

# Development of a quality grading system for the honeybush (*Cyclopia* spp.) tea industry

by

Brigitte Von Pressentin Du Preez

Dissertation presented for the degree of

Doctor of Philosophy  
(Food Science)

at

Stellenbosch University  
Department of Food Science, Faculty of AgriSciences

*The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at are those of the author and are not necessarily to be attributed to the NRF.*



Supervisor:

Prof. E. Joubert, Agricultural Research Council, Stellenbosch, South Africa

Co-supervisors:

M. Muller and Dr. E.I. Moelich, Department of Food Science, Stellenbosch University,  
Stellenbosch, South Africa

December 2020

## Declaration

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dissertation includes one original paper published in a peer-reviewed journal (*Food Research International*) and two unpublished publications. The development and writing of the papers (published and unpublished) were the principle responsibility of myself and, for each of the cases where this is not the case, a declaration is included in the dissertation indicating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

December 2020

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## Abstract

Honeybush tea, produced from *Cyclopia* species endemic to South Africa, has attained an international footprint within the global herbal tea sector. As demand is exceeding supply to a primarily export market, all production batches should meet optimum quality standards. The lack of standardised sensory quality criteria and assessment methods within the commercial sector has resulted in tea of variable sensory quality reaching the market. The trade of inconsistent and inferior quality products will be detrimental to the reputation of honeybush tea and consumer acceptance, and ultimately the honeybush industry. The need for a scientifically founded quality grading system to evaluate, differentiate and communicate the sensory quality of honeybush tea was addressed through four quality control elements, i.e. a sensory lexicon and wheel, sensory quality standard, quality scoring method, and rapid quality classification methods.

The previously developed honeybush aroma lexicon and wheel were revised, based on a newly established comprehensive sensory and physicochemical dataset. Data of samples of the main commercial *Cyclopia* species (*C. intermedia*, *C. subternata* and *C. genistoides*), processed on laboratory- and commercial-scale, were incorporated to represent the sensory space in terms of different qualities. Universal chemical-based reference standards were developed and validated to replace food-based reference standards in the aroma lexicon to facilitate standardised assessment of honeybush sensory quality.

The established sensory quality standard was founded on the comprehensive dataset and input from industry. Sensory quality parameters for the tea infusions and dried plant material were identified, and parameter specifications for 'high', 'moderate', 'low' and 'poor' quality classes were defined through expert focus groups. A user-friendly quality scoring method that incorporates a scorecard and colour reference card, was developed and validated for the assessment and classification of production batches based on obtained parameter and total score values and citation frequencies of specific attributes.

The validity of reference-based rapid methods, polarised sensory positioning (PSP) and polarised projective mapping (PPM), were investigated for their discrimination ability as time-efficient classification tools to distinguish between infusions of large samples sets of variable sensory quality within commercial and research context. The efficacy of the use of *physical* (*p*) poles (tea infusions) and novel *theoretical* (*t*) poles (descriptions), representative of the four sensory quality classes, as references, were compared within each method, using a trained panel. Product configurations similar to that of a classical sensory profiling method, descriptive sensory analysis, demonstrated the validity of the method variations for broad quality classification based on key sensory quality parameters. PPM-*p* indicated the highest discrimination ability between the quality classes. Recommended amendments to *theoretical* pole descriptions would improve feasibility for commercial application. The quality scoring method and PPM-*t*

were tested by a panel of industry representatives, and the need for industry assessor training in sensory quality parameters was emphasised. Implementation of the proposed integrated quality grading system will equip honeybush industry role-players in delivering a final product of consistent sensory quality within the honeybush value chain.



## Opsomming

Heuningbostee, geproduseer van eg Suid-Afrikaanse *Cyclopia* spesies, het 'n internasionale voetspoor in die kruietee-sektor bereik. Soos aanvraag die aanbod oorskry, is dit belangrik dat alle produksielotte aan optimum kwaliteitstandaarde voldoen. Die gebrek aan gestandaardiseerde sensoriese kwaliteitskriteria en toetsmetodes het daartoe gelei dat tee van wisselende sensoriese kwaliteit die mark bereik. Die handel van produkte van wisselende en minderwaardige kwaliteit is skadelik vir die reputasie van heuningbostee en impakteer negatief op verbruikersvertroue en uiteindelik ook die heuningbosteebedryf. Die behoefte aan 'n wetenskaplik-gegronde kwaliteitsgraderingsstelsel om die sensoriese kwaliteit van heuningbostee te evalueer, is aangespreek deur vier kwaliteitskontrole elemente te ontwikkel, naamlik 'n sensoriese leksikon en wiel, 'n sensoriese kwaliteitstandaard, 'n kwaliteitsgraderingsmetode, en 'n vinnige kwaliteitsklassifikasie metode.

'n Bestaande heuningbos aromaleksikon en -wiel is hersien deur dit op 'n nuwe, omvattende sensoriese en fisies-chemiese datastel te baseer. Die omvattende datastel wat monsters van die primêre kommersiële *Cyclopia* spesies (*C. intermedia*, *C. subternata* en *C. genistoides*), geprosesseer op laboratorium- en kommersiële skaal, is gebruik om 'n spektrum van verskillende sensoriese kwaliteite te verteenwoordig. Universele chemiese verwysingsstandaarde is ontwikkel om voedselgebaseerde verwysingsstandaarde te vervang om sodoende die gestandaardiseerde assessering van die sensoriese kwaliteit van heuningbos te fasiliteer.

Die vasgestelde sensoriese kwaliteitsklasse van heuningbostee is gegrond op die gemelde omvattende datastel, asook insette van die bedryf. Sensoriese kwaliteitsparameters is vir tee-infusies en droë blare geïdentifiseer en parameterspesifikasies is deur middel van fokusgroepe vir die 'hoë', 'gematigde', 'lae' en 'swak' kwaliteitsklasse gedefinieer. 'n Gebruikersvriendelike kwaliteitsgraderingsmetode wat 'n tellingkaart en kleurverwysingskaart insluit, is ontwikkel vir die assessering en klassifikasie van produksielotte heuningbostee, gebaseer op die parameters en totale tellingwaardes van spesifieke eienskappe.

Die geldigheid van verwysingsgebaseerde vinnige sensoriese metodes, naamlik gepolariseerde sensoriese posisionering (GSP) en gepolariseerde projektiewe kartering (GPK), is ondersoek vir hul onderskeidingsvermoë om groot getalle monsters van wisselende sensoriese kwaliteit effektief te klassifiseer. Die effektiwiteit van die gebruik van *fisiese (f)* pole (tee infusies) en nuwe *teoretiese (t)* pole (verbale beskrywings) as verwysingsstandaarde, elk verteenwoordigend van 'n sensoriese kwaliteitsklas, is vergelyk binne elke metode met behulp van 'n opgeleide sensoriese paneel. Produkkonfigurasies soortgelyk aan dié van 'n klassieke sensoriese profileringsmetode, kwantitatiewe beskrywende analise, het beide vinnige metodes se geldigheid vir die oorsigtelike kwaliteitskategorisering, gedemonstreer. GPK-*f* het die hoogste onderskeidingsvermoë tussen kwaliteitsklasse aangedui. Aanbevole wysigings tot die teoretiese

poolbeskrywings sal die uitvoerbaarheid vir kommersiële toepassing verbeter. Die kwaliteitsgraderingsmetode en GPK-t is deur 'n paneel van verteenwoordigers uit die bedryf getoets en die behoefte aan assessoropleiding is beklemtoon. Die implimentering van die voorgestelde geïntegreerde kwaliteitsgraderingstelsel sal rolspelers van die heuningbosbedryf toerus om 'n finale produk van konsekwente sensoriese kwaliteit binne die heuningboswaardeketting te lewer.

This dissertation is dedicated to

*George, our sons, Stephan and Alexander,  
and my parents, Klaus-Gerd and Magda*

## Acknowledgements

My most sincere gratitude goes to my supervisor Prof. Elizabeth Joubert and co-supervisors, Nina Muller and Dr. Erika Moelich. Thank you for your guidance, expert advice, encouragement, time and immense help offered throughout this study. It has been a privilege to work with such experienced researchers. Thank you for introducing me to honeybush tea research and sensory science, and for providing me with invaluable opportunities. Thank you for your invaluable inputs and time to successfully complete this dissertation.

Prof. Martin Kidd (Centre for Statistical Consultation, Department of Statistics and Actuarial Sciences, Stellenbosch University) and Mrs. Marieta van der Rijst (Biometry Unit, ARC Infruitec-Nietvoorbij, Stellenbosch) are thanked sincerely for statistical data analyses, guidance in the interpretation thereof and kind assistance throughout this study.

Neil Wiltshire and Dr. Martin Dovey (Kerry EMEA) are thanked sincerely for GC-MS analysis of honeybush infusions and assistance in identifying and supplying potential chemical reference standards for the revised honeybush aroma lexicon.

South African honeybush tea industry role-players (producers, processors, buyers and marketers) are thanked sincerely for their support towards this study through participation in interviews and surveys and supplying plant material and tea samples.

My sincere gratitude goes to Colette Cronjé (Food Safety and Quality Systems Consultancy) for her time, expert advice on the sensory quality of honeybush and invaluable inputs in the focus group sessions.

The panel of industry representatives are thanked for their time and invaluable contribution to this study.

The South African Honeybush Tea Association (SAHTA) is acknowledged for their interest and support towards this study.

Dinesh Wijeyawardana (National Brands Limited) is thanked for his time and expert advice in sensory assessment of tea.

Prof. Dalene De Beer (Post-Harvest and Wine Technology Division, ARC Infruitec-Nietvoorbij, Stellenbosch) is thanked for her advice on physicochemical analyses and kind support throughout this study.

The trained sensory panel are thanked sincerely for their commitment, interest and dedication towards sensory research.

Lené Erasmus is thanked for her practical assistance in sample preparation during screening and validation of chemical-based reference standards.

John Achilles and Natashia Achilles (Stellenbosch University) are thanked for their invaluable assistance in the sensory laboratory. Your hard work, dedication, and kind support are appreciated sincerely.

George Dico, Louise Robertson and Neliswa Matrose (Post-Harvest and Wine Technology Division, ARC Infruitec-Nietvoorbij, Stellenbosch) are thanked for their practical assistance in physicochemical analyses.

Ashlyn Meyer, Anna-Marie Leygonie and Alicia Petrus (Stellenbosch University) are thanked for their practical assistance in the preparation and presentation of tea infusions.

Personnel and students at the Department of Food Science, Stellenbosch University, for their continuous interest and kind support.

FlavorActiV™ Ltd (Aston Rowant, United Kingdom) and Sense of Nambitha (Durban, South Africa) for their assistance in supplying certain nano-encapsulated reference samples.

The financial assistance of the National Research Foundation (NRF) of South Africa for a Freestanding Doctoral Scholarship to BDP (grant 112570) is hereby acknowledged. The opinions, findings, and conclusions or recommendations expressed are those of the author and not to be attributed to the NRF.

Financial support for the project was provided by the Department of Science and Technology (DST/CON 0023/2015) and Western Cape Department of Agriculture (Alternative Crops Fund allocation to South African Honeybush Tea Association).

My friends and fellow students – I am grateful for your loyal support and encouragement throughout this study. A very special thank you to Theresa de Villiers, Ilona Steenkamp and Alex Schulze.

My family – your unconditional love, continuous encouragement, support and prayers carried me throughout this journey. There are no words to express my sincere gratitude.

*'Quality means doing it right when no one is looking' – Henry Ford*

## Preface

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 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. Ethical approval for this study was obtained through the Research Ethics Committee, Stellenbosch University, South Africa (Project no. 9204).

### *Results from this dissertation that have been submitted for publication:*

Du Preez, B. V. P., De Beer, D., Moelich, E. I., Muller, M., & Joubert, E. (2020). Development of chemical-based reference standards for rooibos and honeybush aroma lexicons. *Food Research International*, 127, 1–14. <https://doi.org/10.1016/j.foodres.2019.108734>

### *Results from this dissertation that have been presented at conferences:*

Du Preez, B. V. P., De Beer, D., Moelich, E. I., Muller, M., & Joubert, E. (2018). Development of chemical-based reference standards for rooibos and honeybush aroma lexicons. Poster presented at EuroSense 2018, 8<sup>th</sup> European Conference on Sensory and Consumer Research, 2 – 5 September 2018, Verona, Italy.

Du Preez, B. V. P., Moelich, E. I., Muller, M., & Joubert, E. (2017). Selection of chemical-based reference standards for a honeybush aroma lexicon as quality control and grading tool. Oral presentation at the 22<sup>nd</sup> South African Association for Food Science and Technology (SAAFoST) Biennial Congress and Exhibition, 3 – 6 September 2017, Cape Town, South Africa.

### *Dissemination to industry:*

Du Preez, B. V. P. Development of a quality grading system for the honeybush industry: Research progress update. Information session with producers and processors at the South African Honeybush Association (SAHTA) Annual General Meeting (AGM), 16 July 2019, George, South Africa.

Du Preez, B. V. P. Development of a quality grading system for the honeybush industry: Research progress update. Information session with producers and processors at SAHTA AGM, 19 June 2018, George, South Africa.

Du Preez, B. V. P. Development of a quality grading system for the honeybush industry: Introduction. Information session with producers and processors at SAHTA AGM, 20 June 2017, George, South Africa.

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# Chapter 1

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## Introduction

Honeybush (*Cyclopia* spp.) belongs to the fynbos biome, endemic to specific climatic zones of the Western and Eastern Cape regions (Joubert, Joubert, Bester, De Beer, & De Lange, 2011). Traditional or ‘fermented’ herbal tea is produced through high temperature oxidation (‘fermentation’) of the plant material of several *Cyclopia* species, of which *C. intermedia*, *C. genistoides* and *C. subternata* are currently of major commercial interest (Joubert et al., 2019). Although the formal honeybush industry, established in 1998, is still underdeveloped, honeybush tea has already achieved an international footprint (Joubert et al., 2019), contributing to an increasing market share for herbal and speciality teas (Euromonitor, 2019). This herbal tea is listed as herbal plant on the Tea and Herbal Infusions Europe Inventory (THIE, 2019), and has been included in the Geographical Indication (GI) Protocol of the Economic Participation Agreement with the European Union (European Commission, 2019) for protection against name misappropriation (Biénabe & Marie-Vivien, 2017). Honeybush tea has a vast growth potential provided that a consistent supply of a good quality product could be achieved (Bester, Joubert, & Joubert, 2016).

Demand is currently exceeding supply. Only production batches of optimum quality should reach the market as the trade in inconsistent and inferior quality products could be detrimental for the sustainability and expansion of the honeybush industry (DAFF, 2016; Joubert et al., 2011). At present, there is lack of standardised sensory quality criteria and assessment methods, in spite of the need for a quality grading system recognised with the inception of the formal industry (Du Toit, Joubert, & Britz, 1998). To date no provision has been made for sensory quality in the export regulations for honeybush tea (DAFF, 2019). However, progress made on process optimisation for optimal sensory quality and the development of sensory lexicons and wheels (Joubert et al., 2019) has laid a sound foundation for the development of a quality grading system for honeybush tea. Translation of the research outcomes into a user-friendly quality grading system is required to provide role-players in all industry sectors with the essential tools for reliable assessment, and effective differentiation and communication of honeybush tea sensory quality.

A quality grading system should be able to identify, define and measure the quality parameters of a product (Feria-Morales, 2002). The envisaged quality grading system for fermented honeybush tea should consist of the following scientifically founded elements: 1) a sensory lexicon and wheel to facilitate assessor training and calibration in sensory quality, and to communicate sensory quality throughout all industry sectors, 2) a sensory quality standard and defined specifications based on key sensory attributes and

physicochemical parameters, and 3) validated methods to evaluate and classify production batches according to the established sensory quality specifications within commercial and research environments.

Previous research focussing on the establishment of optimum fermentation time and temperature conditions for several *Cyclopia* species, generated sensory profiles in terms of aroma, flavour, taste and mouthfeel attributes through descriptive sensory analysis (DSA) of hot water infusions (Bergh, Muller, Van der Rijst, & Joubert, 2017; Erasmus, Theron, Muller, Van der Rijst, & Joubert, 2017; Theron et al., 2014). Development of previous honeybush sensory lexicons and wheels were based on the DSA data using a smaller sample set of several *Cyclopia* species, but excluding *C. intermedia* in the most recent generic sensory wheel. Furthermore, these samples were pre-dominantly processed on laboratory-scale and not representative of commercially processed samples. A comprehensive data set, representative of the entire honeybush product category, is required (Lawless & Civille, 2013). Therefore, to establish a foundation for the development of quality grading elements, including a revised aroma lexicon and wheel, DSA analyses of a large sample set, representative of the major commercial *Cyclopia* spp., is required. It is important to include samples that represent product variability (especially in terms of negative sensory attributes) in such a sample set.

Other quality parameters that could be included are the colour and turbidity of the infusions. Infusion colour gives an indication of infusion strength, whereas the presence of turbidity (haze) is associated with poor quality (Bergh et al., 2017). To date, limited research has been conducted on colour (Bergh et al., 2017; Du Toit & Joubert, 1999) and turbidity (Bergh et al., 2017) of honeybush infusions as indicators of herbal tea quality. Similar as for sensory profiling, physicochemical analyses of infusions, prepared from a large sample set of commercial production batches of varying quality are required.

The current honeybush lexicon uses mainly food-based reference standards to describe the respective sensory attributes. Food-based reference standards have disadvantages in terms of unavailability and inconsistency over time (Drake & Civille, 2003). The need exists for universal, reproducible chemical-based reference standards for the lexicon attributes to aid in concept alignment and standardisation of sensory quality assessment.

The common approach in the development of food quality control systems is to define product specifications for a quality standard and to develop methods for the reliable assessment of product compliance to the quality standard (Costell, 2002; Lawless & Heymann, 2010). For the present study, key sensory and physicochemical attributes and the corresponding specifications of the major commercial *Cyclopia* species need to be identified. This should be based on a comprehensive data set and conducted in consultation with industry experts. Experts with thorough product and industry knowledge have accurate and comprehensive sensory vocabularies, compared to consumers (Ballester, Dacremont, Le Fur, &

Etiévant, 2005; Ojeda et al., 2015), and the definition of sensory quality standards by experts is evident in literature (Etaio et al., 2012; Pérez-Elortondo et al., 2007).

Quality scoring methods are commonly used in food quality control (Rogers, 2010), mostly in the form of scorecards based on product standards and specifications (Costell, 2002). A valid and user-friendly quality scoring method for fermented honeybush tea would equip industry assessors to classify production batches according to sensory quality. This quality scoring method could also include corrective actions for dealing with batches of inferior quality within a tea processing and/or packing environment.

A quality scoring method could furthermore form a foundation for sensory quality certification of fermented honeybush tea in the regulatory control sector. A demand exists for the standardisation and accreditation of sensory quality assessment methods for product certification, to guarantee the reliable assessment of food products with specific sensory characteristics, specifically products with quality distinctiveness labels (e.g. GI or Protected designation of Origin (PDO)) (Pérez-Elortondo et al., 2018). Application for the registration of honeybush tea as PDO is in progress (D. Troskie, Western Cape Department of Agriculture, 2019, personal communication).

The assessment of honeybush quality within commercial and research environments requires a simple, rapid and cost-effective method to identify the most important broad-based sensory differences between products and to ascertain overall product quality (Joubert et al., 2019). Although a quality scoring method would be essential for routine quality control and to provide detailed information for record-keeping purposes, analyses of many production samples in a short time period is required to facilitate screening and blending of production batches within a commercial environment. Recent evaluation of rapid sensory methodologies for application on honeybush (Moelich, 2018; Moelich, Muller, Joubert, Næs, & Kidd, 2017) have indicated the potential of these methods to screen and classify production batches according to sensory quality. The potential of rapid sensory methodologies, especially reference-based methods, for quality control purposes have been indicated in literature (Ares, Antúnez, De Saldamando, & Giménez, 2018). However, limited research has been done on the validation of these methods as part of a quality control system within commercial context. A time- and cost-efficient alternative to DSA would be valuable in honeybush breeding/cultivation research programmes of the Agricultural Research Council (Bester et al., 2016; Robertson et al., 2018). These alternative methods could be used to evaluate and screen tea infusions of large numbers of genotypes and selections according to sensory quality.

In view of the above, the aim of the research is to develop a user-friendly quality grading system for the honeybush industry that encompasses essential quality elements required to evaluate, differentiate and communicate the sensory quality of fermented honeybush tea within commercial and research context. The research objectives are 1) to revise the current honeybush aroma lexicon and wheel based on a newly established comprehensive DSA data set and to identify and validate aroma chemicals to replace

existing food-based lexicon reference standards, 2) to identify key sensory quality parameters and to establish a sensory quality standard with specifications for selected parameters, 3) to develop a quality scoring method to assess and classify a production batch according to sensory quality, and 4) to assess rapid sensory methods for application as time-efficient quality classification tools of large sample sets.

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# Chapter 2

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## Literature review

### 1 Introduction

The international herbal tea market is one of the most rapidly growing segments of hot beverages (Euromonitor, 2019a). Over the past few years market trends have shifted more towards herbal teas, whereas traditional black tea sales volumes declined dramatically (Arthur, 2020). These trends have been ascribed to consumers' increasing interest in naturally healthy and functional beverages such as herbal teas to support the movement towards wellness-orientated lifestyles (Euromonitor, 2019a). Worldwide tea companies are expanding their range of tea flavours in response to the new industry trends, i.e. new flavours, differentiated teas, health and wellness (Valduga, Gonçalves, Magri, & Delalibera Finzer, 2019). Honeybush tea, produced from *Cyclopia spp.* endemic to South Africa, has benefitted from these trends, gaining international popularity over the past two decades for its unique, sweet aroma and taste and numerous associated health-promoting properties (Joubert et al., 2019; Joubert, Joubert, Bester, De Beer, & De Lange, 2011).

In this review, background on the honeybush industry, industry role-players, current statutory regulations, and honeybush sensory research to date will provide context for the present study. Sensory quality control methods, specifically quality scoring, used within the agricultural commodity and food and beverage sector, as well as stepwise approaches in developing such methods, will also be reviewed to establish a basis for the development of a scientifically founded sensory quality assessment and classification method. A brief review of sensory lexicons as quality control tools with the emphasis on universal chemical reference standards will be given. A review of rapid sensory profiling methods as potential quality control screening tools will provide insight for selecting suitable methods for honeybush sensory quality control and research.<sup>1</sup>

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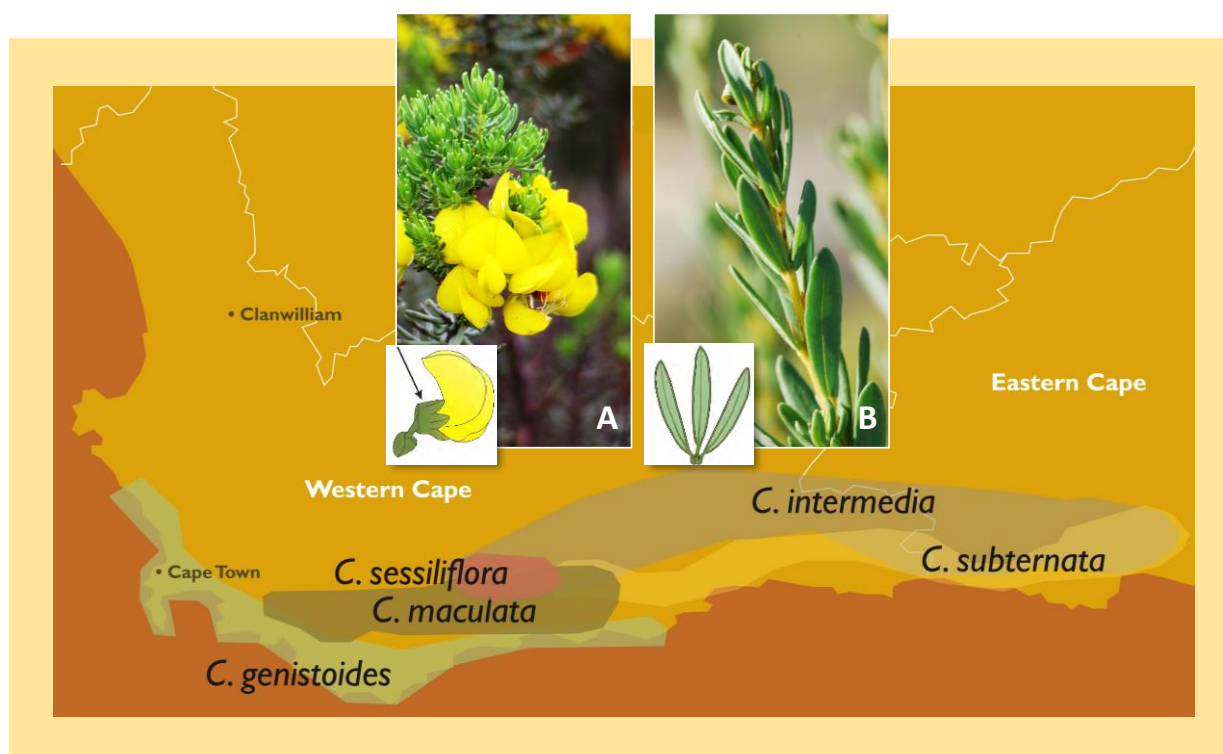
<sup>1</sup>For clarity, the term 'sensory profile' in the text denotes the aroma, flavour, taste and mouthfeel of honeybush tea infusion. Aroma refers to odours perceived through orthonasal analysis, while flavour refers to the retronasal perception of aromas in the mouth. Similar to flavour, the basic taste modalities, i.e. sweet, sour, and bitter and the mouthfeel attribute, astringency (described as the tactile sensation in the oral cavity), are perceived in the oral cavity (Lawless & Heymann, 2010). Flavour, basic tastes and astringency are often referred to as palate attributes (Moelich et al., 2017; Parr, Ballester, Peyron, Grose, & Valentin, 2015). 'Honeybush sensory quality' in the text refers to the overall sensory quality of honeybush tea in terms of aroma, flavour, taste, mouthfeel and appearance.



## 2 Honeybush tea

### 2.1 Background

Honeybush (*Cyclopia* Vent.; family *Fabaceae*; tribe *Podalyrieae*) is an indigenous South African fynbos shrub, endemic to specific climatic zones of the Western and Eastern Cape regions (Joubert et al., 2011). Of the 23 *Cyclopia* species identified to date, species currently of commercial importance include *C. intermedia*, *C. genistoides* and *C. subternata* (Joubert et al., 2019). Their natural habitat varies from sandy coastal to mountainous regions. *Cyclopia* spp. are distinguished from related genera by its trifoliate leaves and the circle-shaped depression ('cyclopia' is derived from the Greek word 'cyclops' meaning 'round-eyed') in the base of the calyx where the pedicel is attached to the yellow flower (Kies, 1951; Schutte, 1997) (Fig. 1).



**Figure 1** Natural distribution of *Cyclopia* species (adapted from SAHTA (2020)). *Picture insert*: Distinctive yellow flowers (A) and trifoliate leaves (B) (photos supplied by E. Joubert, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa; sketches from McGregor (2018)).

General, descriptive names for *Cyclopia* spp. such as 'honigtee', honeybush and 'heuningbostee' are derived from the sweet, honey-like scent of the plant when in full bloom with yellow flowers (Joubert et al., 2011). Traditionally, harvesting for tea production took place during flowering as the bushes could easily be identified in the wild (Du Toit, Joubert, & Britz, 1998). Although the flowers improve the aroma and flavour of the herbal tea, they were found not to be essential for the characteristic sweet aroma and flavour of honeybush tea, contrary to popular belief (Du Toit & Joubert, 1999). The leaves and stems are subjected to a high-temperature oxidation step to produce honeybush tea. This 'fermentation' step, as referred to by

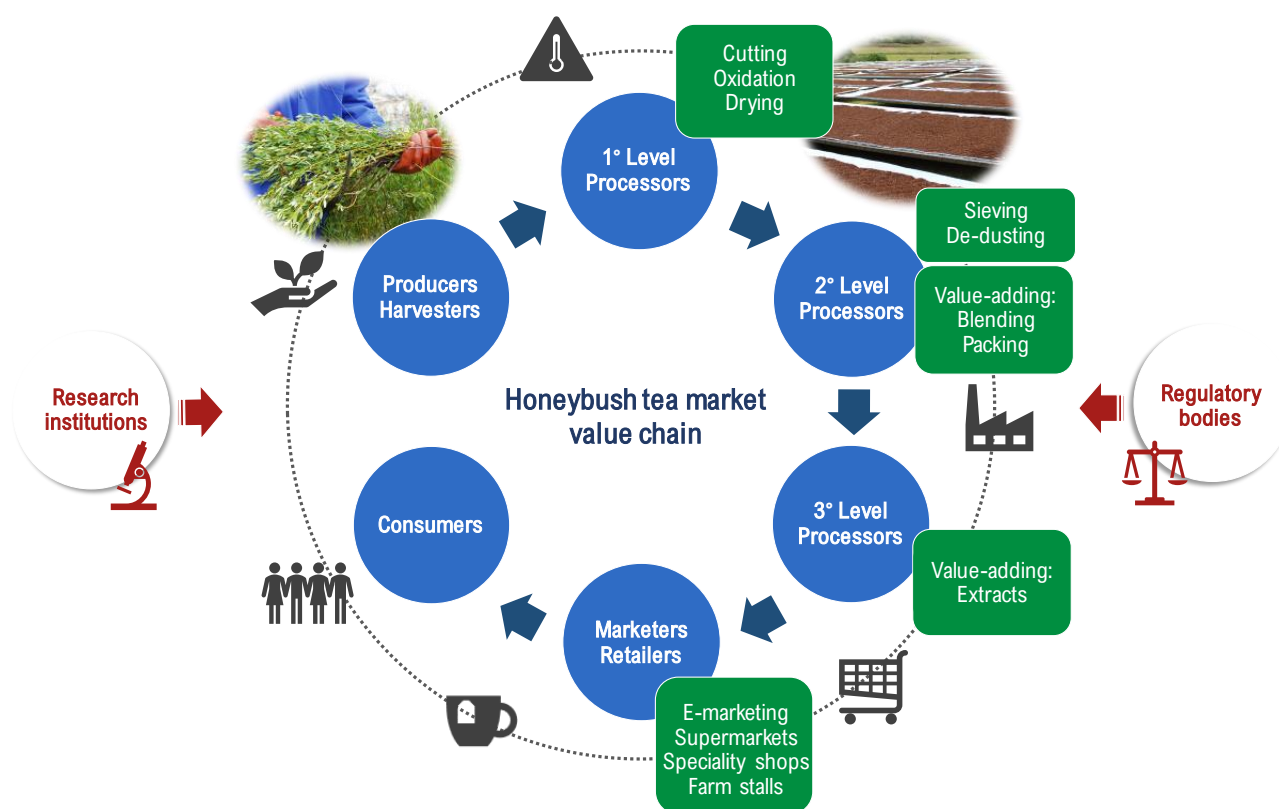
industry, is essential for the development of the characteristic and sought-after sweet aroma and flavour, as well as the brown colour of this herbal tea (Du Toit & Joubert, 1999; Joubert, Gelderblom, Louw, & De Beer, 2008). Green ('unfermented') honeybush tea, produced at lower volumes, has found niche markets as alternative tea product and source for the production of phenolic-rich extracts for the functional food, nutraceutical and cosmetic industries (Joubert et al., 2011).

Honeybush tea has a long history of regional use as medicinal plant or herbal tea that predates the 1800's, with the earliest records indicating its use as restorative and expectorant in chronic catarrh and pulmonary tuberculosis (Bowie, 1830). Continuing research on its numerous associated health-promoting bio-activities, including phytoestrogenic, chemopreventive, antiobesity and antidiabetic properties (reviewed by Joubert et al. (2019)) and recent reconfirmation of its caffeine-free status (Stander, Joubert, & De Beer, 2019), contribute to the increased appreciation of this herbal tea by consumers globally. The elucidation of the phenolic composition of *Cyclopia* species remained a key focus of product-orientated honeybush research (Joubert et al., 2019). Apart from distinguishing honeybush from rooibos and other herbal teas, the phenolic profiles of *Cyclopia* species also give direction to potential value-addition opportunities and the development of niche products. The major phenolic compounds present in all *Cyclopia* species quantitatively analysed to date are the xanthenes, mangiferin and isomangiferin, and the flavanone, hesperidin (Joubert et al., 2019).

Contrastingly to the established ca. 120-year old industry of its fynbos counterpart, rooibos (*Aspalathus linearis*), the formal honeybush tea industry is still relatively young as it remained mainly a small cottage industry until 're-discovered' in the mid-1990's (Du Toit et al., 1998; Joubert et al., 2011). In 1992 the foundation for a formal agricultural and agro-processing industry was laid with the launch of a propagation research project ('*Cyclopia* species: Initiation of commercial plantings and studying of its conservation') by Dr J.H. de Lange of the South African National Botanical Institute (SANBI), and funded by the Agricultural Research Council (ARC) (Joubert et al., 2011). Subsequently, additional honeybush projects by the ARC and several universities followed, and interest and participation of farmers, processors and marketers fuelled the development of an industry. In 1999 the industry was formalised with the establishment of the South African Honeybush Producers Association (SAHPA), later re-named to the South African Honeybush Tea Association (SAHTA) to include all stakeholders (Joubert et al., 2011). In 2019, the formal honeybush tea industry has reached a 20-year milestone and the major post-harvest research advances achieved during this period was reviewed by Joubert et al. (2019). Although the industry is still under-developed, honeybush as herbal tea is advanced in the process of commercialisation compared to other traditional South African herbal teas and functional food ingredients (excluding rooibos, buchu (*Agathosma betulina* and *A. crenata*) and hoodia (*Hoodia gordonii*)) (Van Wyk & Gorelik, 2017). It has already achieved an international footprint (Joubert et al., 2019), with a vast growth potential provided that a consistent supply of a quality product could be achieved (Bester, Joubert, & Joubert, 2016).

## 2.2 Honeybush industry role-players

The honeybush tea market value chain from producer to consumer is depicted in **Fig. 2**. Honeybush industry role-players play an essential part in delivering a final product of acceptable and consistent quality. For the commercial success of the industry, quality should be communicated throughout the value chain, i.e. from the producer of cultivated honeybush, harvester of wild and/or cultivated honeybush, primary level processor (on-farm/commercial oxidation and drying), secondary level processor (commercial blending and packing), tertiary level processor (extract production), marketer/retailer and lastly the consumer (DAFF, 2016). The development and current practice of activities for main role-players, including the role of research institutions and regulatory bodies in improving and controlling honeybush sensory quality, will be reviewed in the following sections. Both traditional (oxidised) and green honeybush tea are produced from honeybush plant material. Within context of the present study, emphasis will be on traditional honeybush tea.



**Figure 2** Honeybush tea market value chain from ‘crop to cup’ depicting the key industry role-players (adapted from DAFF (2016); photos from SAHTA (2020)).

### 2.2.1 Producers

The bulk of *Cyclopia* plant material (ca. 70%) (SAHTA, 2020) sourced for honeybush tea production, is harvested from the slopes of the Cape Fold Mountains, of which *C. intermedia* (‘bergtee’, mountain tea) represents ca. 85% of the wild-harvested crop (McGregor, 2017b, 2017a). Guidelines for sustainable wild-harvesting have been developed, based on interval harvesting of less than 50% of plants in a honeybush-

bearing site, every 2 to 5 years (McGregor, 2017b). However, increased commercial production is required to supply in the growing demand, to ensure market growth, as well as aid conservation of species (North, Joubert, De Beer, De Kock, & Joubert, 2017). Cultivation/plant improvement research on *C. genistoides* ('kustee', coastal tea) and *C. subternata* ('vleitee', marsh tea), amongst other species, were initiated by the Agricultural Research Council (ARC) to address the need for stable and sustainable sources of high quality plant material (Bester et al., 2016; Joubert et al., 2011). Two survival strategies for *Cyclopia* spp. exist based on their adaptation to the frequent fires in their natural habitat: sprouting from a woody rootstock ('sprouters') or recruiting from seed ('non-sprouters'/'re-seeders') after fire (Schutte, Vlok, & Van Wyk, 1995). Approximately 147 ha of cultivated honeybush land in the Western and Eastern Cape exists, of which the bulk comprises of *C. subternata* (non-sprouter) and *C. genistoides* (sprouter) (McGregor, 2017a). *Cyclopia longifolia* (non-sprouter) also emerged as a highly productive cultivated crop as vigorous grower. *Cyclopia intermedia* (sprouter), a slow grower, has shown poor potential for commercial cultivation, as too frequent harvesting prohibits build-up of sufficient energy reserves in the rootstock, resulting in dieback (Joubert et al., 2011).

The on-going ARC honeybush breeding research programme aims to increase to breed and assess cultivars with improved intrinsic quality and horticultural traits (i.e. increased biomass yield) properties (Bester et al., 2016). Sensory and phytochemical analyses of selections and progenies form part of the evaluation of genotypes and selections to ensure that the quality of the plant material and tea is not compromised. The high costs of descriptive sensory analyses (DSA) and the of quantity biomass required for processing into herbal tea according to a standardised process allow analyses of only advanced selections (Bester et al., 2016; Robertson et al., 2018). The need for a time- and cost-efficient sensory quality grading system (method) has been identified for screening of large numbers of genotypes and selections, processed into herbal tea.

Harvesting aspects relating to quality have been addressed by North et al. (2017). Annual harvesting is encouraged to ensure that the shoots stay relatively thin, i.e. less woody, which is more suitable for honeybush tea processing (North et al., 2017). Highly coarse fractions of processed tea may be considered an agricultural waste product which is unfavourable in terms of sustainable and optimal product utilisation (North et al., 2017). Although special cutting machines may be used to reduce the coarse plant material (stems) size for blending, these processed stems have a light tan colour which is detrimental to product appearance. Stems have lower soluble matter than leaves (De Beer et al., 2012; Du Preez, De Beer, & Joubert, 2016), which will affect 'cup-of-tea' strength. Their effect of aroma, flavour and taste of the infusions has not yet been characterised. In the case for rooibos, the stem waste-product was found to contribute a negative aroma attribute to infusions, described as 'pencil-shavings' (Sishi, Muller, De Beer, Van der Rijst, & Joubert, 2019). Harvesting should be conducted during summer to late autumn before its flowering period (August to September), as flowering places the plant under stress. In addition, processed

flowers, classified as 'dust' (particle size fraction is smaller than 40 mesh) contribute very little to the final product yield (North et al., 2017). Spring harvests delivered plant material with of the highest mangiferin levels for *C. genistoides*. Furthermore, hot water-soluble solids and total polyphenol content of the fermented tea increased with years of harvest. Research is still required on the effect of harvesting and irrigation in commercial plantations for optimum production on honeybush tea sensory quality (North et al., 2017). A recent study by Mabizela et al. (2020) on the effect of genotype and harvest season on the phenolic content of the leaves of *C. subternata* and the sensory profile of the herbal tea, indicated that the summer harvest delivered the better product. Through descriptive sensory analysis (DSA) of the herbal tea infusions, certain genotypes could be highlighted in terms of higher intensities of positive sensory attributes, compared to other genotypes.

## 2.2.2 Processors

### 2.2.2.1 General

Primary level honeybush tea processing, namely cutting, high-temperature oxidation ('fermentation') in the case of the fermented product, but not for green honeybush tea and drying, is performed either on-farm or at commercial factories (**Fig. 2**). Secondary level processing (sieving, de-dusting, blending and packing) is mainly performed at commercial factories. These industry role-players include processors that are involved in processing of plant material supplied by honeybush producers, and final product packing, or buyers of bulk processed tea who blend different batches for consistent quality, followed by packing (bulk or for retail). In addition, some secondary level processors and buyers produce and/or pack herbal/fruit tea infusion blends. Many of these companies handle both rooibos and honeybush as part of their product portfolio. At present six major processing facilities exist of which two on-farm tea-processing companies are involved in the entire value chain from 'crop to cup' of single species, namely wild-harvested *C. intermedia* and cultivated *C. genistoides*, respectively (McGregor, 2017a). Numerous small-scale honeybush tea processors are emerging (E. Smith, SAHTA, 2019, personal communication). Tertiary level processing includes bottling of infusions (natural and flavoured) as ready-to-drink iced teas by a small-scale processor, but it is mainly honeybush extract production for the functional beverage, pharmaceutical and cosmetic markets. Extract production is performed by either major South African herbal tea processing or pharmaceutical companies focussing on natural products (Afriplex, 2020; Rooibos Ltd, 2020).

### 2.2.2.2 Primary level processing

Producers are situated in close vicinity (ca. 100 km radius) to a processing plant, as harvested plant material deterioration during prolonged transport could negatively influence final product quality. Shredding of plant material, commonly performed by mechanised fodder cutters or tobacco cutters, ensures the disruption of cellular integrity and facilitates fermentation (Du Toit & Joubert, 1999). However, no research has been done to date to investigate the effect of cut size on fermentation.

Processing of traditional honeybush tea (i.e. fermented product) developed from primitive on-farm fermentation ('curing') heaps or baking ovens and sun-drying, delivering products of poor microbial and sensory quality (Du Toit et al., 1998), to high temperature rotary fermentation and drying under controlled conditions (Joubert et al., 2011). Batch rotary fermentation, a concept developed initially for rooibos (Joubert & Müller, 1997), was adopted by the honeybush industry to ensure consistent fermentation conditions, including even heat distribution and temperature (Du Toit & Joubert, 1999). Fermentation at >60 °C was found adequate to inhibit growth of thermophilic moulds (Du Toit, Joubert, & Britz, 1999). Stainless steel batch rotary fermentation tanks are commonly used or in certain instances, drums with rotating paddles (Bergh, 2014). During natural fermentation, heat is slowly generated from exothermic reactions and temperatures of up to 60 °C have been reported over a 72 h period (Du Toit & Joubert, 1999). Processors may achieve temperature and humidity control with heating elements or direct steam injection and/or water pre-treatment, which are monitored thermostatically (Bergh, 2014; Du Toit & Joubert, 1998). High fermentation temperatures require water pre-treatment which aid characteristic flavour and colour development, uniform colour development of dried product (less white stem pieces) and improved release of tea soluble solids for enhanced infusion characteristics (Du Toit & Joubert, 1998b). Reported fermentation temperature/time regimes vary from ca. 70 °C/60 h to 80-90 °C/18-24 h (Joubert et al., 2011).

Following fermentation, drying either takes place in rotary driers, on fluid bed driers using hot air, or through sun-drying on drying racks (canvas) in the open or enclosed (greenhouses) to achieve a moisture content of ca. 10% (Agulhas, 2020; Du Toit & Joubert, 1998a; L. Slabber, Rooibos Ltd, 2020, personal communication). Contrastingly to rooibos tea that is processed at ca. 38 °C and requires decontamination, normally by steam pasteurisation (Joubert & Schulz, 2006), no steam pasteurisation is performed on honeybush tea after drying, provided that bacterial counts (including *Escherichia coli* and *Salmonella*) are below maximum permitted levels according to export regulations (N. Joubert, Agulhas Honeybush Tea, 2020; E. Nortjé, Melmont, 2020; personal communication). Specifications for total viable bacterial count levels were recently amended from 75 000 CFU (colony forming unit) to 300 000 CFU for honeybush and to 1 000 000 CFU for herbal tea blends in the South African export regulations for honeybush tea (DAFF, 2019b). One of the processors noted that steam pasteurisation might affect honeybush tea sensory quality (Q. Nortjé, Melmont, 2019, personal communication), as seen for rooibos (Koch, Muller, De Beer, Næs, & Joubert, 2013), however, this has not been investigated.

High-temperature fermentation and drying under controlled conditions is important to produce honeybush tea of high sensory quality (Du Toit & Joubert, 1998a, 1999). With the inception of the formal honeybush industry, honeybush tea of poor and inconsistent quality, especially poor flavour or the presence of off-flavours, were identified as major obstacles in successful commercialisation and advancement of the industry (Du Toit et al., 1998). The combination of low temperatures and extreme long fermentation periods results in the development of off-odours or taints associated with poor quality (Du



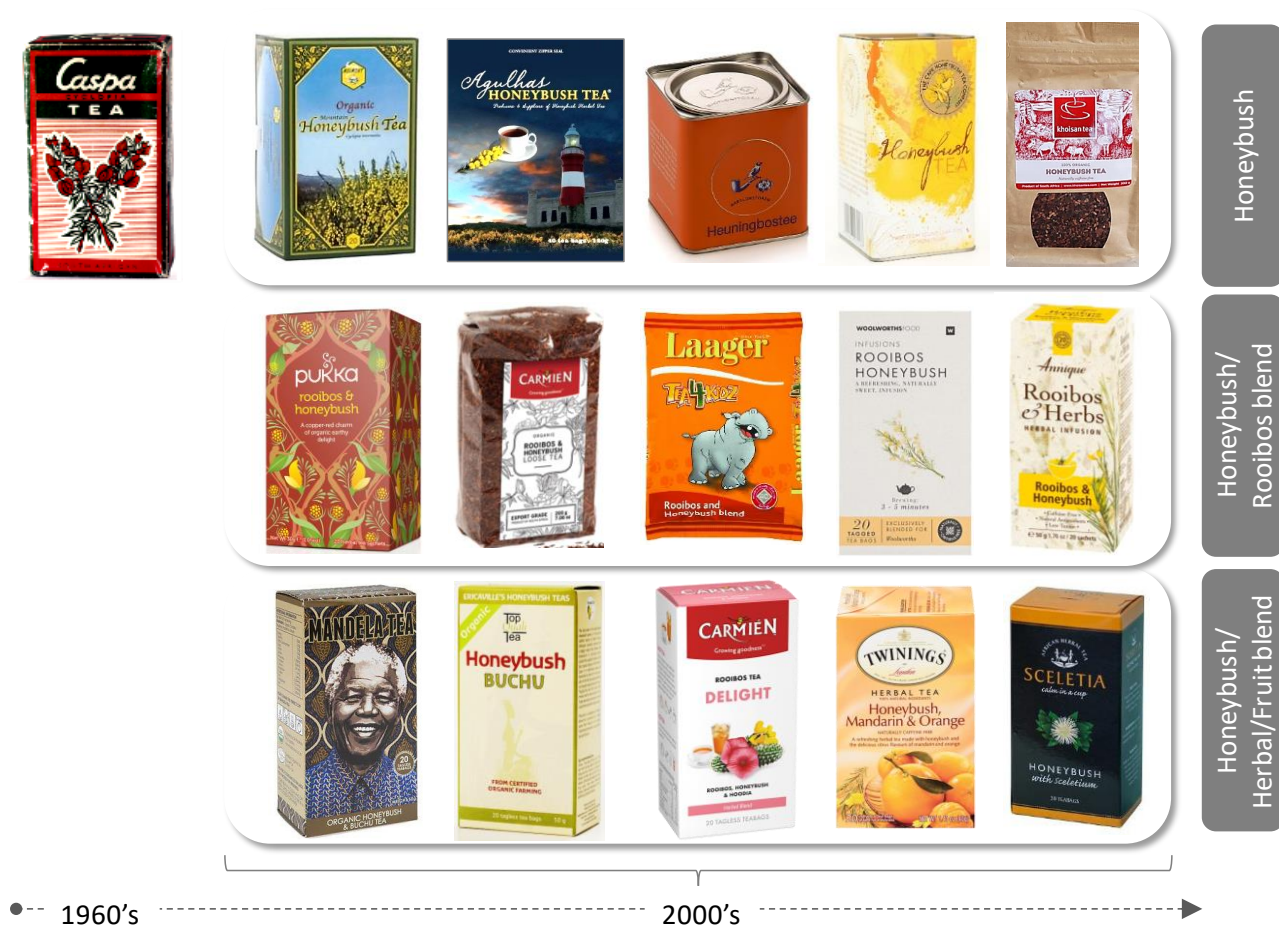
Toit et al., 1998). Even with major improvements in sensory quality achieved through process optimisation and control (Du Toit & Joubert, 1998a, 1999), the prevalent supply of honeybush tea of variable and inferior sensory quality available on the market is ascribed to poor adherence to standardised processing techniques and is of major concern to industry (C. Cronjé, Food Safety and Quality Systems Consultancy, 2018; C. Gass, Gass Co. Ltd, 2018; E. Smith, SAHTA, 2019; personal communication). 'Burnt' or 'smoky' off-flavours have been associated with over-processing or uneven heat distribution in the fermentation tank that results in hot spots, whereas 'grassy' aromas have been associated with under-fermentation (Bergh et al., 2017; Du Toit et al., 1998). Furthermore, infusions of high turbidity and poor sensory quality ('sour' taste and 'rotten'/'vegetable-like' aroma and flavour) have been associated with excessive moisture during processing ('wet fermentation'), as well as too slow initial increase in fermentation temperature using steam injection (C. Cronjé, Food Safety and Quality Systems Consultancy, 2018; personal communication). Refer to Section 2.2.6 for sensory research on optimisation of fermentation temperature-time regimes for optimal sensory quality.

### 2.2.2.3 Secondary level processing

Honeybush tea was traditionally sold mostly as an unrefined mixture of coarse leaves and stems. However, the export market required a finer product (Du Toit et al., 1998). Today, dried honeybush tea is mechanically sieved to desired cut sizes, namely 'fine cut' and 'superfine cut' for teabag production and 'coarse cut' for tea sold as 'loose leaf' or used as base for fruit and herbal tea blends. The latter are either packed in 'pyramid' teabags or loose tea packaging (Carmién, 2020; Rooibos Ltd, 2020). Recently, honeybush 'espresso' also appeared on the local supermarket shelves. It is extra fine fermented leaves and stems for use in espresso machines. For research purposes, sensory analysis of the processed plant material is standardised on the 'tea bag' fraction (< 12 mesh and > 40 mesh) (Bergh et al., 2017; Erasmus, Theron, Muller, Van der Rijst, & Joubert, 2017; Mabizela et al., 2020; Robertson et al., 2018; Theron et al., 2014).

Honeybush tea, an agricultural product with natural variation in plant material, is blended by secondary level processors to improve product consistency in terms of quality and sensory profile. Blending of different batches is common practice in the black tea (Alasalvar et al., 2012; Joliffe, 2003; Liang, Lu, Zhang, Wu, & Wu, 2003) and wine (Cáceres-Mella et al., 2014) industries. As demand exceeds supply, tea processors are forced to blend batches of different *Cyclopia* species to supply a well-rounded commercial product for the increasingly growing markets (Moelich, 2018). Inconsistent blending ratios of species for bulk export lead to inconsistent sensory profiles which impact negatively on the honeybush reputation in the export market (C. Gass, Gass Co. Ltd, 2018, personal communication). In addition, buyers are required to blend batches of variable quality from processors to supply a commercial product that complies to an acceptable quality standard. Buyers have raised concern over the lack in supply of bulk tea of good sensory quality (C. Cronjé, Food Safety and Quality Systems Consultancy, 2018; C. Gass, Gass Co. Ltd, 2018; M.

Nieuwoudt, Khoisan Tea, 2019; E. Smith, SAHTA, 2019; personal communication). The lack of a quality grading system is a concern of buyers (C. Cronjé, Food Safety and Quality Systems Consultancy, 2018; C. Gass, Gass Co. Ltd, 2018; personal communication).



**Figure 3** First branded honeybush tea, ‘Caspa Cyclopia tea’ (Joubert et al., 2011), and examples of more recent commercial honeybush tea, herbal and fruit tea blends (products are either packed in teabags or as loose tea).

The first branded honeybush product, ‘Caspa Cyclopia tea’, appeared in the 1960s on the South African market (Joubert et al., 2011). With the revival of the industry in the 1990s, honeybush tea was sold in bulk form to international clients. However, the industry recognised the importance of local value-addition in terms of packaged products ready for the retail market (Joubert et al., 2011). Over the past two decades various branded honeybush tea, herbal and/or fruit tea blend products have seen the light, of which honeybush and rooibos blended products are prevalent (Fig. 3). In addition, the subtle differences between the sensory profiles of the herbal teas, produced from different *Cyclopia* species, could create opportunities for niche markets with specific taste requirements (Du Toit et al., 1998; Erasmus et al., 2017; Robertson et al., 2018). However, limited supply of fermented honeybush tea restricts expansion of product differentiation on the basis of species and branded products consists predominantly of blends of different honeybush species (Joubert et al., 2011). Honeybush tea packaging is consistent with the global herbal tea market, including bulk tea, loose tea and tea bags (Transparency Market Research, 2017). The



subtle sweet taste of honeybush tea provides a good carrier for combination with other indigenous South African herbal/medicinal plants ('botanicals') including buchu (*Agathosma betulina* and *A. crenata*), hoodia (*Hoodia gordonii*), sutherlandia or cancer bush (*Lessertia frutescens*) and sceletium (*Sceletium tortuosum*) (Cape Honeybush Tea, 2020; Van Wyk, 2011).

### 2.2.3 Marketers / Retailers

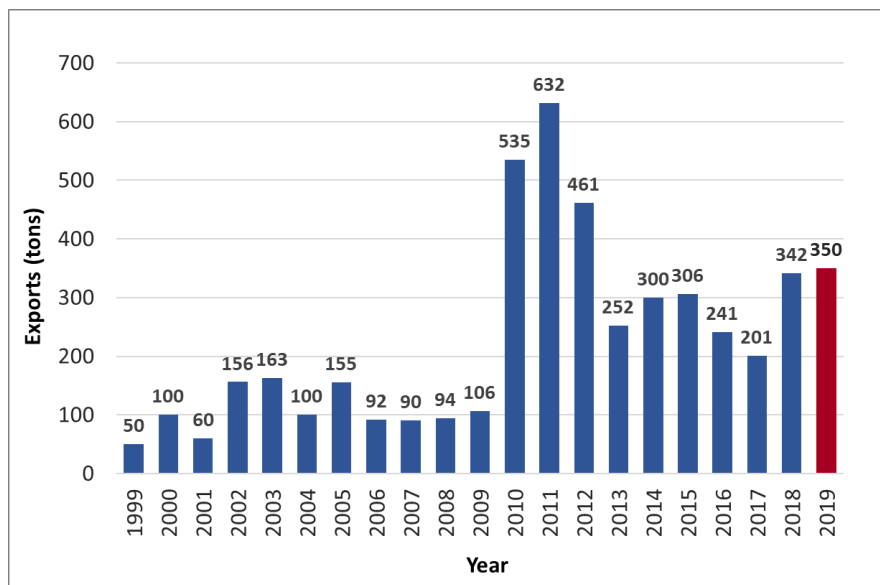
Marketers of honeybush tea products include sales division representatives of processing and blending/packing companies, local companies of which primary and secondary level processing are outsourced entirely for private label products, and marketers of export bulk products for value-addition by international buyers. International food and beverage trade fairs contribute to honeybush tea marketing (Cape Honeybush Tea, 2020; SIPPO, 2014). Honeybush products are sold as speciality and/or health products in major retail supermarkets, health shops, pharmacies, up-market farm stalls, as well as through online marketing (DAFF, 2016; Joubert et al., 2011). The involvement of major rooibos tea marketing companies contributed to the presence of honeybush products on supermarket shelves (Joubert et al., 2011). Individually packaged teabags in envelopes add value as a premium product for the hospitality industry such as hotels and guest houses (Carmién, 2020).

### 2.2.4 Consumers

Honeybush tea was exported for the first time in 1993 and 1995 to Japan and Germany, major international rooibos markets, respectively (Du Toit et al., 1998). The export market has expanded substantially with ca. 632 tons recorded in 2011, although plant material shortages due to severe droughts and veld fires have negatively affected export volumes (Joubert et al., 2011) (**Fig. 4**). Major export countries include the Netherlands, Germany, USA, Canada and UK. Honeybush is also exported to traditional tea-drinking countries such as Japan, Sri Lanka, Malaysia, and China (DAFF, 2016; Joubert et al., 2011). European consumers prefer honeybush in fruit tea blends, whereas the Asian market (especially Japanese consumers) consume honeybush as is, or in iced tea beverages (C. Cronjé, Food Safety and Quality Systems Consultancy, 2018; C. Gass, Gass Co. Ltd, 2018; personal communication). In addition, consumer preferences for certain species have been indicated, e.g. *C. subternata* ('apricot jam' aroma) and *C. intermedia* ('floral' and 'fruit-cake' aroma) by the European and Asian market, respectively (C. Cronjé, Food Safety and Quality Systems Consultancy, 2018, personal communication).

Formerly, many South African consumers were not aware of the existence of honeybush which may be ascribed to localised production for mainly home consumption and no active marketing (Du Toit et al., 1998). A study based on interviews with consumers representative of Living Standards Measure (LSM) segments 6 to 10 in South Africa, indicated 100% consumer awareness and 30% consumption of honeybush tea of the participants (N = 140) interviewed (Vermeulen, 2015). Strong retail volume growth for speciality

herbal/fruit tea and green tea products with health and wellness positioning within the South African market have been recorded recently, despite their higher than average unit prices (Euromonitor, 2019b).



**Figure 4** Honeybush export of bulk tea for 1999-2019 (amended from McGregor (2017a); data provided by Perishable Products Export Control Board).

### 2.2.5 Regulatory bodies

Du Toit et al. (1998) indicated that a quality grading or classification system would be beneficial to both the processor that follow good manufacturing practices (GMP) and consumer to obtain the best quality product. However, to date no standardised honeybush sensory quality specifications and assessment methods have been established, as each primary and secondary processor apply their individual measures to assess sensory quality per production batch (E. Joubert, ARC Infruitec-Nietvoorbij, 2017, personal communication). **Figure 5** depicts current statutory and non-statutory control measurements applied to honeybush tea.

Honeybush tea intended for export is subjected to the regulatory standards of the Department of Agriculture, Forestry and Fisheries (DAFF), as described in the Agricultural Product Standards Act 119 of 1990 (DAFF, 2019b). These regulations were recently amended to exclude any form of sensory quality control. Current export regulations only state specifications for moisture content, microbial content, agro-chemical residue limits and percentage of foreign matter. The previous version (DAFF, 2000) specified classification according to cut size and specifications for moisture content, microbial content, agro-chemical residue limits and percentage of foreign matter were provided. Although no provision was made for sensory quality, the regulations at least stated that the product 'shall have the clean, characteristic taste and aroma and clear, distinctive colour of honeybush' and should be 'free from any foreign flavours and odours which detrimentally affect the characteristic of the product'. No specifications or standardised method for sensory quality assessment were indicated by these regulations. The Perishable Products Export

Control Board (PPECB), an independent service provider of quality certification and cold chain management services, conducts the inspections. Microbial and pesticide content analyses are mainly outsourced to accredited analytical laboratories (Cape Honeybush Tea, 2020; Carmién, 2020; Melmont, 2020; Rooibos Ltd, 2020; M. Joubert, Agulhus Honeybush Tea, 2020, personal communication). No sensory quality assessment (aroma, flavour, taste, mouthfeel or appearance) of the final product (dried tea material and/or tea infusion) is conducted during inspections (M. Joubert, Agulhas Honeybush Tea, 2020, personal communication). Major processing companies have food safety management systems such as Food Safety System Certification 22000 (FSSC 22000) or Hazard Analysis and Critical Control Points (HACCP) in place to assure product safety (Cape Honeybush Tea, 2020; Carmién, 2020; Melmont, 2020; Rooibos Ltd, 2020), but no sensory evaluation is required and specified for certification.



**Figure 5** Summary of honeybush statutory and non-statutory control for which no sensory quality control is specified or applied at present (depicted by crossed-out teacup icons).

Product differentiation and conformity to new generations of quality requirements represent an important source for large tea manufacturers to stimulate demand and maintain their market position (Oxfam, 2002). Many honeybush processors' products are certified as organic, meeting the requirements of the United States Department of Agriculture (USDA), European Commission for organic certification and Japanese Agricultural Standards (JAS). However, organic regulations do not address sensory quality of the product. Sustainability certification through the UTZ and Union for Ethical BioTrade (UEBT) herbal tea programme (part of Rainforest Alliance), currently only applied to rooibos, also does not guarantee sensory quality (UTZ, 2020).

The declining growth rate of traditional tea markets required international tea companies to follow new strategies directed at value-adding and development of more differentiated tea products, as well as the promotion of tea with geographical indicators (Larsen, 2015). Marketing of place-based names has resulted in more opportunities for product differentiation, opportunities created by increased globalisation and liberalisation of agricultural products, and increased consumer demand for quality worldwide (Barham, 2003). Quality distinctiveness labels include Protected Geographical Indication (PGI) and protected designation of origin (PDO) (Etaio et al., 2010b; European Commission, 2020; Pérez-Elortondo et al., 2018). For PDO food, agricultural products and wines, every part of the production, processing and preparation process must take place in the specific region. PGI emphasises the relationship between the specific geographic region and the name of the product, where a particular quality, reputation or other characteristic is essentially attributable to its geographical origin, i.e. at least one of the stages of production, processing or preparation takes place in the region (European Commission, 2020).

Honeybush has been included in the GI Protocol of the Economic Participation Agreement (EPA) with the European Union (EU) and is fully protected as a GI in Europe (European Commission, 2019). GIs not only serve to designate goods with a quality characteristic, or reputation attributed to its geographical origin, but are considered an instrument to protect product names, such as 'honeybush' against misappropriation (Biénabe & Marie-Vivien, 2017). To be included in the GI Register of the EU, the required specification sheet ('Single Document') still needs to be developed. The advantages of inclusion of honeybush in the GI Register include increased visibility within the EU and protection against other trademarks (D. Troskie, Department of Agriculture, Western Cape, 2019, personal communication). Regulations relating to the protection of GIs used on agricultural products intended for sale within SA require a product specification that describes *'the product's main physical, chemical, microbiological and organoleptic (where applicable) characteristics, provided that existing quality and/or compositional requirements prescribed in the regulations published under the Act for the agricultural product, shall be taken into consideration'* (DAFF, 2019a). As for SA export regulations (DAFF, 2019b), no sensory (so-called 'organoleptic') specifications or method to evaluate sensory quality exist to ensure a product complies with the applicable GI regulations.

Both rooibos and honeybush (*C. intermedia*, *C. genistoides*, *C. subternata* and *C. sessiliflora*) are listed as herbs on the Tea and Herbal Infusions Europe (THIE) Inventory List (THIE, 2019). THIE represents the interests of producers and traders of tea (*Camellia sinensis*) and herbal infusions, as well as extracts thereof, in the EU and quality assurance and food safety are key activities. THIE defines herbal and fruit infusions as foodstuffs which are traditionally consumed due to their health and sensory properties. THIE regards sensory characteristics, apart from physical and chemical parameters, as essential for overall product quality. Basic guidelines to assess the sensory quality of herbal and fruit infusions in terms of infusion colour, aroma and flavour (taste) (including possible 'off-flavour') are provided (THIE, 2018).

However, currently no THIE sensory specifications for honeybush exists (L. Mönch-Sander, THIE, 2018, personal communication).

### 2.2.6 Research institutions

Research has played an important catalytic and supporting role in the commercialisation of honeybush tea (Joubert et al., 2011). A recent review gave an updated summary of the honeybush product-orientated research. A key driver of research was the improvement of herbal tea quality by the ARC and Stellenbosch University as collaborating institution (Joubert et al., 2019). Considering the gaps in knowledge and needs of the honeybush industry, research to date aimed at improving the quality of a cup of honeybush tea will be reviewed in the following section.

