral", "woody" and "fynbos-sweet" aroma and flavour, with a slight sweet taste and slight astringent mouthfeel (Erasmus, Theron, Muller, Van Der Rijst, & Joubert, 2017). *Cyclopia* species investigated to date are all associated with this generic profile, however, each species shows higher intensities for specific sensory attributes, making it possible to distinguish one species from another. *Cyclopia genistoides* is associated with a strong "rose-geranium" flavour and perceptible bitter taste. *Cyclopia longifolia* has a similar sensory profile to that of *C. genistoides,* but with a less prominent "rose-geranium" flavour and no perceptible bitter taste (Erasmus et al., 2017). *Cyclopia subternata* and *C. maculata* both associate with "caramel" and "sweet-associated" aroma notes and a slight astringent mouthfeel. The most prominent aroma attributes associated with *C. intermedia* are "fynbos-floral", "fynbos-sweet" and "woody" and to a lesser extent, "fruity-sweet" and "apricot jam" (Bergh, Muller, Van der Rijst, & Joubert, 2017).

The majority of the honeybush harvest is exported, with export volumes increasing on a year to year basis (Joubert et al., 2011). The current production is insufficient to supply in the local and international demand, forcing the honeybush industry to mostly use blends of different *Cyclopia* species (Joubert et al., 2011). The limited regulatory measures to control the quality of this export product do not include sensory quality. Rapid sensory profiling methods that are efficient, flexible and less time consuming than traditional descriptive sensory analysis are required to improve quality and product consistency.

1.2 Sensory profiling

Descriptive sensory analysis (DSA) is regarded as the most comprehensive and informative sensory tool (Lawless & Heymann, 2010; Murray, Delahunty, & Baxter, 2001) that provides results that are robust, consistent and reproducible within time and a specified sensory context (Moussaoui & Varela, 2010). However, the main drawbacks of DSA are the cost and time involved in maintaining well-trained panels. These factors, together with the fact that trained assessors might perceive the sensory profile of products different than consumers, led to the development of sensory tools that have a holistic approach and are more flexible and less time consuming (Varela & Ares, 2014).

One of the alternatives to traditional DSA is projective mapping (PM), a method originating from psychology and first introduced to the field of sensory science by Risvik and co-workers (Risvik, McEwan, Colwill, Rogers, & Lyon, 1994; Risvik, McEwan, & Rødbotten, 1997). PM entails that all products are presented simultaneously and assessors are asked to place products in a two-dimensional space according to perceived similarities and dissimilarities. Each assessor applies his own individual set of criteria, making this a relatively spontaneous procedure. The PM technique evolved to include an attribute collection step, similar to ultra-flash profiling (Perrin et al., 2008). Comparison of PM to DSA for a range of products shows good agreement between these methods (Barcenas, Elortondo, & Albisu, 2004; Cadena et al., 2014; Dehlholm,

Brockhoff, Meinert, Aaslyng, & Bredie, 2012; Hopfer & Heymann, 2013; Louw et al., 2013; Nestrud & Lawless, 2008; Nestrud & Lawless, 2010; Perrin et al., 2008).

Variations of PM have been proposed: global PM has a holistic approach where all attributes are taken into account, while partial PM can direct the assessor to focus on a specified sensory modality such as aroma (Dehlholm, 2014). PM is a comparative technique where all samples need to be presented simultaneously, thereby limiting the number of samples per session. PM studies reported that between 5 (Risvik et al., 1994) and 18 samples (Hopfer & Heymann, 2013) could be used, however, the optimum number of samples to include in a PM task is 12 (Pagès, 2005). Hopfer and Heymann (2013) reported on the effect of the geometrical shape of the evaluation sheet on the product configuration and concluded that better results are obtained when using a rectangular space since assessors regard the horizontal axis as the main dimension to indicate dissimilarity between products. Assessors with different levels of training can be used for PM (Ares, Deliza, Barreiro, Giménez, & Gámbaro, 2010; Moussaoui & Varela, 2010; Nestrud & Lawless, 2008).

The aim of the present study was to determine the validity of three variations of PM for the rapid sensory profiling of honeybush infusions. Sample configuration obtained with partial PM on aroma attributes, partial PM on palate attributes and global PM using all attributes was compared to that of descriptive sensory analysis (DSA). The effect of replication on the PM task and assessor repeatability was also addressed. Based on these results, recommendations on best practice for applying PM in the herbal tea industry could be made.

2. Materials and methods

2.1 Descriptive sensory analysis

2.1.1 Samples

Randomly selected samples (n = 36) representing different production batches of five *Cyclopia* species, namely *C. genistoides*, (n = 7), *C. subternata* (n = 9), *C. maculata* (n = 6), *C. intermedia* (n = 8) and *C. longifolia* (n = 6) of harvest year 2014/2015 were obtained. *Cyclopia* species of commercial interest were selected (Joubert et al., 2011), furthermore different batches were included to address batch variation within species. All samples, except those of *C. intermedia*, were processed on laboratory-scale at the Post-Harvest and Wine Technology Division of ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, using optimum conditions for characteristic aroma development (Erasmus et al., 2017). Samples of *C. intermedia* were sourced from a commercial processor. All samples were mechanically sieved to obtain the "tea bag" fraction (<12 mesh and > 40 mesh) as described by Theron et al. (2014). The sieved plant material was stored at ambient temperature (21ºC) in sealed glass jars, until analysed (Theron et al., 2014). Sample codes for the batches of the respective *Cyclopia* species used in DSA and PM are presented in Table 1.

2.1.2 Preparation of infusions

Infusions were prepared by pouring 1000 g freshly boiled distilled water onto 12.5 g of the sieved plant material. After infusing for 5 min, each infusion was strained through a fine-mesh stainless steel strainer directly into a 1 L pre-heated stainless steel thermos flask (Woolworths, Bellville, South Africa). The infusions were served in white porcelain mugs coded with 3-digit random codes. The mugs were pre-heated in an industrial oven (Hobart, France) at 70°C before aliquots of each infusion (ca. 100 mL) were poured into the mugs and covered with plastic lids. The mugs were arranged in a random order per assessor as generated by the Compusense® five software program (Compusense version 5.6, Guelph, Canada) where all assessors evaluated all the samples while controlling for first order carry-over effects. The samples were served in temperature controlled (65°C) water baths (Scientific Manufacturing Company, Cape Town, South Africa).

2.1.3 Sensory analysis

An existing sensory panel consisting of 9 trained panellists (all female between the ages of 40 and 65) with several years of experience in the descriptive sensory analysis of rooibos (Jolley, Van der Rijst, Joubert, & Muller, 2016; Koch, Muller, Joubert, Van der Rijst, & Næs, 2012) and honeybush (Bergh et al., 2017; Erasmus et al., 2017; Theron et al., 2014) were selected. Panel members completed an official consent form before commencing with DSA. The panel was further trained on the samples and the generic DSA technique, as described by Lawless and Heymann (2010), during eight one-hour sessions. The attributes and references generated during the development of the revised generic honeybush sensory wheel were used as the basis for training (Erasmus, 2015) and consisted of 22 aroma, 17 flavour, 4 taste and mouthfeel attributes (Table 2). Aroma was defined as the aromatics perceived through orthonasal analysis, flavour as the retronasal perception and taste as the basic taste modalities. Astringency was described as the tactile sensation that occurred in the oral cavity caused by the precipitation of salivary proteins (Green, 1993). Attribute intensities were rated on unstructured line scales (0 – 100), using the Compusense® five software program. The experimental design was a balanced complete block design. Five samples, one sample per *Cyclopia* species, were presented in a random order to each assessor per session. Each set of five samples was evaluated in triplicate on the same day with a rest-period of 15 min between each test session. The 36 samples were tested during 21 sessions over a period of 2 weeks. During the last three sessions, 6 samples were presented to be able to analyse all batches obtained. Unsalted water biscuits (Woolworths, Stellenbosch, South Africa) and still natural spring water (Woolworths, Stellenbosch, South Africa) were used as palate cleansers between samples. Assessors were seated at individual booths in a temperature- (21ºC) and light-controlled room.

2.2 Projective mapping

2.2.1 Sample selection and preparation

The sample configuration obtained through principal component analysis (PCA) of the DSA data was used to select two samples per species that could be regarded as most representative of the respective species. Two blind duplicate samples (INT_B3 and LON_B2) were included to aid in evaluating panel performance

(Table 1). This resulted in a total of 12 samples presented simultaneously to each assessor for evaluation per session. The infusions were prepared and served in the same manner as for DSA.

2.2.2 Partial and global projective mapping

The same panel used for the DSA with one additional female panel member in the same age group, participated in the PM task that commenced 2 weeks after completion of DSA. Panel members completed an official consent form before commencing with the PM study. A short explanation of the PM method followed by a training exercise, as described by Hopfer and Heymann (2013), was used to introduce the panel to this rapid profiling technique. Some assessors had previous experience of this method. Three variations of PM were applied: partial PM according to aroma attributes, partial PM according to palate attributes and global PM (all attributes). Partial PM on palate included all flavour attributes, as well as taste (sweet, sour and bitter) and the mouthfeel attribute, astringency. The three variations of PM were performed on three consecutive days, with three replications per PM method per day. Assessors were instructed to place the samples on a rectangular sheet of paper (60 cm x 40 cm) according to perceived similarities or dissimilarities. The PM task was followed by an ultra-flash profiling task where descriptors were added, as described by Perrin et al. (2008). To aid assessors in this step, a list of descriptors were provided, as suggested by Perrin et al. (2008). The list was based on published research on honeybush (Erasmus et al., 2017; Theron et al., 2014) and the revised honeybush sensory wheel (Erasmus, 2015). Assessors were asked to put sample containers back into the water bath after tasting to ensure that the temperature of the infusions remained constant. For spatial positioning of the samples on the paper, the assessors used "sticky notes", each representing a specific coded sample container. Assessors were requested to take a 10 min break between replicates to avoid panel fatigue. Unsalted water biscuits (Woolworths, Stellenbosch, South Africa) and still natural spring water (Woolworths, Stellenbosch, South Africa) were used as palate cleansers. Assessors were seated in a temperature- (21°C) and light-controlled room, at individual tables rather than booths as this provided enough space for the water bath and sheet of paper. All the assessors completed the three replicates of the PM task within a 2-hour period of time. An example of the instruction sheet for global projective mapping is provided in Addendum A.

Data collection was done by measuring the coordinates for each sample placement per assessor using a ruler. The lower left corner of the paper served as the zero point (0, 0). The data were constructed in such a manner where one row represented one sample and consecutive columns, the repetition, sample name, assessor name and the x and y coordinates per assessor x sample for all 10 assessors. The assessor coordinates of the data table were followed by a descriptor block where each column represented a descriptor. Occurrence of descriptors was indicated per sample x assessor in rows. A value of 1 was allocated to a sample if the descriptor was used for that sample and a value of 0 if the descriptor was not used. The number of citations per descriptor was summed. An example of how the data for global PM were captured, is provided in Addendum B.

2.3 Statistical procedures

2.3.1 Descriptive sensory analysis

PanelCheck Software (Version 1.3.2, http://www.panelcheck.com) was used to monitor DSA panel performance. Pre-processing of DSA data were performed to test for panel reliability by means of a model that includes assessor, replication and sample effects and interactions (Næs, Brockhoff, & Tomic, 2010). The Shapiro-Wilk test was performed to test for non-normality of residuals (Shapiro & Wilk, 1965). In the event of significant non-normality ($P \leq 0.05$), outliers were removed when the standardised residuals for an observation deviated more than three standard deviations from the model value. Following confirmation of panel reliability and normality of data, statistical analyses were performed on means over triplicate infusions and assessors of DSA data. The data were subjected to analysis of variance (ANOVA) according to the experimental design to test for treatment differences. Treatments means were compared by calculating Fisher's LSD where a probability level of 5% was considered significant. Univariate analyses were performed using SAS® software (Statistical Analysis System 2006, Version 9.2, SAS Institute Inc., Cary, NC, USA). Principal component analysis (PCA), using the correlation matrix, was conducted using XLStat (Version 7.5.2, Addinsoft, New York, USA) to visualise and elucidate the relationships between the samples and the attributes (Næs, Brockhoff, & Tomic, 2010).

2.3.2 Projective mapping

Data obtained with PM were analysed using multiple factor analysis (MFA) as described by Escofier and Pagès (1994). The co-ordinate data of each assessor were regarded as a separate data table, i.e. the 10 assessors participating in the PM task resulted in 10 data tables with two variables per assessor (x, y co-ordinates) in the MFA calculation. The table with descriptor citations was added as supplementary data. When adding descriptor citations as supplementary data, the product configuration is based on the individual configurations of assessors and is therefore not determined by the number of citations of descriptors.

RV coefficients were calculated to determine the degree of similarity between the PCA bi-plot resulting from DSA and the MFA product configurations of the three variations of PM. The first two dimensions of the respective configurations were considered. The RV coefficient is a multivariate similarity coefficient used to measure the extent to which two product configurations are similar (Abdi, Valentin, Chollet, & Chrea, 2007; Louw et al., 2013). RV coefficients range between 0 and 1, with values closer to 1 indicating a higher degree of similarity (Abdi et al., 2007; Risvik et al., 1997). A RV coefficient of 0.7 is indicative of an acceptable level of similarity (Cartier et al., 2006; Nestrud & Lawless, 2008).

The effect of replication on the product configuration was also measured by calculating RV coefficients for the three replications of each PM method. RV coefficients were further calculated to compare assessors' repeatability over replications. Dimension 1 and 2 of the respective configurations were considered. RV coefficients between assessors and the MFA consensus plot >0.5 were regarded as sufficient consensus (Louw et al., 2013). In the event of insufficient consensus between an assessor's data and the overall MFA map, the assessor's data were removed and recalculated. If the subsequent product configuration differed considerably

from the original, the assessor's data were excluded from the final data analysis. The repeatability of the panel was measured by calculating a people performance index (PPI) as described by Hopfer and Heymann (2013).

The validity of the product configuration of PM could, in addition to RV coefficients, also be determined by calculating a similarity index (SI) (Tomic, Berget, & Næs, 2015). This similarity index is based on MFA and test assessor repeatability over replications of the PM task. This method entails projecting each assessor's product configuration onto the subspace defined by the consensus. The SI measures how well the consensus matrix represents each individual's product configuration. If the difference between the consensus configuration and the individual configuration is large, it would result in a higher SI for that specific assessor. If the projected assessor product configuration is similar to the consensus product matrix, the SI will be close to zero.

Data analyses were performed using R 3.2.0 (R Core team, 2015). FactoMineR was used to perform MFA and to compute the RV coefficients (Lê, Josse, & Husson, 2008).

3. Results and discussion

MFA analysis of the PM data was performed using two approaches: firstly, the conventional approach where descriptors were added as supplementary data, and secondly, where descriptors were added as a table of variables. The explained variance for the two methods of analyses was almost identical. The total explained variance for analysis of global PM according to the first and second approach was 62.8% and 62.7%, respectively. The same results were obtained for MFA of partial PM on aroma and partial PM on palate attributes. Results for MFA with descriptors added as supplementary data will therefore be reported.

3.1 Validation of partial and global projective mapping

Validation of the PM results was done by visually comparing the product configurations obtained for the different PM tasks with the PCA bi-plot of the DSA results. The validity was further determined by comparing the RV coefficients for the product configurations of the PCA bi-plot of the DSA data with those of the different PM configurations.

3.1.1 Descriptive sensory analysis

The PCA bi-plot of the DSA results (Fig. 1), based on the full sample set (n = 36) shows a product configuration with clear differentiation between the different *Cyclopia* species. *Cyclopia intermedia* samples formed a group to the upper right quadrant of the PCA plot, indicating that this species is described with floral and sweet-associated aromas and flavours, including "rose geranium". *Cyclopia subternata* samples, grouped in the lower right quadrant of the PCA plot, are associated with "woody", "cooked apple", "sweet spice/cassia" and "dusty" aroma and flavour, "honey" and "walnut" aroma and sweet taste. *Cyclopia genistoides* samples grouped to the upper left quadrant and are associated with the aroma attributes "apricot/apricot jam" and "rotting plant water", as well as with bitter and sour taste and astringent mouthfeel. *Cyclopia maculata* and *C. longifolia* samples grouped together in the lower left quadrant. These species are associated with the aroma

and flavour attributes "burnt caramel", "green grass" and "cooked vegetables", as well as with "hay/dried grass" aroma and "rotting plant water" flavour. The latter attributes are not desirable in the commercial product.

The first two components explained 71.71% of the variability with 52.81% of the variance explained by component 1 and 18.90% by component 2. The main differentiation between samples on the first principal component (PC1) is therefore the floral, sweet and spicy notes toward the positive end, and the green and vegetative notes, as well as bitter and sour taste and astringent mouthfeel towards the negative side of PC1. These results are in accordance with the findings of Erasmus et al. (2017) in a study of the effect of high-temperature oxidation of *Cyclopia* species on their sensory profiles.

3.1.2 Global projective mapping

The product configuration obtained with DSA of samples selected for the PM task (n = 10), is presented in Fig. 2 with 91% of the variance explained by the first and second components. *Cyclopia intermedia* samples, grouped towards the positive end of PC1, were associated with a sweet taste and aromas of "pine", "caramel" and "fynbos sweet" and "fynbos floral" aroma and flavour. *Cyclopia subternata* samples, grouped in the upper right quadrant, were associated with "cooked apple" aroma and "woody" and "sweet spice/ cassia" aroma and flavour. The remaining three species (*C. genistoides, C. maculata and C. longifolia*) formed one group towards the left of PC 1 and associated with "green grass" and "cooked vegetables" aroma and "hay/dried grass" aroma and flavour. *Cyclopia subternata* and *C. intermedia* were therefore associated with the sweeter and spicy notes towards the positive end of PC1, while *C. genistoides, C. maculata and C. longifolia* were associated with the more green-associated attributes positioned towards the negative side of PC1.

The product configuration and associated correlation circle on attributes obtained from the average of three global PM evaluations by 10 trained assessors are depicted in Fig. 3. Dimensions 1 and 2 account for 46.4% and 16.3% of the explained variance of the individual factor map of global PM data, respectively. According to Fig. 3, the samples on the positive side of dimension 1, namely *C. subternata* (Sub_B2, Sub_B3) and *C. intermedia* (Int_B1, Int_B3) are associated with the floral, fruity, spicy, woody and sweet-associated attributes. Samples to the negative side of dimension 1, namely *C. genistoides* (Gen_B1, Gen_B4), *C. longifolia* (Lon_B2, Lon_B5) and *C. maculata* (Mac_B1, Mac_B2), were associated with the attributes "apricot/apricot jam", "hay/dried grass", "green grass" and "cooked vegetables", as well as with bitter and sour taste and an astringent mouthfeel. When comparing the sample configuration obtained for DSA (Fig. 2) and global PM (Fig. 3), similar sample configurations and association of samples with attributes are evident. Similar product plots for PM and DSA are in accordance with findings for other products (Dehlholm et al., 2012; Hopfer & Heymann, 2013; Kennedy & Heymann, 2009; Risvik et al., 1994).

3.1.3 Partial projective mapping on aroma

Fig. 4 represents the product configuration and the associated correlation circle on attributes obtained for three replications of partial PM, based on the aroma of the *Cyclopia* infusions. Dimensions 1 and 2 account for 46.3% and 15.2% of the explained variance, respectively. *Cyclopia subternata* and *C. longifolia* samples,

placed towards the positive end of dimension 1, were associated with the floral, fruity and spicy attributes described as "rose perfume", "fynbos-floral", "sweet spice/cassia" and "cooked apple". The herbal teas towards the negative end of dimension 1 (*C. maculata*, *C. genistoides* and *C. longifolia*) were associated with the green and vegetative notes represented by "green grass", "hay/dried grass" and "cooked vegetables", as well as with "apricot/apricot jam" and "burnt caramel". These observations were similar to the selected DSA results as presented in Fig. 2.

3.1.4 Partial projective mapping on palate

Fig. 5 represents the product configuration and corresponding attribute correlation circle for three replications of partial PM of *Cyclopia* infusions, according to palate attributes. Dimensions 1 and 2 account for 44.4% and 19.0% of the explained variance, respectively. The product configuration in Fig. 5 is similar to that of DSA (Fig. 2). *Cyclopia subternata* samples were associated with the attributes "cooked apple", "sweet spice/cassia" and "woody", positioned towards to the positive end of dimensions 1 and 2. *Cyclopia intermedia* samples were associated with floral and lime-like attributes. Once again, *C. genistoides, C. longifolia* and *C. maculata*, were associated with green and vegetative notes represented by "hay/dried grass" and "green grass", as well as "apricot/apricot jam" and "burnt caramel". These samples were also associated with a bitter and sour taste and an astringent mouthfeel.

3.1.5 Comparison of the product configurations using RV coefficients

The RV coefficients for the product configurations according to results for DSA, global PM, partial PM on aroma and partial PM on palate are summarised in Table 3. RV coefficients should be interpreted with great care, as emphasised in a recent study by Ares et al. (2014). The value of the RV coefficients is influenced by the number of products and variables when comparing two data matrices (Smilde, Kiers, Bijlsma, Rubingh, & Van Erk, 2009). Furthermore, the RV coefficient puts the greatest importance on the dimension with the largest explained variance (Tomic et al., 2015). Dimensions 1 and 2 accounted for 91% of the explained variance of the PCA bi-plot (subset of samples; Fig. 2) and for >60% of the explained variance of the different variations of PM. Only the first two dimensions of the respective configurations were therefore considered when calculating the RV coefficients. As already mentioned, RV coefficients >0.7 are regarded as an indication of a good level of agreement (Cartier et al., 2006; Nestrud & Lawless, 2008). The RV coefficients between the three variations of PM and DSA were high (RV $\geq$ 0.86), thus PM appears to be a reliable method for the sensory profiling of infusions of these *Cyclopia* species. The global PM method showed the highest RV coefficient (RV = 0.90), indicating that this variation of the PM method resulted in the most reliable product configuration when evaluating all attributes associated with infusions of *Cyclopia* species. This contradicts the results by Dehlholm et al. (2012) who found higher RV coefficients for partial PM when comparing partial PM and global PM to conventional DSA when profiling commercial liver pâtés. Louw et al. (2013), profiling a large sample set of brandies, also concluded that better results were obtained using partial PM.

3.2 Panel performance of the PM task

3.2.1 Positioning of blind duplicates

Two blind duplicate samples were included to determine assessors' variability and the difficulty in performing the PM task (Hopfer & Heymann, 2013). In the overall MFA product configurations, as presented in Figs. 3 to 5, samples Lon_B2 and Lon_B2_dup and Int_B3 and Int_B3_dup represented the blind duplicate samples. The samples and their blind duplicates were positioned close to each other for global PM (Fig. 3) and partial PM on palate (Fig. 5), but when only aroma was considered the *C. longifolia* duplicates were further apart (Fig. 4). A good level of accuracy could therefore be obtained when applying global PM and partial PM on palate, while partial PM on aroma seemed to be less accurate. It was anticipated that the panel would find partial PM on aroma easier to conduct than global PM or partial PM on palate. However, the lower accuracy of PM on aroma could be explained by the fact that one of the duplicate samples (Lon_B2_dup) formed part of the group of samples to the left of the product map, associating with the green notes and possible taints. Assessors might have found it more difficult to distinguish between these slightly tainted samples when considering only aroma attributes. Although PM is regarded as a spontaneous, easy to conduct task, it seemed that some assessors might have found the task difficult to perform.

Inclusion of blind duplicate samples could furthermore be valuable to measure the accuracy of individual assessors. Bertuccioli (2011), as cited by Hopfer and Heymann (2013), proposed the use of the people performance index (PPI) to test repeatability of assessors. The PPI is the ratio of the Euclidian distance between two replicated products and the maximum Euclidian distance between two different products on a PM plot. The PPI ranges between 0 and 1 and a smaller value indicates that the assessor placed identical products together on the map. Table 4 gives the calculated PPI values per assessor for the three variations of PM. PPI 1 and PPI 2 were calculated for the Int_B3 and Lon_B2 samples, respectively. The majority of assessors can be regarded as accurate, given that the PPI values for both PPI 1 and PPI 2 were low for all assessors, except for assessors 2 and 7. A PPI 1 value of 0 was calculated for assessor 2, indicating similar placements for this duplicate sample when conducting global PM. This assessor, however, struggled with the placement of the second duplicate sample for both global and partial PM on aroma. According to Table 4, high PPI values for both PPI 1 and PPI 2 for global PM were calculated for assessor 7. These results indicate that this assessor had difficulty in executing the PM task when evaluating all attributes.

3.2.2 Consistency of assessors using RV coefficients

The consistency of assessors over replications can be evaluated by comparing the RV coefficients of assessors per replication. RV coefficients > 0.5 between individual assessors and the MFA consensus plot per replication are regarded as sufficient consensus (Louw et al., 2013). RV coefficients for the MFA configurations of assessors for three replicates of global PM, partial PM on aroma and partial PM on palate compared to the overall MFA configuration per task were calculated and are presented in Table 5. Of the ten assessors, six were introduced to the PM task for the first time (assessors 1, 2, 3, 7, 9 and 10).

The RV values for the majority of assessors indicated a high similarity between replicate sessions of the respective PM tasks for these assessors. However, low RV coefficients for all replications of PM on aroma, as well as some replications of the other two variations of PM are apparent for assessors 2 and 7. Low RV coefficients for global PM and partial PM on palate were also observed for assessor 3. The lower RV coefficients for assessors unfamiliar to the PM task, could be an indication that these assessors found the task difficult or used different placing criteria when carrying out replications of the task. Removing the data of assessors with low RV coefficients did not influence the overall MFA configuration and data for all the assessors were therefore included in the final analysis. This is in accordance with research on projective mapping where analysis was found to be very similar with and without inclusion of lower performing assessors (Valentin, Cholet, Hervé, & Nestrud, 2016).

3.2.3 Repeatability of assessors using a similarity index

Tomic et al. (2015) proposed the use of a similarity index (SI) based on MFA to measure the degree to which the consensus product configuration represents each assessor's product matrix. In the current study, the similarity index as proposed by Tomic et al. (2015) was modified to determine assessor repeatability over replications. It measures the degree to which the consensus map per assessor represents the product maps of the three replications of the same assessor. The SI will be zero if the projected product configuration per replication is similar to the consensus product matrix, whereas a higher value indicates increased dissimilarity. In this study SI values <0.8 was regarded as sufficient consensus between the projected configuration of a replication and the consensus matrix per assessor. The similarity indices for the repeatability per assessors over three replications of global PM, partial PM on aroma and partial PM on palate are represented in Fig. 6.

Comparison of the MFA SI values of partial PM on aroma showed acceptable repeatability for six of the 10 assessors (SI values <0.8). However, assessors 2, 3, 7 and 9 showed low repeatability over three replications of partial PM on aroma. When comparing the SI values for partial PM on palate, seven assessors showed acceptable repeatability (SI values <0.8) while assessors 2, 3 and 7 showed low repeatability. The same performance was achieved for global PM. A comparison of MFA SI values for the three variations of PM indicated that assessors were more consistent when executing global PM and partial PM on palate than partial PM on aroma.

When comparing the SI results (Fig. 6), the RV coefficients (Table 5) and the calculated PPI (Table 4), low repeatability for the same three assessors, i.e. assessors 3 and 7, and assessor 2 to a lesser extent, is indicated. This study was the first introduction to the PM task for these assessors. The PM task is generally regarded as spontaneous and easy to conduct, but these three assessors might have found the task intricate. Furthermore, these assessors could have used different placement criteria in the replicate sessions of the PM task. However, removal of the data of these assessors did not change the consensus product configuration.

3.3 Effect of replications

RV coefficients were calculated between each of the three replicates for global PM, partial PM on aroma and partial PM on palate, considering the first two dimensions of each configuration (Table 6). The RV coefficients for a set of replications within a PM method were >0.8, indicating good repeatability. The similarity of the product configurations for the different replications was also evident from visual inspection of the respective spatial maps obtained for a PM method (data not shown). Considering the high RV coefficients (>0.8) for replications, it could be argued that one replicate per PM task would be sufficient to obtain a valid product configuration. The samples in the current study showed distinct sensory differences and most assessors were able to distinguish between samples on the main attributes. If the aim is to apply a rapid method for broad profiling of samples that have distinct differences, one replication would be sufficient. When samples are very similar, i.e. only subtle differences are evident, more replications would be advisable. Hopfer and Heymann (2013) recommend replicated PM tasks to ensure that judges can identify similarities and dissimilarities in repeated sessions. Louw et al. (2014) also advised replicate sessions to reduce the risks associated with PM and to ensure valid product configurations. Future research needs to address the efficacy and validity of PM for the rapid profiling of honeybush samples with only subtle differences in sensory profile.

4. Conclusions

Projective mapping can successfully be used for the broad sensory profiling of a complex product such as honeybush infusions. Although current RV coefficients indicate high repeatability over replications within a PM variation, at least two replications are recommended to ensure valid product configurations. A comparison of the variations of PM indicated that global PM is the most effective method for the rapid sensory profiling of herbal tea infusions; therefore, this method could be applied as a valid and reliable tool in quality control programmes in the honeybush herbal tea industry.

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Table 1 *Cyclopia* species with sample codes used for DSA and PM tasks. B denotes batch, 1–9 refer to batch numbers.

Cyclopia species	Total set of samples used for DSA	Subset of samples used for PM
C. genistoides	Gen_B1 to Gen_B7	Gen_B1, Gen_B4
C. subternata	Sub_B1 to Sub_B9	Sub_B2, Sub_B3
C. maculata	Mac_B1 to Mac_B6	Mac_B1, Mac_B2
C. longifolia	Lon_B1 to Lon_B6	Lon_B2, Lon_B5
C. intermedia	Int_B1 to Int_B8	Int_B1, Int_B3
Blind duplicates		Lon_B2_dup*, Int_B3_dup**

* Blind duplicate sample for *C. longifolia*, batch 2

** Blind duplicate sample for *C. intermedia*, batch 3.

Table 2 Sensory lexicon describing aroma, flavour, taste and mouthfeel characteristics of infusions of *Cyclopia* species.

Aroma attributes	Descriptors	Flavour, taste and mouthfeel attributes	Descriptors
Floral	Fynbos-floral[a]	*Floral*	Fynbos-floral[a]
	Rose geranium		Rose geranium
	Rose/Perfume		Rose/Perfume
Fruity	Lemon/Lemongrass	*Fruity*	Lemon/Lemongrass
	Apricot jam/Apricot		Apricot jam/Apricot
	Apple cooked		Apple cooked
Woody	Woody	*Woody*	Woody
	Pine		Pine
Sweet-associated	Fruity-sweet	*Spice*	Sweet spice / Cassia
	Caramel		Coconut
	Honey	*Taints*	Dusty
	Fynbos-sweet		Medicinal
Spice	Sweet spice / Cassia		Burnt caramel
Nutty	Walnut		Rotting plant water
	Coconut		Hay/dried grass
Taints	Dusty		Green grass
	Medicinal		Cooked vegetables
	Burnt caramel		
	Rotting plant water	*Taste*	Sweet
	Hay/dried grass		Sour
	Green grass		Bitter
	Cooked vegetables	*Mouthfeel*	Astringent

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

Table 3 RV coefficients for the correlation between the product configurations obtained with DSA, global PM, partial PM on aroma and partial PM on palate of five *Cyclopia* species.

	DSA	Global PM	Partial PM on aroma	Partial PM on palate
DSA	1.00	0.90	0.89	0.86
Global PM	0.90	1.00	0.93	0.95
Partial PM on aroma	0.89	0.93	1.00	0.95
Partial PM on palate	0.86	0.95	0.95	1.00

Table 4 People performance indices (PPI) for all assessors for three variations of PM.

Judge	Global PM		Partial PM on aroma		Partial PM on palate	
	PPI 1[a]	PPI 2[b]	PPI 1[a]	PPI 2[b]	PPI 1[a]	PPI 2[b]
1*	0.00	0.20	0.05	0.05	0.00	0.41
2*	0.00	0.55	0.16	0.63	0.25	0.15
3*	0.41	0.31	0.46	0.47	0.26	0.15
4	0.21	0.30	0.24	0.32	0.22	0.19
5	0.00	0.18	0.06	0.11	0.00	0.23
6	0.14	0.13	0.04	0.28	0.16	0.19
7*	0.71	0.66	0.27	0.47	0.37	0.43
8	0.00	0.17	0.00	0.00	0.00	0.00
9*	0.00	0.25	0.00	0.03	0.00	0.40
10*	0.05	0.09	0.15	0.15	0.03	0.14

*First introduction to the PM task for these assessors

[a]PPI 1, PPI calculated for *C. intermedia*, batch 3

[b]PPI 2, PPI calculated for *C. longifolia*, batch 2

Table 5 RV coefficients for the correlation of MFA configurations of assessors for three replications (Rep 1–3) of global PM, partial PM on aroma and partial PM on palate compared to the overall MFA solution per task.

	MFA PM global			MFA PM on aroma			MFA PM on palate		
Judge	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1*	0.83	0.83	0.86	0.75	0.72	-**	0.63	0.79	0.87
2*	0.72	0.61	0.49	0.49	0.43	0.29	0.54	0.18	0.82
3*	0.40	0.32	0.25	0.56	0.34	-	0.41	0.67	0.28
4	0.79	0.70	0.72	0.49	0.72	0.77	0.70	0.79	0.75
5	0.80	0.79	0.79	-	0.73	0.73	0.82	0.89	0.84
6	0.76	0.80	0.77	0.76	0.80	0.84	0.71	0.81	0.82
7*	0.17	0.55	0.35	0.31	0.27	0.34	0.52	0.39	0.50
8	0.83	0.66	0.75	0.67	0.75	0.77	0.73	0.72	0.76
9*	0.62	0.83	0.72	0.78	0.76	0.76	0.70	0.81	0.76
10*	0.65	0.44	0.56	0.70	0.67	0.83	0.62	0.50	0.43

*First introduction to the PM task for these assessors

**Incomplete PM task, all samples not placed

Table 6 RV coefficients for the correlation between the product configurations per PM technique and replicate sessions (Rep 1–3) of global PM, PM on aroma and PM on palate of five *Cyclopia* species.