#### 2.2.6.1 Process optimisation

##### 2.2.6.1.1 Sensory and physicochemical characterisation

The first research conducted to determine optimum fermentation and drying parameters for improved herbal tea product quality was done on *C. intermedia*, *C. genistoides* and *C. buxifolia* (initially classified as *C. maculata* ex Du Toitskloof) (Du Toit & Joubert, 1998a, 1998b, 1999; Du Toit et al., 1998). The practice by industry then (Du Toit & Joubert, 1999) and even now, was visual assessment of fermenting tea leaf colour as an indication of the extent of fermentation. However, infusion colour plays an important role in infusion strength assessment and consumer appeal. Therefore, the effect of different fermentation time and temperature regimes were investigated based on wet leaf, dry leaf and infusion colour (CIEL\*a\*b\* colour measurement system), as well as broad-based sensory characterisation (rating of aroma, flavour and overall quality) (Du Toit & Joubert, 1999). Subsequently, optimum fermentation conditions of 70 °C/60 h or 90 °C/36 h were established for *C. intermedia* and *C. buxifolia* (initially classified as *C. maculata* ex Du Toitskloof) (Du Toit & Joubert, 1999), which led to the application of 70 °C/60 h by certain processors. Others preferred to use shorter fermentation times (18–24 h) at 80–85 °C to reduce production costs (Joubert et al., 2019). Since the afore-mentioned study, in which only *C. intermedia* and *C. buxifolia* were investigated, *C. subternata*, *C. genistoides*, *C. maculata* (ex Genadendal) and *C. longifolia* grew in prominence, and the optimum fermentation temperature-time regimes were recently determined for these *Cyclopia* species (Erasmus et al., 2017). Bergh et al. (2017) reinvestigated the fermentation of *C. intermedia* for improved characterisation of sensory profile changes during processing.

Theron and co-workers (2014) were the first to address the comprehensive characterisation of the full sensory profile (including negative sensory attributes) of fermented honeybush tea infusion. Included in the study were the fermented plant material of the *Cyclopia* species, namely *C. sessiliflora*, *C. longifolia*, *C. genistoides*, *C. intermedia*, *C. subternata* and *C. maculata*. Sensory profile characterisation using descriptive sensory analysis (DSA) was essential to define subtle, yet critical sensory profile changes during

fermentation for the identification of optimum fermentation conditions. The broad-based sensory characterisation employed by Du Toit and Joubert (1999) did not address specific positive and negative attributes, as tea infusions were only ranked on a 4-point scale for aroma, flavour and overall quality (i.e. 'the characteristic sweet, honey-like flavour, with no grassy undertones'). DSA is regarded as one of the most sophisticated sensory profiling methods in sensory science which allows for the development of a scientific language for a product to describe and quantify the perceived sensory similarities and differences between samples (Lawless & Heymann, 2010).

Theron et al. (2014) identified generic aroma attributes, i.e. 'floral', 'sweet-associated', 'fruity', 'plant-like' and 'woody', and grouped species according to their sensory profiles. This formed the basis for sensory characterisation of the herbal tea infusions of a selection of *Cyclopia* species, employing larger sample sets to determine attributes common to the characteristic honeybush sensory profile, namely 'fynbos-floral', 'woody', 'fynbos-sweet' aroma with a sweet taste and slightly astringent mouthfeel, but also to identify attributes more prominent in some species. Temperature-time regimes for optimum development of the characteristic aroma attributes in honeybush infusions were established on a laboratory scale for *C. subternata*, *C. genistoides*, *C. maculata*, *C. longifolia* (80 °C/24 h or 90 °C/16h) (Erasmus et al., 2017) and *C. intermedia* (90 °C/24 h or 90 °C/36 h) (Bergh et al., 2017). In addition, Bergh et al. (2017) validated that comparable herbal tea quality could be achieved on factory scale. 'Woody', 'fynbos-floral', 'fynbos-sweet', 'fruity-sweet' and 'apricot' aroma notes that developed at the optimum conditions were identified as primary positive aroma attributes, whereas secondary attributes such as 'cooked apple' and 'rose geranium' were prominent in *C. intermedia* and *C. genistoides*, respectively (Bergh et al., 2017; Erasmus et al., 2017). Additionally, negative attributes associated with un- or under-fermented honeybush tea ('green grass' and 'hay/dried grass'), over-fermentation ('burnt caramel') and poor processing practices ('smoky', 'musty', 'dusty' and 'rotting plant water') were identified (Bergh et al., 2017; Theron et al., 2014). 'Hay/dried grass' aroma was consistently present in all samples analysed and therefore regarded as intrinsic of the generic honeybush aroma profile; however, at high intensities it affects the sensory quality negatively. A decrease of 'hay/dried grass' and 'green grass' aroma intensities was critical considerations for establishment of optimum fermentation temperature-time regimes for the different *Cyclopia* species (Bergh et al., 2017; Erasmus et al., 2017). In addition, bitter taste was most notable in fermented *C. genistoides* infusions, and although fermentation over time showed to reduce bitter intensity (Alexander, 2018; Erasmus et al., 2017), it may negatively influence consumers' acceptance of fermented honeybush tea as herbal tea, since they associate honeybush tea with 'slightly sweet and honey-like' flavour and taste (Vermeulen, 2015).

To date, limited attention has been given to colour (Bergh et al., 2017; Du Toit & Joubert, 1999) and turbidity (Bergh et al., 2017) of honeybush infusions as indicators of herbal tea quality. Infusions of numerous commercially processed honeybush samples gave unacceptably high nephelometric turbidity

unit (NTU) values (> 150). Although linked to poor processing techniques (sub-optimal fermentation conditions), the cause for haze formation in honeybush tea has not yet been elucidated. Haze formation ascribed to interaction of polyphenols and caffeine in black tea is undesirable, and numerous studies report on methods to remove haze, so-called 'tea cream' (Jöbstl, Fairclough, Davies, & Williamson, 2005; Liang, Lu, & Zhang, 2002; Liang & Xu, 2003). Honeybush tea has been specified as caffeine-free (Stander et al., 2019), therefore, turbidity cannot be ascribed specifically to the interaction of its phenolic constituents with caffeine. Similarly, turbidity in caffeine-free rooibos tea (Stander et al., 2019) is regarded as unacceptable and has been linked to over-fermented tea (Joubert & De Villiers, 1997). Bergh et al. (2017) recommended further research to determine specifications for acceptable NTU values to include turbidity as a quality parameter in a quality grading system for honeybush tea.

In addition to fermented honeybush tea, research on processing optimisation of green (unfermented) honeybush as herbal tea has been conducted in terms of sensory characterisation, steam treatment for improved quality (Alexander, De Beer, Muller, Van der Rijst, & Joubert, 2018; Alexander, De Beer, Muller, Van Der Rijst, & Joubert, 2017; Joubert, Manley, Maicu, & De Beer, 2010), storage stability (Alexander, De Beer, Muller, Van der Rijst, & Joubert, 2019b) and elucidation of phenolic compounds responsible for the bitter taste of *C. genistoides* (Alexander, De Beer, Muller, Van der Rijst, & Joubert, 2019c, 2019a). The results of these studies are also useful to identify negative sensory attributes in fermented honeybush, e.g. vegetative aromas and flavours such as 'green grass' and 'hay/dried grass', and bitter taste.

#### 2.2.6.1.2 Volatile composition

Aroma is regarded as one of the most important factors influencing tea character and quality as demonstrated for *Camellia sinensis* (Yang, Baldermann, & Watanabe, 2013). Chemical characterisation of the distinct aroma of honeybush tea infusion commenced with gas chromatography–mass spectrometry (GC–MS) analyses of the changes in the volatile fraction of fermented *C. genistoides* tea (fermentation at 90 °C/16 h) (Le Roux et al., 2008). Changes in volatile organic compounds (VOCs) included formation of terpenoids and a decrease in saturated and unsaturated alcohols, aldehydes and methyl ketones (Le Roux et al., 2008). The major VOCs analysed in green and fermented *C. genistoides* were methyl-5-hepten-2-one and linalool, respectively, and relatively increased levels of the terpenoids,  $\alpha$ -terpineol, geraniol and nerol were observed. Numerous of the terpenoids identified, e.g.  $\alpha$ -terpineol, hexahydrofarnesylacetone, 2,6-dimethyl-1,7-octadien-3,6-diol, *Z*- and *E*-geraniol, linalool, linalool oxide isomers, pseudoionone,  $\beta$ -damascone, and eugenol are known to have floral, sweet, sweet-woody, floral-woody, or spicy aromas (Arctander, 1969).

The analytical methodology developed for the sampling and analysis of extremely low concentrations of VOCs provided a basis for a subsequent study by Le Roux and co-workers (2012) to identify odour-impact



VOCs. Fermented *C. subternata* was selected for analyses due to its strong, 'fruity-sweet' and 'apricot jam-like' aroma. Numerous VOCs (n = 183) were identified or tentatively identified by GC–MS. Gas chromatography–olfactometry (GC-O) detection frequency (DF) by a 15-member panel and aroma extract dilution analysis (AEDA) were employed to identify 37 odour-active compounds, i.e. aroma flavour dilution factor (AFD)  $\geq 2$ . (*E*)- $\beta$ -Damascenone, (*R/S*)-linalool, (*E*)- $\beta$ -damascone, geraniol, (*E*)- $\beta$ -ionone and (7*E*)-megastigma-5,7,9-trien-4-one were identified with the highest AFD factors of  $\geq 512$  (**Table 1**).

Theron and co-workers (2014) analysed a *C. maculata* infusion with a prominent 'cassia/cinnamon' aroma, using GC-O and GC-MS analyses. Eugenol was identified as the only compound described by the GC-O panel members as having a sweet, spicy or clove aroma. Ntlhokwe and co-workers (2017) employed comprehensive GC  $\times$  GC equipped with a single-stage thermal modulator to *C. maculata*, *C. subternata* and *C. genistoides* tea (fermented at 90 °C/16 h) to determine its applicability for profiling of honeybush tea volatiles. A total of 84 compounds were identified using reference standards and eugenol was also shown to be present in these infusions. Furthermore, association of the 'cassia/cinnamon' aroma note with *C. subternata* samples and an unidentified compound, were determined through principal component analysis (PCA) of the descriptive sensory data of the samples (n = 5/species) and the peak data obtained through non-targeted GC  $\times$  GC analysis with flame ionisation detection of the headspace. PCA indicated clear differentiation between the species based on their volatile profiles. VOCs identified for the first time in honeybush tea were 5-methylfurfural and butyl benzoate, whereas 4-vinylanisole was detected in *C. genistoides*, but absent in *C. subternata* and *C. maculata*.

In a subsequent study, Ntlhokwe and co-workers (2018) explored comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC  $\times$  GC-TOF-MS) for the detailed qualitative analysis of honeybush tea volatiles, which enabled the tentative identification of 147 compounds for the first time (total of 287 VOCs were identified)<sup>2</sup>. Most of the compounds identified were common to *C. genistoides*, *C. maculata* and *C. subternata*, although there were differences in their relative levels, and certain compounds were unique to each of the species, emphasising the complexity of honeybush tea volatile composition. Eugenol was present in relatively high levels in *C. genistoides* providing further indication that the 'cassia/cinnamon' note of *C. maculata* samples cannot be ascribed to this VOC. A strong association of two *C. maculata* samples with the descriptors, 'cinnamon' and 'cooked apple' were indicated through PCA analysis of sensory data. The tentative identification of (*E*)-cinnamaldehyde, a major component of cinnamon essential oil, in specific *C. maculata* samples indicates probable contribution of this compound to the prominent 'cassia/cinnamon' notes perceived. In addition, the 'cooked apple' aroma that was only perceived in the *C. maculata* samples, was ascribed to benzyl propanoate. Geraniol, a major

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<sup>2</sup> Experimental work for Chapter 3: *Development of chemical-based reference standards for honeybush aroma lexicon*, of the present study were completed before publication of this article.



compound identified, may contribute to the perceived 'rose geranium' aroma. Maltol (3-hydroxy-2-methylpyrone) was identified for the first time in honeybush and may contribute to the perceived 'caramel' aroma, whereas 'honey' aroma may be ascribed to benzeneacetaldehyde (Ntlhokwe et al., 2018).

**Table 1** Aroma-active volatile organic compounds detected in fermented *C. subternata*. Compounds with typical honeybush aroma (i.e. perceived as distinctively honeybush-like by GC-O assessors) are indicated in bold (adapted from Cronje (2010), Le Roux et al. (2012) and Joubert et al. (2019)).

Compound name	Aroma description <sup>a</sup>	DF <sup>b</sup>	ADF <sup>c</sup>
( <i>E</i> )- $\beta$ -damascenone	woody, sweet, fruity, earthy, green-floral, honey-like, dried prune	100	32.768
linalool	refreshing, floral, floral-woody	100	16.384
( <i>E</i> )- $\beta$ -damascone	fruity (apple-citrus), tea-like with slight minty notes	93	4096
geraniol	sweet, floral, floral-woody, rose, citrus-like	93	512
( <i>E</i> )- $\beta$ -ionone	warm, woody, floral, fruity, raspberry-like; resembles cedarwood	87	512
component C178 (C <sub>9</sub> H <sub>14</sub> O <sub>2</sub> )	<i>aroma descriptor not available</i>	60	512
<b>(7<i>E</i>)-megastigma-5,7,9-trien-4-one</b>	tea-like, spicy and resembling dried fruit	60	512
( <i>E,E</i> )-2,4-decadienal	fried, waxy, fatty, orange-like	33	64
3,4-dehydro- $\beta$ -ionone	ionone-damascone and saffron-like, fruity and slightly leathery	87	64
<b>10-<i>epi</i>-<math>\gamma</math>-eudesmol</b>	woody, floral, sweet	40	64
<b><i>epi</i>-<math>\alpha</math>-cadinol</b>	herbaceous, woody	60	64
<b><i>epi</i>-<math>\alpha</math>-muurolol</b>	herbaceous, slightly spicy	60	64
( <i>E,Z</i> )-2,6-nonadienal	green-vegetable, cucumber or violet leaf	100	32
2,3-dehydro- $\gamma$ -ionone	<i>aroma descriptor not available</i>	87	32
3-methylbutanoic acid	acid acrid, cheesy, unpleasant	93	8
nerol	fresh, sweet-rosy	67	8
2,3-dehydro- $\alpha$ -ionone	tobacco-like	33	8
geranyl 2-methylbutanoate	pleasant sweet	47	8
<b>(6<i>E</i>,8<i>E</i>)-megastigma-4,6,8-trien-3-one</b>	tobacco-like, woody, balsamic	40	8
cadalene	<i>aroma descriptor not available</i>	33	8
( <i>Z</i> )- $\beta$ -ocimene	warm-herbaceous, sweet, floral	60	4
( <i>E,E</i> )-3,5-octadien-2-one	fatty, fruity, mushroom	93	4
2-phenylethanol	mild, warm, rose-honey-like	73	4
4-acetyl-1-methylcyclohexene	spicy	67	4
( <i>E</i> )-2-nonenal	green, cucumber, aldehydic and fatty	100	4
<i>p</i> -anisaldehyde	sweet, floral, 'hay-like'	53	4
( <i>R</i> )-octan-5-olide	peach, coconut-like, sweet, creamy	60	4
eugenol	warm-spicy, dry	80	4
bovolide	celery- and lovage-like, fruity and pleasant	80	4
( <i>R</i> )-2-methylbutanoic acid	cheesy, sweaty, sharp	73	2
$\alpha$ -terpineol	floral, sweet, lilac-type	93	2
(+)- <i>p</i> -menth-1-en-9-al	powerful spicy, herbaceous odour	93	2
$\beta$ -cyclocitral	green, grassy, hay-like	40	2
component 162	<i>aroma descriptor not available</i>	40	2
geranyl formate	fresh, green-rosy, fruity	33	2
( <i>S</i> )-( <i>Z</i> )-7-decen-5-olide	sweet, floral, fruity	93	2
( <i>R</i> )-decan-5-olide	sweet, creamy, nut-like, fruity	87	2
<b>(6<i>E</i>,8<i>Z</i>)-megastigma-4,6,8-trien-3-one</b>	tobacco-like, woody, balsamic	67	2

<sup>a</sup> Acree and Arn (2004); Arctander (1969); Demole and Enggist (1974); Leffingwell (2002); Mosciano et al. (1991a, 1991b); Ohloff (1994); Serra et al. (2006); Yamazaki et al. (1988); Boldingh and Taylor (1962); Buttery et al. (1979); JECFA (2020); Kreck and Mosandl (2003); Mookherjee and Wilson (1990); Näf and Velluz (2000); Oomah and Liang (2007); Tachihara et al. (2006); Takahashi et al. (1980)

<sup>b</sup> Detection frequency; <sup>c</sup> Aroma dilution factor

## 2.2.6.2 Sensory quality control tools

The lack of a quality grading system for honeybush herbal tea product remains a major limitation in the quality control of honeybush as herbal tea product (Joubert et al., 2019). A sensory quality grading system should be able to identify, define and measure the quality parameters of a product (Feria-Morales, 2002). Research progress has been made in terms of grading elements to communicate sensory quality between researchers, processors, quality control personnel and marketers, but limitations have been indicated as reviewed in the following sections.

### 2.2.6.2.1 Sensory lexicons and wheels

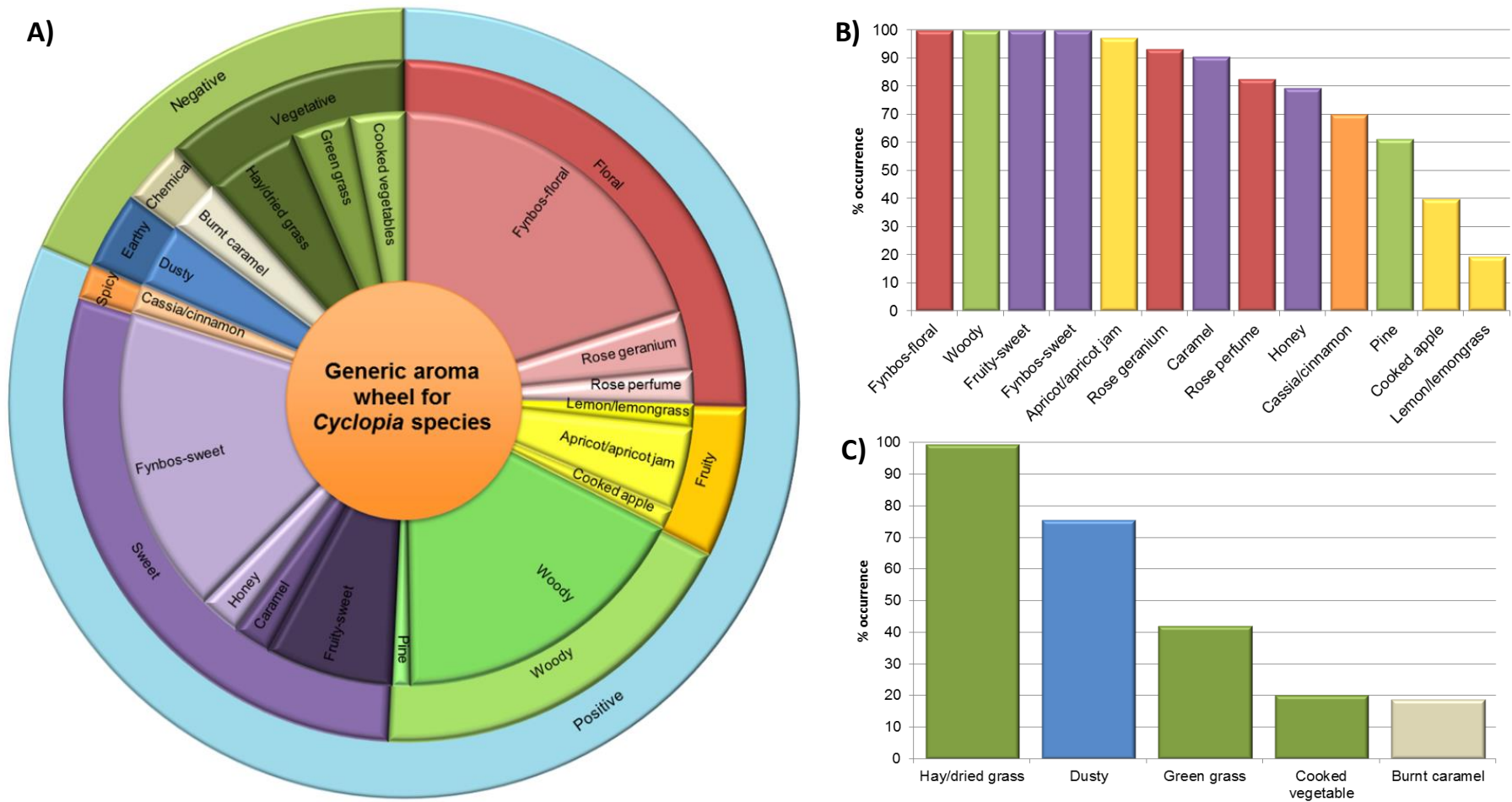
The need to improve and standardise honeybush tea processing and sensory quality was addressed through the development of the first generic honeybush sensory lexicon and wheel (Theron et al., 2014). The inclusion of honeybush sensory attributes as quality parameters in the ARC breeding strategy to develop high-quality plant material (Bester et al., 2016) also directed research on such quality control tools. Sensory lexicons are standardised descriptive vocabularies that consist of sensory attributes, definitions, as well as qualitative and/or quantitative reference standards (food and/or chemical) which can be used to characterise or identify sensory attributes and in certain cases, attribute intensities, perceived in a product (Lawless & Civille, 2013; Muñoz & Civille, 1998).

The first honeybush lexicon was developed based on descriptive sensory analysis (DSA) of 58 different honeybush tea infusions of *C. sessiliflora*, *C. longifolia*, *C. genistoides*, *C. intermedia*, *C. subternata* and *C. maculata* (Theron, 2012). The comprehensive list of original descriptors (68 aroma and 51 flavour, taste and mouthfeel attributes) compiled for honeybush was reduced to 28 aroma, 23 flavour and 3 taste attributes, and one mouthfeel attribute, after considering relevance and redundancies. A generic honeybush tea sensory wheel was created as a simple graphical representation of the sensory lexicon. These terms were assembled to form a three-tiered wheel consisting of an inner tier, representing specific attributes such as 'fruity-sweet', and 'caramel', a middle tier that comprises ten primary attributes ('floral', 'fruity', 'spicy', 'nutty', 'sweet', taste and mouthfeel, 'earthy', 'chemical' and 'vegetative'), and the outer tier that groups the attributes as positive or negative. Negative attributes were included to aid honeybush processors in the identification of batches of poor quality (Theron et al., 2014).

Subsequently, Erasmus (2014) validated the afore-mentioned generic lexicon and wheel with a larger sample set ( $n = 150$ ) of *C. genistoides*, *C. maculata* and *C. subternata* that differed in season, climate, producer and processing conditions (80 °C/24 h and 90 °C/16 h), as well as *C. longifolia* that differed in climate, producer and processing conditions (80 °C/24 h and 90 °C/16 h). A generic aroma wheel (**Fig. 6**) and flavour, taste and mouthfeel wheel were developed in which the average intensity of each specific attribute is indicated, i.e. each slice width corresponds with the specific attribute intensity. Both wheels are accompanied by bar graphs indicating the percentage occurrence of the respective attributes to identify

the most and least prominent attributes in honeybush tea (Erasmus, 2014). In addition, species-specific aroma wheels were also developed for *C. subternata*, *C. genistoides*, *C. maculata* (Robertson et al., 2018) and *C. intermedia* (Bergh et al., 2017), to provide communication tools to describe subtle differences in the aroma profiles of herbal teas of the different *Cyclopia* species.

Development of afore-mentioned quality control tools were based on the assessment of limited number of sample sets and on samples that were pre-dominantly processed on laboratory-scale. In addition, to date, data of *C. intermedia* samples, a major commercial honeybush species, has not been included in the development of a generic honeybush sensory wheel. A comprehensive data set is required that is representative of the entire honeybush product category (Lawless & Civille, 2013), i.e. based on the analyses of a large sample set, and in this case, also the inclusion of commercially processed samples. Additionally, to date the honeybush sensory lexicon consists of mainly food references (Erasmus, 2014) (**Table 2**). The development of more universal reference standards such as chemicals would improve the industry's use of the honeybush lexicon and wheel as quality control tools. The study of honeybush VOCs data (Section 2.2.6.1.2) could aid in the selection of potential chemical-based reference standards.



**Figure 6** **A)** Generic honeybush sensory wheel illustrating the mean intensities of the aroma attributes. Graphs **B)** and **C)** illustrate the average percentage that each attribute appeared in the honeybush infusions ( $n = 150$ ) of *C. genistoides*, *C. maculata*, *C. subternata* and *C. longifolia* analysed (Erasmus, 2014).

**Table 2** Honeybush lexicon used to date in sensory research (food or plant material reference standards are highlighted in red) (Erasmus, 2014).

Category	Attributes	Definition	Reference standard
Floral	Fynbos-floral	Sweet, floral aroma note associated with the flowers of fynbos <sup>a</sup> vegetation.	Honeybush tea prepared from <i>C. intermedia</i> (3 g/100 mL)
	Rose geranium	Floral aroma note associated with the rose geranium plant.	Fresh rose geranium leaf (10 mm x 10 mm) or rose geranium oil (0.005%)
	Rose perfume	Floral aroma note associated with rose petals.	Crushed petals of one rose
Fruity	Lemon/lemongrass	Aromatics associated with general impression of fresh lemons or lemongrass.	Lemon juice (5%)
	Apricot/apricot jam	Sweet-sour aroma reminiscent of apricot jam.	Superfine apricot jam (15 g/100 mL hot water)
	Cooked apple	The flat, slightly sour aroma of cooked apples.	Apple puree (2.5 g/100 mL)
Plant-like	Plant-like	Slightly sour aromatic characteristic of freshly cut fynbos plant material.	Honeybush prepared from <i>C. sessiliflora</i> (3 g/100 mL)
	Woody	Aromatics associated with dry bushes, stems and twigs of the fynbos vegetation.	Honeybush tea prepared from <i>C. maculata</i> (3 g/100 mL)
	Pine	Aroma reminiscent of pine needles.	Fresh pine needles
Sweet-associated	Fruity-sweet	Sweet-sour aromatic reminiscent of non-specific fruit, especially berries and	Superfine apricot jam and strawberry jam (5 g each/100 mL hot water)
	Caramel	Sweet aromatics characteristic of molten sugar or caramel pudding.	Caramel, natural flavour (0.4%)
	Honey	Aromatics associated with the sweet fragrance of fynbos honey.	Wild flower honey
	Fynbos-sweet	Aroma note reminiscent of the fynbos plant.	Honeybush tea prepared from <i>C. intermedia</i> (3 g/100 mL)
Spicy	Cassia/cinnamon	The sweet, woody, spicy aromatic of ground cinnamon/cassia bark.	Soak cinnamon/cassia bark in water overnight
Nutty	Walnuts	Aroma note associated with fresh walnuts (not rancid).	Freshly chopped walnuts
	Coconut	Aromatics associated with desiccated coconut.	Desiccated coconut
Negative	Dusty	Earthy aroma associated with wet hessian or wet cardboard or dry dirt road.	Old, dry tree bark ( <i>Jacaranda mimosifolia</i> ) (1 piece/100 mL hot water, infuse for 5 min filter)
	Medicinal	Aromatic characteristic of Band-aid®, disinfectant-like (phenolic).	Place a Band-aid® adhesive bandage in a petri dish and cover
	Rotting plant water	Slightly sour aromatic characteristic of rotting plant water.	Grass ( <i>Pennisetum clandestinum</i> ) (30 shredded blades/100 mL hot water.
	Hay/dried grass	Slightly sweet aroma associated with dried grass or hay.	Hay or dried grass
	Green grass	Aroma associated with freshly cut green grass.	<i>cis</i> -3-hexen-1-ol (0.005%) or green grass ( <i>Pennisetum clandestinum</i> )
	Cooked vegetable	An overall aroma note associated with canned/cooked vegetables.	Brine from canned green beans (5%)
	Burnt caramel	Aroma associated with blackened/acrid carbohydrates.	Caramel, natural flavour (0.4%)

<sup>a</sup> Fynbos is natural shrubland vegetation occurring in the Western Cape, South Africa.

### 2.2.6.2.2 Rapid sensory profiling methodologies

A honeybush quality control programme within commercial and research environments requires time- and cost-effective method to identify the most important broad-based sensory differences between products and to ascertain overall product quality (Joubert et al., 2019). DSA is a very effective method to determine the detailed sensory profile of a product, but it would be too laborious, costly and unfeasible for regular quality control within a commercial environment (Joubert et al., 2019). Analyses of many production samples in a short time period is required at honeybush tea processing, blending and/or packing facilities. Erasmus (2014) assessed the viability of sorting, a rapid sensory categorisation method, as a simple and user-friendly alternative to DSA for processors to quickly identify the profile of tea samples to ensure consistent blending. Instructed sorting was identified as a possible quality control tool, especially when samples need to be classified according to a selected list of sensory attributes (Erasmus, 2014); however, further validation to determine method stability and repeatability was required.

Moelich (2018) demonstrated the validity of sorting, projective mapping (PM) and polarised sensory positioning (PSP) for broad sensory profiling of fermented *C. genistoides*, *C. subternata*, *C. maculata*, *C. intermedia* and *C. longifolia* infusions. These methods were validated by comparing results of the samples assessed with that of DSA, which is regarded as 'gold standard' of sensory methods. Sorting and PM are categorisation (similarity-based) methods for the assessment of global similarities and differences among samples, whereas PSP is a reference-based method for comparison of samples to product references (Valentin, Chollet, Lelièvre, & Abdi, 2012). The efficacy of partial (aroma or palate attributes) or global (all attributes) assessment was compared within each rapid method. Trained assessors were instructed to focus on one modality or both, respectively. Assessors' differentiation between samples with only subtle differences was improved through concentration on only one modality and the provision of a list of relevant sensory attributes.

Moelich (2018) recommended sorting as a screening tool for the assessment of numerous tea batches, as it demonstrated to be the most effective and user-friendly method for the broad sensory profiling of honeybush infusions. The compilation of a list of key sensory attributes, as well as assessor training on these attributes using sensory wheels (Erasmus, 2014), were also recommended. Furthermore, Moelich (2018) recommended PSP for application in routine quality control programmes, specifically for its advantage of aggregating data of different sessions for monitoring quality over time. In PSP, samples are compared with a fixed set product references, so-called 'poles', which allow assessors to quantify the overall degree of difference between a sample and each of the chosen poles using scales, ranging from 'exactly the same' to 'totally different' (Teillet, Schlich, Urbano, Cordelle, & Guichard, 2010). Moelich (2018) selected poles (tea infusion samples) representative of five different *Cyclopia* species to distinguish between different species. For application of PSP in quality control, Moelich (2018) recommended the use of poles that represent specific quality grades (classes), i.e. tea infusions representative of the key sensory

attributes of different quality grades. To date, no rapid sensory profiling methods have been evaluated specifically for application in screening and grading (classification) of honeybush production batches for quality control within commercial or research context (e.g. honeybush breeding/cultivation programmes).

### 3 Sensory quality control

#### 3.1 Defining sensory quality

A broad range of definitions for 'sensory quality' exists in the food and beverage industry: from simply the 'absence of defects', the minimum requirement for an acceptable standard in certain traditional sensory quality assessment methods (Bodyfelt, Drake, & Rankin, 2008), to 'the composite of those characteristics that differentiate among individual units of a product and have significance in determining the degree of acceptability of that unit by a user' (Kramer, 1959). In literature, a distinction is made between authors that regard sensory quality only as product-oriented, as determined by product experts, and those that regard sensory quality also as consumer-orientated, as determined by consumers in terms of consumer acceptability (Costell, 2002). Overall, product quality can be classified in terms of intrinsic and extrinsic factors. Intrinsic product factors are related to the product itself, i.e. sensory properties such as aroma, palate attributes and appearance; whereas extrinsic product factors refer to properties which are not physically part of the product, e.g. packaging design and format, region of origin, price, etc. (Sáenz-Navajas et al., 2016).

Research on sensory quality estimation through analytical means is prevalent in literature, for example for black tea (*C. sinensis*), from chemical and infusion colour difference analyses (Liang et al., 2003) to rapid and 'green' analytical techniques for in-line quality monitoring, such as near infrared (NIR) spectroscopy, electronic tongue (E-tongue), electronic nose (E-nose) and computer vision-based algorithms for colour and texture analysis, as reviewed by Chen et al. (2011). Djokam and co-workers (2017) recently demonstrated the application of hyperspectral imaging coupled with chemometric modelling as a rapid quality control method for the authentication of herbal tea blends. However, it has been argued that instrumental and chemical measures lack the capability to integrate sensory perceptions and the accuracy of human senses (Aparicio, Morales, & Alonso, 1996). According to Bleibaum et al. (2002), sensory analysis can be regarded as the only method that provides integrated, direct measurements of perceived intensities of target sensory attributes of a product, and therefore the ultimate method for sensory quality assessment. Although instrumental measurements of quality parameters cannot replace sensory analysis, there are instances in which certain quality parameter measurements may complement sensory information (Kilcast, 2010a). For example, accredited sensory testing laboratories perform VOC analyses to confirm detection of olive oil defects for the accurate classification of extra virgin olive oil (Aparicio-Ruiz, Morales, & Aparicio, 2019).



Effective quality assurance and control systems are required to produce products of consistent and acceptable quality (Muñoz, 2002). Minimum requirements for implementing a sensory quality control (QC) programme are 1) the selection and use of a sound sensory method, 2) the definition of critical sensory attributes and tolerance limits or specifications, 3) assessor training and 4) sound product preparation and presentation protocols, i.e. consistent sensory practices to ensure sound product assessment and reliable results (Muñoz, 2002).

## 3.2 Defining sensory quality specifications

### *3.2.1 Development approach*

The common approach when developing food quality control systems is to define product specifications or quality standards (i.e. the characteristics of the ideal or average product), and to develop and test methods to assess, in a reliable manner, whether the product complies with the requirements of the quality standard(s) (Costell, 2002; Lawless & Heymann, 2010). Standards and specifications are designed to determine the acceptable or tolerable variation in a product with reference to a previously selected product or an established written standard (Costell, 2002). The intricate process to establish sound specifications encompasses the following: 1) selection of samples representative of the variability within the market, 2) assessment of the perceived magnitude of the attribute and/or defects through direct comparison to a product standard or through descriptive sensory analysis (DSA), 3) defining the attributes and their variability ranges, and 4) establishment of sensory specifications/limits based on managements' criteria and/or consumer responses on product acceptability using a large consumer panel (Costell, 2002; Muñoz, 2002). For small- to medium-sized companies, it is important that management's input should be ascertained when defining a product's quality control sensory limits, particularly if it is not viable to establish consumer-based specifications (Muñoz, 2002). This process should include the all-important elements in quality control, i.e. representative sampling, incorporation of product variability and consideration of the consumer or particularly management input.

The question of whether quality standards should be defined by industry experts or consumers is a controversial topic. The input of trained sensory professionals and industry experts with thorough product knowledge to establish specifications in the development of sensory quality standards and scoring methods, especially products with quality distinctiveness labels (PGI or PDO), has been reported in recent research (Etaio et al., 2010a, 2012, 2013; Ojeda et al., 2015; Pérez-Elortondo et al., 2007). Sensory quality standards defined by product experts are considered as more comprehensive and accurate than by naive assessors or consumers (Ballester, Dacremont, Le Fur, & Etiévant, 2005; Ojeda et al., 2015). The involvement of different disciplines (including product development, production, and sales and marketing) in the development of specifications is recommended as different experts are able to identify potential product variability (Beeren, 2010).



### 3.2.2 Identification of sensory quality parameters and key attributes

The objective of a quality control programme determines the selection of sensory quality parameters (Costell, 2002). For example, a standard was developed by the International Olive Oil Council (IOC) to classify virgin olive oil (*extra virgin*, *virgin* and *lampante*) according to the intensity of perceptible sensory defects. The standard incorporates the measurement of chemical parameters (free acidity and peroxide values) and absorbance in the UV region as indicators of oxidation, and median intensity scores for sensory attributes, namely defects and key positive characteristics (mainly 'fruity' aroma) (EU Reg no 1348/2013) (Langstaff, 2014). For sensory quality control of products with quality distinctiveness labels (e.g. PDO or PGI), the standard should not only include positive key attributes defining its sensory profile, and defects that affect acceptability, but also those attributes that can establish differences with other similar products from other designations of origin. However, often the latter is measured by differences in intensities of the same positive key attributes (Costell, 2002).

Considering the minimum requirement for sensory quality 'absence of defects', taints as a sensory quality parameter can be defined as 'a taste or aroma foreign to the product' or 'an unpleasant aroma or flavour caused by contamination from sources external to the product' (Kilcast, 2010b). An 'off-flavour or off-aroma' may be defined as 'an unpleasant aroma or flavour resulting from deteriorative change' (Kilcast, 2010b). The latter is often referred to as a 'sensory defect' that is ascribed to poor processing and storage (Langstaff, 2014).

The sensory quality parameters of black tea assessed by professional tasters according to a Chinese grading system (GB/T 23776-2018) (Qu et al., 2019) are the appearance of dry and infused tea leaves and liquor (visual assessment), as well as liquor aroma and flavour (**Table 3**). The International Organisation for Standardisation (ISO) standards for black tea vocabulary provide a list of terms and definitions, applicable to the techniques of processing and assessment of black tea (ISO, 1982). Negative attributes (defects) include 'burnt' liquor flavour ascribed to abnormally high temperatures during firing and 'grassy' and 'green' liquor flavour ascribed to inadequate withering or fermentation. Negative attributes for liquor appearance include undesirable 'dull' and 'muddy' appearance, as opposite to 'bright' which is associated with careful processing. The South African herbal tea, rooibos, is graded (AA, A, B, C, D, E or F) by a major processing company based on the assessment of infusion flavour and the visual assessment of the dry and wet leaf, as well as infusion appearance (Bergh, 2014).

An important appearance parameter of many beverages is how clear or cloudy the product is. Turbidity (cloudiness or haze) occurs when small suspended particles divert light from a straight path through the material and scatter it in different directions (Lawless & Heymann, 2010). Clarity, i.e. the absence of haze, is regarded as a desirable quality parameter in hibiscus tea (Monteiro et al., 2017). Both infusion clarity and colour (intensity and red hue) were selected as appearance modalities for visual assessment in the development of a hibiscus tea lexicon for quality control (Monteiro et al., 2017). Hibiscus

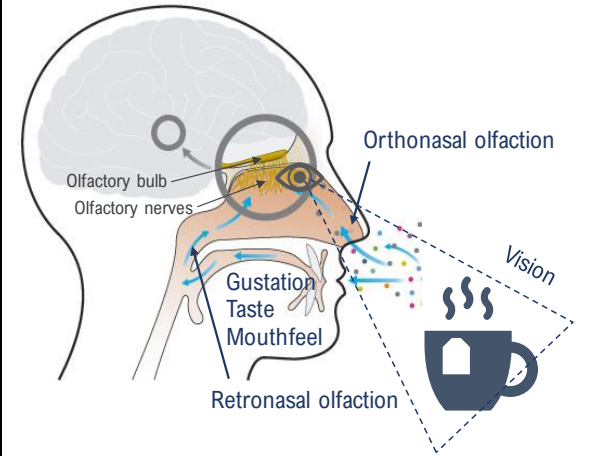
tea with 'diminished clarity' (i.e. cloudy/milky appearance), is regarded as unacceptable and would not reach the market. Haze formation in hibiscus tea is associated with microbial contamination and/or deficient filtration of extracts (Monteiro et al., 2017). As mentioned previously, haze (so-called 'tea cream') is also an undesirable quality in black tea (Jöbstl et al., 2005; Liang et al., 2002; Liang & Xu, 2003). Similarly, turbidity has been regarded as undesirable in white and red wine wines and has been associated with low quality (Etaio et al., 2010a, 2012; Sáenz-Navajas et al., 2016). Wine batches are rejected merely on the basis of presence of haze through visual assessment, irrespective of the level of turbidity (Etaio et al., 2010a). Visual attributes cited by expert wine professionals scoring Spanish red wines, such as clarity (limpidity), depth (colour intensity), and red-purple colour are related to high quality, whereas oxidised-brown colour, turbidity and light in colour are related to low quality (Sáenz-Navajas et al., 2016). Haze is also an important quality parameter in beer (Steiner, Becker, & Gastl, 2010) and is descriptively categorised as 'brilliant' (0-2 NTU), 'almost brilliant' (2-4 NTU), 'very slightly hazy' (4-8 NTU), 'slightly hazy' (8-16 NTU), 'hazy' (16-32 NTU), and 'very hazy' (>32 NTU) (Briggs, Boulton, Brooks, & Stevens, 2004).

### 3.3 Defining assessors of sensory quality

Traditionally, a single expert taster or grader was responsible for assessing the sensory quality of a product. Today, such 'expert assessors' are limited to a few specialised branches of the food and beverage industry, including tea, coffee and wine production. The reliability and consistency of their individual assessment and that in comparison to other experts, specifically their subjectivity and operation under varying individual mental standards (criteria), has been criticised widely in literature (De Vos, 2010; Feria-Morales, 2002; Lawless & Heymann, 2010).

However, quality assessment by 'real experts' in accordance with their mental standards, specifically to distinguish between good and exceptional quality, is still regarded as valid for products such as coffee and wine (Costell, 2002). Wine industry professionals, i.e. oenologists, wine producers and brand managers, are referred to as wine experts that perform routine wine quality assessments which are especially important for high-quality wines (Brand et al., 2018). Dairy experts are highly trained to recognise product defects and assign overall quality scores (Bodyfelt, Tobias, & Trout, 1988). The traditional 'cupping' method ('cup tasting') to assess tea and coffee quality is still performed by tea and coffee evaluation experts, respectively (Feria-Morales, 2002). Apprenticeships within tea and coffee industries exist to educate these assessors in the sensory quality attributes found in the product category and with extensive training, it is possible that the quality assessments of successive tea tasters would be relatively similar (Langstaff, 2010). In the wine industry, experts used in wine judging would not have had a similar 'apprenticeship' in assessing wine quality leading to judgments that could be very dissimilar from each other.

**Table 3** Sensory perception of black tea (*Camellia sinensis*).

Participating sense(s)	Attributes relevant for sensory quality assessment	Compounds linked to attributes <sup>1</sup>
	Appearance: dry leaf colour Appearance: wet leaf colour Appearance: infusion colour Appearance: infusion clarity	Oxidation of catechins leads to formation of theaflavin (yellow-orange/yellowish-brown colour) and thearubigins (black-brown/reddish-brown colour) Haze formation ('tea cream') – complex of caffeine with theaflavins and thearubigins (Roberts, 1963)
<b>Orthonasal perception (Smell)</b>	Infusion aroma	Volatile organic compounds
<b>Retronasal perception</b>	Infusion flavour	Volatile organic compounds
<b>Taste<sup>2</sup></b>	Sweet, sour, bitter taste	Non-volatile compounds e.g. organic acids, sugars, free amino acids, caffeine, etc.
<b>Chemesthesis<sup>3</sup></b>	Astringent mouthfeel <sup>4</sup>	Theaflavins; thearubigins

<sup>1</sup> Alasalvar et al. (2012)

<sup>2</sup> Taste reflects the gustatory perceptions in mouth (Regueiro, Negreira, & Simal-Gándara, 2017)

<sup>3</sup> Trigeminal stimulation based on the chemical stimulation of free nerve endings of trigeminal and other nerves in mouth, nose and eyes (Green, 2002)

<sup>4</sup> Complex sensation combining three distinct aspects: drying of the mouth, roughing of oral tissues, and puckery or drawing sensations felt in the cheeks and muscles of the face (Green, 1993)

\*Image adapted from Foodpairing® (2020)

Alternative sensory techniques have been established that aim to minimise bias and improve reliability by using a group of assessors, i.e. a sensory panel as true 'measuring instrument' (De Vos, 2010). Therefore, the recruitment, training and monitoring of panel members should be carried out with great care, as indicated by ISO 8586:2012 (ISO, 2012). Sensory assessment can be performed by assessors with different levels of knowledge and experience of the product to be evaluated (ISO, 2012). The ISO standard defines 'sensory assessors' as any individuals taking part in a sensory test, from 'naive assessors' who do not have to meet any precise criterion, to 'initiated assessors' who have already participated in sensory tests. 'Selected assessors' are chosen for their ability to perform a sensory test, whereas 'expert sensory assessors' are selected assessors with a demonstrated sensory sensitivity and with considerable training and experience in sensory testing. 'Expert sensory assessors' are able to make consistent and repeatable sensory assessments of various products, have proved particular acuity and reproducibility in panel work, and have developed a good long-term sensory memory, allowing reliable comparative judgements, possibly in the absence of control samples. In literature, 'expert sensory assessors' as defined by ISO, are generally referred to as 'trained professional sensory assessors' or 'trained assessors' as part of a trained sensory panel. For example, panels of trained assessors are employed for the descriptive analysis of specific sensory attributes for the classification of virgin olive oil (Langstaff, 2014).

In a previous standard version (ISO 8586-2:2012) product experts/product-specialised experts were excluded in the definitions for sensory assessors as 'their expertise does not lie within sensory competence'. However, the standard states that a product expert/product-specialised expert who is trained as an expert sensory assessor may be regarded as an 'expert sensory assessor'. These assessors may draw on additional knowledge, such as that of the product, process or market, to interpret sensory data and make conclusions. In addition, Ares and Varela (2017) emphasised the importance of using trained assessors, and not naive assessors or consumers, in sensory quality control.

### 3.4 Methods in sensory quality control

The selection of an appropriate method for sensory quality control is based on the capacity of the method to measure variations in those characteristics that influence product quality with adequate precision (Costell, 2002). However, it is not always the most precise and costly methods that are most suitable. There are two extreme alternatives, namely 1) detailed, complete specification of a product that is difficult to apply in practice, or 2) the selection of only those characteristics that has high impact on quality which allows for simpler and more user-friendly method to assess whether the product fulfils the requirements of a quality grade (Costell, 2002).

As sensory science has developed as a more recent discipline during the second half of the 20<sup>th</sup> century, compared to chemical and microbial analyses, methods developed over time differ with variable scientific base and cannot be all considered adequate to evaluate and control sensory quality (Costell,

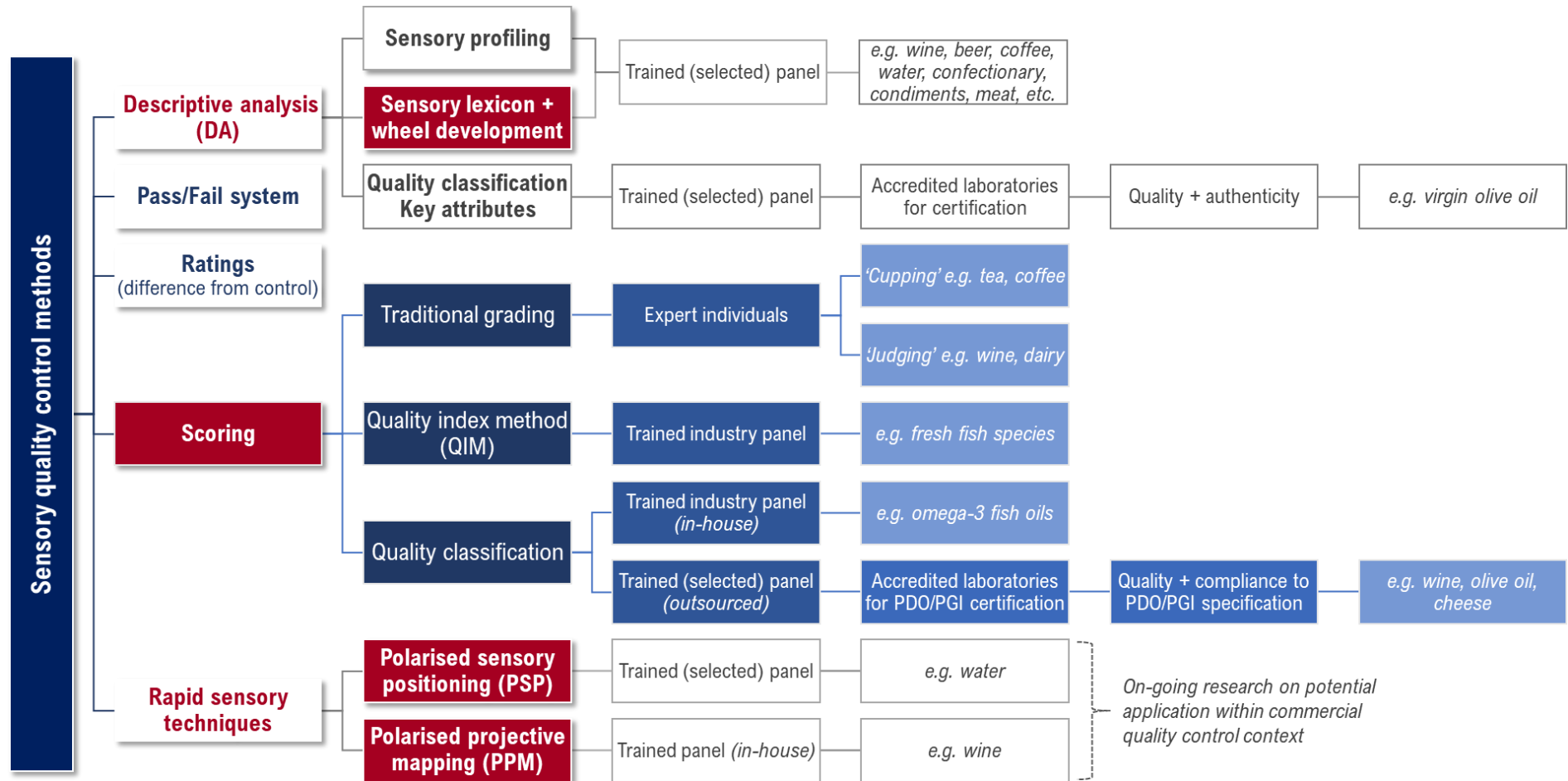
2002). Four main methods are applied in sensory quality control, namely 1) descriptive analysis, 2) in/out (pass/fail) system, 3) rating systems (degree of difference or difference from control test) and 4) quality scoring (also referred to as quality rating/grading methods) (Costell, 2002; Muñoz, 2002; Rogers, 2010).

An overview of sensory methods applied to quality control in food and beverage industries are presented in **Fig. 7**. Quality control methods and elements assisting in quality control addressed in the current study are highlighted in red. Research on rapid sensory profiling methods have grown exponentially over the past two decades and although certain methods have been recommended for quality control application, limited examples exist in literature (rapid sensory profiling methods are reviewed in Section 5). A summary of the characteristics of the four main sensory methods (and hybrids thereof), including advantages and limitations, are presented in **Table 4**. In addition, scales and extracts from ballots or scorecards used in descriptive sensory analysis, quality rating (difference from control) and quality scoring are depicted in **Fig. 8**.

### *3.4.1 Classic sensory methods*

Conventional descriptive analysis refers to the sensory method by which identification, quantification, and description of sensory attributes of food by a panel of trained assessors are obtained (Piggott, Simpson, & Williams, 1998). It is regarded as one of the most sophisticated tools in sensory science which allows for the development of scientific language of products, or product categories, to describe and objectively quantify the perceived sensory similarities and differences between samples (Lawless & Heymann, 2010).

Descriptive sensory analysis (DSA) (also referred to as descriptive analysis (DA)) is commonly carried out with a panel of trained assessors (8 – 20) in three phases, namely descriptor generation, assessor training and evaluation (Lawless & Heymann, 2010). Assessors are trained by a professional sensory leader who guides the panel to generate a list of attributes for sensory profiling of a product and may further be anchored by references or reference definitions for each attribute (Drake & Civille, 2003). Meticulous, precise, consistent and reproducible results are obtained, and data (overall panel mean score for each attribute) are analysed employing analysis of variance (ANOVA) to determine significant differences between treatment means. Multivariate techniques can also be used to establish an association between sensory attributes and samples, as well as whether the attributes can act as drivers of preference or quality (Lawless & Heymann, 2010).



**Figure 7** Overview of sensory quality control in food and beverage industries: 1) classic sensory profiling (DA), 2) common practice (highlighted in blue) and 3) rapid sensory techniques (methods and other elements highlighted in red will be addressed in the current study).

In addition, DSA also enables the development of sensory lexicons and wheels, which are valuable tools commonly applied in research and quality control for product categorisation and quality assessment within a sensory quality control programme. A lexicon is a list of standardised terms, definitions, and references (either food or chemical) for sensory attributes that can be present in a product to aid assessors in systematically identifying and quantifying the differences among products within a category (Lawless & Civille, 2013). Well-defined sensory lexicons may be used by trained panels to provide accurate and reproducible results for calibration and validation in sensory research (Lawless & Civille, 2013). Lexicons may also act as communication tools within panels and across diverse industry role-players as indicated in the recently published sensory lexicons for hibiscus tea (Monteiro et al., 2017) and yerba mate tea (Godoy, Chambers, & Yang, 2020). The role of sensory lexicons, specifically chemical reference standards, in quality control will be reviewed in more detail in Section 4.

In a commercial environment, DSA can be regarded as costly and extensive with regard to the required resources of employing and maintaining a well-trained, calibrated sensory panel (Varela & Ares, 2012). Popular quality control methods are founded on a form of simplified descriptive analysis that is condensed (reduced) to the assessment of only key attributes (Everitt, 2010). An example is the sensory profile sheet that is used by a panel of trained assessors in the so-called 'panel test' for classification of virgin olive oil (IOC, 2018; Langstaff, 2014). This standard sensory assessment method aids in the global homogenisation of virgin olive oil quality and the authentication of their quality categories, as well as detection of the presence of other crude oils at very low levels (Aparicio-Ruiz et al., 2019).

### *3.4.2 Quality control in practice*

#### 3.4.2.1 General

In/out and quality rating (difference from standard or control product) systems are popular methods within a commercial production environment, although these methods pose several disadvantages or challenges, as indicated in **Table 4**. Even though these methods are simple to perform, limited or no descriptive information can be obtained from these methods to provide reasons for rejection or difference from product control ('gold standard') to aid in problem-solving (Lawless & Heymann, 2010).

Quality grading methods (also referred to as quality scoring or rating) are the most commonly used (Rogers, 2010). The information collected during the development of a product standard and establishment of the specifications are encompassed in a scorecard that includes a scoring system with points assigned to each grade and a description of the sensory attributes that define the quality of each grade (Costell, 2002). High levels of assessor training and experience are usually required as the method relies in part on the assessors' sensory memory of the ideal product (often seen in scales of 'typicality' as indicated in **Fig. 8**). Assessors are required to have thorough knowledge of the product in question, its manufacturing process and possible variations that may occur (Rogers, 2010).

**Table 4** Main sensory quality control methods and their key characteristics (recommended number of highly trained assessors required are indicated in brackets) (Costell, 2002; Lawless & Heymann, 2010; Rogers, 2010).

Sensory QC method	Principle	No of assessors; Training/ experience level; Training requirements	Advantages	Disadvantages / Limitations	Application
<b>DESCRIPTIVE ANALYSIS</b>	<ul style="list-style-type: none"> <li>– Intensity ratings for individual sensory attributes (including critical attributes);</li> <li>– Emphasis on perceived intensity of single attributes (not quality or overall difference);</li> <li>– Specifications set via consumer testing and/or management input (range of allowable intensity scores for key attributes; cut-off known to management &amp; not assessors).</li> </ul> <p><b>Data generated:</b> <i>Intensity rating of single sensory characteristics.</i></p>	<p><b>(10); High</b></p> <ul style="list-style-type: none"> <li>– Extensive training;</li> <li>– Analytical frame of mind and focused attention on individual sensory characteristics;</li> <li>– Reference standards to understand key attributes' definition and intensity standards to anchor quantitative ratings on intensity scale.</li> </ul>	<ul style="list-style-type: none"> <li>– Data detailed and quantitative (can be correlated with instrumental analysis);</li> <li>– Lower cognitive burden on assessors once analytical frame of mind adopted;</li> <li>– Report only intensity perception of key attributes (no integration of sensory experiences into overall score required);</li> <li>– Specific characteristics are rated: easier to conclude defect and corrective actions associated with ingredients/process factors than with overall quality score).</li> </ul>	<ul style="list-style-type: none"> <li>– Extensive training required;</li> <li>– Data handling and statistical analysis required;</li> <li>– Higher number of assessors required;</li> <li>– Not suitable for in-line QC on production plant level;</li> <li>– Extensive sample preparation time to select references for range of intensities per sensory attribute.</li> </ul>	<ul style="list-style-type: none"> <li>– Suitable for quality assessment of final products, e.g. virgin olive oil (IOC, 2018; Langstaff, 2014).</li> </ul>
<b>PASS/FAIL (IN-OUT) SYSTEM</b>	<ul style="list-style-type: none"> <li>– Differentiates products considered different/ outside specifications from norm.</li> </ul> <p><b>Data generated:</b> <i>Simple 'Yes/No' or 'In/Out' answer.</i></p>	<p><b>25 (10); Moderate</b></p> <ul style="list-style-type: none"> <li>– Training in recognition of characteristics that define 'in-or out-of-spec' products;</li> <li>– Blind control samples important.</li> </ul>	<ul style="list-style-type: none"> <li>– Method simplicity;</li> <li>– Decision-making tool.</li> </ul>	<ul style="list-style-type: none"> <li>– Criterion-setting challenges;</li> <li>– No provision of diagnostic reasons for rejection and lack of direction for problem-solving;</li> <li>– Difficulty to relate data to instrumental analyses of food quality;</li> <li>– Difficulty to assess detail (defects) and integrated overall quality.</li> </ul>	<ul style="list-style-type: none"> <li>– QC during in-line production (production plant level);</li> <li>– Raw materials/basic products with few key attributes.</li> </ul>
<b>DIFFERENCE FROM CONTROL RATINGS</b>	<ul style="list-style-type: none"> <li>– Ratings used for overall degree of difference from a standard/control product;</li> <li>– Small number of key attributes;</li> <li>– Cut-off known to management (not to assessors).</li> </ul> <p><b>Data generated:</b> <i>Single scale 'extremely different from standard' to 'the same as standard' (or other verbal descriptions).</i></p>	<p><b>30 (18); Low to Moderate</b></p> <ul style="list-style-type: none"> <li>– Training with range of reference samples that represent points along the scale;</li> <li>– Blind control samples important.</li> </ul>	<ul style="list-style-type: none"> <li>– Scale provides for a range of differences that are acceptable.</li> </ul>	<ul style="list-style-type: none"> <li>– Difficult to establish nature and conditions to reproduce control;</li> <li>– No provision of diagnostic reasons for difference if only single scale is used (<i>could provide open-ended reasons for difference or questions/scales/checklists for attributes that are common problems or show common variation</i>);</li> <li>– Constant 'gold standard' product for comparison to be maintained.</li> </ul>	<ul style="list-style-type: none"> <li>– Basic products with one or few key attributes.</li> <li>– To compare products from different production sites.</li> </ul>



Table 4 (continued)

Sensory QC method	Principle	No. of assessors; Training/ experience level; Training requirements	Advantages	Disadvantages / Limitations	Application
<b>QUALITY SCORING (RATINGS WITH DIAGNOSTICS)</b>	<p>– Assess differences and how they are weighted in determining product quality.</p> <p><b>Data generated:</b></p> <p>– <b>Scale directly represents quality judgement</b> (not sensory difference) e.g. ‘poor’ to ‘excellent’;</p> <p>– Specific characteristics can be rated in addition to overall quality (quality of texture, flavour, appearance, etc.)</p> <p>– Integrated quality score;</p> <p>– Quality score for individual attributes are added to give overall score for certain schemes (e.g. wine);</p> <p>– Point deduction scheme (e.g. dairy).</p>	<p><b>8-12 (5); Moderate to High</b></p> <p>– Extensive training required</p> <p>– Critical main abilities of expert/trained assessor:</p> <p>1) to maintain mental standard of ideal product (sensory characteristics);</p> <p>2) to anticipate + identify common defects ascribed to poor production/GMPs;</p> <p>3) to know weight influence of each defect at different levels / how it detracts from overall quality.</p>	<p>– Time and cost advantages;</p> <p>– ‘Quality grading works best when there is management or industry consensus on what is good’.</p> <p>(Lawless &amp; Heymann, 2010)</p>	<p>– Complex judgment procedure for assessors - intensive training required for ability to recognise all defects and integrate into quality score; Liability of individual subjectivity (likes/dislikes) influence on assessment;</p> <p>– Specialised terms for technical defects difficult to understand by non-technical managers;</p> <p>– Small panels: statistical difference tests rarely applied - method qualitative; Quality scoring approach prone to abuse - small panels, poorly trained judges.</p>	Traditional food commodities, e.g. wine judging – UC Davis 20-point scorecard for wine (Langstaff, 2010); dairy judging (Kraggerud, Solem, & Abrahamsen, 2012).
<b>QUALITY RATINGS WITH DIAGNOSTICS (hybrid approach)</b> (Beckley & Kroll, 1996)	<p>– Compromise between quality rating method and descriptive analysis approach.</p> <p><b>Data generated:</b></p> <p>– <b>Overall quality rating scale;</b></p> <p>– <b>Diagnostic scales</b> for individual key attributes known to vary in production to include descriptive information.</p>	<p><b>Not specified</b></p> <p>– Training in concept boundaries (specific product references) required;</p> <p>– Tolerance ranges and gold standard to be established before training.</p>	<p>– Simplicity in using overall rating;</p> <p>– Addition of attribute scales to supply reasons for product rejection;</p> <p>– Situations recognised for products to be still acceptable when no match with ‘gold standard’.</p>	<p>– Method may give an assessor the perception of being responsible for decision for rejection of product: e.g. quality scale: 1-2 = Reject; 3-5 = Unacceptable to ship, to be blended/re-worked; 6-8 = Acceptable; 9-10 = Near identical/match to standard.</p>	Commercial food products (Beckley & Kroll, 1996).
<b>DIFFERENCE SCORING WITH KEY ATTRIBUTE SCALES (recommended method)</b> (Lawless & Heymann, 2010)	<p>– Similar to hybrid approach: overall difference scale used instead of quality rating scale, with descriptive analysis.</p> <p><b>Data generated:</b></p> <p>– <b>Degree-of-difference scale and diagnostic scales</b> for rating of individual key attributes known to vary in production;</p> <p>– <b>Just-right scales</b> for attributes that can be too strong or too weak;</p> <p>– <b>Intensity scales</b> for key attribute and defects problematic at higher levels;</p> <p>– <b>Checkboxes</b> for intolerable defects.</p>	<p><b>Ideally ≥ 8 highly trained assessors</b> for statistical analyses (mean ratings)</p> <p>– Extensive assessor screening, selection and training recommended;</p> <p>– assessors for statistical analyses (mean ratings);</p> <p>– Action criteria should take negative minority opinions in consideration.</p>	-	-	Commercial food products, e.g. apple juice (Lawless & Heymann, 2010).



Various forms of quality grading (scoring) methods have become popular for online production sensory control as they provide a quick, reliable (provided that assessors have been trained fully against reference products) and practical approach to measure deviations from the target quality (Everitt, 2010). Unstructured line scales or semantic category scales may be used (Beeren, 2010). Score ranges that are linked to descriptions such as 'reject', 'unacceptable', 'acceptable' and 'match' (Beckley & Kroll, 1996) can place an unnecessary burden on assessors for feeling directly responsible for actions to be taken and assessors are often inclined to use higher scores, associated with 'acceptable' score ranges (Lawless & Heymann, 2010). Lawless and Heymann (2010) recommended a method that applies a combination of scales within one scorecard, namely an overall degree-of-difference scale from 'extremely different' to 'match', diagnostic attribute ratings and intensity scales for key attributes and defects that are problematic at high intensities, and checkboxes for those defects that are intolerable.

The use of a combination of an overall quality scale and other scales to assess individual attribute quality or intensities within the same scorecard, may be difficult to perform as it requires different mental attitudes, and may lead to erroneous results (Costell, 2002). Therefore, screening of assessors for sensory acuity and a good assessor training programme is required (Lawless & Heymann, 2010). Trained or expert assessors of a quality grading system should have the following skills: 1) the ability to maintain a mental standard of the ideal product in terms of its sensory attributes, 2) the ability to anticipate and identify common defects that may occur due to lack of good manufacturing processes (GMPs), and 3) to know the influence or weight of each defect at different intensity levels and how they detract from overall quality (commonly for point deduction schemes such as traditional dairy grading) (Lawless & Heymann, 2010). Another critical challenge is that assessors are required to perform an analytical task simultaneous to a qualification task ('accept' vs 'reject') which could result in deviations in results (Costell, 2002).