	Global PM				Partial PM on aroma				Partial PM on palate		
	Rep 1	Rep 2	Rep 3		Rep 1	Rep 2	Rep 3		Rep 1	Rep 2	Rep 3
Rep 1	1.00	0.86	0.89	Rep 1	1.00	0.88	0.94	Rep 1	1.00	0.84	0.84
Rep 2	0.86	1.00	0.90	Rep 2	0.88	1.00	0.90	Rep 2	0.84	1.00	0.92
Rep 3	0.89	0.90	1.00	Rep 3	0.94	0.90	1.00	Rep 3	0.84	0.92	1.00

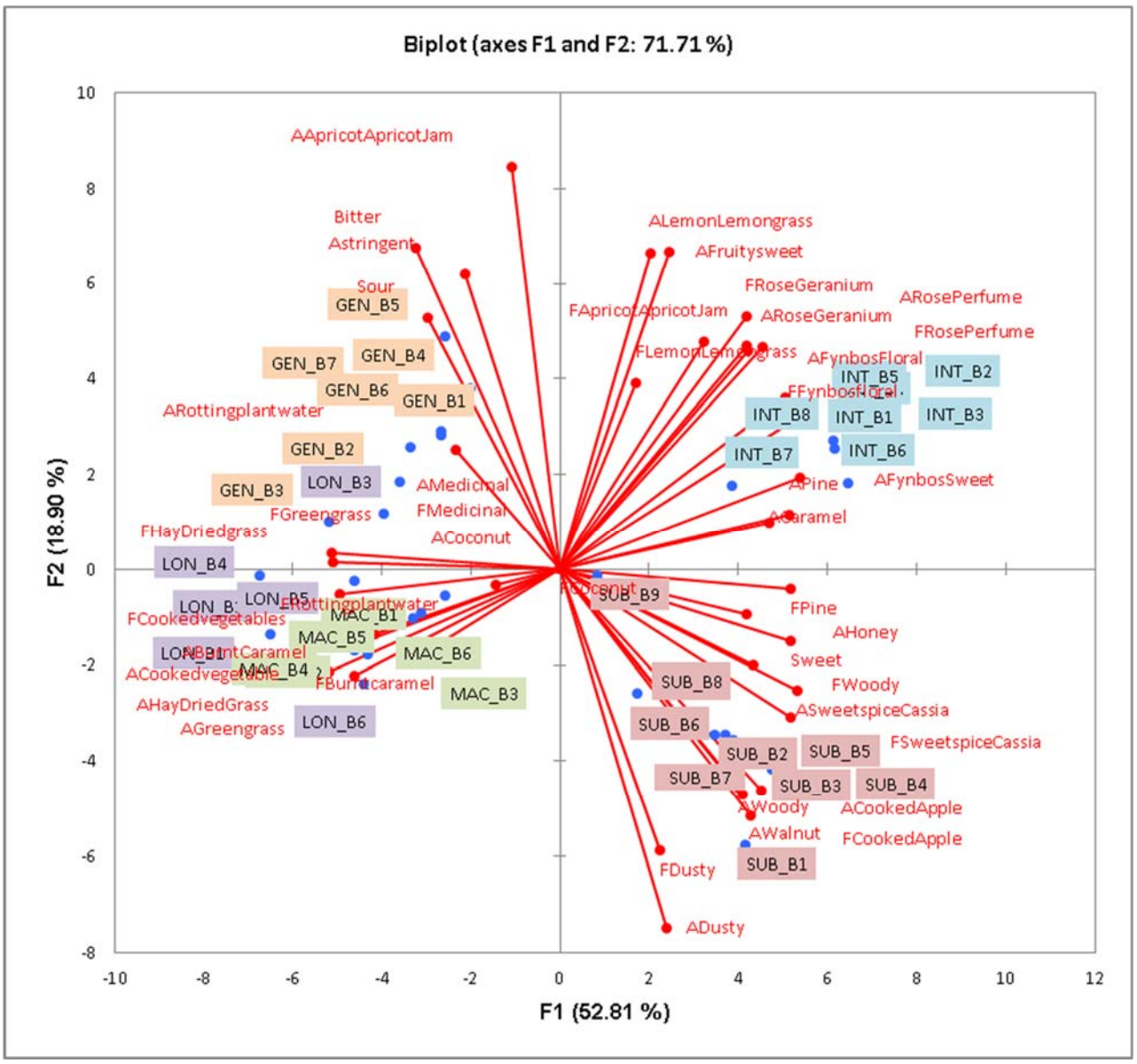


Fig. 1. PCA bi-plot representing the differentiation among five *Cyclopia* species obtained with DSA (total sample set). Capital letter added to attributes indicate A: aroma (orthonasal) and F: flavour (retronasal). The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia,* respectively. B refers to batches, 1–9 refer to the batch number.

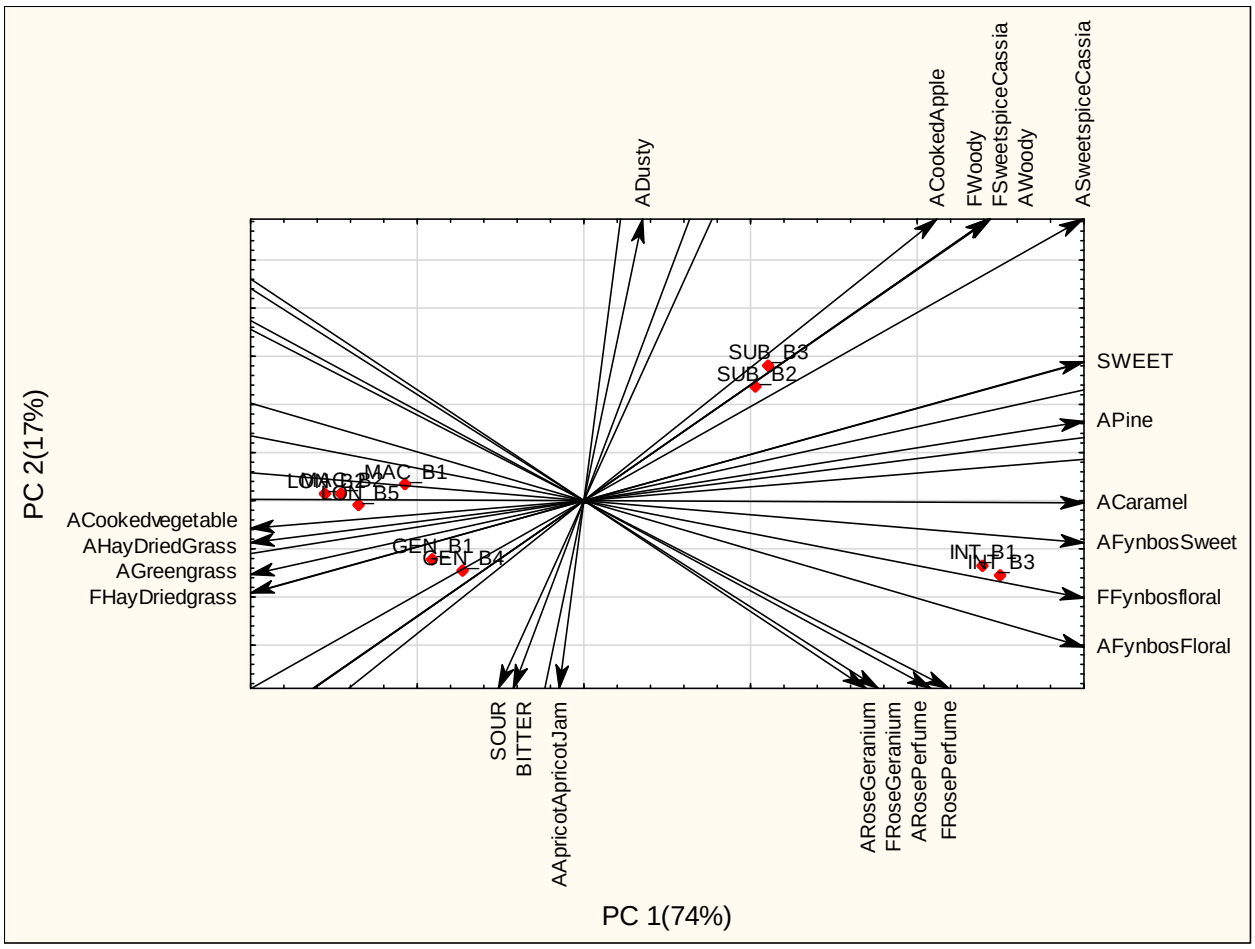


Fig. 2. PCA bi-plot representing the differentiation among five *Cyclopia* species obtained with DSA. Samples selected for PM task are included. Capital letter added to attributes indicate A: aroma (orthonasal) and F: flavour (retronasal). The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia,* respectively. B refers to batches, 1–5 refer to the batch number.

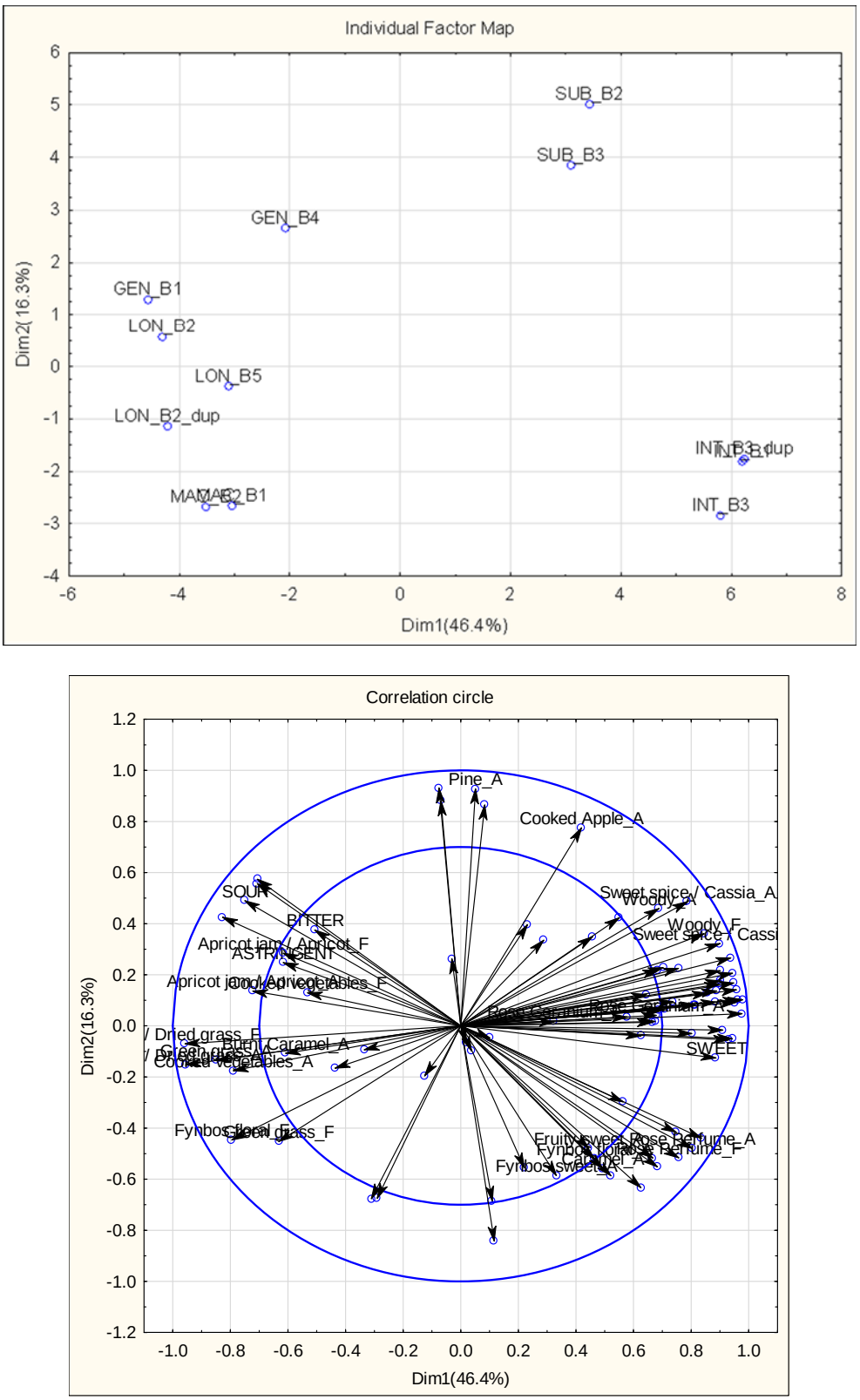


Fig. 3. Individual factor map and correlation circle of attributes obtained from global PM of five *Cyclopia* species. Capital letters added to attributes indicate A: aroma (orthonasal) and F: flavour (retronasal). The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia,* respectively. B refer to batches, 1–5 refer to the batch number.

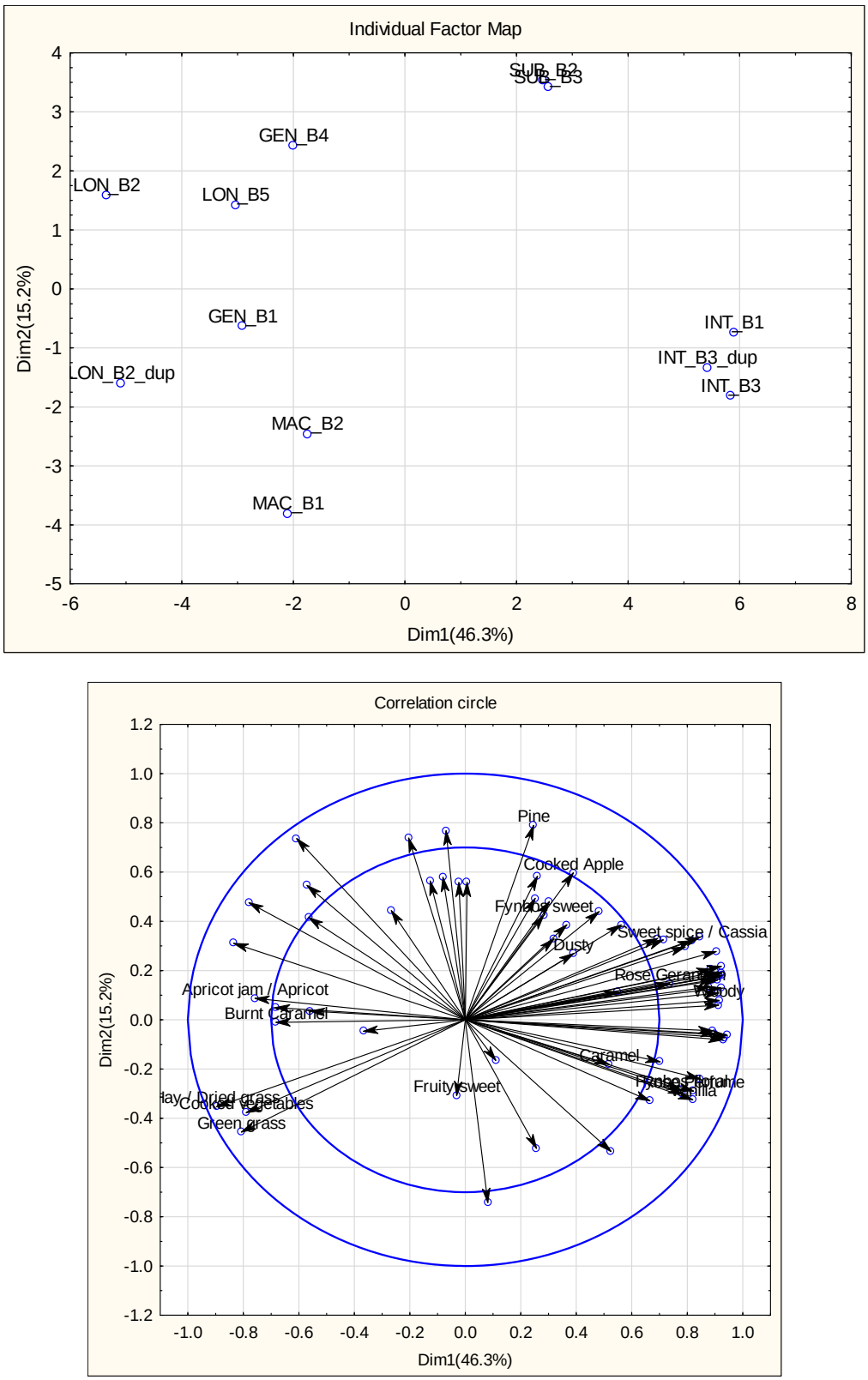
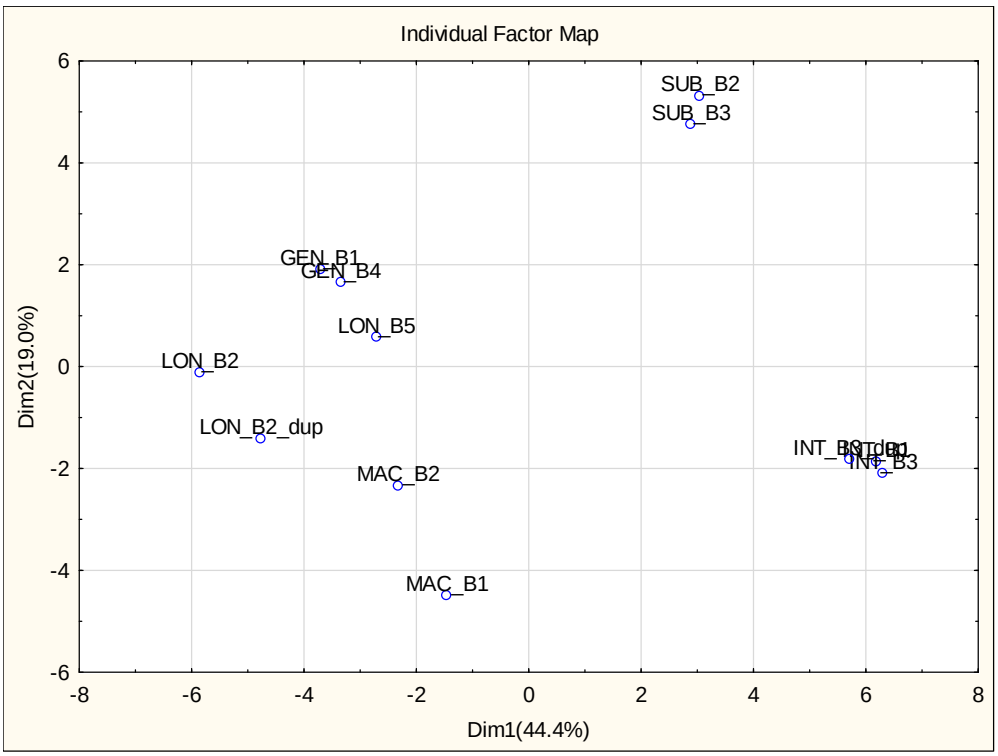


Fig. 4. Individual factor map and correlation circle of attributes obtained from partial PM on aroma of five *Cyclopia* species. The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides*, *C. maculata*, *C. longifolia*, *C. subternata* and *C. intermedia*, respectively. B refers to batches, 1–5 refer to the batch number.



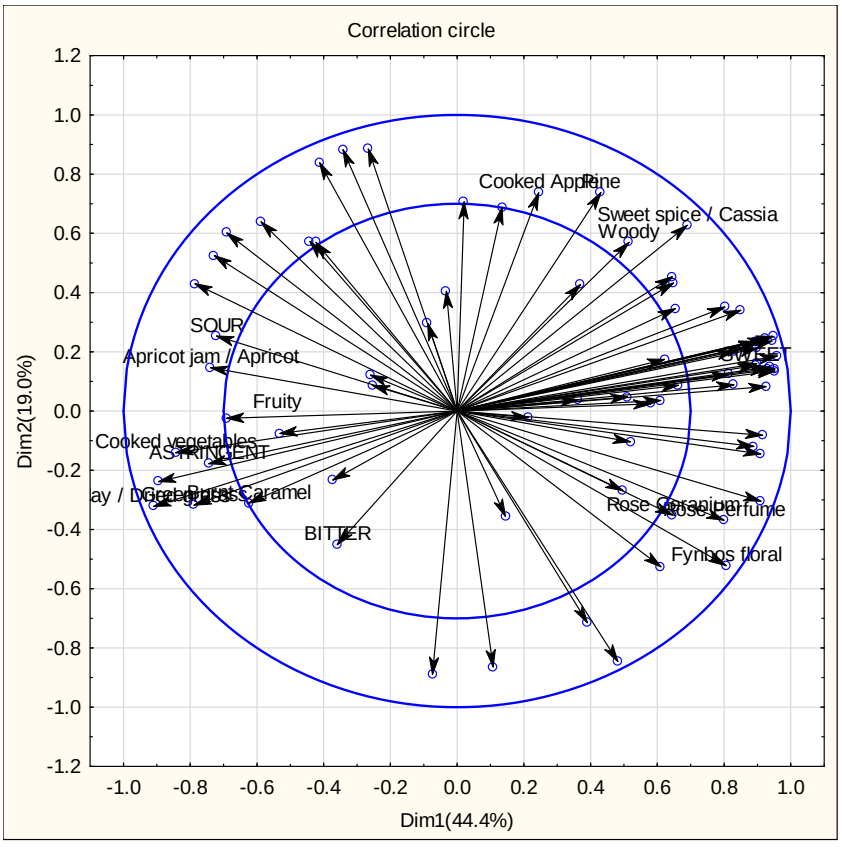


Fig. 5. Individual factor map and correlation circle of attributes obtained from partial PM on palate attributes of five *Cyclopia* species. The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia,* respectively. B refers to batches, 1–5 refer to the batch number.

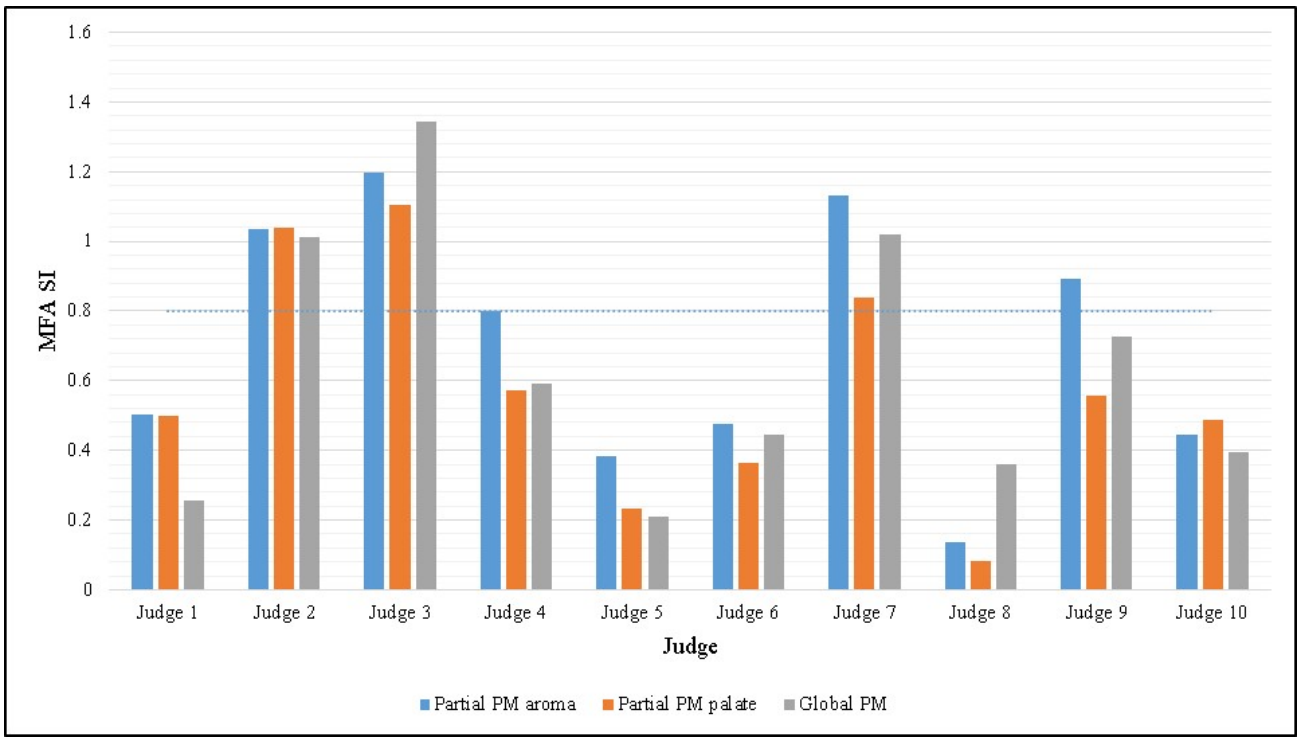


Fig. 6. Similarity indices (SI) based on MFA for the repeatability of assessors over three replications of global PM, partial PM on aroma and partial PM on palate. A SI value <0.8 is regarded as an acceptable level of accuracy.

ADDENDUM A

Instruction sheet: Global projective mapping

HONEYBUSH 2015

Day 3 – Monday, 22nd June 2015

SESSION 3: GLOBAL PROJECTIVE MAPPING

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

INSTRUCTIONS

- You have been presented with **12 honeybush samples** labelled with a three-digit code.
- Please evaluate the samples and place them on the provided space according to how **SIMILAR or DISSIMILAR** they are for you.
    - You are allowed to **smell** and **taste** the samples as many times as you like and in any order.
    - On the large A2 paper that is provided, position the samples according to how similar or dissimilar they are for you. The more similar the samples are, the closer they should be positioned to each other, the more dissimilar they are the further apart they should be positioned.
    - You may form **as many groups as you wish,** but **a minimum of TWO and NOT MORE THAN 10 GROUPS.**
- Once you have evaluated all samples:
    - **Write down the sample codes** on the A2 paper where you have positioned them.
    - W**rite down the criteria** and/or descriptive terms for your groups. There is no right or wrong answer! You may use the list with aroma and palate attributes provided.
    - Use the label with your name and session no and stick that in any open space on the A2 paper.
    - **NOTE**: Please try to work as quickly as possible to **prevent the samples from cooling down** too much.

ADDENDUM B

Raw data: Samples with co-ordinates (x; y) per assessor, followed by a descriptor block, for global projective mapping

DAY 3 - GLOBAL NAPPING ON 2014 HB SAMPLES

Samples 10 samples (2/species) + 2 blind duplicates per rep = 12 samples / rep

3 reps on same 12 samples

Paper size: rectangular 640 mm x 455 mm

22-Jun-15

Rep	Sample	Judge	x	y	Fynbos floral_A	Rose Geranium_A	Rose Perfume_A	Lemon / Lemon grass_A	Apricot jam / Apricot_A	Cooked Apple_A	Woody_A	Fruity sweet_A
1	GEN_B1	1	280	50	0	0	0	0	0	0	0	0
1	INT_B1	1	540	400	1	1	1	0	0	0	0	0
1	MAC_B1	1	280	50	0	0	0	0	0	0	0	0
1	LON_B2	1	105	261	0	0	0	0	0	0	1	0
1	SUB_B2	1	165	342	0	0	0	0	0	0	1	0
1	GEN_B4	1	280	210	0	0	0	1	1	0	0	0
1	INT_B3	1	540	400	1	1	1	0	0	0	0	0
1	MAC_B2	1	280	50	0	0	0	0	0	0	0	0
1	LON_B5	1	305	136	0	0	0	0	1	0	0	0
1	SUB_B3	1	165	380	0	0	0	0	0	0	1	0
1	DUP_INT_B3	1	540	400	1	1	1	0	0	0	0	0
1	DUP_LON_B2	1	280	50	0	0	0	0	0	0	0	0
1	GEN_B1	2	380	225	0	0	0	0	0	0	0	0
1	INT_B1	2	80	34	0	1	0	0	0	0	1	0
1	MAC_B1	2	380	225	0	0	0	0	0	0	0	0
3	SUB_B3	10	570	254	0	0	0	0	0	0	1	0
3	DUP_INT_B3	10	410	353	1	0	0	0	0	0	1	0
3	DUP_LON_B2	10	160	356	1	0	0	0	1	0	0	0

Chapter 6

Directed sorting for sensory characterisation of a complex product: "cup-of-tea" infusions of *Cyclopia* species as case study

Abstract

The validity of sorting as rapid method for the broad sensory characterisation of food and beverages has been addressed in recent years, but research on its effectiveness for the sensory profiling of complex products where temperature control is needed, is limited. Directed sorting was investigated using infusions prepared from five *Cyclopia* species (honeybush) at "cup-of-tea" strength. These species produce herbal teas with similar, but also distinct differences in their overall sensory profiles, respectively linked to characteristic honeybush and species-specific sensory attributes. A trained panel of 9 assessors, provided with a list of attributes, sorted 15 samples representing five *Cyclopia* species based on aroma, palate or all attributes (global sorting). Descriptive sensory analysis (DSA) provided a detailed sensory profile of the same samples for comparison. Sorting data were analysed using cluster analysis, DISTATIS and correspondence analysis (CA). Similar product configurations when comparing the sorting DISTATIS plots and DSA PCA bi-plot were confirmed by high RV coefficients. Although RV coefficients for replicate sessions within a sorting method were high, more stable results were obtained by conducting replicating sessions. A novel analysis was performed, comparing descriptors used in DSA to that of CA using multiple factor analysis (MFA), revealing high similarity in descriptors used for DSA and sorting. Differentiation between samples with only subtle differences were obtained with sorting on palate. Global sorting was less effective to differentiate between different honeybush infusions. The longer list provided with global sorting, could have complicated the task for assessors. Comparison of the three variations of directed sorting indicated directed sorting on aroma and palate to be valid methods for the sensory characterisation of a complex product such as honeybush infusions when applied by trained assessors. Directed sorting on aroma or palate could find application in quality control programmes of the herbal tea industry.

Keywords: Sorting task, directed sorting, trained panel, *Cyclopia* species, cluster analysis, DISTATIS, correspondence analysis

1. Introduction

The sensory characterisation of food products plays a vital role in the food industry and is applied in different areas including research and development, quality control, shelf-life studies and marketing research. Monitoring the sensory quality of products is an essential part of routine quality control programmes to

maintain the sensory integrity of the product (Varela & Ares, 2014). Classic descriptive sensory analysis (DSA) relies on an analytical approach to product characterisation and has been extensively applied for detailed, qualitative and quantitative analysis (Lawless & Heymann, 2010; Murray, Delahunty, & Baxter, 2001) of products. The disadvantage of DSA is that the maintenance of a well-trained panel, a pre-requisite for valid DSA results, is costly and time consuming (Ares, 2015; Murray et al., 2001; Varela & Ares, 2014). The need for sensory methods that are time- and cost-effective, and which can incorporate consumers' perceptions, led to the development of rapid sensory profiling methods. The sorting task, one of the most applied rapid sensory profiling methods, has a holistic approach and the sensory configuration of the products is based on the similarity or dissimilarity of the overall sensory perception (Chollet, Lelièvre, Abdi, & Valentin, 2011).

The sorting task, also referred to as "free sorting" (Chollet, Valentin, & Abdi, 2014), was introduced to the sensory domain to determine the perceptual structure in odours (Lawless, 1989; Lawless & Glatter, 1990; MacRae, Howgate, & Geelhoed, 1990) and later applied to a range of food products including vanilla beans (Heymann, 1994), cheese (Lawless, Sheng, & Knoops, 1995), breakfast cereals (Cartier et al., 2006), beer (Lelièvre, Chollet, Abdi, & Valentin, 2008) and wine (Bécue-Bertaut & Lê, 2011). It is a categorisation method where products with perceived similarities are grouped together (Chollet et al., 2011; Lawless et al., 1995). The method originated in psychology since sorting of products into groups which share similar perceived characteristics is a natural cognitive process, routinely applied in everyday life (Chollet et al., 2011).

The sorting task is conducted in one session; all samples are presented simultaneously with each assessor receiving the samples in a different order. Assessors are instructed to evaluate the samples and then sort them into groups according to perceived similarities, however, the groups should be mutually exclusive (Chollet et al., 2011). Assessors use their own criteria to form groups of similar products; they can put as many products into a group and form as many groups as they wish. The sorting task can end at this point or a descriptive step can be added where assessors are asked to add descriptive terms to each group, referred to as "labelled sorting" (Bécue-Bertaut & Lê, 2011). Assessors can use their own set of descriptors but this poses some difficulty to the sensory scientist during data analysis when interpretation of descriptive terms would be required (Bécue-Bertaut & Lê, 2011). Such difficulty can be overcome by providing assessors with a list of descriptors applicable to the set of samples being evaluated (Lelièvre et al., 2008).

The sorting task is easy to conduct and understand, and can be performed by trained and untrained assessors; furthermore, product configurations obtained are comparable to that of classic descriptive analysis (Cartier et al., 2006; Chollet et al., 2011). Although conventional profiling and sorting result in similar product maps, classic descriptive analysis provides a more detailed and precise product description which is easier to interpret than the sorting data (Chollet et al., 2011). The sorting task cannot be applied if quantification of product differences is required (Cartier et al., 2006).

One of the limitations of sorting is that all samples have to be presented simultaneously. Furthermore, only products that remain stable with regard to temperature and structure throughout the sensory session can be used for the sorting task (Cartier et al., 2006; Chollet et al., 2014). The number of products that can be evaluated successfully in one session depends on the complexity of the product, as well as on the ability and level of training of the assessors. Assessors need to re-taste when they cannot remember the sensory character of a product and re-tasting increases with increased number of samples. Assessors also tend to re-taste when products are very similar. Chollet et al. (2011) recommends between 9 and 20 samples for the sorting task, with the optimum number of samples suggested being 12.

Different variations of the sorting task have been introduced to the sensory domain. The first variation, directed sorting, entails that assessors are restricted with regard to the number and/or type of groups formed. Assessors could be asked to sort products to specific modalities (aroma, flavour or global attributes) or to form groups according to specified aromas (Lawless, 1989).

Research is still needed to determine the validity of rapid methods for the characterisation of complex products or products with only small perceptual differences (Valentin, Chollet, Lelièvre, & Abdi, 2012). Limited research has been published on the efficacy of the sorting task as applied to a complex product where temperature control is essential. The first objective of the current study was therefore to determine how effective is sorting for the sensory characterisation of a complex product such as honeybush "cup-of-tea" infusions that must be evaluated at a constant hot temperature, simulating a consumer's drinking experience of a hot beverage.

Several *Cyclopia* species are used for the preparation of honeybush herbal tea (Joubert, Joubert, Bester, De Beer, & De Lange, 2011). Previously we demonstrated that projective mapping (PM) could be a suitable sensory tool for screening infusions prepared from different *Cyclopia* species (Chapter 5). Although both PM and sorting are categorisation techniques, Veinand, Godefroy, Adam and Delarue (2011) reported that PM was difficult to perform especially for panel members having difficulty with handling spatial information. Sorting thus offers an alternative to PM, meriting its investigation as a potential rapid sensory method for evaluation of honeybush infusions. Applying the sorting task for the broad sensory characterisation of the different *Cyclopia* species and identifying production batches that are atypical could save the industry time and money. The second objective of the study was to determine if sorting and DSA would result in similar sensory characterisation, thus confirming the validity of sorting as rapid characterisation method for such a complex product as heoneybush. The validity of directed sorting on aroma, palate or global sorting (all attributes) was determined by comparing product configurations to that obtained with DSA. The effect of replication of the sorting task was also addressed to clarify whether results would be improved or unnecessarily be complicated, especially when applied in industry.

2. Materials and methods

2.1 Descriptive sensory analysis

DSA was used for the sensory profiling of randomly selected, independent batches of five *Cyclopia* species of harvest year 2014/2015, as described in Chapter 5. A standardised protocol for sample preparation and serving of samples at 65°C is described in Chapter 5. Sample codes for the respective *Cyclopia* species used in DSA and the sorting task are presented in Table 1.

2.2 Sorting

2.2.1 Samples and sample selection

The sample configuration obtained through principal component analysis (PCA) of the DSA results was used as basis to select three batches per *Cyclopia* species for inclusion in the sorting task. Batches that could be regarded as most representative of the respective species, based on previous research on sensory profiling of *Cyclopia* species (Bergh, Muller, Van der Rijst, & Joubert, 2017; Erasmus, Theron, Muller, Van der Rijst, & Joubert, 2017; Theron, Muller, Van der Rijst, Cronje, Le Roux, & Joubert, 2014) were selected (Fig. 1). This resulted in a total of 15 samples (5 species x 3 batches per species) being presented simultaneously to each panel member for evaluation per session. The infusions were prepared in the same manner as for DSA (described in Chapter 5).

2.2.2 Directed sorting task

The same panel used for DSA performed the sorting task. Following a brief explanation of the sorting task and a short training exercise, panel members were instructed to smell and/or taste the samples in the order presented. Thereafter they were allowed to smell and taste the samples as many times as they wanted to and in any order. The entire set of samples was presented simultaneously to each panel member with each panel member receiving the samples in a specific randomised order. Three variations of the sorting task were applied: directed sorting according to aroma attributes (sorting on aroma), directed sorting according to palate attributes (sorting on palate) and directed sorting on all attributes (global sorting). Panel members were asked to sort samples into as many groups as they wanted, but they were limited to five groups. No limitation was placed on the number of samples per group, nor was the time to complete the task, limited. Upon completion of the sorting task, assessors had to record the three-digit codes of the samples in each group on an answering sheet.

After completion of the sorting task, assessors were instructed to add descriptors to each group. The same list of attributes applied during DSA was provided to ensure uniformity in descriptors. For sorting aroma, a list comprising of 22 aroma descriptors was provided, while a list of 21 terms describing flavour, taste (sweet, sour and bitter) and mouthfeel (astringency) was provided for sorting on palate. Global sorting required that all attributes (n = 43) be taken into account when adding descriptors to groups of samples. Groups were mutually exclusive. The complete list of descriptors used for DSA and for the respective sorting tasks is

provided in Chapter 3. An example of the instruction sheet and questionnaire for global sorting is provided in Addendum A of this chapter. Paper, pencil and sticky notes were provided to make notes or as memory aids to reduce re-tasting. The three variations of directed sorting were performed on three consecutive days, with three replications per method per day. Assessors were requested to take a 15 min break between replicates to avoid panel fatigue. The same palate cleansers as for DSA were used. Assessors were seated in a temperature-(21°C) and light-controlled room, at individual tables rather than booths as this provided enough space for the water bath and paraphernalia required for the sorting task. All the assessors completed the three replicates of the sorting within a two-hour-period.

2.3 Statistical procedures

2.3.1 DSA

The statistical analysis of the DSA data was performed as described in Chapter 5.

2.3.2 Sorting

2.3.2.1 Cluster analysis

Cluster analysis is a statistical classification method to construct dendograms that give an indication of the structure of the data. Objects are classified into groups or clusters where objects in a group are more similar to each other than to objects in another group. Cluster analysis was used as an exploratory procedure to reveal the structure in the sorting data. Ward's method, based on minimum variance cluster analysis, was applied and is designed to generate clusters in such a way as to minimise the within-cluster variance (Punji & Stewart, 1983). Cluster analysis was performed on the three sets of data representing sorting on aroma, sorting on palate and global sorting.

2.3.2.2 DISTATIS

The sorting data were subsequently analysed using DISTATIS as described by Abdi, Valentin, Chollet and Chrea (2007). A schematic illustration of the DISTATIS analysis of sorting data is provided in Fig. 2. The data were first captured in an indicator matrix per assessor per replication where samples were indicated in rows and groups in columns. Thereafter, the indicator matrix of the sorting task of each assessor was encoded in an individual co-occurrence matrix where the rows and the columns represent the products and a value of 1 across a row and a column indicates that the assessor grouped the products together while a value of 0 indicates that the products were not grouped together. The co-occurrence matrix per assessor was then transformed into a distance matrix where rows and columns are products and a value of 0 across a row and a column indicates that these products were grouped together by the assessor while a value of 1 indicates that the products were not grouped together. The individual cross-product matrices were aggregated to form a compromise cross-product configuration representing a consensus between assessors' individual matrices. The compromise cross-product matrix is a weighted average of the individual assessors' matrices (Abdi, O'Toole, Valentin, & Edelman, 2005). Assessors that show the highest agreement, contribute larger weights

to the compromise configuration. The weights of assessors were determined by computing RV coefficients between all pairs of assessors.

The RV coefficient is a multivariate similarity coefficient used to measure the extent to which two product configurations are similar (Abdi et al., 2007). An RV coefficient of 1 indicates that assessors grouped their samples in exactly the same way while a value of 0 would indicate that they used a totally different way of grouping their products. Assessors that grouped their samples in a similar way will therefore contribute a larger weight to the compromise cross-product matrix. An extract of an indicator matrix is provided in Addendum B.

Analysis of sorting data using DISTATIS results in two maps: the first map is a map of the assessors, indicating the assessors' agreement while the second map is a map indicating the relative placement of products. In both cases, the proximity between two points on the map (assessors or products), reflects their similarity (Abdi et al., 2007).

2.3.2.3 Correspondence analysis

During sorting, assessors described each group of products using the provided attribute list. The assumption was made that descriptors assigned to a group of products were applicable to each product in the group. A contingency table was constructed with descriptors in rows and products in columns. The values in the contingency table indicate the frequency of use of terms to describe a product. As assessors were provided with a pre-determined list of attributes, no interpretation of attributes prior to data analysis was necessary. Descriptors used at low frequency were discarded and only descriptors with a count >20 were included in the analysis. Some assessors added terms such as low, medium and high to indicate the intensity of the attributes. When an attribute and intensity were mentioned >20 times, it was regarded as a separate attribute and included as such in the correspondence analysis. Correspondence analysis (CA) was used to elucidate the association between products and descriptors onto a two-dimensional sensory space. Confidence ellipses on the CA of the sorting data were calculated by performing a bootstrap methodology similar to that reported by Cadoret and Husson (2013) using R software (R Core Team, 2015).

2.3.2.4 Comparison of the sorting tasks to DSA

RV coefficients were calculated to determine correlations between the PCA bi-plot obtained for DSA data and the product configuration of the different variations of the sorting task as obtained through DISTATIS analyses. The first two components of the respective plots were taken into account when calculating the RV coefficients.

In addition to comparing results of DSA and sorting by visual inspection of product configurations and calculating RV coefficients, a novel analysis was performed, comparing descriptors used in DSA to that of CA using multiple factor analysis (MFA). The data matrix for the MFA calculation consisted of two data tables: the DSA attributes were input variables in the first table followed by CA descriptors, with a descriptor count of >20, in the second table. The variables included for the MFA calculation were therefore the intensities per product and attribute, obtained with DSA, followed by the standardised deviates per product and descriptor

obtained with CA of the sorting task. The MFA resulted in an individual factor map with products and a correlation circle with attributes.

2.3.2.5 Effect of replication

The effect of replication was evaluated by two approaches, firstly, by visually comparing the DISTATIS plots of the replications per sorting task, and secondly, by calculating RV coefficients. An RV coefficient closer to 1 indicates high similarity between two maps while a value closer to 0 indicates dissimilar configurations. A RV coefficient of >0.7 was regarded as an indication of sufficient agreement between product configurations (Cartier et al., 2006; Faye et al., 2004).

Data analyses were performed using different packages of R software (R Core Team, 2015). DISTATIS analysis was performed using the DISTATISR package while FactoMineR was used to perform MFA and to compute RV coefficients (Lê, Josse, & Husson, 2008).

3. Results

3.1 Directed sorting on aroma, palate or global attributes

3.1.1 Cluster analysis

Ward's method of cluster analysis on the first three components was used as an exploratory procedure to reveal the structure in the sorting data. Cluster analyses of the three sorting variations revealed fairly similar dendograms with four distinct clusters. Cluster analysis of global sorting (Fig. 3) revealed four clusters, three formed by *C. subternata, C. intermedia and C. longifolia* samples, respectively, while samples of *C. genistoides* and *C. maculata* formed the fourth cluster. Directed sorting on aroma and palate (provided in Addendum C) similarly resulted in distinct clusters formed by *C. subternata* and *C. intermedia* samples while the *C. longifolia* samples were either grouped with the *C. genistoides* infusions or with the *C. maculata* infusions. These results revealed that the panel could differentiate between *C. subternata* and *C. intermedia* when sorting honeybush infusion in groups according to perceived similarity according to aroma and/or palate attributes, but the differentiation between the remaining three species were less distinct. The data were subsequently analysed using DISTATIS.

3.1.2 DISTATIS

The DISTATIS product configurations obtained with directed sorting on aroma, palate and global attributes of five *Cyclopia* species revealed similar spatial maps with the explained variance on the first two components of these plots being 44.3%, 47.5% and 48.7%, respectively. The DISTATIS configuration for global sorting is presented in Fig. 4, while DISTATIS plots for directed sorting on aroma and palate are provided in Addendum D. Three separate groups were observed in the DISTATIS plot for global sorting, with a clear distinction between *C. subternata* and *C. intermedia* samples towards the positive end of PC1. The third group, towards the left of the PC1, comprised of the *C. genistoides*, *C. maculata* and *C. longifolia*

samples. These results indicated that the panel were able to distinguish between *C. subternata* and *C. intermedia* and sorted these samples into two distinct groups when taking all attributes into account. The remaining three species were regarded as more similar, and the consensus sorting resulted in one group with an overlap of species.

3.1.3 Correspondence analysis

Correspondence analysis (CA) was used to visualise the relationship between the categories of two qualitative dependant variables (Abdi & Valentin, 2007), in this case the sorting data and the descriptor counts. Descriptors with counts >20 were included. The CA of sorting based on aroma (Fig. 5) revealed three distinct groups with no overlap of confidence ellipses between these groups. The first two dimensions account for 74.8% of the explained variance in the data. Samples representing *C. intermedia* formed a group toward the upper right of the plot and were associated with "rose perfume", "floral" and "fynbos sweet" aroma attributes and to a lesser extent with a "rose geranium" and "fruity sweet" aroma. Samples representing *C. subternata*, grouped towards the lower right of the plot, were associated with "sweet spice", "spicy", "pine", "dusty" and "woody" aroma attributes and to a lesser extent with "caramel" and "fynbos floral" aroma attributes. Samples representing the remaining species (*C. genistoides*, *C. maculata* and *C. longifolia*) formed one group towards the negative side of dimension 1, and were associated with "burnt caramel", "cooked vegetable", "grass", "hay" and "apricot" aroma attributes.

Correspondence analysis of sorting data on palate revealed four groups, however, the groups were not distinctly different as evident from slight overlapping of confidence ellipses (Fig. 6). Dimensions one and two account for 43.5 and 27.2% of the explained variance, respectively. Samples representing *C. subternata* and *C. intermedia* formed two groups towards the negative side of dimension 1 with only a slight overlap of confidence intervals. *Cyclopia intermedia* samples were associated with a "high fynbos floral", "fruity" and "rose geranium" flavour, while *C. subternata* associated with "spicy", "woody high", "fynbos floral" and "floral high" flavour attributes. Both these two species associated with a sweet and no bitter taste. Two groups, although not distinctly different as there is a slight overlap of confidence ellipses, were observed towards the right of the plot. The three *C. genistoides* samples and one *C. longifolia* sample formed one group, associated with bitter and sour taste, and "apricot" flavour. The *C. maculata* and remaining *C. longifolia* samples formed a group toward the upper right quadrant, and were associated with "hay", "fynbos", "grass" and "cooked vegetable" flavours.

In the correspondence analysis of global sorting (Fig. 7), dimensions one and two account for 73.1% of the explained variance. *Cyclopia subternata* and *C. intermedia* samples formed two groups towards the positive end of dimension 1 with a slight overlap of confidence ellipses. *Cyclopia subternata* associated with "fynbos" and "woody" aroma and flavour and to a lesser extent with "spicy" and "caramel" aroma attributes and a sweet taste. *Cyclopia intermedia* associated with a "spicy", "rose geranium", "fynbos floral" and "fynbos sweet" aroma and a sweet taste. During sorting of aroma, "spicy" associated more with *C. subternata,* therefore indicating a slight shift in association between samples and some attributes when comparing the

different variations of sorting. No clear differentiation between the remaining three species (*C. genistoides*, *C. maculata* and *C. longifolia*) was evident in the CA plot for global sorting.

Although a slight shift of attributes was observed when comparing CA plots for sorting on aroma and on palate, the overall description of samples and association of samples with attributes, were similar for these variations of sorting of honeybush infusions.

3.1.4 Effect of replications

Visual inspection of the three replications within sorting on palate or global sorting revealed similar product maps and a configuration similar to that of the consensus configuration of palate and global sorting. However, differences in the three replications for sorting on aroma were observed (Fig. 8) and therefore only results for directed sorting on aroma were included. The DISTATIS plots of the three replications of directed sorting on aroma of different *Cyclopia* species revealed three distinct groups of samples for both replication (rep) 1 and 2, while four groups were observed for rep 3 (Fig. 8). Based on aroma, assessors could distinguish between *C. subternata* and *C. intermedia* in replication 1 and 2, while the remaining three species were regarded as more similar in aroma in these two replications. However, for rep 3, assessors again sorted *C. subternata* and *C. intermedia* into separate groups, but further sorted the remaining samples into two groups, totalling four groups. One *C. longifolia* sample was grouped with *C. genistoides* to form a group towards the lower left quadrant of the DISTATIS plot, while the remaining two *C. longifolia* samples were grouped with *C. maculata*, forming a group towards the upper left quadrant.

RV coefficients were calculated between the DISTATIS plots of the replicate sessions and the consensus plot for directed sorting on aroma, palate and global sorting (Table 2). RV coefficients should be interpreted with caution as this correlation measurement puts the greatest emphasis on the dimension with the largest explained variance (Tomic, Berget, & Næs, 2015). The first two dimensions of the respective plots were taken into account when calculating the RV coefficients. High repeatability for directed sorting on aroma was obtained when comparing rep 1 and 2 (RV = 0.94), while RV <0.7 was obtained when comparing rep 3 with rep 1 and 2, thus indicating a poor level of agreement for rep 3.

In the case of directed sorting on palate and global sorting, RV coefficients calculated on the first two dimensions of the DISTATIS plots revealed high repeatability between replicate sessions and the consensus plot for these variations of the sorting task (RV $\geq$ 0.85).

3.2 Comparison of DSA and sorting

Validation of the sorting task as tool for the sensory description of honeybush infusions was done by visually comparing the product configurations obtained for the different sorting tasks with that of the DSA results as presented in the PCA bi-plot for the same set of samples (Fig. 9). The validity of the sorting task for sensory characterisation of a complex product was further determined by calculating the RV coefficients for the PCA bi-plot of the DSA data and DISTATIS plots of the sorting data.

3.2.1 Comparison of product configurations

The PCA bi-plot (Fig. 9) obtained from DSA data shows a product configuration with a clear differentiation between *C. intermedia* and *C. subternata* and the remaining three *Cyclopia* species. *Cyclopia intermedia* samples formed a group to the upper right quadrant of the plot and this species associated with "fynbos floral", "caramel", "fynbos sweet" and "pine" aroma and "fynbos floral" flavour. *Cyclopia subternata* samples grouped in the lower right quadrant of the plot, and associated with a "woody" and "cooked apple" aroma and flavour, "sweet spice/cassia" flavour and "walnut" aroma. *Cyclopia longifolia* and *C. maculata* were associated with a "cooked vegetables", "green grass" and "hay/dried grass" aroma and flavour and a "burnt caramel" aroma. Two of the *C. genistoides* samples (GEN_B4 and GEN_B1) associated with a sour and bitter taste and "apricot/apricot jam" aroma. There was no clear differentiation between *C. genistoides*, *C. maculata* and *C. longifolia*, positioned towards the left of the PCA bi-plot (Fig. 9). The first two components of Fig. 9 account for 78% of the explained variance, describing the most important sensory attributes associated with this set of samples. The main differentiation between samples on the first component is therefore the floral, woody, sweet and spicy notes toward the positive end of PC1 and the green and vegetative aromas towards the negative side of PC1.

Comparison of the PCA bi-plot obtained through DSA (Fig. 9) and the DISTATIS plots of the three variations of the sorting task, revealed highly similar product configurations. The DISTATIS product configuration of global sorting is presented in Fig. 4, while DISTATIS plots for sorting on aroma and palate are provided in Addendum D. Each of these product maps revealed a clear separation between *C. subternata* and *C. intermedia* on the one side, as opposed to a group positioned towards the opposite side of the plot, composed of *C. genistoides*, *C. maculata* and *C. longifolia*. Products were therefore grouped similarly in DISTATIS configurations obtained from sorting and in the PCA-bi-plot obtained from DSA.

Comparison of the PCA bi-plot (DSA data) with the CA plots of the different sorting tasks is also useful as CA visualises the relationship between the products and attributes, this is not possible with DISTATIS. Visual inspection revealed fairly similar product configurations for the PCA bi-plot (Fig. 9) and the CA plots of the sorting tasks (Fig. 5, 6 and 7). The respective plots revealed a differentiation between *C. subternata* and *C. intermedia*, where *C. subternata* associated with "woody", "spicy" and sweet attributes, while *C. intermedia* associated with "fynbos floral" attributes. No clear distinction between the remaining species (*C. genistoides*, *C. maculata* and *C. longifolia*) was clear on the respective plots. The PCA bi-plot of the DSA data (all attribues included) were compared to those based only on aroma or palate attributes. These plots were similar in overall product configurations. The PCA bi-plot based on only aroma or only palate attributes are presented in Addendum F.

3.2.2 RV coefficients

The RV coefficients for the similarity of the PCA bi-plot obtained with DSA and DISTATIS configurations for sorting on aroma, palate or global sorting are presented in Table 3. The RV coefficients between the three variations of the sorting task and the PCA bi-plot of DSA were high (RV > 0.86), indicating

that any of these variations of sorting are valid methods for the sensory profiling of infusions of *Cyclopia* species.

3.2.3 Product description

The product configuration and associated correlation circle obtained from MFA of DSA attribute intensities and CA descriptor standardised deviates of sorting aroma are depicted in Fig. 10. Dimensions 1 and 2 account for 69.9% of the explained variance in the individual factor map. The spatial orientation of samples in the individual factor map is similar to that obtained with PCA of DSA and CA of sorting aroma. Of interest is the correlation circle with attributes. Descriptors used to describe groups of similar product in sorting according to aroma, are depicted in blue while attributes used in DSA are depicted in red. According to the DSA results, assessors associated the samples on the left of dimension 1 (*C. genistoides*, *C. maculata* and *C. longifolia*) with "hay/dried grass", "green grass" and "cooked vegetable" aroma and flavour attributes. During sorting based on aroma, "cooked vegetable", "grass" and "hay" were used to describe the same samples. Similarly, attributes of DSA correspond to terms used in sorting to describe *C. intermedia* species. DSA attributes used to describe this species were "rose perfume", "rose geranium", "fynbos floral" and "fynbos sweet" corresponding to "floral", "rose perfume", "rose geranium" and "fynbos sweet" descriptors added during sorting on aroma.

The product configuration and corresponding correlation circle obtained with MFA performed on the merged data of DSA and CA standardised deviates of sorting on palate are presented in Fig. 11. Dimension 1 and 2 account for 65.5% of the explained variance. Inspection of the MFA product map revealed four clusters of products, mainly based on species, except in the case of *C. longifolia*. From the MFA correlation circle on attributes it is clear that attributes used for sorting correspond to those used during DSA. Descriptors used to describe groups of similar products during sorting are depicted in blue, while attributes used in DSA are depicted in red. DSA illustrated that *C. intermedia* were associated with high intensities of "rose geranium". In sorting the descriptor, "rose geranium" was also used to describe this *Cyclopia* species. The group of samples towards the upper left quadrant of the MFA factor map comprised of three *C. genistoides* samples and one *C. longifolia* sample (LON_B3) and were described with the terms bitter, sour and astringent during sorting. The latter result is consistent with the attributes associated with this group of samples based on DSA data. Similarly, the group of samples towards the lower left quadrant of the plot (samples representing *C. maculata* and two *C. longifolia* samples) were associated with a "green grass", "hay / dried grass" and "cooked vegetable" aroma and flavour based on DSA data. The same terms were used during the descriptive step of the sorting task to describe this group of samples. The MFA product configuration and corresponding correlation circle obtained from sorting on palate data confirmed the results obtained with CA of sorting on palate which revealed four separate groups of samples. The MFA product map and corresponding correlation circle obtained from DSA and CA of sorting on global data (provided in Addendum E) were similar to data obtained for sorting on aroma and sorting on palate.

The results obtained from the MFA configurations from sorting on aroma, sorting on palate and global sorting indicated the similarity between terms added during the descriptive step of sorting and attributes used during DSA, confirming the consistent use of terms by the panel and panel reliability.

4. Discussion

In recent years, the food and beverage industry expressed the need for rapid methods suitable for the broad sensory profiling of products using assessors with different levels of training. The information obtained through sensory profiling is useful, if not essential, for research and development, quality control and insight in consumer behaviour. Sorting has gained popularity as a rapid sensory profiling method, mainly because of its ease of implementation, and numerous studies report on its application on different food and beverage products (Campo, Do, Ferreira, & Valentin, 2008; Cartier et al., 2006; Chollet et al., 2011; Fleming, Ziegler, & Hayes, 2015; Lelièvre et al., 2008; Mielby, Hopfer, Jensen, Thybo, & Heymann, 2014; Nestrud & Lawless, 2010). Limited research on the application of sorting on more complex products or products where temperature control is essential, has been published. When proposing new methodologies to industry, it is vitally important that these techniques provide results that are reliable, valid and easy to interpret. In the current study, three variations of directed sorting, namely directed sorting based on aroma, palate and global sorting for the sensory characterisation of a complex product where temperature control is necessary, were investigated. The research revealed promising results with potential for application in quality control in the herbal tea industry.

4.1 Comparison of DSA and sorting product configurations

Descriptive sensory analysis is regarded as the "gold standard" of sensory techniques that provides detailed results on all the sensory attributes associated with a product and describes differences in the sensory profile between products (Lawless & Heymann, 2010). DSA is usually used as basis to compare the suitability of new methods. The validity of the sorting task for the sensory characterisation of a complex product such as honeybush tea infusions was evaluated by relating results obtained with sorting to that of DSA. Similar results were obtained when comparing DISTATIS product configurations of the three variants of the sorting task to the PCA bi-plot obtained with DSA. This is in accordance to research that found similar product maps for sorting and DSA when evaluating breakfast cereals (Cartier et al., 2006) and beer (Chollet et al., 2011). Comparison of CA plots of sorting data and the PCA plot of DSA data revealed similar product configurations and associations between products and attributes for sorting aroma and sorting palate, and to a lesser extent for global sorting. Similarity of product configurations were confirmed with high RV coefficients (> 0.86). Results of the current study thus indicated directed sorting to be a valid tool for the sensory characterisation of a complex product, such as infusions of honeybush tea.

4.2 MFA for comparing descriptors used in DSA and sorting

A novel technique for analysing and comparing DSA and sorting data was proposed, and merits further discussion. Multiple factor analysis was performed on the merged data set, comprising that of DSA and CA

of sorting, allowing a direct comparison of description of products obtained through DSA and sorting. Inspection of the MFA product configuration and corresponding correlation circle, calculated for each of the sorting tasks, revealed a high correlation between DSA attributes and similar descriptors for the same products used in the sorting task. Assessors were therefore consistent in using terms to describe products when comparing results for DSA and sorting. These results are important to take into account when applying sorting in the herbal tea industry. Erasmus (2015) compared product configurations obtained with instructed and uninstructed sorting of infusions of three *Cyclopia* species to that obtained through DSA. During instructed sorting, assessors were provided with a list of attributes while no list or guidelines were provided during uninstructed sorting where assessors were allowed to use their own descriptors. Erasmus (2015) reported that similar product configurations were obtained when comparing instructed sorting and DSA, while results obtained with uninstructed sorting were not similar to that obtained with DSA. Moreover, the latter results were difficult to interpret with no logical explanation for the resulting sample configurations. Results of the current study illustrated that providing assessors with a pre-determined list when evaluating honeybush infusions, simplified the sorting task for assessors. Assessors sorted samples into groups and added attributes that were interpretable and consistent to that used during DSA.

The proposed technique demonstrates potential for combining DSA and sorting data, obtained with different groups of assessors, e.g. trained assessors and a group of consumers. It would allow researchers to link attributes, defined by trained assessors, to descriptors applied by consumers. Research on the development of a quality grading tool suitable for the honeybush herbal tea, is ongoing. This technique could also find application in quality grading for analysing and comparing descriptors used by a trained panel and that used by a tea grading panel in the herbal tea industry.

4.3 Length of list of attributes provided to assessors

Several researchers have discussed the usefulness of providing assessors with a list of attributes, as well as the effect of the length of the list on assessor performance when conducting the sorting task (Chollet et al., 2011; Hughson & Boakes, 2002; Lelièvre et al., 2008). Chollet et al. (2011) emphasised the importance of using a shorter list of relevant attributes which assessors are familiar with and are trained on. Indeed, Hughson and Boakes (2002) found that a shorter list (14 terms), as opposed to a longer list of 125 terms, aided untrained assessors in completing the sorting task and these researchers postulated that a shorter list of attributes would improve expert assessor's performance.

In the current study, trained assessors were provided with a list of 22 terms for sorting on aroma, 21 terms were included for sorting on palate and 43 terms for global sorting. Correspondence analysis, performed on the sorting data, was used to elucidate the relationship between products and descriptors. Results for CA performed on data of sorting on aroma revealed three distinct groups while CA of sorting on palate revealed four groups. The differentiation between samples, and description of samples, were similar to that obtained with DSA. However, results for CA of global sorting were more difficult to interpret and differentiation between species were less distinct. It appears that assessors found it easier to group samples on perceived

similarity, when directed to focus on one modality, e.g. aroma or palate. Furthermore, providing trained assessors with a shorter list of attributes (less than 25 terms) which they were familiar with, resulted in improved differentiation between products.

The configuration obtained with CA of sorting on palate merits further discussion. This sensory map revealed four groups of samples, firstly a differentiation between *C. subternata* and *C. intermedia*, and secondly a differentiation between the remaining samples. The three *C. genistoides* samples and one *C. longifolia* sample formed a group towards the lower left of the plot and these samples associated with bitter and sour taste, and "apricot" flavour. The three *C. maculata* samples and remaining two *C. longifolia* samples formed a group towards the upper right of the plot and these samples associated with "cooked vegetable", "fynbos", "grass" and "hay" flavour. The bitter taste, associated with *C. genistoides* and to a lesser extent with *C. longifolia*, could be the main driver for the differentiation on dimension two for samples towards the right of the plot. This differentiation into four groups was not obtained with sorting on aroma or with DSA. Refer to Addendum F for the PCA bi-plot based on DSA palate attributes. These results demonstrated that, when focused on one modality and provided with a shorter list of attributes, trained assessors were able to distinguish between samples with only subtle differences.

4.4 Replications within the sorting task

The sorting task is a rapid sensory method and replicating sessions reduces the swiftness of the technique. Several researchers reported high similarity of repeated sorting tasks (Cartier et al., 2006; Chollet et al., 2011; Lawless & Glatter, 1990). Results of the current study also demonstrated high repeatability, confirmed by high RV coefficients, for replicate sessions of directed sorting on a complex product such as honeybush with the added complication of testing the product hot. These results could lead to the conclusion that one replication would be sufficient. Visual inspection of the DISTATIS plots for the three replications of sorting on aroma indicated that assessors' ability to distinguish between the samples based on aroma, might have improved with subsequent sessions. This was the first introduction to the sorting task for some of the assessors and one could postulate that assessors grew accustomed to and more confident with the task with subsequent sessions. Chollet and Valentin (2000) reported that replicate sessions of sorting of beer by trained assessors revealed different product configurations, suggesting that assessors changed their classification criteria with subsequent sessions. Although all variations of directed sorting indicated high repeatability, a minimum of two replications with a trained panel are recommended to ensure stable results.

5. Conclusions

The results of the current research indicated directed sorting to be a valid method for the broad sensory characterisation of infusions of *Cyclopia* species. These samples represent a complex product with a large array of descriptors with the additional complication that products need to be evaluated hot. Although more detailed qualitative and quantitative results were obtained with DSA, it is important to keep in mind that the sorting task is more cost-effective and less time consuming to conduct.

Correspondence analysis of sorting data revealed product configurations that were easier to interpret and of more practical value than DISTATIS plots, primarily as descriptors were added to the CA plots. Comparison of CA configurations of the three variants of sorting revealed distinct differentiation between groups of samples when conducting directed sorting on aroma and palate attributes. Moreover, differentiation and description of samples with only subtle differences were obtained with sorting on palate. The bitter taste, associated with *C. genistoides* and to a lesser extent, with *C. longifolia*, could be the main driver for the differentiation between the samples with only subtle differences. The shorter list of attributes provided for sorting on aroma or palate, which simplifies the process for assessors, could explain the higher degree of differentiation compared to that obtained with CA of global sorting.

Multiple factor analysis was performed on the merged data of DSA and CA of sorting data, a novel technique for comparing results of different sensory methods. These results indicated similarity of descriptive terms used during sorting and the description of products with DSA. This underlines the reliability of the panel and the validity of the sorting method for the sensory characterisation of honeybush infusions.

Three variations of the sorting task (based on aroma, palate or global attributes) were investigated in the current study. Infusions of *Cyclopia* species, the product selected for the current study, show distinct product differences on aroma. Directed sorting on aroma was thus demonstrated to be a valid method for characterisation of this complex product. Although sorting is regarded as a holistic method, results of the current study indicated global sorting to be less effective to differentiate between different honeybush infusions. The longer list provided with global sorting, could have complicated the task for assessors. If *a priori* knowledge of the product under question is available, the choice of sorting on aroma, palate or global sorting should be selected based on the intrinsic product characteristics. If product differences are more pronounced in aroma, directed sorting on aroma would be more suitable while directed sorting on palate would be more suitable for products with distinct differences in palate attributes.

If the sensory characteristics of a product category and sub-groups within a category are known, the sorting task can be a very useful tool to determine where and how new products fit into the group or match the sensory profile of subgroups. The sorting task could be well suited for incorporation in quality control where the sensory characteristics of different batches of products need to be determined. In this sense, the sorting task can find application in the herbal tea industry as batches of different species and originating from different areas and suppliers could be used to blend a product of consistent quality.

An extensive database on the sensory characteristics of the major *Cyclopia* species has previously been established. This database is ideal when the aim is to characterise new batches of samples within a quality control programme. The sorting procedure could therefore be applied to first categorise the batches according to sensory attributes relating to species and to identify batches that are not regarded as typical of the species or to identify batches that are of lower grade or inferior quality.

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Table 1 *Cyclopia* species with sample codes used for DSA and sorting tasks.

Cyclopia species	Sample code
Cyclopia genistoides	GEN_B1
	GEN_B3
	GEN_B4
Cyclopia subternata	SUB_B2
	SUB_B3
	SUB_B5
Cyclopia maculata	MAC_B1
	MAC_B2
	MAC_B5
Cyclopia longifolia	LON_B2
	LON_B3
	LON_B5
Cyclopia intermedia	INT_B1
	INT_B3
	INT_B5

Table 2 RV coefficients for the correlation between the DISTATIS product configurations per sorting task and replicate sessions of directed sorting on aroma, palate or global sorting (replicate 1, 2, 3) of five *Cyclopia* species.

	Sorting: Aroma			Sorting: Palate			Sorting: Global		
	Rep 2	Rep 3	Consensus plot	Rep 2	Rep 3	Consensus plot	Rep 2	Rep 3	Consensus plot
Rep 1	0.94	0.57	0.98	0.85	0.82	0.92	0.91	0.90	0.94
Rep 2		0.66	0.98		0.93	0.97		0.97	0.99
Rep 3			0.66			0.96			0.98

Table 3 RV coefficients for the correlation between the PCA bi-plot obtained with DSA and DISTATIS product configurations for directed sorting on aroma, palate and global sorting of five *Cyclopia* species.