#### 3.4.2.2 Quality grading of commodities

In literature, a distinction is made between general quality grading or scoring within commercial product context and traditional grading of commodities such as tea, coffee and wine. Traditional grading has been widely criticised for the use of individual or small numbers of expert tasters without the necessary training experience who, as mentioned previously, could make subjective judgements on a product (Costell, 2002; Feria-Morales, 2002; Langstaff, 2010; Lawless & Heymann, 2010; Muñoz, 2002). A persistent debate exists over the relevance of quality scoring methods, specifically for wine, ascribed to the difficulty of establishing a concrete definition of quality and the lack of standardised methods for evaluating assessor training and expertise (Langstaff, 2010).

The UC Davis 20-point scorecard (**Fig. 8**) was developed for the young California wine industry in the 1960's in an attempt to standardise and objectify general wine quality assessment (Langstaff, 2010), and is still used today by wine industry professionals world-wide, including in South-Africa (Brand et al., 2018).

The overall sensory impression of wine is divided into different quality attributes (weighted based on the perception of their relative importance) for which scores are assigned with the sum to total the arbitrary number of twenty (Langstaff, 2010). The wine scorecard is an additive scheme for giving overall quality, whereas traditional dairy judging is based on a point-deduction scheme. Appearance, texture, and flavour defects are listed together with their point deduction values for ‘slight’, ‘definite’, and ‘pronounced’ levels of sensory intensity as indicated in **Fig. 8** (Lawless & Heymann, 2010).

**Table 5** Black tea sensory specifications and parameter weights (score coefficients) in overall quality for a Chinese grading system (reproduced from Qu et al. (2019)).

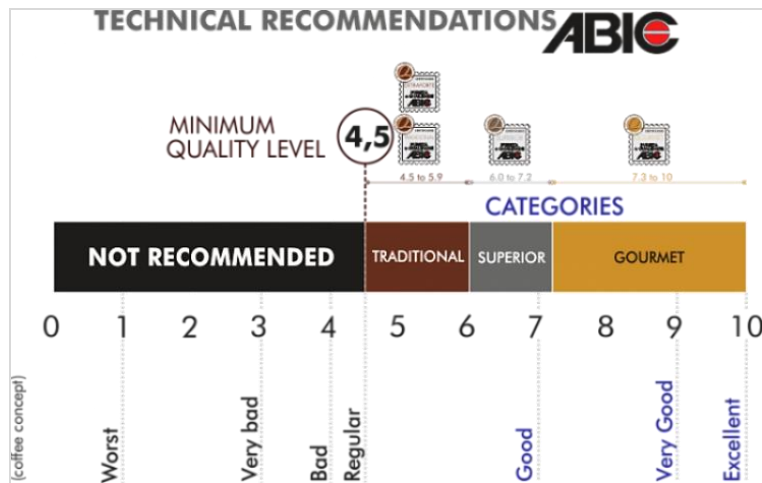
Factors	Description	Scores	Score coefficient
<b>Appearance</b>	Black and bright colour, tight and tender streak	90-99	<b>20%</b>
	Black and little bright colour, little tight and tender streak	80-89	
	Black and dull colour	70-79	
<b>Liquor colour</b>	Bright and red colour, loose streak	90-99	<b>10%</b>
	A little bright and red colour	80-89	
	Red and dull colour	70-79	
<b>Aroma</b>	Floral, fruity and lasting aroma	90-99	<b>30%</b>
	Sweet and lasting aroma	80-89	
	Pure with no peculiar smell	70-79	
<b>Taste</b>	Soft, mellow, and refreshing taste	90-99	<b>30%</b>
	Mellow taste	80-89	
	Brisk taste	70-79	
<b>Infused leaf</b>	Bright, red and tender leaves with lots of buds	90-99	<b>10%</b>
	A little bright, red and tender leaves with little buds	80-89	
	Dull, red leaves with tea stems	70-79	

Traditional tea grading systems are based on the sensory assessment of key sensory attributes by expert tasters. The standard for a Chinese tea grading system (GB/T 23776-2018: *Methodology for sensory evaluation of tea*) used by expert tasters within research and practice is presented in **Table 5**. The sensory attributes are divided into three grades, namely 90-99, 80-89 and 70-79, to rank different levels of black tea (Qu et al., 2019). In another Chinese grading system, a similar weight distribution is assigned to taste (35%), aroma (30%) and infusion colour (15%), followed by the appearance of dry (10%) and infused (10%) tea leaves for the black tea quality (Liang et al., 2003). Statutory classification of black tea quality is however based on dry leaf particle size, determined by sorting after processing, of which the main grades are ‘leaf’, ‘brokens’, ‘fannings’ and ‘dust’ (Alasalvar et al., 2012; ISO, 1982). Current grading weights assigned to rooibos tea by a major South African herbal tea processing/blending company are 40% for infusion taste, 30% for infusion appearance and 30% for dry leaf appearance (C. Cronjé, Food Safety and Quality Systems Consultancy, 2018, personal communication). Blending of tea plant material of different origins, grades and quality to obtain a product of consistent quality, is standard practice within the tea industry (Joliffe, 2003; Liang et al., 2003).

In a recent study to develop sensory quality control tools for Hunan fuzhuan brick tea, an indigenously microbial fermented tea, Quantitative Descriptive Analysis (QDA) by a trained panel was compared to the standard Chinese ‘cupping’ method by professional tasters to compare methods, as well as to assess the validity of a developed sensory lexicon (Li, Luo, Wang, Fu, & Zeng, 2019). Assessment results showed similar descriptions and ratings of the test samples, indicating that both QDA and the ‘cupping’ method can be effectively employed for quality assessment of this tea (Li et al., 2019). Authors concluded that these results would form a basis for developing a Hunan fuzhuan brick tea sensory assessment method for quality control.

As for black tea, no global standardised method for sensory assessment for coffee exists as different coffee producing countries apply their own sensory quality measures in developed classification systems (Feria-Morales, 2002). Coffees are often not tasted at source and ‘cup quality’ is implied based the assessment of the appearance and defect counts of coffee bean samples during quality grading. Only a few exceptions measure ‘cup profile’ according ISO specifications for the sensory evaluation of green coffee (ISO 6668:2008) (ISO, 2008) (Giacalone et al., 2019). ‘Clean cup’ is the quality attribute associated with the absence of defects, including common roast defects, e.g. ‘dark’, ‘light’, ‘scorched’, ‘baked’ and ‘underdeveloped’ (Giacalone et al., 2019).

The pressing need for a harmonised global grading system for coffee with the emphasis on sensory quality has been highlighted by Feria-Morales (2002). The movement towards ‘gourmet’ and ‘speciality’ coffees have recognised the importance of the full sensory profile of coffees as indicated by Brazilian Coffee Quality Program (launched by the Brazilian Coffee Industry Association (ABIC)) in which the sensory quality of the brewed coffee obtained through roasted and ground coffee, is certified (De Alcantara & Freitas-Sá, 2018). The sensory analysis method (validated by the ABIC) is performed by trained assessors of accredited laboratories who evaluate *inter alia* aroma, acidity, body, astringency and bitterness. To be certified, the coffee must score  $\geq 80$ , and no parameter can be equal to zero (BSCA, 2020). The method classifies and differentiates coffees into quality categories that are determined according to a so-called ‘Global quality score’ on a scale of 0 to 10 (**Fig. 9**). The minimum score of a recommended beverage corresponds to 4.5 points and, according to the score obtained, coffee is classified as ‘Extra Strong’, ‘Traditional’, ‘Superior’ or ‘Gourmet’ (ABIC, 2020).



**Figure 9** Brazilian Coffee Industry Association 'global quality score' scale for coffee quality certification (ABIC, 2020).

Although the afore-mentioned traditional grading methods are not regarded as suited for processed engineered food products (i.e. other than standardised commodities), they may provide a valuable starting point for development of a quality control system for closely related products (Lawless & Heymann, 2010). Quality scoring methods allow for the rapid qualification of a product and the detection of possible causes for rejection (Costell, 2002). Quality scoring methods may be economical, and aid in quality decisions, provided that the correct controls are in place and methods are backed by industry standards and linked to consumer acceptability, according to Rogers (2010). With small panels ( $\leq 5$  assessors) within industry context, the method is primarily qualitative as statistical difference tests are seldom applied to such data (Lawless & Heymann, 2010).

Notwithstanding different criticisms, two types of sensory quality scoring methods have been recognised in literature for their use as objective tools in sensory quality classification and development approach with a scientific foundation, namely the Quality Index Method (QIM) for assessment of freshness and shelf-life of fish (Hyldig & Green-Petersen, 2005; Sveinsdottir, Hyldig, Martinsdottir, Jørgensen, & Kristbergsson, 2003), and specific quality scoring methods of products with quality distinctiveness labels, including traditional wines and cheeses (Lawless, 2017). The development approach for quality scoring methods with a scientific foundation for PDO products is reviewed in Section 3.5.

QIM is a standardised quality grading system used by the European fresh fish sector and inspection services to estimate the sensory quality and freshness of different fish species. It was developed in close cooperation between the seafood industry and European fisheries research institutes to provide a quality tool to perform sensory quality assessment in a practical, systematic and objective manner. It was developed to improve communication of sensory quality between different industry role-players in the European fishery chain (i.e. research, fishermen, processors, fishmongers and marketing) to facilitate trade and traceability of quality information (Hyldig & Green-Petersen, 2005; Sveinsdottir et al., 2003). QIM is a scaling method used by trained assessors based on the assessment of key sensory parameters in fish

deterioration for the whole fish using numerous weighted quality parameters and a score system (0 [fresh] – 3 demerit points) for which resulting scores are cumulated to establish a Quality Index (QI) (**Fig. 8**). For example, the QIM for farmed Atlantic salmon was developed by a panel of trained sensory assessors and QIM experts and includes the colour and/or aroma of skin, eyes, gills and abdomen as quality parameters. QDA of the salmon after cooking was employed as a reference to enable prediction of the remaining storage time of raw salmon in ice using the QIM (the calculated QI evolved linearly with storage time in ice) (Sveinsdottir et al., 2003).

#### *3.4.3 Visual aids in sensory quality assessment*

Although instrumental measurement of colour (e.g. CIEL\*a\*b\* colour system) and turbidity (NTU) measurements is performed in quality control, most visual and appearance attributes can be evaluated using standard descriptive analysis techniques, provided assessment is performed under controlled conditions (Lawless & Heymann, 2010). The human as instrument has the ability to assess visual differences when samples are placed next to each other or next to a standard (Lawless & Heymann, 2010). In certain sensory quality control studies, appearance reference cards with digital images (Hyldig & Green-Petersen, 2005; Ojeda et al., 2015; Zannoni & Hunter, 2013) or printed transparent film (Etaio et al., 2010a) were developed as visual aid in quality scoring to anchor individual scores to objective values. For example, the PDO Idiazabal cheese quality scoring method is accompanied by a catalogue of photographs representing the optimum characteristics and possible defects for the quality parameters 'shape, rind, paste colour and hole' (Pérez-Elortondo et al., 2007). The advantage of appearance reference standards for universal assessment in different laboratories have been indicated, provided that the display and viewing conditions for products and reference cards are identical (Lawless & Heymann, 2010). The use of visual standards for turbidity is not as common in literature, although a 100 NTU turbidity meter standard has been included as reference for 'clarity' for a sensory lexicon for hibiscus tea that was developed for quality control purposes (Monteiro et al., 2017).

#### *3.4.4 Good sensory practice*

Although cost, resource and time constraints may limit the number of assessors available in quality control divisions within commercial context, assessor screening and training is still regarded as important for small panels (De Vos, 2010). The application of good sensory practices, even within a small panel set-up has been emphasised (Lawless, Liu, & Goldwyn, 1997). Apart from assessor training and the professional attitude and motivation of assessors, other standard sensory practical factors such as a good testing environment, consistent sample handling, temperature and volume control, sample randomisation, blind coding and inclusion of blind duplicates (including the control) are important (Lawless & Heymann, 2010; Lawless et al., 1997). Implementation of good sensory practices, even in small operations, is critical in maintaining the integrity of the sensory test and ensuring the objectivity of the results (Lesschaeve & Noble, 2010). For



example, Endrizzi et al. (2013) demonstrated the viability of implementing basic sensory analysis principles in quality control of typical products such as Trentingrana cheese (PDO Grana Padano variety). This included the introduction of a balanced experimental design, monitoring of expert assessors' performance and control of sample presentation to avoid systematic effects and psychological errors in order to collect reliable data.

In terms of data analyses in sensory quality control, data should consist of interval scale measurements where possible. Statistical analysis is not performed with very small panels as it is difficult to apply mean ratings to panels with less than eight assessors. Such data should be treated qualitatively, i.e. frequency counts of individual scores should be reported and considered in standards of actions to be taken. Although outliers can be omitted, it is important to consider that a few low scores, i.e. a minority opinion, may be indicative of an important problem. In instances where there is strong disagreement between assessors or high panel variability, re-tasting may be required (Lawless & Heymann, 2010).

### 3.5 Development approaches for quality scoring methods

Limited scientific publications on the development of sensory quality classification methods that incorporate quality scorecards are available in the literature. Apart from QIM (Hyldig & Green-Petersen, 2005) and a quality scoring method for calf's meat (Etaio et al., 2013), only a few publications are available on the systematic approach to quality scorecard development, namely for traditional wine and cheese products with quality distinctiveness labels.

#### *3.5.1 Products with quality distinctiveness labels*

The sensory properties of PDO products are quoted in EU official documents and should be controlled to ensure conformity of a product with its official sensory characteristics (Pérez-Elortondo et al., 2018). Pérez-Elortondo and co-workers (2018) addressed the pressing need for the harmonisation of sensory quality control methods used for the certification of PDO food products and wines within EU. The current status of a wide range of sensory methods that exist within the EU could lead to unfair competition among these products. The lack of standardisation of valid sensory methods and databases for sensory descriptors (including defects), technical standards for assessor training and performance monitoring for control of PDO by accredited sensory laboratories was highlighted. Sensory analysis should form an integral part of quality control since perceived sensory characteristics represent the most important key factors for typicality of PDO products, and are therefore essential for preserving the market position and profitability, and for maintaining consumer confidence and loyalty toward the product (Delahunty & Drake, 2004). Two main methods for products performed by small panels (5-12 assessors) exist, namely 1) the identification of positive and negative attributes (defects) with use of citation frequencies, resulting in qualitative data, and

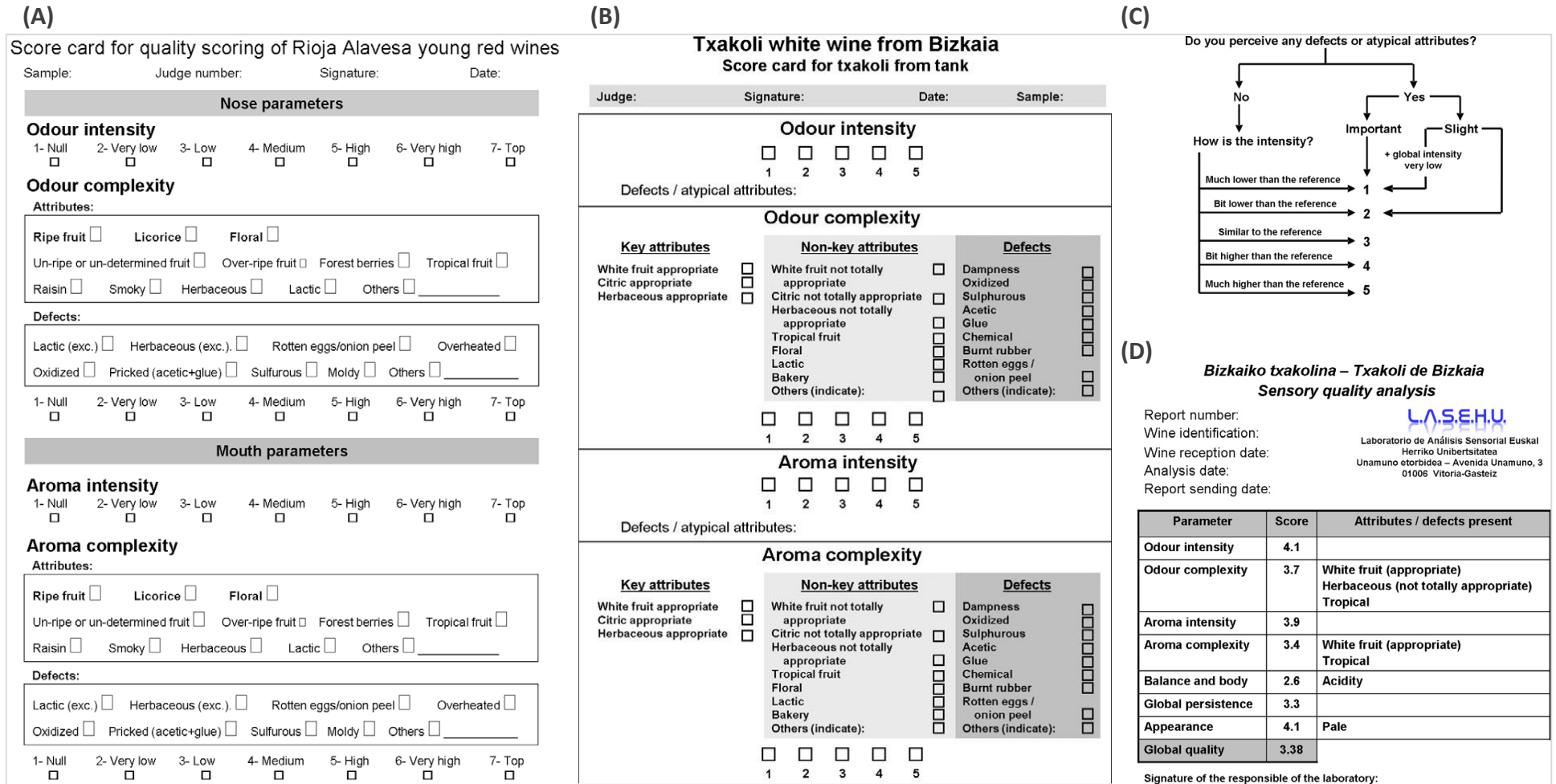


2) the quantification of attribute intensities on continuous/discontinuous scales, resulting in quantitative data (Pérez-Elortondo et al., 2018).

To address the afore-mentioned need for accredited methods to score the sensory quality of PDO products by certification bodies, sensory quality scoring methods using scorecards have been developed for Idiazabal cheese (Ojeda et al., 2015; Pérez-Elortondo et al., 2007), Rioja Alavesa young red wine (Etaio et al., 2010a) and Bizkaia txakoli white wine (Etaio et al., 2012). Successful accreditation of these methods not only assures the technical competence by an external institution but also increases the guarantee and quality image of the product (Etaio et al., 2010a). Lawless (2017) commended these method development approaches of PDO products for their disciplined and logical manner, and for following good sensory practice principles in the development process, including sensory lexicon development, use of reference standards and assessor training and screening. According to Lawless (2017), quality scoring systems have merit provided that they are founded on a sound scientific basis.

The scorecard development steps include 1) the selection of a panel of expert assessors familiar with the product and its sensory attributes, 2) sourcing of products ( $n > 50$ ) that represent the product's sensory space, i.e. a range of sensory characteristic and common defects, 3) assessment of samples to produce a list of sensory attributes, 4) identification of defects and the selection of parameters that define the characteristic sensory quality of the product, 5) definition of 'ideal or top situation' per parameter and establishing of scoring criteria based on the presence of desired quality parameters for each major sensory category (appearance, aroma, flavour, etc.), 6) allocation of parameter weights (%) based on whether categories are critical or non-critical for overall sensory quality, 7) enumeration of categories and scoring criteria, 7) standardisation of the sensory quality assessment protocol, and 8) the development of reference standards per quality attribute to train assessors (Etaio et al., 2010a, 2012; Lawless, 2017; Ojeda et al., 2015; Pérez-Elortondo et al., 2007). The repeatability, reproducibility and discrimination ability in scores and attribute identification of assessors are considered as the validation criteria of these sensory quality control methods (Etaio et al., 2010b).

For certain sensory parameters, decision tree diagrams instead of line scales are applied which allow the assessor to allocate scores for the presence of key attributes in each sensory category, and lower scores are assigned if any defects are present. **Figure 10** presents extracts of the developed scorecards for Rioja Alavesa young red wine and Bizkaia txakoli white wine, respectively, as well as an example of a decision tree diagram used for scoring aroma intensity. An extract of the report compiled by the accredited Sensory Laboratory of the University of the Basque Country (LASEHU) (Etaio et al., 2012) is also presented. The report is used for the qualification of a product and includes the information about the analysis as per ISO/IEC 17025:2017 (ISO, 2017) requirements (sample code, sample reception date, analysis date or report delivery date), score mean of each parameter and the sensory characteristics of each parameter shown as the percentage of assessors indicating them (citation frequencies).



**Figure 10 A)** An extract of the scorecard for PDO Rioja Alavesa young red wines; Official sensory quality control documents of PDO Bizkaia txakoli white wine used by a panel of trained assessors: **B)** an extract of the scorecard, **C)** decision tree diagram for scoring aroma attribute intensities and **D)** an extract of the report provided by the accredited Sensory Laboratory of the University of the Basque Country (LASEHU) (Etaio et al., 2010a, 2012).

### 3.5.2 An industry case study

A good example of the development of a scientifically founded classification system was recently published (Larssen et al., 2019). The cooperation between industry and research institutes with the aim to establish a common industry sensory standard and 'flavour guarantee' for omega-3 marine (fish) oil products was demonstrated (Larssen et al., 2019). Although strict regulations for chemical quality exist, the need for a defined method and vocabulary to assess the sensory quality of omega-3 oil was identified for improved quality control within production facilities, and to differentiate and highlight the unique quality of Norwegian-produced omega-3 oils in an increasingly competitive market. A systematic approach with a sound scientific foundation was followed, and industry input through surveys on method requirements and sensory vocabulary establishment contributed to the development of a classification system that provides the industry with a simple, convenient and valuable quality control tool to communicate quality throughout the value chain (Larssen et al., 2019). As an initial step to harmonise sensory quality control within different omega-3 production companies, the so-called 'quality control test' incorporates a sensory lexicon and wheel for positive and negative sensory attributes that was further developed from a preliminary study (Larssen, Monteleone, & Hersleth, 2018) and the quality classification method (Larssen et al., 2019).

The development steps included the 1) collection of omega-3 fish oil production batch samples (n = 46) representative of different products from eight Norwegian omega-3 fish oil producing companies, 2) Descriptive Analysis (DA) (Lawless & Heymann, 2010) using a highly trained panel (N = 10) to further develop and establish a detailed sensory nomenclature lexicon and aroma wheel for application in quality control and to describe sensory deviations, 3) chemical analysis (fatty acid composition, primary and secondary oxidation parameters and GC-MS) to study correlations between sensory attributes and the quality of raw materials, chemical oxidation parameters and fatty acid profiles, 4) identification of market requirements and consumer acceptance regarding sensory quality of omega-3 marine oils and the benefit of and requirements for a sensory quality standard and method and sensory wheel through industry surveys and interviews with omega-3 oil producing companies and buyers, 5) selection of key sensory parameters and adjustment of DA intensity scales to semantic intensity category scales ('Very low' [0] – 'Very Strong' [4]) and 6) establishment of a sensory quality standard and quality classes, namely *Gold*, *Silver* and *Regular*.

For quality classification of omega-3-oils, sensory assessment would be performed using an adjusted quality control tests (NMKL:201 2017) for which the points (5 to 1) corresponds to the descriptions for the quality classifications (**Table 6**). A product specification that refers to an odour-and flavour-less oil with no specific sensory characteristics, instead of a physical product reference (omega-3 fish oil) was recommended. From industry interviews and DA data, four sensory aroma and flavour attributes were accepted in oils regardless of intensity or classification, namely 'sourness', 'grassy', 'butter' and 'nut', whereas three attributes were accepted at only very low [ $\leq 0$ ] to moderate [ $\leq 2$ ] intensities, namely

'fermented', 'rancid', 'process' or 'other (i.e. chemical, metal, pungent and fruit)'. 'Fish' aroma and flavour were allowed in all classifications, although at low [ $\leq 1$ ] to moderate [ $\leq 2$ ] intensities (**Table 7**). Another important outcome of this study was the recommendation of the development of 'synthetic' (chemical) aroma references as sensory quality control tool for industry assessor training in sensory quality classification (Larssen et al., 2019).

**Table 6** Specifications for the sensory quality standard and deviations thereof used for the quality control tests NMKL 201 (NMKL:201 2017) (reproduced from Larssen et al. (2019)).

Point	Deviation to product specification	Accepted deviation	Classification
5	No deviation.	Odour- and flavourless	<b>GOLD</b> <i>(Extra high sensory quality)</i>
4	Minimal deviation from product specification.	Sourness, grass, nut and butter (present) Fish (low intensity)	
3	Weak deviation from product specification.	Sourness, grass, nut and butter (present) Fish, rancid, fermented and process (low intensity) Other (e.g. chemical, metal, fruit) (low intensity)	<b>SILVER</b> <i>(High sensory quality)</i>
2	Moderate deviation from product specification.	Sourness, grass, nut and butter (present) Fish, rancid, fermented and process (moderate intensity) Other (e.g. chemical, metal, fruit) (moderate intensity)	<b>REGULAR</b> <i>sensory quality</i>
1	Distinct deviation from product specification.	Deviation not included in the standard	<i>Not commodity</i>

**Table 7** Proposal of classification of fish oils including chemical specifications and accepted aroma and flavour attributes (reproduced from Larssen et al. (2019)).

Sensory characteristics and chemical parameters	<b>GOLD</b> <i>Extra high sensory quality</i>	<b>SILVER</b> <i>High sensory quality</i>	<b>REGULAR</b> <i>Sensory quality</i>
Peroxide <sup>1</sup> value	$\leq 5$	$\leq 5$	$\leq 5$
Anisidine <sup>1</sup> value	$\leq 20$	$\leq 20$	$\leq 20$
Sourness, grassy, butter, nut	Allowed	Allowed	Allowed
Fish (fresh)	$\leq 1$	$\leq 1$	$\leq 2$
Fermented, rancid, process	0	$\leq 1$	$\leq 2$
Other (i.e. chemical, metal, pungent, fruit)	0	$\leq 1$	$\leq 2$

<sup>1</sup> Fat oxidation products

## 4 Role of sensory lexicons in quality control

### 4.1 Sensory lexicons as quality control tools

Sensory lexicons are developed by highly trained assessors using descriptive analysis (Lawless & Heymann, 2010). The American Society for Testing and Materials (ASTM) (ASTM Stock #DS72 2011) summarises the systematic development approach of any lexicon into the following phases: 1) to establish a frame of reference from a wide array of products within a category, i.e. the background information and reference points (frame of comparison) that assessors mentally refer to when assessing products (Muñoz & Civille, 1998), 2) to develop and generate sensory terms that describe products, 3) the use of references to clarify the sensory terms and definitions, 4) the use of examples so the panel fully understands the sensory terms, and 5) to develop the final list of sensory descriptors for the lexicon (Lawless & Civille, 2013).

Well-defined sensory lexicons and wheels may be used by trained panels to describe the sensory attributes associated with a product consistently and correctly for calibration and validation in sensory research (Drake & Civille, 2003; Lawless & Civille, 2013). These tools may serve as a powerful qualitative frame of reference when conducting DSA, as well as for assessing the broad-based quality of a product (Drake & Civille, 2003). Standardised, descriptive vocabularies enable that the sensory attributes of a specific product or commodity are related accurately and objectively to technical (research and development, processing and quality control) and non-technical (marketing and sales) business audiences, and ultimately the consumer (Drake & Civille, 2003; Lawless & Civille, 2013).

The development of sensory lexicons and wheels using DSA is well-documented for a wide range of products (**Table 8**). Its continued publication is encouraged to allow for increased standardisation of sensory science protocols that will benefit the discipline through increased reproducibility (Lawless & Civille, 2013). The importance and relevance of sensory lexicons for application in quality control, as well as for plant breeding programmes to develop or select superior quality cultivars, have been highlighted recently in a review by Suwonsichon (2019). Monteiro and co-workers (2017) developed a hibiscus tea sensory lexicon with the aim to provide practical support for product optimisation processes, including hibiscus cultivar selection and breeding, as well as tea processing methods.

Sensory lexicons and/or wheels have been published for black tea (Bhuyan & Borah, 2001), green tea (Lee & Chambers, 2007), Hunan fuzhuan brick tea (Li et al., 2019) and herbal teas such as rooibos (Koch, Muller, Joubert, Van der Rijst, & Næs, 2012), honeybush (Bergh et al., 2017; Erasmus, 2014; Robertson et al., 2018; Theron et al., 2014), hibiscus (Monteiro et al., 2017) and yerba mate (Godoy et al., 2020). A 'living' lexicon for brewed coffee has been developed as an important tool to improve the understanding of coffee quality throughout all industry sectors, for which additional terms may be included as required for expansion of the lexicon (Chambers et al., 2016; World Coffee Research, 2017). Furthermore, defect wheels

have been developed for the wine, beer and olive oil industries to facilitate assessor training in recognition in defects related to poor processing or poor GMPs (Langstaff, 2016).

Sensory lexicon development and reference standard selection were regarded as an essential first step in the development of quality scoring methods for PDO Rioja Alavesa young red wines (Etaio et al., 2010a) and omega-3 fish oil (Larssen et al., 2019, 2018). Various lexicons have been developed to define the specific quality characteristics of indigenous or traditional products that have PDO or PGI status (Monteiro et al., 2014; Pereira, Dionísio, Matos, & Patarata, 2015; Rétiveau, Chambers, & Esteve, 2005; Stolzenbach, Byrne, & Bredie, 2011; Vázquez-Araújo et al., 2012) (**Table 8**). The application of these standardised vocabularies may facilitate both the definition and protection of the product reputation and market share of high quality traditional products from inferior ones, as well as encourage the successful promotion of these products based on their unique sensory qualities, i.e. to differentiate them from atypical and/or inferior quality products (Rétiveau et al., 2005).

## 4.2 Reference standards

### 4.2.1 *General*

Qualitative references allow assessors to associate with the concept of the sensory attribute, whereas quantitative reference standards assist in defining the intensity limits (anchor points) in which the panel of assessors rate the intensity of a specific product attribute (Muñoz & Civille, 1998; Rainey, 1986). Where some lexicon definitions lack in accurately describing an unfamiliar concept, reference standards may relay a concept and common description that panellists could grasp readily (Drake & Civille, 2003). References that are inexpensive and common across a wide range of geographic areas are generally preferred (Lawless & Civille, 2013). Ideally one would use a reference standard that is simple, reproducible and represents only one attribute term; however, in some instances a single ingredient or chemical may not fully describe the perceived aroma or flavour in a product (Rainey, 1986).

The provision of reference standards for each perceived attribute aids in the development and clear understanding of lexicon terms among assessors to ultimately achieve concept alignment in panels, as well as shorten panel training time (Drake & Civille, 2003; Murray & Delahunty, 2000; Murray, Delahunty, & Baxter, 2001; Rainey, 1986). Reference standards are defined as any chemical, ingredient or product to be used for the characterisation or identification of an attribute or attribute intensity perceived in a product (Rainey, 1986). It may include other non-food related substances that illustrate sensory stimuli, e.g. grass for 'grassy' or 'green' (Murray et al., 2001). For a clear and distinct demonstration of a specific attribute term, it is essential that the attribute in question is the dominant trait exhibited in the reference (Civille & Lyon, 1996). The provision of examples or products has been recommended to increase assessors' understanding of significant attributes, e.g. for 'vanillin' attribute, specific commercial brands of vanilla ice cream in which vanillin was very prominent, were used as references (Lawless & Civille, 2013). Murray and

Delahunty (2000) noted that assessors identified the smoky cheese product reference for the attribute 'smoky' as more representative than the chemical reference, guaiacol. However, commercial food products may be re-formulated over time and are, therefore, not as consistent and reliable as chemicals (Rainey, 1986).

The importance of reference standards to clarify the meaning of sensory attribute descriptors has been highlighted in an increasing interest in cross-cultural sensory research (Cherdchu, Chambers, & Suwonsichon, 2013). Discrepancies that may develop from language misinterpretation by different assessors, panels or cultures may be reduced by the appropriate reference standards (Murray & Delahunty, 2000). A standardised lexicon with definitions and references increases accurate communication among different research groups and research locations, improves cross panel validation and ensures effective universal interpretation and reproduction of results, especially as globalisation spreads companies across the world and DSA is becoming more outsourced to different business entities (Drake & Civille, 2003; Lawless & Civille, 2013). A study on the development of a soya sauce lexicon indicated the value of using reference standards to overcome language barriers and to aid assessors in understanding sensory characteristics of products across cultures (Cherdchu et al., 2013).

#### *4.2.2 Development of reference standards*

Often underemphasised is the establishment of concrete reference standards as an integral step in the process of descriptive language development. In the past, many descriptive sensory studies reveal no or little information on the method of reference standard development. In many instances reference standards were either not specified or merely selected by the panel leader and imposed on assessors during their training phase to demonstrate sensory concepts (Noble et al., 1987, 1984; Piggott, Jardine, Piggott, & Jardine, 1979; Rainey, 1986; Stolzenbach et al., 2011). However, the involvement of assessors in the development of reference standards is increasingly evident (Murray et al., 2001). During training assessors are encouraged to discuss their individual descriptions of each reference to allow for a panel to reach consensus over attribute terms (Civille & Lyon, 1996). Numerous studies indicate that once the panel has reached agreement on selected descriptors, assessors were involved in the recommendation and selection process of representative references (Bárcenas, Pérez Elortondo, Salmerón, & Albisu, 1999; Drake & Civille, 2003; Lee & Chambers, 2007; Pe et al., 2002; Rétiveau et al., 2005). Lawless and Civille (2013) recommend a 'validate, then revalidate' process that could determine the suitability of a reference for unique attributes.



**Table 8** Examples of product-specific sensory lexicons in order of publication year.

Product	References standard type	Qualitative references	Quantitative references	Reference preparation indicated	Assessment in product matrix	Assessors involved in development	Publication reference
Yerba mate tea	Food (+ commercial brands)	Yes	Yes	Yes	No	Yes	Godoy et al. (2020)
Hunan fuzhuan brick tea	Food, chemical	Yes	Yes	Yes	No	Yes	Li et al. (2019)
Fish oils	Food; chemical	Yes	No	Yes	<b>Yes</b>	<i>not specified</i>	Larssen et al. (2018)
Green Spanish-style table olives	Food; chemical	Yes	No	Yes	No	<i>not specified</i>	López-López et al. (2018)
Hibiscus tea	Food	Yes	No	Yes	No	Yes	Monteiro et al. (2017)
Seasoning soy sauce	Food (+ commercial brands)	Yes	Yes	Yes	No	Yes	Pujchakarn et al. (2016)
Coffee	Food (+ commercial brands); chemical	Yes	Yes	Yes	No	Yes	Chambers et al. (2016)
Soy milk	Food	Yes	Yes	No	No	Yes	Xia et al. (2015)
Portuguese cooked blood sausage <sup>1</sup>	Food	Yes	Yes	Yes	No	Yes	Pereira et al. (2015)
Pomelo fruit	Food (+ commercial brands)	Yes	Yes	Yes	No	Yes	Rosales and Suwonsichon (2015)
Pink port <sup>2</sup>	Food (+ commercial brands; chemical)	Yes	Yes	Yes	No	Yes	Monteiro et al. (2014)
Soy sauce <sup>3</sup>	Food (+ commercial brands); chemical	Yes	No	<b>No</b>	No	Yes	Cherdchu et al. (2013)
Bottled natural mineral water <sup>3</sup>	Food, chemical	Yes	No	Yes	<b>Yes</b>	<i>not specified</i>	Rey-Salgueiro et al. (2013)
Nutty aroma/flavour	Food (+ commercial brands); chemical	Yes	Yes	Yes	No	Yes	Miller et al. (2013)
Beef	Food; chemical	Yes	Yes	Yes	<b>Yes</b>	Yes	Maughan et al. (2012)
Mango	Food; chemical	Yes	Yes	Yes	No	Yes	Suwonsichon et al. (2012)
Nougat ( <i>Turrón</i> ) <sup>2</sup>	Food (+ commercial brands); chemical	Yes	Yes	Yes	No	Yes	Vázquez-Araújo et al. (2012)
Bizkaia txakoli white wine	Food (+ commercial brands)	Yes	No	Yes	No	Yes	Etaio et al. (2012)
Finish apples	Food; chemical	Yes	No	Yes	No	Yes	Seppä et al. (2012)



Table 8 *continued*

Product	References standard type	Qualitative references	Quantitative references	Reference preparation indicated	Assessment in product matrix	Assessor involved in development	Publication reference
Danish honeys <sup>2</sup>	Food; chemical	Yes	No	Yes	No	<i>not specified</i>	Stolzenbach et al. (2011)
Rioja Alavesa young red wine <sup>2</sup>	Chemicals (predominantly); food	Yes	No	Yes	Yes	Yes	Etaio et al. (2010a)
Pomegranate juice	Food (+ commercial brands); chemical	Yes	Yes	Yes	No	Yes	Koppel and Chambers (2010)
Processed cheese	Food (+ commercial brands); chemical	Yes	No	Yes	No	Yes	Drake et al. (2010)
Rice	Food (+ commercial brands); chemical	Yes	Yes	Yes	No	Yes	Limpawattana and Shewfelt (2010)
Green aroma/flavour	Food (+ commercial brands) vs chemical <sup>4</sup>	Yes	Yes	Yes	No	Yes	Hongsoongnern and Chambers (2008)
Green tea	Food (+ commercial brands); chemical	Yes	Yes	Yes	No	Yes	Lee and Chambers (2007)
Floral honeys <sup>3</sup>	Food (+ commercial brands); chemical	Yes	No	Yes	No	Yes	Galán-Soldevilla et al. (2005)
French cheese <sup>2</sup>	Food (+ commercial brands); chemical	Yes	Yes	Yes	No	Yes	Rétiveau et al. (2005)
Smoke flavour	Chemical (apart from 'burnt bread')	Yes	No	Yes	No	Yes	Pe et al. (2002)
Cheddar cheese	Food (+ commercial brands) vs chemical <sup>4</sup>	Yes	No	Yes	No	Yes	Murray and Delahunty (2000)
Ewes milk cheese (including Idiazabal cheese) <sup>2,3</sup>	Food (+ commercial brands); chemical	Yes	No	Yes	No	Yes	Bárcenas et al. (1999)
Wine	Food (+ commercial brands); chemical	Yes	No	Yes	Yes	<i>not specified</i>	Noble et al. (1987)
Beer	Chemical	Yes	No	Yes	Yes	Yes	Meilgaard et al. (1979; 1982)

<sup>1</sup> Lexicon developed for PDO/PGI application<sup>2</sup> PDO or PGI product<sup>3</sup> Bibliography for references standards included in lexicon<sup>4</sup> Two lexicons provided and compared

To the author's knowledge, only one study on the selection of reference standards through descriptive analysis by a trained panel has been published. Murray and Delahunty (2000) established a method which allowed sensory assessors to select references for a Cheddar cheese lexicon using suitability-scaling. Assessors scored four potential standards (blind-coded) per attribute on 100 mm continuous line scales. These references included food products and chemicals and were previously selected during an extensive screening process on how representative they were of the sensory attribute under evaluation. Therefore, sensory assessors who best understood the meaning of their selected attributes, could equally contribute to reference standard selection (Murray & Delahunty, 2000).

To allow the assessor to understand the attribute as it appears in the product, reference standards are often added to a neutral base (matrix) of the specific product or a model system being analysed (Drake & Civille, 2003) (**Table 8**). For example, to provide a wine aroma background Noble et al. (1987) prepared standards in neutral red or white wines, i.e. wines that were free of defects and had aromas of low intensities. Similarly, Meilgaard et al. (1979) indicated better agreement between assessors when reference standards were presented in beer. Chemicals or commercial products have been prepared and assessed in relevant product matrixes (e.g. cheese or wine) to develop qualitative and quantitative reference standards for assessor training (Etaio et al., 2010a, 2012; Ojeda et al., 2015). Murray and Delahunty (2000) recommended the application of chemical references in a modified cheese matrix to best represent the aroma and flavour attributes of Cheddar. An omega-3 fish oil lexicon was developed that provides the preparation and/or dosing instructions of food (e.g. hazelnut oil for 'nut and seed' aroma) or chemical (e.g. 2,3-butanedione for 'butter' aroma) references in sunflower oil (Larssen et al., 2018).

#### *4.2.3 Chemical-based reference standards*

Chemical-based reference standards in particular allow for enhanced elucidation of terms as not all food-based references are available globally (Drake & Civille, 2003). The use of chemical standards, particularly chemical components analysed in the product involved, may allow for a clearer and more grounded lexicon (Drake & Civille, 2003). A character impact compound for a specific aroma represents the volatile chemical compound (VOC) that is responsible for the aroma contributions that is reminiscent of the principle sensory identity of the food that it was derived from (Molnár, 2009). For example, of the first character impact compounds that have been identified and synthesised, include benzaldehyde ('cherry' aroma), vanillin ('vanilla' aroma), methyl-salicylate ('wintergreen' aroma) and cinnamaldehyde ('cinnamon' aroma) (Fischetti, 2010).

Gas chromatography–olfactometry (GC-O) may relate gas chromatographic data and chemical compounds to sensory impact and its application in food flavour analysis represents to be a valuable technique for the characterisation of aroma-active compounds (Zellner, Dugo, Dugo, & Mondello, 2008). GC-O uses human assessors as sensitive and selective detectors to determine the odour activity of VOCs in

a sample extract, and assign a relative importance to each VOC through either detection frequency, dilution to threshold or direct intensity (Delahunty, Eyres, & Dufour, 2006). However, to establish a link between VOCs and sensory language remains a challenge as the relative amount of a chemical compound determined in a food may not necessarily be a measure of its sensory impact (Drake & Civille, 2003; Regueiro et al., 2017). These compounds do not contribute equally to the overall sensory profile of a product, and the interaction of the food matrix and different thresholds of the compounds can have an impact on the perceived aroma (Drake & Civille, 2003; Friedrich & Acree, 1998; Zellner et al., 2008). Research for improved understanding of the association between aroma/flavour and chemical composition is ongoing, as reviewed by Chambers IV and Koppel (2013) and Regueiro and co-workers (2017).

Since the handling of single concentrated chemicals or essences used for chemical-based reference standards may be problematic and impractical within industry context (Noble et al., 1987), commercial reference standard kits comprising of spray-dried and nano-encapsulated stable chemicals have been developed ([www.aroxa.com](http://www.aroxa.com); [www.flavoractiv.com](http://www.flavoractiv.com)). Off-flavours and taints detected in food products can have a significant impact on the quality and consumers' acceptability of products and remain a major concern to the food and beverage industry (Ridgway, Lalljie, & Smith, 2010). Many commercial aroma kits using chemical-based reference standards are available to train and calibrate assessors in the recognition and scaling of the intensities of aroma notes, most notably off-odours (defects) in beer, cider, wine, water, etc. ([www.aroxa.com](http://www.aroxa.com); [www.flavoractiv.com](http://www.flavoractiv.com)). A coffee lexicon that includes pharmaceutical grade, shelf-stable and food-safe chemical reference standards ([www.flavoractiv.com](http://www.flavoractiv.com)) has been published by World Coffee™ Research (World Coffee Research, 2017). The importance of replacing commercial branded food products (e.g. 'Lorna Doone' brand cookies or 'Green Giant' brand cut green beans) and other food product references that may not be readily available or change over time, with more universal chemical-based references, has been recognised (World Coffee Research, 2017).

## 5 Rapid sensory methods for quality control

### 5.1 General

The description of the intrinsic properties of food products to obtain sensory profiles is a key requirement within the food industry, and plays a significant role during product development, production, quality control, advertising and marketing (Lawless & Heymann, 2010). Conventional descriptive sensory analysis is widely used for sensory characterisation of food products using trained assessors, as reviewed by Lawless and Heymann (2010), and provides robust, detailed and consistent information with high reproducibility, even when minor differences in sensory attributes of products are perceived (Moussaoui & Varela, 2010). However, the method is regarded as time-consuming and costly, impairing its use by smaller companies in terms of financial constraints, as well as larger companies in terms of a wide range of different products to

be analysed (Varela & Ares, 2012). To address the increased demand from food and beverage industries for faster profiling of products, the development of rapid sensory profiling methods and optimised statistical tools have become one of the most active and dynamic areas of sensory science research in recent years (Nestrud & Lawless, 2008, 2010; Tomic, Berget, & Næs, 2015; Varela & Ares, 2014). The wide application for these sensory techniques has been indicated, especially to ascertain the relative sensory positioning of products within research and industry context such as product development/re-formulation, market positioning, and quality control (Ares, Antúnez, De Saldamando, & Giménez, 2018; Horita et al., 2017; Valentin et al., 2012). These methods could be conducted by product experts or naive consumers (Valentin et al., 2012; Varela & Ares, 2012).

Rapid sensory profiling methods can be categorised into three main groups, namely 1) verbal-based methods, e.g. flash-profile (FP) and check-all-that-apply (CATA), for the assessment of individual attributes, 2) holistic or similarity-based methods, e.g. sorting and projective mapping (PM) or Napping® for the assessment of global similarities and differences among samples, and 3) reference-based methods, e.g. polarised sensory positioning (PSP), polarised projective mapping (PPM) and Pivot® profiling for the comparison with product references (Ares et al., 2013; Valentin et al., 2012; Varela & Ares, 2012, 2014).

Similarity-based methods such as projective mapping (PM) have become prominent to obtain a quick overview of the similarities or dissimilarities in a sample set by projecting samples onto a two-dimensional space. The comparison of samples is based on a holistic assessment as the assessor evaluates global differences, according to the assessor's own criteria, without any prior indication on which sensory attributes should be focussed on, or their relative importance. In PM, each assessor is instructed to position samples onto a paper sheet (Valentin et al., 2012). PM is also referred to as Napping® (Pagès, 2005; Perrin & Pagès, 2009; Perrin et al., 2008), which denotes the French for 'tablecloth', namely 'nappe'.

## 5.2 Application in quality control

Although application of rapid profiling methods in quality control has been proposed, to date literature of application of these methods as quality control tools for specifically routine quality assessment of production batches within commercial context is limited. However, the use of rapid sensory profiling techniques in determining key sensory quality drivers of products has been indicated.

Perrin and co-workers (2008) reported on Napping®, followed by Ultra-flash profiling (UFP) (a descriptive step in which terms are added to describe the products) to provide the criteria for wine that were specifically important to discriminate between products according to wine professionals. Napping® with a subsequent UFP step was recommended for application by the wine industry for pre-sorting of wines before blending (Perrin et al., 2008). Sáenz-Navajas and co-workers (2016) recommended the combination of a categorisation task with subsequent flash-profiling step and GC-O analysis as a rapid sensory-directed

tool to screen distinctive and quality wine aroma profiles for research on optimisation of various technical processes for wines.

Brand and co-workers (2018) demonstrated the potential of sorting in combination with quality scoring (using a 20-point scoring system) in a single assessment session as a rapid tool for the identification of drivers of wine quality by industry professionals. The method proposed by Brand and co-workers (2018) resulted in the identification of those sensory attributes that were most frequently cited in wines that have received the highest average quality scores. Sensory drivers of quality can be the key factors in guiding the blending process during wine production (Brand et al., 2018). The reference-based method, PSP, was used in combination with a description step to differentiate between coffee quality according to the Coffee Quality Program of the Brazilian Coffee Industry Association (ABIC) and to establish key sensory quality drivers by consumers (De Alcantara & Freitas-Sá, 2018).

The potential of PM (Moelich, Muller, Joubert, Næs, & Kidd, 2017) and PSP (Moelich, 2018) to distinguish between honeybush infusions for quality control or grading purposes has been indicated. The use of PSP with references representative of different honeybush quality grades was recommended (Moelich, 2018).

### 5.3 Reference-based methods

Holistic methods such as PM has attained much attention as a rapid approach to obtain a quick overview of similarities or dissimilarities in a sample set; however, the comparative nature of the method requires that all samples in a test set need to be evaluated simultaneously and conclusions derived from different sessions on the main sensory attributes of samples, cannot be compared to one another (Ares et al., 2018; Hopfer & Heymann, 2013). Furthermore, a limited number of samples can be assessed in one session, especially for sensory-complex products that may affect assessors' sensory fatigue or products that necessitate strict temperature-control (Ares et al., 2018).

One of the key advantages of reference-based methods is that a large set of samples may be evaluated over consecutive sessions. Through data aggregation, different data sets of smaller test subsets may be combined owing to the use of a fixed set of reference products (so-called 'poles'), with the prerequisite that the references remain constant over consecutive sessions (Antúnez et al., 2015; Ares et al., 2018). Therefore, PSP and PPM have been regarded as attractive alternatives to other holistic sensory methods such as sorting and PM (or Napping®) for the application of sensory-complex beverages with a wide range of sensory attributes (e.g. wine), products with intense or persistent sensory attributes (e.g. chilli or distillates), as well as products that require consistent high temperature-control (Ares et al., 2018; De Saldamando, Antúnez, Torres-Moreno, Giménez, & Ares, 2015).

### 5.3.1 Polarised sensory positioning and extensions thereof

The PSP methodology was initially developed by Teillet and co-workers (2010) to establish a method for a French supplier of tap water to routinely assess the taste of one or more water samples. For PSP, samples are compared with a fixed set product references, so-called 'poles', which is based on a holistic assessment as assessors evaluate global differences without an indication regarding the specific sensory attributes that should be considered in the assessment or their relative importance (Ares et al., 2018).

Two approaches for PSP exist, namely continuous-scale PSP and triad PSP (Ares et al., 2018; Teillet, 2015). Continuous scale PSP is based on rating scores that reflect the distance between test samples and poles. Assessors are instructed to rate the similarity of a test sample to each of the poles using an unstructured line scale, i.e. degree of difference scale, that is anchored from 'exactly the same' to 'totally different'. Any type of intensity scale, including structured scales, may be used (Ares et al., 2018). Triad PSP is an alternative approach to PSP in which assessors are instructed to simply indicate to which pole a sample resembles the most, and to which pole it resembles the least, without indicating the distance from the pole (Teillet, 2015). PSP has been recommended for commercial application such as new product development (prototype comparison) and quality control (batch control), specifically for its advantage of aggregating data of different sessions, and its application to sensory-complex products (Antúñez et al., 2015; Teillet, 2014). A summary of published PSP and PPM studies is provided in **Table 9**.

Ares and co-workers (2013) proposed PPM as an extension of PSP. This method combines the advantage of PSP of data aggregation over consecutive sessions and the holistic character and intuitive approach of conventional PM. Assessors firstly evaluate the respective poles that have been placed onto a two-dimensional map, and then locate the test samples relative to the poles and each other, based on the key sensory characteristics memorised from the respective poles. To date, only one study on the discrimination efficacy of PPM compared to PSP have been published (Ares et al., 2013). Product applications of PPM have been limited to orange-flavoured powdered drink samples (Ares et al., 2013), low sodium frankfurter containing garlic products (Horita et al., 2017) and old-vine Chenin Blanc wine (Wilson, Brand, Du Toit, & Buica, 2018). Although not generally combined with PSP, the descriptive step, Ultra flash profiling (UFP), has found application in rapid sensory characterisation of sensory-complex products such as wine by industry professionals as complementary task for Napping® (Perrin et al., 2008) and PPM (Wilson et al., 2018), and its application in pre-sorting of wines before blending has been indicated (Perrin et al., 2008), as mentioned previously.

Pivot® profile is another extension of PSP, which was initially developed by Thuillier and co-workers (2015) to characterise the sensory attributes of champagne using wine experts. It is derived from the free description method, popular among wine professionals, which allows to record assessors' free expression in an ordinal manner (Thuillier et al., 2015). The method was developed to address the disadvantage of PSP to provide indirect descriptions of the product through the description of known references. For this

method, assessors are instructed to provide free descriptions of the differences between test samples and a reference product, so-called 'pivot', in terms of the sensory attributes (including appearance, aroma, flavour and texture) they perceive less or more intense in the test sample compared to the pivot. Another more recent application of this method was to characterise a large variety of honey from different geographic origins (Deneulin, Reverdy, Rébénague, Danthe, & Mulhauser, 2018).

### 5.3.2 Practical considerations

#### 5.3.2.1 Pole selection

An important prerequisite of referenced-based methods is the use of standards or reference products that are stable over time (Ares et al., 2018; Teillet, 2015). The selection of poles is regarded as the most important step when conducting reference-based rapid sensory methods (Ares et al., 2018, 2015; Teillet, 2014). Poles should represent the key sensory characteristics responsible for the anticipated similarities and differences among samples (De Saldamando, Antúnez, Giménez, Varela, & Ares, 2015) to ensure that the complete sensory space is represented in the two-dimensional map determined by the poles (Ares et al., 2015).

The sensory space of a representative sample set of a product category should be known prior to the task to be able to select optimal product references, which may be regarded as a limitation of reference-based methodologies (Valentin et al., 2012). Notwithstanding, the sensory space of a product category may be obtained from previous sensory characterisation studies with samples of the product category, or preliminary studies using rapid methods. For example, Moelich (2018) selected poles based on Principle Component Analysis (PCA) of their DSA data, to obtain five poles that represent the key sensory attributes of different *Cyclopia* species, i.e. the poles selected were based on a representation of the total sensory space of the products under question. Similarly, Horita and co-workers (2017) selected poles based on PCA and Hierarchical Cluster Analysis (HCA) performed on DSA data to investigate the application of PPM in product re-formulation of low sodium frankfurter products.

A PM task was used to identify the sensory characteristics responsible for the main differences among commercial orange-flavoured powdered drinks (Ares et al., 2013). Based on the results from PM, three poles could be selected representative of the three main sample groups identified, namely 'economy sector' pole (characterised by its low total flavour intensity), 'premium sector with sugar' pole (characterised by its sweet and intense orange flavour), and 'premium sector with sweetener' pole (characterised by its sourness) (Ares et al., 2013). Similarly, in a study on rapid sensory assessment of South African Chenin Blanc wines using PPM, Wilson and co-workers (2018) used PM to obtain a sensory space and selected poles accordingly.

**Table 9** Summary of studies on polarised sensory positioning (PSP) and polarised projective mapping (PPM) in order of publication year.

Publication	Products	Assessors	Applied methods	No of poles	Statistical analysis	Major findings and/or recommendations
Teillet et al. (2010)	<b>mineral/tap water</b> (n = 16/13)	trained panel (n = 15/16)	DA	n/a	Multiple analysis of variance (Manova) Canonical Variables Analysis	1) Sensory profiling unsuitable for products with low sensory stimuli. 2) PSP result in higher discrimination than DA for analysis of water.
	<b>water</b> (n = 10)	consumers (n = 32)	<b>PSP</b>	3 poles	Multidimensional scaling (MDS) unfolding Statis	
	<b>water</b> (n = 29)	consumers (n = 389)	Sorting	n/a	MDS	
De Saldamando et al. (2013)	<b>make-up foundations</b> (n = 8)	consumers (n = 30)	<b>PSP</b>	3 poles (2 sets)	Multiple factor analysis (MFA)	1) Similar sample configurations obtained with different sets of poles. 2) Poles should be representative of full sensory space of product category for better differentiation of samples. 3) Different poles affected conclusions regarding similarities and dissimilarities of products to certain extent. 4) Careful selection of stable poles for PSP recommended.
	<b>orange-flavoured powdered drinks</b> (n = 8)	consumers (n = 92)	<b>PSP</b>	3 poles (2 sets)	MFA	
Ares et al. (2013)	<b>orange-flavoured powdered drinks</b> (n = 9)	consumers (n = 45)	<b>PSP with scales</b>	3 poles	MFA	
		consumers (n = 45)	<b>Triadic PSP</b>	3 poles	Multiple correspondence analysis	
		consumers (n = 45)	<b>PPM</b>	3 poles	MFA	
Cadena et al. (2014)	<b>low-fat probiotic yoghurts</b> (n = 8)	trained assessors (n = 9)	DSA	n/a	ANOVA and PCA	1) CATA, PM and PSP provided similar results on main differences between products. 2) Product configuration of CATA most similar to DSA. 3) Bootstrapping resampling method indicated sample configurations of PSP and CATA to be highly reliable.
		consumers (n = 81)	CATA	n/a	Cochran's Q test Correspondence analysis	
		consumers (n = 81)	PM	n/a	MFA	
		consumers (n = 81)	<b>PSP</b>	3 poles	MFA	



Table 9 continued

Publication	Products	Assessors	Applied methods	No of poles	Statistical analysis	Major findings and/or recommendations
Antúñez et al. (2015)	<b>orange-flavoured powdered drinks</b> (n = 7)	consumers (n = 120) [divided into 3 groups (n = 40) to assess sample subsets]	<b>PSP with scales</b>	3 poles	PCA	1) Data aggregation (different sessions with different assessors) provides similar information compared to assessment of samples in a single session. 2) Some differences in conclusions obtained from whole set vs split set, specifically for PSP with scales - care should be taken when aggregating data from assessment of similar sample by different consumer groups. 3) Data aggregation feasible for samples that are markedly different. 4) Better agreement between sample configuration obtained with triadic PSP - ascribed to fact that method deals not with heterogeneity in consumers' use of scale.
		consumers (n = 120) [divided into 3 groups (n = 40) to assess sample subsets]	<b>Triadic PSP</b>	3 poles	Correspondence analysis	
	<b>chocolate milk beverages</b> (n = 7)	consumers (n = 120) [divided into 3 groups (n = 40) to assess sample subsets]	<b>PSP with scales</b>	3 poles	PCA	
		consumers (n = 120) [divided into 3 groups (n = 40) to assess sample subsets]	<b>Triadic PSP</b>	3 poles	Correspondence analysis	
Fleming et al. (2015)	<b>astringent agents</b> (n = 10)	consumers (n = 41)	CATA	n/a	Cochran's Q test Correspondence analysis	1) Visually similar product configurations when comparing methods. 2) Mixed approach of CATA with subsequent PSP recommended (CATA results to be used to characterise sensory space to guide pole selection for PSP).
		consumers (n = 30)	Sorting	n/a	Multidimensional scaling	
		consumers (n = 41)	<b>PSP</b>	3 poles	MFA	
De Saldamando et al. (2015)	<b>orange-flavoured powdered drinks</b> (n = 12) [divided into 2 sample subsets: Set 1: medium degree of differences; Set 2: small degree of differences]	consumers (n = 206) [divided into 4 groups to assess sample subsets: 2 groups assessed samples over 2 sessions; 2 groups assessed samples over 3 sessions]	<b>PPM</b>	3 poles per sample set	MFA	1) PPM regarded as a repeatable and reproducible method. 2) Preliminary evidence of validity of data aggregation from different sessions (further research required on influence of degree of difference between samples on feasibility of data aggregation). 3) Reliable method for assessment of simple products with moderate difference among them.

Table 9 continued

Publication	Products	Assessors	Applied methods	No of poles	Statistical analysis	Major findings and/or recommendations
De Saldamando et al. (2015)	<b>orange-flavoured powdered drinks</b> (n = 12) [divided into 2 sample subsets: Set 1 represented a wider range of sensory characteristics than Set 2]	consumers (n = 44) consumers (n = 45) consumers (n = 43)	<b>PPM</b>	2 sets of 3 poles (main characteristics) 2 sets of 3 poles (main characteristics) 2 sets of 3 poles (narrow part of sensory space)	MFA	1) Set of poles had no large effect on sample configurations and consumers' descriptions. 2) Some differences in sample configurations identified for sets of poles that did not represent the whole sensory space was observed. 3) Selection of poles that represent the whole sensory space defined by the samples under question recommended. 4) An intermediate degree of difference among poles are recommended.
Ares et al. (2015)	<b>chocolate flavoured milk drinks</b> (n = 8)  <b>vanilla flavoured milk drinks</b> (n = 8) <b>orange flavoured powdered drinks</b> (n = 6)	consumers (n = 40)  consumers (n = 40) consumers (n = 40)	<b>PSP</b>	3 sets of poles: 3 poles; 2 poles; 2 poles	MFA	1) Poles should clearly represent the sensory characteristics responsible for the key differences among samples provided. 2) Two well-selected poles are sufficient to obtain reliable product categorisation, provided that they represent the key sensory characteristics associated with the sensory space. 3) The number of poles to be selected is based on the main sensory characteristics that discriminate among samples
Horita et al. (2017)	<b>Low sodium frankfurter containing garlic products</b> (n = 8)	trained assessors (n = 10)  consumers (n = 50)	DA  <b>PPM</b>	n/a  3 poles	ANOVA and PCA  MFA	1) Adequate correlation between methods for product characterisation. 2) PPM better discrimination between samples when compared to DSA. 3) Discriminative vocabulary of sensory characteristics generated with descriptive step. 4) PPM for rapid sensory characterisation and application in product re-formulation (sodium reduction using garlic products) indicated.
Wilson et al. (2018)	<b>SA Chenin Blanc wines</b> (n = 12)  4 subsets (n = 10) using n = 17 samples including poles	trained assessors (n = 15)  trained assessors (n = 10-15)	PM  <b>PPM</b> 4 experiments	n/a  3 poles	MFA  MFA	1) Overall groupings in 'global' MFA (4 experiments) consistent with those in PM. 2) Consistent results in terms of repetitions, blind duplicates, explained variance, confidence ellipses and grouping trends. 3) Choice of poles critical (foreknowledge of products under question and their sensory space is required). 4) Data aggregation demonstrated (constant expert panel over long period of time recommended).

**Table 9** *continued*

Moelich (2018)	<b>honeybush tea</b> <i>Cyclopia</i> <i>intermedia</i> , <i>C. subternata</i> , <i>C. genistoides</i> , <i>C. maculata</i> and <i>C. longifolia</i>	trained assessors (n = 10)	DSA	n/a	ANOVA and PCA	<p>1) Similar product configurations for DSA, partial and global PSP. Validity of methods for broad sensory profiling of <i>Cyclopia</i> species demonstrated.</p> <p>2) Global and partial PSP on aroma were more effective to differentiate between samples than partial PSP on palate.</p> <p>3) Application of partial PSP on aroma by trained assessors indicated for honeybush quality control.</p> <p>4) PSP on one modality (i.e. aroma) reduces assessment time and sensory fatigue and effective.</p>
(n = 15) [3 samples per species]		<hr/> <p><b>'partial' PSP (on aroma)</b>      5 poles      MFA Correspondence <b>'partial' PSP (on palate)</b>      5 poles      analysis <b>'global' PSP (on aroma and palate)</b>      5 poles</p> <hr/>				

Another consideration in pole selection is the degree of difference among the poles that can affect discrimination among samples. De Saldamando and co-workers (2015) studied the effect of different sets of poles ( $n = 3$ ) representing the distinctive characteristics, namely 'sweetness', 'sourness' and 'total flavour' intensity, and variations thereof, including sets of poles representing a narrower part of the sensory space. The authors concluded that apart from selecting poles that represent the entire sensory space, there should be an intermediate degree of difference among poles. These results were also regarded as applicable to other reference-based methodologies, such as PSP. Correspondingly, Ares et al. (2015) recommended that poles that are perceived as distinctly different from each other should be selected, and that each pole should be markedly representative of one or two sensory characteristics of the product that is studied.

Usually, the number of poles required for a reference-based method is at least equal to the number of sensory dimensions required to present the perceptual space (Ares et al., 2018). However, if two poles are very close to one another within the sensory space, one of the poles may be omitted, as recommended by Teillet (2014). Furthermore, the selection of only a small number of poles that are distinctly different in terms of specific sensory characteristics (or lack thereof) is recommended to limit assessors' sensory and cognitive fatigue. This is especially an important consideration for PSP in which assessors have to taste and re-taste test samples against each pole (Moelich, 2018). Moelich (2018) studied the application of PSP to honeybush tea by selecting five poles, representing five different *Cyclopia* species, respectively. Poles for *C. subternata* and *C. intermedia* were distinctly different in sensory profiles and represented specific sensory characteristic, whereas poles for *C. genistoides*, *C. maculata* and *C. longifolia* did not differ distinctly and indicated an overlap of sensory characteristics. As assessors could not distinguish between samples of the afore-mentioned three species, the selection of only one pole for each of *C. genistoides*, *C. subternata* and *C. intermedia*, respectively, was recommended. Each of these species represented high intensities of one or two specific, distinctly different sensory characteristics (Moelich, 2018).