	DSA	*p*-value
Sorting: aroma	0.86	p<0.001
Sorting: palate	0.87	p<0.001
Sorting: global	0.84	p<0.001

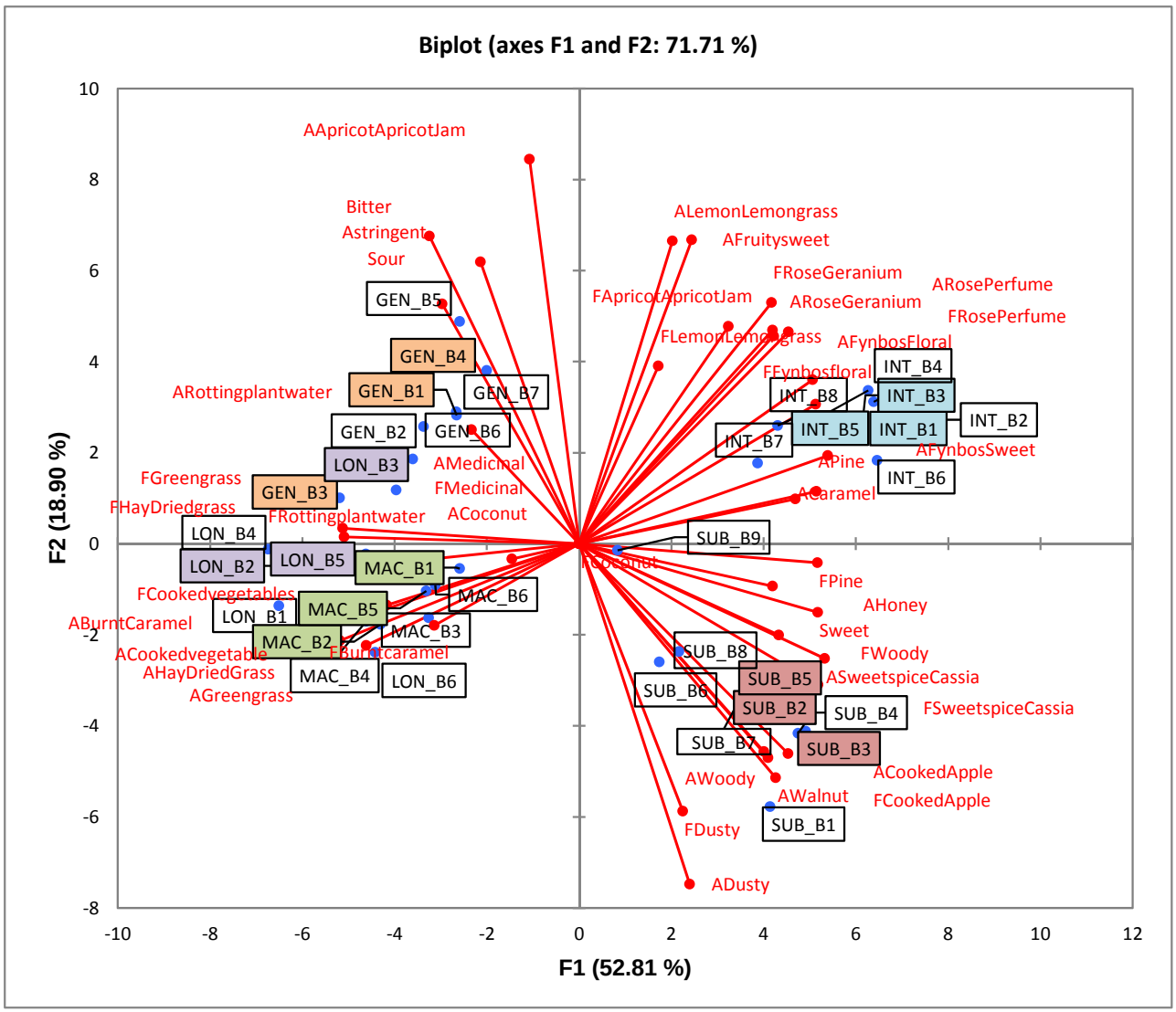
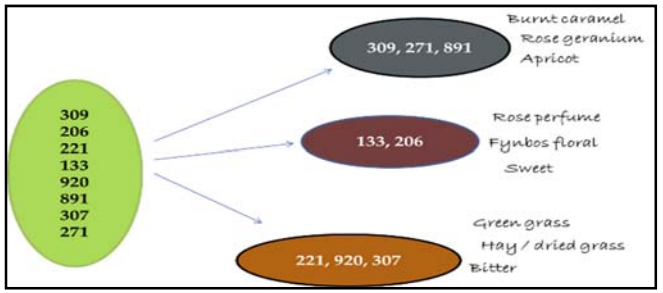


Fig. 1. PCA bi-plot obtained with DSA of five *Cyclopia* species (total sample set). Samples selected for inclusion in the sorting task are marked in colour per species. Capital letters added to attributes indicate A: aroma (orthonasal) and F: flavour (retronasal). The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides*, *C. maculata*, *C. longifolia*, *C. subternata* and *C. intermedia*, respectively. B refers to batches, 1–9 refer to batch number.

a)



b)

Judge	Sample	Gr 1	Gr 2	Gr 3	Gr 4	Gr 5
1	GEN_B1	0	1	0	0	0
1	INT_B1	0	0	1	0	0
1	MAC_B1	1	0	0	0	0
1	LON_B2	1	0	0	0	0
1	SUB_B2	0	0	0	1	0
1	GEN_B3	0	1	0	0	0
1	INT_B3	0	0	1	0	0
1	MAC_B2	1	0	0	0	0

c)

Judge	Sample	GEN_B1	INT_B1	MAC_B1	LON_B2	SUB_B2
1	GEN_B1	1	0	0	0	0
1	INT_B1	0	1	0	0	0
1	MAC_B1	0	0	1	1	0
1	LON_B2	0	0	1	1	0
1	SUB_B2	0	0	0	0	1
1	GEN_B3	1	0	0	0	0
1	INT_B3	0	1	0	0	0
1	MAC_B2	0	0	1	1	0

d)

Judge	Sample	GEN_B1	INT_B1	MAC_B1	LON_B2	SUB_B2
1	GEN_B1	0	1	1	1	1
1	INT_B1	1	0	1	1	1
1	MAC_B1	1	1	0	0	1
1	LON_B2	1	1	0	0	1
1	SUB_B2	1	1	1	1	0
1	GEN_B3	0	1	1	1	1
1	INT_B3	1	0	1	1	1
1	MAC_B2	1	1	0	0	1

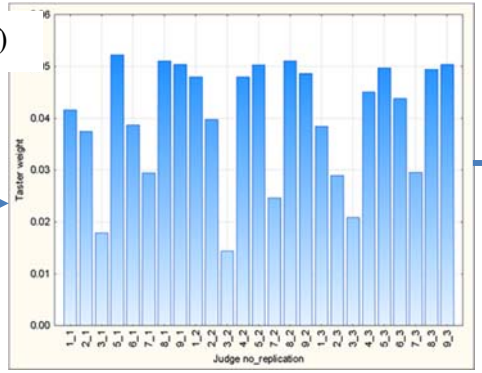
e)

Judge	Sample	GEN_B1	INT_B1	MAC_B1	LON_B2	SUB_B2
3	GEN_B1	0	1	1	1	1

Judge	Sample	GEN_B1	INT_B1	MAC_B1	LON_B2	SUB_B2
2	GEN_B1	0	1	1	1	1

Judge	Sample	GEN_B1	INT_B1	MAC_B1	LON_B2	SUB_B2
1	GEN_B1	0	1	1	1	1
1	INT_B1	1	0	1	1	1
1	MAC_B1	1	1	0	0	1
1	LON_B2	1	1	0	0	1
1	SUB_B2	1	1	1	1	0
1	GEN_B3	0	1	1	1	1
1	INT_B3	1	0	1	1	1
1	MAC_B2	1	1	0	0	1

f)



g)

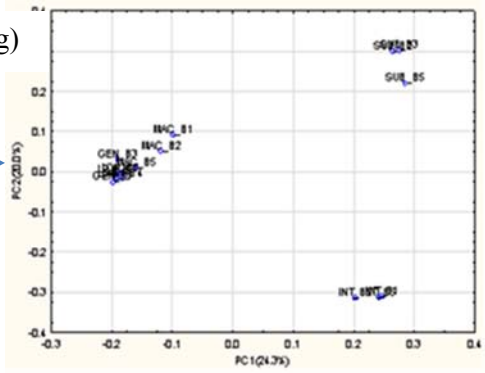


Fig. 2. Schematic illustration of DISTATIS analysis of sorting data. a) Individual sort per assessor, b) Indicator matrix per assessor, c) Co-occurrence matrix per assessor, d) Distance matrix per assessor, e) Individual cross-product matrices for assessors 1, 2, etc., f) Taster weights of assessors g) Compromise cross-product configuration.

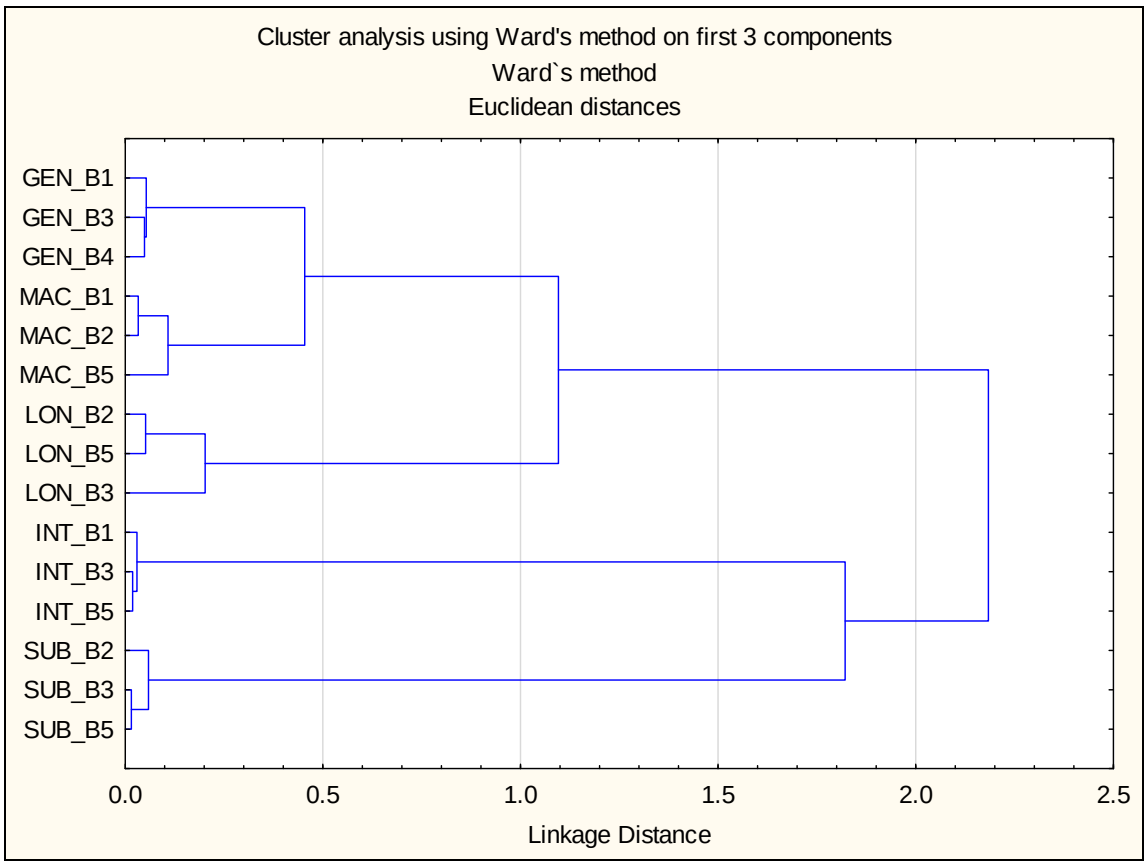


Fig. 3. Cluster analysis of directed sorting based on global attributes of five *Cyclopia* species. The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides*, *C. maculata*, *C. longifolia*, *C. subternata* and *C. intermedia*, respectively. B refers to batches, 1–5 refer to the batch number.

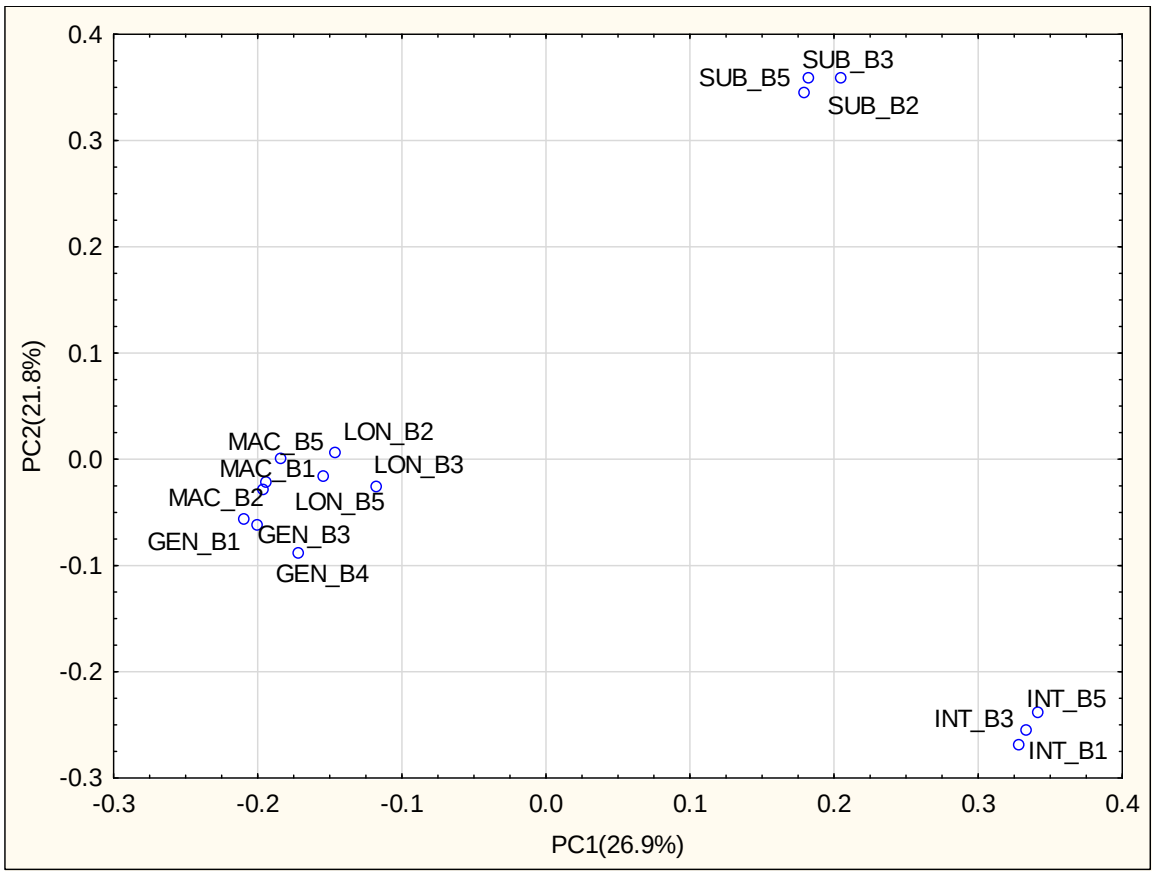


Fig. 4. DISTATIS product configuration of three replications of global sorting of five *Cyclopia* species. The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia,* respectively. B refers to batches, 1–5 refer to the batch number.

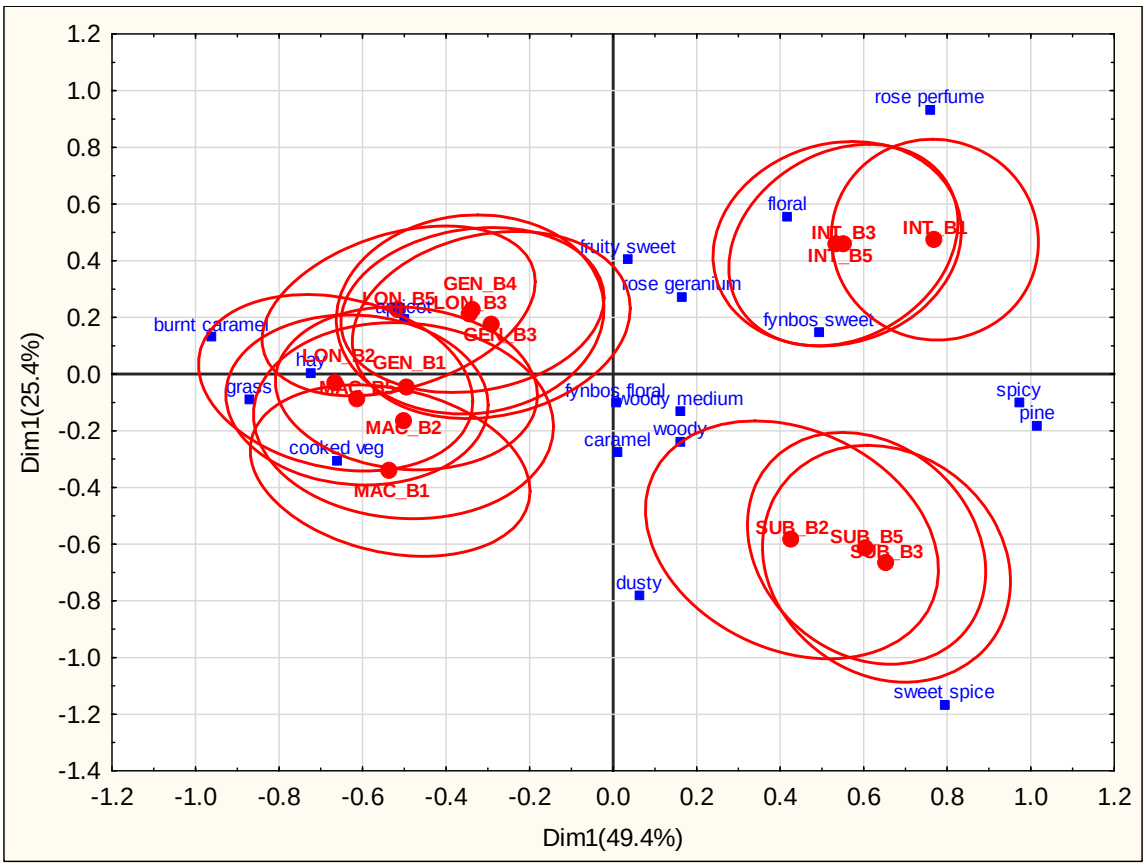


Fig. 5. CA plot of directed sorting (three replications) according to aroma of five *Cyclopia* species. The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia,* respectively. B refers to batches, 1–5 refer to the batch number.

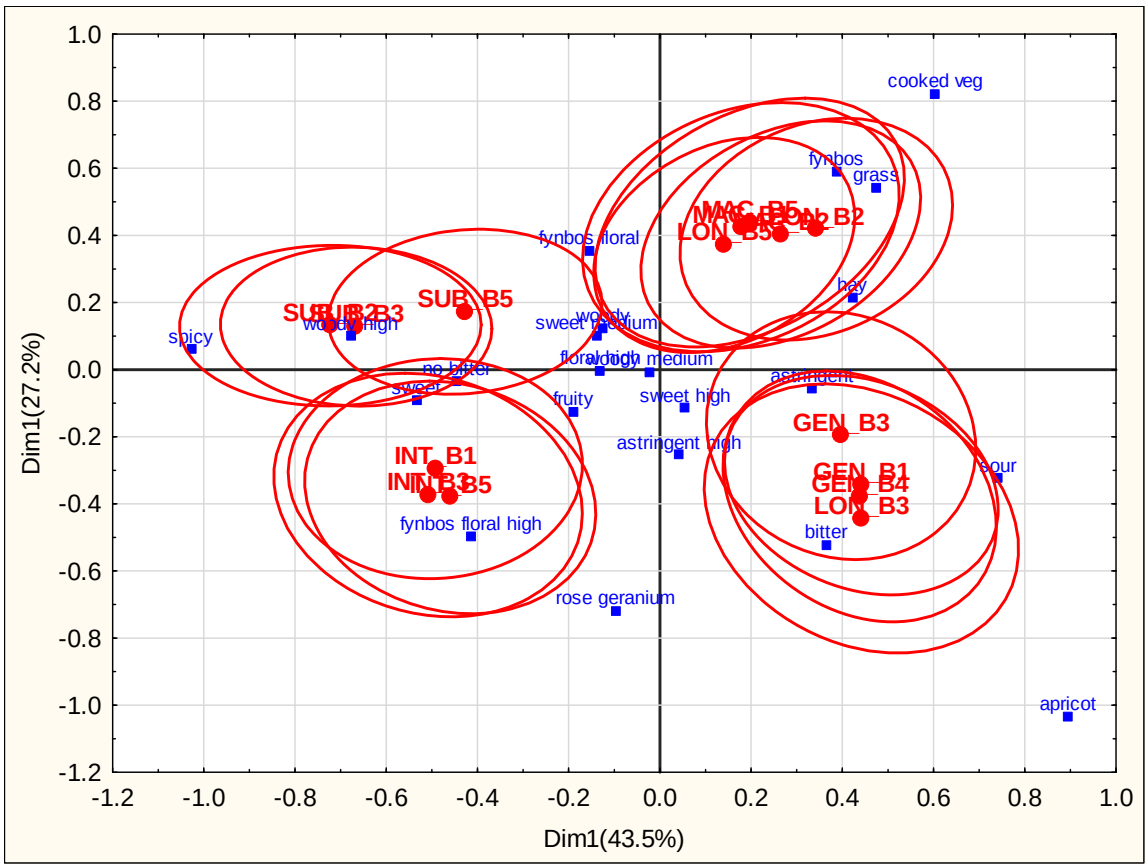


Fig. 6. CA plot of directed sorting (three replications) on palate of five *Cyclopia* species. The abbreviations GEN, MAC, LON, SUB and INT refer *C. genistoides*, *C. maculata*, *C. longifolia*, *C. subternata* and *C. intermedia*, respectively. B refers to batches, 1–5 refer to the batch number.

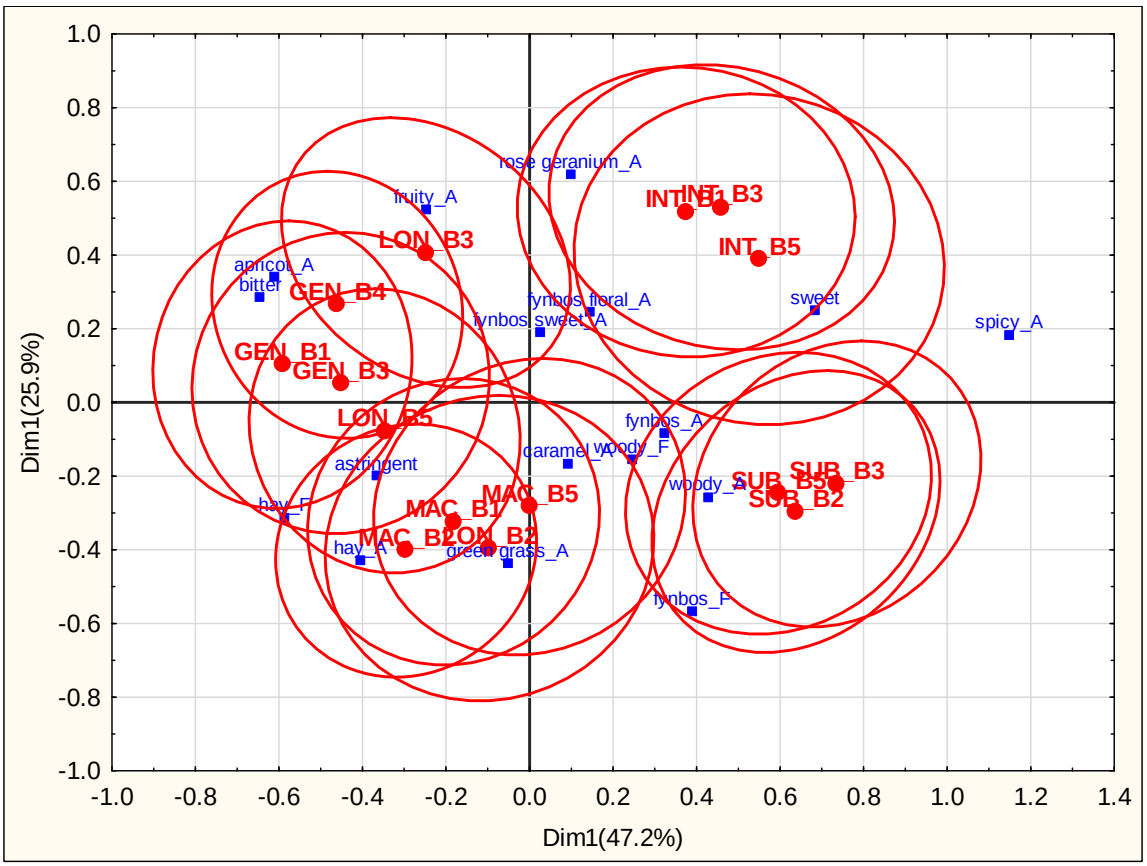
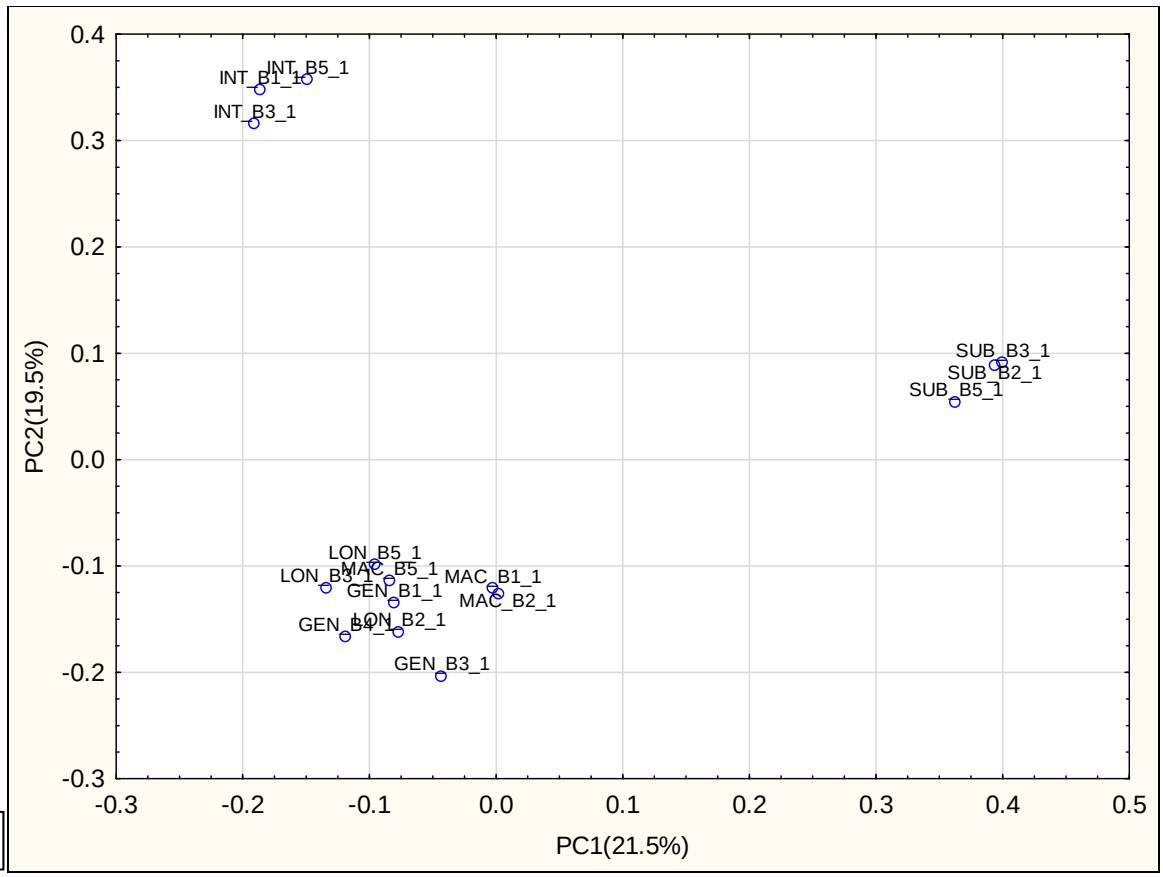
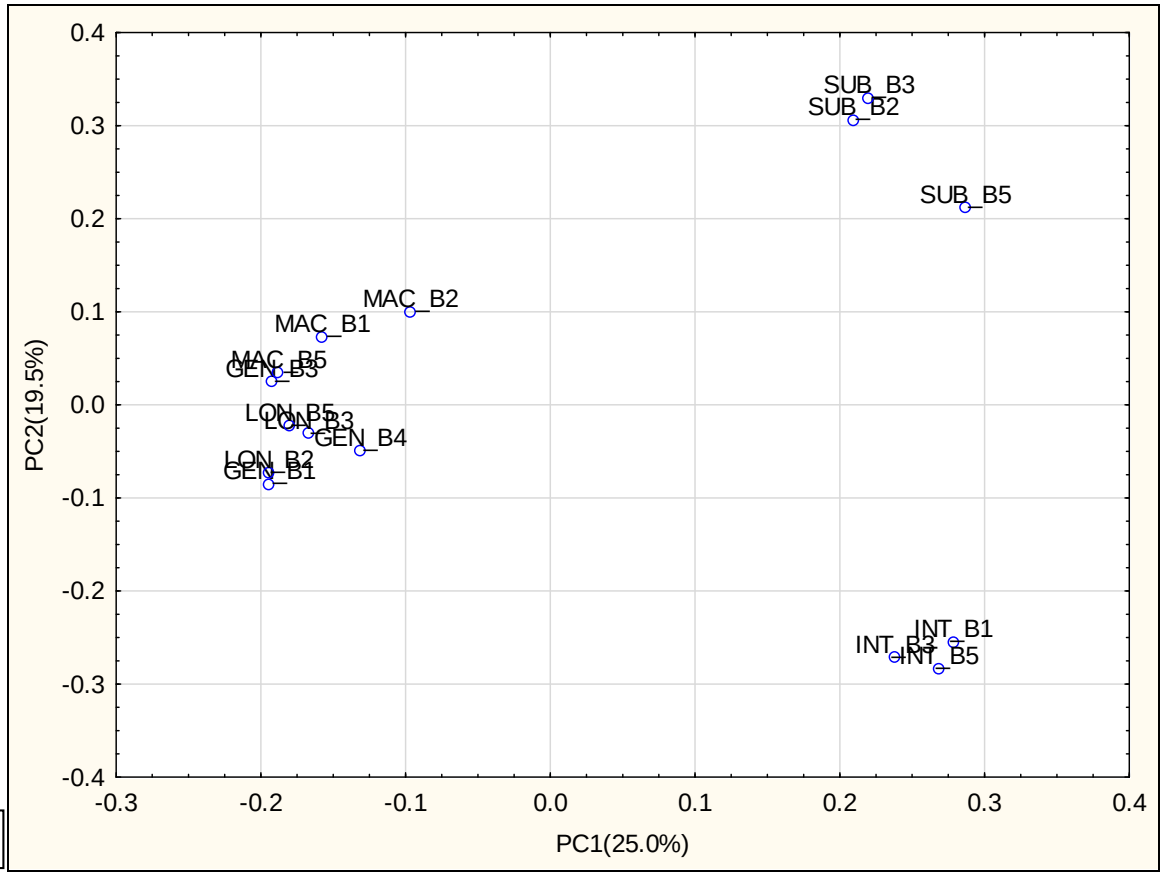


Fig. 7. CA plot of global sorting (three replications) of five *Cyclopia* species. Capital letters added to attributes indicate A: aroma (orthonasal) and F: flavour (retronasal). The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia,* respectively. B refers to batches, 1–5 refer to the batch number.

Rep 1



Rep 2



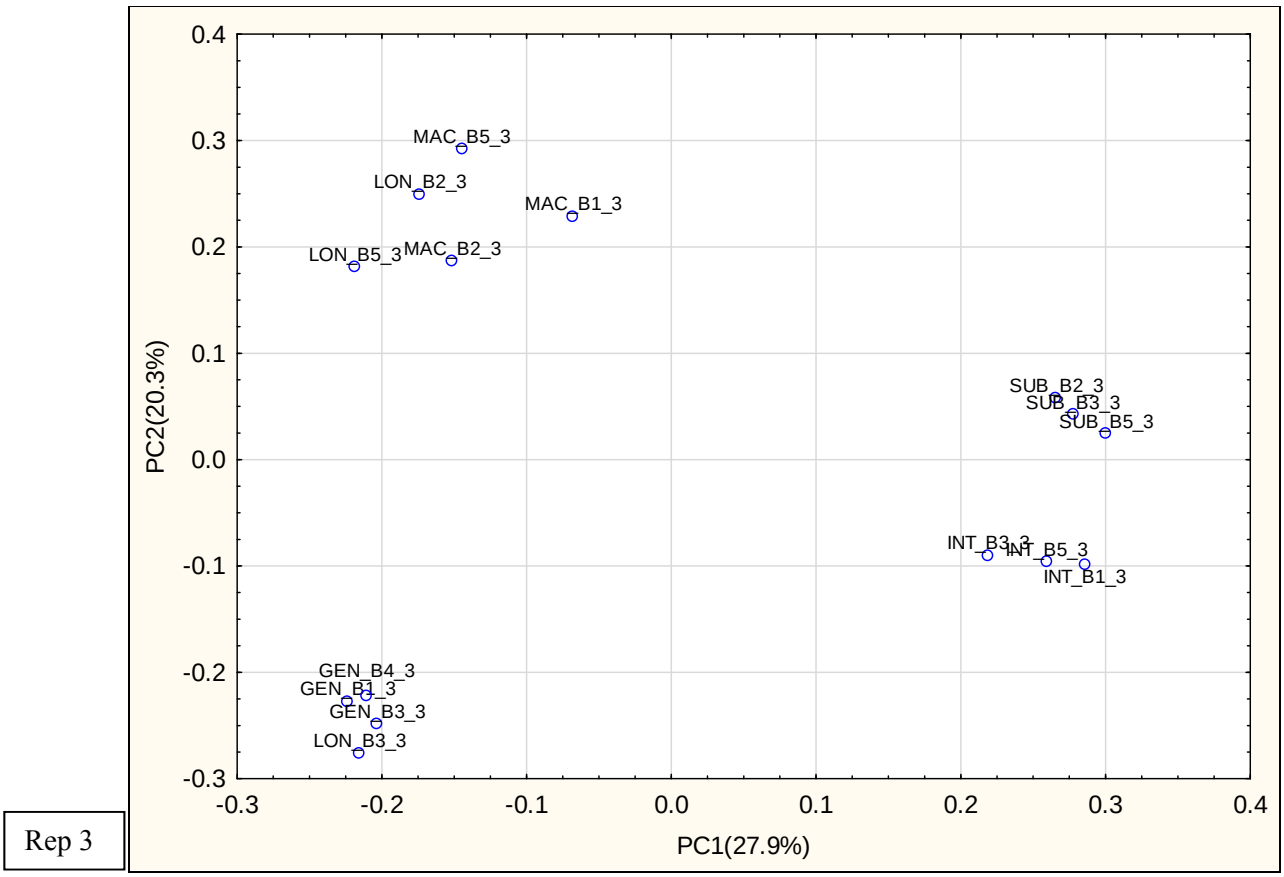


Fig. 8. DISTATIS plots of three replications (rep 1-3) of directed sorting according to aroma of five *Cyclopia* species. The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia*, respectively. B refers to batches, 1–5 refer to the batch number.