#### 5.3.2.2 Assessor level of experience and training

Studies have indicated that reference-based rapid sensory methods may be successfully performed by naive or semi-trained assessors/consumers for PSP (De Alcantara & Freitas-Sá, 2018) and PPM (Ares et al., 2013; De Saldamando, Antúñez, Torres-Moreno, et al., 2015; Horita et al., 2017). However, consumer-based studies have generally not been recommended for the identification of subtle differences or for quality control application (Varela & Ares, 2015). Limited studies using trained assessors for PSP (Moelich, 2018; Teillet et al., 2010) and PPM (Horita et al., 2017; Wilson et al., 2018) are available. Only a few rapid sensory profiling method studies using industry professionals, specifically wine experts, have been published, namely for sorting (Ballester, Patris, Symoneaux, & Valentin, 2008; Brand et al., 2018) and

PM/Napping® (Perrin et al., 2008; Torri et al., 2013). To the authors' knowledge, no studies on the application of PSP or PPM using industry professionals have been published to date.

Industry professionals, specifically wine experts, are found to be repeatable (Perrin et al., 2007) and to perform as well as trained assessors, even when experts are required to use sensory attributes used in DA to evaluate wines (Zamora & Guirao, 2004). Assessors' sensory experience of a product and their level of product knowledge (especially for experts and professionals) has shown to significantly influence product differentiation (Maitre, Symoneaux, Jourjon, & Mehinagic, 2010). In a study on how wine aroma differences are perceived by naive and experienced assessors using PM, Torri and co-workers (2013) suggested that product differentiation by experts was mainly based on the perceived overall quality rather than on specific individual sensory attribute differences. The authors concluded that PM could be a valuable method for assessing the perceived similarities or dissimilarities among samples with subtle sensory differences, provided that assessors share a high level of knowledge and experience of the product.

Research on the minimum number of assessors required to perform PSP (and PPM) tasks is still lacking (Ares et al., 2018). Generally, the number of trained assessors considered for conventional DA is similar for rapid profiling methods, i.e. 10 to 15 trained assessors for DA and sorting, for example (Lawless & Heymann, 2010; Varela & Ares, 2012). However, the number of naive assessors (consumers) is expected to increase from the recommended minimum of 30 to 60 consumers, as the product's sensory complexity increases, as well as the degree of difference among samples is reduced (Ares et al., 2018).

### 5.3.2.3 Number of samples and test replicates

The major advantage of PSP and PPM over other rapid methods is that there is no limitation on the number of samples that can be evaluated in a study, as samples can be divided into subsets and evaluated in different sessions. Data aggregation has been demonstrated to be valid for PSP (Antúñez et al., 2015; Teillet, 2014) and PPM (De Saldamando, Antúñez, Torres-Moreno, et al., 2015; Wilson et al., 2018). However, at least five samples are required to obtain an adequate sample representation in a two-dimensional sensory space (Ares et al., 2018) and a maximum of 15 samples (Varela & Ares, 2015) per session, have been recommended. Previously, in reference-based method studies on sensory-complex products, 7 honeybush tea samples for PSP (Moelich, 2018), 3 to 4 coffee samples for PSP (De Alcantara & Freitas-Sá, 2018) and 7 Chenin Blanc wine samples for PPM (Wilson et al., 2018) were used per test session, and data aggregation for consecutive sessions was applied. Generally, tests are not replicated in PSP tasks due to time constraints and/or available resources to conduct a study and analyse the results (Ares et al., 2018).

### 5.3.3 Data analyses and method validation

For PSP with continuous scale, data consist of the degree of difference scores for each pole and are collected in the form of a dissimilarity matrix between test samples and poles for each assessor. Analysis of PSP data using multi-block statistical techniques (Abdi, Williams, & Valentin, 2013) are highly recommended as each assessor evaluate the degree of difference between test samples and poles using their own personal criteria (Ares et al., 2018). Therefore, consensus representation of the similarities and differences among test samples can be obtained with two established methods, namely multiple factor analysis (MFA) or generalised Procrustes analysis (GPA) (Ares et al., 2018). These methods provide information regarding the consensus product configuration, which represents the 'mean' product configuration across all individual assessors and provides important insight on the overall perception of products (Tomic et al., 2015).

Multiple factor analysis is essentially a multi-block PCA of concatenated matrices (Tomic et al., 2015). For sample configurations obtained from MFA, confidence ellipses around test samples may be constructed to represent the area of the space with a certain probability of containing the real position of test samples. This enables the identification of significant differences among samples (Dehlholm, Brockhoff, & Bredie, 2012). As for PM, PPM data is collected by measuring the X and Y coordinates on the bi-dimensional space for each test sample and pole relative to the zero point (usually the lower left corner of a paper or screen) per assessor. MFA has been applied to PPM data (Ares et al., 2013; Horita et al., 2017; Wilson et al., 2018). On-going development of web-based sensory software programmes has enabled electronic data capturing and analyses for rapid sensory methodologies within research or commercial context (Compusense®; EyeQuestion®). Sensory software programmes have enabled computer screens, tablets, and other devices to constitute the projective response area for PM or Napping® (Dehlholm, 2014), replacing the use of paper sheets.

DSA is regarded as the most advanced method in the sensory descriptive toolbox (Lawless & Heymann, 2010) and has been used as 'gold standard' for comparison to test the descriptive efficiency of new sensory methods (Lestringant, Delarue, & Heymann, 2019). Graphic representations of results are interpreted by visually comparing product configurations obtained from rapid method tasks and PCA bi-plot of the DSA results, as well as with RV coefficients (Cadena et al., 2014; Horita et al., 2017; Louw et al., 2013; Moelich et al., 2017). RV coefficients are multivariate correlation coefficients that measure the similarity between two-factorial product configurations (Abdi, 2010). RV coefficients closer to 1 indicate high similarity between respective configurations, whereas 0 indicates uncorrelated configurations. RV coefficients depend on the relative position of the points in the product configuration and is, therefore, independent of rotation and translation (Robert & Escoufier, 1976).

## 6 Concluding remarks

Globally increased consumer appreciation for natural products such as herbal teas, with sensory appeal as one of the key drivers of choice, has contributed to the rapidly growing market for fermented honeybush tea (Joubert, De Beer, & Malherbe, 2017). As the demand for this uniquely South African herbal tea is exceeding supply to a predominantly export market, it is essential that all production batches should be of optimum quality. However, inconsistent quality in the current local and international market is a major concern for the reputation of honeybush, and the pressing need for standardised control of honeybush sensory quality has been emphasised (DAFF, 2016; Joubert et al., 2019, 2011).

Over the past two decades good progress has been made in honeybush sensory research in terms of process optimisation and development of quality control tools (Joubert et al., 2019). However, limitations such as the development of sensory lexicons that describe mainly food-based reference standards, as well as developed sensory wheels that are based on limited datasets and an updated generic wheel that does not represent all three major commercial *Cyclopia* species (i.e. *C. intermedia*, *C. subternata* and *C. genistoides*), have been highlighted. Furthermore, despite the wealth of information generated from the comprehensive study of positive and negative sensory characteristics of several *Cyclopia* species, these results have not yet been transformed into defined sensory quality parameters for establishment of a quality standard and classification method to assess the sensory quality of a product batch.

The advantages of sensory lexicons, and especially chemical-based reference standards, for assessor training and calibration in quality control and communication of sensory quality between different industry role-players in various food and beverage industries, have been highlighted in this review. Chemical-based reference standards have the advantage of global availability, convenience and homogeneity (Bárcenas et al., 1999; Drake & Civille, 2003; Pe et al., 2002), compared to food-based reference standards. Universal chemical reference standards would improve the industry's use of the honeybush lexicon and wheel as sensory quality communication and training tools, especially as it would be globally available to all industry role-players.

An in-depth review on sensory quality control within food and beverage sectors, including commodities such as tea and coffee, has emphasised the universal need for standardised sensory quality control methods. The relevance of quality scoring methods that incorporate scorecards, provided that they are based on a sound scientific research approach, has been highlighted. The role of industry experts in the development of such methods and the importance of applying good sensory practice such as assessor training has also been highlighted. The importance of sensory quality control of products with quality distinctiveness labels (e.g. PDO and PGI) to provide products of high quality, to assure their authenticity and to differentiate them from similar commercial products (Bertozzi, 1995; Bertozzi & Panari, 1993), has also been emphasised. The development approaches for establishing sensory quality specifications and

methods reviewed in this chapter will form a foundation for the development of a sensory quality scoring method for fermented honeybush tea.

In addition, the need for alternative time- and cost-efficient sensory methods that can be applied as rapid quality classification tools to identify the most important broad-based sensory quality differences between products within research (e.g. honeybush cultivation/breeding programmes of the Agricultural Research Council) and commercial (e.g. blending of tea batches for consistent quality) environments, has been indicated. Recent advances in sensory science, especially the development of reference-based rapid sensory profiling methods, such as polarised sensory positioning and polarised projective mapping, that allow data aggregation over different sessions for batch comparison, has been highlighted in this review. To date, limited studies on the application of rapid sensory profiling methods for quality control within commercial context are available.

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# Chapter 3

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## Development of chemical-based reference standards for honeybush aroma lexicon

### Abstract

The existing generic honeybush sensory wheel, originally developed using several *Cyclopia* species and a sample set of  $n = 150$ , was revised with the focus on positive and negative aroma attributes of the major commercial *Cyclopia* species, i.e. *C. intermedia*, *C. subternata* and *C. genistoides*. For the revision, sample sets of  $n = 195$ ,  $n = 260$  and  $n = 130$  of the respective *Cyclopia* species were used. Candidate aroma chemicals that could potentially serve as reference standards for the honeybush aroma lexicon were identified through a comprehensive literature search and gas chromatography–mass spectrometry analysis. These chemicals were screened by an expert panel for their suitability in terms of typicality of the target aroma. Each chemical was evaluated in a ‘base tea’ and compared to a specific ‘reference tea’ exhibiting a high intensity of the target aroma. A total of 44 chemicals were selected for validation by a trained panel. Descriptive sensory analysis was conducted to assign typicality and intensity scores on unstructured line scales (0 – 100) for each chemical representing a target aroma attribute. Chemicals with typicality score values that did not differ significantly ( $p \geq 0.05$ ) from the target aroma in the respective reference teas were regarded as highly representative reference standards, i.e. 2-acetyl-5-methylfuran (‘woody’), levulinic acid (‘fynbos-sweet’), maltyl isobutyrate (‘caramel’), and 2-acetylpyrrole (‘nutty’). High typicality scores of  $\geq 70$ , although significantly different ( $p < 0.05$ ), were obtained for geranyl acetone (‘fynbos-floral’), nerol and geraniol (‘rose geranium’), maltyl isobutyrate and 2-methylbutanal (‘apricot’), (*E*)-2-hexen-1-al (‘apple’),  $\beta$ -cyclocitral (‘raisin’), propyl propionate and geranyl isovalerate (‘fruity-sweet’), ethyl maltol (‘caramel’), benzaldehyde (‘nutty’), *o*-cresol (‘hay/dried grass’), (*Z*)-3-hexen-1-ol (‘green grass’), *p*-cresol (‘medicinal/rubber’) and 3-ethylpyridine (‘smoky’). The inclusion of chemicals as universal reference standards in the revised honeybush aroma lexicon would aid industry role-players in the recognition and communication of the respective sensory attributes as an integral part of honeybush quality control.

# 1 Introduction

Sensory lexicons provide standardised, descriptive vocabularies that allow for accurate and objective communication of sensory attributes of products among industry role-players (Lawless & Civille, 2013) across all cultures and language barriers (Cherdchu, Chambers, & Suwonsichon, 2013; Monteiro et al., 2017). Sensory wheels are graphical representations of the attributes captured in lexicons and indicate how the sensory attributes are related (Lawless & Civille, 2013). Generally, descriptive sensory analysis is used for the development of a lexicon to describe and quantify the full range of perceived sensory attributes associated with a specific product (Lawless & Heymann, 2010; Muñoz & Civille, 1998). A sensory lexicon consists of a list of sensory attributes with a definition for each attribute, as well as qualitative and/or quantitative reference standards. Qualitative reference standards facilitate concept alignment, whereas quantitative reference standards assist in defining intensity limits and panel calibration (Muñoz & Civille, 1998; Murray & Delahunty, 2000).

Reference standards can be chemical- and/or food-based (Drake & Civille, 2003). A disadvantage of commercial food products as reference standards is that they may be reformulated or become unavailable over time (Rainey, 1986). Chemical standards have the advantage of global availability, consistency and shelf-life stability (Drake & Civille, 2003). For the evaluation and selection of suitable chemical reference standards a neutral base (product matrix) is recommended to assist assessors to understand an attribute as it appears in the product in question (Drake & Civille, 2003; Etaio et al., 2010, 2012). The application of gas chromatography–olfactometry (GC–O) analysis to characterise odour-active and character impact compounds, i.e. those volatile organic compounds responsible for the characteristic aroma of a food product, has been indicated (Zellner, Dugo, Dugo, & Mondello, 2008). GC analysis of the volatile fraction of the product of interest, may aid selection of suitable chemical reference standards. The use of chemical-based reference standards, especially chemical compounds present in the product of interest, may allow for a clear and grounded lexicon (Drake & Civille, 2003).

The role of lexicons in defining the specific quality characteristics of indigenous or traditional products that have Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI) status, i.e. to distinguish them from atypical and/or inferior quality products, is evident in literature (Monteiro et al., 2014; Pereira, Dionísio, Matos, & Patarata, 2015; Rétiveau, Chambers, & Esteve, 2005; Stolzenbach, Byrne, & Bredie, 2011; Vázquez-Araújo et al., 2012). For example, Etaio et al. (2010) developed sensory reference standards as a critical step in the development of a quality scoring method for PDO Rioja Alavesa young red wines. Honeybush has been included in the GI Protocol of the Economic Participation Agreement (EPA) with the European Union (EU) and is fully protected as a GI in Europe (European Commission, 2019). GIs not only serve to designate goods with a quality characteristic, or reputation attributed to its geographical origin, but are considered an instrument to protect product names, such as ‘honeybush’ against misappropriation (Biénabe & Marie-Vivien, 2017). The current South African regulatory guidelines

for export of honeybush (DAFF, 2019) do not make provision for sensory specifications in terms of its characteristic aroma, taste and colour. Potential misinterpretation of the sensory quality of honeybush by industry role-players emphasises the need for a validated product-specific sensory lexicon. It is important that lexicons include both positive and negative sensory attributes as positive attributes are typical of the product, while negative attributes are generally a result of poor quality, most likely caused by poor processing practices. Infusions prepared from under-fermented plant material usually have, by comparison to optimally fermented plant material, perceptible intensities of negative aroma attributes such as 'green grass' (Bergh, Muller, Van der Rijst, & Joubert, 2017; Erasmus, Theron, Muller, Van der Rijst, & Joubert, 2017). A further result of poor processing practices is the development of a 'smoky' taint, usually caused by over-heating of the plant material during contact with a hot surface (Bergh et al., 2017).

The need for a sensory lexicon and wheel for fermented honeybush was initially addressed by Theron and co-workers (2014). The first generic honeybush sensory wheel was developed, based on the aroma, flavour, taste and mouthfeel attributes of six *Cyclopia* species. The latter species included the primary commercialised species, as well as some minor *Cyclopia* species, with the number of samples per species varying between 7 and 11. Further development focused on species-specific aroma wheels for *C. intermedia* (Bergh et al., 2017), *C. subternata*, *C. genistoides* and *C. maculata* (Robertson et al., 2018), as well as an updated generic aroma wheel, based on a set of 150 samples (*C. genistoides*, *C. subternata*, *C. maculata* and *C. longifolia*) (Erasmus, 2014). The revision of the generic honeybush aroma wheel, which is based on a comprehensive sample set of the main commercial species, *C. intermedia*, *C. subternata* and *C. genistoides*, would allow for a more representative quality control tool in the market.

The sensory lexicon for fermented honeybush was developed using verbal descriptions and predominantly food-based reference standards. However, there is a need for the identification of universal chemical-based reference standards to illustrate individual aroma attributes and to improve global understanding of the respective aroma descriptions. The aim of the present study was therefore to identify, screen and validate aroma chemicals as potential reference standards for the revision of the honeybush lexicon. The generic honeybush aroma wheel was updated based on the large set of honeybush samples used to validate the attributes for the lexicon.

## 2 Materials and methods

The present study is divided into two phases, namely 1) the establishment of a comprehensive sensory dataset for the revision of the honeybush aroma lexicon and wheel, and 2) the identification, screening and validation of aroma chemicals as potential reference standards in the revised honeybush aroma lexicon. The experimental lay-out of the study is presented in **Fig. 1**.

## 2.1 Comprehensive dataset for revised honeybush aroma lexicon and wheel

A comprehensive data set was compiled, obtained by sensory analysis of the major commercial *Cyclopia* species, i.e. *C. intermedia* (n = 195), *C. subternata* (n = 260) and *C. genistoides* (n = 130). These samples spanned several production years and included samples produced at the optimum fermentation conditions (Bergh et al., 2017; Erasmus et al., 2017; Robertson et al., 2018), as well as commercially processed samples to represent the entire product category. The comprehensive data set comprised of 1) existing baseline sensory data from previous honeybush research and 2) new sensory data from the analyses of samples from mainly commercially processed honeybush batches performed in the present study (**Table 1**).

### 2.1.1 Samples for the baseline sensory dataset

Sensory data of fermented honeybush samples collected from honeybush research (2010 – 2016) at the Department of Food Science, Stellenbosch University, South Africa, and Post-Harvest and Wine Technology Division of Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Stellenbosch, South Africa, formed the baseline data set to represent the variation within the major commercial honeybush species. *C. subternata* and *C. genistoides* samples were processed on laboratory-scale at ARC Infruitec-Nietvoorbij, using optimum fermentation conditions (80 °C/24 h and/or 90 °C/16 h) for characteristic aroma development (Erasmus et al., 2017). *C. intermedia* samples were either sourced from commercial processors, or produced on laboratory-scale, using a range of fermentation temperature/time regimes for processing optimisation studies for *C. intermedia* (Bergh et al., 2017).

### 2.1.2 Samples for new sensory dataset

To expand the baseline data and increase its robustness, fermented honeybush batches of *C. intermedia* (n = 87), *C. subternata* (n = 38) and *C. genistoides* (n = 32) were sourced from reputable processors who produce only single-species batches. Additional samples produced on laboratory scale at optimum fermentation conditions (90 °C/24 h) for *C. intermedia* (Bergh et al., 2017) were also included, primarily to ensure that the comprehensive dataset also represents *C. intermedia* samples of optimum quality. However, due to severe drought conditions only a limited quantity of fresh plant material could be sourced to produce a further 10 batches of *C. intermedia* at the ARC Infruitec-Nietvoorbij, Stellenbosch.

### 2.1.3 Sample preparation

The commercially processed samples (n = 157) and the additional *C. intermedia* samples processed on laboratory scale (n = 10) were stored at ambient temperature (21 °C) in sealed glass jars, until analysis (Erasmus et al., 2017). Infusions of samples were prepared at 'cup-of-tea' strength before serving according to a standard protocol, as described by Erasmus et al. (2017), i.e. 1000 g freshly boiled distilled water 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). White porcelain tasting mugs (Woolworths, Bellville, South Africa) were pre-heated in an industrial oven (Hobart, France) at 70 °C before ca. 100 mL aliquots of each infusion 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 programme (Compusense version 5.6, Guelph, Canada). The samples were served in temperature controlled (65 °C) water baths (Scientific Manufacturing Company, Cape Town, South Africa).

#### 2.1.4 Sensory analysis

Twelve female assessors (aged 35 – 65) with extensive experience in descriptive sensory analysis (DSA) of fermented honeybush (Bergh et al., 2017; Erasmus et al., 2017; Theron et al., 2014) served on the panel. Each of the three *Cyclopia* species sample sets were assessed in separate experimental blocks. Attributes that were assessed are listed in **Table A.1** (Bergh et al., 2017; Erasmus et al., 2017). Aroma refers to odours perceived through orthonasal analysis, while flavour refers to the retronasal perception of aromas in the mouth. Similar to flavour, the basic taste modalities, i.e. sweet, sour, and bitter and the mouthfeel attribute, astringency, are perceived in the oral cavity (Lawless & Heymann, 2010). Astringency is described as the tactile sensation that occurs in the oral cavity due to the precipitation of salivary proteins (Green, 1993). Flavour, basic tastes and astringency are often referred to as palate attributes (Moelich et al., 2017; Parr, Ballester, Peyron, Grose, & Valentin, 2015). The panel was trained in separate sessions on each of the sample sets based on the generic DSA technique, as described by Lawless and Heymann (2010). For each experimental block five to six coded samples were presented in a random order to each assessor per testing session. Each set of five to six samples was evaluated in triplicate on the same day with a 15 min break between each test session. Attribute intensities were rated on unstructured line scales (0 = none; 100 = extremely high) and scores were captured electronically with the aid of Compusense® five programme (Compusense, Guelph, Canada). Unsalted water biscuits (Woolworths, Stellenbosch, South Africa) and still natural spring water (Woolworths, Stellenbosch, South Africa) were used as palate cleansers between samples. All analyses were conducted in individual tasting booths in a sensory laboratory under standard lighting and controlled temperature (21 °C) conditions. Trained assessors signed an informed consent form before commencement of the study.

## 2.2 Configuration of aroma wheel and occurrence frequency bar graphs

The complete comprehensive sensory dataset (based on DSA of 585 samples) was compiled from the aforementioned two datasets. For the development of the aroma wheel, the average intensity of an aroma attribute was calculated. Similar to a pie chart, the percentage of the wheel that each attribute should represent was obtained by expressing this average for an attribute as a percentage of the sum of the average intensities for all attributes. The occurrence of an attribute in the full sample set was counted



when present at an average intensity  $\geq 1$  on a 100-point scale. This count value was used to calculate occurrence frequency as a percentage of the total number of samples. The data were presented in two bar graphs, displaying the positive and negative aroma attributes, respectively.

## 2.3 Aroma chemicals for revised honeybush aroma lexicon

### 2.3.1 Chemicals

Chemicals were supplied by Sigma-Aldrich (St Louis, MO, USA) and Kerry EMEA (Durban, South Africa). Kerry EMEA supplied chemicals diluted in propylene glycol or triacetin at 0.001, 0.01, 0.1, 1% or 10% (w/v). Other chemicals were also diluted with propylene glycol to 0.1% or 1% (w/v) solutions prior to use. Nano-encapsulated chemicals in plastic capsules, sourced from FlavorActiV™ Ltd (Aston Rowant, UK), were added directly to the infusions.

### 2.3.2 Tea samples

A honeybush 'base tea' sample was selected to serve as matrix when assessing the respective aroma chemicals. The criterium for selection of the base tea was the typicality of its overall aroma profile, yet without overt prominence of any positive aroma notes. The base tea was thus considered to be 'neutral' in aroma profile yet providing the typical matrix of the tea. A 1:1:1 blend of fermented, commercial *Cyclopia* spp. (*C. intermedia*, *C. genistoides* and *C. subternata*) samples from our in-house sample library (Department of Food Science, Stellenbosch University) was selected as base tea. The base tea without the addition of a chemical also served as control ('calibration tea') during screening and validation of the aroma chemicals (**Fig. 2**).

Specific 'reference teas' for honeybush were selected from the in-house sample collection, previously identified to exhibit a high intensity of a specific target aroma (Bergh et al., 2017; Erasmus et al., 2017; Jolley, Van der Rijst, Joubert, & Muller, 2017; Robertson et al., 2018). The reference teas were used to familiarise assessors with the respective aroma attributes and to ascertain to what extent the perceived aroma of a chemical was typical of the target aroma attribute illustrated by the reference tea. Infusions of all honeybush samples were freshly prepared before serving, as described by Erasmus et al. (2017).

### 2.3.3 Identification and screening of potential chemical reference standards

Selection of honeybush chemical reference standards was based on 23 aroma attributes and their lexicon descriptions (Bergh et al., 2017; Erasmus et al., 2017). These aroma attributes were also represented in the revised honeybush aroma wheel in the present research. Literature, chemical databases and aroma chemical supplier data were studied to identify chemicals that could potentially serve as reference standards for the aroma lexicon descriptors. Additionally, potential odorant compounds in volatile fractions of honeybush infusions, identified by GC-MS analyses (Kerry EMEA) (**Table 2**), were included for evaluation.

Aroma chemicals were first screened by a panel of expert assessors (N = 4) during 18 sessions (ca. 1.5 h per session) to eliminate atypical chemicals. This panel had extensive experience with DSA of honeybush. During each screening session, different chemicals for two to three honeybush lexicon descriptors were evaluated. Descriptors evaluated per session were selected based on their category (e.g. 'honey' and 'caramel' in the sweet-associated category) or whether they complement each other (e.g. 'apple' and 'sweet spice' or 'apricot' and 'fruity-sweet').

A freshly prepared base tea infusion (1000 mL) was spiked with a nano-encapsulated chemical capsule directly before each session. Similarly, the diluted chemical was added in 20 µL increments until its aroma was perceived clearly. Subsequently, ca. 100 mL aliquots of the base tea, base tea dosed with chemical and reference tea, representing the target aroma (**Fig. 2**), were transferred to white porcelain mugs, covered with plastic lids to limit loss of volatiles, and placed in water baths controlled at 65 °C for the duration of the screening period. The chemicals were assessed in terms of their typicality and intensity. Typicality of a chemical refers to the similarity of the perceived target aroma of the chemical in the dosed base tea compared to that of the aroma attribute of the specific reference tea. Based on the aforementioned, the chemical compound was eliminated or selected for validation. The chemical concentration (dose) was amended where applicable. Preparation and presentation of the respective infusions are depicted in **Addendum A, Fig. A1**.

### *2.3.4 Validation of chemical reference standards using DSA*

#### 2.3.4.1 Panel training

Eight female assessors (aged 35 to 65) with extensive experience in DSA of fermented honeybush served on the panel (these assessors also served on the panel described in Section 2.3). A maximum of three chemicals per aroma descriptor was presented to the panel in six training sessions. For each aroma descriptor, the panel was presented with a base tea, base tea dosed with the chemical compounds and corresponding reference tea (**Fig. 2**). Tea infusions were prepared and presented as for screening.

At the start of each training session, the panel was informed of the target descriptor (e.g. 'fynbos-floral') to be assessed to focus assessors on the relevant descriptor. Assessors were instructed to remove the sample from the water bath, remove the plastic lid and swirl the infusion several times before analysing the aroma. Each assessor evaluated the different infusions individually, followed by a group discussion in which the group reached consensus on the suitability of each chemical as a potential chemical reference standard, based on typicality and intensity. Firstly, assessors assessed and described the base tea to calibrate their sensory perception. This was followed by the assessment of the chemical (e.g. (*R/S*)-linalool) by comparing the base tea dosed with the chemical with the corresponding reference tea (honeybush sample exhibiting a high intensity of e.g. 'fynbos-floral' aroma). Descriptions of the perceived target aroma in each sample were noted. The typicality (0 = atypical to 100 = extremely typical) and intensity (0 = not

detectable to 100 = extremely high intensity) of the target aroma attribute was scored on unstructured line-scales once consensus was reached.

#### 2.3.4.2 Analysis of samples

For DSA testing, one target aroma was analysed per session to limit panel fatigue and carryover effects. Samples were tested in triplicate with a 15 min break between each sample set. Two chemicals were tested per target aroma attribute, apart from 'green grass' and 'honey' for which only one chemical was tested. Blind testing of samples, labelled with 3-digit codes, was conducted with presentation order randomised per assessor. In addition, a clearly labelled mug with base tea (labelled as 'base') was included to serve as a fixed point to calibrate assessors at the start of each session. The specific reference tea (labelled as such) for the target aroma (e.g. 'fynbos-floral') was also included to sensitise assessors in terms of typicality and intensity. Scores for the perceived typicality and intensity of the target aroma were captured, using Compusense® five software (Compusense, Guelph, Canada). All analyses were conducted in individual tasting booths in a sensory laboratory under standard lighting and controlled temperature (21 °C) conditions.

### 2.4 Statistical analyses

DSA data were analysed separately for each of the three *Cyclopia* species with three replicates of each sample served to each assessor in random order. The DSA data were subjected to various statistical techniques to confirm panel reliability (Næs, Brockhoff, & Tomic, 2010) and data normality (Shapiro & Wilk, 1965). In the event of the Shapiro–Wilk test indicating significant deviation from normality ( $p \leq 0.05$ ), outliers were removed. Data were subjected to analysis of variance (ANOVA), using the GLM (General Linear Model) procedure of SAS statistical software (Version 9.4, SAS Institute, Cary, NC, USA) according to the model for the study design. When effects were significant, Fisher's least significant difference was calculated to compare the means of typicality and intensity of an aroma chemical to that of the specific reference tea.  $P < 0.05$  was considered significant.

## 3 Results and discussion

In the present study, the development of chemical-based reference standards for honeybush entailed identification, screening and validation of aroma compounds with the aid of expert and trained assessors.

### 3.1 Revised honeybush aroma wheel

The revised aroma wheel, compiled from *C. intermedia*, *C. subternata* and *C. genistoides* samples, is depicted in **Fig. 3**. The relative intensity of each of the aroma attributes is reflected by the width of a

wedge. The major aroma notes are 'woody', 'fynbos-floral', 'fynbos-sweet', and to a lesser extent, 'fruity-sweet', agreeing with the relative intensities indicated for the species-specific aroma wheels of *C. intermedia* (Bergh et al., 2017), *C. genistoides*, *C. subternata* and *C. maculata* (Robertson et al., 2018). The positive aroma attributes, 'orange', 'plant-like' and 'coconut' included in the first generic honeybush sensory wheel (Theron et al., 2014) were removed, 'raisin' was added and 'walnut' and 'cassia/cinnamon' were changed to the more generic terms, 'nutty' and 'sweet spice', respectively. The negative aroma attributes remained the same except for the exclusion of 'yeasty' and the addition of 'smoky'. The new honeybush aroma wheel is accompanied by two bar graphs, indicating the occurrence frequency (%) of the positive and negative aroma attributes in the full sample set, respectively. The bar graphs give another dimension to the relative importance of each attribute within the overall sensory profile of honeybush.

### 3.2 Identification of potential chemical reference standards

Volatile compounds ( $n = 33$ ) that may contribute to the perceived aroma attributes in freshly brewed *Cyclopia* spp. blend were identified by GC-MS analysis (**Table 2**). Major compounds identified were 6-methyl-5-hepten-2-one, linalool,  $\beta$ -ionone, eugenol and several isomers of megastigmadienes and megastigmatrienones (not specified). Other compounds that were present in smaller quantities included hexanal,  $\beta$ -cyclocitral,  $\beta$ -damascenone and geranyl acetone. Le Roux, Cronje, Burger and Joubert (2012) identified (*E*)- $\beta$ -damascenone, (*R/S*)-linalool, (*E*)- $\beta$ -damascone, geraniol, (*E*)- $\beta$ -ionone, and (*7E*)-megastigma-5,7,9-trien-4-one by GC-olfactometry (GC-O) analysis as the major odour-active volatile compounds in fermented *C. subternata*. In addition, the GC-O assessors perceived the aromas of (*6E,8Z*)-megastigma-4,6,8-trien-3-one, (*6E,8E*)-megastigma-4,6,8-trien-3-one, (*7E*)-megastigma-5,7,9-trien-4-one, 10-*epi*- $\gamma$ -eudesmol, *epi*- $\alpha$ -muurolol, and *epi*- $\alpha$ -cadinol as 'typically honeybush-like'. However, only commercially available megastigma-4,6,8-trien-3-one could be sourced for screening. Ntlhokwe, Muller, Joubert, Tredoux and De Villiers (2018) identified 3-hydroxy-2-methylpyrone (maltol) and (*E*)-cinnamaldehyde, volatiles associating with caramel and cinnamon aroma, respectively, for the first time in honeybush. For our investigation maltol and *trans*-cinnamaldehyde could be sourced from a commercial supplier. A total of 25 chemical compounds identified from honeybush GC-MS data were subsequently sourced for screening.

From an extensive literature search and GC-MS data, a total of 79 potential chemical-based reference standards were identified and screened for honeybush (**Table 3**). The objective was to test at least two chemicals per aroma attribute. Only chemical compounds assigned with food grade status and/or a FEMA (Flavor and Extract Manufacturers Association) number, i.e. compounds that are 'generally recognised as safe' (GRAS) for their intended use as flavour ingredients (Marnett et al., 2013), were sourced for testing.

### 3.3 Screening of chemical reference standards by expert panel

Selected chemicals were screened based on perceived typicality of the positive characteristic sensory attributes ascribed to freshly prepared honeybush tea infusions. In addition, chemicals were screened for typicality of negative aroma attributes (often associated with taints related to poor processing and storage methods) as perceived in the respective reference teas. Certain compounds were screened for more than one aroma attribute within the lexicon (**Table 3**).

#### *3.3.1 Sensory profile of base tea*

Use of a base tea for evaluation of potential chemical reference standards is essential to improve concept alignment among assessors, i.e. to understand each lexicon attribute as it is perceived in the product (Drake & Civille, 2003). For example, to provide a wine aroma background, Noble et al. (1987) prepared standards in neutral red or white wines, i.e. wines that were free of defects and with typical aromas at low intensities. Similarly, white wine (Etaio et al., 2012) and red wine (Etaio et al., 2010) bases were used in the development of chemical reference standards for PDO Bizkaiko txakolina white wine and Rioja Alavesa young red wine, respectively. Meilgaard, Reid and Wyborsk (1982) assessed potential chemical-based reference standards for beer in a 'relatively bland' beer matrix. Murray and Delahunty (2000) recommended the application of chemical references in a modified cheese matrix to best represent the aroma and flavour attributes associated with Cheddar cheese. In the present study, the expert panel described the honeybush base tea, a combination of three *Cyclophia* species, as 'woody with subtle floral, fruity and sweet-associated aroma notes'.

#### *3.3.2 Selection of chemical reference standards from literature (aroma description vs. perception)*

The aroma character of each chemical was assessed in the base tea at an intensity level which provided a perceptible aroma. Noble et al. (1987) suggested that for training purposes, the intensity of a reference standard should be representative of the levels at which an aroma characteristic may be encountered. However, to illustrate a specific note, an intensity which provides an obvious aroma perception is recommended.

Lawless and Civille (2013) proposed that an initial literature search could disclose useful references for descriptors from different product categories. However, the aroma description of a chemical given by different literature sources is not consistent, and the aroma description from literature was often found to be misleading in the present study. In several instances, the literature description of the aroma associated with a chemical differed from that perceived by the expert panel in the honeybush base tea infusion. For example, ethyl isobutyrate is categorised in the 'sweet spices' class by Acree and Arn (2004), but described as 'sweet, ethereal, fruity and floral' by Arctander (1969) and 'ripe or fermented apple' by the expert panel when added to the honeybush base tea. The aroma characteristics of chemicals may change as the product

matrix and composition changes, as reviewed by Chambers and Koppel (2013) for hexanal and 3-methyl-1-butanol. Similarly, San-Juan et al. (2011) demonstrated changes in Spanish red wines from a 'fresh fruit' to 'dry fruit' character due to the suppressing effect of 4-ethylphenol, acetic acid, phenylacetaldehyde, and methional, especially in the presence of  $\beta$ -damascenone and  $\beta$ -ionone. For the present study, interactions of the aroma chemicals with the honeybush matrix are unknown and may have influenced the perceived aroma and aroma intensity of the chemicals.

In addition, the perceived aroma of a chemical may change at different concentration levels. Only limited literature sources and chemical databases ([www.thegoodscentscompany.com](http://www.thegoodscentscompany.com)) specify the aroma description of a chemical with details of concentration and solvent, for example, at 1% in propylene glycol vs. 10% in propylene glycol. The aroma of (*E*)-2-hexenal identified for the 'apple' attribute is described by Arctander (1969) as 'powerful green-fruity, pungent vegetable-like' which becomes 'pleasant fruity and fresh-green' at dilutions < 0.1%. Similarly, Hongsoongnern and Chambers (2008) reported notable changes in the aroma character of certain chemicals at different concentrations during the development of a 'green' aroma lexicon. These findings illustrated that it is critical to specify the concentration and solvent when a chemical is used as a reference standard. Several lexicons indicate the preparation method for specific reference standards (Etaio et al., 2010, 2012; Larssen, Monteleone, & Hersleth, 2018; López-López, Sánchez-Gómez, Montañó, Cortés-Delgado, & Garrido-Fernández, 2018; Pérez-Elortondo et al., 2007; Suwonsichon, Chambers IV, Kongpensook, & Oupadissakoon, 2012).

### 3.3.3 Selecting reference standards from literature (character-impact compounds)

Volatile chemicals that contribute to the aroma of the principal sensory identity of a product are commonly referred to as character-impact compounds (Molnár, 2009). These compounds can be classified into four groups: 1) the characteristic aroma conclusively determined by a single character-impact compound; 2) the characteristic aroma due to a combination of a small number of compounds; 3) the characteristic aroma replicated, using a large number of compounds; and 4) no character-impact compounds have been identified, and therefore the aroma cannot be reproduced adequately (Molnár, 2009). Groups 3 and 4 are relevant in the case of thermally processed foods (coffee and bread) and fermented foods (red wine, beer, cocoa and tea), and pose a challenge in selecting an appropriate chemical as reference standard. Processing of honeybush tea entails a high temperature oxidation process of the plant material (Bergh et al., 2017).

Several single character-impact compounds identified from literature or from GC-MS data were screened for lexicon attributes. (*E*)-Cinnamaldehyde, the character-impact compound for cinnamon (Fischetti, 2010) perceived as 'subtle spice' by the expert panel, was screened as potential reference standard for 'sweet spice'. However, it was not included in the final validation set as the expert panel regarded the aromas of eugenol and 2,4-heptadienal (both identified in honeybush by GC-MS analysis) more characteristic. Eugenol is the character-impact compound for cloves (Fischetti, 2010) and was also

perceived by the expert panel as 'clove-like'. Benzaldehyde (identified in honeybush by GC-MS analysis), the character-impact compound for almond (Fischetti, 2010), was perceived as 'almond, marzipan' and selected for the 'nutty' attribute. Contrastingly,  $\delta$ -decalactone identified as an odour impact compound for apricot in sweet Fiano wine (Genovese, Gambuti, Piombino, & Moio, 2007), were perceived as 'peach, butter, coconut' in honeybush base tea. Similarly,  $\beta$ -damascenone, also considered as a character-impact compound for apple (Cunningham, Acree, Barnard, Butts, & Braell, 1986) and identified in honeybush, was perceived 'peach-like', and rejected for further validation as reference standard for 'apple'. In a study on the characterisation of aroma-active compounds in fermented *C. subternata* by GC-O and GC-MS analysis, Le Roux et al. (2012) suggested that  $\beta$ -damascenone probably contributes to the sweet background of the tea infusion rather than representing a character impact compound.

The selection of chemicals for the fruity aroma attributes, such as 'apple' and 'apricot' represented challenges as a character impact aroma for fruit is often elicited by a synergistic blend of several aroma chemicals. The aroma of *n*-hexanal (component of natural apple flavour) is reminiscent of 'green, painty, rancid oil'. However, in combination with character-impact compounds, ethyl 2-methyl butyrate and 2-hexenal, the characteristic aroma note of 'apple' is perceived (Flath, Black, Guadagni, McFadden, & Schultz, 1967). Both *n*-hexanal and (*E*)-2-hexen-1-al (perceived as 'fresh, green apple') were identified in honeybush by GC-MS, and the latter was selected for further validation as potential chemical reference standard for the 'apple' attribute. Furthermore, the chemical references standards were required to represent a processed fruit character, i.e. 'dried apricot or apricot jam' and 'cooked apple or apple pie' as per lexicon descriptions for 'apricot' and 'apple' attributes, respectively. For the 'apricot' attribute eight chemicals were screened of which 2-methylbutanal (perceived as 'dried-fruit') and methyl isobutyrate (perceived as 'cooked fruit, caramel-like') were selected for their 'processed apricot' character perceived in the specific reference tea for 'apricot'.

For the 'lemon/lemongrass' attribute, 6-methyl-5-hepten-2-one (perceived as 'lemon') and the character-impact compound for lemon, citral (Fischetti, 2010) (perceived as 'artificial lemon-flavoured sweets/candy'), were screened but only the former chemical was selected for further validation. Both chemicals were identified in honeybush. GC-MS results indicated the presence of 6-methyl-5-hepten-2-one at high quantities (**Table 2**) even though the intensity and occurrence frequency of the 'lemon/lemongrass' attribute are normally extremely low (Erasmus et al., 2017), as also depicted in the revised honeybush wheel (**Fig. 3**).

Screening of chemicals for the negative honeybush attributes (taints) is particularly important since their presence is detrimental to the quality and ultimately consumer acceptance of the herbal tea. For example, the 'green grass' attribute is associated with under-fermented honeybush (Du Toit & Joubert, 1999) and (*Z*)-3-hexen-1-ol, character-impact for 'green leafy' (Fischetti, 2010), was selected for further validation. Furthermore, a combination of low temperatures and excessive long fermentation periods



favours the development of off-odours, or taints associated with poor quality products (Bergh et al., 2017). Chemicals associated with 'burnt' may impart an undesired 'tobacco' or 'smoky' aroma. These aroma notes are not typical of the 'burnt caramel' attribute associated with honeybush. The character-impact compound for *inter alia* burnt sugar aroma, 4-hydroxy-2,5-dimethyl-3(*H*)-furanone imparts a sweet caramel, burnt-sugar flavour with noticeable fruitiness to beer, Arabica coffee and white bread crust (Acree & Arn, 2004; Hodge, Mills, & Fisher, 1972). This compound was therefore selected for further validation of the 'burnt caramel' attribute of honeybush. For the 'smoky' attribute, guaiacol, character-impact for 'smoke' (Fischetti, 2010) was selected. Interestingly, guaiacol was used at 350 µL for 'smoky' attribute, whereas it was perceived as 'woody' at a lower concentration (100 µL), confirming that the perceived aroma of a chemical may change at different concentration levels.

Several chemicals associated with a 'rotten' or 'sulfurous/vegetable' character were screened for the 'rotting plant water' attribute. Thiols, in particular, impart off-flavours to beer (Vermeulen, Gijs, & Collin, 2005; Walker, 1995) and wine (Swiegers, Bartowsky, Henschke, & Pretorius, 2005). San-Juan et al. (2011) indicated that the combined impact from dimethylsulfide (DMS), 1-hexanol and methanethiol could be related to the 'vegetal' character of Spanish red wines. Ethanethiol was selected for 'rotting plant water' for further validation as it is regarded as the chemical responsible for the 'putrefaction' taint typically found in beer (Baxter & Hughes, 2001), and dimethyl trisulfide (perceived as 'drain-like and rotting cabbage') was also selected. Methional and methionol, perceived by the expert panel as 'potato or cooked vegetable water' and 'cabbage-like', respectively, were selected for the 'cooked vegetables' attribute.

Methyl salicylate, the character-impact compound for wintergreen (Fischetti, 2010), was screened for the negative attribute, 'medicinal/rubber' ('Band-aid®') but rejected for its prominent 'bubble gum' aroma and low intensity of the 'Band-aid®' character. However, *p*-ethylphenol (also known for imparting *Brettanomyces* character in wine (Suárez, Suárez-Lepe, Morata, & Calderón, 2007)) and *p*-cresol (perceived as 'medicinal, plaster-like' by the expert panel) were selected for 'medicinal/rubber' attribute. Geosmin is an off-odour-impact compound for 'earthy-musty', imparting an undesirable 'earthy' taint to drinking water (Parker, 2015). (*R/S*)-Geosmin is available as reference standard in commercial aroma kits ([www.aroxa.com](http://www.aroxa.com); [www.flavoractiv.com](http://www.flavoractiv.com)) and was selected for the 'dusty' attribute of the honeybush lexicon.

The 'hay/dried grass' attribute is associated with under-fermented honeybush (Bergh et al., 2017) and is regarded as a taint when present at high intensities. Further research is still required to establish at which intensity level 'hay/dried grass' is acceptable as intrinsic to the characteristic aroma profile of honeybush and at which concentration level it should be classified as a taint (Bergh et al., 2017). Nonanal is used in certain commercial aroma kits as a standard for 'hay' character. Its aroma has been described as 'hay, like dried grass or cucumber skin' according to the AROXA™ flavour ingredients range ([www.aroxa.com](http://www.aroxa.com)) and as 'dry hay or straw' according to FlavorActiV™ ([www.flavoractiv.com](http://www.flavoractiv.com)). In the



present study, both nonanal and *o*-cresol were perceived as ‘hay-like’ during screening and therefore selected for further validation.

A total of 11 compounds identified from honeybush GC-MS data, were selected as potential chemical reference standards for further validation by the trained panel (**Table 3**).

### 3.4 Validation of chemical reference standards for the honeybush lexicon

DSA was conducted to determine how representative the aroma chemicals were of the target aromas in the respective reference teas. Given the complexity of the honeybush aroma profile, the focus was on typicality as it forms the basis of selecting a suitable chemical standard. It was important that the intensity of the respective aroma notes was not particularly high in the base tea, as it served as a neutral matrix. When a chemical was added to the base tea, the objective was to achieve a high typicality score at an intensity similar to the target aroma note for which the reference tea was selected.

The mean intensity and typicality scores for the honeybush attributes are presented in **Table 4**. The majority of the typicality scores for the perceived aroma of the chemicals differed significantly ( $p < 0.05$ ) from that of the target aroma in the respective specific reference teas. One could argue that the aroma of one chemical compound does not necessarily elicit a similar aroma perception to that of the reference tea. In contrast, the typicality scores of aroma chemicals evaluated for several lexicon attributes, i.e. 2-acetyl-5-methylfuran (‘woody’), levulinic acid (‘fynbos-sweet’), maltol isobutyrate (‘caramel’), and 2-acetylpyrrole (‘nutty’) did not differ significantly ( $p \geq 0.05$ ) from that of the respective reference teas. Subsequently, these aroma chemicals could, therefore, be regarded as a better match and thus highly suitable reference standards for the lexicon. Many chemicals had high typicality scores of  $\geq 70$ , yet were significantly different from the reference tea ( $p < 0.05$ ), e.g. geranyl acetone for ‘fynbos-floral’, nerol and geraniol for ‘rose geranium’, maltol isobutyrate and 2-methylbutanal for ‘apricot’, (*E*)-2-hexen-1-al for ‘apple’,  $\beta$ -cyclocitral for ‘raisin’, propyl propionate and geranyl isovalerate for ‘fruity-sweet’, ethyl maltol for ‘caramel’ and benzaldehyde for ‘nutty’ (**Table 4**). These chemicals merit further investigation in terms of the effect of dosage on typicality. Chemicals for ‘rose perfume’, ‘lemon/lemon grass’, and ‘honey’ had lower ( $\leq 65$ ) typicality scores and may be revised by either amending their concentration levels, or other chemicals could be investigated.

For the negative attributes (taints), only a few chemicals received high typicality scores ( $\geq 70$ ) compared to those for the positive attributes, i.e. *o*-cresol for ‘hay/dried grass’, (*Z*)-3-hexen-ol for ‘green grass’, *p*-cresol for ‘medicinal/rubber’ and 3-ethylpyridine for ‘smoky’. The typicality scores of chemicals for ‘dusty’, ‘cooked vegetables’, ‘rotting plant water’ and ‘burnt caramel’ were particularly low and should be revised by either amending their concentration levels, or alternative chemicals should be investigated. In many instances character-impact is produced by a synergistic blend of several aroma chemicals that contribute to a recognisable sensory impression when evaluated, as discussed previously. More than one

compound may be responsible for off-odours present, for example Masanetz and co-workers (1998) reported that (*Z*)-1,5-octadien-3-one and methional impart the 'fishy' taint found in dried spinach, although neither had a 'fishy' character when assessed individually.

In addition, the most suitable chemical-based reference standard(s) per attribute can also be derived from the results. For instance,  $\beta$ -cyclocitral had a significantly ( $p < 0.05$ ) higher typicality score than  $\beta$ -damascenone and the former compound could be regarded as a more suitable reference standard for 'raisin'. Both nerol and geraniol may be regarded as suitable reference standards for 'rose geranium' as their typicality scores did not differ significantly ( $p \geq 0.05$ ).

For five honeybush attributes the mean intensity values of one/both aroma chemical(s) in the base tea and the respective reference teas did not differ significantly ( $p \geq 0.05$ ). It is important to note that reference samples that exhibited the highest perceived intensity of the attributes were selected from the in-house sample collection.

Additionally, Lawless and Civille (2013) emphasised that original literature references should be cited to allow researchers to cross-reference attributes and corresponding reference standards across studies. For example, Galán-Soldevilla et al. (2005) and Bárcenas et al. (1999) cited references for floral honey and ewes milk cheese, respectively. Furthermore, it is essential to specify the concentration and/or preparation method of the chemical references for assessment in the lexicon, as discussed previously. The updated honeybush lexicon is presented in **Table 5**, illustrating general and specific sensory attributes, attribute descriptions, chemical reference standard information (compound and concentration) and sources of information.

## 4 Conclusions

Chemical reference standards have been tested and validated for the honeybush lexicon, using the typicality score as parameter for inclusion. For several target aroma notes high typicality scores were obtained, yet single aroma chemical compounds did not fully represent complex target aromas such as 'fynbos-floral', 'rose perfume', 'burnt caramel' or 'rotting plant water'. An important outcome was the high typicality scores for aroma notes that are generally unknown, but typical of honeybush, namely geranyl acetone for 'fynbos-floral' and levulinic acid for 'fynbos-sweet'. The use of a large sample set of honeybush also enabled updating of the generic honeybush aroma wheel. Future research could evaluate different concentrations of selected chemicals to improve the perceived aromas of the reference standards. Specifically, chemicals for negative attributes such as 'dusty', 'cooked vegetables', 'rotting plant water' and 'burnt caramel' should be revised for improved assessor training in taint recognition for quality control.

Furthermore, the addition of a complementary nominal scale for each reference standard could aid scoring of the perceived intensity of a target aroma.

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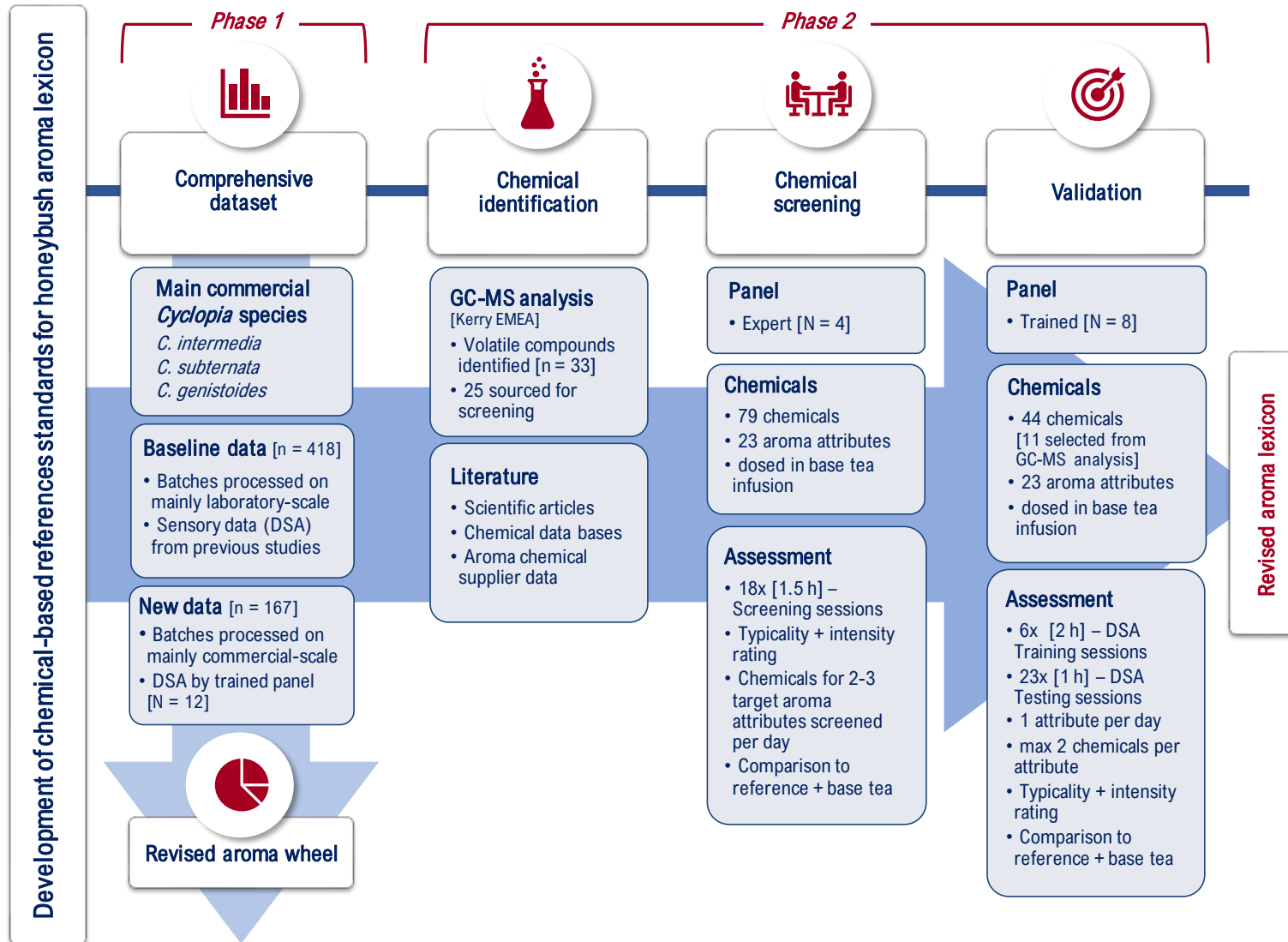
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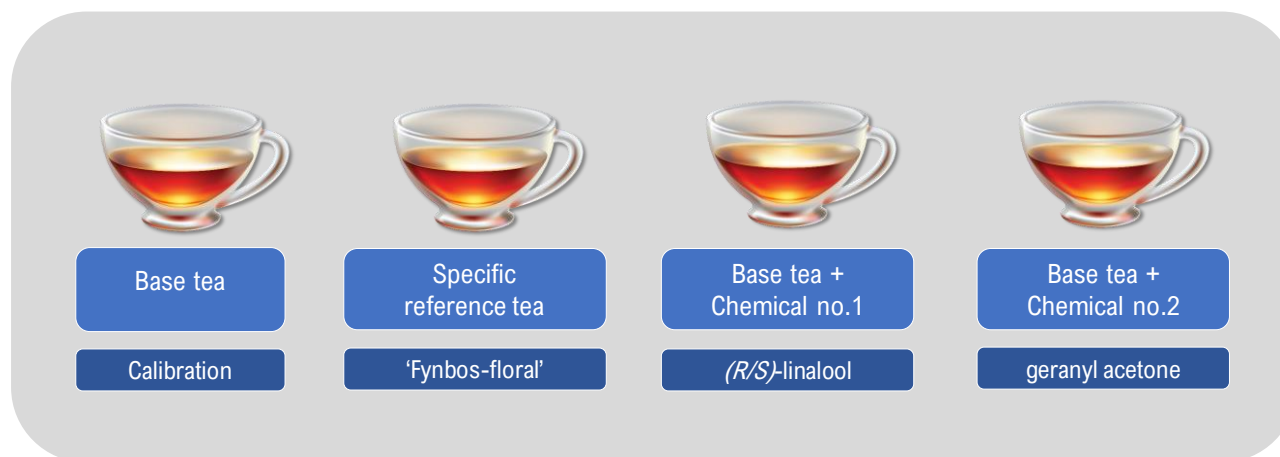
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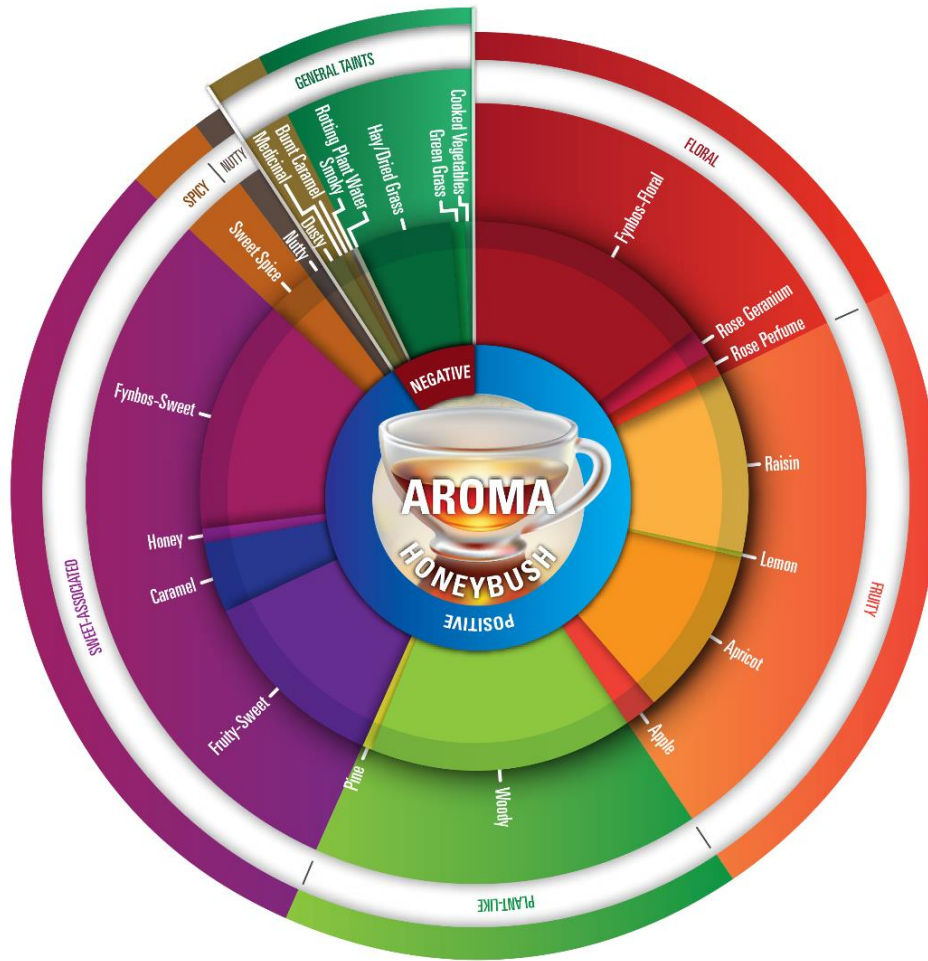


**Figure 1** Experimental lay-out for the revision of the honeybush aroma lexicon and wheel.

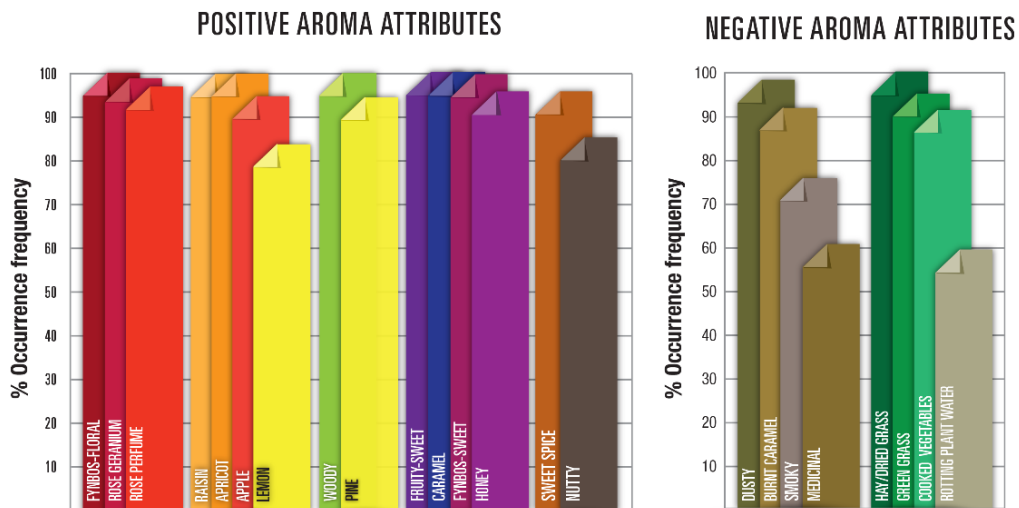


**Figure 2** Assessment of a target aroma descriptor, e.g. 'fynbos-floral' of the honeybush lexicon. 'Base tea' without the addition of a chemical served as calibration sample. The perceived aroma typicality and intensity of each chemical in the 'base tea' were compared to that of the specific 'reference tea' selected for a high intensity of the target aroma, e.g. 'fynbos-floral'.

A)



B)



**Figure 3** The revised generic honeybush aroma wheel (A), compiled from sensory data for *C. intermedia*, *C. subternata* and *C. genistoides*, depicts relative intensities (width of a wedge) of 23 aroma attributes. The bar graphs (B) indicate the occurrence frequency (%) of positive and negative aroma attributes in the full sample set (n = 585) (graphic design by TBND Design Studio, Stellenbosch, South Africa).

**Table 1** Fermented honeybush samples (n = 585) used to establish a comprehensive dataset on major commercial *Cyclopi*a species through combination of data from previous research (2010–2016) and the current study.

Spp.	SAMPLES IN BASELINE DATASET ( <i>previous research</i> )					SAMPLES IN NEW DATASET ( <i>current study</i> )				
	Analysis date	N	Processing date	Processing scale	Processing temp/time	Analysis date	N	Processing date	Processing scale	Processing temp/time
<i>C. intermedia</i>	2013	3	2013 <sup>a</sup>	Laboratory	80 °C/16 h	2017	5	2011	Commercial	unknown
		3	2013 <sup>a</sup>	Laboratory	80 °C/24 h		7	2012	Commercial	unknown
		3	2013 <sup>a</sup>	Laboratory	80 °C/36 h		4	2013	Commercial	unknown
		3	2013 <sup>a</sup>	Laboratory	80 °C/48 h		4	2014	Commercial	unknown
		3	2013 <sup>a</sup>	Laboratory	90 °C/16 h		5	2015	Commercial	unknown
		3	2013 <sup>a</sup>	Laboratory	90 °C/24 h		7	2016	Commercial	unknown
		3	2013 <sup>a</sup>	Laboratory	90 °C/36 h		12	2017	Commercial	unknown
		3	2013 <sup>a</sup>	Laboratory	90 °C/48 h		10	2017	Laboratory	90 °C/24 h
	2014	4	2014 <sup>a</sup>	Commercial	90 °C/16 h	2018	14	2017	Commercial	unknown
		4	2014 <sup>a</sup>	Commercial	90 °C/24 h		29	2018	Commercial	unknown
		4	2014 <sup>a</sup>	Commercial	90 °C/36 h					
		4	2014 <sup>a</sup>	Commercial	90 °C/48 h					
		4	2014 <sup>a</sup>	Laboratory	90 °C/16 h					
		4	2014 <sup>a</sup>	Laboratory	90 °C/24 h					
		4	2014 <sup>a</sup>	Laboratory	90 °C/36 h					
4		2014 <sup>a</sup>	Laboratory	90 °C/48 h						
2016	42	2016 <sup>b</sup>	Commercial	unknown						
<i>C. subternata</i>	2010	6	2010 <sup>c,d</sup>	Laboratory	80 °C/24 h	2018	38	2018	Commercial	unknown
		6	2010 <sup>c,d</sup>	Laboratory	90 °C/16 h					
	2012	8	2012 <sup>d</sup>	Laboratory	80 °C/24 h					
		8	2012 <sup>d</sup>	Laboratory	90 °C/16 h					
	2013	8	2013 <sup>d</sup>	Laboratory	80 °C/24 h					
		8	2013 <sup>d</sup>	Laboratory	90 °C/16 h					
	2014	29	2014 <sup>e,f</sup>	Laboratory	90 °C/16 h					
	2015	68	2015 <sup>e,f</sup>	Laboratory	90 °C/16 h					
2016	57	2016 <sup>e,f</sup>	Laboratory	90 °C/16 h						
	24	2016 <sup>g</sup>	Laboratory	90 °C/16 h						
<i>C. genistoides</i>	2010	6	2010 <sup>c,d</sup>	Laboratory	80 °C/24 h	2018	32	2018	Commercial	unknown
	2010	6	2010 <sup>c,d</sup>	Laboratory	90 °C/16 h					
	2012	8	2012 <sup>d</sup>	Laboratory	80 °C/24 h					
	2012	8	2012 <sup>d</sup>	Laboratory	90 °C/16 h					
	2013	8	2012 <sup>d</sup>	Laboratory	80 °C/24 h					
	2013	8	2012 <sup>d</sup>	Laboratory	90 °C/16 h					
	2015	17	2015 <sup>e</sup>	Laboratory	80 °C/24 h					
	2015	13	2015 <sup>e</sup>	Laboratory	90 °C/16 h					
	2016	24	2016 <sup>g</sup>	Laboratory	80 °C/24 h					
TOTAL	418				TOTAL	167				

<sup>a</sup> Bergh (2014), Bergh et al. (2017); <sup>b</sup> unpublished; <sup>c</sup> Theron et al. (2014); <sup>d</sup> Erasmus (2014), Erasmus et al. (2017); <sup>e</sup> Bester, Joubert and Joubert (2016); <sup>f</sup> Robertson et al. (2018); <sup>g</sup> unpublished

**Table 2** Volatile organic compounds identified by GC–MS analysis in the aroma of a blend (1:1:1:1) of fermented *Cyclopia genistoides*, *C. subternata*, *C. maculata* and *C. longifolia* (major compounds are highlighted in **bold**).

Compound	% <sup>a</sup>	Aroma description <sup>b</sup>
3-methylbutanal	0.23	malt, cocoa, peach-apricot (dried)
2-methylbutanal	0.15	malt, cocoa, peach-apricot (dried)
2-ethylfuran	0.35	solvent-acetone, earthy-muddy, musty water, cocoa
hexanal	3.19	fatty, rancid oil, green, old apple core, nutty (raw peanut)
<i>trans</i> -2-hexenal	0.68	leafy-grassy-green, green apple, slightly fatty
heptanal	0.51	green, fatty, slightly goaty
benzaldehyde	0.24	almond, cherry
anhydrolinalool oxide (6-ring form)	0.23	distilled lime note, woody, slightly floral
<b>6-methyl-5-hepten-2-one</b>	<b>14.09</b>	<b>herbal-green-fruity, citrus-lemongrass notes</b>
myrcene	2.64	green-resinous, citrus-lime
ocimene quintoxide	0.69	distilled lime note, camphor, minty
2,4-heptadienal	1.15	fatty, spicy-cinnamon, citrus
limonene	1.38	mild citrus, terpenic (solvent-like, paint thinners), slightly pungent
2,2,6-trimethylcyclohexanone	0.42	characteristic tobacco, camphor, woody
$\beta$ -pinene	0.58	mild, resinous, pine, woody
ocimene	1.14	citrus (lime), herbal-floral, anise notes
isophorone	0.84	sweet, woody-tea, tobacco, honey, camphor, green
3,5-octadien-2-one	1.60	woody-nutty, fatty-creamy-dairy, fresh green tea, mushroom
<b>linalool</b>	<b>40.79</b>	<b>floral (jasmine, lavender, tea), woody, coriander, citrus</b>
nerol oxide	1.24	green-floral, geranium
<i>trans</i> -2-nonenal	0.37	fatty-green, cucumber-melon, woody-planty, cardboard-like
safranal	0.59	characteristic saffron, tobacco, tea, woody-medicinal-phenolic
$\beta$ -cyclocitral	2.71	fruity (dried), berry, grassy-green-hay, floral-saffron, woody, tobacco
camphene	1.68	camphor, dusty-earthly ('starting to rain')
citral	2.01	characteristic lemon, floral-rose, fruity
<b>eugenol</b>	<b>4.13</b>	<b>characteristic clove, woody-metallic, spicy, phenolic-medicinal, floral-carnation</b>
$\beta$ -damascenone	2.19	dried fruit (apricot, raisin), tobacco, floral (red rose), berry, woody, apple/plum
<i>cis</i> -jasmone	0.32	characteristic jasmine-floral, tea
$\beta$ -damascone	1.34	berry, plum, floral (red rose), apple, tea, tobacco
geranyl acetone	2.44	floral, rosy-green-fruity
<b><math>\beta</math>-ionone</b>	<b>4.45</b>	<b>woody, fruity-berry-raspberry, violet-floral</b>
$\delta$ -decalactone	1.89	buttery-creamy, peach-apricot, coconut
<b>several isomers of megastigmadi</b> <b>/trienones</b>	<b>3.75</b>	<b>fruity (dried fruit/dates), tobacco, woody, characteristic boronia flowers</b>

<sup>a</sup> Based on corrected GC peak area (Kerry EMEA, Durban, South Africa).<sup>b</sup> Aroma descriptors (Kerry EMEA, Durban, South Africa).

**Table 3** Chemicals screened for the 23 generic honeybush (*Cyclopia* spp.) aroma lexicon attributes in order of testing.

General attribute	Specific attribute	Chemical	FEMA <sup>a</sup> no	
Floral	Fynbos-floral	geranyl acetone <sup>b</sup>	3542	
		( <i>E</i> )- $\beta$ -ionone <sup>b</sup>	2595	
		( <i>R/S</i> )-linalool <sup>b</sup>	2635	
		( <i>R/S</i> )- $\beta$ -damascone <sup>b</sup>	3243	
		( <i>Z</i> )-jasnone <sup>b</sup>	3196	
		geranyl formate	2514	
		2-nonanone	2785	
	Rose geranium	nerol	2770	
		geraniol	2507	
	Rose perfume	phenethyl acetate	2857	
		phenylacetaldehyde	2874	
	Plant-like	Woody	isophorone <sup>b</sup>	3553
			3,5-octadien-2-one <sup>b</sup>	4008
2,2,6-trimethylcyclohexanone <sup>b</sup>			3473	
anhydrolinalool oxide <sup>b</sup>			3759	
guaiacol			2532	
2-acetyl-5-methylfuran			3609	
Pine			camphene <sup>b</sup>	2229
		ocimene quintoxide <sup>b</sup>	3665	
		bornyl acetate	2159	
		D-camphor	2230	
Fruity		Apricot	$\delta$ -decalactone <sup>b</sup>	2361
			$\beta$ -cyclocitral <sup>b</sup>	3639
			$\beta$ -damascenone <sup>b</sup>	3420
			2-methylbutanal <sup>b</sup>	2691
			geranyl isovalerate	2518
	maltol isobutyrate		3462	
	isoamyl isobutyrate		3507	
	heptyl acetate		2547	
	Apple	( <i>E</i> )-2-hexen-1-al <sup>b</sup>	2560	
		ethyl isobutyrate	2428	
		( <i>E</i> )-2-heptenal	3165	
	Raisin	$\beta$ -cyclocitral <sup>b</sup>	3639	
		$\beta$ -damascenone <sup>b</sup>	3420	
		( <i>R/S</i> )- $\beta$ -damascone <sup>b</sup>	3243	
		megastigma-4,6,8-trien-3-one <sup>b</sup>	4663	
	Lemon	( <i>E/Z</i> )-citral <sup>b</sup>	2303	
		6-methyl-5-hepten-2-one <sup>b</sup>	2707	
		( <i>R/S</i> )-ocimene <sup>b</sup>	3539	
myrcene <sup>b</sup>		2762		
$\alpha$ -terpinene		3558		
( <i>E</i> )-2-octenal	3215			

Table 3 (continued)

General attribute	Specific attribute	Chemical	FEMA <sup>a</sup> no	
Sweet-associated	Fynbos-sweet	( <i>R/S</i> )- $\delta$ -decalactone <sup>b</sup>	2361	
		phenethyl alcohol	2858	
		levulinic acid	2627	
	Fruity-sweet	(R/S)- $\delta$ -decalactone <sup>b</sup>	isoamyl isobutyrate	2361
			geranyl isovalerate	3507
			2-nonanone	2518
			2-pentylfuran	2785
			diethyl succinate	3317
			maltol	2377
			ethyl propionate	3462
			propyl propionate	2456
			2958	
	Honey	methyl phenylacetate	2733	
	Caramel	maltol	ethyl maltol	2656
			furfuryl alcohol	3487
			levulinic acid	2491
			maltol	2627
			maltol isobutyrate	3462
	Spicy	Sweet spice	eugenol <sup>b</sup>	2467
( <i>E,E</i> )-2,4-heptadienal <sup>b</sup>			3164	
( <i>E</i> )-cinnamaldehyde			2286	
dihydrocoumarin			2381	
Nutty	Nutty	2-acetyl-5-methylfuran	3609	
		benzaldehyde <sup>b</sup>	2127	
		5-methyl-2-hepten-4-one	3761	
		2-acetylpyrrole	3202	
Vegetative taint	Hay/dried grass	octanal	2797	
		( <i>E</i> )-2-nonenal <sup>b</sup>	3213	
		camphene <sup>b</sup>	2229	
		<i>o</i> -cresol	3480	
		( <i>Z</i> )-4-heptenal	3289	
		3-methyl-2,4-nonanedione	4057	
		nonanal	2782	
	Green grass	( <i>Z</i> )-3-hexen-1-ol	2563	
	Rotting plant water	2-methoxythiophenol	ethanethiol	4159
			dimethyl trisulfide	4258
			3275	
	Cooked vegetables	methional	methionol	2747
			2-pentylfuran	3415
			benzyl acetate	3317
			S-methylthioacetate	2135
			3876	
	General taint	Burnt caramel	3-ethyl-2,5-dimethylpyrazine	3149
			4-hydroxy-2,5-dimethyl-3(2 <i>H</i> )-furanone	3174
		Medicinal/rubber	methyl salicylate	<i>p</i> -ethylphenol
<i>p</i> -cresol				3156
2337				
Dusty		L-borneol	( <i>R/S</i> )-geosmin	2157
			4682	
Smoky		guaiacol	3-ethylpyridine	3532
			3394	
			furfurylmethyl disulphide	3362

<sup>a</sup> Flavor and Extract Manufacturers Association (FEMA) numbers sourced from FEMA (Washington DC, USA; [www.femaflavor.org](http://www.femaflavor.org)), The Good Scents Company Information System (Oak Creek, WI, USA; [www.thegoodscentscompany.com](http://www.thegoodscentscompany.com)) and Sigma Aldrich (St Louis, MO, USA; [www.sigmaaldrich.com](http://www.sigmaaldrich.com)).

<sup>b</sup> Chemicals identified in 1:1:1:1 blend of fermented *Cyclopia genistoides*, *C. subternata*, *C. maculata* and *C. longifolia* by GC-MS, analyses conducted by Kerry EMEA (Durban, South Africa).



**Table 4** Descriptive sensory analysis (DSA) results for potential chemical reference standards evaluated for honeybush aroma attributes (chemicals that did not differ significantly from the target aroma in the specific reference tea<sup>a</sup> are highlighted in **bold**).

General attributes	Specific attributes	Sample	Typicality	Intensity	
Floral	Fynbos-floral	base tea (control)	92.67a ± 1.15	64.88ab ± 0.92	
		fynbos-floral reference tea	89.21a ± 2.72	64.11b ± 3.56	
		<i>(R/S)</i> -linalool <sup>b</sup>	67.24c ± 0.35	68.10a ± 1.59	
		geranyl acetone <sup>b</sup>	73.67b ± 2.92	52.90c ± 0.38	
	Rose geranium	base tea (control)	94.45ab ± 1.71	48.16b ± 2.21	
		rose geranium reference tea	98.06a ± 1.76	51.25ab ± 1.85	
		nerol	90.76b ± 0.35	50.13b ± 0.33	
		geraniol	90.89b ± 6.59	54.23a ± 1.61	
	Rose perfume	base tea (control)	94.35a ± 0.69	29.76d ± 2.46	
		rose perfume reference tea	95.88a ± 1.48	41.76c ± 1.68	
		phenethyl acetate	48.44c ± 2.48	75.38b ± 1.87	
		phenylacetaldehyde	61.44b ± 1.87	82.49a ± 0.55	
Plant-like	Woody	base tea (control)	76.47b ± 11.14	51.10c ± 1.13	
		<b>woody reference tea</b>	<b>96.31a ± 0.16</b>	68.31b ± 0.63	
		guaiacol	47.92c ± 3.63	75.18a ± 2.69	
		<b>2-acetyl-5-methylfuran</b>	<b>85.44ab ± 9.83</b>	52.29c ± 1.63	
	Pine	base tea (control)	93.11a ± 0.72	20.18d ± 2.71	
		pine reference tea	93.85a ± 1.59	66.94b ± 0.77	
		bornyl acetate	67.71b ± 1.36	75.83a ± 0.91	
		camphene <sup>b</sup>	51.36c ± 4.49	50.13c ± 1.90	
	Fruity	Apricot	base tea (control)	83.25bc ± 1.07	50.75c ± 1.73
			apricot reference tea	95.11a ± 0.25	77.16a ± 2.62
maltol isobutyrate			75.84c ± 0.53	55.02b ± 2.79	
2-methylbutanal <sup>b</sup>			87.17b ± 7.78	43.94d ± 1.47	
Apple		base tea (control)	97.60a ± 2.08	23.66c ± 2.58	
		apple reference tea	93.92b ± 1.84	48.83b ± 3.43	
		<i>(E)</i> -2-hexen-1-al <sup>b</sup>	72.31c ± 0.45	78.22a ± 0.49	
		ethyl isobutyrate	52.59d ± 0.82	77.85a ± 2.68	
Raisin		base tea (control)	96.18a ± 2.57	27.97c ± 1.88	
		raisin reference tea	96.87a ± 1.62	45.47a ± 1.21	
		β-cyclocitral <sup>b</sup>	89.30b ± 2.15	32.23b ± 2.81	
		β-damascenone <sup>b</sup>	59.43c ± 1.17	28.60c ± 0.69	
Lemon/lemon grass		base tea (control)	19.11c ± 3.50	10.49c ± 2.44	
		lemon/lemon grass reference tea	88.39a ± 5.19	54.10b ± 0.99	
	6-methyl-5-hepten-2-one <sup>b</sup>	63.69b ± 5.31	68.81a ± 1.66		
	<i>(R/S)</i> -ocimene <sup>b</sup>	62.05b ± 5.08	65.70a ± 5.92		
Sweet-associated	Fynbos-sweet	base tea (control)	95.45a ± 0.86	48.71b ± 2.78	
		<b>fynbos-sweet reference tea</b>	<b>91.99b ± 2.60</b>	52.06b ± 1.84	
		phenethyl alcohol	64.03c ± 0.87	62.27a ± 1.38	
		<b>levulinic acid</b>	<b>90.29b ± 2.21</b>	49.44b ± 2.93	
	Fruity-sweet	base tea (control)	90.20a ± 3.85	37.89d ± 3.19	
		fruity-sweet reference tea	95.85a ± 3.59	75.91a ± 2.55	
		geranyl isovalerate	71.41c ± 1.36	49.23b ± 0.19	
		propyl propionate	81.57b ± 2.56	44.35c ± 0.78	
	Honey	base tea (control)	96.01a ± 3.45	20.56c ± 1.13	
		honey reference tea	97.71a ± 2.06	37.25b ± 0.47	
		methyl phenylacetate	65.76b ± 2.19	86.37a ± 1.01	
	Caramel	base tea (control)	94.27a ± 0.70	35.27c ± 5.26	
		<b>caramel reference tea</b>	<b>91.28a ± 2.63</b>	43.22b ± 1.53	
		ethyl maltol	78.15b ± 1.95	73.75a ± 5.58	
		<b>maltol isobutyrate</b>	<b>94.19a ± 1.52</b>	42.01bc ± 2.77	
	Spicy	Sweet spice	base tea (control)	69.68b ± 10.75	17.93d ± 1.81
sweet spice reference tea			95.20a ± 3.03	83.65a ± 2.89	
dihydrocoumarin			67.75b ± 2.03	69.26b ± 1.84	
<i>(E/E)</i> -2,4-heptadienal <sup>b</sup>			67.91b ± 2.48	54.72c ± 6.41	
Nutty	Nutty	base tea (control)	91.50a ± 5.79	22.68c ± 2.07	
		<b>nutty reference tea</b>	<b>99.03a ± 0.91</b>	35.74a ± 3.09	
		benzaldehyde <sup>b</sup>	72.22b ± 2.12	28.79b ± 1.63	
		<b>2-acetylpyrrole</b>	<b>90.93a ± 6.37</b>	23.86c ± 1.68	

**Table 4** (continued)

General attributes	Specific attributes	Sample	Typicality	Intensity
Vegetative taint	Hay/dried grass	base tea (control)	94.75a ± 0.92	21.20c ± 2.45
		hay/dried grass reference tea	94.61a ± 0.97	42.33b ± 0.99
		<i>o</i> -cresol	70.40b ± 2.54	44.04b ± 2.17
		nonanal	58.82c ± 2.76	49.31a ± 1.69
	Green grass	base tea (control)	2.08c ± 3.61	0.00c ± 0.00
		green grass reference tea	95.77a ± 3.66	51.56b ± 0.22
		( <i>Z</i> )-3-hexen-1-ol	79.71b ± 0.13	78.13a ± 0.22
	Rotting plant water	base tea (control)	0.00c ± 0.00	0.00c ± 0.00
		rotting plant water reference tea	96.88a ± 2.71	53.02b ± 0.75
		dimethyl trisulfide	44.26b ± 1.81	93.65a ± 0.09
		ethanethiol	46.23b ± 3.48	95.44a ± 3.27
	Cooked vegetables	base tea (control)	0.00d ± 0.00	0.00d ± 0.00
		cooked vegetables reference tea	98.19a ± 1.58	79.65a ± 0.67
methional		52.38c ± 2.54	74.00b ± 1.15	
methionol		60.94b ± 0.98	43.09c ± 1.60	
General taint	Burnt caramel	base tea (control)	13.32d ± 2.59	10.76d ± 1.23
		burnt caramel reference tea	94.99a ± 0.21	38.41c ± 1.07
		4-hydroxy-2,5-dimethyl-3( <i>H</i> )-furanone	53.48b ± 3.20	56.89b ± 2.58
		3-ethyl-2,5-dimethylpyrazine	29.16c ± 6.76	76.73a ± 3.63
	Medicinal/rubber	base tea (control)	6.46d ± 0.83	6.27c ± 0.04
		medicinal/rubber reference tea	96.71a ± 0.53	69.80b ± 0.12
		<i>p</i> -ethylphenol	69.81c ± 1.08	82.19a ± 4.02
		<i>p</i> -cresol	74.75b ± 0.33	72.13b ± 4.06
	Dusty	base tea (control)	95.42a ± 3.97	16.89d ± 1.33
		dusty reference tea	94.88a ± 4.45	35.24c ± 1.28
		L-borneol	57.81b ± 1.69	84.88a ± 0.84
		( <i>R/S</i> )-geosmin	59.72b ± 1.19	58.96b ± 1.44
	Smoky	base tea (control)	8.32d ± 3.50	0.92d ± 1.04
		smoky reference tea	99.96a ± 0.03	59.90a ± 0.85
		guaiacol	40.22c ± 5.67	29.95b ± 0.62
		3-ethylpyridine	71.23b ± 16.79	11.96c ± 2.19

<sup>a</sup> Honeybush tea previously identified to exhibit a high intensity of the specific target aroma attribute.

<sup>b</sup> Chemicals identified in fermented honeybush by GC-MS.

**Table 5** Updated honeybush aroma lexicon with chemical-based reference standards.

General attributes	Specific attributes	Description of aroma attributes	Reference standards <sup>a</sup>	Information sources <sup>b</sup>
Floral aroma	Fynbos-floral	Sweet, floral aroma note associated with the flowers of fynbos <sup>c</sup> vegetation	1) ( <i>R/S</i> )-linalool (1% in propylene glycol); 400 µL/L tea 2) geranyl acetone (1% in propylene glycol); 240 µL/L tea	A; B; C; D; E B; C; D; E
	Rose geranium	Floral aroma note associated with the rose geranium plant	1) nerol (1% in propylene glycol); 160 µL/L tea 2) geraniol (1% in propylene glycol); 240 µL/L tea	A; B; D; E A; B; D; E
	Rose perfume	Floral aroma note associated with rose petals or rosewater ( <i>Turkish Delight</i> )	1) phenethyl acetate (1% in propylene glycol); 200 µL/L tea 2) phenylacetaldehyde (1% in propylene glycol); 120 µL/L tea	A; B; D; E A; B; D; E
Plant-like aroma	Woody	Aromatics associated with dry bushes, stems and twigs of the fynbos <sup>c</sup> vegetation	1) guaiacol (1% in propylene glycol); 100 µL/L tea 2) 2-acetyl-5-methylfuran (1% in propylene glycol); 200 µL/L tea	A; B; D; E B; D; E
	Pine	Aroma reminiscent of pine needles	1) bornyl acetate (1% in propylene glycol); 200 µL/L tea 2) camphene (1% in propylene glycol); 800 µL/L tea	B; D; E A; B; C; D; E
Fruity aromas	Apricot	Sweet-sour aroma reminiscent of apricot jam or dried apricot	1) methyl isobutyrate (1% in propylene glycol); 920 µL/L tea 2) 2-methylbutanal (0.1% in triacetin); 740 µL/L tea	D; E B; C; D; E
	Apple	The sweet, slightly sour aroma of cooked apples	1) ( <i>E</i> )-2-hexen-1-al (1% in propylene glycol); 140 µL/L tea 2) ethyl isobutyrate (1% in propylene glycol); 160 µL/L tea	B; C; D; E B; D; E
	Lemon/lemongrass	Aromatics associated with general impression of fresh lemons or lemongrass	1) 6-methyl-5-hepten-2-one (1% in propylene glycol); 500 µL/L tea 2) ( <i>R/S</i> )-ocimene (1% in propylene glycol); 640 µL/L tea	B; C; D; E B; C; D; E
	Raisin	Sweet aroma note reminiscent of 'hanepoot' raisin	1) β-cyclocitral (0.1% in propylene glycol); 200 µL/L tea 2) β-damascenone (1% in propylene glycol); 80 µL/L tea	B; C; D; E B; C; D; E
Sweet-associated aromas	Fruity-sweet	Sweet-sour aromatic reminiscent of non-specific fruit	1) geranyl isovalerate (1% in propylene glycol); 320 µL/L tea 2) propyl propionate (1% in propylene glycol); 320 µL/L tea	B; D; E B; C; D; E
	Honey	Aromatics associated with the sweet fragrance of fynbos <sup>c</sup> honey	1) methyl phenylacetate (1% in propylene glycol); 120 µL/L tea	B; D; E
	Caramel	Sweet aromatics characteristic of molten sugar or caramel pudding	1) ethyl maltol (1% in propylene glycol); 240 µL/L tea 2) methyl isobutyrate (1% in propylene glycol); 600 µL/L tea	D; E D; E
	Fynbos-sweet	The sweet aroma note reminiscent of the fynbos <sup>c</sup> plant	1) phenethyl alcohol (1% in propylene glycol); 400 µL/L tea 2) levulinic acid (1% in propylene glycol); 1.36 mL/L tea	D; E D; E
Spicy aroma	Sweet spice	Sweet, woody and spice aroma, including ground cinnamon/cassia bark	1) dihydrocoumarin (1% in propylene glycol); 200 µL/L tea 2) ( <i>E/E</i> )-2,4-heptadienal (0.1% in triacetin); 500 µL/L tea	D; E C; E
Nutty aroma	Nutty	Aromatics associated with fresh walnuts or chopped almonds	1) benzaldehyde (1% in triacetin); 150 µL/L tea 2) 2-acetylpyrrole (1% in propylene glycol); 1 mL/L tea	C; E D; E

Table 5 (continued)

General attributes	Specific attributes	Description of aroma attributes	Reference standards <sup>a</sup>	Information sources <sup>b</sup>
Vegetative taints	Hay/dried grass	Slightly sweet aroma associated with dried grass or hay	1) <i>o</i> -cresol (0.1% in propylene glycol); 640 µL/L tea 2) nonanal ('dry hay' capsule); 1 capsule/L tea	A; C; E F; G
	Green grass	Aroma associated with cut green grass or decomposing cut grass	1) ( <i>Z</i> )-3-hexen-1-ol (1% in propylene glycol); 800 µL/L tea	A; B; D; E
	Rotting plant water	Aromatics associated with the old and rotting vase water of cut flowers	1) dimethyl trisulfide (0.1% in propylene glycol); 10 µL/L tea 2) ethanethiol ('mercaptan' capsule); 1 capsule/1 L, then 800 mL base tea added to 200 mL of spiked solution	D; E E; G
	Cooked vegetables	An overall aroma note associated with canned/cooked vegetables	1) methional (0.1% in propylene glycol); 160 µL/L tea 2) methionol (0.1% in propylene glycol); 940 µL/L tea	D; E D; E
General taints	Burnt caramel	Aroma associated with burnt carbohydrates, especially burnt sugar	1) 4-hydroxy-2,5-dimethyl-3(2 <i>H</i> )-furanone (10% in triacetin); 40 µL/L tea 2) 3-ethyl-2,5-dimethylpyrazine ('burnt caramel' capsule); 1 capsule/1 L, then 750 mL base tea added to 250 mL of spiked solution	A; E A; E; G
	Medicinal/rubber	Aromatic characteristic of Band-aid <sup>®</sup> and antiseptic (TCP)	1) <i>p</i> -ethylphenol (1% in propylene glycol); 200 µL/L tea 2) <i>p</i> -cresol (1% in propylene glycol); 600 µL/L tea	A; B; D; E A; B; D; E
	Dusty	Earthy aroma associated with dry dirt road	1) L-borneol (1% propylene glycol); 5 µL/L tea 2) ( <i>R/S</i> )-geosmin ('dry earth' capsule); 1 capsule/L tea	B; E E; F; G
	Smoky	Smoky aroma note associated with burning hay/grass or tobacco	1) guaiacol (1% in propylene glycol); 350 µL/L tea 2) 3-ethylpyridine (0.1% in propylene glycol); 40 µL/L tea	A; B; D; E D; E

<sup>a</sup> Chemical, and its dilution in brackets; volume of diluted chemical added to base tea.

<sup>b</sup> Information sources: A, Acree and Arn (Acree & Arn, 2004); B, Arctander (Arctander, 1969); C, Kerry EMEA (Durban, South Africa; [www.kerry.com](http://www.kerry.com)); D, Sigma-Aldrich (St Louis, MO, USA; [www.sigmaaldrich.com](http://www.sigmaaldrich.com)); E, The Good Scents Company (Oak Creek, WI, USA; [www.thegoodscentscompany.com](http://www.thegoodscentscompany.com)); F, AROXA<sup>™</sup> (Cara Technology, Leatherhead, UK; [www.aroxa.com](http://www.aroxa.com)); G, FlavorActiv<sup>™</sup> (Aston Rowant, UK; [www.flavoractiv.com](http://www.flavoractiv.com)).

<sup>c</sup> Fynbos is natural shrubland vegetation occurring in the Western Cape, South Africa.

Addendum A (*Supplementary material Chapter 3*)**Table A1** Aroma, flavour, taste and mouthfeel attributes assessed in descriptive sensory analysis of honeybush infusion samples.

General attributes	Aroma descriptors	Flavour descriptors	Taste and mouthfeel descriptors
<b>Floral</b>	<i>Fynbos-floral</i> <sup>a</sup>	<i>Fynbos-floral</i> <sup>a</sup>	Sweet
	<i>Rose geranium</i>	<i>Rose geranium</i>	Sour
	<i>Rose perfume</i>	<i>Rose perfume</i>	Bitter
<b>Plant-like</b>	<i>Woody</i>	<i>Woody</i>	Astringent
	<i>Pine</i>	<i>Pine</i>	
<b>Fruity</b>	<i>Apricot</i>	<i>Apricot</i>	
	<i>Apple</i>		
	<i>Lemon/lemongrass</i>		
<b>Sweet-associated</b>	<i>Raisin</i>	<i>Raisin</i>	
	<i>Fruity-sweet</i>		
	<i>Honey</i>		
	<i>Caramel</i>		
<b>Spicy</b>	<i>Fynbos-sweet</i> <sup>a</sup>		
	<i>Sweet spice</i>	<i>Sweet spice</i>	
<b>Nutty</b>	<i>Nutty</i>	<i>Nutty</i>	
<b>Vegetative taints</b>	<i>Hay/dried grass</i>	<i>Hay/dried grass</i>	
	<i>Green grass</i>	<i>Green grass</i>	
	<i>Rotting plant water</i>	<i>Rotting plant water</i>	
	<i>Cooked vegetables</i>	<i>Cooked vegetables</i>	
<b>General taints</b>	<i>Burnt caramel</i>	<i>Burnt caramel</i>	
	<i>Medicinal/rubber</i>	<i>Medicinal/rubber</i>	
	<i>Dusty</i>	<i>Dusty</i>	
	<i>Smoky</i>	<i>Smoky</i>	

<sup>a</sup> Fynbos is natural shrubland vegetation growing in the Western Cape, South Africa.



**Figure A.1** A) Preparation of honeybush tea infusions, B) dosing of 'base tea' infusions with diluted chemicals or commercial nano-encapsulated chemicals in plastic capsules, C) presentation of infusion samples in a temperature-controlled (65 °C) water bath for screening, training and testing sessions, and D) individual tasting booth in the temperature- and light-controlled sensory research laboratory.

# Chapter 4

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## Classification of fermented honeybush tea sensory quality: development and validation of a quality scoring method

### Abstract

A scientifically founded quality scoring method that incorporates a quality scorecard and colour reference card was developed to evaluate and classify fermented honeybush infusions according to sensory quality. A systematic approach was followed: 1) industry consultation on perceived honeybush sensory quality attributes (survey and interviews), 2) establishment and study of a comprehensive sensory attribute and physicochemical parameter dataset of fermented honeybush samples (n = 585), 3) scorecard development and refinement by expert panel focus groups, and 4) scorecard validation by panels of industry professionals and trained assessors. Appearance ('dry leaf' and 'infusion'), aroma and palate attributes were identified as key sensory quality parameters. Semantic category scales were allocated to facilitate intensity scoring of parameter sub-categories and checkboxes were assigned for citation of 'dry leaf appearance', 'infusion haze' and the presence of positive and negative attributes. Score values (0 to 3) were allocated to the parameter category scales and weights (%) were assigned to the respective parameter sub-categories to obtain a total score (%). *High*, *moderate*, *low* and *poor* sensory quality classes were each defined by the intensity level of parameter sub-categories. DSA data of commercially processed honeybush batches (n = 20) of variable sensory quality was used as 'gold standard' for method validation. Quality classes were pre-assigned to the respective samples according to their DSA attribute intensities. Application of the quality scoring method by the industry and trained panel resulted in classification of samples based on total score values (%), similar to their pre-assigned classes. Product configurations obtained from DSA, scorecard scores and citation frequencies data were compared to assess the discrimination ability of the method. Results from industry and trained panels indicated distinction between 'moderate-high' and 'poor-low' quality groupings, irrespective of expertise' level. The need for industry assessor training in parameter and scale recognition and calibration to facilitate quality classification using the scorecard, was identified. Citation of critical parameter sub-categories such as 'infusion haze' and 'taints' ('smoky', 'medicinal' and 'musty' flavours) would guide processors and blenders in quality control actions. Method user-friendliness and its relevance to address sensory quality within commercial context were indicated.



# 1 Introduction

The advantage of a honeybush quality classification system to obtain the best quality product has long been recognised (Du Toit, Joubert, & Britz, 1998). Current export regulations only specify food safety standards in terms of microbiological and foreign matter content, but no specifications for sensory quality is included (DAFF, 2019). The pressing need for sensory quality control of honeybush tea to foster consumer confidence in the product has been emphasised (Joubert et al., 2019; Joubert, Joubert, Bester, De Beer, & De Lange, 2011).

Good progress has been made in the development of a standardised descriptive vocabulary for honeybush in the form of sensory lexicons and wheels for effective communication between processors, quality control personnel and marketers (Joubert et al., 2019). This includes the recently revised honeybush aroma wheel and lexicon with universal chemical-based reference standards (Chapter 3). However, despite the wealth of information generated from several studies of the sensory characteristics of the herbal teas of a number of *Cyclopia* species over the past 10 years (Joubert et al., 2019), the results have not yet been transformed into defined sensory quality parameters for establishment of a quality standard and classification method. The specific aim of such a classification method would be to assess whether product batches fall within acceptable specifications.

Effective quality assurance and control systems are required to produce consistent products of acceptable quality (Muñoz, 2002). The common approach to food quality control is to define product specifications or quality standards, and to develop and test methods to assess, in a reliable manner, whether the product complies with the requirements of the quality standards (Costell, 2002; Lawless & Heymann, 2010). A distinction has been made between sensory methods for 1) *establishing* of standards and specifications, and methods for 2) *testing* product compliance to these specifications.

The intricate process to establish sound specifications encompasses 1) the selection of samples representative of the variability within the market, 2) the assessment of the perceived magnitude of attributes and/or defects through direct comparison to a product standard or by application of descriptive sensory analysis (DSA), 3) defining the attributes and their variability ranges, and 4) establishment of sensory specifications/limits based on managements' criteria and/or consumer response by assessing consumer acceptability by a large consumer panel (Costell, 2002; Muñoz, 2002). This process signifies the link to all important elements in quality control, i.e. representative sampling, incorporation of product variability and consideration of the management and/or consumer input.

Scientific publications on the systematic approach to the development of a sensory quality classification method that encompasses a quality scorecard, have been limited to calf's meat (Etaio et al., 2013), and wine and cheese products with quality distinctiveness labels, e.g. protected designation of origin (PDO) products (Etaio et al., 2010a, 2012; Ojeda et al., 2015; Pérez-Elortondo et al., 2007). The

development steps employed were 1) acquirement of an expert panel familiar with the product and its sensory attributes, 2) sourcing of products (usually  $n > 50$ ) that represent a range of sensory characteristics and common defects, 3) assessment of samples to produce a list of sensory attributes, 4) selection of parameters that define the typical quality and identification of defects, 5) definition of 'ideal/top situation' per parameter and establishment of scoring criteria based on the presence of desired quality parameters for each major sensory category, 6) allocation of weights based on categories critical and non-critical to overall quality, 7) standardisation of assessment protocol, and 8) development of reference standards per quality attribute for panel training. The relevance and importance of sensory reference development were highlighted in these methods. In Chapter 3 of the present study, the development and verification of chemical reference standards for assessor training was described.

In literature an ongoing debate exists on whether establishment of quality standards should be based on the opinion of experts or that of consumers. The input of sensory professionals and experts with thorough product knowledge to define specifications in development of sensory quality standards and scoring methods has been reported in recent literature (Etaio et al., 2010a, 2012, 2013; Larssen et al., 2019; Ojeda et al., 2015; Pérez-Elortondo et al., 2007). Definition of sensory quality standards by experts is more comprehensive and accurate (Ballester, Dacremont, Le Fur, & Etiévant, 2005; Ojeda et al., 2015).

Reviews of different sensory quality control methods exist, including those that combine rating/scoring with DSA (Lawless & Heymann, 2010; Muñoz, 2002; Rogers, 2010). Lawless and Heymann (2010) recommended the use of 'difference scoring with key attribute scales'. This approach includes degree-of-difference scales (from 'extremely different' to 'match'), intensity or diagnostic scales for scoring of individual key attributes and problematic defects, and checklists or boxes for defects that are intolerable at any level. Two main method development approaches for products with distinctiveness labels performed by small panels (5-12 assessors) exist, namely 1) identification of positive and negative attributes (defects) with use of citation frequencies, resulting in qualitative data, and 2) quantification of attribute intensities on continuous/discontinuous scales, resulting in quantitative data (Pérez-Elortondo et al., 2018). The use of a combination of line/category scales and checkboxes is evident in literature on the development of scorecards for olive oil (Langstaff, 2014), Idiazabal cheese (Ojeda et al., 2015), Rioja Alavesa young red wines (Etaio et al., 2010a), Bizkaia txakoli white wine (Etaio et al., 2012) and other products with quality distinctiveness labels (Pérez-Elortondo et al., 2018). Quality scoring systems have merit if founded on a sound scientific basis (Lawless, 2017). Successful accreditation of such sensory quality scoring methods has been reported (Etaio et al., 2010b; Ojeda et al., 2015; Pérez-Elortondo et al., 2007). These sensory quality scoring methods were aimed at the control and improvement of the quality characteristics of products with PDO status.

In sensory science, the perceived intrinsic quality of a product is often defined as 'the absence of defects' (Lawless & Heymann, 2010). Although this would be the minimum requirement for an acceptable

standard in certain traditional sensory quality assessment methods, for example, dairy products (Bodyfelt, Drake, & Rankin, 2008; Kraggerud, Solem, & Abrahamsen, 2012; Ojeda et al., 2015), this definition does not allow for quality assessment of a product with a complex sensory profile such as honeybush (Moelich, Muller, Joubert, Næs, & Kidd, 2017; Ntlhokwe, Muller, Joubert, Tredoux, & De Villiers, 2018).

To ensure the sustained growth of local and international honeybush tea markets and to add value to the current honeybush breeding programme for the development of 'superior' plant material (Joubert et al., 2019), quality control of honeybush is critical, not only to deliver a consistent quality product, but also to ensure that good quality is not forfeited during the selection process. The aim of the present study was, therefore, to develop and validate a scientifically founded quality scoring method for the evaluation and classification of fermented honeybush tea in terms of generic sensory quality, i.e. irrespective of *Cyclopia* species. In view of the common industry practice of blending production batches of different *Cyclopia* species and of variable quality to supply in demand, sensory classes and specifications for 'high', 'moderate', 'low' and 'poor' quality should be defined. Furthermore, such a method should be user-friendly and provide information on honeybush production batches for effective communication of product quality between industry role-players, i.e. processors, buyers (tea packing companies), and marketers.

The aim of the present study was to develop and validate a quality scoring method for fermented honeybush tea. The objectives were 1) to consult with industry role-players to ascertain the current sensory quality control (QC) practices and requirements, 2) to establish and study a comprehensive sensory attribute and physicochemical parameter dataset reflecting the natural variation within production batches, 3) to develop a quality scorecard through quality parameter identification and specification based on the comprehensive dataset and industry responses, and 4) to validate the method by a panel of industry representatives and trained assessors, respectively.

## 2 Materials and methods

A systematic approach was followed which entailed four phases, namely 1) *consultation* with honeybush industry representatives through a survey and interviews, 2) *establishment* and *study* of a comprehensive sensory attribute and physicochemical parameter dataset of fermented honeybush samples, 3) *development* of sensory quality scoring method that incorporates a quality scorecard and colour reference card, and 4) *validation* of the quality scoring method, using an industry panel and trained assessors. During phase 3, the quality scorecard was refined by expert panel focus groups. The current industry practice of blending tea batches was considered for quality class definitions, namely 'high' (premium quality), 'moderate' (standard quality), 'low' (acceptable to blend with caution into batches of 'moderate' or 'high' quality) and 'poor' (unacceptable). The experimental lay-out of the study is presented in **Fig. 1**.

## 2.1 Industry consultation

Honeybush industry role-players were consulted through a survey and interviews to 1) identify key sensory quality parameters (modalities) for the development of the scorecard and to 2) establish the level of sensory quality assessment and requirements within the current industry.

### 2.1.1 Survey

A survey was conducted to collect information on the sensory quality perspectives and requirements by honeybush industry role-players for the envisaged quality classification method. The role-players consisted of producers, processors, buyers (blending/packing companies) and marketers (national and international), as well as relevant government officials and researchers. The survey was conducted at the annual general meeting of the South African Honeybush Tea Association (SAHTA, George, South Africa, 2018). Attendees were invited to complete the questionnaire on paper (**Addendum B, Fig. B1**). The questionnaire included questions on 1) industry representative type, 2) *Cyclopia species* and origin of plant material (wild-harvested or plantation) processed, 3) perceived importance of sensory quality parameters, 4) quality classification system required (generic or for individual *Cyclopia species*), and 5) indication to partake in the current study, e.g. supply of commercially processed tea samples, testing of quality scoring method as industry assessors, etc. Quality parameters listed in the questionnaire were based on sensory parameters generally evaluated for black tea (*Camellia sinensis*) (ISO, 1982; Liang, Lu, Zhang, Wu, & Wu, 2003; Qu et al., 2019), herbal teas (THIE, 2018) and honeybush (Bergh, 2014). The same survey was also conducted electronically using Checkbox® surveys (Watertown, MA, USA). Survey data of participants (N = 41) were analysed with Statistica data analysis software system (Statistica, version 13, 2018, TIBCO Software Inc., Palo Alto, CA, USA).

### 2.1.2 Interviews

Representatives of honeybush tea processing (N = 4), blending/packing (N = 7) and international marketing (N = 1) companies were interviewed via telephone and/or e-mail to collect information on the current status of the sensory quality of honeybush within industry, in-house sensory quality assessment methods, and market requirements. A tea manager ('tea master') of a national tea packing company of black tea and herbal teas was interviewed on site. Assessment (traditional 'cupping') of honeybush infusions of variable quality by expert tasters of the same company is shown in **Fig. B2 (Addendum B)**. Discussions were also held with a sensory quality control expert with extensive experience in honeybush sensory quality, obtained at a local honeybush processing and marketing company.

## 2.2 Comprehensive dataset

A comprehensive dataset was compiled from a large sample set of fermented honeybush ( $n = 585$ ), comprising the data of the three main commercial *Cyclophia* species, namely *C. intermedia*, *C. subternata* and *C. genistoides*. Samples from production batches were sourced from commercial processors. Batches of honeybush, processed on laboratory-scale using optimum fermentation temperature/time regimes (Bergh, Muller, Van der Rijst, & Joubert, 2017; Erasmus, Theron, Muller, Van der Rijst, & Joubert, 2017) were included in the sample set to ensure samples of good quality. DSA, colour (CIEL\*a\*b\*) and turbidity measurement (NTU) data were studied to 1) identify quality parameter sub-categories within the respective key quality parameters (modalities) identified through industry correspondence and to 2) establish parameter specifications.

### 2.2.1 Samples

Two sets of data were combined to compile the comprehensive dataset, namely 1) baseline data from previous honeybush sensory research (2011-2016;  $n = 418$ ) and 2) new data from analyses conducted during the current study (2017-2018;  $n = 167$ ). Sample details of these two datasets are presented in **Chapter 3 (Table 1)**.

Baseline data comprised of attribute intensities obtained through DSA of fermented honeybush samples (*C. intermedia*,  $n = 98$ ; *C. subternata*,  $n = 222$ ; *C. genistoides*,  $n = 98$ ) in previous honeybush studies. These samples were mainly processed (fermented) on laboratory-scale. For the new dataset in the current study, fermented honeybush samples were mainly sourced from different commercial processors (*C. subternata*,  $n = 38$ ; *C. genistoides*,  $n = 32$ ; *C. intermedia*,  $n = 97$ ). These samples were included in the sample set to increase its robustness and to establish a library of commercially processed samples for the development and validation of the quality scoring method.

### 2.2.2 Descriptive sensory analysis of new samples

DSA was conducted by a trained panel ( $N = 12$ ) to quantify the intensity of infusion aroma, flavour, taste and mouthfeel attributes of the commercial honeybush sample set ( $n = 167$ ), as described in Chapter 3. Aroma refers to odours perceived through orthonasal analysis, while flavour refers to the retronasal perception in the mouth. Similar to flavour, the basic taste modalities, i.e. sweet, sour, and bitter and the mouthfeel attribute, astringency (described as the tactile sensation in the oral cavity), are perceived in the oral cavity (Lawless & Heymann, 2010). Flavour, the basic taste modalities and astringency are often referred to as palate attributes (Moelich et al., 2017; Parr, Ballester, Peyron, Grose, & Valentin, 2015). Infusions were prepared at 'cup-of-tea' strength according to a standard protocol, as described by Erasmus et al. (2017). Samples were tested in triplicate over six consecutive weekdays.

### 2.2.3 Physicochemical analysis

Infusion appearance as a key quality parameter was investigated in terms of colour as an indication of infusion strength, and turbidity. Analyses were conducted on the infusions of all samples. Infusions for each sample were prepared in triplicate, as described by Erasmus et al. (2017).

#### 2.2.3.1 Colour analysis

An aliquot (ca 100 mL) of each infusion was filtered (Whatman no 4) prior to analysis. A Konica Minolta CM-5 chroma meter (Konica Minolta Inc., Osaka, Japan) was used to conduct CIEL\*a\*b\* colour measurements. Manual zero (0%) and white (100%) calibrations were performed using a transmittance zero calibration plate (10 mm) and deionised water, respectively. The L\*, a\* and b\* values of infusions were measured directly in transmittance mode using 10 mm path length polystyrene cuvettes. Each reading was repeated in triplicate, and an average was calculated by the instrument software. Measuring conditions were standardised on D65 illuminant and 10° standard observer. Chroma (C) and hue (h) values of infusions were generated using SpectraMagic NX software (Version 2.5, Konica Minolta Inc.).

#### 2.2.3.2 Turbidity analysis

The turbidity of a 30 mL aliquot of each unfiltered infusion was measured using a HACH 2100N turbidity meter (HACH, Colorado, USA) in nephelometric turbidity units (NTU). The turbidity meter was calibrated using four stabilised formazin turbidity standards, i.e. < 0.1, 20, 200, 1000 NTU (HACH).

### 2.2.4 Statistical analyses

Analyses on the new DSA dataset (Section 2.2.2) were conducted on sample means over assessors, as described in Chapter 3. The experimental design was completely random, with three replicates of each sample served in random order to assessors in three consecutive sessions per day. The reliability of the panel members was evaluated using Panelcheck software (Nofima, Ås, Norway). Univariate analyses were performed using SAS statistical software (Version 9.4; SAS Institute, Cary, USA) to confirm panel reliability (Næs, Brockhoff, & Tomic, 2010) and normality (Shapiro & Wilk, 1965) of data. In the event of the Shapiro–Wilk test indicating significant deviation from normality ( $p < 0.05$ ), outliers were removed when the standardised residuals for an observation deviated more than three standard deviations from the model value. Sensory data were thereafter subjected to analysis of variance (ANOVA) according to the model of the study design. Sample means were compared by calculating Fisher's LSD where a probability level of 5% was considered significant. **Table 1** presents a summary of the distribution of attribute intensities, as well as the physicochemical parameter values in terms of mean, median, maximum, minimum and quartiles (upper and lower) values for the total comprehensive dataset using the full sample set ( $n = 585$ ).

Data distribution was visualised using XLStat (Version 2019.2.1, Addinsoft, Paris, France) by computing scatter plots for the positive and negative sensory modalities, indicating the attribute intensities (x-axis) and the occurrence frequency (expressed as a percentage) of the respective attributes (y-axis). Following confirmation of normal distribution of the data, normal distribution curves of the data of each *Cyclophia* species were constructed. These curves were fitted on the intensity distribution for each aroma attribute to illustrate species differences. In addition, normal distribution curves for the total dataset were fitted to compare total distribution to that of individual species. Distribution of attribute intensities captured in the scatter and normal distribution plots were considered to identify the main (generic) attributes and attributes that are more prominent in certain species, as potential quality parameters for the quality scoring method. Box-and-whisker plots, illustrating the mean, minimum, maximum and range of attribute intensity scores, were computed to graphically summarise the distribution and variation of the sensory and physicochemical data. Confidence intervals and upper/lower bounds from normal distribution curves, as well as the quartile groups in box- and whisker-plots were studied to determine specifications for different intensity ranges per attribute.

### 2.3 Development of sensory quality scoring method (scorecard)

A quality scoring method to assess and classify the sensory quality of fermented honeybush was developed through focus groups. Method ease-of-use in an industry QC environment, QC panel size, industry assessor expertise level and cost implications were considered in the development phase. As previously mentioned, the method encompassed the use of a **quality scorecard** and complementary **infusion colour reference card**. Conclusions derived from industry consultation (Section 2.1) and the comprehensive dataset (Section 2.2) were taken into account in the development of the scorecard.

#### 2.3.1 Scorecard development steps

The development process of the scorecard encompassed the following main steps:

- I) Identification and definition of **key parameters** (modalities) and **parameter sub-categories**
- II) Parameter specifications for four sensory quality classes ('high', 'moderate', 'low' and 'poor')
  - Allocation of **category scales** to parameter sub-categories
    - Allocation of score values (0, 1, 2 or 3) to semantic category scales ('absent/barely perceptible', 'low', 'moderate' and 'high')
  - Assignment of **weights** (%) to parameter sub-categories
  - Allocation of **checkboxes** to specific parameters, i.e. 'dry leaf appearance' and 'infusion turbidity/ haze'
  - Allocation of **checkboxes** to indicate the presence of individual positive attributes, and negative sensory attributes for taints and/or the 'overall character on palate'



### III) Quality scoring method protocol

- Parameter assessment order
- Allocation of **actions** to be followed for specific parameters and attributes when indicated by checkboxes

The final scorecard is presented in **Fig. 2.1** and comprised of the following elements: i) 3 key quality parameters (modalities), namely 'appearance', 'aroma' and 'palate' attributes, ii) 12 parameter sub-categories, iii) 10 category scales assigned to specific parameter sub-categories, so-called 'scores parameters', iv) 29 checkboxes assigned to specific parameter sub-categories (7) and specific positive (12) and negative (10) attributes, so-called 'citation parameters', v) 6 actions to be performed if specific checkboxes have been indicated by an assessor. Citation of critical parameter sub-categories, i.e. 'infusion haze', and critical negative attributes (taints), i.e. 'smoky', 'medicinal' and 'musty/mouldy', were included to provide important information to the processor and blender/packer in terms of actions to be taken.

A more cost-effective alternative to instrumental colour measurement was considered, i.e. the visual assessment of the 'infusion appearance' parameter, colour. A colour reference card with digital images of honeybush infusions in white porcelain mugs, as per preparation protocol (Erasmus et al., 2017), was compiled and assessed in the expert focus groups. Infusions ranged in colour strengths and CIEL\*a\*b colour measurements. Digital images of three infusions for each of the four colour intensity criteria, 'yellow/green', 'light red-brown', 'red-brown', 'dark red-brown', were selected and incorporated into the colour reference card. The infusion colour reference card is presented in **Fig. 2.2**.

## 2.3.2 Focus groups

### 2.3.2.1 Panel

The panel of expert assessors (N = 5) consisted of one industry QC professional with extensive experience in sensory quality control of honeybush within a commercial context and four researchers. Three of the researchers are sensory scientists with extensive knowledge of the sensory space of fermented honeybush, while the fourth researcher has vast practical experience of honeybush processing and the effect on its sensory profile.

### 2.3.2.2 Focus group sessions

Three separate focus group sessions of ca. 4 h each, spanning over a period of three months, were conducted. The focus groups entailed 1) identification, selection and evaluation of the respective scorecard elements, 2) testing of the scorecard with honeybush infusions of variable quality, including presence of taints and 3) refinement of the scorecard. The focus groups were conducted in a light and temperature-controlled (21 °C) sensory research laboratory. During each session fermented honeybush samples of different quality levels were individually rated by the assessors according to four different quality classes

(‘poor’ to ‘high’), using the relevant draft scorecard. Samples that represented the sensory space of positive and negative attributes were selected from the in-house library of samples, specifically samples of commercially processed honeybush production batches (Section 2.2). Coded infusions were prepared at ‘cup-of-tea’ strength and presented in temperature-controlled water baths (65 °C), as described by Erasmus et al. (2017). The quality scores assigned by the individual assessors to samples and the efficacy of the scorecard elements to differentiate between quality classes were discussed. Following each session, the draft scorecard was amended and refined until the final scoring method was established. Sessions were therefore both theoretical and practical, a procedure followed by Etaio et al. (2010a, 2012) and Etaio et al. (2013) in the development of scorecards for the sensory quality evaluation of PDO wines and calf chops, respectively.

## 2.4 Validation of sensory quality scoring method (scorecard)

The developed quality scoring method for honeybush tea was tested on commercially processed honeybush batches of variable quality levels to determine its efficacy to distinguish between the different quality classes (‘poor’ to ‘high’). Two panels with different levels of expertise in sensory analysis of honeybush tea were used to evaluate the discrimination ability of the scorecard.

### 2.4.1 Samples

Commercially processed samples of *C. intermedia*, *C. genistoides* and *C. subternata* with variable sensory attribute intensities, colour (CIEL\*a\*b\*) and turbidity (NTU) values were selected from the in-house sample library (**Chapter 3, Table 1**). Samples with high intensities of positive or negative attributes, irrespective of species, were identified. Infusions of samples were prepared as described by Erasmus et al. (2017) and screened using the scorecard to confirm differences in appearance and sensory attribute intensities. Subsequently, a total of 36 samples were selected for further analyses.

As the individual commercial samples (n = 36) were previously analysed in different experimental blocks over different time periods, a repeat of DSA of the selected sample set (n = 36) was required to improve the reliability of the data. DSA data was required to assign quality classes to individual samples based on their attribute intensity data, as well as for comparison of product configurations with DSA as the ‘gold standard’. Following DSA, quality classes as defined in focus groups (Section 2.3) were assigned to the samples. Subsequently, a subset of 18 samples was selected to serve as the validation sample set. This sample set comprised of four quality groups, i.e. ‘high’ (n = 4), ‘moderate’ (n = 5), ‘low’ (n = 5) and ‘poor’ (n = 4). In addition, one sample of each of the ‘high’ and ‘low’ quality groups, respectively, were selected as blind duplicates to evaluate assessor reliability within a test replicate.

### 2.4.2 Descriptive sensory analysis

A trained panel (N = 13), aged 35 – 65, with extensive experience in descriptive sensory analysis (DSA) of fermented honeybush, performed sensory analysis on the full sample set (n = 36). Infusions were prepared, as described by Erasmus et al. (2017), and DSA was conducted as described in Chapter 3. The panel was instructed to score the intensity of 'infusion strength' (0 = extremely light; 100 = extremely dark) and 'overall positive character on palate' (0 = extremely weak; 100 = extremely strong), in addition to scoring the intensity of 23 aroma (orthonasal aroma), 19 flavour (retronasal aroma), 3 taste attributes and 1 mouthfeel attribute. 'Overall positive character on palate' was defined during the focus groups as a 'balance of positive flavours, sweet taste and astringency'. For objective intensity rating of infusion strength, each assessor was presented with a colour reference card (**Fig. 2.2**). Assessors were trained in the sensory attributes in four 1 h sessions before testing commenced.

### 2.4.3 Physicochemical analyses

CIEL\*a\*b\* colour and turbidity measurements of infusions were conducted as described in Section 2.2.3, directly after sensory analysis. Instrumental analyses were required to compare results to visual assessment of colour and turbidity for research purposes.

### 2.4.4 Assessors

#### 2.4.4.1 Industry panel

Honeybush industry professionals were invited to test the efficacy of the quality scoring method for application in industry. The objective was to acquire a panel representative of relevant industry role-players. These assessors (N = 14) were predominantly honeybush industry professionals working in a QC capacity; however, some assessors were also representatives of processing, procurement and marketing divisions, i.e. individuals taking part in ad hoc sensory quality assessment of fermented honeybush. The level of expertise in general sensory analyses practices, and sensory quality assessment of fermented honeybush tea specifically, varied extensively within the industry panel. The majority of the industry professionals were representatives of rooibos processing plants that also blend and pack honeybush tea, or buyers/packers of these South African herbal teas and therefore had experience in assessment of both herbal teas. The assessors signed a register to indicate their consent to participate in the study.

#### 2.4.4.2 Trained panel

An existing sensory panel consisting of 13 trained assessors (aged 35 to 65) with several years of experience in the descriptive sensory analysis of fermented honeybush was selected to test the efficacy of the quality scoring method for application in research projects. Assessors were screened, selected and trained according to standardised protocols to participate in various honeybush studies (Bergh et al., 2017;

Erasmus et al., 2017; Theron et al., 2014). Panel training focussed on the recognition and scoring of positive and negative honeybush attributes in terms of infusion intensity on unstructured line scales (0 = none; 100 = extremely high).

#### *2.4.5 Assessment*

*The following procedure was followed for both industry and trained panels with exceptions indicated:*

Assessment of the validation sample set (n = 20, including 2 blind duplicates), using the developed scorecard, was repeated in two separate sessions per panel. The tests by the industry panel were performed on the same day in two consecutive sessions with a 20-minute break between sessions, due to practical constraints, whereas the tests by the trained panel were performed on two separate days. Assessors conducted each test replicate under controlled conditions as described for DSA in Chapter 3. Sample infusions were prepared and presented, as described by Erasmus et al. (2017). For each session all samples (n = 20) were presented simultaneously, in a randomised order. Assessors were presented with 20 scorecard paper sheets per session and a colour reference card. Each sheet was numbered with a 3-digit number corresponding to the sample code. 'Dry leaf appearance' parameter was not assessed due to limited availability of plant material.

Prior to assessment, each panel participated in a short briefing session to introduce the quality scoring method and the use of the scorecard. As the industry panel did not receive any prior training in the sensory quality descriptions associated with fermented honeybush infusions (Chapter 3), industry assessors participated in a brief calibration ('warm-up') session in which four samples of different quality classes were evaluated using the scorecard, followed by a brief discussion.

Both panels were instructed to complete a questionnaire on the user-friendliness of the quality scoring method after completion of the test. In addition, the industry assessors were also presented with a questionnaire that included questions on their expertise of sensory quality assessment, current in-house sensory QC practices and nomenclature used, as well as concerns regarding the sensory quality of honeybush tea. Questionnaire responses from the industry panel were captured electronically using EyeQuestion® software (Elst, The Netherlands).

#### *2.4.6 Statistical analyses*

**Figure 3** summarises the workflow for the selection of the samples used for validation based on their DSA data and the statistical analyses of the scorecard data.

##### *2.4.6.1 Descriptive sensory analysis data*

Data analyses were conducted on sample means over assessors, as described in Section 2.2.4. After confirmation of normality, principal component analysis (PCA) was conducted using XLStat (Version

2019.2.1, Addinsoft, Paris, France) to visualise and elucidate the association between the samples ( $n = 36$ ) and positive and negative sensory attributes (Næs et al., 2010). Quality classes ‘high’, ‘moderate’, ‘low’ or ‘poor’ (Section 2.3) were assigned to individual samples based on sample configuration in the constructed PCA scores and loading plot, as well as the intensity data of the individual sensory attributes. The validation sample set ( $n = 18$ ) and blind duplicates ( $n = 2$ ) were selected accordingly.

#### 2.4.6.2 Scorecard validation data

Distinction was made between parameters that were scored using category scales, so-called ‘scores parameters’, and specific parameters (‘dry leaf appearance’ and ‘infusion haze/turbidity’) and positive and negative sensory attributes that were indicated with checkboxes, so-called ‘citation parameters’. Scores data (i.e. total score and ten individual parameter scores (%)) and citation frequency data (i.e. citation with checkboxes for specific parameter sub-categories and sensory attribute identification) from the industry and trained panel, respectively, were analysed. Incomplete scorecards, i.e. scorecards for which one or more parameters were not scored by an assessor, were not included in analyses.

The validation criteria considered for the quality scoring method were 1) discrimination between samples based on final classification derived from total score (%) compared to pre-assigned classification of samples based on the DSA PCA bi-plot, 2) discrimination between samples based on individual parameter scores, 3) discrimination between samples based on citation frequencies of parameters, especially that of specific negative attributes (taints), 4) association between DSA variables and ‘scores parameters’ and ‘citation parameters’, respectively, and 5) association between scorecard variables. In addition, assessor reliability per panel was assessed. Statistical analyses were conducted using Statistica version 13 (2018) (TIBCO Software Inc.).

##### 2.4.6.2.1 Scorecard quantitative data (scores)

Scores data were subjected to analysis of variance (ANOVA) with assessor type (industry/trained panel) and products (test samples) as effects. Mixed model ANOVA was used to analyse unbalanced data (panels of different sizes), focussing on both products and assessors, in which assessor effect and interaction between the assessor and product can be considered random (Næs et al., 2010). Sample means were compared by calculating Fisher’s LSD where a probability level of 5% was considered significant. Least square (LS) means line plots were computed for total score (%) as well as for individual parameter scores (%) to compare scores data for each sample between panels. PCA was conducted to visualise and elucidate the relationships between the samples and *scores parameters* (Næs et al., 2010). PCA bi-plots were computed for average scores across assessors and replicates (STATIS). Regression vector (RV) coefficients were calculated between the first two dimensions of the product configurations obtained with the PCA bi-plots of the DSA and scorecard data. RV coefficients are multivariate similarity (correlation) coefficients that measure the similarities between two-factorial product configurations (Abdi, 2010). High similarity

between respective configurations is indicated by RV coefficients closer to 1, whereas 0 indicates uncorrelated configurations. Visual inspection of product configurations in the two-dimensional space of the respective DSA and scorecard PCA bi-plots, as well as RV coefficients were considered to determine method validity.

#### *2.4.6.2.2 Scorecard qualitative data (citation frequencies)*

The presence of the appearance descriptors 'turbid/hazy', 'clear' and 'dust/sediment' of the infusion, as well as positive and negative aroma and flavour descriptors, so-called 'citation parameters' were indicated (cited) using checkboxes. For each assessor a data matrix (contingency table) was created containing descriptors, i.e. *citation parameters*, in columns and samples in rows, and each cell represented whether the *citation parameter* term was checked/identified (1) or not (0). Standardised residuals (also known as Pearson residuals) were calculated for each parameter to indicate the magnitude of deviation between samples and *citation parameters*. Negative values indicate a lesser association of a sample with a specific *citation parameter* whereas positive values indicate a higher association of a sample with a specific *citation parameter*. Correspondence analysis (CA) was applied to standardised residuals to elucidate the association between samples and *citation parameters* onto a two-dimensional sensory space. RV coefficients were calculated for the product configurations obtained with the PCA bi-plot of the DSA data and the CA of the scorecard data. Visual inspection of product configurations in the two-dimensional space of the respective DSA bi-plots and scorecard CA plots, and RV coefficients were considered to determine method validity.

#### *2.4.6.2.3 Association between scorecard variables*

The relationship between scorecard variables (scores and citation frequencies) were visualised and assessed with canonical correspondence analysis (CCA) and multiple factor analysis (MFA).

#### *2.4.6.2.4 Association between DSA and scorecard variables*

The relationship between sensory attribute data determined by DSA and scores- and citation parameters, respectively, were visualised using MFA. RV coefficients were computed for the product configurations obtained for DSA and for the parameter scores and citation frequencies, respectively.

#### *2.4.6.2.5 Assessor reliability*

Two blind duplicate samples were included to determine assessors' repeatability within a test replicate as an additional measure of method validity by evaluating projection of replicates on the respective two-dimensional PCA and CA maps. Intraclass correlation coefficients (ICC) as a reliability index in intra-rater and inter-rater reliability analyses were calculated. ICC agreement for each assessor was calculated based on total score (%) assigned to each sample to evaluate assessor intra reliability (between replicates) and assessor inter reliability (between assessors within a panel). ANOVA was applied to determine mean ICC

agreement between replicates over assessors to compare repeatability between panels. Data interpretation was based on ICC agreement as a stringent measure of reliability as it accounts for the variability in the repetition ascribed to the biological nature of the sample ( $ICC \text{ (agreement)} = \text{subject variability} / (\text{subject variability} + \text{variability in repetition} + \text{measurement error})$ ).