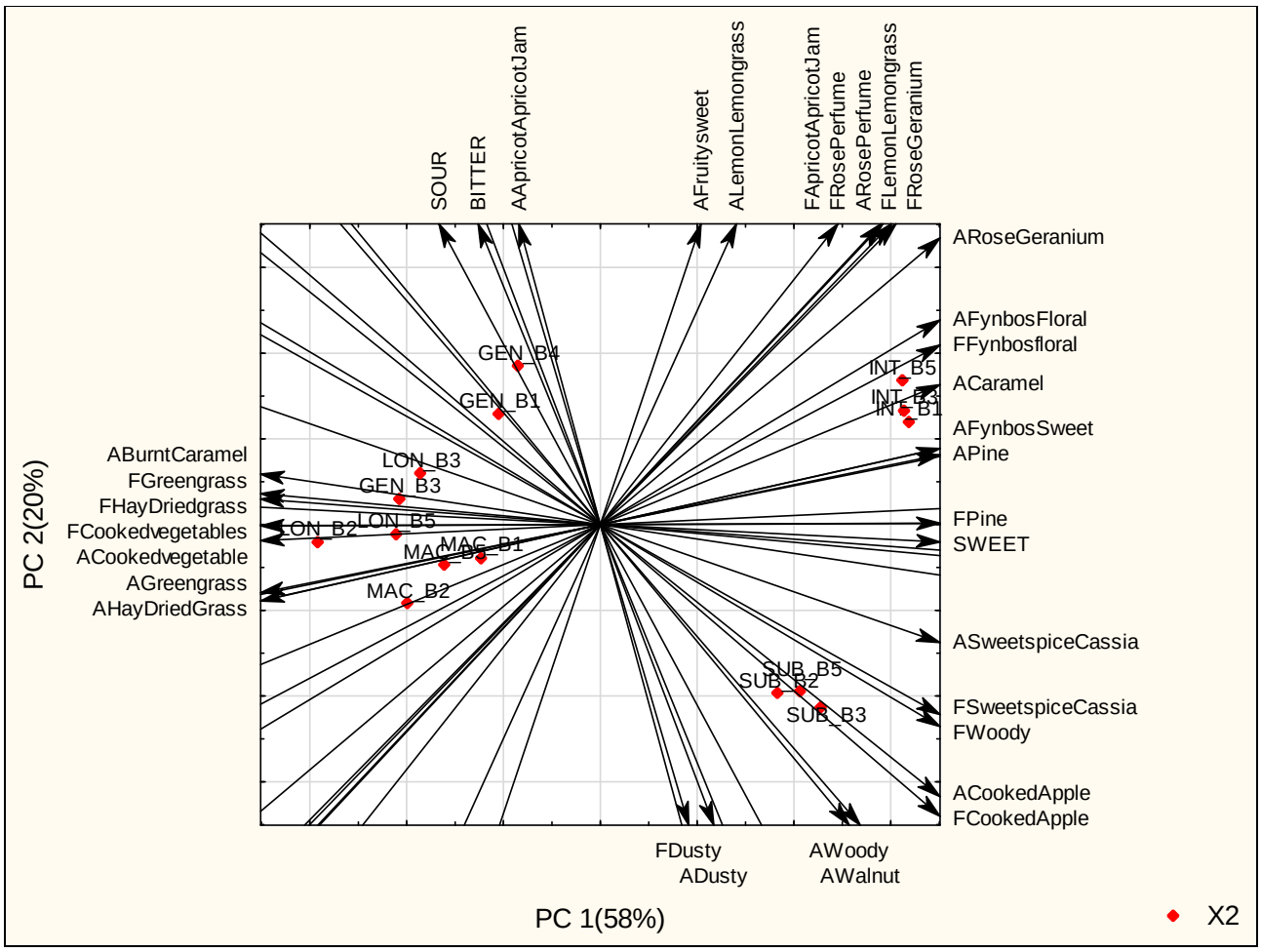
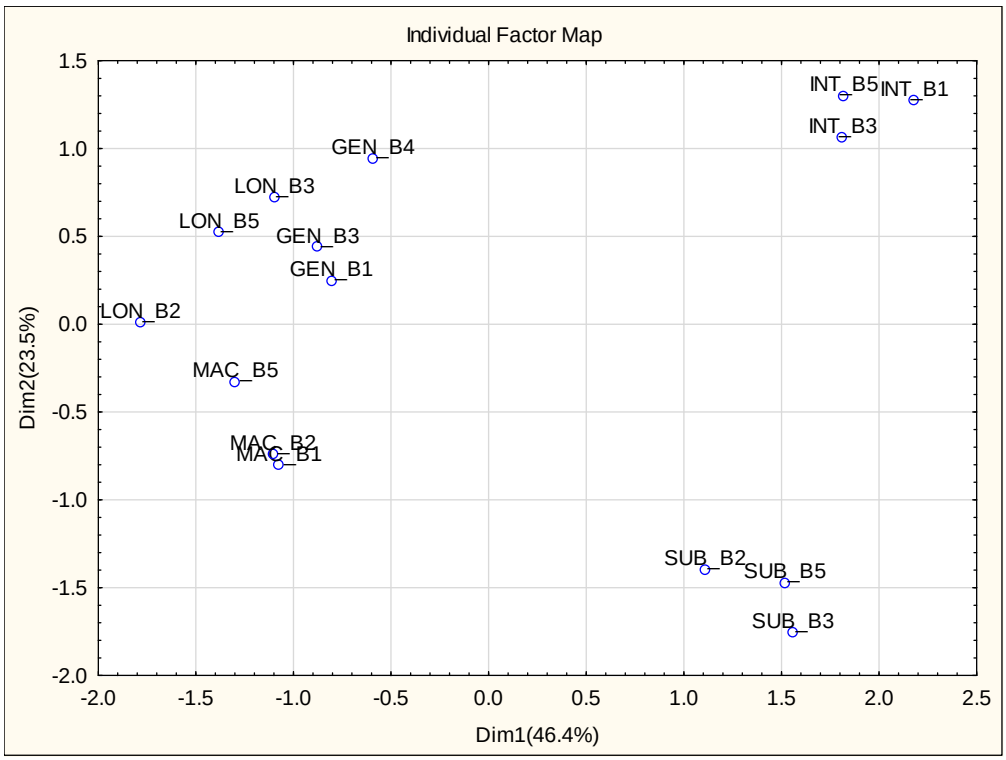


Fig. 9. PCA bi-plot obtained with DSA presenting the differentiation among five *Cyclopia* species. Samples selected for the sorting task are included. Capital letters added to attributes indicate A: aroma (orthonasal) and F: flavour (retronasal). The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia*, respectively. B refers to batches, 1–5 refer to the batch number.



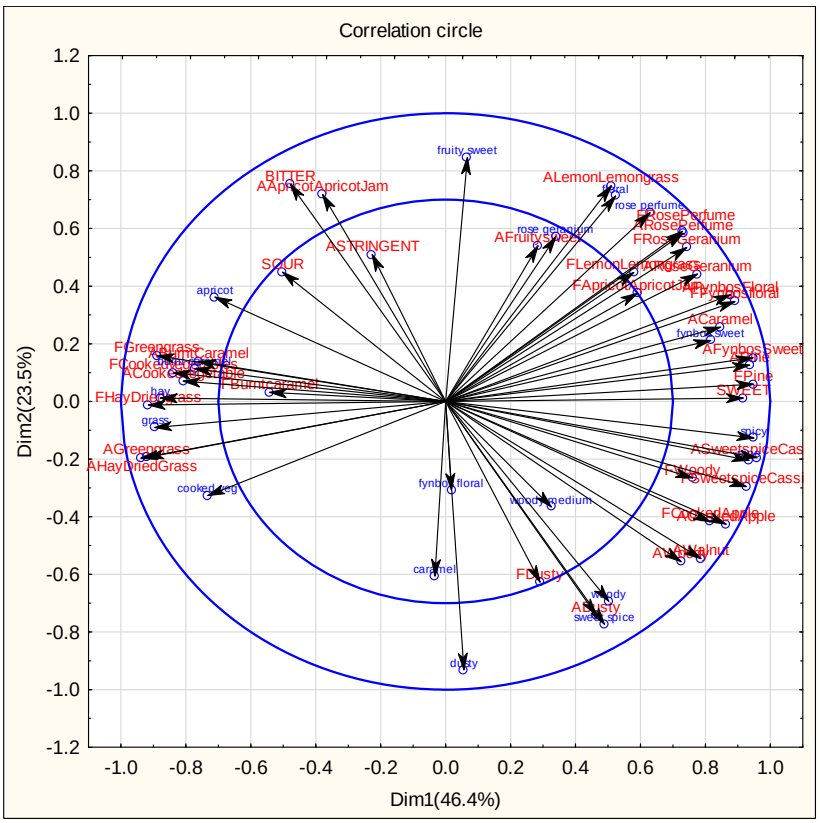


Fig. 10. Individual factor map and correlation circle of attributes obtained with MFA of merged data on DSA and CA of sorting on aroma. Capital letters added to attributes indicate A: aroma (orthonasal) and F: flavour (retronasal). The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides*, *C. maculata*, *C. longifolia*, *C. subternata* and *C. intermedia*, respectively. B refers to batches, 1–5 refer to the batch number. Sorting attributes are marked in blue and DSA attributes in red.

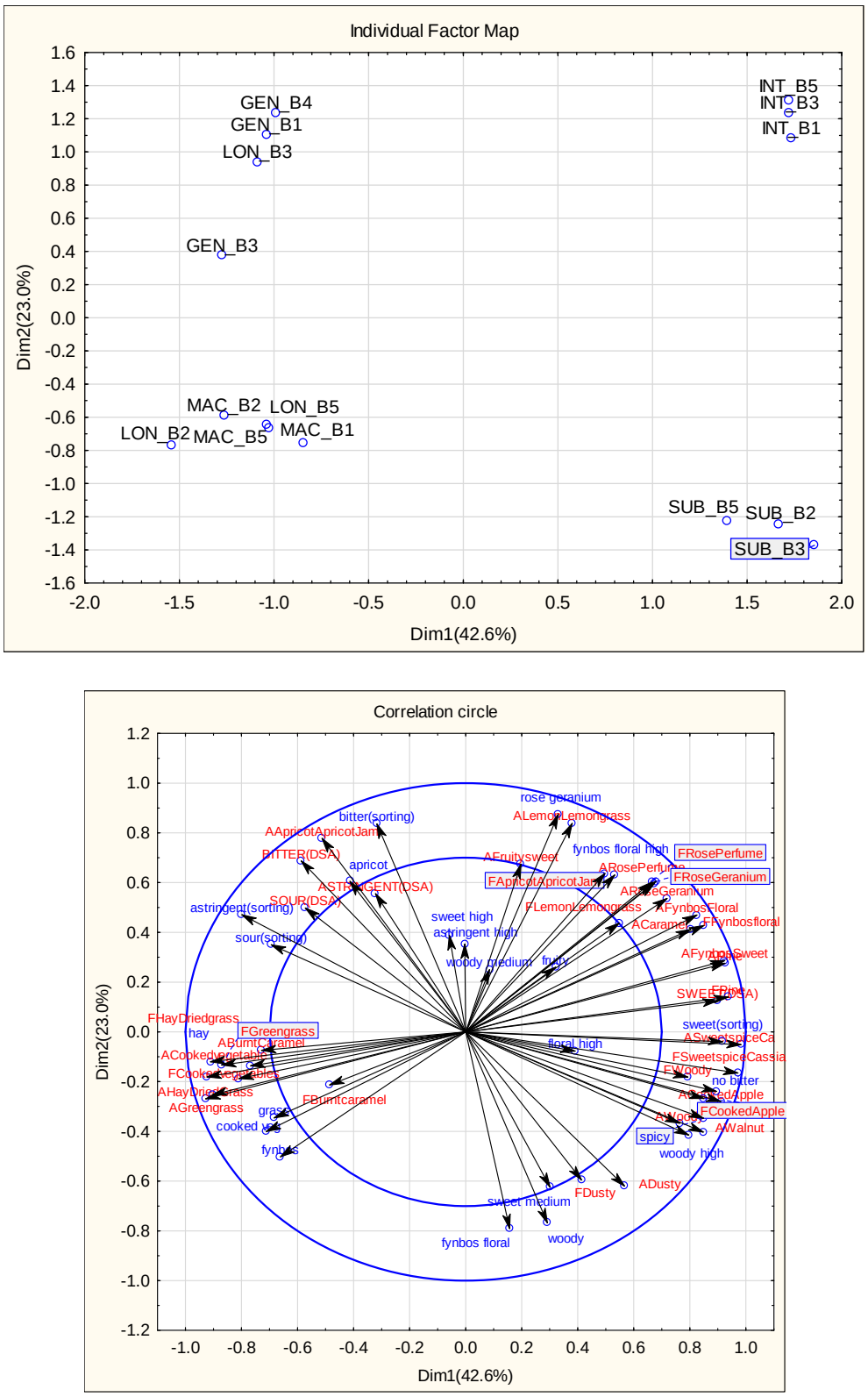


Fig. 11. Individual factor map and correlation circle of attributes obtained with MFA of merged data on DSA and CA of sorting palate. Capital letters added to attributes indicate A: aroma (orthonasal) and F: flavour (retronasal). The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides*, *C. maculata*, *C. longifolia*, *C. subternata* and *C. intermedia*, respectively. B refers to batches, 1–5 refer to the batch number. Sorting attributes are marked in blue and DSA attributes in red. Borders around attributes or samples indicate that these have been moved from original position to make it more legible.

ADDENDUM A

Instruction sheet and questionnaire: Directed sorting task

HONEYBUSH 2015 SORTING

Day 3 –Wednesday, 17 June 2015

SESSION 3 DIRECTED SORTING according to AROMA & PALATE ATTTIBUTES

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

INSTRUCTIONS

- You have been presented with **15 honeybush samples** labelled with a three digit code.
- Please sort the samples according to the **SIMILARITY OF THEIR SENSORY PROFILES**
    - You are allowed to **smell & taste** the samples as many times as you like and in any order.
    - On the large A3 paper that is provided, group together the samples that have a similar sensory profile
    - You may form **as many groups as you wish, but NOT MORE THAN 5 GROUPS.**
    - Each group may contain **as many samples as you like**
    - Once you have assigned all samples to a group, use the **table** provided on the **separate A4 page** to indicate which samples you have grouped together
- Using the list with attributes provided, write down the major **sensory attributes associated with each of the sample groups**. D**o not use more than 5 attributes** to describe the sensory characteristics of each group.
    - **NOTE**: Please try to work as quickly as possible to **prevent the samples from cooling down** too much. Place samples back in the water-bath while you are the tasting the other samples.
    - **Groups are mutually exclusive - one sample can only be present in one group.**

Name:		Date:	Session 3 Rep 3
Complete the table below by indicating which samples you have placed in which group. Then please write down the major AROMA AND PALATE attributes associated with each group in the column on the right.			
Group	Samples		AROMA AND PALATE attributes associated with each group
1		1.	4.
		2.	5.
		3.	
2		1.	4.
		2.	5.
		3.	
3		1.	4.
		2.	5.
		3.	
4		1.	4.
		2.	5.
		3.	
5		1.	4.
		2.	5.
		3.	

ADDENDUM B

Raw data: Indicator matrix (samples and groups) for directed sorting on all attributes (global)

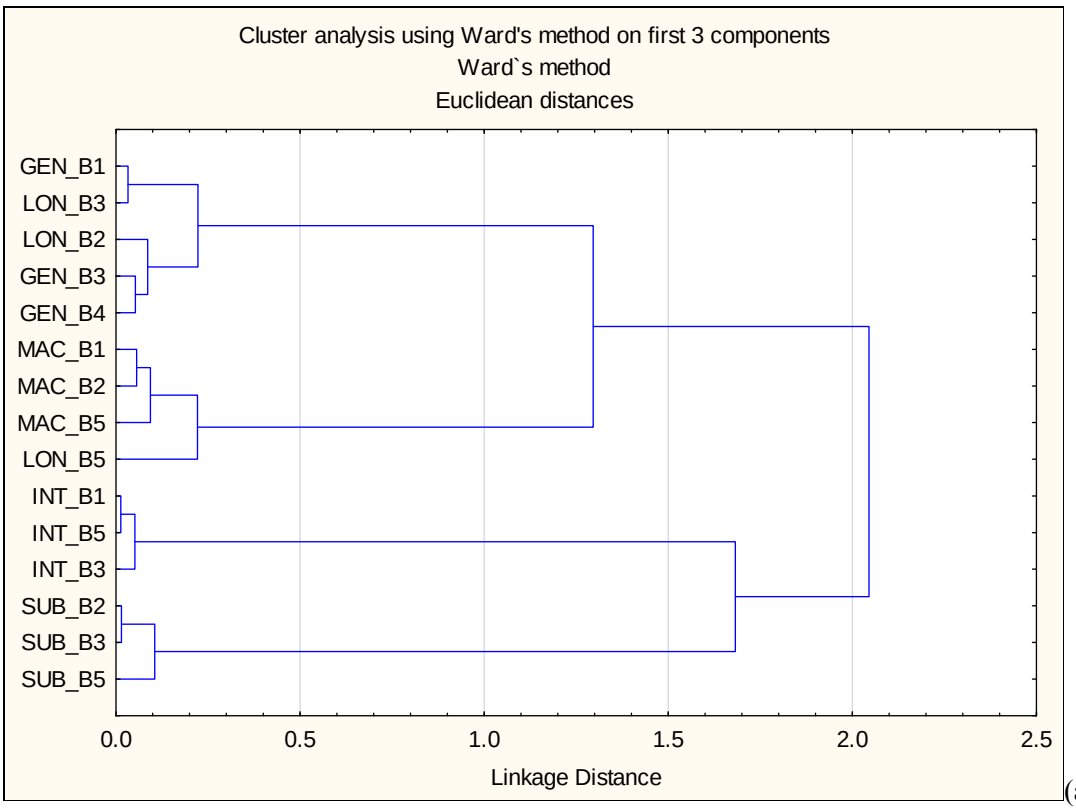
DAY 3 - DIRECTED SORTING ON 2014 HB SAMPLES – GLOBAL 17-Jun-15

Samples 5 species x 3 samples / specie = 15 SAMPLES

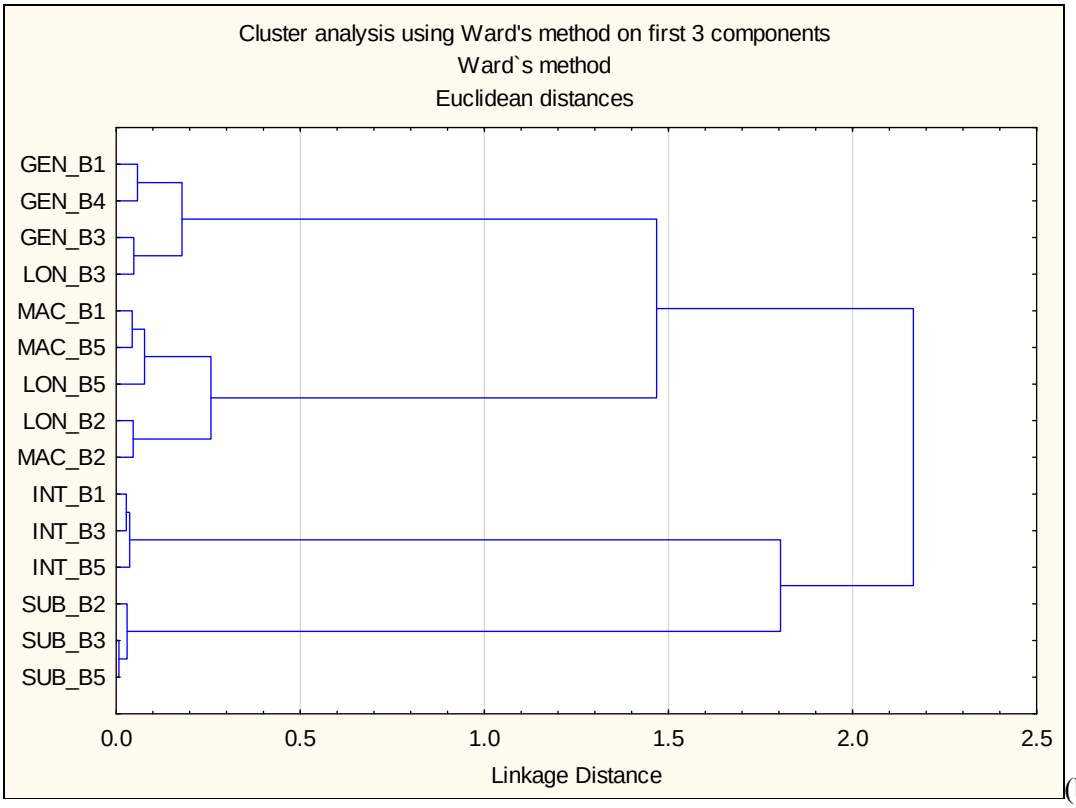
Rep	Judge	Sample	Gr 1	Gr 2	Gr 3	Gr 4	Gr 5	Desc.	Desc.	Desc.	Desc.	Desc.
1	1	GEN_B1	0	1	0	0	0	fruity_A	apricot_A			
1	1	INT_B1	0	0	1	0	0	floral_A				
1	1	MAC_B1	1	0	0	0	0	cooked veg_A				
1	1	LON_B2	1	0	0	0	0	cooked veg_A				
1	1	SUB_B2	0	0	0	1	0	woody_A				
1	1	GEN_B3	0	1	0	0	0	fruity_A	apricot_A			
1	1	INT_B3	0	0	1	0	0	floral_A				
1	1	MAC_B2	1	0	0	0	0	cooked veg_A				
1	1	LON_B3	0	1	0	0	0	fruity_A	apricot_A			
1	1	SUB_B3	1	0	0	0	0	cooked veg_A				
1	1	GEN_B4	0	1	0	0	0	fruity_A	apricot_A			
1	1	INT_B5	0	0	1	0	0	floral_A				
1	1	MAC_B5	1	0	0	0	0	cooked veg_A				
1	1	LON_B5	1	0	0	0	0	cooked veg_A				
1	1	SUB_B5	0	0	0	1	0	woody_A				
1	2	GEN_B1	1	0	0	0	0	fynbos floral_A	apricot	fruity sweet	fynbos sweet_A	
1	2	INT_B1	0	1	0	0	0	fynbos floral_A	rose geranium_A	woody	spicy	caramel
1	2	MAC_B1	0	0	1	0	0	fynbos floral_A	woody	hay	fynbos sweet_A	
3	9	SUB_B3	0	1	0	0	0	sweet spice_A	cooked apple_A	sweet	woody_F	
3	9	GEN_B4	0	0	1	0	0	hay_A	sour	plantlike_A	hay_F	
3	9	INT_B5	1	0	0	0	0	rose perfume_A	fruity sweet_A	fynbos floral_A	sweet	
3	9	MAC_B5	0	0	0	0	1	astringent	sour	green_A	plantlike_A	bland_F
3	9	LON_B5	0	0	0	1	0	burnt caramel_A	apricot_A	bitter	woody_F	
3	9	SUB_B5	0	1	0	0	0	sweet spice_A	cooked apple_A	sweet	woody_F	

ADDENDUM C

Cluster analysis performed on data from directed sorting on (a) aroma and (b) palate of five Cyclopia species.



(a)

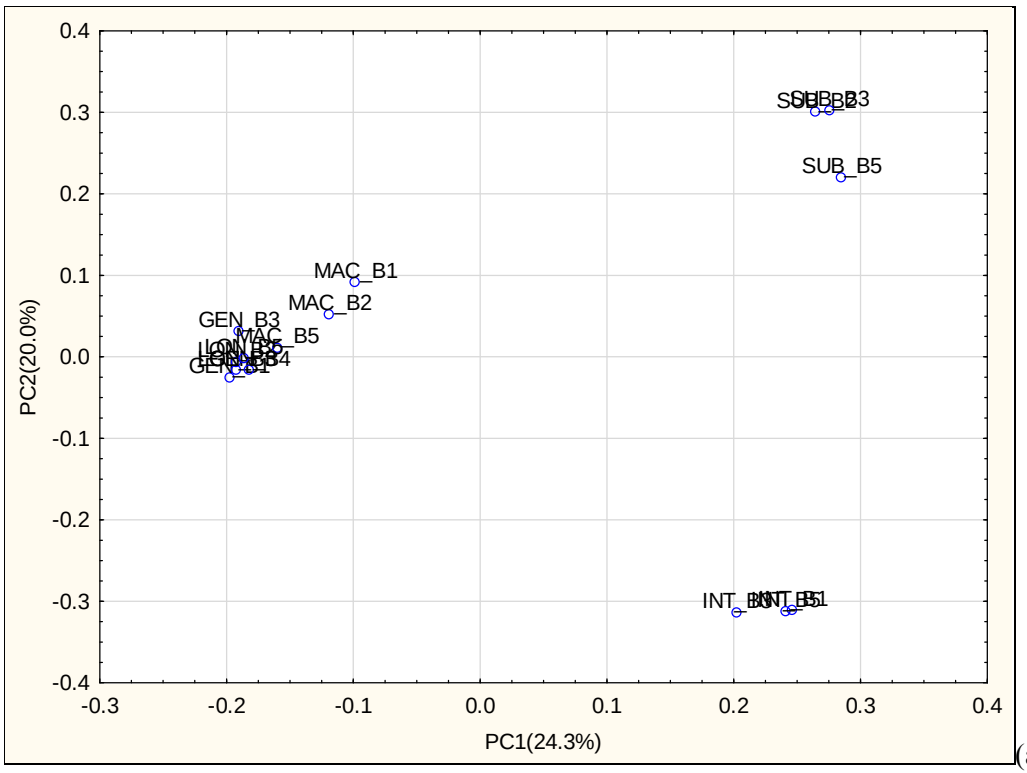


(b)

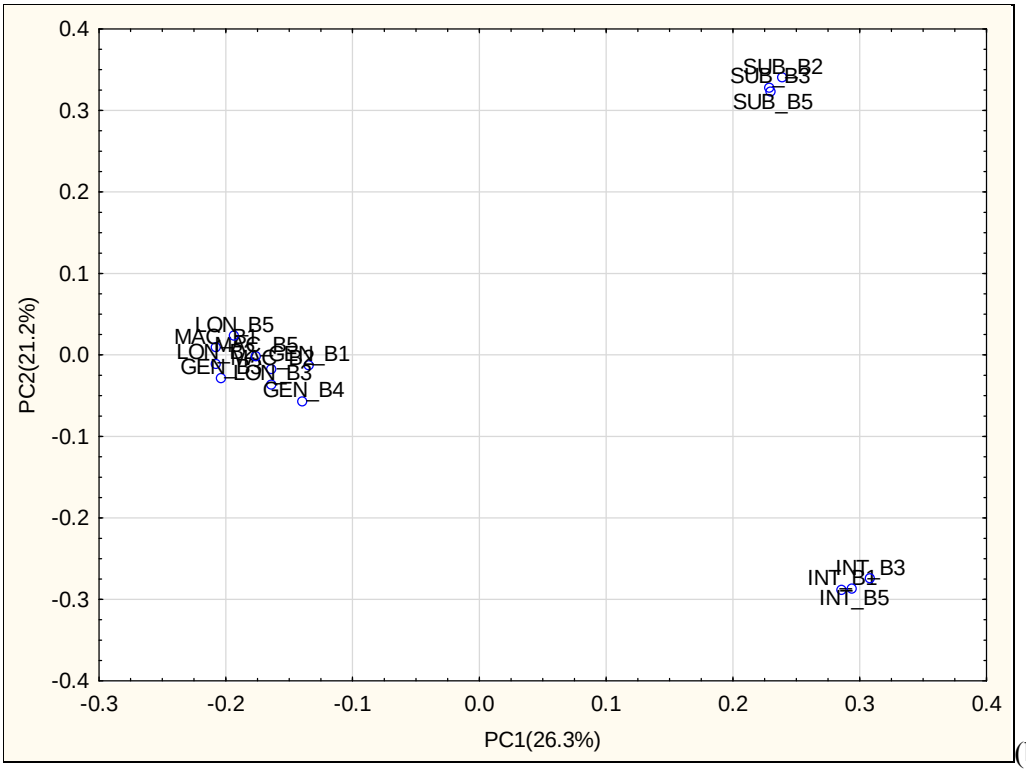
The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia,* respectively. B refers to batches, 1–5 refer to the batch number.

ADDENDUM D

DISTATIS product configurations of three replications of directed sorting according to (a) aroma and (b) palate attributes of five Cyclopia species.



(a)

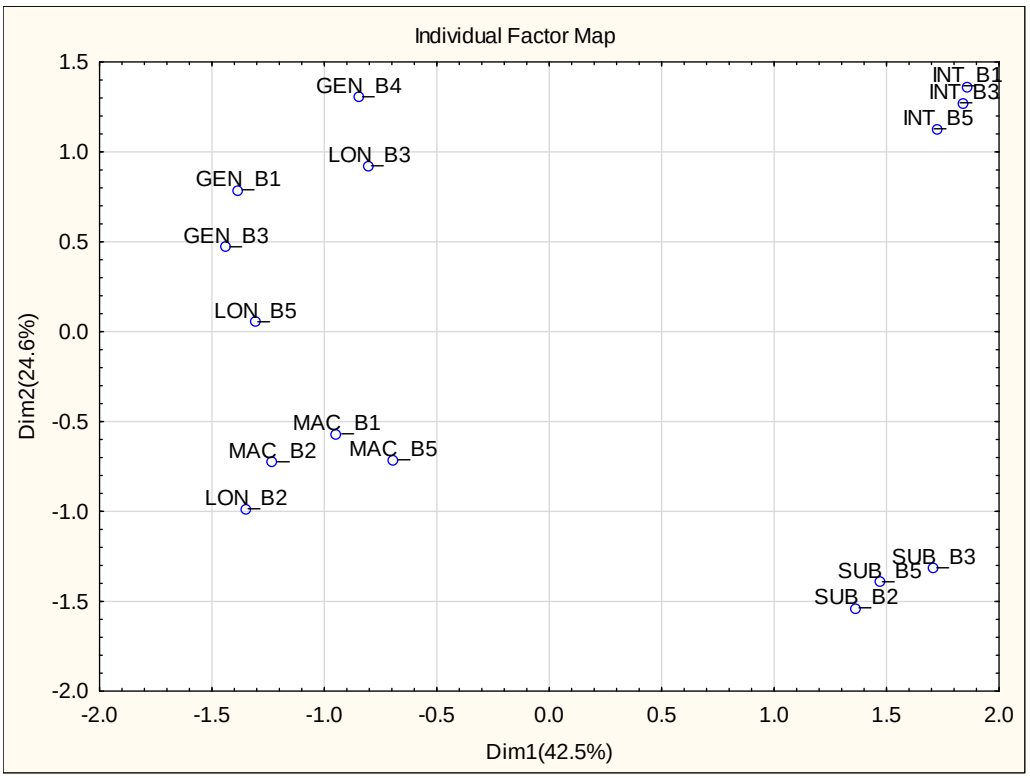


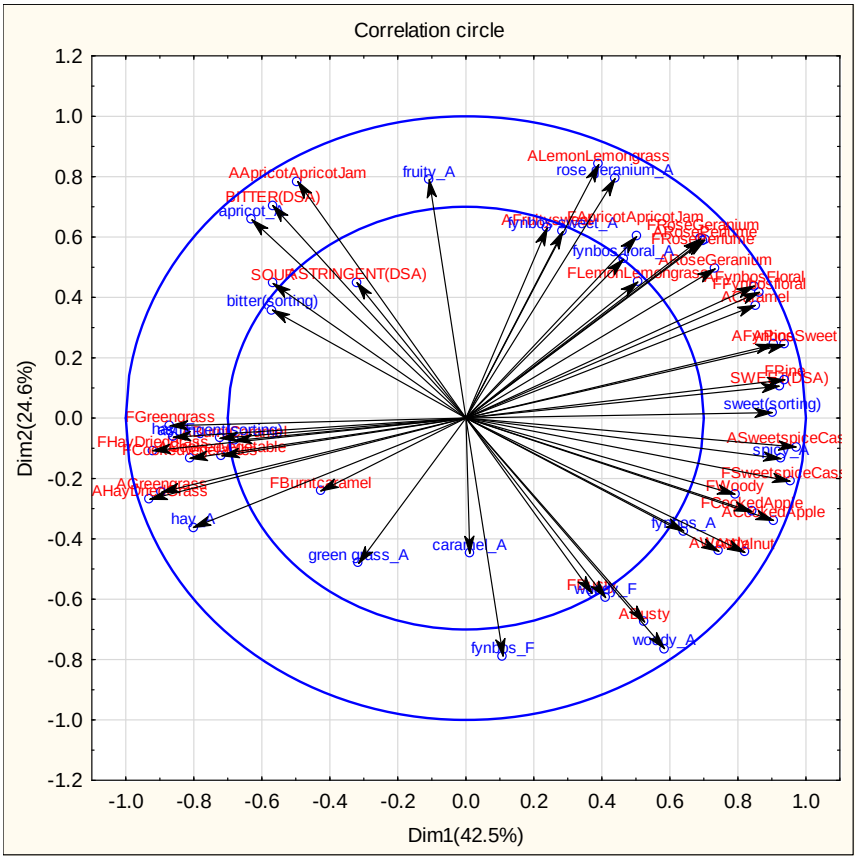
(b)

The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia,* respectively. B refers to batches, 1–5 refer to the batch number.

ADDENDUM E

Multiple factor analysis: individual factor map and correlation circle on attributes obtained from merged data of DSA and standard deviates of correspondence analysis on sorting global.