### 3 Results and discussion

The aim of this study was to develop and validate a generic quality scoring method for fermented honeybush tea, irrespective of *Cyclopia* species. Industry responses from a survey and interviews and a comprehensive sensory and physicochemical dataset were studied to identify and specify elements that need to be scored on a scorecard. An informed decision-making process was followed through expert panel focus groups to identify and specify the sensory quality parameters in terms of four quality classes, namely 'high', 'moderate', 'low' and 'poor' quality. Selected scorecard parameter sub-categories for assessment of aroma and palate attributes were derived from DSA attributes. A combination of *category scales* for parameter intensity scoring and *checkboxes* for citation of parameter/attribute identification was selected based on scorecard development approaches described in literature (Etaio et al., 2010a, 2012; Langstaff, 2014; Ojeda et al., 2015). The aim of the envisaged classification/grading system was not to address liking or preference (consumer appeal) of honeybush tea but quality. Therefore, considering the relative short existence of the formal honeybush industry (Joubert et al., 2019) in which consumers have been exposed to products of variable quality, input from experts, and not consumers, were used in the development steps to identify quality parameters and to define parameter specifications per quality class.

#### 3.1 Industry survey responses

Role-players of the South African honeybush industry were approached to specify the perceived key sensory quality parameters (modalities). Additionally, they had to indicate the current status of sensory quality control (QC) as applied to honeybush tea by industry. It was also important that they identify their requirements for the development of a formal sensory QC system. This approach has previously been used for the development of a classification system for omega-3 fish oils (Larssen et al., 2019).

A total of 41 representatives of the honeybush industry completed the survey, with 25 participants indicating their willingness to participate in the study in terms of information sharing, supply of samples and testing of the developed scoring method (scorecard). Survey respondents were producers (N = 21), processors (N = 8), QC personnel (N = 2), marketers/distributors (N = 3) and researchers/government officials (N = 7). Preference for a species-specific or generic honeybush sensory quality classification system was divided equally between survey respondents (5 respondents did not indicate any preference). The preference for a species-specific classification system could be ascribed to the concern of some producers



that a generic quality control system would be prejudicial to certain *Cyclopia* species, especially as species differ in sensory characteristics and anecdotal evidence suggests consumer preference for certain species. No consumer studies have been conducted to confirm this generalisation, although the bitter taste intrinsic of *C. genistoides* has been regarded as unfavourable, particularly when present at high intensities (Alexander, De Beer, Muller, Van der Rijst, & Joubert, 2019; Moelich, 2018). Furthermore, the survey results indicated that most participants predominantly produce and/or process *C. subternata* (40%), followed by *C. intermedia* (20%), *C. maculata* (14%), *C. longifolia* (14%), *C. genistoides* (6%) and *C. plicata* (4%). *Cyclopia subternata* is cultivated and harvested from natural populations whereas only few producers have access to wild-harvested *C. intermedia*. In view of the feedback from industry, it was decided to develop a generic sensory quality scoring system. In all likelihood, the development of species-specific sensory quality scoring system will ensue in future.

All information obtained from industry responses through the survey and interviews, whether relevant or not to the study, i.e. cut size of leaf and rate of oxidation, are summarised in **Table 1**. From the feedback it is evident that not all of the respondents could distinguish between relevant and irrelevant issues relating to sensory quality control of honeybush tea. Yet, in spite of this, important themes emerged. Most importantly, 'infusion taste', i.e. industry terminology describing the overall/collective perception of the palate attributes, i.e. flavour, basic taste and mouthfeel attributes, was regarded as the most important quality parameter, followed by infusion aroma, colour and turbidity, and dry leaf and wet leaf appearance. The lack of a standardised sensory quality system for the quality control of honeybush tea was confirmed. Respondents agreed with the objective that the envisaged quality scorecard would improve communication between processors, buyer/packers and marketers, as well as in-house communication between company divisions, i.e. quality control, production, procurement and marketing/sales. It was emphasised that the successful implementation of a sensory quality control system for classification of honeybush tea would aid production of products of consistent quality, fair marketing and price negotiation, and ultimately industry growth.

Respondents acknowledged the natural variation of honeybush tea as an agricultural product, and therefore blending of batches is common practice. The concern was, however, raised that processors supply inconsistent blends of different *Cyclopia* species to packers, whereas single batches (not previously blended) are preferred by certain packers as they wish to conduct blending according to their own in-house blending ratios. Respondents stressed that a market cannot be developed and sustained when the product shows large variation in quality and sensory profile as this will harm the reputation of honeybush tea. Variation in quality has also been ascribed to the entry of new processors or blending/packing companies, particularly if they are not experienced in honeybush processing and/or the sensory quality of the product. Certain respondents also indicated that, due to inexperience in sensory quality assessment of honeybush tea, an aroma lexicon with chemical-based reference standards for honeybush (Chapter 3) would be an

useful QC and communication tool, with the provision that relatable terms are used to describe the sensory characteristics of honeybush tea.

### 3.2 Study of comprehensive dataset

The comprehensive honeybush dataset obtained from the analyses of a large sample set ( $n = 585$ ), comprised of the intensity values of sensory modalities, CIEL\*a\*b\* colour values and turbidity values. The sensory modalities encompassed 1) *positive* aroma, flavour, taste and mouthfeel attributes (characteristics intrinsic of honeybush) and 2) *negative* aroma, flavour and taste attributes (characteristics that may be ascribed to poor tea processing conditions or the lack of good manufacturing processes (GMPs), e.g. poor storage conditions). CIEL\*a\*b\* colour and turbidity values were included as the infusion colour gives an indication of infusion strength, while the presence of turbidity/haze is associated with poor quality (Bergh et al., 2017). Infusion colour could be an important cue of sensory quality as colour was shown to influence the perceived intensity of taste modalities (Carvalho & Spence, 2019). At this stage, colour and turbidity values are intended for application in research.

**Table 2** presents a summary of the distribution of sensory attribute intensities and colour (CIEL\*a\*b\*) and turbidity (NTU) values in terms of mean, median, maximum, minimum and quartiles (upper and lower) values for the samples of the comprehensive dataset. Median values were used to compute percentage occurrence frequency plots to identify attributes that are present in all samples (100%) at relative high intensities for the selection of parameters to be included in the scorecard (**Fig. 4**). Normal distribution curves were studied to confirm that the selected parameters showed similar intensity distributions for *C. intermedia*, *C. subternata* and *C. genistoides* (**Fig. 5; Addendum B, Fig. B3**). Normal distribution curves were also studied to confirm attributes that are more prevalent in specific species or are present at higher intensities compared to other species (**Fig. 5.2**). Ranges in box- and whiskers plots (**Addendum B, Fig. B4**) were studied to determine 1) parameter specifications for 'high', 'moderate', 'low' and 'poor' quality classes, and 2) intensity descriptions for category scales ('absent/barely perceptible', 'low', 'moderate' and 'high' intensity). The interquartile ranges between  $Q_1$  and  $Q_3$  for CIEL\*a\*b\* colour and turbidity values were studied to determine specifications thereof for research application (**Addendum B, Fig. B5**). Information obtained from the comprehensive dataset is summarised in **Table 3**, and key points will be discussed in Sections 3.2.1 and 3.2.2.

#### 3.2.1 Aroma, flavour, taste and mouthfeel attributes as indicators of infusion quality

For the compilation of the scatter plots (**Fig. 4**), the occurrence of an attribute in the complete sample set was counted when present at a mean intensity  $\geq 1$  on a 100-point scale. This count value was used to calculate occurrence frequency as a percentage of the total number of samples. Preliminary identification of the key attributes present in all three *Cyclopia* species were based on median attribute intensity values  $\geq$

5 and 100 percent occurrence in the sample set. Intensity values  $< 5$  can be regarded as being barely perceptible (Erasmus et al., 2017) and thus of negligible importance. Erasmus (2014), Bergh et al. (2017) and Robertson et al. (2018) used a similar approach for the identification of key attributes of honeybush.

*Positive modalities* present in 100% of the samples with high intensities ( $>30$ ) were 'fynbos-floral', 'fynbos-sweet' and 'woody' aroma, which is in agreement with previous studies on a number of *Cyclopia* spp. (Bergh et al., 2017; Erasmus, 2014; Erasmus et al., 2017; Robertson et al., 2018). In the current study, 'fruity-sweet', 'raisin', 'apricot' and 'caramel' aroma were also present in 100% of the samples but at lower intensities ( $20 > x < 30$  for the first three attributes;  $<15$  for 'caramel'). Previously, 'fruity-sweet' and 'caramel' (Bergh et al., 2017) and 'fruity-sweet' and 'apricot' aroma (Robertson et al., 2018) have also been identified in 100% of the samples, but at lower intensities. In the current study the major taste and mouthfeel attributes were identified as sweet taste and astringent mouthfeel, as found by Theron et al. (2014) and Erasmus (2014).

Previous studies have indicated that flavour attributes of honeybush tea illustrate similar trends to their corresponding aroma notes, but these attributes were perceived at lower intensities (Bergh et al., 2017; Erasmus et al., 2017); therefore, the focus of these studies was only on aroma as modality for the sensory characterisation of different *Cyclopia* species. However, based on sensory parameters evaluated for black tea (*C. sinensis*) (ISO, 1982; Liang et al., 2003) and herbal teas (THIE, 2018), as well as current practice for South African herbal teas (Section 3.1), flavour as modality on palate was also considered in the current study. 'Fynbos-floral' and 'woody' flavour were present in 100% of samples at high median intensities ( $>30$ ), whereas 'fynbos-sweet' flavour was present in all samples but at a lower intensity (13).

*Negative modalities* (i.e. negative flavours, bitter and sour tastes) were present in  $<100\%$  samples at intensities  $< 5$ , except for 'hay/dried grass' aroma and flavour, which were present in 100% of the samples at mean intensities of 15 and 16, respectively. Given its consistent presence, 'hay/dried grass' aroma and flavour are regarded as intrinsic characteristics of honeybush tea. However, the intensity of 'hay/dried grass' aroma and flavour would determine whether 'hay/dried grass' is perceived as negative or positive. Bergh et al. (2017) recommended consumer testing to clarify at what intensity level these attributes may be considered unacceptable. To establish at which intensity ranges 'hay/dried grass' aroma and flavour could be regarded as negative, the interquartile ranges representing intensity values of 50% of sample set were studied (**Table 2**). From the interquartile ranges for 'hay/dried grass' aroma (11–19) and flavour (13–19), one could derive that these values represent the average intensity values for honeybush infusions, and higher intensity values ( $> 20$ ) may be regarded as negative for both aroma and flavour. Box-and-whisker plots (**Addendum B, Fig. B4**) were used to define ranges for the scorecard category scales (Section 3.3.2).

Major generic attributes were further confirmed by similar normal distributions for *C. genistoides*, *C. subternata* and *C. intermedia* over all intensity categories for each major aroma, flavour, taste and mouthfeel attribute. Using this approach, attributes identified as common to the three *Cyclopia* species

were 'fynbos-floral', 'fynbos-sweet', 'woody', 'hay/dried grass' aroma and flavour, 'fruity-sweet', 'apricot', 'raisin' and 'caramel' aroma and sweet taste and astringent mouthfeel (**Fig. 5.1** and **Fig. B3, Addendum B**). Robertson et al. (2018), investigating *C. genistoides*, *C. subternata* and *C. maculata*, followed a similar approach to identify generic attributes and attributes more prominent in specific species through assessment of the normal distributions of the intensity values of the different attributes. Normal distributions of attribute intensities in *C. intermedia* have not yet been studied, and in the present study, normal distribution plots of the attribute intensities for the three major commercial species were compared for the first time (**Figs. 5.1 to 5.2; Fig. B3, Addendum B**).

Maximum and minimum intensity values for negative attributes (**Table 2**) depict the large variation in intensities, i.e. from absent to values > 25 for 'musty/mouldy', 'hay/dried grass', 'green grass' and 'smoky' aroma, 'musty/mouldy', 'hay/dried grass' and 'smoky' flavour and sour taste. Negative aroma attributes have been previously associated with underfermented honeybush tea ('sour', 'green grass', 'cooked vegetables' and 'hay/dried grass'), over-fermented honeybush tea ('burnt caramel') and poor processing practices ('smoky', 'musty', 'medicinal', 'dusty' and 'rotting plant water') (Bergh et al., 2017; Du Toit & Joubert, 1998, 1999; Erasmus et al., 2017; Theron et al., 2014). Although not regarded as major generic attributes in terms of occurrence frequency and normal distribution intensity ranges, the presence of negative attributes would be critical in quality assessment of production batches and the scorecard therefore made provision for scoring taints.

The scorecard was devised for scoring of parameters that are generic to the main commercial *Cyclopia* species. Attributes that are more prominent in specific species, indicated by checkboxes, will provide additional information to industry role-players. The presence of these positive attributes would be useful to buyers and blenders, especially if production batches high in specific aroma notes or flavours are sought. For example, some buyers show preference for honeybush tea with a prominent 'apricot' note (A. Redelinghuys, Rooibos Ltd, 2019, personal communication). For the identification of attributes that define a species, i.e. that are more prominent in a species, the individual samples of a species should be well-distributed over all intensity categories (Robertson et al., 2018). This is exemplified for 'rose geranium' aroma, showing a wide distribution range across intensity categories for *C. genistoides*, whereas a narrow distribution range was evident for both *C. subternata* and *C. intermedia* (**Fig. 5.2**). Therefore, 'rose geranium' aroma may be regarded as specific to *C. genistoides*, which is in agreement with Robertson et al. (2018). However, it should be noted that the distribution over intensity categories for 'rose geranium' aroma was wider for *C. intermedia* than for *C. subternata*. Based on distribution in the respective sample sets of the *Cyclopia* species (**Fig. 5.2**), 'rose perfume', 'nutty' and 'sweet spice' aroma can be regarded as more specific to *C. intermedia*, and 'apple' and 'sweet spice' aroma for *C. subternata*. Furthermore, although 'raisin' aroma was identified as a major attribute based on the fact that this attribute was present in all samples (100%) at a high median intensity of 27, the intensity categories for *C. genistoides* was

narrower and lower than for *C. subternata* and *C. intermedia*, implicating that 'raisin' aroma is more prominent in the latter two species (**Addendum B, Fig. B3**).

From **Fig. 5.2** it is evident that bitter taste is specific to *C. genistoides*. Bitterness of *C. intermedia* and *C. subternata* showed a very narrow distribution with mean intensity values of 1 and 2, respectively. The prominence of bitterness in *C. genistoides* is also in agreement with Erasmus et al. (2017). High bitter intensities may impact negatively on the acceptability of *C. genistoides* as herbal tea by the average honeybush-drinking consumer, given that the consumer has the perception of honeybush as naturally sweet-tasting (Vermeulen, 2015). Therefore, although regarded as more specific to one species, bitter taste was also included in the scorecard.

### 3.2.2 Colour and turbidity as indicators of infusion quality

Box-and-whisker plots depict the intensity distribution of objective colour values for parameters,  $L^*$ ,  $a^*$ ,  $b^*$ , chroma and hue, and turbidity units as indication of the infusion quality of the samples ( $n = 585$ ) (**Addendum B, Fig. B5**). Infusion colour as defined by the respective parameters, varied substantially: lightness ( $L^*$ ) from 70.2–97.0, red ( $a^*>0$ ) from 0.3–24.9, yellow ( $b^*>0$ ) from 6.9–67.3, saturation/chroma (C) from 6.9–70.8, and hue (h) from 62.1–88.7 (**Table 2; Addendum B, Fig. B5**). As the combination of these individual values represent the colour space (three-dimensional colour coordinate system) of an infusion, 'low', 'moderate' and 'high' intensity ranges could not simply be derived as for the aroma, flavour, taste and mouthfeel attributes (Section 3.2.1). However, one could conclude from the data that higher  $+a^*$ -values (i.e. degree of redness) and lower  $L^*$  values (i.e. degree of lightness) would both contribute to a more desirable infusion 'strength'. The high-temperature oxidation process of honeybush plant material results in the development of the distinctive dark brown colour of the 'fermented' plant material, and red-brown colour of the brewed infusion. Red-brown infusion colour has been associated with honeybush tea of acceptable overall quality (Du Toit & Joubert, 1999). Similarly, 'red-brown' infusion colour was regarded as desirable according to industry responses in the present study (Section 3.1). In a study to evaluate the effect of fermentation temperature/time regimes on colour development, browning (darkening) of the fermenting plant material resulted in a decrease in the  $L^*$  (lightness) and  $b^*$  ( $b^*>0 =$  yellow) values and an increase in  $a^*$  ( $a^*>0 =$  red) values with increasing fermentation time and temperature. Higher fermentation temperature (90 °C) resulted in a noticeable darker and more red-brown infusion than honeybush fermented at lower temperatures (Du Toit & Joubert, 1999). In a study on objective colour measurement (CIEL $^*a^*b^*$  system) for predicting rooibos tea infusion quality, the red colour component of infusions was indicated as critical in visual grading, with higher  $a^*$  values resulting in higher colour grading (Joubert, 1995).

In view of the afore-mentioned, honeybush infusion colour can be regarded as an important parameter that has a marked influence on the perception of infusion strength and therefore quality.

Although the CIEL\*a\*b\* data provide some insight into the variation that exists in production samples, which is valuable within research context, specific cut-off points that translate to the visual colour observed, cannot yet be determined. Further research is required to determine the  $\Delta E$ -values that result in visual differences. As an initial starting point to select optimum CIEL\*a\*b\* colour ranges, digital images of aliquots of 100 mL infusions in white porcelain mugs (as prepared by Erasmus et al. (2017)) were compared to the respective CIEL\*a\*b\* values. Visual colour assessment as cost-effective and practical alternative to instrumental analyses was considered for the development of the quality scoring method. Therefore, a colour reference card was developed to aid the objective quality scoring of infusion colour, as described in Section 2.3.1. Similarly, reference cards with digital images (Hyldig & Green-Petersen, 2005; Ojeda et al., 2015; Zannoni & Hunter, 2013) and printed transparent films (Etaio et al., 2010a) were developed to aid visual appearance quality scoring. These visual aids anchored individual scores to objective values. According to Lawless and Heymann (2010), the human assessor as instrument has the ability to assess visual differences when samples are placed next to each other or next to a standard.

The interquartile range for turbidity was 24.3-91.5 NTU with a maximum value as high as 532 NTU (**Table 2**). This latter value is clearly an outlier (**Addendum B, Fig. B5**). When this outlier value of 532, and another high value of 523, was omitted from the dataset, the descriptive statistics did not change significantly as an interquartile range of 24.2–91.1 NTU was obtained. Even when all data values > 300 (7 values) were omitted from the dataset, an interquartile range of 43.9–89.15 NTU was obtained. Median and mean values also did not change significantly. High turbidity is unacceptable in honeybush tea and is contributed to sub-optimal fermentation conditions (Bergh et al., 2017). In the present study, the presence of sediment/dust in some commercial samples that were not sieved sufficiently after processing could have resulted in turbidity of their infusions and thus higher NTU values. Therefore, in these instances, high turbidity levels are ascribed to insufficient sieving of the processed plant material and not haze formation. Notwithstanding, Bergh et al. (2017) was the first to attempt quantification of turbidity of honeybush infusions and high NTU values for several commercial samples were observed. Specifications for acceptable NTU values were therefore recommended for turbidity as a quality parameter in a quality grading system for honeybush tea (Bergh, 2014; Bergh et al., 2017). Turbidity correlated strongly with poor sensory quality. As for the present study, Bergh (2014) observed a large variation of NTU values for *C. intermedia* infusion samples (n = 54), which ranged from a mean value of 39.8 NTU (associated with 'good' quality), 72.1 NTU (associated with 'average' quality), to 313.9 NTU (associated with 'poor' quality).

As confirmed for honeybush tea in the current study, infusion 'clarity' (described as 'related to the ability of seeing through the sample') is regarded as a desirable quality parameter in hibiscus tea (Monteiro et al., 2017). Haze formation in hibiscus tea is associated with microbial growth and/or deficient filtration of extracts (Monteiro et al., 2017). Both infusion haze and colour (intensity and red hue) were included as appearance modalities for visual assessment in the development of a hibiscus tea sensory lexicon

(Monteiro et al., 2017). A 100 NTU standard was selected as lexicon reference for infusion haze, indicating that the presence of haze formation would be visible at this NTU value. Therefore, for the present study, the NTU value for honeybush infusions should be less than 100. However, the exact acceptable cut-off value still needs to be determined. The study on hibiscus tea did not provide any additional information in this regard (Monteiro et al., 2017). Haze is also an important quality parameter in beer (Steiner, Becker, & Gastl, 2010) and is descriptively categorised as ‘brilliant’ (0-2 NTU), ‘almost brilliant’ (2-4 NTU), ‘very slightly hazy’ (4-8 NTU), ‘slightly hazy’ (8-16 NTU), ‘hazy’ (16-32 NTU), and ‘very hazy’ (>32 NTU) (Briggs, Boulton, Brooks, & Stevens, 2004). Should these levels be applied to honeybush tea, any infusions with values >4 NTU that are not ascribed to dust/sediment, should be regarded as unacceptable. Similarly, to honeybush, haze/turbidity has also been regarded as undesirable in white and red wine wines and has been associated with low quality (Etaio et al., 2010b, 2012; Sáenz-Navajas et al., 2016). Wine batches are rejected merely on basis of the presence of haze, irrespective of level, and was therefore not included as quality parameter in the scorecard development of red wine (Etaio et al., 2010a). Considering the afore-mentioned, for the present study, honeybush infusions should be clear, and any presence of turbidity/haze (which is not ascribed to dust particles that settle to the bottom after a time period) would be unacceptable to secondary level processors and end consumers.

Digital images of clear infusions and their corresponding NTU values are presented in **Addendum B, Fig. B6**. Black lines in the background indicate that one can see through the samples and therefore no haze is present. Higher NTU values (> 4) were ascribed to the presence of dust particles (or sediment when dust particles have settled to the bottom), since the infusions were clear. Current industry practice for traditional ‘cupping’ is the visual assessment of haze (‘cup clarity’) in a teacup or tasting spoon, as mentioned previously (Section 3.1). Therefore, in the current study, the visual assessment of an infusion in a white porcelain mug or tasting spoon for the presence or absence of haze was considered in scorecard development.

### 3.3 Development of sensory quality scoring method

The development of the scorecard followed a stepwise approach as described in Section 2.3.1.

#### *3.3.1 Parameter identification and allocation of scales/checkboxes*

The key parameters determining honeybush quality were APPEARANCE, AROMA and PALATE attributes. Their selection and that of the parameter sub-categories were based on literature, industry consultation (**Table 1**) and the comprehensive dataset (**Table 3**). Icons of an eye, nose and mouth/tongue denoted the key parameters, thereby making the scorecard more user-friendly (**Fig. 2.1**).

The selected parameter sub-categories and specific DSA attributes, as well as definitions thereof, were derived from previous studies on honeybush tea and/or established through expert focus groups.



Parameter sub-categories selected to be scored using *category scales* (referred to as '**scores parameters**'), and parameter sub-categories and individual attributes selected to be cited using *checkboxes* (referred to as '**citation parameters**') are indicated in **Table 4**.

The APPEARANCE parameter sub-categories were dry leaf appearance and the turbidity and colour of the infusion. As dry leaf appearance was considered non-critical for final classification of the quality of a production batch, no category scale was allocated. Specification for dry leaf appearance was defined by cut size homogeneity ('even' vs 'uneven') and presence or absence of light stem particles ('acceptability' vs 'unacceptable') (**Table 4**). Checkboxes were used to indicate dry leaf appearance. Examples for dry leaf appearance are given in **Addendum B, Fig. B7**. The presence of haze, at any level, was considered unacceptable. In this case, a simple 'in/out' selection was incorporated into the scorecard by means of checkboxes for 'turbid/hazy' and 'clear'. As it is important to distinguish between turbidity ascribed to the presence of dust particles (temporary defect to be solved by sieving steps) and haze formation as a result of processing (permanent/semi-permanent defect) the checkbox 'dust/sediment' was included.

AROMA parameter sub-categories encompassed the generic DSA aroma attributes, i.e. 'fynbos-floral', 'fynbos-sweet', 'fruity-sweet', 'apricot', 'raisin', 'caramel' and 'woody' aroma. Their descriptions were further condensed/simplified to broader descriptions, i.e. 'floral', 'fruity and sweet', 'spicy and/or nutty' and 'woody'. This was based on the main segments of the revised generic honeybush aroma wheel (Chapter 3), namely '*floral*', '*fruity*', '*sweet-associated*', '*spicy*' and '*nutty*'. Although 'sweet spice' and 'nutty' aroma were not regarded as generic aroma attributes (i.e. occurrence frequency <100% and mean intensities <10 and <5, respectively), these two attributes contribute positively to the overall aroma quality of honeybush (Chapter 3).

PALATE parameter sub-categories embodied the generic **positive** DSA palate attributes, i.e. 'fynbos-floral', 'fynbos-sweet' and 'woody' flavour, sweet taste and astringent mouthfeel. Feedback from the honeybush industry indicated that it is common practice to assess only infusion 'taste' ('infusion taste' is the industry terminology describing the overall/collective perception of the palate attributes, i.e. flavour, basic taste and mouthfeel attributes), and not infusion aroma during traditional 'cupping'. Reasons provided were 1) impracticality (i.e. in practice test samples are not kept at a high constant temperature (65 °C) to limit loss of aroma volatiles during assessment) and 2) infusion 'taste' was considered more important than infusion aroma. In view of the latter, **positive** flavour, taste and mouthfeel attributes were combined into one parameter sub-category, namely 'overall positive character on palate' and was defined by the expert panel as the 'balance of positive flavours, sweet taste and astringent mouthfeel' (**Table 4**). Low intensity scores for 'overall positive character on palate' would be assigned to infusions that are perceived as 'weak', 'watery' or 'insipid', whereas high intensity scores would be assigned to infusions with high intensities of positive flavours, a sweet taste, and low to moderate astringency. In red and white wine scorecard development, a combination of flavour and mouthfeel attributes were captured as 'balance and

body' (Etaio et al., 2010a, 2012). Furthermore, for the present study, the allocation of checkboxes for indication of the presence of specific positive aroma attributes is granted, as it allows for the provision of additional information to industry role-players to communicate quality issues in the supply chain. The indication of the presence of specific positive attributes such as 'rose perfume' and species-specific 'rose geranium' aroma may emphasise 'high' quality of specific production batches.

Only **negative** DSA palate attributes, and not negative aroma attributes, were selected and included as PALATE parameter sub-categories, i.e. 'hay/dried grass' flavour, bitter and sour taste. The descriptors 'weak/watery' next to the 'overall character on palate' parameter and 'vinegar-like' next to sour taste were added to the scorecard as supplementary checkboxes for additional indication of inferior sensory quality to aid assessors in the assessment process. The PALATE parameter sub-category 'taints' reflects the negative flavours (other than 'hay/dried grass'). The category, 'taints', was defined by the expert panel as 'undesirable taints or defects atypical of the characteristic sensory profile of honeybush' (**Table 4**). These taints are associated with poor processing or lack of good manufacturing practices (GMPs). The individual negative flavour attributes, namely 'green grass', 'rotting plant water', 'cooked vegetables', 'burnt caramel', 'dusty', 'medicinal', 'smoky', and 'musty', were again indicated with checkboxes. The expert focus groups considered 'medicinal', 'smoky' and 'musty' flavour as critical taints. These negative flavour attributes were indicated on the scorecard as 'CRITICAL' with the recommendation that production batches that contain 'medicinal' and 'smoky' flavours cannot be blended with batches of higher quality to mask these taints, even when perceived at low intensity levels. 'Musty' flavour could be indicative of a potential safety risk due to microbial contamination. Therefore, the scorecard provides a guideline to the processor or blender/packer in subsequent actions to be taken.

### *3.3.2 Assignment of sensory quality classes*

The sensory quality of honeybush tea was assigned four quality categories, namely 'high', 'moderate', 'low' and 'poor' by the expert panel (**Table 5**). 'High' quality represents top/premium quality, 'moderate' quality represents the minimum accepted standard for a final product for consumption by the consumer, and 'low' quality represents the minimum accepted standard to be used for batch blending. In practice, it could be expected that only a few production batches of 'high' quality would be available. Such batches are ideally reserved for niche markets. The bulk of the production would be classified as 'moderate' quality. 'Low' quality represents samples with a low intensity in 'overall quality on palate' (so-called 'flat' taste), high intensities of 'hay/dried grass' flavour or 'bitter' taste and/or the presence of certain taints, namely 'green grass', 'rotting plant water', 'cooked vegetables' and 'burnt caramel'. The intention should be that 'low' quality batches should not be sold "as is", but they could be blended with either 'high' quality batches or in small quantities with 'moderate' quality batches to obtain an acceptable product of 'moderate' quality. 'Poor' quality batches ascribed to high intensities of taints, and/or the presence of specific critical taints, i.e.

'smoky', 'medicinal' and 'musty', should be rejected and not be blended in with higher quality batches as these taints could be perceived after blending.

Standards and specifications are designed to determine the acceptable or tolerable variation in a product with reference to a previously selected product or an established written standard (Costell, 2002). Similar to the present study, expert focus groups determined the 'top situation' for the classification of omega-3 fish oils, and formulated a written product specification, i.e. an odour- and flavourless oil, although the presence of 'sourness', 'grassy', 'butter', 'nut' and 'fresh fish' at intensities  $\leq 1$ , are allowed (Larssen et al., 2019). The proposed quality classes for omega-3 fish oils were 'GOLD' (extra high sensory quality), 'SILVER' (high sensory quality), and 'REGULAR' (standard sensory quality) (Larssen et al., 2019). The 'GOLD' class is awarded for omega-3 fish oil free of any defects (Larssen et al., 2019). For honeybush tea, free of defects, 'high' or 'moderate' quality would depend on the intensity on the sensory attributes (**Table 5**).

For the establishment of the specifications for the respective sensory quality classes of honeybush tea, criteria for scoring of the intensities of parameter sub-categories or citation of their presence had to be determined. Descriptions for the category scales (i.e. semantic category scales) (Beeren, 2010) allocated to specific parameter sub-categories, and not the actual score values (i.e. numbers) were considered. Specific descriptions such as 'absent', 'low', 'moderate' or 'high' (for negative attributes), would ensure ease of use by assessors without any cognitive ambiguity. This is especially relevant for negative parameters when reversed scores are assigned. Descriptors as opposed to numerical scores would still permit effective product discrimination.

The use of decision trees for a scorecard (Etaio et al., 2010a, 2012, 2013; Ojeda et al., 2015) was not considered in the current study. Although the link of sensory situations to quality scores in such decision trees have indicated to homogenise the criteria used by assessors and to minimise subjectivity in scoring, their application in the current study would reduce the user-friendliness of the honeybush scorecard, as well as the speed to score a sample. Similarly, Lawless (2017) regarded scorecards with individual decision trees for each parameter as too complicated. In addition, overall quality rating scales for which score ranges are linked to descriptions such as 'reject', 'unacceptable', 'acceptable' and 'match' (Beckley & Kroll, 1996) were not considered, primarily to avoid the placement of unnecessary burden on assessors for feeling directly responsible for actions to be taken. In such instances, assessors are often inclined to use higher scores, associated with 'acceptable' score ranges (Lawless & Heymann, 2010).

For investigation of scale ranges for the '*scores parameters*', attribute intensity ranges within the box- and whiskers plots (i.e. the interquartile group,  $Q_1$  and  $Q_3$  groups and ranges below and above the whiskers' lower and upper bonds) from the comprehensive dataset were studied (**Table 6**). The selected 4-point semantic category scale was based on these aforementioned attribute intensity ranges, namely 'absent/barely perceptible' ( $< 5$ ), 'low' (range below  $Q_1$ ), 'moderate' (interquartile range) and 'high' (range

above  $Q_3$ ). As the interquartile groups represent attribute intensities of 50% of the sample set, these groups were linked to 'moderate' intensity ranges of the respective attributes. The link between the continuous unstructured 100-point intensity rating scale used by the trained panel for the DSA of the comprehensive dataset and semantic category scale (4-point intensity scale) is indicated in **Table 6**. A short 4-point category scale was regarded as user-friendly by the expert panel. Similarly, a 4-point scale was better accepted by assessors for defect Parmigiano-Reggiano cheese than 7-point scale used for positive descriptors (Zannoni & Hunter, 2013).

The final 4-point category scales and scores allocation per 'scores parameter' are presented **Table 7**. Scores (0 to 3) were allocated to the 4-point category scale from 'absent/barely perceptible' to 'high' for positive aroma parameter sub-categories, namely 'floral', 'fruity/sweet' and 'spicy and/or nutty' aroma and 'overall character on palate'. Reversed scores (3 to 0) were allocated for the negative parameter sub-categories, namely 'hay/dried grass' flavour, sour taste, bitter taste and 'taints'. The 'top/optimum' specification is linked to highest intensity per positive parameter sub-category and the lowest intensity per negative parameter sub-category. An exception is the non-linear score allocation for 'woody' aroma. High intensities (i.e. above the interquartile group [ $> Q_3$ ]) for 'woody' aroma, namely  $>45$  on a 100-point unstructured line scale, is regarded as undesirable in terms of sensory quality (similar for 'woody' flavour) (**Table 6**). Therefore, for 'woody' aroma a lower score of '2' was allocated for 'high' intensity in the category scale. Similarly, with the scorecard development of Idiazabal cheese, the attribute 'toasty' aroma was considered as 'totally appropriate' at 'slight-medium' intensities, whereas at excessively 'high' intensities, it was regarded a defect, and scores were allocated accordingly (Ojeda et al., 2015). In the current study, similar to 'woody' aroma, astringent mouthfeel as positive mouthfeel attribute was considered as undesirable at high intensities above the interquartile group, namely  $> 30$  (**Table 6**). However, as this attribute was incorporated into the parameter sub-category 'overall character on palate', astringent mouthfeel would not affect the score allocation of 'high' intensity in this category scale. In addition, a 4-point semantic category scale was selected for infusion colour. Based on industry consultation, descriptions for the four points were defined by the expert panel as 'green/yellow', 'light red-brown', 'red-brown' and 'dark red-brown'. As for 'woody' aroma, the score allocation for infusion colour parameter sub-category was non-linear with colour intensity above the upper limit, 'red-brown', regarded as undesirable. Therefore, a low score of '1' was allocated to 'dark red-brown', whereas '3' was allocated to the desirable 'red-brown' colour.

**Table 7** also presents the weights (%) applied to all 'scores parameters'. The weights represent the contribution of each 'scores parameter' to the overall sensory quality of a production batch. Assignment of weights was based on the relative importance of the 'scores parameters' as expressed by industry role-players and the focus group participants. The three 'scores parameters' that were regarded as critical to the overall sensory quality of honeybush tea were 'infusion colour' (20%), 'overall character on palate' (15%)

and 'taints' (20%). The highest total % weight was allocated to the 'palate' modality (total of 53%, sum of all flavour, taste and mouthfeel descriptors), followed by 'aroma' modality (total of 27%, sum of all aroma descriptors). For a black tea quality grading system, commonly used in China (Liang et al., 2003), a similar distribution of weights were awarded to taste (35%), aroma (30%) and infusion colour (15%), followed by the appearance of dry (10%) and infused (10%) tea leaves. Weighting of the importance of quality parameters for the calculation of an overall sensory quality score was also reported for scorecard development of PDO wines (Etaio et al., 2010a, 2012). Current grading weights assigned to rooibos by a major South African herbal tea processing and blending company are 40% for infusion 'taste' (palate attributes), 30% for infusion appearance and 30% for dry leaf appearance (C. Cronjé, Food Safety and Quality Systems Consultancy, 2018, personal communication).

An example of the calculation of the total score (%) using the optimum score value per category scale (i.e. maximum score value of 3), is presented in **Table 7**. Therefore, should a maximum score of 3 be allocated to each '*scores parameter*', the total score assigned to a batch would be 100%. Through expert panel consultation, a total score (%) range was selected per sensory quality class, namely 80–100 ('high' quality class), 60–79 ('moderate' quality class), 40–59 ('low' quality class) and 0–39 ('poor' quality class).

### 3.3.3 Quality scoring method protocol

To enhance method user-friendliness, actions '*blend with caution*', '*further processing*' and '*continue scoring*' (**Fig. 2.1**) were allocated on the scorecard next to specific quality parameters. This is intended to guide processors and assessors in practical steps to be followed when certain checkboxes are selected. Furthermore, the order of assessment was selected based on the order of standard 'cupping' protocol, i.e. firstly assessment of appearance, followed by aroma and palate. Similarly, Beeren (2010) specifies that appearance attributes are generally assessed first, followed by aroma, taste/flavour, texture/mouthfeel and in the final stage of consumption (after swallowing), specific aftertastes should be perceived. The assessment of aroma prior to the appearance to avoid loss of important aroma volatiles has been recommended for hot-served products such as coffee (Beeren, 2010). However, in the current study the expert focus groups indicated that the short time period needed to assess infusion appearance had no detrimental effect on the aroma perception. Scorecard assessment order varies in literature from appearance first for the Davis 20-point wine quality scorecard (Langstaff, 2010) to last for certain PDO wine scorecards (Etaio et al., 2010a, 2012). Etaio et al. (2010a) specifically indicated that red wine colour should not influence the assessment of other sensory parameters, and therefore aroma and taste are assessed in darkness. However, for the present study this approach was regarded as impractical and not user-friendly.

The colour reference card (**Fig. 2.2**) that was developed would aid assessors in recognition of the intensity descriptors of the infusion colour category scale, i.e. from 'yellow/green' to 'dark red-brown'. The advantage of appearance reference standards for universal assessment in different laboratories have been

indicated, provided that the display and viewing conditions for products and reference cards are identical (Lawless & Heymann, 2010). In the current study, the developed colour reference card forms a basis for the colour assessment of honeybush infusions, but it may be amended in future by replacing images of infusions in white porcelain mugs with infusions in standardised glass containers with standardised lighting specifications provided. Similarly, a protocol for visual assessment of turbidity in standardised glass containers marked with black lines, may also be developed in future, as an alternative to visual turbidity assessment in a porcelain mug and/or tasting spoon as performed in the current study.

### 3.4 Validation of sensory quality scoring method

An industry panel was selected to test the efficacy of the quality scoring method for application in industry. In addition, a trained panel also tested the method for application in research, specifically in the honeybush breeding programme (Joubert et al., 2019). Similarly, Kraggerud et al. (2012) compared DSA data by a trained panel to quality scores assigned by an expert panel to determine the suitability of a quality scoring method for cheese, whereas Etaio et al. (2013) used a trained panel to test suitability of a sensory quality scoring method for calf's meat, based on discrimination ability with scores and citation frequencies. Results of the current study will be discussed in terms of the criteria considered for method validation (Section 2.4.6.2) (**Fig. 3**). Similarly, 'repeatability, reproducibility and discrimination capacity' were considered as validation criteria for the development of a sensory quality scorecard for Idiazabal cheese (Pérez-Elortondo et al., 2007).

#### 3.4.1 Selection of validation sample set

The PCA bi-plot of the DSA data of the 36 samples is presented in **Fig. 6**. The subset of 18 samples that were selected for the validation sample set and their pre-assigned quality classes are highlighted in red ('high' quality), yellow ('moderate' quality), green ('low' quality) and grey ('poor' quality). Samples associated with positive aroma and flavour attributes, sweet taste and 'overall character on palate' towards the positive end of principle component 1 (PC1) represented moderate and high sensory quality. Mean intensity values of DSA data (**Addendum B, Table B1.1-1.3**) were also considered, i.e. high quality samples showed higher positive attribute intensity values, compared to moderate quality samples. Samples associated with negative aroma and flavour attributes, bitter and sour taste, and 'infusion strength' (determined using colour reference card, **Fig. 2.2**) towards the negative end of PC1, represented low and poor quality. Low quality samples specifically associated with high intensities in astringency and 'infusion strength' (dark red-brown colour), 'burnt caramel' aroma/flavour and vegetative taints, whereas poor quality samples specifically associated with 'smoky' and 'medicinal' aromas and flavours, and low intensities of 'infusion strength' (light yellow colour) and 'overall character on palate'. CIEL\*a\*b\* colour and turbidity data are presented in **Addendum B, Table B1.4**. It should be noted that although 'dust/sediment' was observed in

certain samples, none of these samples were turbid due to haze. Digital images of infusion colour of the selected 18 samples are presented in **Addendum B, Fig. B8**.

**Figure 7A** depicts the PCA bi-plot of PC1 and PC2, showing the association of the selected 18 samples with the sensory attributes, obtained by DSA. The PCA bi-plot of PC1 and PC3 (**Fig. 7B**) indicates the association of selected 'poor' quality samples with 'medicinal' and 'smoky' aroma and flavour towards the positive end of PC3. The samples selected for method validation (including 2 blind duplicates) and their pre-assigned quality classes are listed in **Table 8**.

### *3.4.2 Discrimination ability based on classification (% total score) vs pre-assigned classes (DSA)*

The mean total scores (LS means, %) for the validation sample set assigned by each of the two panels, and the pre-assigned vs scorecard classifications are presented in **Table 9**. The key for quality classification based on the total quality score (%) range is presented in **Table 7**. Samples highlighted in green indicate a complete match between the pre-assigned quality class and the classification by the panel based on the total score values (%) for each sample. Samples highlighted in yellow indicate that although not a complete match, the sample was classified either in the 1) 'moderate to high' (positive) grouping as 'high' instead of 'moderate' or *vice versa*, or in the 2) 'poor to low' (negative) grouping as 'low' instead of 'poor', or *vice versa*. Samples highlighted in red indicate no match between the pre-assigned class and classification using the scorecard, although total scores assigned were close to the cut-off range for the correct class for some samples.

Evaluation of the validation samples (n = 18) and the two blind duplicates by the industry panel produced 11 matched classifications and 6 matched classifications based on 'moderate-to-high' or 'low-to-poor' groupings. Classification of only three samples was not matched based on the aforementioned criteria, although both 10\_L/Sub and 9\_M/Gen were close to the cut-off (i.e. 60%) between 'low' and 'moderate' class. The trained panel classified 9 samples correctly and 8 samples correctly based on 'moderate-high' or 'low-poor' groupings. Both panels classified 10\_L/Sub as 'moderate' which could be an indication that the pre-assigned quality class of this sample, based on its DSA data, may have been too low. Therefore, based on their total scores (%), the sensory quality scoring method was able to distinguish and classify batches of variable sensory quality of honeybush tea relative effectively, according to their pre-assigned classes.



### 3.4.3 Discrimination ability based on parameter scores or citation frequencies vs pre-assigned classes (DSA)

The PCA bi-plot, based on the parameter scores and total score awarded by industry panel to the samples ( $n = 18 + 2$ ), depicts a positive association between the parameters scores, total score and samples with black arrows pointing in the same direction of increased quality (**Fig. 8A**). It is important to note that reverse scores allocation has been incorporated in the scoring for the negative parameters, therefore 'hay/dried grass' flavour, bitter taste, sour taste and 'taints' parameter scores are in the same direction as the positive parameters scores, opposite from low and poor quality samples. The first two components accounted for > 90% of the explained variance, describing the total and parameter scores associated with the samples. The main differentiation between samples on the first principal component (PC1) are samples associated with higher total and parameter scores on the left, i.e. samples pre-classified as 'high' and 'moderate' quality, and samples associated with lower scores, i.e. samples pre-classified as 'low' and 'poor' quality on the right. Samples in proximity with each other on the two-dimensional space were assigned similar scores.

The PCA bi-plot (**Fig. 8A**) further indicates that higher positive parameter scores, and lower negative parameter scores resulted in higher quality products, therefore leading to higher quality classification (the blue arrow indicates the direction of increased quality parameter and total scores). Accordingly, the validity of the method to classify between 'moderate' to 'high' and 'poor' to 'low' quality was demonstrated, although no distinct differentiation between 'moderate' and 'high', and 'poor' and 'low' quality, respectively, could be obtained. Furthermore, although 10\_L/Sub was pre-classified as 'low' quality (**Table 9**), this sample correlated positively with higher total and parameter scores (**Fig. 8A**) for both panels (data for trained panel; **Addendum B, Fig. B9**). This may indicate that although the DSA data of 10\_L/Sub represented lower intensities of positive attributes, particularly of 'overall character on palate', the respective panels did not regard this sample as 'low' quality. In addition, although misclassified by the industry panel based on total scores (**Table 9**), samples, 9\_M/Gen and 2\_H/Int (2), correlated with higher positive individual parameter scores as indicated on the left of PC1 (**Fig. 8A**).

Similar sample discrimination was obtained by the trained panel with > 90% of the explained variance on PC1 (**Addendum B, Fig. B9**), although better discrimination between 'moderate-high' and 'poor-low' quality groupings was obtained by the trained panel, i.e. groupings were further apart in the two-dimensional space. The RV coefficients computed for the comparison between sample configuration of PCA bi-plot of the DSA data (**Fig. 7A**) and that of the respective PCA bi-plots based on the scorecard data (**Fig. 8A; Fig. B9, Addendum B**) are given in **Table 10**. A lower RV coefficient for the industry panel (0.53) compared to that for trained panel (0.71) was obtained. Therefore, considering the respective sample configurations and RV coefficients compared to DSA as 'gold standard', assessor training level affected the discrimination ability of the quality scoring method, although its effect on classification based on % total

score vs pre-assigned quality classes was not as obvious. The effect of the level of training of assessors on method discrimination ability is discussed in Section 3.5.

The CA plot represents the differentiation among the samples based on parameter citation frequencies by the industry panel (**Fig. 8B**). Clear distinction between samples of both 'poor' and 'low' quality, and samples of 'moderate' and 'high' on dimension 2 was observed. 'Poor' and 'low' quality samples were further distinguished on dimension 1. The 'poor' quality samples associated with 'weak/watery', 'smoky', 'medicinal', 'rotting plant water' and 'musty' flavour attributes on the left, whereas 'low' quality samples associated with 'burnt caramel' and 'turbid/hazy' and 'dust/sediment'. Accordingly, the validity of method to classify between 'moderate' to 'high' and 'poor' to 'low' quality based on citation frequencies, was demonstrated. Similar differentiation between 'moderate-high' and 'poor-low' quality was observed for the trained panel (**Addendum B, Fig. B9**). In this case, better distinction between 'poor' and 'low' quality was obtained by the industry panel. The trained panel was less effective in scoring the 'poor' quality samples. Samples 17\_P/Sub and 18\_P/Sub did not associate with the negative attributes, but rather with the positive attributes, 'apple' and 'nutty' aroma. It should be noted that although DSA data for 17\_P/Sub and 18\_P/Sub indicated high intensities of 'apple' and 'nutty' aroma, their intensities for 'overall character on palate' were very low (**Addendum, Table B1**). Therefore, trained assessors distinguished these samples more on infusion aroma attributes, than on infusion palate attributes. These two very insipid samples were further distinguished with the *citation parameter* 'weak/watery' by both panels.

The RV coefficients computed for the comparison between sample configuration of the PCA bi-plot of the DSA data (**Fig. 7A**) and that of the respective CA plots of the scorecard data (**Fig. 8B; Fig. B9, Addendum B**) are indicated in **Table 10**. Relatively high RV coefficient of 0.67 and 0.66 for the industry and trained panel, respectively, were obtained which further demonstrates method validity. *Citation parameter* results may therefore be useful in the classification of poor and low quality batches, especially in terms of identifying specific taints in specific batches.

#### 3.4.4 Association of parameter scores with citation frequencies

Canonical correspondence analysis (CCA) was applied to the individual parameter scores and citation frequencies obtained for the validation sample set. The data of the industry panel for the association between positive '*scores parameters*' and positive '*citation parameters*', both indicating good quality, is depicted with arrows pointing in opposite direction of negative '*citation parameters*' (**Fig. 9**). As for PCA bi-plots of the scorecard data, it should be highlighted that reverse scores allocation has been incorporated in the scoring of negative parameters, therefore arrows for 'hay/dried grass', 'bitter', 'sour' and 'taints' point in the same direction as the positive parameters.

The positive correlation between positive '*scores parameters*' and positive '*citation parameters*', and negative correlation between positive '*scores parameters*' and negative '*citation parameters*' was perhaps

to be expected. In scorecard development, positive and negative '*citation parameters*' indicated with checkboxes, were aligned mainly next to the respective positive and negative '*scores parameters*'. Although similar associations were observed for both panels, the industry panel associated 'woody' *scores parameter* more with the negative *citation parameters*, indicating industry assessors' negative connotation with 'woody' aroma (**Fig. 9**). This was also indicated from the industry survey responses (Section 3.1). 'Hay/dried grass' flavour and bitter taste *scores parameters* associated more to positive parameters for the trained panel (CCA plot not shown), than for the industry panel, indicating that industry assessors either did not recognise/perceive these parameter attributes or they seem to interpret the scale descriptions differently. This may be improved with adequate training in recognition and scaling of these parameters. Furthermore, the association of 'turbid/hazy' and 'dust/sediment', and 'weak/watery', with negative *scores parameters* by both panels further demonstrates the validity of the method. As mentioned previously, validation samples showed no turbidity due to haze. Their higher NTU values are ascribed to the presence of dust particles due to insufficient sieving. The need for training in the visual assessment of infusion haze, and distinction between presence of haze and dust/sediment, was indicated.

#### 3.4.5 Association of DSA attributes with scorecard parameters

The positive association between positive sensory attributes used for DSA (including 'overall character on palate' and 'infusion strength') and the positive *scores parameters* of the scorecard (including 'overall character %', and 'colour %') by the industry panel, is depicted in **Fig. 10.1**. These variables correlate positively with positive quality, i.e. samples of 'moderate' to 'high' quality (**Fig. 10.1A**). Similarly, **Fig. 10.2** depicts positive association between positive DSA attributes and positive *citation parameters*, whereas negative DSA attributes clearly associate with negative *citation parameters*, namely 'weak/watery' and specific negative flavours (taints). These negative variables correlate positively with negative quality, i.e. samples of 'poor' to 'low' quality. Similar results were achieved for the trained panel (results not shown), although higher RV coefficients for DSA attributes vs *scores* and *citation parameters*, respectively, were obtained for the trained panel (0.85 and 0.82, respectively) than for the industry panel (0.65 and 0.62, respectively) (**Table 10**). These results were to be expected as the trained panel had extensive training in DSA attributes of honeybush tea which were similar to those used for the *scores* and *citation parameters*.

These results may be regarded as further validation of the quality scoring method, especially as the industry panel was not previously trained in the nomenclature and recognition of the specific scorecard parameters. Similarly, the relationship between DSA data of cheese samples from trained assessors and its quality scores from expert assessors have been assessed (Hersleth, IIseng, Martens, & Næs, 2005; Kraggerud et al., 2012). Hersleth et al. (2005) reported that the expert assessors' scores for consistency (body and texture), flavour (odour and taste) and overall quality correlated positively with DSA attributes such as 'mature' flavour, 'firmness', 'graininess' and 'dryness' of Norwegian cheese samples. Kraggerud et

al. (2012) reported significant regression correlations between DSA variables and quality scoring variables, e.g. the flavour/taste attribute 'aromatic' correlated positively with overall quality score.

### 3.4.6 Assessor reliability

Two blind duplicate samples were included to determine assessors' repeatability within a test replicate. The projection of infusion replicates on the respective two-dimensional PCA plots (**Fig. 8A**; **Fig. B9.A, Addendum B**) and CA plots (**Figs. 8B**; **Fig. B9.B, Addendum B**) was evaluated to determine assessors' reliability. The close proximity in the 2-dimensional sensory space for 2\_H/Int and 11\_L/Int and their respective blind duplicates, 2\_H/Int(2) and 11\_L/Int(2), indicated good repeatability for both trained and industry assessors within a test replicate; thus, both panels were considered reliable which further demonstrated the validity of the method.

A degree of product variation within replicates is expected, particularly due to the biological nature of honeybush tea samples, i.e. although all measures were taken during infusion preparation to assure representative replicates from the same sample, variation may have occurred due to the inhomogeneous nature of the plant material. Therefore, the variation in repeatability is due to a combination of assessor variation between replicates, as well as biological variation of the plant material within replicates. ICC agreement between replicates for industry panel assessors was lower, although not significantly, than that of the trained panel assessors.

Furthermore, inter-assessor reliability, i.e. degree of agreement between assessors within each panel, was also assessed. Multidimensional scaling (MDS) plots illustrated higher agreement between each other for trained assessors than for industry assessors (plots not shown). This was to be expected from a trained panel that was trained as a unit, compared to the industry panel with large variation in expertise and no formal training of the individual assessors in terms of the range of sensory attributes associated with honeybush tea.

## 3.5 Effect of assessor training

The ANOVA results for scores (%) of the samples used in method validation showed that the product effect for total and all 10 individual *scores parameters* were significant ( $p < 0.05$ ) (**Table 11**), i.e. samples varied in quality and both panels were able to discriminate significantly between samples based on total and individual parameter scores (%). Significant assessor type  $\times$  product interaction effects, for the *scores parameters* 'floral', 'fruity and sweet', 'spicy and/nutty', 'woody', 'hay/dried grass' and 'taints', indicate that the panels used the category scales differently for these attributes. LS means line plots for 'woody' aroma (**Fig. 11.1B**) and 'hay/dried grass' flavour and 'taints' (**Fig. B10, Addendum B**) depict these differences. However, panels scored 'infusion colour' and 'overall character on palate' similarly (**Fig. 11.2**).

The use of the colour reference card may have aligned the panels to discriminate similarly on infusion colour.

Furthermore, the industry panel assigned significantly lower total scores to the majority of the samples compared to that of the trained panel (**Fig. 11.1A**). This may indicate that industry assessors were stricter (i.e. less lenient) on quality during scoring as the intention was quality assessment and not purely discrimination based on parameter intensity (Jackson, 2009), or as a result of a different interpretation of parameter descriptions and scale descriptions by the industry panel. Conversely, the higher total scores for the trained panel may be ascribed to their experience in parameter descriptions, particularly as most of the scorecard descriptions were similar to that used for DSA, or their lack of experience in quality assessment, particularly in terms of parameters that are key to sensory quality of honeybush tea. Only 11\_L/Int and its blind duplicate 11\_L/Int (2) received similar total scores by both panels. Based on its DSA data, 11\_L/Int had a sour taste with high intensities of several vegetative taints ('green grass', 'hay/dried grass', 'rotting plant water' and 'cooked vegetables' flavours). Similar scores for this sample could possibly be ascribed to the extreme intensity value of these recognisable taints.

Similar trends of significantly lower scores by the industry panel are noted for the individual scores parameter, 'woody' aroma (**Fig. 11.1B**), except for sample 11\_L/Int (2). The significant lower scores for 'woody' could be ascribed to the trained panel's experience in recognition of the specific parameter description (which was the same as the DSA attribute description) or a different interpretation of parameter description and scale by the industry panel. Industry assessors' negative association with 'woody' aroma was also noted in the association between scorecard parameters (Section 3.4.4).

For the negative parameters, 'hay/dried grass' flavour, bitter taste, sour taste and 'taints', the score allocation to category scales was reversed, i.e. highest score (3) was allocated to 'absent/barely perceptible' and lowest score (0) allocated to 'high'. Therefore, samples with high scores for negative parameters indicate low perception of the respective parameters and *vice versa*. The industry panel gave significantly higher scores for 'hay/dried grass' flavour parameter, i.e. they seemed to perceive this parameter at a lower intensity than the trained panel (**Addendum B, Fig. B10**). This tendency may be ascribed to the industry panel's inability to identify this attribute as they were unfamiliar with parameter nomenclature (i.e. they were not trained in parameter descriptions).

The need for training of industry assessors in the recognition and scoring of parameters (*scores and citation parameters*) is identified from afore-mentioned results, method validation results (Section 3.4) and industry panel responses from the questionnaire completed after assessment of samples. Assessors specified their experience level in the sensory quality assessment of honeybush tea as 'novice' (N = 5), 'low' (N = 4) and 'moderate' (N = 5). Comments relating to current challenges experienced by QC personnel include the lack of standardised in-house vocabulary for sensory attributes and the need for adequate training for effective sensory assessment. Additionally, the current supply of production batches of poor

and inconsistent quality supplied by processors, ascribed to poor processing, was confirmed. This emphasises the need for training on production level. The revised generic honeybush aroma wheel and lexicon with chemical-based reference standards (Chapter 3) would aid in assessor training to communicate honeybush sensory quality within company divisions, and to suppliers and marketers in the supply chain. Similarly, Larssen et al. (2019) identified the need for 'synthetic' reference standards for omega-3 fish oil lexicon for sensory panel training. The development of defect wheels to facilitate assessor training in recognition in defects related to poor processing or poor GMPs, as developed for wine, beer and olive oil industries (Langstaff, 2016), may be considered for training of assessors in recognition of honeybush taints. Additionally, the development of quantitative reference standards, by amending the concentration levels of developed chemical reference standards (Chapter 3) to represent 'low', 'moderate' and 'high' intensity levels, would aid assessors in anchoring of the scorecard parameter scales. Quantitative reference standards representative of 'moderate' or 'high' intensities, specifically for parameter sub-categories that are regarded as defects at higher intensities such as 'woody' and 'hay/dried grass', may be developed. Quantitative reference standards for the more intricate parameter sub-category 'overall character on palate', would also facilitate parameter recognition and scale anchoring. This approach was followed for wine and cheese. Quantitative reference standards for 'lactic' and 'herbaceous' were developed for a red wine scorecard to allow assessors to recognise when attributes should be categorised as defects, i.e. when intensities perceived in a batch are higher than a reference standard intensity (Etaio et al., 2010a). For Idiazabal cheese, quantitative reference standards for both 'medium' and 'high' intensities for specific attributes have been developed (Ojeda et al., 2015).

Although cost, resource and time constraints may limit the number of assessors available in the production or quality control divisions, assessor screening and training is still regarded as important for small panels (De Vos, 2010). Furthermore, the application of good sensory practices even within a small panel set-up has been emphasised (Lawless & Heymann, 2010; Lawless, Liu, & Goldwyn, 1997). Therefore, considering the availability of small panels within the present honeybush industry, assessor training and application of standard sensory practices, as recommended by Lawless and Heymann (2010), are important for effective sensory quality assessment of honeybush tea.

Allocation of scores to category scales for specific parameters may be re-considered for scorecard improvement. A non-linear score allocation for 'hay/dried grass' flavour is recommended. As an intrinsic characteristic of honeybush, this parameter will be very seldom 'absent/barely perceptible' as per semantic category scale description and only at higher intensities is it regarded as a defect. Therefore, score allocation may be changed to '0' for 'absent/barely perceptible', '3' for 'low' and '2' for 'moderate' intensity.

In addition, to enhance method validity, it is recommended that the validation test is repeated with a different set of samples, as well as with industry and trained panel assessors after training, respectively.

### 3.6 User-friendliness of scorecard and practical considerations

Questionnaire responses from the industry panel (N = 14) on the user-friendliness of the scorecard are presented in **Fig. 12**. More than 50% of assessors regarded the method as 'very easy' or 'easy'. Industry assessors who regarded the method as difficult remarked that they were unfamiliar with the sensory attributes of honeybush tea and regarded it difficult to recognise and to distinguish between descriptions of positive and negative *scores* and *citation parameters*. Industry assessors commented that although it takes a level of concentration and time to complete the scorecard, the method provides detailed information of the product batch which will aid in record-keeping and traceability. In addition, positive feedback was given on the scorecard lay-out and order of assessment. One industry assessor remarked that the inclusion of actions to be taken is applicable as it is current practice to blend tea batches of different quality, i.e. the quality scoring method produces 'actionable results' that can be used to improve product quality in the commercial environment.

The questionnaire results from the trained panel (N = 13) indicate that > 70% of assessors regarded the quality scoring method as 'easy' or 'very easy' and two assessors as 'neither easy/difficult' (data not shown). This may be ascribed to the fact that trained assessors are familiar with the sensory attributes of honeybush tea which were translated into *scores* and *citation parameters* for the scorecard. Trained assessors regarded the scorecard lay-out as clear, concise and easy to understand, and the semantic category scales from 'absent/barely perceptible' to 'high' were regarded as easy to distinguish and to rate.

Information on current QC practices within the honeybush industry relating to panel size, sensory parameters assessed and QC during processing is provided in **Fig. 12**, i.e. the use of small QC panels (mainly < 5 representative of different divisions), the main steps in production chain in which sensory assessment is performed, namely predominantly on receipt of batches from suppliers, and key quality parameters tested. The industry predominantly assesses infusion taste (sensory attributes on palate), followed by infusion colour and aroma. Similar information was obtained from industry consultation (Section 3.1). In addition, assessors indicated different examples of attribute nomenclature, from 'steamed/baked fruit', 'dried apple rings', 'Christmas cake' for positive attributes to 'dish water', 'herb' and 'vegetative' for negative attributes, or simply 'acceptable' and 'unacceptable'. Several attributes similar to that included in the revised sensory lexicon for fermented honeybush (Chapter 3) and used in the present study were cited by two or more assessors for positive attributes, 'apricot', 'sweet', 'caramel', 'honey' and 'floral'/'fynbos-floral'/'flowery'/'fynbos', and negative attributes, 'smoky'/'burnt', 'musty', 'rot water'/'rotten', 'green'/'green grass', 'hay' and 'medicinal'. This may be ascribed the use of a rooibos sensory lexicon (Koch, Muller, Joubert, Van der Rijst, & Næs, 2012) by some of the assessors who were representatives of rooibos packing companies. In addition, one assessor indicated that they use the descriptors of a previous version of the generic honeybush wheel (Theron et al., 2014).



The aim of the quality scoring method for honeybush was not simply to provide a single overall quality score but to use the scorecard results as a report document ('certificate of analysis') that provides an overview of all the individual parameter scores and citation frequencies of a production batch. This was also confirmed by the industry panel feedback. Correspondingly, the final report for sensory assessment by an accredited laboratory for certified products with PDO status includes the mean score of each parameter, as well as an indication of the presence of specific defects and positive attributes based on the citation frequency by assessors (Etaio et al., 2010a, 2012; Ojeda et al., 2015; Pérez-Elortondo et al., 2007).

An electronic scorecard is recommended for future use to prevent unnecessary loss of data due to incomplete scorecards as seen for the validation test in the present study, as well as to facilitate automatic data processing and analyses. Highly developed web-based sensory programmes exist for electronic sensory data capturing and analyses (Compusense®, EyeQuestion®). Similarly, the UC Davis scorecard for olive oil certification has been configured for internet use to speed up the data capturing process, for ease of data analyses and graph computation and to reduce errors involved in manual entry (Langstaff, 2014). In addition, to support results obtained from visual assessment of appearance parameters, instrumental turbidity and colour measurements are recommended for companies that would like to implement instrumental methods in addition to sensory analysis.

## 4 Conclusions

The current research demonstrated the effective use of the quality scoring method to assess and classify the sensory quality of fermented honeybush by industry assessors and researchers. Collectively, the information captured from the comprehensive dataset and industry survey directed the choice of parameters and scales for inclusion in the sensory quality scorecard. DSA sensory attributes were effectively translated into scorecard parameters to provide representative descriptions for the sensory quality of honeybush tea.

Specifications for the optimum sensory quality standard, namely 'high' quality class, were defined as 1) *dry leaf appearance parameter*: even 'cut size' and absence of 'light stem particles', 2) *infusion appearance parameter*: 'red-brown' colour and absence of 'haze', 3) *infusion aroma parameter*: high intensities for 'floral', 'fruity and sweet', 'nutty and/or spicy' and moderate intensity of 'woody' aroma, and 4) *infusion palate attribute parameter*: high intensity for 'overall character on palate', low intensity of 'hay/dried grass' flavour, low intensity/absence of bitter taste, and absence of sour taste and taints.

Scorecard scores and citation parameters allowed for effective distinction between 'poor' to 'low' and 'moderate' to 'high' quality products. The application of citation parameters on the scorecard was regarded as essential to aid in the classification of 'poor' and 'low' quality batches, especially related to

critical taints, namely 'medicinal', 'smoky' and 'musty' flavour. Industry and trained assessors were able to discriminate samples, similarly, based on critical *scores parameters*, 'infusion colour' and 'overall character on palate', irrespective of expertise' level. The accurate interpretation of semantic category scales for these two parameters and use of a colour reference card for assessor alignment were indicated. Although similar citations of negative attributes (taints) by industry and trained assessors were observed, training in parameter sub-categories, especially 'woody' aroma and 'hay/dried grass' flavour would improve parameter recognition and interpretation, and subsequent discrimination between batches. Furthermore, the provision of checkboxes for citation of specific positive aroma attributes such as 'rose perfume' and 'raisin' aroma would aid as a distinguishing factor amongst samples of 'moderate' to 'high' quality. The latter feature of the scorecard could also form the basis for distinguishing and reporting differences between honeybush species for exploitation of niche markets.

The developed quality scoring system for honeybush tea could find application as valuable tool to monitor and optimise sensory quality during processing and blending, which would improve communication of sensory quality between company divisions and throughout the honeybush supply chain. Industry responses confirmed that assignment of quality classes to product batches would empower processors in price negotiation and marketing of their products. Application of the developed quality scoring method to blends of individual batches for which the quality classes are known, is recommended for further validation. In addition, considering time constraints, the investigation of novel rapid sensory profiling methods as time-efficient quality screening and classification tools within commercial and research environments, is granted.

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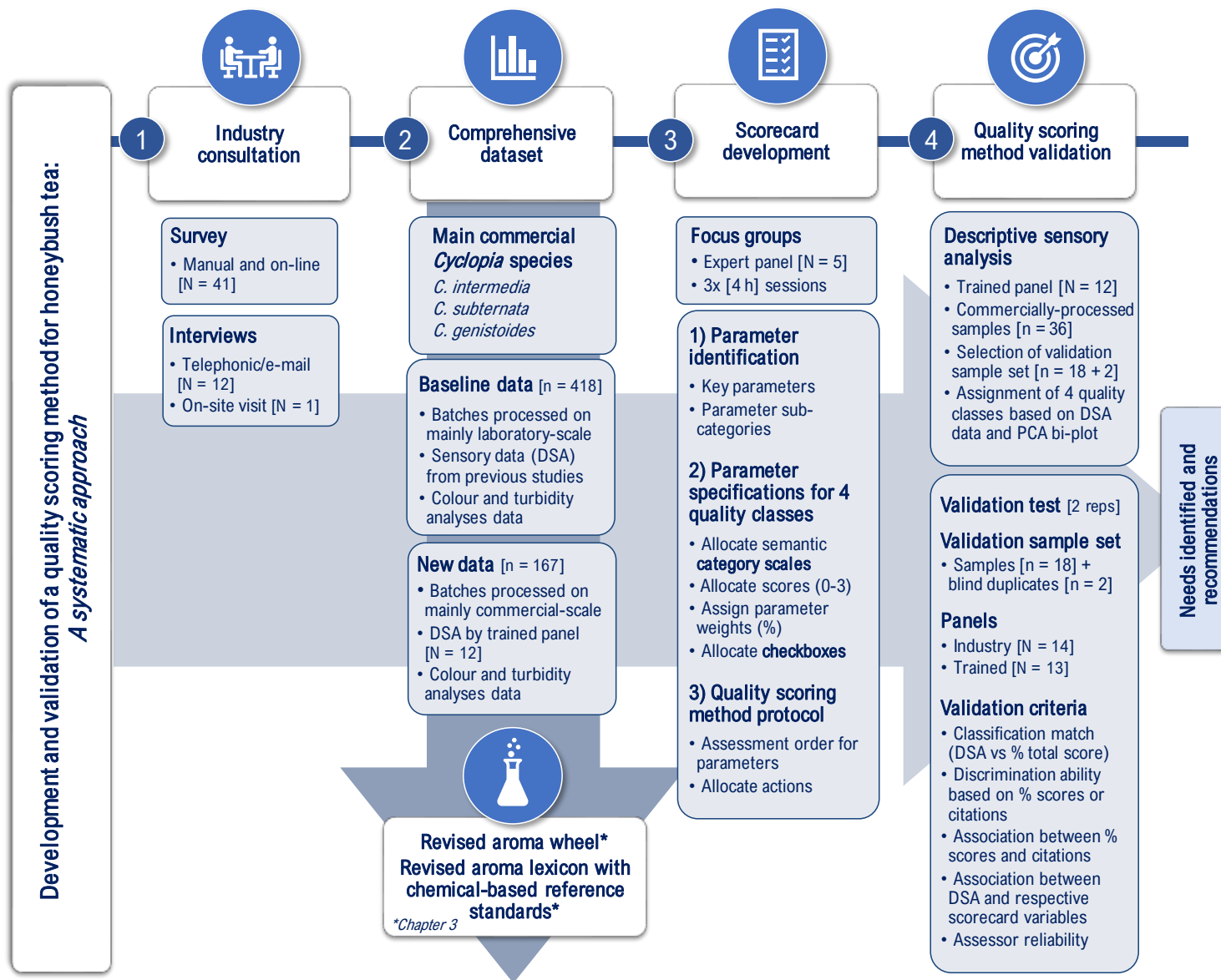


Figure 1 Experimental lay-out for the development of a sensory quality scoring method for fermented honeybush tea.

SENSORY QUALITY SCORECARD FOR FERMENTED HONEYBUSH TEA				
Analysis Date:		Supplier (if applicable):		
Assessor Name:		Species:		
Assessor Signature:		Batch Number:		
		Production Date:		

 <b>APPEARANCE</b>	<b>DRY LEAF APPEARANCE</b>	<input type="checkbox"/> UNEVEN CUT <input type="checkbox"/> STEM UNACCEPTABLE		<i>ACTION: Further Processing</i>								
		<input type="checkbox"/> EVEN CUT <input type="checkbox"/> STEM ACCEPTABLE		<i>ACTION: Continue scoring</i>								
	<b>INFUSION TURBIDITY / HAZE</b>	<input type="checkbox"/> TURBID / HAZY	<i>ACTION: Further Processing</i>									
		<input type="checkbox"/> DUST / SEDIMENT	<i>ACTION: Sieving step(s)</i>									
<input type="checkbox"/> CLEAR		<i>ACTION: Continue scoring</i>										
	<b>INFUSION COLOUR</b>	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 25%; text-align: center;">Yellow/Green</td> <td style="width: 25%; text-align: center;">Light Red-brown</td> <td style="width: 25%; text-align: center;">Red-brown</td> <td style="width: 25%; text-align: center;">Dark Red-brown</td> </tr> <tr> <td style="height: 20px;"></td> <td></td> <td></td> <td></td> </tr> </table>	Yellow/Green	Light Red-brown	Red-brown	Dark Red-brown						
Yellow/Green	Light Red-brown	Red-brown	Dark Red-brown									

 <b>AROMA (NOSE)</b>		Absent/Barely Perceptable	Low	Moderate	High	<i>Check boxes if present:</i> <input type="checkbox"/> Fynbos-Floral <input type="checkbox"/> Rose Geranium <input type="checkbox"/> Rose Perfume <input type="checkbox"/> Apricot <input type="checkbox"/> Raisin <input type="checkbox"/> Cooked Apple <input type="checkbox"/> Fynbos-Sweet <input type="checkbox"/> Fruity-Sweet <input type="checkbox"/> Caramel <input type="checkbox"/> Honey <input type="checkbox"/> Sweet-spice <input type="checkbox"/> Nutty
	<b>FLORAL</b>					
	<b>FRUITY and SWEET</b>					
	<b>SPICY and/or NUTTY</b>					
		<b>WOODY</b>	Absent/Barely Perceptable	Low	Moderate	High

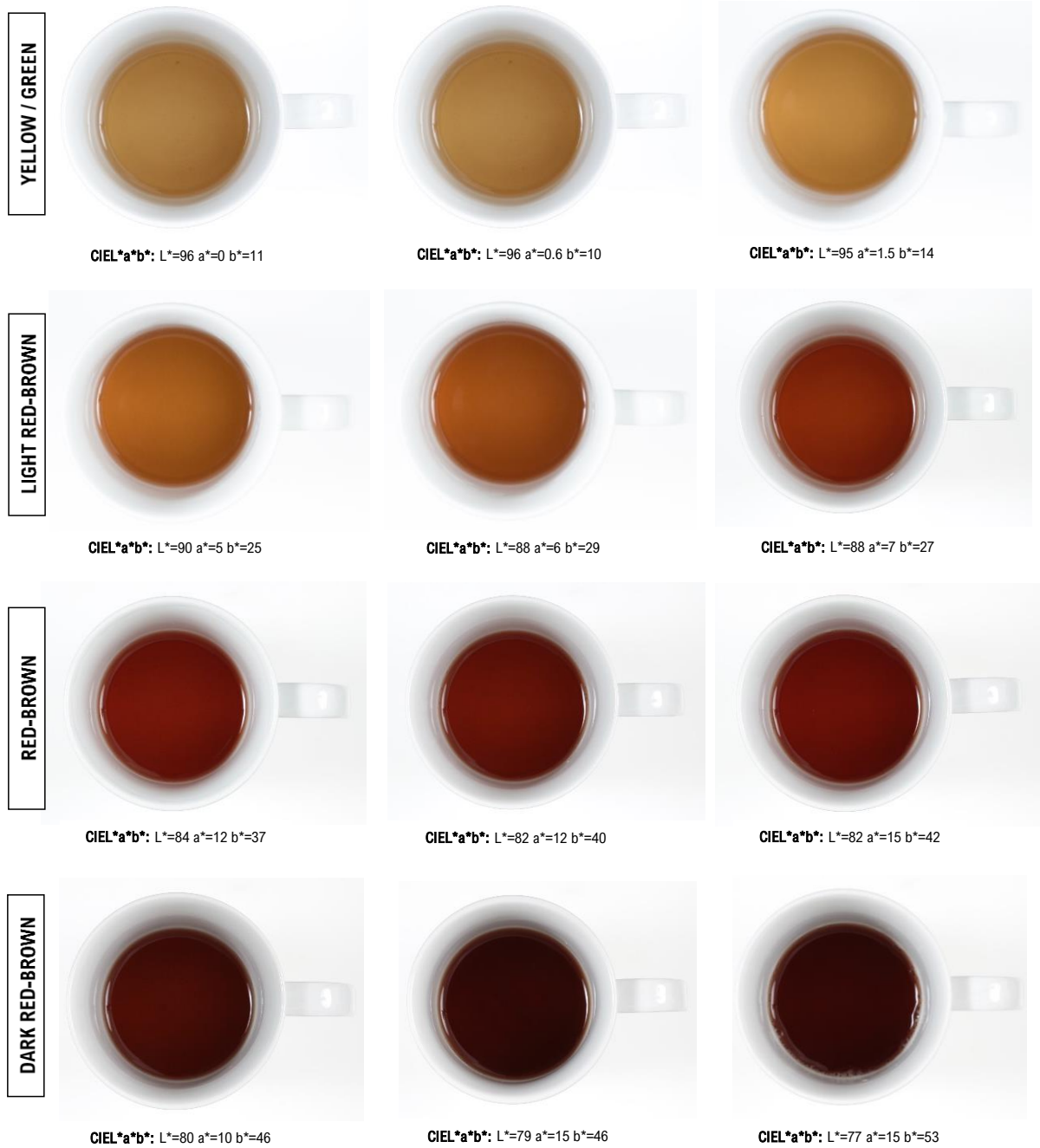
 <b>FLAVOUR / TASTE / MOUTHFEEL (PALATE)</b>	<b>OVERALL CHARACTER*</b>	Absent/Barely Perceptable	Low	Moderate	High	<i>Check boxes if present:</i> <input type="checkbox"/> Weak / Watery
	<b>HAY/DRIED GRASS</b>	Absent/Barely Perceptable	Low	Moderate	High	
	<b>BITTER</b>					
	<b>SOUR</b>					<input type="checkbox"/> Vinegar-like
		<b>TAINT(S)</b>	Absent/Barely Perceptable	Low	Moderate	High

<b>OVERALL COMMENTS:</b>

Figure 2.1 Sensory quality scorecard for fermented honeybush tea.

**COLOUR REFERENCE CARD FOR INFUSION APPEARANCE ASSESSMENT OF  
FERMENTED HONEYBUSH TEA**



**Figure 2.2** Colour reference card for infusion appearance assessment of fermented honeybush tea. Samples (100 mL) are presented in porcelain mugs (diameter 75 mm at 100 mm depth)<sup>3</sup> (images by Anton Jordaan Photography, Stellenbosch, South Africa).

<sup>3</sup> CIEL\*a\*b\* values are indicated only for research purposes and will not be included for industry application.

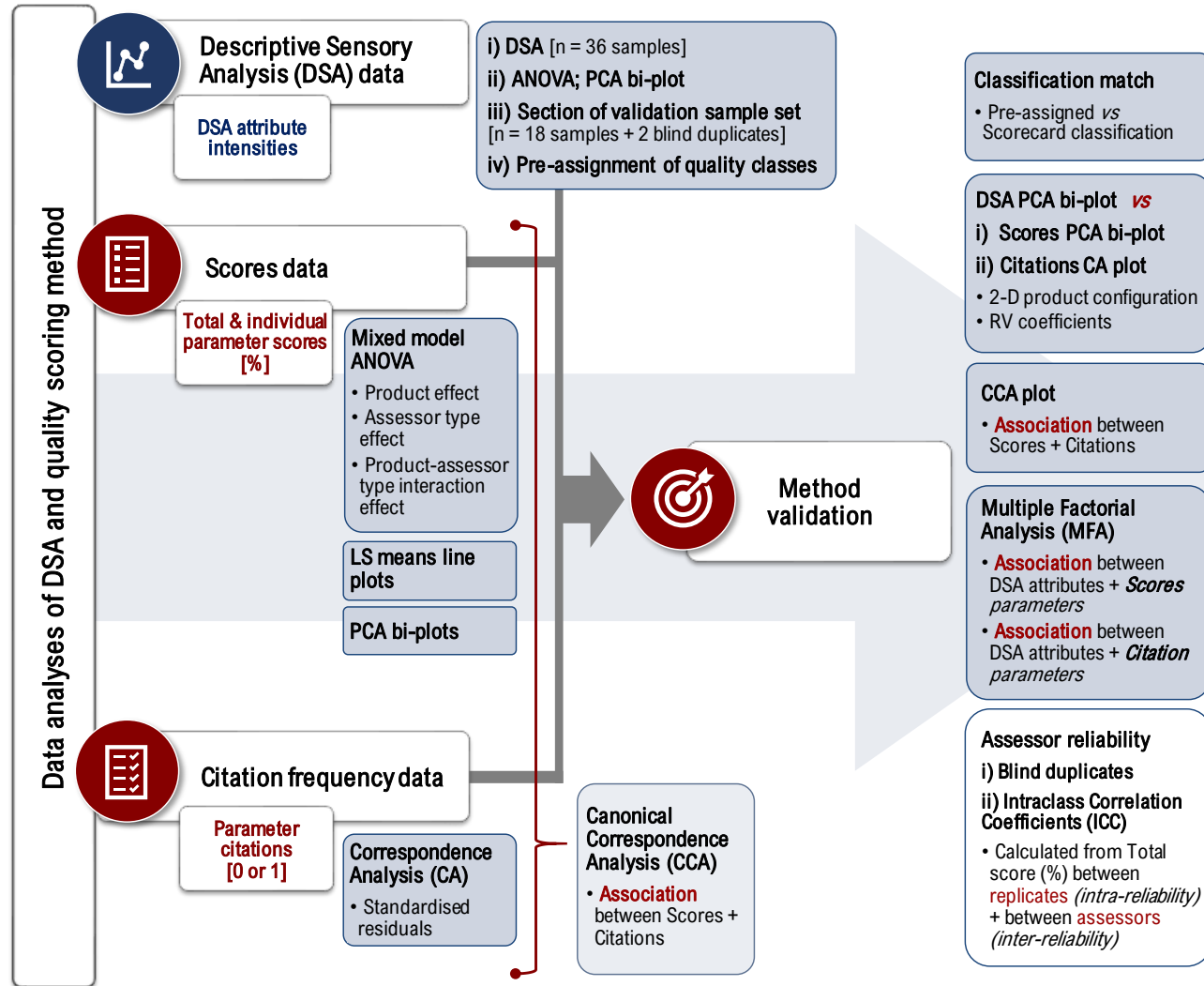
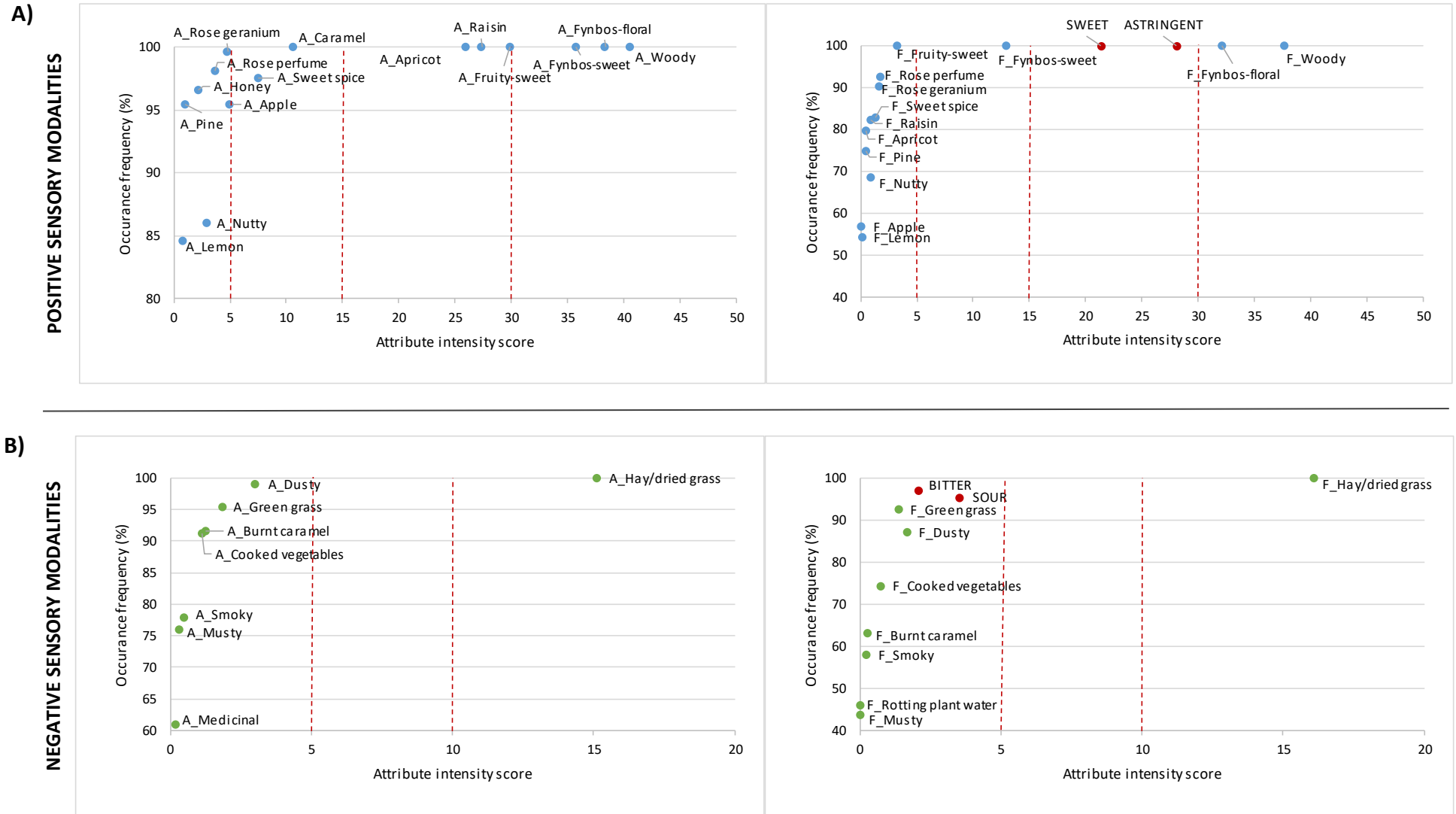
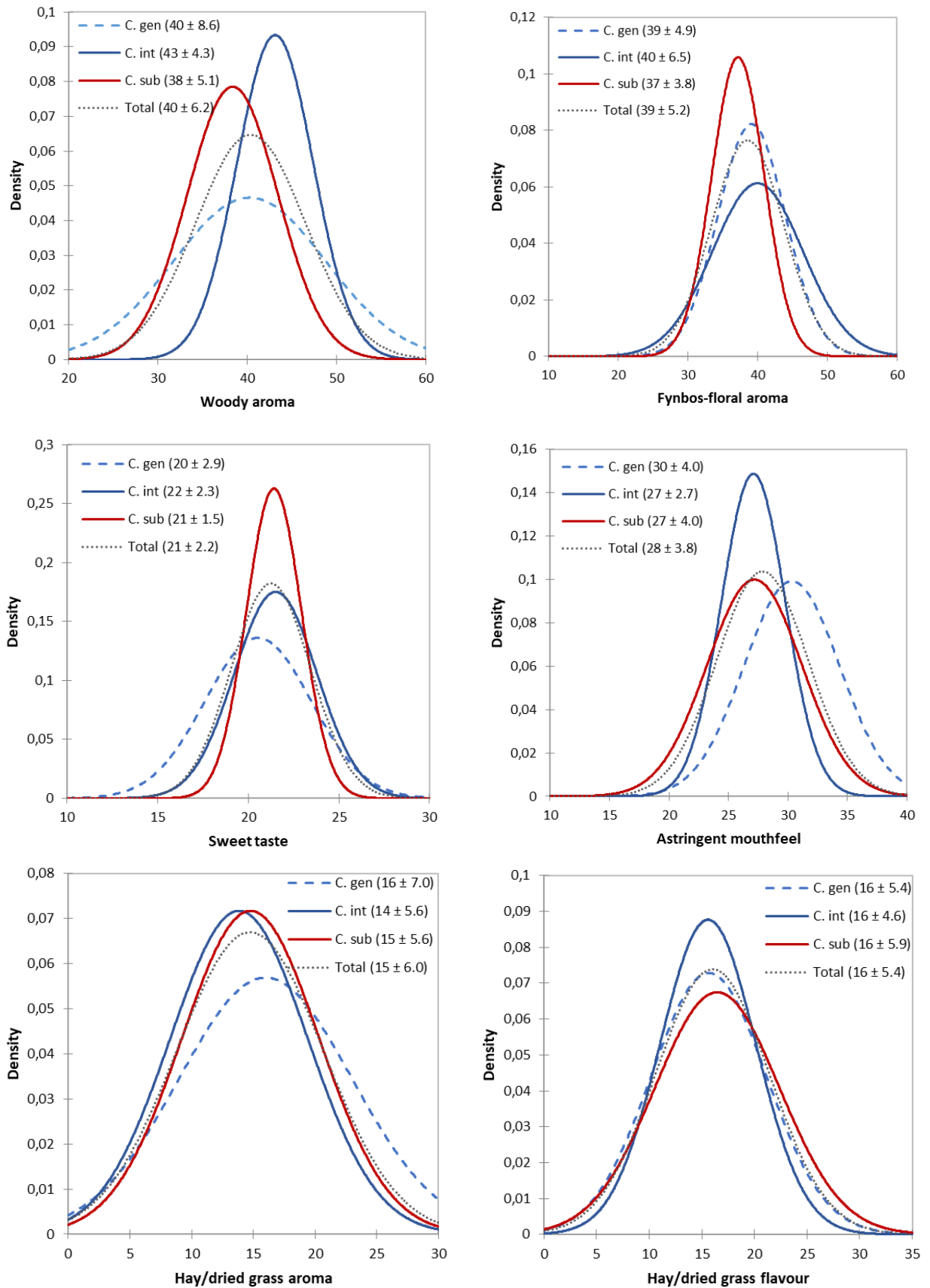


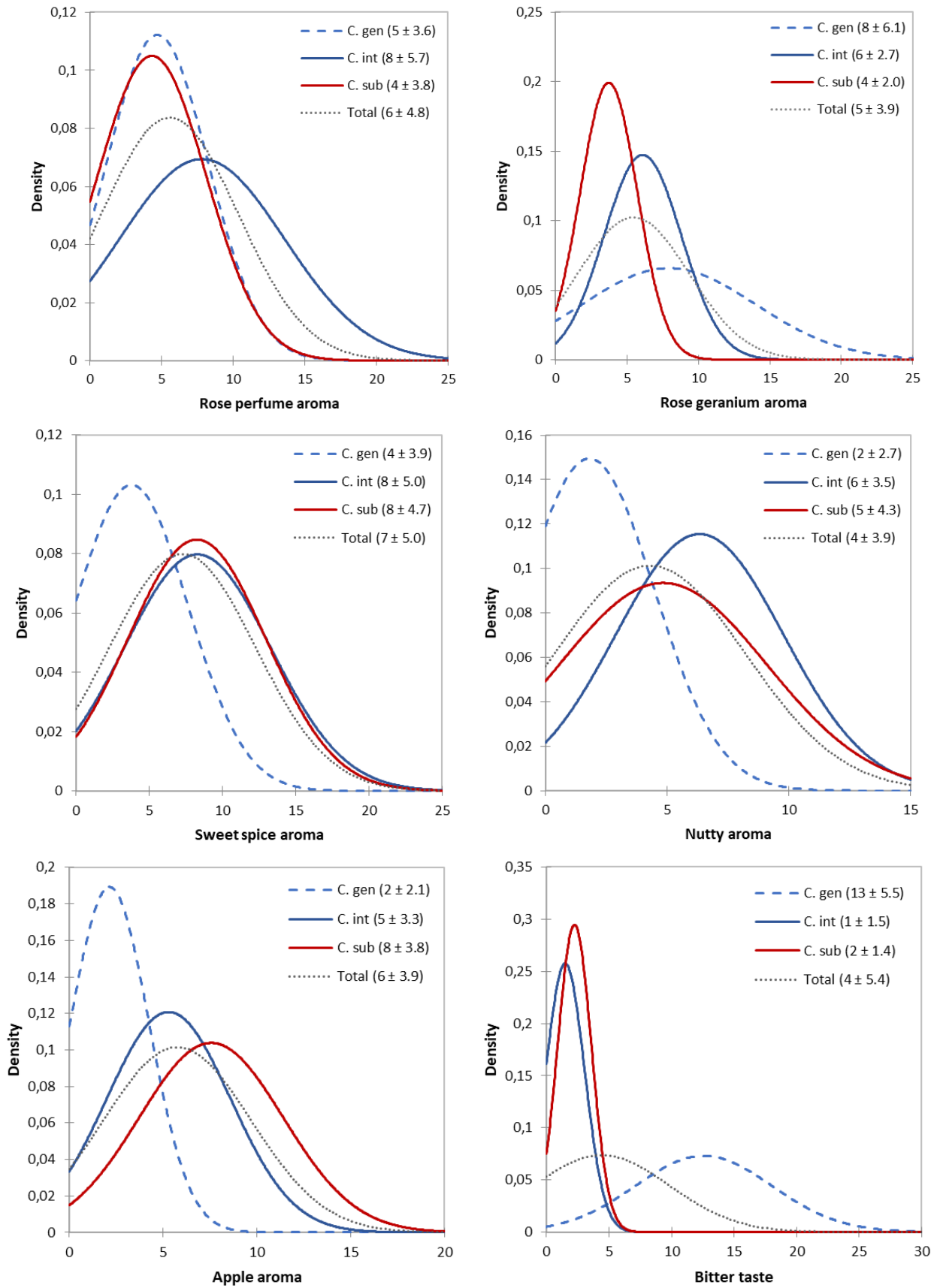
Figure 3 Schematic presentation of the statistical analyses conducted for validation of the sensory quality scoring method.



**Figure 4** Occurrence frequency plots based on comprehensive dataset for intensity scores of **A) positive sensory modalities**, i.e. aroma (*A*), flavour (*F*), sweet taste and astringent mouthfeel and **B) negative sensory modalities**, i.e. negative aroma (*A*), flavour (*F*), bitter and sour taste.

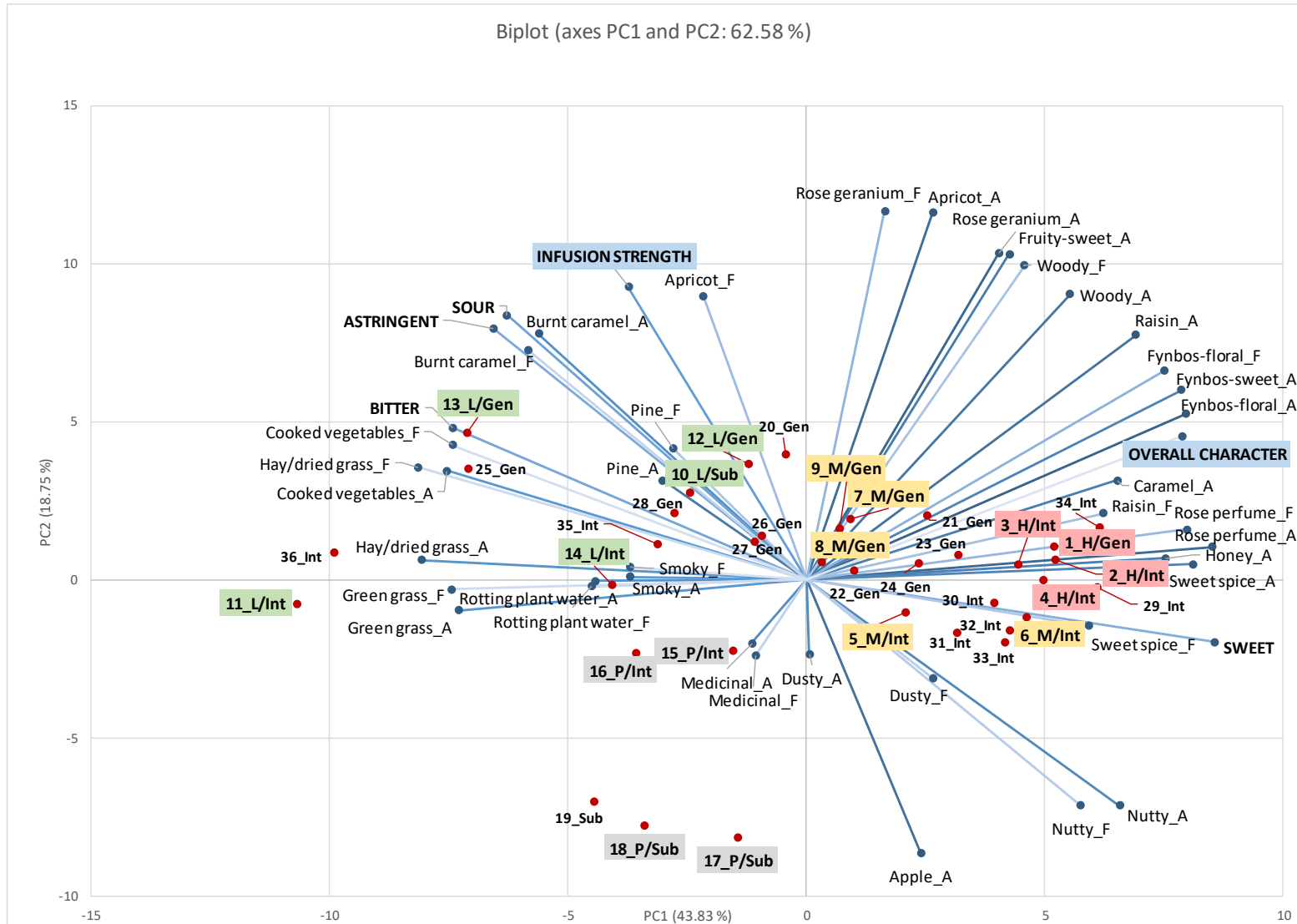


**Figure 5.1** Normal distribution plots depicting intensity distribution for **intrinsic sensory attributes** for hot water infusions of fermented *C. genistoides* (*C. gen*), *C. subternata* (*C. sub*) and *C. intermedia* (*C. int*) at 'cup-of-tea' strength. Data in brackets in legends represent mean intensities ± standard deviations.



**Figure 5.2** Normal distribution plots depicting intensity distribution for **sensory attributes more prominent in specific species** for hot water infusions of fermented *C. genistoides* (*C. gen*), *C. subternata* (*C. sub*) and *C. intermedia* (*C. int*) at ‘cup-of-tea’ strength. Data in brackets in legends represent mean intensities ± standard deviations.



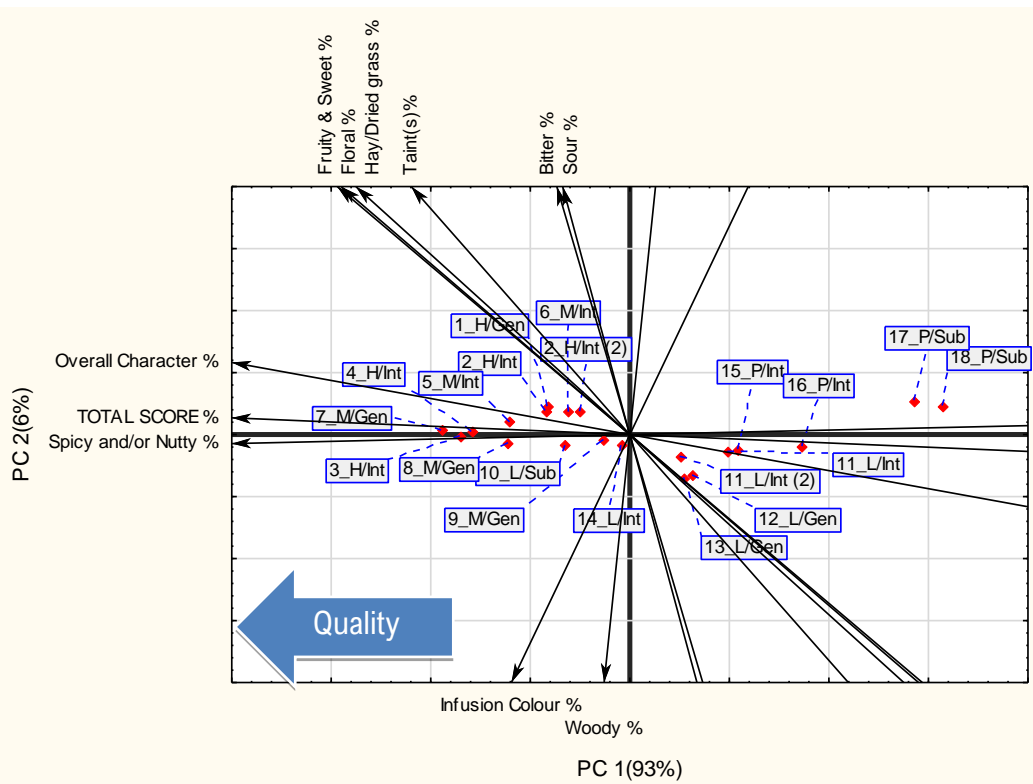


**Figure 6** PCA bi-plot of DSA data indicating sample configuration of sample set (n = 36) analysed for selection of validation sample set [1–36 represent sample numbers; Int, Sub, Gen represent *C. intermedia*, *C. subternata* and *C. genistoides*, respectively; H (red), M (yellow), L (green), P (grey) represent high, moderate, low and poor quality, respectively; aroma (A) or flavour (F) attributes are indicated after descriptors].

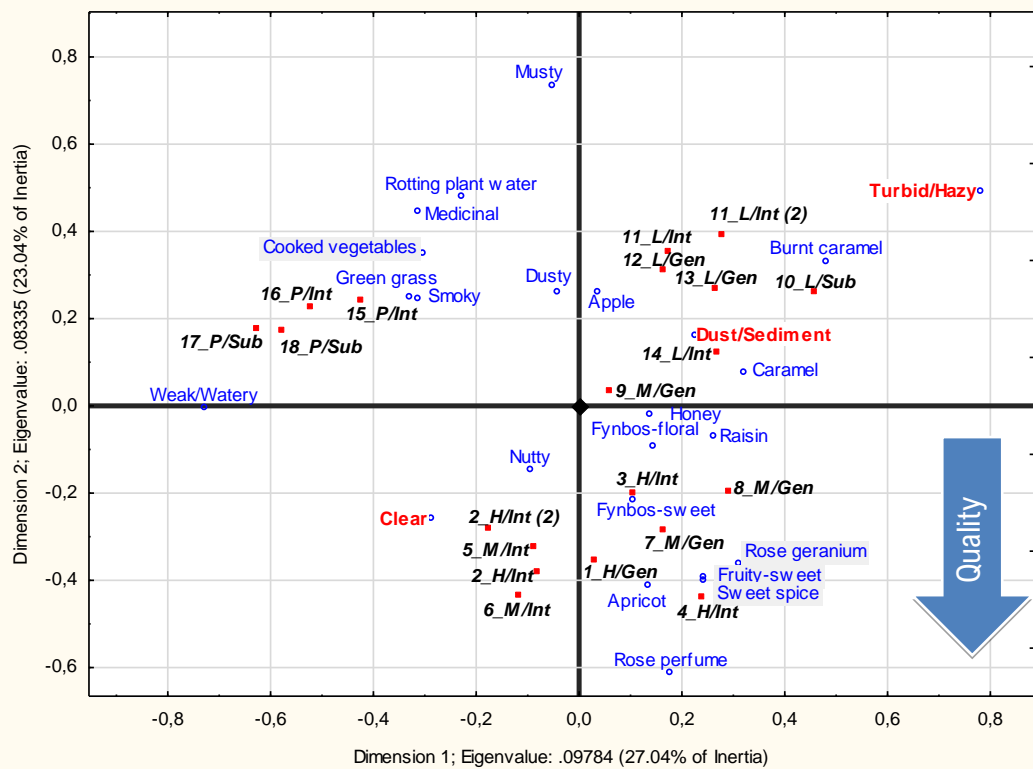


**Figure 7** PCA bi-plot of DSA data indicating sample configuration of validation sample set (n = 18) for **A) PC1 and PC2** and **B) PC1 and PC3** [1–18 represent sample numbers; Int, Sub, Gen represent *C. intermedia*, *C. subternata* and *C. genistoides*, respectively; H (red), M (yellow), L (green), P (grey) represent high, moderate, low and poor quality, respectively; aroma (A) or flavour (F) attributes are indicated after descriptors].

A)



B)



**Figure 8** A) PCA bi-plot of scorecard data for **total and individual parameter scores**<sup>4</sup>, and B) CA plot of **standardised residuals** calculated from citation frequencies for the **industry panel** [infusion appearance (red) and sensory attribute parameters (blue)]; 1–18 represent sample numbers; Int, Sub, Gen represent *C. intermedia*, *C. subternata* and *C. genistoides*, respectively; H, M, L, P represent high, moderate, low and poor quality, respectively; (2) after sample code indicates blind duplicate]. Blue arrows indicate direction of increased sensory quality.

<sup>4</sup> Reversed scores allocation has been incorporated for negative parameters: therefore, 'hay/dried grass' flavour, bitter taste, sour taste and 'taints' parameter scores are in opposite direction from low and poor quality samples.

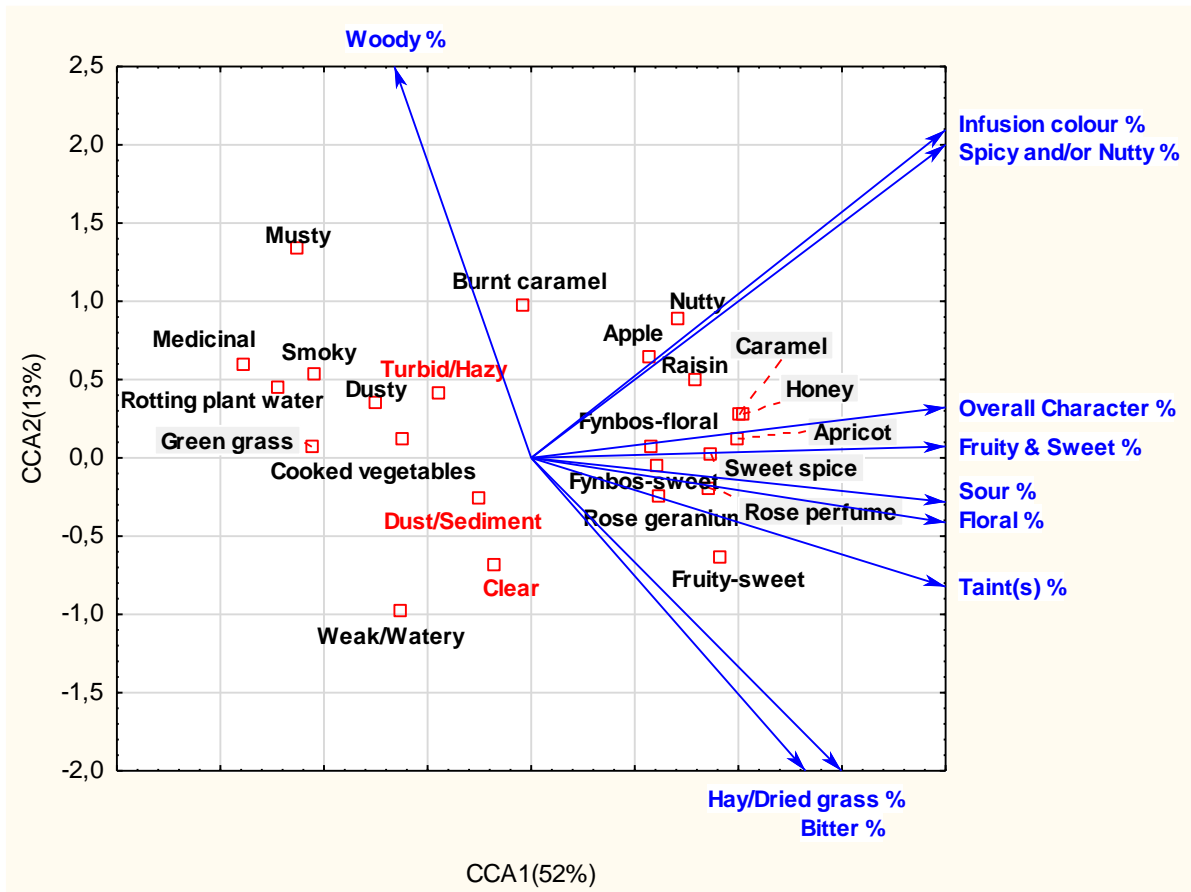
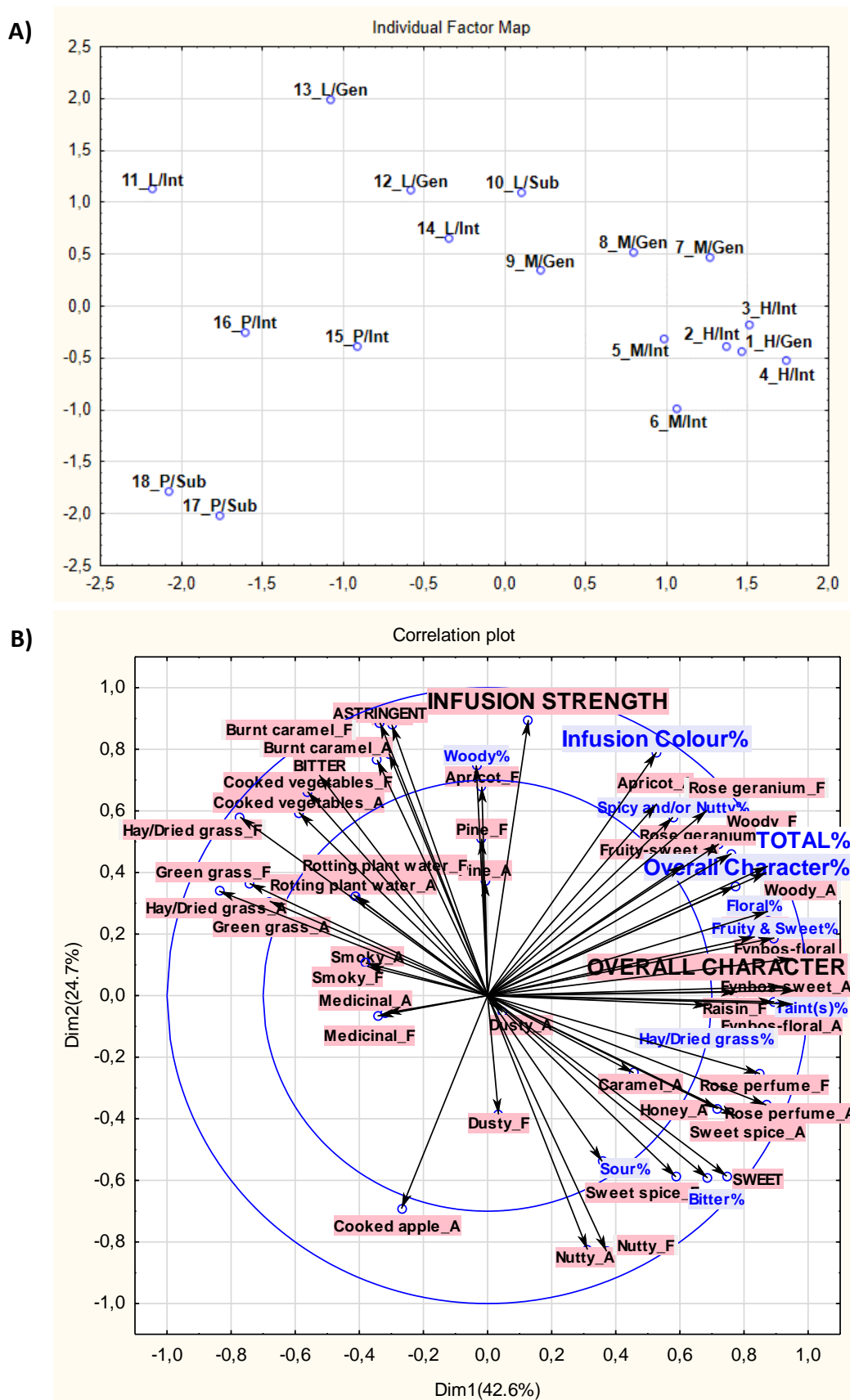
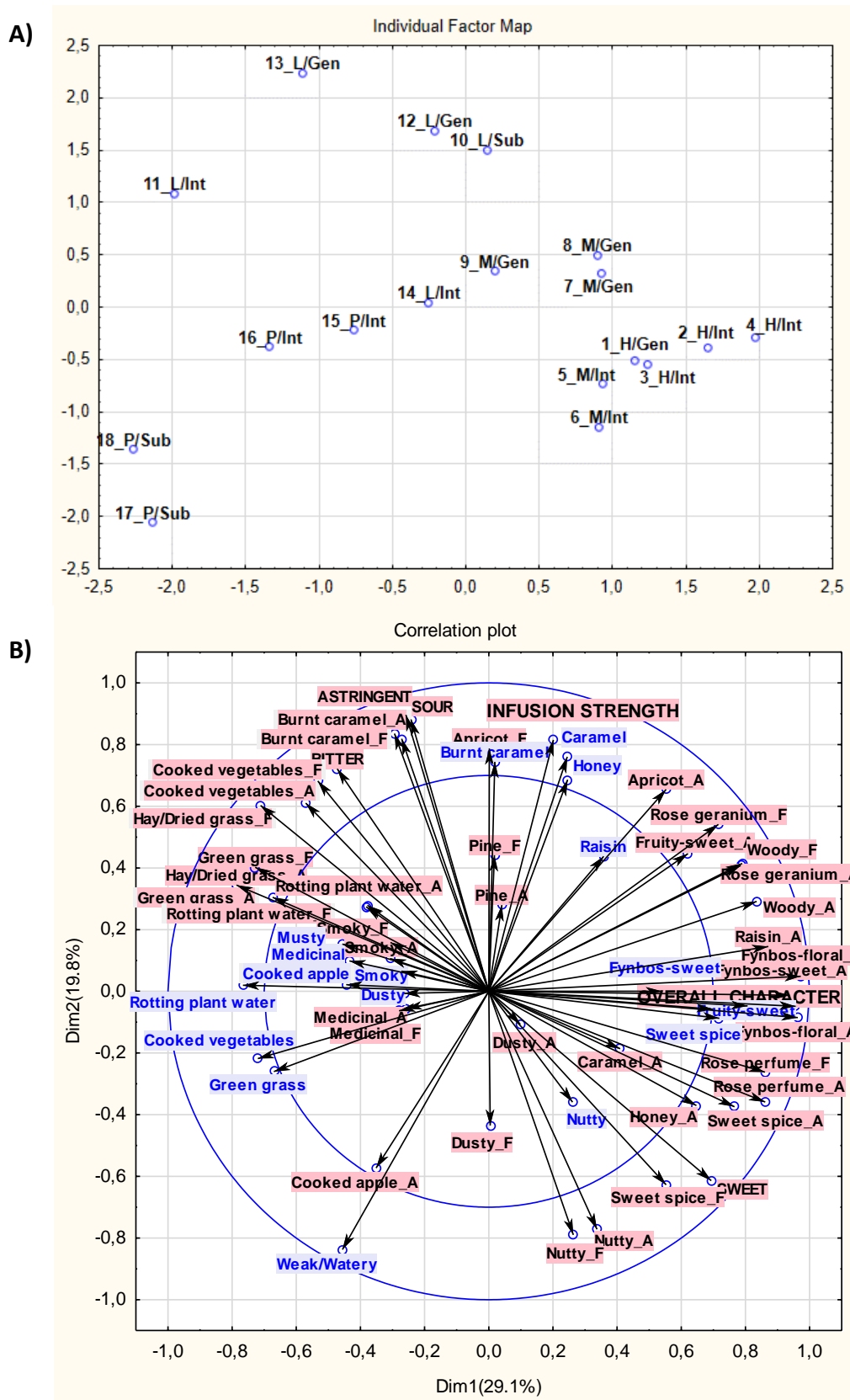


Figure 9 CCA plot of scorecard data for *industry panel* illustrating the association between ‘scores parameters’<sup>5</sup> (blue) and ‘citation parameters’ [infusion aroma/palate parameters (black) and appearance parameters (red)].

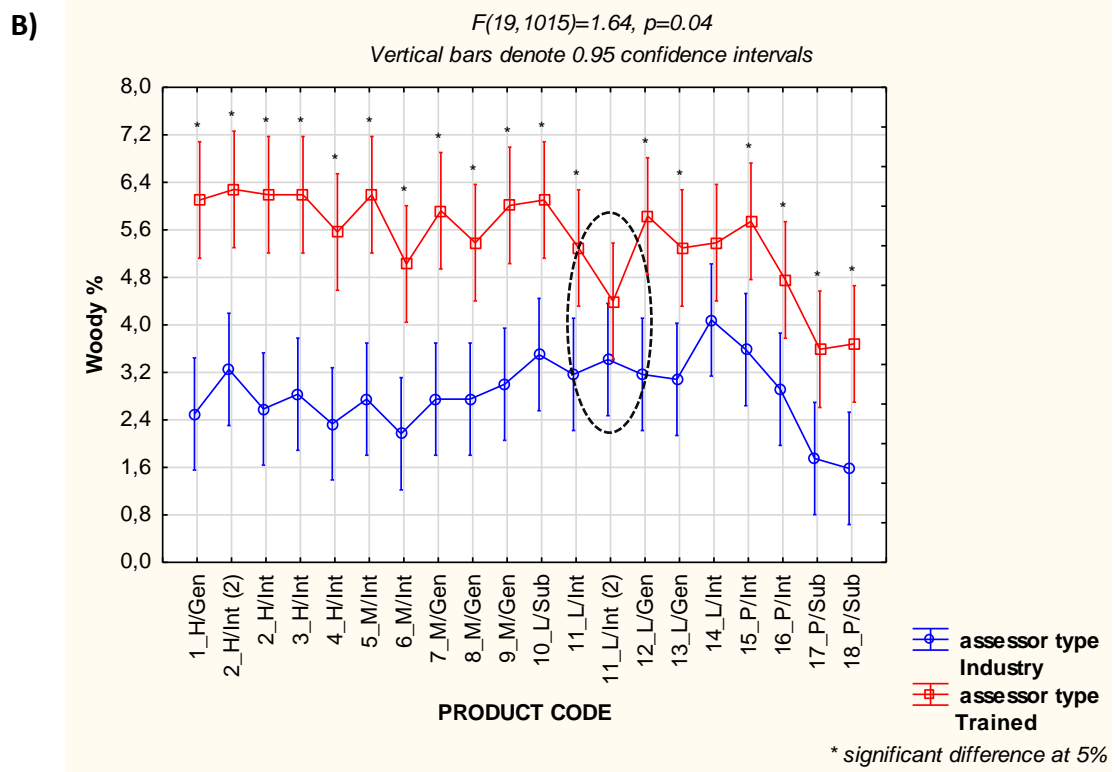
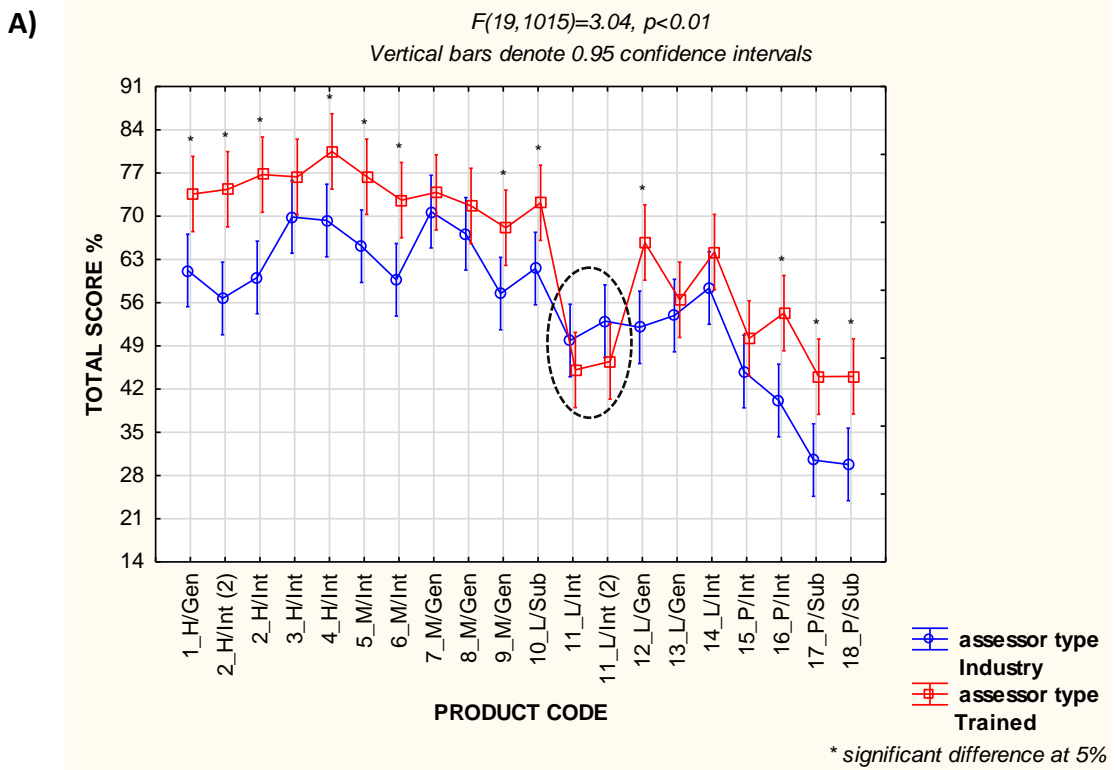
<sup>5</sup> Reversed scores allocation has been incorporated for negative scores parameters: therefore, ‘hay/dried grass’ flavour, bitter taste, sour taste and ‘taints’ associate with positive ‘scores parameters’, in opposite direction from low and poor quality samples.



**Figure 10.1** A) Individual factor map (MFA) and B) correlation plot depicting the association between DSA attributes and scores parameters for the industry panel [DSA attributes (red) and scores parameters (blue); 1–18 represent sample numbers; Int, Sub, Gen represent *C. intermedia*, *C. subternata* and *C. genistoides*, respectively; H, M, L, P represent high, moderate, low and poor quality, respectively].

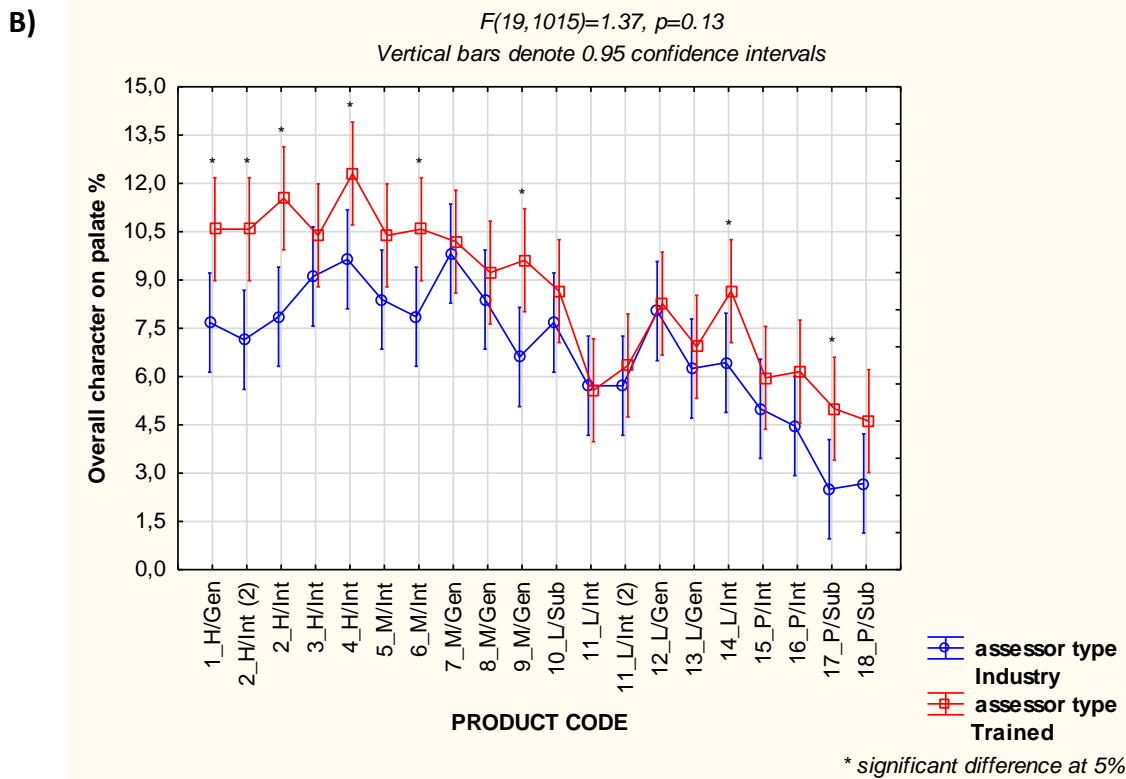
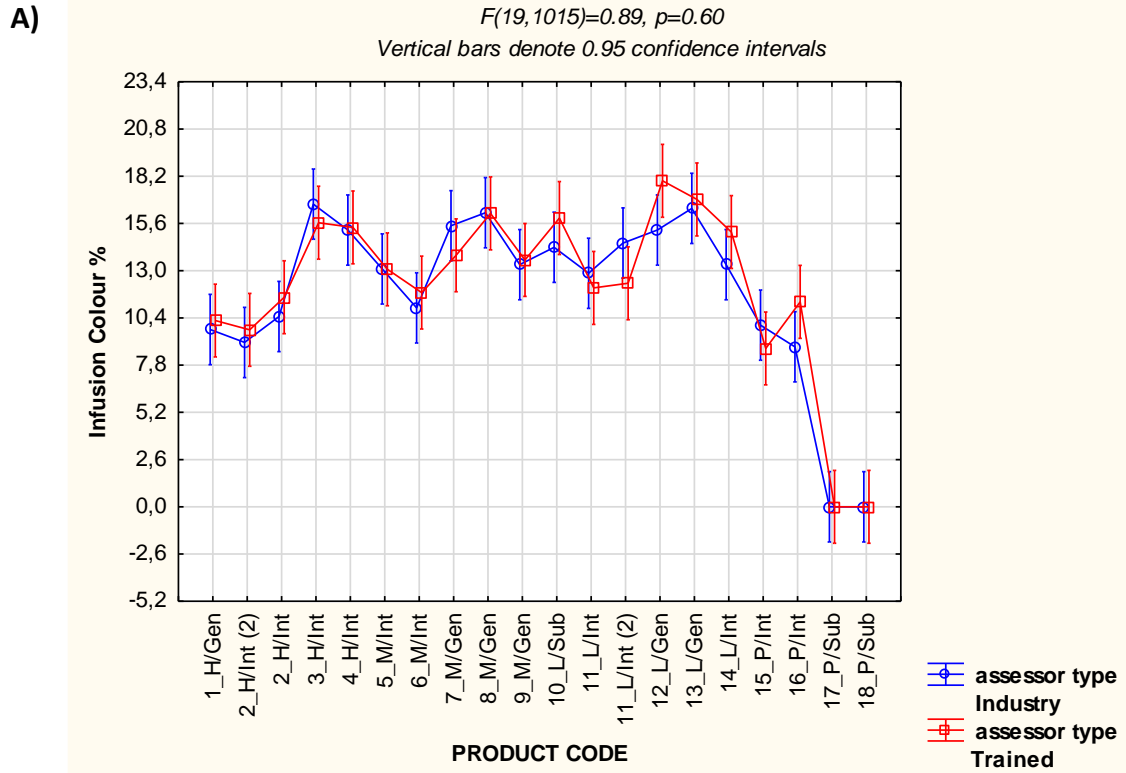


**Figure 10.2** A) Individual factor map (MFA) and B) correlation plot depicting the association between DSA attributes and citation parameters for industry panel [DSA attributes (red) and citation parameters (blue) ('dry leaf appearance' and 'infusion turbidity/haze' citation parameters were not included); 1–18 represent sample numbers; Int, Sub, Gen represent *C. intermedia*, *C. subternata* and *C. genistoides*, respectively; H, M, L, P represent high, moderate, low and poor quality, respectively].

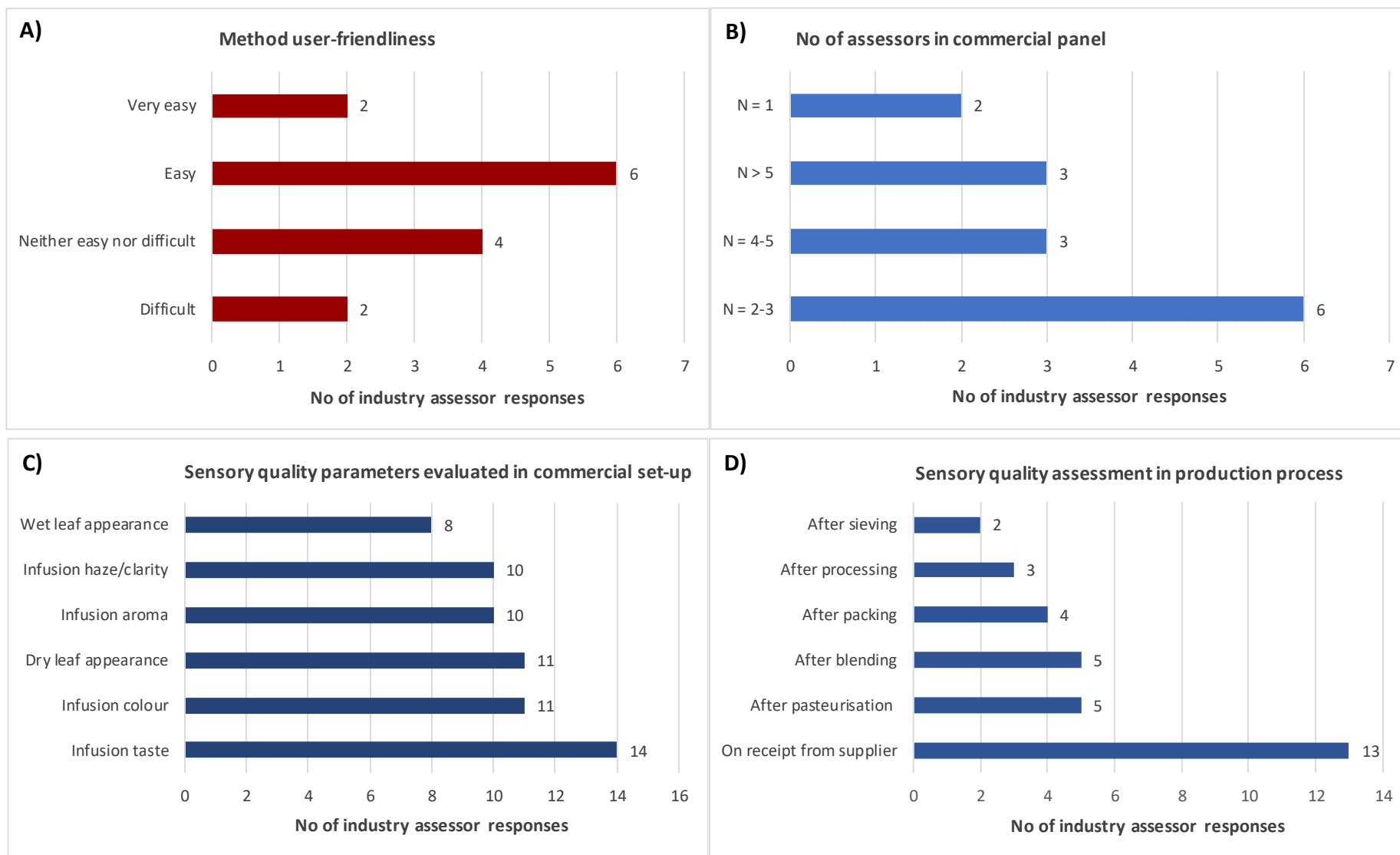


**Figure 11.1** Least squared (LS) means line graphs for **industry** vs **trained panel** for **A) total scores (%)** and **B) 'woody' aroma parameter scores (%)** [1–18 represent sample numbers; Int, Sub, Gen represent *C. intermedia*, *C. subternata* and *C. genistoides*, respectively; H, M, L, P represent high, moderate, low and poor quality, respectively; (2) after sample code indicates blind duplicate].