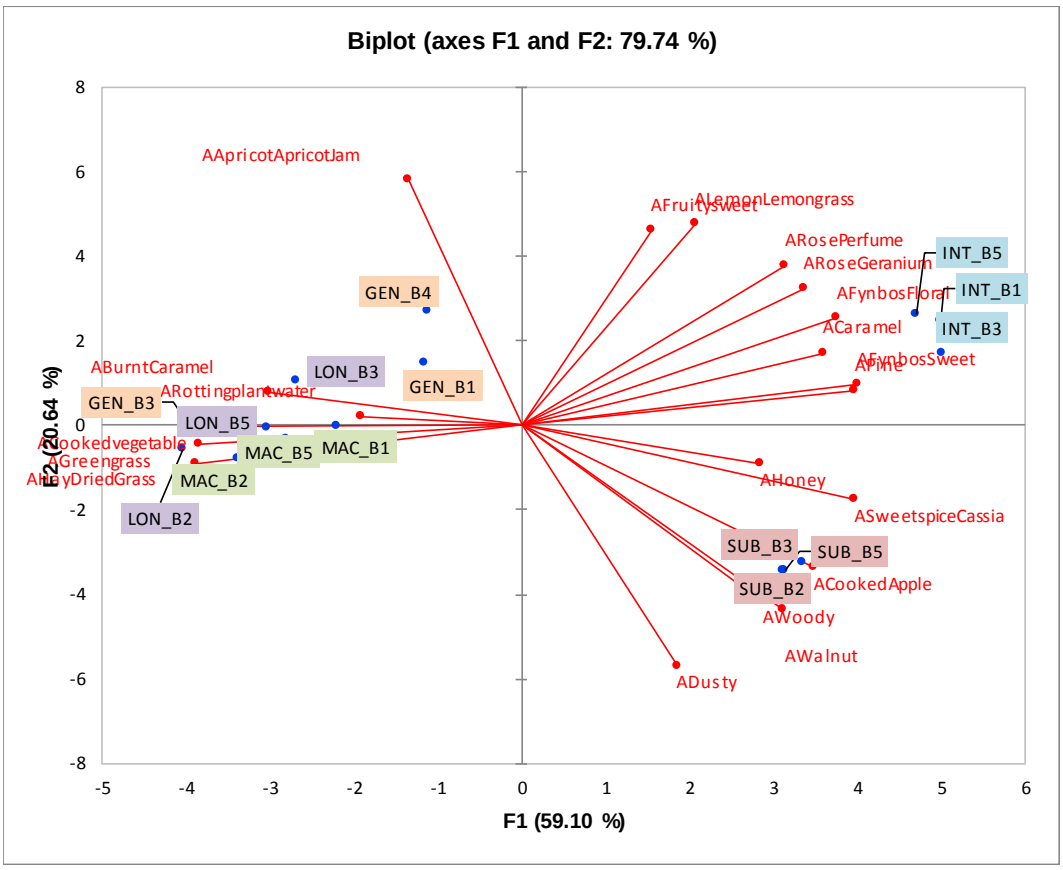




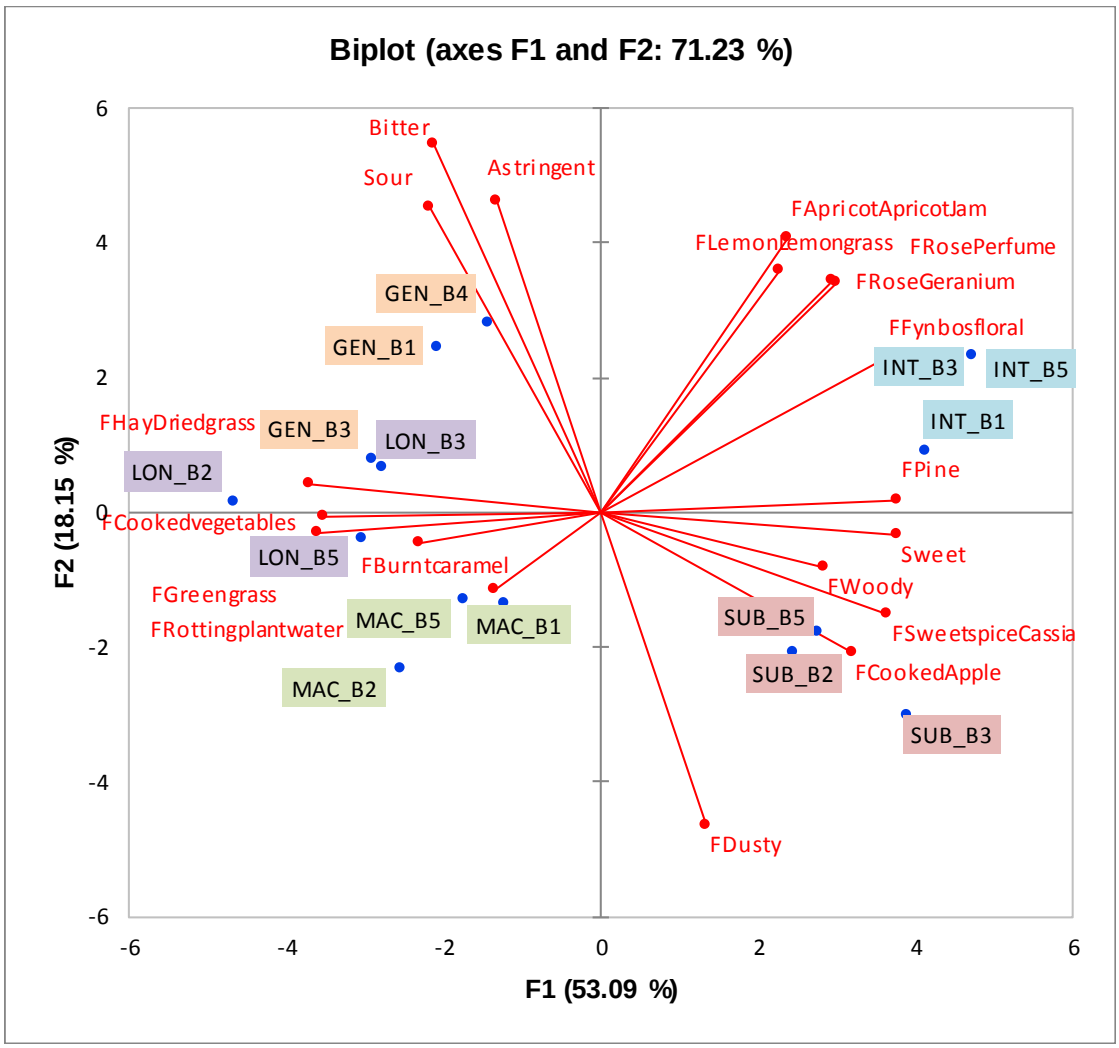
Capital letters added to attributes indicate A: aroma (orthonasal) and F: flavour (retronasal). The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia*, respectively. B refers to batches, 1–5 refer to the batch number. Sorting attributes are marked in blue and DSA attributes in red.

ADDENDUM F

PCA bi-plots based on DSA of samples included in the sorting task, based on aroma or palate attributes respectively.



PCA bi-plot obtained with DSA of five *Cyclopia* species. Samples selected for the sorting task and only aroma attributes are included. Capital letters added to attributes indicate A: aroma. The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides*, *C. maculata*, *C. longifolia*, *C. subternata* and *C. intermedia*, respectively. B refers to batches, 1–5 refer to batch number.



PCA bi-plot obtained with DSA of five *Cyclopia* species. Samples selected for the sorting task and only palate attributes are included. Capital letters added to attributes indicate F: flavour (retronasal). The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides*, *C. maculata*, *C. longifolia*, *C. subternata* and *C. intermedia*, respectively. B refers to batches, 1–5 refer to batch number.

Chapter 7

Polarised sensory positioning: a case study with trained assessors and honeybush as a complex product

Abstract

Polarised sensory positioning (PSP) is a reference-based rapid sensory method, based on the comparison of samples to fixed references that allows data collection across multiple sessions. Projective mapping and sorting, both sensory categorisation methods, were demonstrated to be valid for the sensory characterisation of honeybush infusions, but these methods do not allow for data aggregation. The current study aims to determine the applicability of PSP, with data aggregation over consecutive sessions, for the sensory characterisation of honeybush infusions. The efficacy of partial and global PSP for the sensory characterisation of honeybush infusions was investigated. Trained assessors (n = 10) used partial PSP (P-PSP) on aroma, P-PSP on palate and global PSP to evaluate the infusions (n = 15). PSP were applied using a continuous scale and five poles. Two blind duplicates were included to determine panel performance and reliability. Data, aggregated for three sessions per PSP variation, were subjected to multiple factor analysis (MFA) and correspondence analysis (CA). Similar product configurations were obtained when comparing results of descriptive sensory analysis and MFA and CA plots for partial and global PSP. Similarity of product configuration was confirmed with RV coefficients >0.8. Placement of samples and blind duplicates in close proximity on the perceptual maps indicated good panel reliability. Global and P-PSP on aroma were more effective to differentiate between samples than P-PSP on palate. Application of P-PSP on aroma by trained assessors is recommended as rapid method for quality control in the honeybush industry. P-PSP on aroma focuses assessors on one modality, reduces evaluation time and sensory fatigue and is effective in discriminating between samples based on their sensory profiles.

Keywords: Rapid profiling; Polarised sensory positioning; Reference based method; Multiple factor analysis, Correspondence analysis; *Cyclopia* species

1. Introduction

Sorting and projective mapping (PM) were successfully applied for the sensory characterisation of honeybush herbal tea, providing results comparable to that of DSA, as described in Chapters 5 and 6. These two methods are rapid categorisation techniques for the evaluation of global similarity (or dissimilarity) between products with the further advantage that broad characterisation of products are obtained through the addition of descriptors (Varela & Ares, 2012). However, a disadvantage of these methods is that the entire

sample set has to be presented simultaneously, problematic for the evaluation of large sample sets or comparing data collected in different sessions. The need for a comparative method that addresses these limitations, led to the development of polarised sensory positioning (PSP) by Teillet, Schlich, Urbano, Cordelle and Guichard (2010).

Polarised sensory positioning has a holistic approach, based on measuring the overall similarity or dissimilarity of products in relation to an actual reference or "sensory pole" (Teillet, 2014; Valentin, Chollet, Lelièvre, & Abdi, 2012). Although PSP was originally developed to evaluate the sensory properties of mineral water using trained assessors (Teillet et al., 2010), it has been successfully used with consumers (Antúnez et al., 2015; Ares et al., 2013; Cadena et al., 2014), suggesting that the use with an untrained panel could be feasible.

The choice of poles is regarded as the most critical step when conducting PSP (Ares et al., 2015). The selection of three poles, that are stable over time and represent the total sensory space and main sensory characteristics of the product category in question, is recommended (De Saldamando, Delgado, Herencia, Giménez, & Ares, 2013; Teillet, 2014). The level of training of assessors should be taken into account when selecting a set of poles. The set of poles could have a more pronounced effect when working with consumers and an untrained panel than with trained assessors who have previous knowledge of the sensory characteristics of the product.

Two PSP approaches have been reported: PSP using a continuous scale and triadic PSP (t-PSP) (Teillet, 2014). In the case of PSP with a continuous scale, assessors need to indicate the similarity (or dissimilarity) of each sample relative to each pole using an unstructured line scale where 0 indicates perceived similarity to the pole and 100 indicates dissimilarity to the pole. Triadic PSP requires that assessors indicate to which of the included poles the sample is most similar and to which least similar; no scale values are given in this test variation.

Although PSP has delivered promising results, application of this method on a limited range of products have been published. In addition to the study on mineral water (Teillet et al., 2010), the PSP methodology has been applied on products including make-up foundation (De Saldamando et al., 2013), orange-flavoured drinks (Antúnez et al., 2015; Ares et al., 2013; De Saldamando et al., 2013), functional yoghurts (Cadena et al., 2014), chocolate milk beverages (Antúnez et al., 2015; Ares et al., 2015) and astringent agents (Fleming, Ziegler, & Hayes, 2015). All of these studies used consumers, thus untrained assessors, for the PSP task. Recently one study, presented at the EuroSense Conference 2014, reported on the PSP task with trained assessors (Varela, Svartebekk Myhrer, Naes, & Hersleth, 2014). To date, this is the only study where a trained panel was used.

Similar results were obtained when comparing results of PSP to that of DSA (Cadena et al., 2014), polarised projective mapping (Ares et al., 2013), PM and check-all-that apply (CATA) (Cadena et al., 2014) and sorting (Fleming et al., 2015). PSP can be considered a promising method for the sensory characterisation of products, having the advantage of data collection over multiple sessions and different panels as demonstrated by Teillet and co-workers (Teillet et al., 2010). A disadvantage of this method is that *a priori*

knowledge of the sensory space is necessary for the effective selection of poles (Teillet, 2014). This could pose some difficulty in selecting poles and would entail that some sensory technique be first applied to obtain a representation of the sensory space. A further limitation of the method is that descriptive information about the sensory characteristics of the samples is only obtained if the poles are well described. The data obtained with PSP is not quantitative and therefore it is not possible to link PSP data to physical and chemical data. Furthermore, data analysis can be more complex when compared to that of DSA.

Both the scale and triadic PSP approaches are based on the principle that products are compared to a reference, which are constant across consecutive testing sessions. This provides an excellent opportunity for the food and beverage industry to apply PSP as a tool within quality control. Quality control implies evaluating different products during numerous sessions to determine the degree of compliance of the test products to the set quality standards. The PSP method shows potential for implementation as tool in quality control in the herbal tea industry. Applications would be to ensure batch-to-batch consistency during the production season, especially when blending of different species is required. Due to the distinct differences in the sensory profiles of different *Cyclopia* species (Erasmus, Theron, Muller, Van der Rijst, & Joubert, 2017), special care is required when blending different species to ensure a product with consistent, high quality.

An extensive database on the sensory qualities of the main *Cyclopia* species of commercial interest is available, making effective pole selection possible. Furthermore, dry plant material, used for the preparation of honeybush infusions, is stable over time when stored in sealed containers and therefore suitable to use as poles. The aim of the current study was to evaluate the validity of PSP, with data aggregation over consecutive sessions, for the sensory characterisation of honeybush herbal tea. This herbal tea is a good example of a complex beverage with a wide range of sensory attributes and a product that has to be evaluated at a constant high temperature. A novel variation of PSP was tested: trained assessors were asked to focus on one modality at a time when conducting PSP. Product configurations of partial PSP on aroma, partial PSP on palate and global PSP (all attributes) were compared to that of DSA to determine the validity of the proposed variations for the sensory characterisation of this complex product.

2. Materials and methods

2.1 Descriptive sensory analysis

Descriptive sensory analysis (DSA) was used for the sensory profiling of randomly selected, independent batches of five *Cyclopia* species (n = 36), as described in Chapter 5. A standardised protocol for sample preparation and presentation at 65°C is also described in Chapter 5 (Moelich, Muller, Joubert, Næs, & Kidd, 2017).

2.2 Polarised sensory positioning

2.2.1 Sample selection

The sample configuration of the total sample set, obtained through principal component analysis (PCA) of the DSA data, was used to select three representative samples per species. The three samples per *Cyclopia* species were selected as three independent replications (representing different batches of plant material) and were evaluated in three consecutive sessions, i.e. one sample per *Cyclopia* species was evaluated per session. Two blind duplicates, identical to two of the poles, were included to aid in evaluating panel performance (Falahee & MacRae, 1997; Lim & Lawless, 2005). Five poles, representing the five *Cyclopia* species, were prepared by blending equal amounts of the three batches per species selected for the PSP task. Blending was done to address the variation within species. These poles represented the sensory space associated with the product category, a requirement proposed by previous research on PSP (Ares et al., 2015; De Saldamando et al., 2013). Each assessor received seven samples, one sample per *Cyclopia* species and two blind duplicates, to compare to the five poles per session. The experimental design and sample codes used in the PSP task are presented in Table 1. The hot water herbal tea infusions were prepared according to the standardised protocol as described in Chapter 5.

2.2.2 Partial and global polarised sensory positioning

The same panel used for the DSA, except for one new female panel member, participated in the PSP task, which commenced three weeks after completion of the DSA. Samples were presented simultaneously to assessors for evaluation using PSP with a continuous scale. Coloured labels with letters G, I, S, M and L, representing *C. genistoides*, *C. intermedia*, *C. subternata*, *C. maculata* and *C. longifolia*, respectively, were used to identify the five poles. The test samples were marked with three-digit codes and presented in a randomised order to each assessor. The poles and the test samples were kept at a constant temperature of 65°C throughout the evaluation session. The PSP task was explained to the panel but no retraining on honeybush infusions and attributes were conducted. Panel members were instructed to smell and/or taste each pole and thereafter evaluate the similarity or dissimilarity of each labelled sample in comparison to each pole. A questionnaire was provided on which assessors had to indicate similarity or dissimilarity on a 100 mm line scale where 0 indicated that the test sample were perceived to be similar to the pole (same) and 100 indicated dissimilarity to the pole (different). An example of the instruction sheet and questionnaire for global PSP are provided in Addendum A.

Three variations of the PSP task were applied: partial PSP on aroma (P-PSP aroma), partial PSP on palate (P-PSP palate) and PSP on all attributes (global PSP). These three variations of the PSP task were performed on three consecutive days, with three sessions per method per day. Assessors were requested to take a 15 min break between sessions to avoid panel fatigue. Unsalted water biscuits (Woolworths, Stellenbosch, South Africa) and still natural spring water (Woolworths, Stellenbosch, South Africa) were used as palate cleansers. Assessors were seated in a temperature- (21°C) and light-controlled room at individual tables rather than booths as this provided enough space for the water bath and the completion of the PSP task. All the assessors

completed the three sessions of the respective PSP variations within a 2-hour period. The distance from the "same" to the mark on the continuous scale was measured for each sample and each pole per assessor.

2.3 Statistical procedrues

2.3.1 DSA

The statistical analysis of the DSA data were previously described in Chapter 5.

2.3.2 Polarised sensory positioning

2.3.2.1 Multiple factor analysis (MFA)

PSP data were analysed using multiple factor analysis (MFA) where data from each assessor were considered as a separate group of variables. By using this approach, individual data of assessors are preserved. This analysis thus compensates for individual assessor differences when scoring differences between products and poles (Teillet, 2014). Data of the three consecutive sessions per variation of the PSP task were aggregated. The data matrix consisted of ratings per assessor for each sample across each of the five poles and three sessions, as done in previous PSP studies (Ares et al., 2013; Cadena et al., 2014; De Saldamando et al., 2013). An extract of the data matrix of global PSP is provided in Addendum B. Confidence ellipses were calculated using parametric bootstrapping, as described by Dehlholm, Brockhoff and Bredie (2012).

2.3.2.2 Correspondence analysis

Correspondence analysis (CA) is used as a descriptive technique to analyse multi-way tables containing some measure of correspondence between rows and columns. The primary purpose of CA is to produce a simplified (low-dimensional) representation of the information contained in a large frequency table. The data set describes the association between two qualitative variables (Lê, 2014). CA of the PSP data allowed us to understand the proximity of the infusions relative to the poles, as presented in a two-dimensional space. Products situated closer to the pole on the 2-dimensional space were therefore perceived to be similar to the corresponding pole. The PSP data were thus analysed using CA with poles in rows (10 rows consisting of five poles and five opposites of poles) and products in columns (five species x 3 replications and two blind duplicates). The correspondence analysis of the PSP data was based on a dissimilarity matrix. A dissimilarity matrix is a square and symmetric matrix that expresses similarity by comparing pairs of samples. A value of minus 1 (-1) indicates similarity between a pair of samples while a value of +1 indicates dissimilarity. The values in the dissimilarity matrix were based on the scale (0–100) used by the assessors, where 0 indicated same as or most similar to pole and 100 indicated that the sample were perceived to be dissimilar to the pole. An extract of the dissimilarity matrix of global PSP data used for CA is presented in Addendum C.

2.3.2.3 Comparison of methodologies

The degree of similarity between product configurations in the first two components of the PCA of DSA data and MFA and CA of PSP data were compared using RV coefficients. The RV coefficient is a multivariate similarity coefficient that can be used to measure the extent of which two product configurations are similar

(Abdi, Valentin, Chollet, & Chrea, 2007; Louw et al., 2013). The RV coefficient depends on the relative position of the points in the configuration and is therefore not influenced by rotation and translation (Robert & Escoffier, 1976). RV coefficients closer to 1 indicates high similarity between configurations for the dimensions under question and 0 indicates unrelated configurations. RV coefficients ≥ 0.7 are indicative of an acceptable level of similarity (Cartier et al., 2006; Nestrud & Lawless, 2008).

Data analyses were performed using R 3.2.0 (R Core Team, 2015). FactoMineR was used to perform MFA and to compute RV coefficients (Lê, Josse, & Husson, 2008).

3. Results

3.1 Descriptive sensory analysis

DSA data of the entire sample set (n = 36) have been discussed in full in Chapter 3 (Development of prediction model). Principal component analysis, using the correlation matrix of the DSA data, was used to visualise and elucidate the relationship between samples and attributes (Fig. 1). The first two components explained 71.71 % of the variability in the DSA data. A clear differentiation between *C. subternata* and *C. intermedia*, towards the positive end of principal component 1 (PC1), was observed. *Cyclopia subternata* samples were associated with a "cooked apple", "sweet spice / cassia", "woody" and "walnut" aroma and flavour and a "dusty" aroma. *Cyclopia intermedia* samples associated with a "fynbos floral", "rose perfume" and "rose geranium" aroma and flavour and a "pine", "fynbos sweet" and "caramel" aroma. Samples of *C. genistoides*, *C. maculata* and *C. longifolia* formed one group towards the negative end of PC1, and were associated with bitter, astringent and sour tastes, as well as a "hay / dried grass", "green grass" and "cooked vegetable" aroma and flavour. Samples regarded as representative of the respective species were selected for the three sessions of the PSP task and are indicated on the PCA bi-plot (Fig. 1).

3.2 Polarised sensory positioning

3.2.1 Multiple factor analysis

The sample configurations of the five *Cyclopia* species in the first two dimensions of MFA performed on data of P-PSP aroma, P-PSP palate and global PSP are presented in Fig. 2, 3 and 4. The first and second dimensions of the MFA explained 54.7% of the variance in the P-PSP aroma data, 54.5% in the P-PSP palate data and 58.6% in the global PSP data. The configurations for the three variants of PSP were fairly similar when comparing differentiation between samples on dimension 1 of the respective plots. *Cyclopia subternata* and *C. intermedia* samples, associated with the sweet, spicy and floral attributes, formed groupings towards the positive end of dimension 1, while *C. genistoides*, *C. maculata* and *C. longifolia*, associated with "hay / dried grass" and "green grass" aroma and flavour, formed a group towards the negative end of dimension 1 (Fig. 1, 2 and 3).

Although the MFA plots revealed fairly similar differentiation of samples on dimension 1 for the different versions of PSP, several differences between the configurations were observed and need to be highlighted. Three separate groups of samples were observed in the MFA product configurations of P-PSP on aroma (Fig. 2) and global PSP (Fig. 4), while the differentiation between groups of samples were less distinct for P-PSP on palate. In the case of P-PSP on aroma and global PSP, a clear distinction between *C. subternata* and *C. intermedia* samples were evident, with no overlap of confidence ellipses, while a slight overlap of confidence ellipses of these samples was observed for P-PSP on palate. Tighter group formation indicates a higher degree of similar evaluations among assessors. Assessors were thus well able to differentiate between *C. subternata* and *C. intermedia* when evaluating aroma or global attributes, but differentiation between these species were less distinct evaluating palate attributes.

3.2.2 Correspondence analysis

Correspondence analysis was used to provide information on the structure of the variables included in the data tables for the variations of PSP, resulting in product maps as presented in Fig. 5, 6 and 7. The first two dimensions were included in the respective CA plots, explaining 70.54% of the variance of P-PSP on aroma (Fig. 5), 72.84% of P-PSP on palate (Fig. 6) and 76.02% of global PSP (Fig. 7) plots. Similar to the MFA product configurations, three groups were observed in the CA plots. A clear differentiation between *C. subternata* and *C. intermedia* samples towards the positive side of dimension 1 was evident, but no clear distinction between the remaining three species (*C. genistoides*, *C. maculata* and *C. longifolia*) was apparent. Again, as in the case with MFA, tighter groups were observed in CA plots for P-PSP aroma (Fig. 5) and global PSP (Fig. 7) compared to that of P-PSP palate (Fig. 6).

3.3 Comparison of PSP to DSA

Validation of the PSP task as method for the sensory characterisation of a complex product such as honeybush infusions was done by visually comparing product configurations obtained for the PSP tasks with that obtained through PCA of the DSA data. The validity of the PSP task was further determined by calculating RV coefficients for the PCA bi-plot of the DSA data (Fig. 8, samples selected for PSP included), and the product configurations obtained with MFA (Fig. 2, 3 & 4) and CA (Fig. 5, 6 & 7) of PSP data.

3.3.1 Comparison of product configurations

The DSA results of the subset of samples used for the PSP task were subjected to PCA, and the resulting product configuration is presented in Figure 8. Component 1 and 2 explained 52.81% and 18.90% of the variance in the DSA data, respectively. Visual comparison of the PCA bi-plot obtained from DSA data (Fig. 8) and the MFA plots (Fig. 2, 3 & 4) and CA plots (Fig. 5, 6 & 7) obtained from the PSP data revealed similar product configurations. Three groups were observed in the corresponding MFA and CA product configurations of PSP, similar to those in the PCA bi-plot of DSA. The PCA bi-plot and the MFA and CA product maps revealed a separation between *C. subternata* and *C. intermedia* on the one side, as opposed to a group towards the opposite side of the map, composed of *C. genistoides*, *C. maculata* and *C. longifolia*.

3.3.2 RV coefficients

The RV coefficients for the similarity of product configurations obtained with DSA and MFA and CA of partial and global PSP are presented in Fig. 9. The first two dimensions of the respective plots were taken into account. RV coefficients place the greatest emphasis on the dimension with the largest explained variance and should therefore be interpreted with caution (Tomic, Berget, & Næs, 2015) and not in isolation. RV coefficients ≥ 0.7 are regarded as an indication of a good level of agreement (Cartier et al., 2006; Nestrud & Lawless, 2008). The RV coefficients between MFA and CA of the three variations of the PSP task and PCA of DSA data were high (RV >0.84), indicating that any of these variations are valid methods for the sensory analysis of infusions of *Cyclopia* species. Closer inspection of the RV coefficients calculated for MFA configurations revealed higher values for P-PSP aroma and global PSP compared to P-PSP palate. RV coefficients for the MFA of P-PSP aroma, P-PSP palate and global PSP were 0.94, 0.87 and 0.95, respectively, confirming similar product configurations.

3.4 Panel performance of the PSP task

The reliability of the panel can be assessed by inspecting how well the panel performed when evaluating blind duplicate samples (Falahee & MacRae, 1997; Hopfer & Heymann, 2013). Two blind duplicates were included: Dulp_INT and Dupl_LON were the duplicate samples for Pole_I (pole *C. intermedia*) and Pole_L (pole *C. longifolia*), respectively. Inspection of the MFA product configurations obtained through the three variations of the PSP task (Fig. 2, 3 & 4) revealed that the duplicate samples for the three replications were placed in close proximity of the corresponding poles. The MFA plots also show an overlap of confidence ellipses for the sample in question and its blind duplicate. This was the case for all three variations of the PSP task, indicating that the panel could be regarded as reliable.

Five poles were included in the PSP task, and the relative position of the poles are indicated on the CA plots by Pole S_-1, Pole I_-1, Pole M_-1, Pole L_-1 and Pole G_-1 representing the poles for *C. subternata*, *C. intermedia*, *C. maculata*, *C. longifolia* and *C. genistoides*, respectively. Samples in close proximity to P S_-1 indicate similarity to the *C. subternata* pole while samples in close proximity to Pole I_-1 indicate similarity to the *C. intermedia* pole. Visual inspection of the CA plots for the variations of PSP (Fig. 5, 6 & 7) revealed that the blind duplicate samples (Dupl_INT and Dupl_LON) were in close proximity to the corresponding poles. A panel can be regarded as reliable when the blind duplicate in question is located in close proximity to the corresponding sample on the perceptual map (Falahee & MacRae, 1997; Fleming et al., 2015).

4. Discussion

The current work evaluated the effectiveness of the PSP task for the sensory characterisation of honeybush infusions representing a complex product with a wide range of sensory attributes where temperature control is essential. Promising results were obtained and the validity of this method will be discussed by comparing

results to that of DSA. The relevance of partial and global PSP for the sensory characterisation of honeybush infusions and implications for application in the honeybush herbal industry will also be addressed.

4.1 Comparison to DSA

Similar product configurations were obtained when visually comparing the PCA bi-plot of the DSA data (Fig. 8) and the MFA configurations of the different variants of PSP (Fig. 2, 3 & 4). Similar product configurations were confirmed by RV >0.84. Furthermore, samples representing the same species, and samples with their blind duplicates, were positioned in close proximity in the sensory space and their confidence ellipses overlapped, indicating good reproducibility of the methodology. The results of the current study are in accordance to research by Varela et al. (2014) who reported highly correlated results when comparing partial and global PSP to quantitative descriptive analysis (QDA) when evaluating Norwegian cheeses and formulated lamb and sheep meat products with trained assessors. Cadena et al. (2014) also reported similar product configurations and high RV coefficients when comparing results of DSA and PSP for the sensory characterisation of yoghurts using consumers.

Limited differentiation between *C. genistoides*, *C. longifolia* and *C. maculata* (positioned toward the left of dimension 1) was obtained with all three variants of PSP (Fig. 3, 4 and 5). During the development of *Cyclopia* species-specific aroma wheels for C. *maculata, C. genistoides* and *C. subternata,* the honeybush species, *C. maculata* revealed no distinct sensory profile and the attributes identified in this species were regarded as the "common" attributes shared by all the *Cyclopia* species (Erasmus et al., 2017). The limited number of attributes that distinguish *C. maculata*, could be one of the reasons for the low degree of differentiation between *C. genistoides*, *C. longifolia* and *C. maculata*. In a study focussing on the cognitive aspects behind the PSP task, Ares et al. (2015) reported that consumers try to identify the most important sensory characteristics that differentiate the poles and associate one or two attributes with each pole. When evaluating test samples, they measure the similarity of samples to poles by evaluating the intensity of the identified attributes. These researchers therefore recommend the selection of poles that are distinctly different, and with each pole representing specific sensory attributes. When conducting the PSP task in the current study, the panel might have found it difficult to identify one or two attributes that would distinguish the *C. genistoides*, *C. maculata* and *C. longifolia* poles, i.e. attributes that would aid in the differentiation between these species. Results on PM (Chapter 5) and sorting on aroma and global sorting (Chapter 6) of the same sample set, revealed similar product configurations to DSA and to the configurations obtained with partial and global PSP. Limited differentiation between *C. genistoides*, *C. maculata* and *C. longifolia* were also obtained with all these methods. Only results for sorting on palate demonstrated differentiation between *C. genistoides* and *C. longifolia.* This differentiation was mainly a result of the bitter taste associated with these species and not with the other samples included in the sorting task.

4.2 Global and partial PSP

A second point of interest was to evaluate the efficacy of PSP when focussing assessors on one modality at a time as an alternative to the complete holistic approach as proposed by Teillet et al. (2010). The original PSP methodology requires evaluating the holistic sensory perception of the product in question in relation to that of the reference. In the current study, a new variant of the PSP task was proposed, focusing assessors on one modality at a time (aroma or palate) using P-PSP. Results of the present work indicated that P-PSP aroma illustrated better discriminative ability than P-PSP palate. This is interesting as sorting on palate illustrated good discriminating ability, as discussed previously (Chapter 6). The lower discriminative ability of P-PSP on palate could be a result of the fact that five poles were included in the current study. Inclusion of five poles forced assessors to taste and re-taste many times, causing sensory fatigue and reduced ability to differentiate between samples with only subtle differences.

The MFA configurations of P-PSP aroma (Fig. 2) and global PSP (Fig. 4) revealed a clear differentiation between samples representing *C. subternata* and *C. intermedia* with no overlap of confidence ellipses between these samples. Discrimination between these species was not obtained with P-PSP palate (Fig. 3). These results suggest that assessors could distinguish the similarity between these samples and the respective poles when evaluating aroma or global attributes, but not when focussing on only palate attributes.

In the context of the current research, the results for P-PSP aroma is of interest. As discussed previously, Ares et al. (2015) suggests that assessors should select one or two attributes most representative of each pole ("key attributes") and evaluate similarity of test samples by comparing the "key" attributes of the poles and the test samples. Aroma, and not palate attributes, appears to be the main driver for product differentiation in the samples selected for the current study. Previous research on honeybush infusions (Erasmus et al., 2017) illustrated similar trends for aroma and flavour attributes: aroma attributes were perceived at higher intensities than flavour attributes and it was thus easier for the assessors to distinguish between samples when considering aroma attributes. When discrimination between samples is driven by aroma differences, P-PSP aroma is a practical choice since focussing the assessors on aroma only, could make the PSP task easier for assessors and it would also minimise sensory fatigue. These findings have implications for the current research but could also unlock numerous possible applications of PSP in the honeybush herbal tea industry.

4.3 Selection of poles

Careful selection of poles that cover the sensory space in question, is advised and in most cases, three poles are sufficient to obtain a stable sample configuration (Ares et al., 2013; Cadena et al., 2014; De Saldamando et al., 2013; Teillet et al., 2010). Ares et al. (2015) further recommend the selection of poles that clearly represent specific characteristics of the sensory space in question and that are perceivable different, as discussed previously. In the current study, five poles were selected to represent the five *Cyclopia* species to determine if differentiation between the species would be possible when comparing samples to a known reference. Two of the selected poles (poles for *C. subternata* and *C. intermedia*) were distinctly different in sensory profiles and represented specific sensory characteristic, but the remaining three poles (poles for *C.*

genistoides, *C. maculata* and *C. longifolia*) were not distinctly different and rather show an overlap of sensory characteristics. Their selection was based on DSA results of several samples of each species. Inclusion of the latter three poles that were not clearly different, might have increased the sensory and cognitive fatigue for assessors and consequently did not result in additional differentiation between these species.

Inclusion of only three poles that cover the sensory space in question and that are distinctly different in sensory profiles, might have been sufficient to obtain a valid product configuration. As an alternative for including poles representing *C. genistoides*, *C. maculata* and *C. longifolia* respectively, only one pole representing *C. genistoides* should have been included. Three poles representing *C. subternata*, *C. intermedia* and *C. genistoides* would be sufficient to cover the sensory space associated with the five *Cyclopia* species under investigation. Furthermore, *C. genistoides* is associated with distinct sensory attributes that might make it easier for assessors to complete the PSP task effectively. Future research is necessary to evaluate the efficacy of PSP with three poles for the sensory characterisation of different *Cyclopia* species.

5. Conclusions

The present research demonstrated PSP to be a valid method for the broad sensory categorisation of a complex product such as honeybush herbal tea. Similar product configurations were obtained with DSA and partial and global PSP of infusions of honeybush herbal tea. Data aggregation for consecutive session provided reliable and stable results. Placement of samples and their blind duplicates in close proximity on the perceptual map indicated good panel reliability and confirmed the validity of this method. P-PSP on aroma demonstrated to be an effective method to discriminate between samples when aroma is the main driver in sensory differentiation.

In the sensory domain, PSP is referred to as a sensory characterisation method but description of samples is only possible when poles are well described. The sensory space of the five *Cyclopia* species included in the current study was well defined, based on a detailed quantitate and qualitative description obtained with DSA, and interpretation of product configurations of PSP was therefore possible. Future research could address this limitation by including a descriptive step when conducting PSP where assessors are instructed to add descriptors to each pole. This will allow for some description of the samples related to each pole.

In the current study, inclusion of more poles (five poles were included) did not result in additional differentiation between the samples. Inclusion of only three poles that cover the sensory space in question and that are distinctly different in sensory profiles, might have been sufficient to obtain a valid product configuration.

PSP could find application in the honeybush industry in quality control programs where poles, which represent specific quality attributes, could be selected and included in consecutive sessions to ensure consistent quality. Data aggregation over several sessions could further add valuable information on consistency of products over several production seasons. Research on developing quality grading tools that is practical and

easy to implement in the herbal tea industry, is ongoing. PSP could find application in this context. Future research, focussing on PSP with poles representing different grades of tea, is recommended.

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Table 1 *Cyclopia* species with sample codes used for DSA, PSP task and poles in PSP task

Cyclopia species	DSA	PSP variation (aroma, palate and global)			
		Poles	Session 1	Session 2	Session 3
Cyclopia genistoides	GEN_B1 GEN_B3 GEN_B4	Pole_G	GEN_B1	GEN_B3	GEN_B4
Cyclopia subternata	SUB_B2 SUB_B3 SUB_B5	Pole_S	SUB_B2	SUB_B3	SUB_B5
Cyclopia maculata	MAC_B1 MAC_B2 MAC_B5	Pole_M	MAC_B1	MAC_B2	MAC_B5
Cyclopia longifolia	LON_B2 LON_B3 LON_B5	Pole_L	LON_B2	LON_B3	LON_B5
Cyclopia intermedia	INT_B1 INT_B3 INT_B5	Pole_I	INT_B1	INT_B3	INT_B5

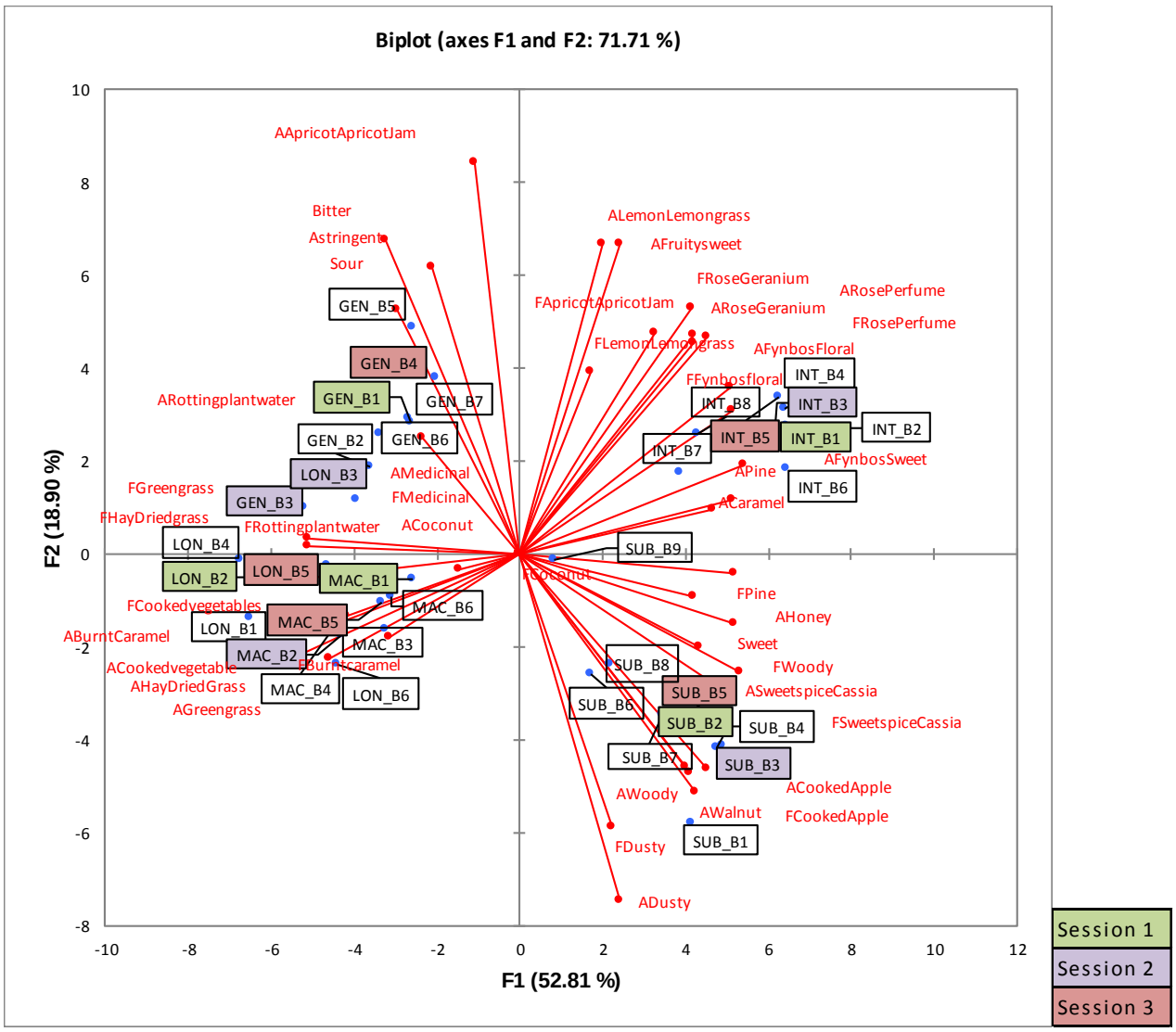


Fig. 1. PCA bi-plot obtained with DSA of five *Cyclopia* species (total sample set). Samples selected for inclusion in replication 1, 2 and 3 of the PSP task are marked with green, purple and red respectively. Capital letters added to attributes indicate A: aroma (orthonasal) and F: flavour (retronasal). The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides*, *C. maculata*, *C. longifolia*, *C. subternata* and *C. intermedia*, respectively. B refers to batches, 1–9 refer to batch number.

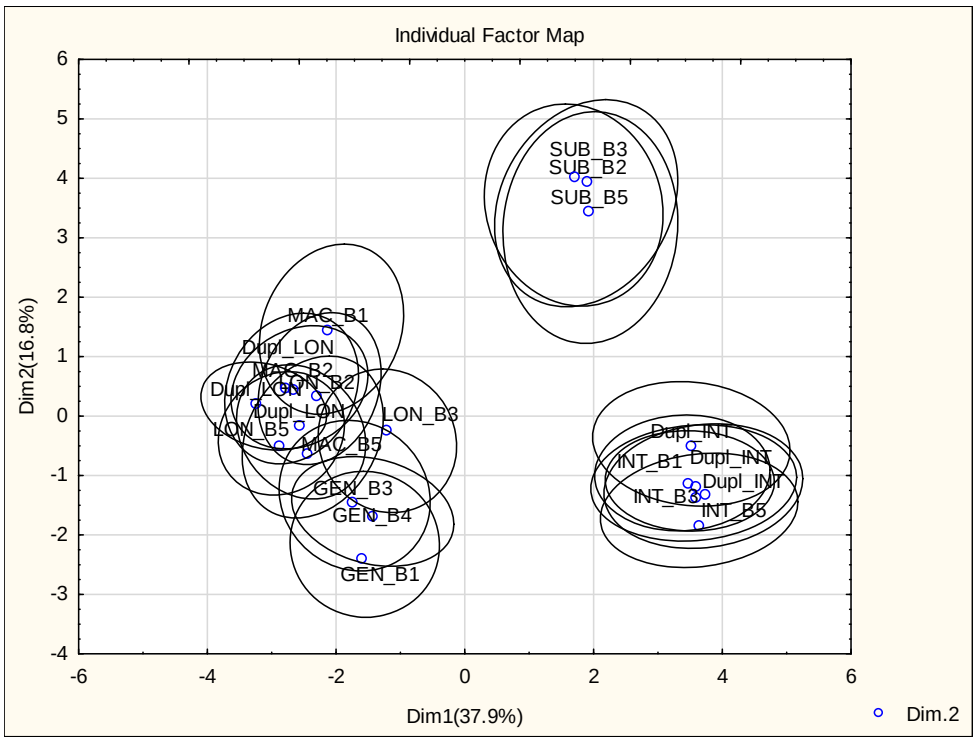


Fig. 2. Sample configuration of five *Cyclopia* species in the first two dimensions of multiple factor analysis performed on data from partial polarised sensory positioning based on aroma. The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides*, *C. maculata*, *C. longifolia*, *C. subternata* and *C. intermedia*, respectively. B refers to batches, 1–5 refer to the batch number. Dupl_INT refers to the sample identical to pole I, Dupl_LON refers to the sample identical to pole L.

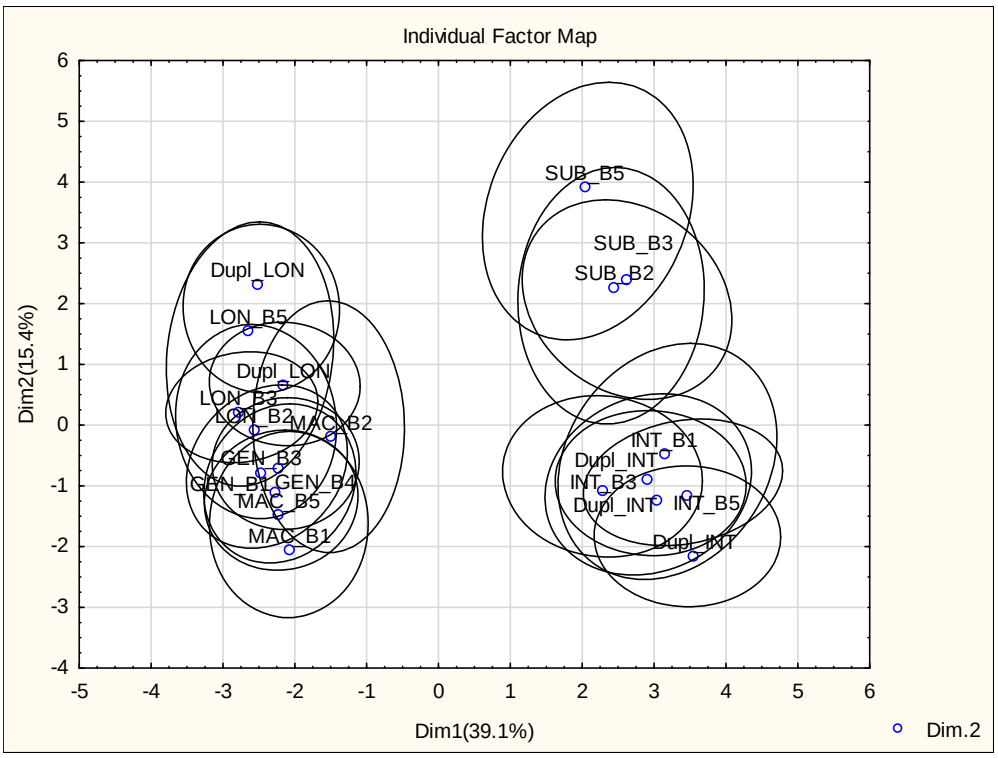


Fig. 3. Sample configuration of five *Cyclopia* species in the first two dimensions of multiple factor analysis performed on data from partial polarised sensory positioning based on palate. The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia,* respectively. B refers to batches, 1–5 refer to the batch number. Dupl_INT refers to the sample identical to pole I, Dupl_LON refers to the sample identical to pole L.

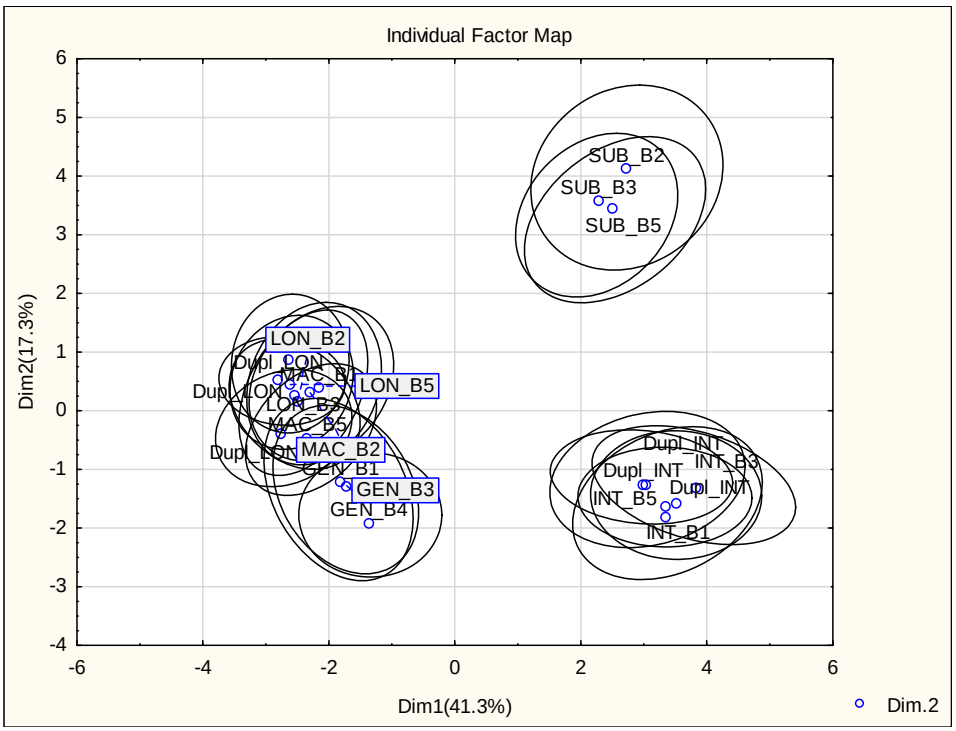


Fig. 4. Sample configuration of five *Cyclopia* species in the first two dimensions of multiple factor analysis performed on data from polarised sensory positioning (global). The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia,* respectively. B refers to batches, 1–5 refer to the batch number. Dupl_INT refers to the sample identical to pole I, Dupl_LON refers to the sample identical to pole L. Samples with borders have been moved on the plot to make it more legible.

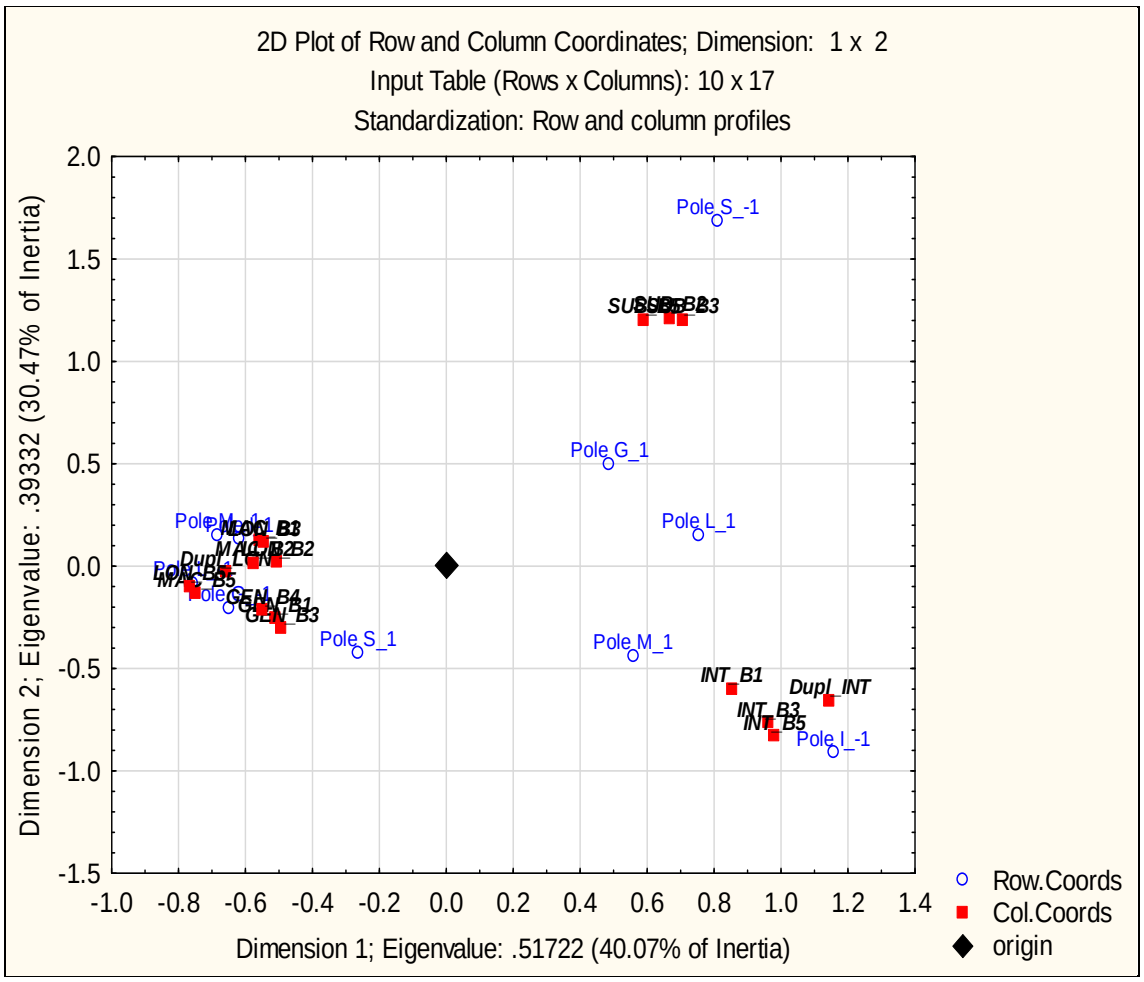


Fig. 5. Sample configuration of five *Cyclopia* species in the first two dimensions of correspondence analysis performed on data from partial polarised sensory positioning based on aroma. The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia,* respectively. B refers to batches, 1–5 refer to the batch number. Dupl_INT refers to the sample identical to pole I, Dupl_LON refers to the sample identical to pole L.

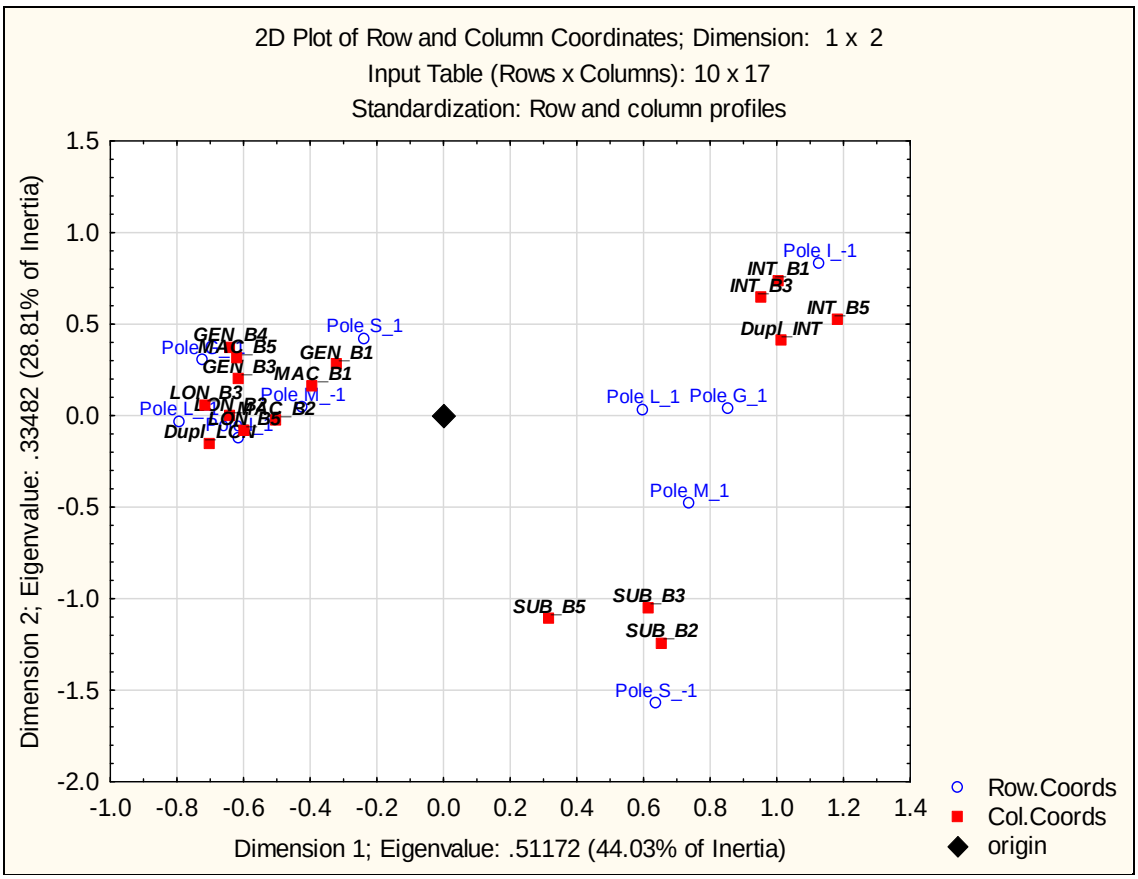


Fig. 6. Sample configuration of five *Cyclopia* species in the first two dimensions of correspondence analysis performed on data from partial polarised sensory positioning based on palate. The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia*, respectively. B refers to batches, 1–5 refer to the batch number. Dulp_INT refers to the sample identical to pole I, Dupl_LON refers to the sample identical to pole L.

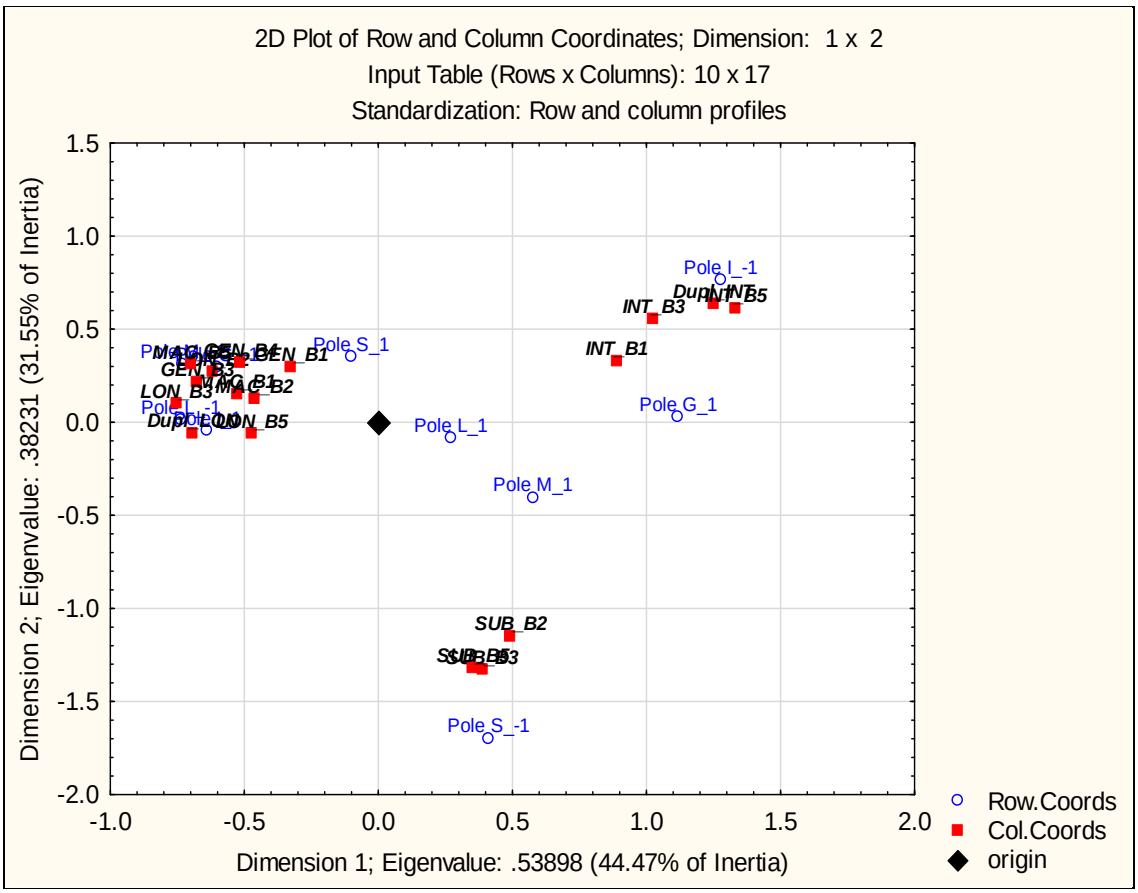


Fig. 7. Sample configuration of five *Cyclopia* species in the first two dimensions of correspondence analysis performed on data from polarised sensory positioning (global). The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia,* respectively. B refers to batches, 1–5 refer to the batch number. Dulp_INT refers to the sample identical to pole I, Dupl_LON refers to the sample identical to pole L.

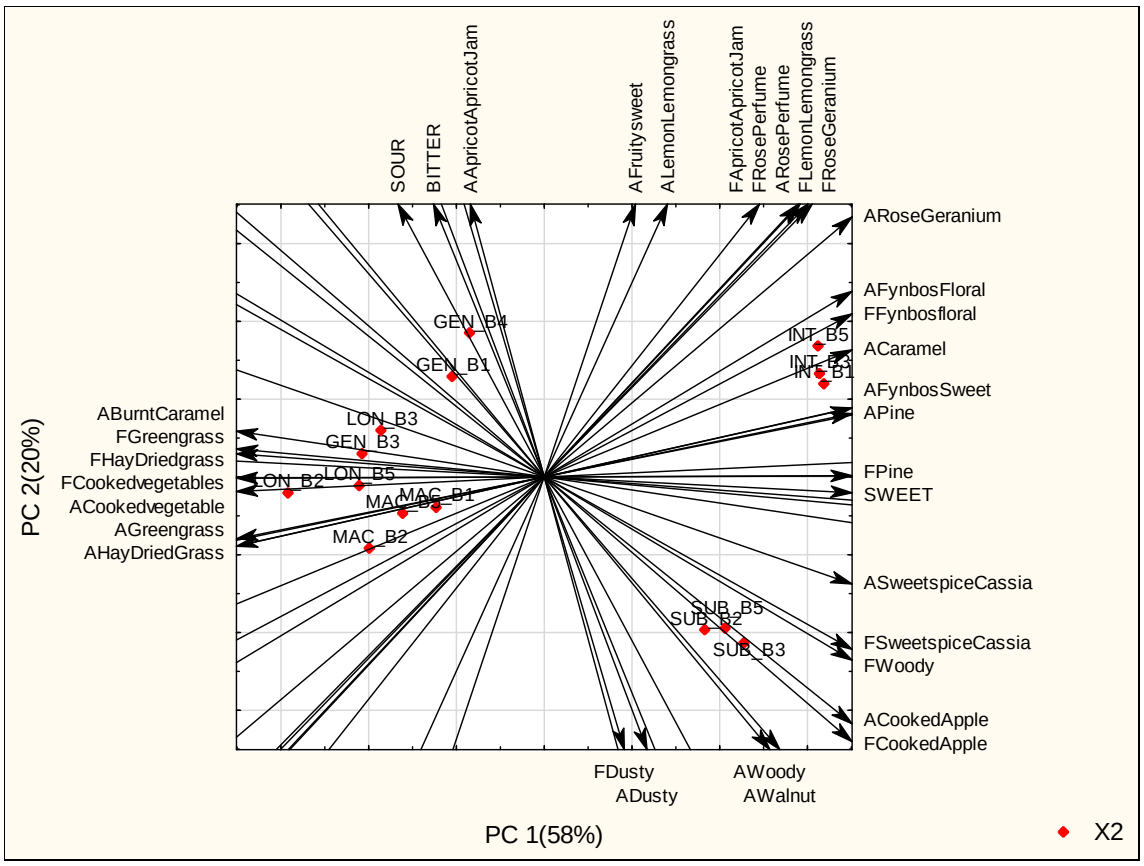


Fig. 8. PCA bi-plot obtained with DSA representing the differentiation among five *Cyclopia* species. Samples selected for the PSP task are included. Capital letters added to attributes indicate A: aroma (orthonasal) and F: flavour (retronasal). The abbreviations GEN, MAC, LON, SUB and INT refer to *C. genistoides, C. maculata, C. longifolia, C. subternata* and *C. intermedia,* respectively. B refers to batches, 1–5 refer to the batch number.

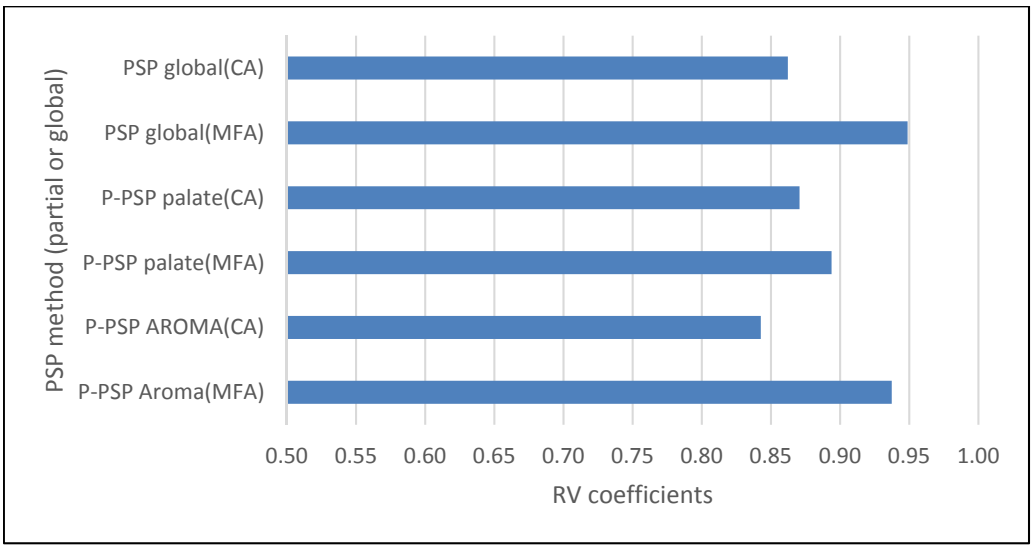


Fig. 9. RV coefficients for the correlation between the product configurations obtained with PCA of DSA data and MFA and CA of partial PSP (aroma or palate) and PSP on global attributes of five *Cyclopia* species

ADDENDUM A

Instruction sheet and questionnaire: Polarised sensory positioning (global)

HONEYBUSH 2015 POLARIZED SENSORY POSITIONING

Day 3 – Thursday, 25th June 2015

SESSION 2 PSP **according to** GLOBAL ATTRIBUTES

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

INSTRUCTIONS

- You have been presented with **5 honeybush samples** labelled with a letter (G, I, S, M, L) on labels with different colours. These samples represent 5 poles or 5 reference samples.
- You have been presented with a further 7 samples labelled with a three digit code.
- Please evaluate each sample in comparison to the five poles or reference samples, according to how **SIMILAR or DISSIMILAR** they are for you.
    - On the questionnaire provided, mark the similarity or dissimilarity of each sample relative to each pole.
    - Use the line scale to indicate similarity or dissimilarity, where 0 indicate that the test sample is similar to the pole (SAME), and 100 indicates that the test sample is dissimilar to the pole (DIFFERENT).
    - You have to test each sample against all 5 the poles.
    - Please evaluate the test samples in the order presented, working from front to back.
    - You are allowed to **taste or smell** the samples as many times as you like.
    - **NOTE**: Please try to work as quickly as possible to **prevent the samples from cooling down** too much.

QUESTIONNAIRE FOR PSP: GLOBAL

Judge no	Judge name	Rep

SAMPLE NO				
	SAME		DIFFERENT	
POLE G	0	|______|		100
POLE I	0	|______|		100
POLE S	0	|______|		100
POLE M	0	|______|		100
POLE L	0	|______|		100
SAMPLE NO				
	SAME		DIFFERENT	
POLE G	0	|______|		100
POLE I	0	|______|		100
POLE S	0	|______|		100
POLE M	0	|______|		100
POLE L	0	|______|		100
SAMPLE NO				
	SAME		DIFFERENT	
POLE G	0	|______|		100
POLE I	0	|______|		100
POLE S	0	|______|		100
POLE M	0	|______|		100
POLE L	0	|______|		100
SAMPLE NO				
	SAME		DIFFERENT	
POLE G	0	|______|		100
POLE I	0	|______|		100
POLE S	0	|______|		100
POLE M	0	|______|		100
POLE L	0	|______|		100

ADDENDUM B

Raw data: Polarised sensory positioning (global)

DAY 3 - PSP ON 2014 HB SAMPLES – GLOBAL 2015/06/25

Samples 1 pole per specie = 5 poles

5 samples (species) + 2 blind duplicates per rep = 7 samples / rep

3 reps

3 sets of 5 species + 2 blind duplicates used for 3 reps

Scale Unstructured line scale of 0-100 mm where 0 = same as pole and 100 = dissimilar to pole

Rep	Judge	Sample	Pole G	Pole I	Pole S	Pole M	Pole L	Judge	Sample	Pole G	Pole I	Pole S	Pole M	Pole L		Judge	Sample	Pole G	Pole I	Pole S	Pole M	Pole L
1	1	GEN_B1	3	44	74	52	25	2	GEN_B1	3	56	31	44	76		10	GEN_B1	3	76	96	49	22
1	1	INT_B1	66	3	39	56	50	2	INT_B1	91	0	91	91	95		10	INT_B1	58	5	78	30	58
1	1	MAC_B1	25	62	30	5	19	2	MAC_B1	74	13	66	74	83		10	MAC_B1	32	76	98	46	32
1	1	LON_B2	34	56	45	21	5	2	LON_B2	85	32	75	41	94		10	LON_B2	30	70	96	7	29
1	1	SUB_B2	35	48	6	18	24	2	SUB_B2	48	72	17	78	15		10	SUB_B2	93	38	5	68	96
1	1	Pole_Int	45	4	28	38	41	2	Pole_Int	70	5	61	79	87		10	Pole_Int	58	3	74	35	58
1	1	Pole_Lon	16	55	35	40	5	2	Pole_Lon	46	69	20	24	62		10	Pole_Lon	9	62	99	36	21
2	1	GEN_B3	6	67	48	55	32	2	GEN_B3	12	22	71	21	79		10	GEN_B3	22	80	99	56	4
2	1	INT_B3	65	5	39	55	60	2	INT_B3	3	19	51	69	79		10	INT_B3	66	3	84	48	66
2	1	MAC_B2	39	66	32	5	24	2	MAC_B2	34	38	46	24	70		10	MAC_B2	42	68	91	4	28
2	1	LON_B3	5	66	44	48	27	2	LON_B3	89	90	14	29	9		10	LON_B3	2	70	94	31	10
2	1	SUB_B3	40	70	5	26	33	2	SUB_B3	84	90	24	71	54		10	SUB_B3	88	74	4	46	91
2	1	Pole_Int	64	4	37	57	53	2	Pole_Int	24	3	82	88	92		10	Pole_Int	60	15	88	32	66
2	1	Pole_Lon	31	79	41	24	11	2	Pole_Lon	87	89	3	34	40		10	Pole_Lon	29	71	96	43	16
3	1	GEN_B4	6	57	44	40	31	2	GEN_B4	20	30	89	18	85		10	GEN_B4	11	48	85	38	28
3	1	INT_B5	53	8	34	49	42	2	INT_B5	82	8	82	28	79		10	INT_B5	55	4	80	33	57
3	1	MAC_B5	49	60	34	7	21	2	MAC_B5	5	53	73	26	92		10	MAC_B5	34	66	82	12	30
3	1	LON_B5	52	68	42	24	8	2	LON_B5	53	68	79	89	4		10	LON_B5	29	78	95	40	19
3	1	SUB_B5	55	64	4	42	30	2	SUB_B5	88	88	19	89	48		10	SUB_B5	98	71	11	62	98
3	1	Pole_Int	58	5	43	50	56	2	Pole_Int	90	62	86	88	90		10	Pole_Int	66	11	94	34	66
3	1	Pole_Lon	46	62	40	26	8	2	Pole_Lon	88	88	44	90	21		10	Pole_Lon	15	60	93	31	5

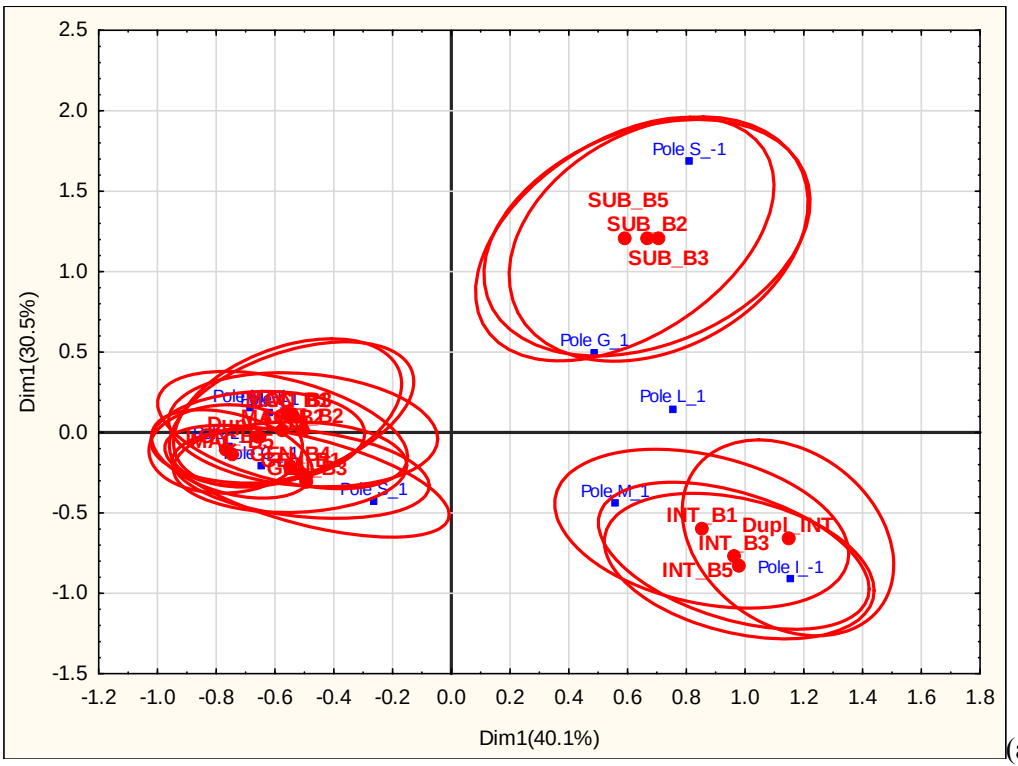
ADDENDUM C

Dissimilarity data matrix for correspondence analysis of polarised sensory positioning (global) data

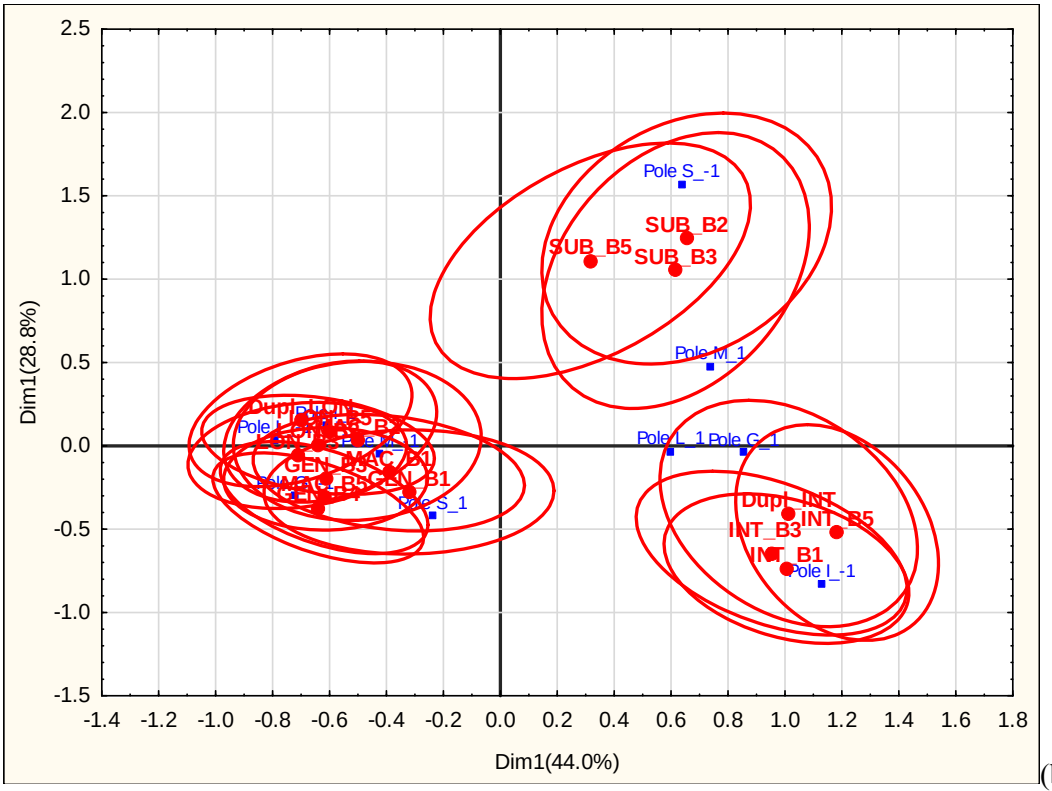
	Rep	Sample	Value	pole/taster	Pole	Judge	minmax	Pole/dir
GEN_B1	1	GEN_B1	3	Pole G(1)	Pole G	1	-1	Pole G_-1
GEN_B1	1	GEN_B1	74	Pole S(1)	Pole S	1	1	Pole S_1
GEN_B1	1	GEN_B1	3	Pole G(2)	Pole G	2	-1	Pole G_-1
GEN_B1	1	GEN_B1	76	Pole L(2)	Pole L	2	1	Pole L_1
GEN_B1	1	GEN_B1	38	Pole G(3)	Pole G	3	-1	Pole G_-1
GEN_B1	1	GEN_B1	65	Pole S(3)	Pole S	3	1	Pole S_1
GEN_B1	1	GEN_B1	92	Pole I(4)	Pole I	4	1	Pole I_1
GEN_B1	1	GEN_B1	11	Pole L(4)	Pole L	4	-1	Pole L_-1
GEN_B1	1	GEN_B1	15	Pole G(5)	Pole G	5	-1	Pole G_-1
GEN_B1	1	GEN_B1	92	Pole S(5)	Pole S	5	1	Pole S_1
GEN_B1	1	GEN_B1	100	Pole G(6)	Pole G	6	1	Pole G_1
GEN_B1	1	GEN_B1	1	Pole L(6)	Pole L	6	-1	Pole L_-1
GEN_B1	1	GEN_B1	14	Pole I(7)	Pole I	7	-1	Pole I_-1
GEN_B1	1	GEN_B1	60	Pole M(7)	Pole M	7	1	Pole M_1
GEN_B1	1	GEN_B1	0	Pole G(8)	Pole G	8	-1	Pole G_-1
GEN_B1	1	GEN_B1	54	Pole I(8)	Pole I	8	1	Pole I_1
GEN_B1	1	GEN_B1	0	Pole G(9)	Pole G	9	-1	Pole G_-1
GEN_B1	1	GEN_B1	80	Pole I(9)	Pole I	9	1	Pole I_1
GEN_B1	1	GEN_B1	3	Pole G(10)	Pole G	10	-1	Pole G_-1
GEN_B1	1	GEN_B1	96	Pole S(10)	Pole S	10	1	Pole S_1
INT_B1	1	INT_B1	66	Pole G(1)	Pole G	1	1	Pole G_1
INT_B1	1	INT_B1	3	Pole I(1)	Pole I	1	-1	Pole I_-1
INT_B1	1	INT_B1	0	Pole I(2)	Pole I	2	-1	Pole I_-1
.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.
LON_B5	3	LON_B5	0	Pole L(9)	Pole L	9	-1	Pole L_-1
LON_B5	3	LON_B5	95	Pole S(10)	Pole S	10	1	Pole S_1
LON_B5	3	LON_B5	19	Pole L(10)	Pole L	10	-1	Pole L_-1
SUB_B5	3	SUB_B5	64	Pole I(1)	Pole I	1	1	Pole I_1
SUB_B5	3	SUB_B5	4	Pole S(1)	Pole S	1	-1	Pole S_-1
SUB_B5	3	SUB_B5	19	Pole S(2)	Pole S	2	-1	Pole S_-1
SUB_B5	3	SUB_B5	89	Pole M(2)	Pole M	2	1	Pole M_1
SUB_B5	3	SUB_B5	71	Pole I(3)	Pole I	3	1	Pole I_1
SUB_B5	3	SUB_B5	31	Pole S(3)	Pole S	3	-1	Pole S_-1
SUB_B5	3	SUB_B5	24	Pole S(4)	Pole S	4	-1	Pole S_-1
SUB_B5	3	SUB_B5	90	Pole M(4)	Pole M	4	1	Pole M_1
SUB_B5	3	SUB_B5	88	Pole G(5)	Pole G	5	1	Pole G_1
SUB_B5	3	SUB_B5	14	Pole S(5)	Pole S	5	-1	Pole S_-1
SUB_B5	3	SUB_B5	100	Pole I(6)	Pole I	6	1	Pole I_1
SUB_B5	3	SUB_B5	28	Pole S(6)	Pole S	6	-1	Pole S_-1
SUB_B5	3	SUB_B5	70	Pole S(7)	Pole S	7	1	Pole S_1
SUB_B5	3	SUB_B5	31	Pole L(7)	Pole L	7	-1	Pole L_-1
SUB_B5	3	SUB_B5	0	Pole S(8)	Pole S	8	-1	Pole S_-1
SUB_B5	3	SUB_B5	56	Pole L(8)	Pole L	8	1	Pole L_1
SUB_B5	3	SUB_B5	0	Pole S(9)	Pole S	9	-1	Pole S_-1
SUB_B5	3	SUB_B5	90	Pole M(9)	Pole M	9	1	Pole M_1
SUB_B5	3	SUB_B5	98	Pole G(10)	Pole G	10	1	Pole G_1
SUB_B5	3	SUB_B5	11	Pole S(10)	Pole S	10	-1	Pole S_-1

ADDENDUM D

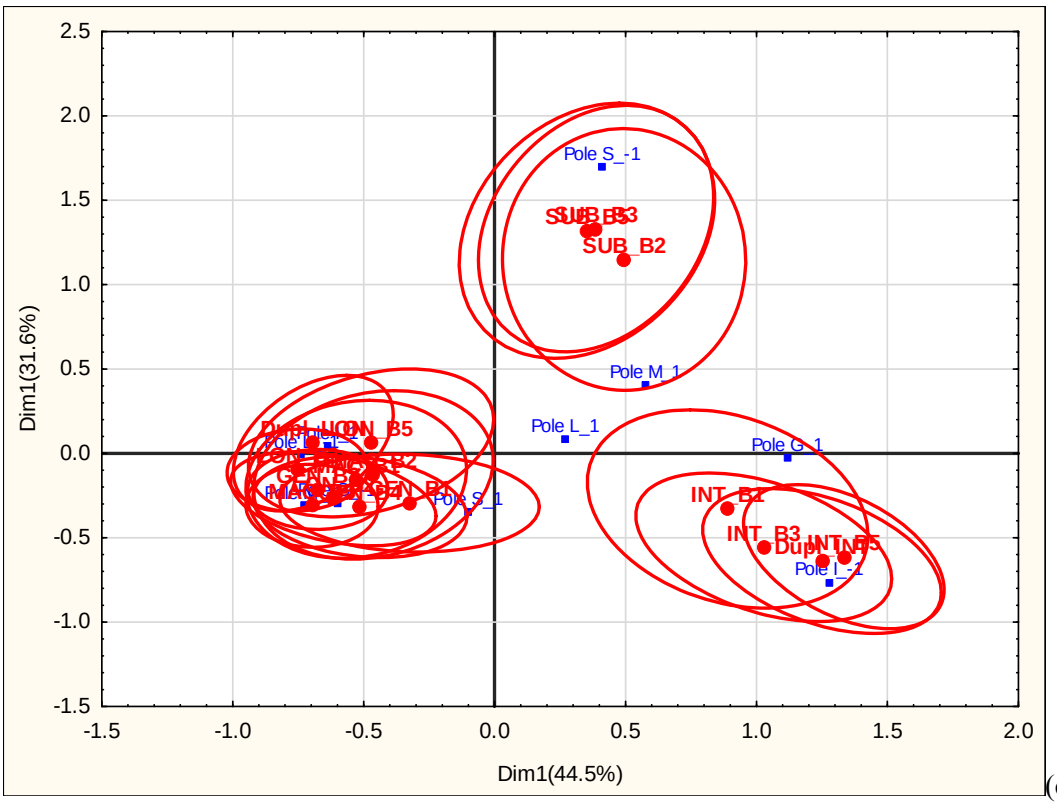
Sample configuration of five Cyclopia species in the first two dimensions of correspondence analysis performed on data from polarised sensory positioning (a) aroma, (b) palate and (c) global.



(a)



(b)



(c)

The abbreviations GEN, MAC, LON, SUB and INT refer to the specific *Cyclopia* species: C. genistoides, C. maculata, C. longifolia, C. subternata and C. intermedia, respectively. B refers to batches, 1-5 refer to the batch number. Dulp_INT refers to the sample identical to pole I, Dupl_LON refers to the sample identical to pole L.

Chapter 8

General discussion and conclusions

Several *Cyclopia* species, endemic to South Africa, are used for the production of honeybush herbal tea (Joubert, Gelderblom, Louw, & De Beer, 2008). Three species provide the bulk of production. *Cyclopia genistoides* and *C. subternata* are produced commercially while *C. intermedia* is mainly harvested from the wild (Joubert, Joubert, Bester, De Beer, & De Lange, 2011). Species under development are *C. maculata* and *C. longifolia.* Production lags behind demand, forcing tea processors to use blends of different species to supply a well-rounded commercial product for the growing local and international markets.

The evaluation of *Cyclopia* plant material, both in research for cultivation programs, and in industry to ensure optimum utilisation of a limited resource, is of utmost importance. The Honeybush Cultivar Development Program of the Agricultural Research Council (ARC), South Africa evaluates and selects plant material for cultivar development. Early screening of plant material to determine its suitability for cultivation, is necessary. A prediction model could find application in this research program for screening large sets of plant material early during the selection and development phase.

The honeybush industry needs to maximise output with the limited available produce to supply a product with consistent quality and high consumer appeal. Different species are blended to supply in the demand, but no standard procedure for blending to obtain a product with consistent, high quality has been formulated. One of the main commercial species often used in blending, *C. genistoides*, is associated with bitterness, which could be detrimental to consumer acceptability. Rapid and efficient sensory methods is needed to implement in industry as part of quality control (QC) programs where the effect of blending on the sensory profile need to be addressed on a regular basis.

In view of this, the objectives of the current research were to develop and validate a prediction model, using individual polyphenol content to predict sensory bitterness in *Cyclopia* species. The second objective was to establish robust blending protocols for *C. genistoides* with other *Cyclopia* species to mask bitterness of infusions. The third objective was to establish the most effective rapid profiling method for application within the honeybush industry.

The first problem was addressed by developing a **prediction model** to predict sensory bitterness associated with *Cyclopia* species, based on the phenolic composition. Theron (2012) investigated the link between the phenolic composition and the sensory taste of honeybush infusions and the results suggested that mangiferin might be associated with the perceptible bitter taste. Erasmus (2015) continued this work, and used step-wise regression analysis to investigate the relationship between phenolic composition and sensory taste attributes. The results indicated that bitterness could be associated with several compounds, including

mangiferin and isomangiferin. Limitations of step-wise regression, as employed Erasmus (2015), were identified, with the main limitation being the inability of this technique to handle collinearity of variables. A further concern was the limited variation in bitterness intensities in the sample set. A more targeted approach to regression analysis was necessary. Furthermore, since limited variation in bitterness intensities were captured with the standard profiling scale, an extended scale for evaluating only taste attributes, were proposed. In the current study, further attempts to that of Theron (2012) and Erasmus (2015) were made to identify phenolic compounds responsible for the bitter taste associated with *Cyclopia* species.

A data set, covering four *Cyclopia* species (*C. genistoides*, *C. subternata*, *C. longifolia* and *C. maculata*), representing the natural variation in sensory quality and phenolic composition, were used. The *Cyclopia* species included in the current study were fermented, thus subjected to a high-temperature oxidation process. The phenolic composition of infusions of these *Cyclopia* species were analysed using high-performance liquid chromatography–diode array detection (HPLC-DAD) while the bitter taste of the same infusions were determined using descriptive sensory analysis (DSA) and the extended scale. Large qualitative and quantitative differences were observed when comparing the phenolic content between species. These results corresponded to studies where the phenolic composition of several *Cyclopia* species was quantified (Schulze et al., 2015). Apart from external factors such as climate and area, the phenolic content is also influenced by processing conditions such as fermentation time and temperature (Du Toit & Joubert, 1999). Considerable variation in the phenolic content and sensory bitterness were observed for *C. genistoides*, although all these samples were processed at optimum fermentation conditions (80°C/24h or 90°C/16h). In the case of *C. longifolia*, samples fermented at different temperature/time regimes (80°C and 90°C for 8, 16 and 24 h) were included, contributing to the variation in sensory bitterness and phenolic content. A longer fermentation time reduced the bitterness in *C. longifolia* and in this species, bitterness is associated with under fermented samples (Erasmus, Theron, Muller, Van der Rijst, & Joubert, 2017).

The relationship between phenolic composition and sensory attributes were investigated using partial least squares (PLS) regression analysis, a technique that can handle numerous and strongly collinear *X*-variables. Data of *C. genistoides* and *C. longifolia* illustrated considerable variation in bitterness intensities and phenolic content, and were included in the regression analysis. Partial least squares regression analysis with variable selection was effective in identifying candidate predictors for sensory bitterness. The xanthones, mangiferin and isomangiferin, were highly correlated to sensory bitterness and were identified as the major predictors of sensory bitterness. Two of the benzophenones, iriflophenone-3-*C*-glucoside-4-*O*-glucoside and iriflophenone-3-*C*-glucoside also contributed to the bitter taste, but illustrated lower predictive ability. External validation of the proposed model demonstrated that 76% of observed bitterness values were within the 95% confidence interval of predicted bitterness. This validated prediction model could find application as screening tool in research programs such as the Honeybush Cultivar Development Program of the ARC, South Africa. When evaluating plant material for cultivar development program, a large number of samples need to be screened. The validated prediction model thus provide a scientific tool to implement in research programs for rapid screening of large sample sets.

The **extended scale**, employed in the current study, demonstrated to aid in capturing variation in bitterness. This scale could find application in future sensory research where it is necessary to capture sensory differences of selected attributes of one sensory modality. Although external validation indicated that the proposed model could accurately predict 76% of the observed bitterness values, the model seems to under predict high bitter samples. Most of the samples with low or moderate bitterness were accurately predicted by the model but the bitterness of infusions that the panel perceived as very bitter, were under predicted. A possible explanation for this problem is the time lapse between the sensory analysis of the training and the validation sets (almost 12 months) and therefore drift in panel performance. In future, such concerns could be addressed by including reference standards with known and defined bitterness.

The prediction model, developed in the current study, identified four compounds as candidate predictors for sensory bitterness, including mangiferin and isomangiferin. This is in accordance to results of Theron (2012) and Erasmus (2015), both employing large sample sets for model development. However, individual phenolic compounds might not be responsible for specific taste and mouthfeel attributes but taste could be elicited by a combination of compounds. The modulating effect of compounds could furthermore influence the final taste perception. For future research on the contribution of individual compounds to bitter taste, taste-guided fractionation could be investigated. The fractions, or individual compounds isolated within specific fractions, driving sensory bitterness, could be investigated using this approach (Sáenz-Navajas et al., 2017). The taste of individual isolated compounds and their contribution to taste could further be investigated using dose-over-threshold (Dot) values (i.e. the ratio of the concentration and the threshold of a compound) (Scharbert & Hofmann, 2005).

The second aim of the current study was to establish robust **blending** protocols for *C. genistoides* with other *Cyclopia* species to mask bitterness of infusions. Infusions representing six blend ratios of fermented *C. genistoides* with *C. subternata*, *C. maculata* and *C. intermedia* respectively, were evaluated using DSA, focussing only on taste modalities and astringency with the extended scale. Blends of *C. genistoides* with any of *C. subternata, C. maculata* or *C. intermedia* in a ratio of 2:3 were effective in reducing bitterness to below perceptible levels. While it is important to reduce bitterness to below perceptible levels in order to supply a product with high consumer acceptability, blending of different *Cyclopia* species may have a significant effect on the species-specific sensory profile. Research to qualify the sensory profiles of several *Cyclopia* species revealed an array of attributes common to all species, defined as the generic sensory profile, which can be described as “fynbos-floral”, “woody” and “fynbos-sweet” aroma and flavour, with a slight sweet taste and slight astringent mouthfeel (Erasmus, 2015). Furthermore, the respective species show higher intensities of specific sensory attributes, contributing to species-specific sensory profiles. *Cyclopia genistoides* is associated with a strong “rose-geranium” flavour and perceptible bitter taste, while *C. subternata* associate with “caramel” and “sweet-associated” aroma notes and a slight astringent mouthfeel. *Cyclopia subternata* demonstrated the highest ability to reduce bitterness associated with *C. genistoides*. The effect of blending on the full sensory profile of the *C. genistoides-C. subternata* blends using DSA indicated that the optimum *C. genistoides-C. subternata* blend (2:3 ratio) associated with a “fynbos floral”, “apricot”, “woody”, “fruity

sweet" and "fynbos sweet" aroma, a sweet taste and no perceptible bitterness. Blending *C. genistoides* with *C. subternata* thus demonstrated to be effective in reducing bitterness to below perceptible levels while retaining the positive aroma and flavour attributes of both these species. Future research needs to determine the effect of blending *C. genistoides* with other *Cyclopia* species (*C. maculata* and *C. intermedia*) on the respective sensory profiles. *Cyclopia longifolia*, when fermented for a short time, is also associated with a bitter taste (Erasmus et al., 2017) and were therefore not included in the blending experiment of the current study. As previously indicated, bitterness is not elicited by a single phenolic compound, but associated with several compounds. The modulating effect of compounds could result in an increase or decrease in perceptible bitter taste. This leads to more research opportunities. Investigating the effect of blending *C. genistoides* with *C. longifolia* on bitter taste, and the possible modulating effect of the polyphenolic compounds, is recommended.

Descriptive sensory analysis of honeybush infusions was used to obtain detailed and robust data which served as basis for the development of a prediction model. Furthermore, DSA was employed to evaluate the effect of blending of different *Cyclopia* species on bitterness perception and to determine the sensory profile of the blended infusions. Although DSA delivered a detailed sensory profile of the products tested, this comprehensive method is considered time-consuming to conduct and can be regarded as too cumbersome for the honeybush industry to use in quality control programs. The industry expressed the need for rapid sensory methods but these methods need to be evaluated before implementing in QC programs in industry.

Preliminary research has demonstrated that *Cyclopia* species can be profiled using sorting (Erasmus, 2015); however, no information on the suitability of other **rapid methods** as applied to honeybush has been published. In the current study, four rapid sensory methods were applied for the sensory characterisation of honeybush infusions, three methods with a trained panel and one method with consumers. Sorting, projective mapping (PM) and polarised sensory positioning (PSP) were employed for sensory characterisation of infusions of five fermented *Cyclopia* species. In all three of these methods, trained assessors were instructed to focus on one modality namely aroma, palate or global attributes when evaluating honeybush. Furthermore, within each method, three replications were conducted per modality. Check-all-that-apply (CATA) questions were employed to determine consumers' degree of liking of *C. genistoides*-*C. subternata* blends.

When selecting new sensory methodologies, it is important to determine the **validity** and **reliability** of these methods. Validity requires that the test methodology or instrument is measuring what it is intended to measure, while reliability is a measure of the stability or consistency of test results (Lawless & Heymann, 2010). In the sensory domain, DSA is regarded as the cornerstone of sensory methods and the validity of novel methods are determined by comparing results to that of DSA by visual comparison of the product configurations and by calculating RV coefficients. The repeatability of novel sensory methodologies has been evaluated at panel level by evaluating the placement of blind duplicate samples in the product configuration (Hopfer & Heymann, 2013). Repeatability can also be evaluated by comparing product configurations of replicate sessions.

The validity of sorting, PM and PSP (and variations within methods) was determined by comparing the respective product configurations to that obtained with DSA on the same sample set. The product configurations for the three variations (aroma, palate or global) within a rapid method were similar to that obtained with DSA, indicating that these methods resulted in valid product configurations. Similarity of configurations were confirmed by high RV coefficients. Repeatability was evaluated by comparing the replications within a method using product configurations and RV coefficients. The three rapid methods demonstrated good repeatability, with one exception namely sorting on aroma. With this specific application, repeatability was lower but could be explained by the fact that this was the first introduction to rapid profiling methods for some assessors. They were thus not familiar with the technique and could have used different categorisation criteria for replicate sessions. Blind duplicate samples were included when conducting PM and PSP and good panel repeatability were obtained for these methods, demonstrated by the fact that the sample and its blind duplicate were positioned in close proximity on the two-dimensional product configurations. Inclusion of blind duplicates to measure panel performance demonstrated to be an easy, straightforward and valid method of evaluating individual panel members. Inclusion of blind duplicate samples in future research on rapid methods, is therefore advised.

In industry where rapid results is required, one **replication** would be sufficient for the broad-based profiling and to obtain an overview of the sensory space associated with the samples. However, if there are only subtle differences between samples, more replications are recommended to ensure stable results. Concerning the application of rapid methods for research purposes, and specifically for sensory characterisation of complex products with a small panel, a minimum of two replications is recommended to ensure stable results.

Sorting and PM are both regarded as categorisation methods, and results of these methods will therefore be compared. The broad sensory profiling of five *Cyclopia* species, obtained with sorting and PM, were fairly similar although some minor differences need to be highlighted. In the case of PM, comparison of product configurations for partial PM (aroma or palate) and global PM (all attributes) resulted in similar configurations, contrary to research that reported partial PM to be more effective (Dehlholm, Brockhoff, Meinert, Aaslyng, & Bredie, 2012; Louw et al., 2013). Any of these variations of PM (aroma, palate or global) could therefore be applied for the broad sensory profiling of *Cyclopia* species. When comparing results for sorting on aroma, palate or global attributes, additional differentiation between samples with only subtle difference were obtained when employing sorting based on palate attributes. This additional differentiation is linked to the bitter taste associated with *C. genistoides* and to lesser extent with *C. longifolia*. Global sorting was less effective to differentiate between different honeybush infusions. The longer list provided with global sorting, could have complicated the task for assessors. In the case of both sorting and PM, the sensory character of the product in question will influence the choice of partial or global evaluation. If the sensory differences between products are mainly driven by aroma attributes, sorting or PM on aroma would be sufficient to obtain a broad idea of the sensory attributes associated with the products. However, if palate attributes are more important, as in the case of blending *Cyclopia* species to reduce bitterness, it is recommended to include palate attributes in the

evaluation process. For both sorting and PM, focusing assessors on only one modality and providing a list of relevant attributes increased the ability of the assessors to differentiate between samples with only subtle differences. Implications of these results for the honeybush industry is that a list of terms describing the key sensory attributes of the products in question, need to be compiled. Furthermore, training assessors on these attributes is recommended. The aroma wheel (Theron, 2012) and species-specific aroma and flavour wheels (Erasmus, 2015) for *Cyclopia* species would serve as valid tools in both these tasks.

In the sensory domain, PSP, a reference-based method, is referred to as a **sensory characterisation** method but only limited description of samples is obtained and description of samples is only possible when poles are well described. The sensory space of the five *Cyclopia* species included in the current study was well defined, based on a detailed quantitate and qualitative description obtained by DSA, and interpretation of product configurations of PSP was therefore possible. PSP was successful in discriminating between *Cyclopia* species with distinct sensory profiles. However, sensory description of species was only obtained by referring to the sensory profile generated through DSA. Future research could address this concern by including a descriptive step when conducting PSP. Assessors could be instructed to describe each pole. This step will then allow for some description of the samples related to each pole. Varela et al. (2014) reported on application of global and partial PSP with a descriptive step and found that partial PSP allowed for more detailed description compared to DSA. Another possible approach to obtain description of samples could be to combine PSP with a CATA task where PSP is followed by a step where assessors have to check the attributes that correspond between the pole and the test sample. Data analysis of this proposal still needs to be clarified.

Distinct differences in the sensory profiles of the sample set used in current study were observed, as illustrated by the results of DSA. Honeybush tea is regarded as a **complex product**, as evident in the large array of sensory descriptors used to profile this product and further underlined by the large number of volatile and non-volatile compounds identified in the infusions of the different species (Ntlhokwe, Muller, Joubert, Tredoux, & de Villiers, 2017; Schulze et al., 2015). The sensory analysis of this product is further complicated by the fact that infusions need to be evaluated at a constant, hot temperature. Limited research on the application of rapid methods for profiling of a complex product such as honeybush infusions, have been published. The current study demonstrated that rapid sensory methods such as sorting, PM and PSP can effectively be applied for the broad profiling of complex products.

The current research further addressed the effect of blending on bitterness. Results, obtained with DSA and a trained panel, illustrated that blending was effective to reduce bitterness below perceptible levels, but no information on consumers' opinion of these blends have been attained. Consumers' sensory description of the proposed *C. genistoides*-*C. subternata* blends were determined by using a rapid sensory profiling method namely check-all-that-apply (CATA). The samples that consumers had to evaluate, were blends of two species. These samples illustrated an overlap of sensory attributes with differences mainly on intensities in attributes, as illustrated with DSA. When applying CATA questions, consumers were unable to differentiate between infusions with only subtle perceptual differences. Future research, where rate-all-that-apply (RATA) questions is employed to determine consumers' opinions of blends of *Cyclopia* species, is recommended.

Four rapid sensory characterisation methods were applied in the current study and it is necessary to compare the validity, **ease of use**, both by the sensory scientist and assessors, as well as ease of data analysis, before making recommendations to the industry on the most applicable method. Table 1 presents a quick overview of the factors influencing the ease of use of the rapid methods evaluated in the current study.

Table 1 Comparison of rapid sensory methods applied on *Cyclopia* species to DSA with regard to level of training of assessors, samples evaluated, replications, validity, and ease of use.

Factor	Sensory method				
	DSA	Sorting	PM	PSP	CATA
Training	Extensive	Limited	Limited	Limited	No
Ease of conducting method	Intricate	Very easy	Fairly easy	Moderately easy	Moderately easy
Ease for assessors	Intricate	Very easy	Fairly easy	Fairly easy	Very easy
Samples / session	6	15	15	12	5
Reps* industry	Several	1	1	1	1
Reps* research	Several	2	2	2	1
Validity vs. DSA	N/A	Yes	Yes	Yes	Yes
Sensory profile	Detailed	Broad	Broad	Broad	Broad
Data aggregation	Yes	No	No	Yes	Yes
Statistical analysis of data	Complicated	Fairly intricate	Fairly intricate	Fairly intricate	Easy

*Replications recommended based on results

Although PM is regarded as a spontaneous, easy to conduct task, some assessors found the task difficult to perform; less intuitive, especially for assessors having difficulty with handling spatial information. In industry, where assessors are not as thoroughly trained in sensory analysis as in a research environment, assessors might find the PM task difficult and cumbersome. Limited product characterisation of products were obtained with PSP. When employing CATA questions consumers were unable to differentiate between samples with subtle differences. The sorting task on the other hand, was regarded as easy to conduct, both from the sensory scientist and the assessors' point of view. Results of the current study indicated that assessors sorted samples into groups and added attributes that were interpretable and consistent to that used during DSA. Comparison of the three variations of directed sorting indicated directed sorting on aroma or palate to be valid methods for the sensory characterisation of a complex product such as honeybush when applied by trained assessors. Directed sorting on aroma or palate could find application in the herbal tea industry.

One of the challenges facing the honeybush herbal tea industry, is the development of **quality grading** tools that are practical and easy to implement. DSA demonstrated to be very useful to quantify the sensory

attributes associated with different *Cyclopia* species and to identify taints (Bergh et al., 2017; Erasmus et al., 2017, Theron et al., 2014), but this method is too laborious to include in regular quality control. PSP could find application in this context. Application of PSP with poles that represent different grades of honeybush tea (again selected to represent specific sensory attributes associated with different grades of tea) needs further investigation and validation.

The honeybush industry expressed the need for rapid sensory methods to implement as part of their quality control programs. When comparing the rapid methods applied in the current study, sorting demonstrate to be valid and the most effective method for characterisation of honeybush. This categorisation method is easy to use, both from the sensory scientist and assessors' point of view and the information obtained with sorting is comparable to that of DSA. Implementation of this method for screening plant material in the honeybush industry, would aid in evaluating large batches of plant material in a valid and scientific manner. PSP on the other hand, could be applied in quality control programs in the honeybush industry. This could be attained by selecting poles with attributes that represent specific quality parameters, and conducting PSP as part of routine QC programs. Data aggregation over consecutive sessions will add valuable information on long term quality monitoring. Implementation of valid, scientific methods, such as sorting and PSP, will aid the honeybush industry in their effort to maximise output with the limited available produce to supply a product with consistent quality and high consumer appeal.

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