**Figure 11.2** Least squared (LS) means line graphs for **industry** vs **trained panel** for **A)** ‘infusion colour’ parameter scores (%) and **B)** ‘overall character on palate’ parameter scores [1–18 represent sample numbers; Int, Sub, Gen represent *C. intermedia*, *C. subternata* and *C. genistoides*, respectively; H, M, L, P represent high, moderate, low and poor quality, respectively; (2) after sample code indicates blind duplicate].



**Figure 12** Questionnaire results for *industry panel* (N = 14) indicating **A)** user-friendliness of quality scoring method; **B)** number of assessors in panel that partake in routine sensory assessment (*representatives of quality control, production and/or procurement/marketing personnel in a company*); **C)** quality parameters assessed in routine sensory quality assessment (*'taste' refers to palate attributes*); and **D)** step in production process when sensory quality of a production batch is assessed.

**Table 1** Summary of main sensory quality-related topics derived from consultation through a survey and interviews with role-players in the honeybush industry.

Main theme	Sub-category	Key points
Current industry practice	Processing	– Batch blending was recognised as common industry practice (secondary level processors)
	Panel size/ assessors	– Small industry panels (N ≤ 5 assessors) are used for routine in-house sensory quality assessment – Often different divisions (QC/procurement/marketing divisions) are used for in-house sensory quality assessment
	Sensory quality assessment	– Assessment is mainly conducted on receipt of supplier batches and after blending – Traditional 'cupping' is performed by two to three expert tasters at facility (2 respondents) – Basic 'cupping' is often performed by marketers when samples presented to international clients – International clients (buyers) apply their own in-house sensory quality assessment procedures – No scorecards or grading documents are used for routine assessment – Samples compared to previous batches or a competitor's product with comments are noted (only for certain respondents) – Monthly scorecard document is completed for quality management system auditing purposes (only for one respondent) – Very few respondents reported visual assessment of infusion turbidity ('cup clarity') in teacup/tasting spoon – No formal sensory quality assessment procedures exist for producers with well-established on-farm processing systems (mainly 1/2 representatives assess final product; no quality issues such as turbidity reported by these producers)
Key quality parameters assessed in order of importance	Infusion	– <b>#1 'Taste'</b> <sup>1</sup> – <b>#2 Aroma</b> (not assessed for all respondents e.g. certain major tea processing/packing companies do not assess aroma) – <b>#3 Appearance (haze/turbidity and colour)</b>
	Dry Leaf	– <b>#4 Appearance (composition, cut size and colour)</b> (1 respondent only assess this parameter, and not aroma/'taste' <sup>1</sup> , for incoming batches in comparison to previous batch as reference)
Notes on parameters	Infusion aroma and 'taste'	– 'Floral', 'fruity' and 'sweet' aroma notes important indication for high sensory quality – 'Woody' aroma at high intensity undesirable – Presence of infusion aroma and 'taste' <sup>1</sup> taints intolerable – 'Burnt' taste ascribed to too dry fermentation conditions – 'Sour' and 'rotten' off-tastes ascribed to too wet fermentation conditions – 'Musty/mouldy' aroma/taste is an indication of poor microbial quality – implies a safety risk
	Infusion appearance	– 'Red-brown' infusion colour indicative of a 'fuller' flavour (preferred by consumers) – Lighter infusions indicative of less intense infusion taste profile and 'body' – Presence of infusion haze (turbidity) intolerable – Turbidity ascribed to poor processing conditions (too wet fermentation conditions)

<sup>1</sup> 'Infusion taste' – industry terminology describing the overall/collective perception of the palate attributes, i.e. flavour, basic taste and mouthfeel attributes.

**Table 1** (continued)

Main theme	Sub-category	Key points
Notes on parameters (continued)	Dry leaf appearance	<ul style="list-style-type: none"> <li>– Composition of dry leaf and cut size good indicators of infusion sensory quality</li> <li>– Darker red stems, finer cut size, and higher leaf-to-stem ratio specifically indicators of good quality</li> <li>– Practical relevance/implication of dry leaf appearance specifications in tea packing facility emphasised</li> <li>– Fine and even small particles allow for better flow which aids mechanised teabag packing</li> <li>– Cut size crucial for optimal oxidation/ 'fermentation' (contact surface for moisture absorption and oxygen availability)</li> <li>– May compromise on dry leaf appearance but not on infusion taste and appearance for batch blending</li> </ul>
	Wet leaf appearance	<ul style="list-style-type: none"> <li>– Least important sensory quality parameter (according to survey)</li> </ul>
Assessment order		<ul style="list-style-type: none"> <li>– 1) dry leaf appearance, 2) infusion appearance, 3) infusion aroma, and 4) infusion'taste'<sup>1</sup></li> </ul>
Concerns		<ul style="list-style-type: none"> <li>– Consumers are exposed to a large variation in product sensory quality</li> <li>– Formalised sensory quality assessment protocols within industry lacking</li> </ul>
Needs identified		<ul style="list-style-type: none"> <li>– Communication of sensory quality within company divisions and throughout industry sectors</li> <li>– Sensory quality control to aid production of consistent quality, fair marketing and price negotiation</li> <li>– Batch traceability</li> <li>– Consistency of product quality (infusion colour and palate attributes) and consistency in product supply critical</li> <li>– Controlled processing critical (including aeration and rotation during fermentation)</li> <li>– Objective sensory quality and colour assessment</li> </ul>
Main markets confirmed	SA/African	<ul style="list-style-type: none"> <li>– Honeybush tea is consumed neat, or in herbal tea blends with rooibos and/or other indigenous plants</li> </ul>
	European	<ul style="list-style-type: none"> <li>– Honeybush tea mainly used in fruit tea blends</li> </ul>
	Asian	<ul style="list-style-type: none"> <li>– Honeybush tea mainly consumed neat or for iced tea beverages (especially Japan)</li> </ul>

<sup>1</sup> 'Infusion taste' – industry terminology describing the overall/collective perception of the palate attributes, i.e. flavour, basic taste and mouthfeel attributes.

**Table 2** Sensory and physicochemical modality data for the comprehensive sample set (n = 585) expressed as mean, median, minimum, maximum, first and third quartile values.

Modality	Attribute	Minimum	Maximum	Interquartile range <sup>1</sup>			Mean	Standard deviation (n-1)
				1st Quartile	Median	3rd Quartile		
<i>Aroma</i>	Woody	20	52	37	41	45	40	6
	Fynbos-floral	15	54	35	38	42	39	5
	Fynbos-sweet	15	47	31	36	39	35	5
	Fruity-sweet	3	48	20	30	33	27	9
	Raisin	4	41	15	27	32	24	9
	Apricot	1	42	15	26	31	23	10
	Caramel	0	23	7	11	14	10	5
	Sweet spice	0	25	3	8	11	7	5
	Apple	0	16	3	5	9	6	4
	Rose geranium	0	32	3	5	7	5	4
	Rose perfume	0	21	2	4	9	6	5
	Nutty	0	14	1	3	8	4	4
	Honey	0	15	1	2	7	4	4
	Pine	0	10	1	1	2	1	1
	Lemon/lemongrass	0	5	0	1	1	1	1
	Dusty	0	14	2	3	4	3	2
	Musty/mouldy	0	35	0	0	1	1	4
	Medicinal	0	12	0	0	1	1	1
	Burnt caramel	0	19	0	1	3	2	3
	Rotting plant water	0	15	0	0	1	1	2
Hay/dried grass	1	29	11	15	19	15	6	
Green grass	0	31	1	2	4	3	4	
Cooked vegetables	0	19	1	1	3	2	3	
Smoky	0	32	0	1	2	1	3	
<i>Taste</i>	Sweet	13	27	20	21	23	21	2
	Sour	0	28	1	4	11	6	6
	Bitter	0	29	1	2	5	4	5
<i>Mouthfeel</i>	Astringent	14	38	26	28	30	28	4
<i>Flavour</i>	Fynbos-floral	9	44	30	32	35	32	5
	Rose geranium	0	17	1	2	4	3	3
	Rose perfume	0	17	1	2	6	4	4
	Raisin	0	6	0	1	2	1	1
	Lemon/lemongrass	0	17	0	0	1	1	2
	Apricot	0	16	0	1	1	1	1
	Apple	0	2	0	0	0	0	0
	Woody	17	49	35	38	42	38	5
	Pine	0	6	0	0	1	1	1
	Honey	0	2	0	0	0	0	0
	Fruity-sweet	2	5	3	3	4	3	1
	Fynbos-sweet	10	17	12	13	14	13	1
	Sweet spice	0	8	0	1	3	2	2
	Nutty	0	8	0	1	3	2	2
	Dusty	0	21	1	2	3	2	2
	Musty/mouldy	0	35	0	0	1	1	4
	Medicinal	0	11	0	0	0	0	1
	Burnt caramel	0	11	0	0	1	1	2
	Rotting plant water	0	8	0	0	1	1	1
	Hay/dried grass	2	30	13	16	19	16	5
	Green grass	0	22	1	1	3	2	3
	Cooked vegetables	0	14	0	1	2	2	2
	Smoky	0	31	0	0	1	1	3

**Table 2** (continued)

Modality	Attribute	Minimum	Maximum	Interquartile range <sup>1</sup>			Mean	Standard deviation (n-1)
				1st Quartile	Median	3rd Quartile		
<i>Appearance</i>	<b>Turbidity (NTU)</b>	5.253	532.000	24.250	<b>44.750</b>	91.517	69.700	72.041
	<b>Colour (L*)</b>	70.243	97.013	78.544	<b>82.530</b>	87.064	82.789	5.798
	<b>Colour (a*)</b>	0.267	24.880	7.786	<b>11.807</b>	15.887	11.764	5.547
	<b>Colour (b*)</b>	6.910	67.257	31.064	<b>39.190</b>	45.420	37.626	10.367
	<b>Colour (C)</b>	6.940	70.800	32.149	<b>41.235</b>	47.883	39.570	11.251
	<b>Colour (h)</b>	62.075	88.670	69.594	<b>72.885</b>	76.333	73.541	5.393

<sup>1</sup> Range between 1<sup>st</sup> quartile (25<sup>th</sup> percentile) and 3<sup>rd</sup> quartile (75<sup>th</sup> percentile) representing attribute intensities for 50% of samples in the comprehensive data set.

**Table 3** Summary of major conclusions derived from the study of the DSA and physicochemical data captured in the comprehensive dataset.

Sensory quality parameters	Graphical visualisation of data distribution	Major conclusions
Infusion aroma, flavour, taste and mouthfeel	% occurrence frequency plots; normal distribution curves; box- and whiskers plots	<b>Generic attributes intrinsic to honeybush sensory profile</b>
		– ‘fynbos-floral’, ‘fynbos-sweet’, ‘woody’ aroma ( <i>high intensities</i> )
		– ‘fruity-sweet’, ‘raisin’, ‘apricot’ aroma ( <i>moderate intensities</i> )
		– ‘caramel’ aroma ( <i>low intensities</i> )
		– ‘fynbos-floral’, ‘woody’ flavour ( <i>high intensities</i> )
		– ‘fynbos-sweet’ flavour ( <i>low intensities</i> )
		– sweet taste, astringent mouthfeel ( <i>moderate intensities</i> )
		– ‘hay/dried grass’ aroma and flavour ( <i>low–moderate intensities</i> )
		– Citation of positive aroma attributes may provide additional information (although not critical for quality): e.g. ‘rose perfume’, ‘rose geranium’, ‘spicy’, ‘nutty’ aroma
		<b>Exceptions</b>
		– ‘woody’ and ‘hay/dried grass’ aroma and flavour, astringent mouthfeel: intrinsic to honeybush sensory profile but undesirable (negative) at high intensities
		<b>Negative attributes</b>
		– ‘green grass’, ‘cooked vegetables’, ‘rotting plant water’, ‘dusty’, ‘musty/mouldy’, ‘smoky’, ‘burnt caramel’, ‘medicinal’ aroma and flavour (taints) undesirable
		– Bitter taste: intrinsic to <i>C. genistoides</i> but undesirable (negative) at any intensity level
		– Sour taste: undesirable at any intensity level
Infusion appearance: colour and turbidity	box-and whiskers plots	–Importance of infusion colour and infusion turbidity as quality parameters with comparison to literature was emphasised –Large variation of infusion colour (CIEL*a*b*) and turbidity (NTU) measurement values in comprehensive dataset exist –Difficult to derive colour intensity ranges for visual colour perceived from individual instrumental CIEL*a*b* values –Turbidity (NTU) values $\geq 90$ undesirable based on data and literature –Visual assessment of colour and turbidity to be considered as cost-effective and practical alternative to instrumental analyses



**Table 4** Parameters and sub-categories selected for classification of fermented honeybush infusion sensory quality and definitions (*attribute citations are highlighted in red*).

Key parameter (modality)	Parameter sub-category	Definition	Measurement	Data generated
<b>Appearance</b>	<b>Dry leaf appearance:</b> <i>even/uneven cut size</i>	Fermented tea plant material particles of different cut sizes ('uneven') or particles of small (< 5 mm), similar cut size ('even') for teabag packing.	Identification (checkbox)	citation (0/1)
	<b>Dry leaf appearance:</b> <i>acceptable/unacceptable stem particles</i>	Absence ('acceptable') or presence ('unacceptable') of light stem particles indicative of poor processing practices.	Identification (checkbox)	citation (0/1)
	<b>Infusion turbidity</b>	Haze or absence thereof (clarity) related to the ability to see through an infusion.	Identification (checkbox)	citation (0/1)
	<b>Infusion appearance:</b> <b>dust/sediment</b>	Presence of fine dust particles ascribed to poor processing or sieving practices, that give the illusion of turbidity but settles at the bottom, so-called sediment.	Identification (checkbox)	citation (0/1)
	<b>Infusion colour</b>	<b>Infusion colour hue and chroma (yellow/green, light red-brown, red-brown to dark red-brown)<sup>1</sup></b>	<b>Intensity scoring (4-point scale)</b>	<b>score (%)</b>
<b>Aroma (orthonasal)</b>	<b>Floral</b>	<b>Overall sweet, non-specific floral aroma.</b>	<b>Intensity scoring (4-point scale)</b>	<b>score (%)</b>
	<i>Fynbos-floral</i>	<i>Sweet, floral aroma note associated with the flowers of fynbos<sup>2</sup> vegetation*.</i>	Identification (checkbox)	citation (0/1)
	<i>Rose geranium</i>	<i>Floral aroma note associated with the rose geranium plant*.</i>	Identification (checkbox)	citation (0/1)
	<i>Rose perfume</i>	<i>Floral aroma note associated with rose petals or rosewater (turkish delight)*.</i>	Identification (checkbox)	citation (0/1)
	<b>Fruity and Sweet</b>	<b>Overall non-specific fruity and sweet aroma.</b>	<b>Intensity scoring (4-point scale)</b>	<b>score (%)</b>
	<i>Apricot</i>	<i>Sweet-sour aroma reminiscent of apricot jam or dried apricot*.</i>	Identification (checkbox)	citation (0/1)
	<i>Apple</i>	<i>Sweet, slightly sour aromatics associated with cooked apples or apple pie*.</i>	Identification (checkbox)	citation (0/1)
	<i>Raisin</i>	<i>Sweet aroma note reminiscent of 'hanepoot' raisin*.</i>	Identification (checkbox)	citation (0/1)
	<i>Fynbos-sweet</i>	<i>The sweet aroma note reminiscent of the fynbos<sup>2</sup> plant*.</i>	Identification (checkbox)	citation (0/1)
	<i>Fruity-sweet</i>	<i>Sweet-sour aromatic reminiscent of non-specific fruit*.</i>	Identification (checkbox)	citation (0/1)
	<i>Honey</i>	<i>Aromatics associated with the sweet fragrance of fynbos<sup>1</sup> honey*.</i>	Identification (checkbox)	citation (0/1)
	<i>Caramel</i>	<i>Sweet aromatics characteristic of caramelised sugar*.</i>	Identification (checkbox)	citation (0/1)
	<i>Fynbos-sweet</i>	<i>The sweet aroma note reminiscent of the fynbos<sup>2</sup> plant*.</i>	Identification (checkbox)	citation (0/1)

<sup>1</sup> Related to CIEL\*a\*b\* colour space from chromatic a\*-axis (+a\* = red) and chromatic b\*-axis (+b\* = yellow)]<sup>2</sup> Fynbos is natural shrubland vegetation occurring in the Western Cape, South Africa.

\* Definitions based on revised fermented honeybush lexicon (Chapter 3)

Table 4 (continued)

Key parameter (modality)	Parameter	Definition	Measurement	Data generated
<b>Aroma</b> ( <i>orthonasal</i> )	<b>Spicy and/or Nutty</b>	<b>Overall non-specific spicy and/or nutty aroma.</b>	<b>Intensity scoring (4-point scale)</b>	<b>score (%)</b>
	<b>Sweet spice</b>	<i>Sweet, woody and spice aroma, including ground cinnamon/cassia bark*.</i>	Identification (checkbox)	citation (0/1)
	<b>Nutty</b>	<i>Aromatics associated with fresh walnuts or chopped almonds*.</i>	Identification (checkbox)	citation (0/1)
	<b>Woody</b>	<b>Aromatics associated with dry bushes, stems and twigs of the fynbos<sup>2</sup> vegetation*.</b>	<b>Intensity scoring (4-point scale)</b>	<b>score (%)</b>
<b>Palate</b> [ <i>flavour</i> ( <i>retronasal</i> ), <i>taste, and</i> <i>mouthfeel</i> ]	<b>Overall character</b>	<b>Balance of positive flavours, sweet taste and astringency.</b>	<b>Intensity scoring (4-point scale)</b>	<b>score (%)</b>
	<b>Weak/Watery</b>	<i>Overall insipid taste with positive flavours, sweet taste and astringency lacking.</i>	Identification (checkbox)	citation (0/1)
	<b>Hay/dried grass</b>	<b>Slightly sweet aroma associated with dried grass or hay*.</b>	Intensity scoring (4-point scale)	score (%)
	<b>Bitter</b>	<b>Fundamental taste factor of which caffeine or quinine is typical<sup>3</sup>.</b>	Intensity scoring (4-point scale)	score (%)
	<b>Sour</b>	<b>Fundamental taste factor of which citric acid in water is typical<sup>3</sup>.</b>	Intensity scoring (4-point scale)	score (%)
	<b>Vinegar-like</b>	<i>Acidic taste associated with vinegar.</i>	Identification (checkbox)	citation (0/1)
	<b>Taints</b>	<b>Undesirable taints or defects atypical of characteristic honeybush sensory profile associated with poor processing or poor good manufacturing practices (GMPs).</b>	<b>Intensity scoring (4-point scale)</b>	<b>score (%)</b>
	<b>Rotting plant water</b>	<i>Flavour associated with the old and rotting vase water of cut flowers*.</i>	Identification (checkbox)	citation (0/1)
	<b>Cooked vegetables</b>	<i>Flavour associated with canned/cooked vegetables*.</i>	Identification (checkbox)	citation (0/1)
	<b>Burnt caramel</b>	<i>Flavour associated with burnt sugar, burnt caramel or burnt caramelised vegetables*.</i>	Identification (checkbox)	citation (0/1)
	<b>Medicinal</b>	<i>Flavour characteristic of Band-aid® and antiseptic*.</i>	Identification (checkbox)	citation (0/1)
	<b>Dusty</b>	<i>Earthy flavour associated with dry dirt road*.</i>	Identification (checkbox)	citation (0/1)
<b>Smoky</b>	<i>Smoky flavour note associated with burning hay/grass or tobacco*.</i>	Identification (checkbox)	citation (0/1)	
<b>Musty</b>	<i>Mouldy flavour associated with mildew or damp cellars**.</i>	Identification (checkbox)	citation (0/1)	

<sup>3</sup> Koppel and Chambers (2010)

\* Definitions based on revised fermented honeybush lexicon (Chapter 3)

\*\* Definition based on revised fermented rooibos lexicon (Du Preez et al. 2020)

**Table 5** Proposed specifications for each parameter sub-category per sensory quality class of fermented honeybush tea.

Key quality parameter	Parameter sub-category	Assessment method	SENSORY QUALITY CLASS			
			HIGH	MODERATE <sup>1</sup>	LOW <sup>2</sup>	POOR
DRY LEAF AND INFUSION APPEARANCE	Dry leaf appearance: <i>Cut size</i>	Visual inspection + citation	Even	Even	Uneven	Uneven
	Dry leaf appearance: <i>Light stem particles</i>	Visual inspection + citation	Absent	Absent	Present	Present
	<b>Infusion turbidity/haze<sup>3</sup></b>	Visual inspection* + citation	Absent	Absent	Absent	Present
	<b>Infusion colour<sup>3</sup></b>	Visual inspection with colour reference card + scoring	Red-brown	Light red-brown to dark red-brown	Light red-brown to dark red-brown	Yellow/Green
INFUSION AROMA	Floral aroma	Sensory assessment + scoring	High	Moderate	Low	Absent to Low
	Fruity and Sweet aroma	Sensory assessment + scoring	High	Moderate	Low	Absent to Low
	Nutty and/or Spicy aroma	Sensory assessment + scoring	Moderate to High	Low to Moderate	Absent to Low	Absent to Low
	Woody aroma	Sensory assessment + scoring	Low to Moderate	Low to Moderate	Absent to Low or High	Absent to Low
INFUSION FLAVOUR, TASTE AND MOUTHFEEL	Overall character on palate <sup>3</sup>	Sensory assessment + scoring	High	Moderate	Low	Absent to Low
	Hay/dried grass flavour	Sensory assessment + scoring	Low	Low to Moderate	Moderate to High	Moderate to High
	Bitter taste	Sensory assessment + scoring	Absent to Low	Absent to Low	Moderate to High	Moderate to High
	Sour taste	Sensory assessment + scoring	Absent	Absent	Low to Moderate	Moderate to High
	<b>Taints (negative flavours)<sup>3</sup></b>	Sensory assessment + scoring	Absent	Absent	Low	Moderate to High

<sup>1</sup> Minimum accepted standard for packing of final product for consumer

<sup>2</sup> Minimum accepted standard for blending with other production batches of high and/or moderate quality

<sup>3</sup> Critical parameters for determining quality class

\* Instrumental analysis (NTU measurement) is recommended

**Table 6** Association between continuous 100-point rating scale used for DSA and semantic 4-point category scale proposed for the quality scoring method (*interquartile ranges linked with 'moderate' in the category scale are highlighted in grey*).

**Descriptive statistics of comprehensive sample set (n = 585)**

Key parameter (modality)	Positive attribute	Min	1st Quartile	Median	3rd Quartile	Max	Mean
<b>Aroma</b>	<b>Woody<sup>1</sup></b>	20.19	37.09	<b>40.59</b>	45.19	51.92	40.39
Aroma	Fynbos-floral	14.82	35.21	<b>38.35</b>	41.86	53.97	38.58
Aroma	Fynbos-sweet	15.27	31.41	<b>35.79</b>	39.28	47.28	35.37
Aroma	Fruity-sweet	2.98	20.35	<b>29.88</b>	33.31	48.41	26.88
Aroma	Raisin	3.76	14.88	<b>27.36</b>	31.67	40.85	24.05
Aroma	Apricot	1.11	14.71	<b>25.89</b>	30.75	41.63	22.88
Aroma	Caramel	0.20	7.12	<b>10.63</b>	13.98	23.04	10.22
Aroma	Nutty	0.00	0.76	<b>2.90</b>	8.00	13.58	4.29
Aroma	Sweet spice	0.00	2.55	<b>7.52</b>	11.05	24.92	7.26
Taste	Sweet	12.72	19.87	<b>21.37</b>	22.67	27.41	21.25
<b>Mouthfeel</b>	<b>Astringent<sup>1</sup></b>	14.02	25.91	<b>28.07</b>	30.42	37.95	27.86
<b>Flavour</b>	<b>Woody<sup>1</sup></b>	17.43	35.31	<b>37.68</b>	41.82	49.10	37.83
Flavour	Fynbos-floral	9.14	29.90	<b>32.17</b>	35.41	44.22	32.50
Flavour	Fynbos-sweet	9.71	11.85	<b>12.90</b>	13.61	16.67	12.86

Key parameter (Modality)	Negative attribute	Min	1st Quartile	Median	3rd Quartile	Max	Mean
Taste	Bitter	0.00	0.95	<b>2.08</b>	5.12	29.35	4.41
Taste	Sour	0.00	1.19	<b>3.53</b>	10.54	28.11	5.94
Aroma	Hay/dried grass	0.58	11.13	<b>15.11</b>	18.78	29.23	14.73
Flavour	Hay/dried grass	1.85	12.74	<b>16.10</b>	18.93	30.23	16.02

<i>Semantic category scale</i>	Absent/ Barely perceptible	Low	Moderate	High
<i>Proposed score</i>	<b>0</b>	<b>1</b>	<b>2</b>	<b>3</b>
	0	< 35	35 ≤ x ≤ 45	<b>&gt; 45</b>
	0	< 35	35 ≤ x ≤ 40	> 40
	0	< 30	30 ≤ x ≤ 40	> 40
	0	< 20	20 ≤ x ≤ 35	> 35
	0	< 15	15 ≤ x ≤ 30	> 30
	0	< 15	15 ≤ x ≤ 30	> 30
	0	< 5	5 ≤ x ≤ 15	> 15
	0	< 5	5 ≤ x ≤ 10	> 10
	0	< 5	5 ≤ x ≤ 10	> 10
	0	< 20	20 ≤ x ≤ 25	> 25
	0	< 25	25 ≤ x < 30	<b>&gt; 30</b>
	0	< 35	35 ≤ x ≤ 40	<b>&gt; 40</b>
	0	< 30	30 ≤ x ≤ 35	> 35
	0	< 10	10 ≤ x ≤ 15	> 15
<i>Proposed score</i>	<b>4</b>	<b>3</b>	<b>2</b>	<b>0</b>
	0	< 5	5 ≤ x ≤ 10	> 10
	0	< 5	5 ≤ x ≤ 10	> 10
	0	< 10	10 ≤ x ≤ 20	> 20
	0	< 10	10 ≤ x ≤ 20	> 20

<sup>1</sup> Score allocation for sensory attribute is non-linear. High intensities are undesirable. A lower score should be therefore allocated to 'high' in the semantic category scale. 'x' = attribute intensity

**Table 7** Proposed 4-point category scales and scores allocation of the scorecard and key for classification based on total score (%).

Key parameter (modality)	Parameter sub-category	Category scale and scores allocated			
		Yellow/Green	Light red-brown	Red-brown	Dark red-brown
<i>Appearance</i>	Infusion colour <sup>1</sup>	<b>0</b>	<b>2</b>	<b>3</b>	<b>1</b>
		Absent/Barely Perceptible	Low	Moderate	High
<i>Aroma</i>	Floral	<b>0</b>	<b>1</b>	<b>2</b>	<b>3</b>
<i>Aroma</i>	Fruity and Sweet	<b>0</b>	<b>1</b>	<b>2</b>	<b>3</b>
<i>Aroma</i>	Nutty and/or Spicy	<b>0</b>	<b>1</b>	<b>2</b>	<b>3</b>
<i>Aroma</i>	Woody <sup>1</sup>	<b>0</b>	<b>1</b>	<b>3</b>	<b>2</b>
<i>Flavour/ Taste/ Mouthfeel</i>	Overall character on palate <sup>2</sup>	<b>0</b>	<b>1</b>	<b>2</b>	<b>3</b>
<i>Flavour</i>	Hay/dried grass	<b>3</b>	<b>2</b>	<b>1</b>	<b>0</b>
<i>Taste</i>	Bitter	<b>3</b>	<b>2</b>	<b>1</b>	<b>0</b>
<i>Taste</i>	Sour	<b>3</b>	<b>2</b>	<b>1</b>	<b>0</b>
<i>Flavour</i>	Taints	<b>3</b>	<b>2</b>	<b>1</b>	<b>0</b>

Example of calculation for allocation of maximum score values (3)			
Score allocated	Weight (%)	Score x Weight	Total score (%)
<b>3</b>	<b>20</b>	60	<b>20</b>
<b>3</b>	<b>7</b>	21	<b>7</b>
<b>3</b>	<b>7</b>	21	<b>7</b>
<b>3</b>	<b>6</b>	18	<b>6</b>
<b>3</b>	<b>7</b>	21	<b>7</b>
<b>3</b>	<b>15</b>	45	<b>15</b>
<b>3</b>	<b>6</b>	18	<b>6</b>
<b>3</b>	<b>6</b>	18	<b>6</b>
<b>3</b>	<b>6</b>	18	<b>6</b>
<b>3</b>	<b>20</b>	60	<b>20</b>
<b>TOTAL</b>	<b>100</b>	<b>300</b>	<b>100</b>

<sup>1</sup> Non-linear scores allocation to parameter sub-category

<sup>2</sup> 'Balance of positive flavours, sweet taste and astringency'

**KEY FOR QUALITY CLASSIFICATION**

Quality class	Total score range %
<b>HIGH</b>	<b>80–100</b>
<b>MODERATE</b>	<b>60–79</b>
<b>LOW</b>	<b>40–59</b>
<b>POOR</b>	<b>0–39</b>

**Table 8** Sample set (n = 20) for validation of sensory quality scoring method and pre-assigned quality classes (blind duplicate samples are indicated by '(2)').

Sample code	Sensory quality class <sup>1</sup>	<i>Cyclopia species</i>
1_H/Gen	High	<i>C. genistoides</i>
2_H/Int	High	<i>C. intermedia</i>
3_H/Int	High	<i>C. intermedia</i>
4_H/Int	High	<i>C. intermedia</i>
5_M/Int	Moderate	<i>C. intermedia</i>
6_M/Int	Moderate	<i>C. intermedia</i>
7_M/Gen	Moderate	<i>C. genistoides</i>
8_M/Gen	Moderate	<i>C. genistoides</i>
9_M/Gen	Moderate	<i>C. genistoides</i>
10_L/Sub	Low	<i>C. subternata</i>
11_L/Int	Low	<i>C. intermedia</i>
12_L/Gen	Low	<i>C. genistoides</i>
13_L/Gen	Low	<i>C. genistoides</i>
14_L/Int	Low	<i>C. intermedia</i>
15_P/Int	Poor	<i>C. intermedia</i>
16_P/Int	Poor	<i>C. intermedia</i>
17_P/Sub	Poor	<i>C. subternata</i>
18_P/Sub	Poor	<i>C. subternata</i>
2_H/Int (2)	High	<i>C. intermedia</i>
11_L/Int (2)	Low	<i>C. intermedia</i>

<sup>1</sup> Sensory quality classes were pre-assigned based on attribute intensities in DSA data, visual inspection of sample configuration in PCA bi-plot and quality class definitions by expert focus groups.

**Table 9** Mean total scores (LS means, %) for the validation sample set (n = 20) assigned by each panel and the pre-assigned vs scorecard classifications (scores are presented in order from highest to lowest values)<sup>1</sup>.

INDUSTRY PANEL			Classification		TRAINED PANEL			Classification	
Product	LS Mean <sup>2</sup>	Standard deviation	Class pre-assigned <sup>3</sup>	Classification by panel	Product	LS Mean <sup>2</sup>	Standard deviation	Class pre-assigned <sup>3</sup>	Classification by panel
7_M/Gen	70.74 <sup>bcdef</sup>	12.29	M	M	4_H/Int	80.49 <sup>a</sup>	11.89	H	H
3_H/Int	69.88 <sup>bcdef</sup>	8.35	H	M	2_H/Int	76.74 <sup>ab</sup>	12.43	H	M
4_H/Int	69.31 <sup>bcdef</sup>	12.67	H	M	5_M/Int	76.40 <sup>ab</sup>	11.27	M	M
8_M/Gen	67.14 <sup>cdefg</sup>	15.99	M	M	3_H/Int	76.37 <sup>ab</sup>	12.49	H	M
5_M/Int	65.14 <sup>defghi</sup>	10.37	M	M	2_H/Int (2)	74.36 <sup>abc</sup>	10.66	H	M
10_L/Sub	61.52 <sup>ghijkl</sup>	18.69	L	M	7_M/Gen	73.86 <sup>abc</sup>	11.64	M	M
1_H/Gen	61.21 <sup>ghijkl</sup>	13.90	H	M	1_H/Gen	73.60 <sup>abcd</sup>	14.30	H	M
2_H/Int	60.08 <sup>ghijklm</sup>	14.78	H	M	6_M/Int	72.60 <sup>bcde</sup>	12.32	M	M
6_M/Int	59.70 <sup>hijklm</sup>	14.00	M	M	10_L/Sub	72.17 <sup>bcde</sup>	11.48	L	M
14_L/Int	58.37 <sup>ijklmn</sup>	19.79	L	L	8_M/Gen	71.67 <sup>bcdef</sup>	11.43	M	M
9_M/Gen	57.46 <sup>ijklmn</sup>	16.02	M	L	9_M/Gen	68.13 <sup>cdefgh</sup>	13.07	M	M
2_H/Int (2)	56.68 <sup>klmno</sup>	15.98	H	L	12_L/Gen	65.73 <sup>efghij</sup>	15.32	L	M
13_L/Gen	53.90 <sup>mnop</sup>	20.76	L	L	14_L/Int	64.21 <sup>fghijk</sup>	14.08	L	M
11_L/Int (2)	53.00 <sup>mnopeq</sup>	17.49	L	L	13_L/Gen	56.46 <sup>lmno</sup>	21.88	L	L
12_L/Gen	52.01 <sup>nopqr</sup>	20.99	L	L	16_P/Int	54.31 <sup>lmno</sup>	14.60	P	L
11_L/Int	49.87 <sup>opqr</sup>	20.80	L	L	15_P/Int	50.21 <sup>nopqr</sup>	15.01	P	L
15_P/Int	44.85 <sup>rs</sup>	15.91	P	L	11_L/Int (2)	46.46 <sup>pqrs</sup>	13.84	L	L
16_P/Int	40.15 <sup>s</sup>	19.41	P	L	11_L/Int	45.10 <sup>qrs</sup>	11.24	L	L
17_P/Sub	30.50 <sup>t</sup>	11.54	P	P	18_P/Sub	44.05 <sup>rs</sup>	10.07	P	L
18_P/Sub	29.80 <sup>t</sup>	15.05	P	P	17_P/Sub	44.01 <sup>rs</sup>	12.83	P	L

<sup>1</sup> Green cells indicate 100% match between pre-assigned class and classification by panel; Yellow cells indicate match based on 'moderate-high' or 'low-poor' groupings between pre-assigned class and classification by panel; Red indicate no match between pre-assigned class and classification by panel (although for certain samples scores were near cut-off range for correct class)

<sup>2</sup> Least squares mean over replicates and assessors (means that have not the same letter are significantly different;  $p < 0.05$ )

<sup>3</sup> Quality classes high (H), moderate (M), low (L) and poor (P) pre-assigned to validation sample set based on their DSA data  
Blind duplicates are indicated by '(2)'



**Table 10** RV coefficients computed between the first two dimensions of the product configurations obtained with the PCA bi-plot of the DSA data and PCA bi-plot, CA plot and MFA individual factor map of the scorecard data, respectively.


DSA	Sensory quality scoring method	RV coefficient ( $p < 0.01$ )
DSA PCA bi-plot	Scores PCA bi-plot ( <i>industry panel</i> )	0.53
DSA PCA bi-plot	Scores PCA bi-plot ( <i>trained panel</i> )	0.71
DSA PCA bi-plot	Citations CA plot ( <i>industry panel</i> )	0.67
DSA PCA bi-plot	Citations CA plot ( <i>trained panel</i> )	0.66
DSA PCA bi-plot	MFA individual factor map (association between DSA attributes and 'scores parameters') ( <i>industry panel</i> )	0.65
DSA PCA bi-plot	MFA individual factor map (association between DSA attributes and 'scores parameters') ( <i>trained panel</i> )	0.85
DSA PCA bi-plot	MFA individual factor map (association between DSA attributes and 'citation parameters') ( <i>industry panel</i> )	0.62
DSA PCA bi-plot	MFA individual factor map (association between DSA attributes and 'citation parameters') ( <i>trained panel</i> )	0.82

**Table 11** Assessor type, product and assessor type × product interaction effects from ANOVA for total and individual parameter scores.

Parameter score	ASSESSOR TYPE (panel) <i>p value</i> <sup>1</sup>	PRODUCT (test sample) <i>p value</i> <sup>1</sup>	ASSESSOR TYPE × PRODUCT <i>p value</i> <sup>1</sup>
% Total	< 0.01	< 0.01	< 0.01
% Infusion colour	0.57	< 0.01	0.6
% Floral	< 0.01	< 0.01	< 0.01
% Fruity and Sweet	0.01	< 0.01	0.01
% Spicy and/Nutty	0.83	< 0.01	< 0.01
% Woody	< 0.01	< 0.01	0.04
% Overall character on palate	0.01	< 0.01	0.13
% Hay/dried grass	< 0.01	< 0.01	< 0.01
% Bitter	< 0.01	< 0.01	0.89
% Sour	0.01	< 0.01	0.73
% Taints	0.3	< 0.01	< 0.01

<sup>1</sup>*p-values of significant effects (< 0.05) are highlighted in blue.*

Addendum B (Supplementary material Chapter 4)



**Fermented Honeybush Tea**  
**Sensory Quality Survey for Honeybush Industry Role-players**

SAHTA AGM, George  
 19 June 2018

Please select check box where applicable:

**1) Industry role-player:**

- a. Honeybush Producer/Farmer .....
- b. Honeybush Processor .....
- c. Honeybush Quality Controller .....
- d. Honeybush Marketer/Distributor.....
- e. Other (specify): \_\_\_\_\_

**2) Origin of plant material and *Cyclopia* spp. used for processing:**

- a. Wild .....
- b. Plantations .....
- c. Species (specify): \_\_\_\_\_

**3) Which quality aspects of fermented honeybush tea do you regard as **important**?**

- a. Aroma (*smell*) of tea infusion .....
- b. Taste/Flavour of tea infusion .....
- c. Colour of tea infusion .....
- d. Turbidity of tea infusion .....
- e. Dry leaf appearance .....
- f. Infused leaf appearance .....
- g. Other (specify): \_\_\_\_\_

**4) Type of Quality Grading System required:**

- a. General (one grading system for all *Cyclopia* spp.) .....
- b. Species-specific (one grading system per *Cyclopia* spp.) .....

**5) Would you be interested to participate in the development of our quality grading system for honeybush tea (e.g. testing of grading method, provide tea samples, etc.)?**


- a. Yes  \* \_\_\_\_\_
- b. No  (\*Please provide name, e-mail address, mobile number)

**6) Comments:** \_\_\_\_\_


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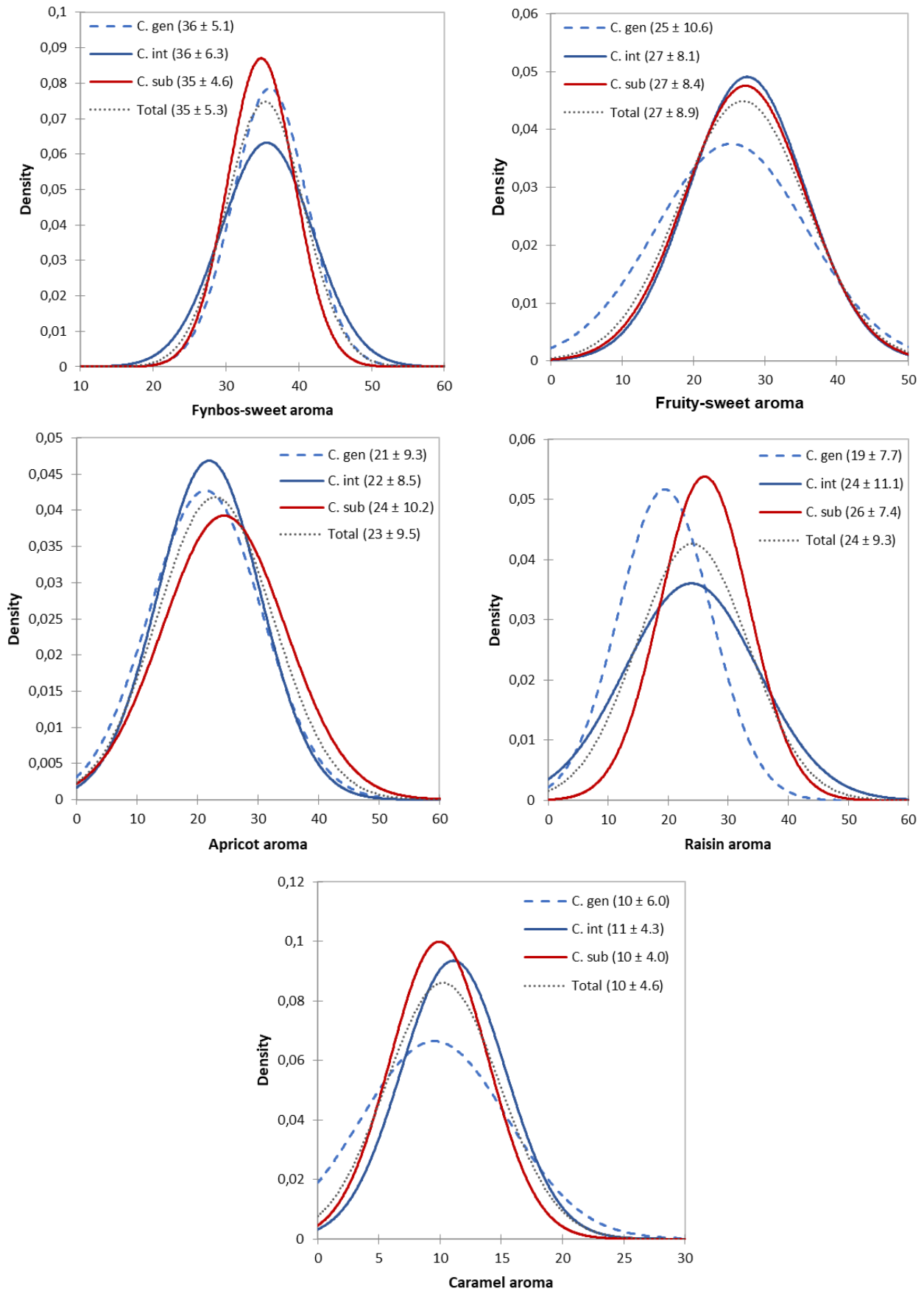
Plant  
Biorefinery  
Group

**Thank you for  
partaking in  
this survey**

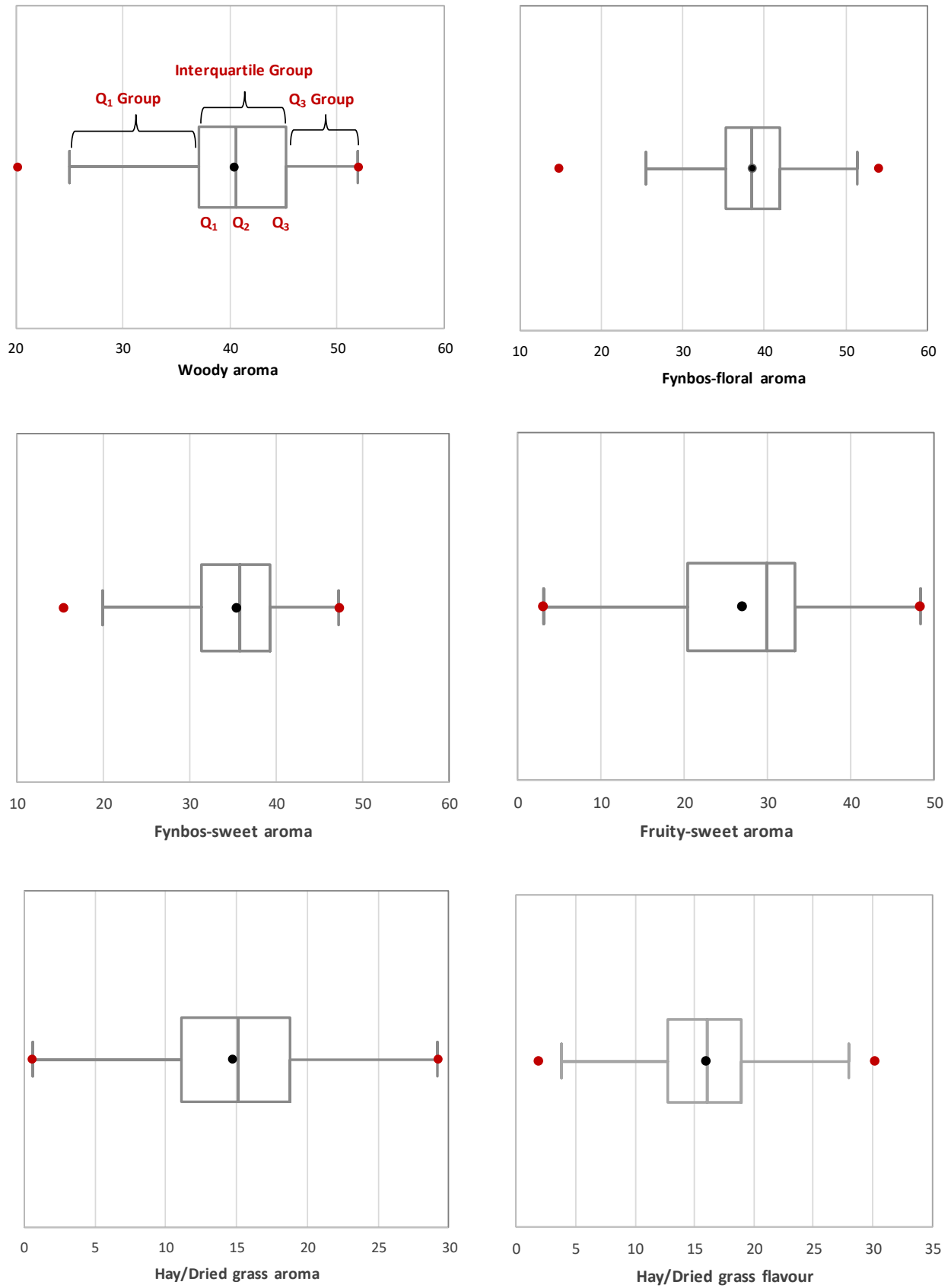
**Figure B1** Survey questionnaire presented to industry role-players at the annual general meeting of the South African Honeybush Tea Association (SAHTA), June 2018, George, South Africa.



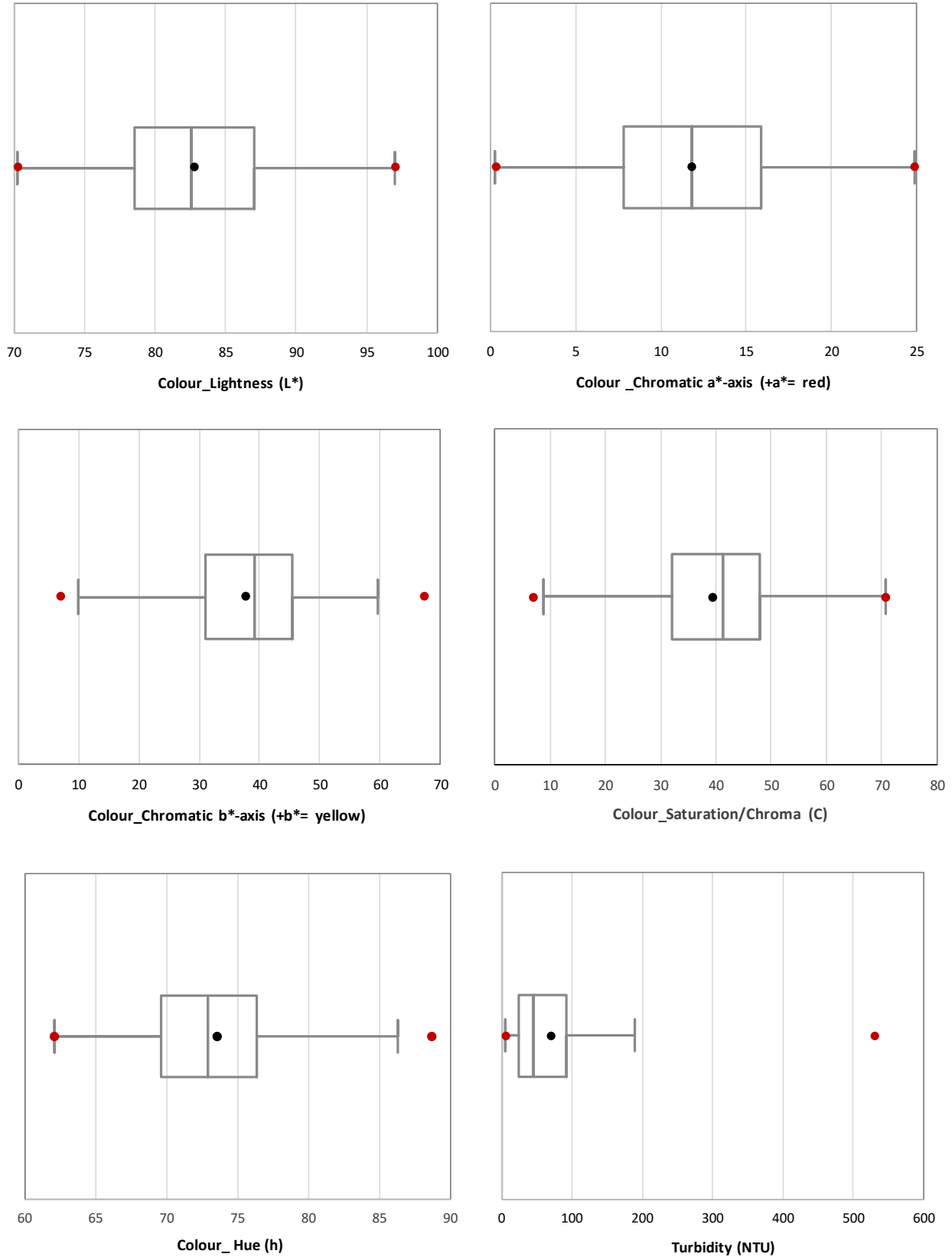
**Figure B2** Assessment ('cupping') of honeybush infusions of variable sensory quality by industry professionals during a visit to a national black and herbal tea blending and packing company.



**Figure B3** Normal distribution plots depicting intensity distribution for **intrinsic sensory attributes** for hot water infusions of fermented *C. genistoides* (*C. gen*), *C. subternata* (*C. sub*) and *C. intermedia* (*C. int*) at 'cup-of-tea' strength. Data in brackets in legends represent mean intensities ± standard deviations.

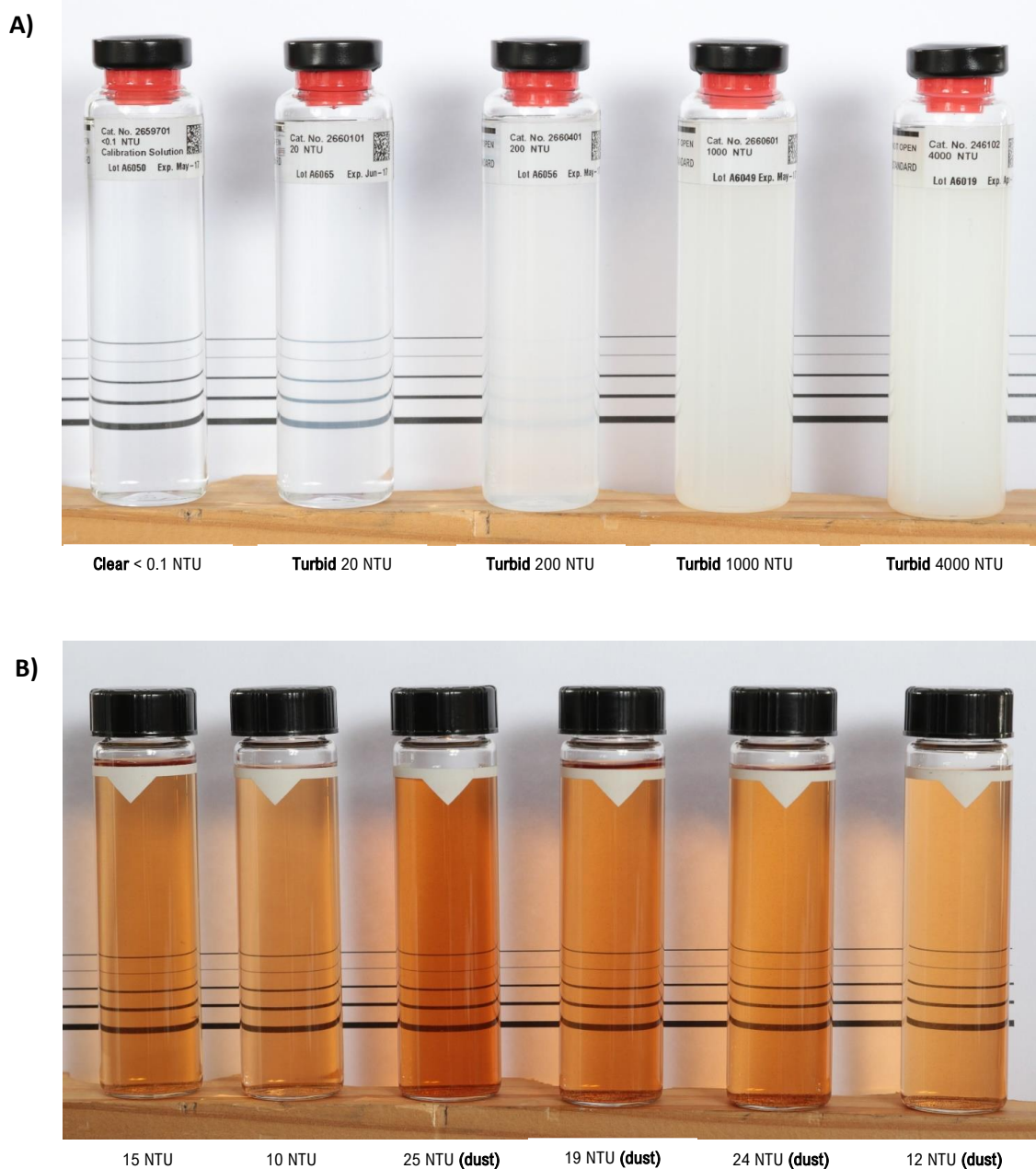


**Figure B4** Box-and-whiskers plots of intensity distributions of DSA attributes in the comprehensive dataset. Quartile and interquartile groups were studied for the specification ranges of 'scores parameters' (means are indicated in black and minimum/maximum values in red).



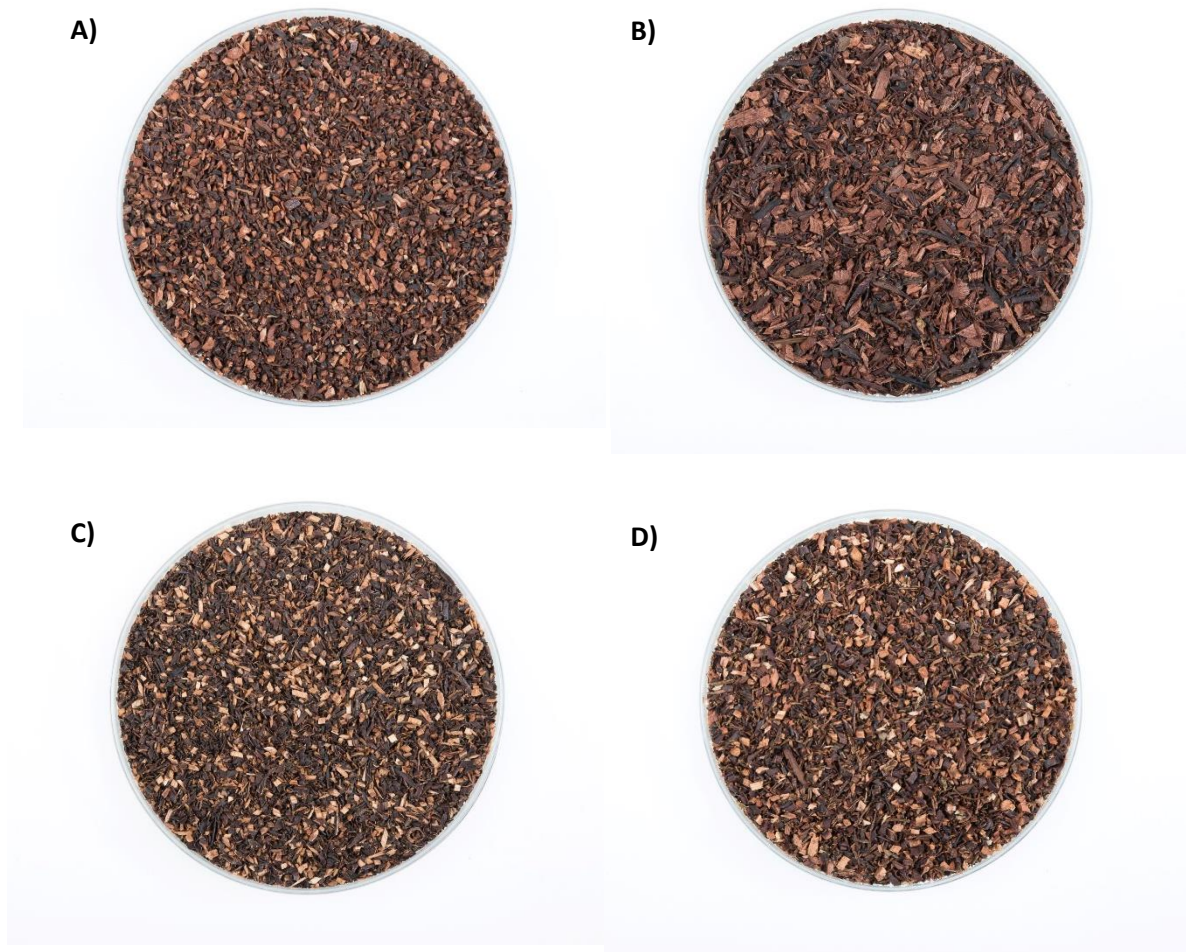
**Figure B5** Box-and-whiskers plots of CIEL\*a\*b\* colour space and turbidity (NTU) values in the comprehensive dataset (means are indicated in black and minimum/maximum values in red).



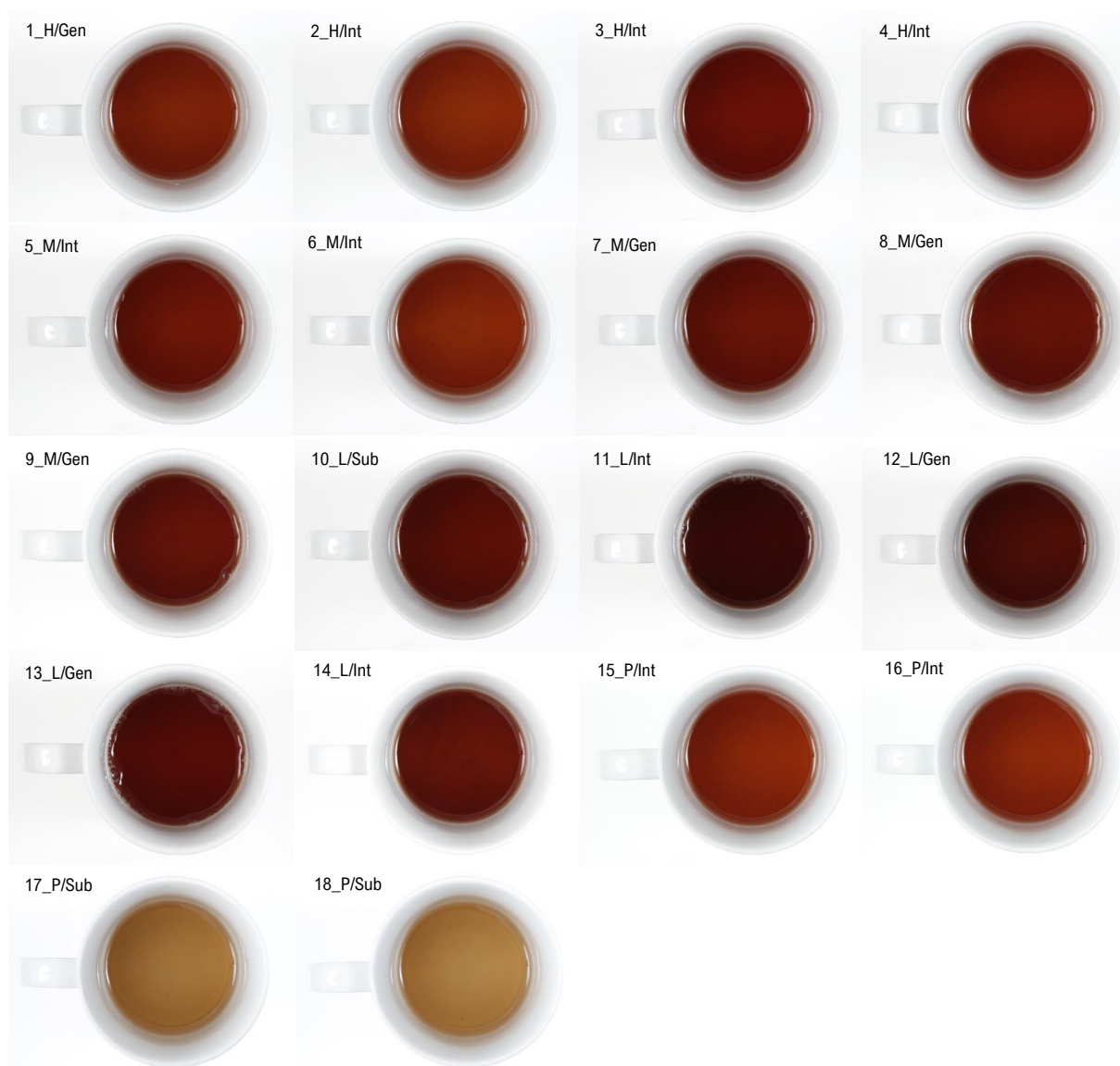


**Figure B6 A)** Digital images of stabilised formazin turbidity standards (<0.1, 20, 200, 1000, 4000 NTU) and **B)** fermented honeybush tea infusions ('dust' in brackets indicate small particles/sediment present in respective samples; black lines on paper sheet behind vials indicate the ability to be able to see through an infusion sample) (images by Anton Jordaan Photography, Stellenbosch, South Africa).



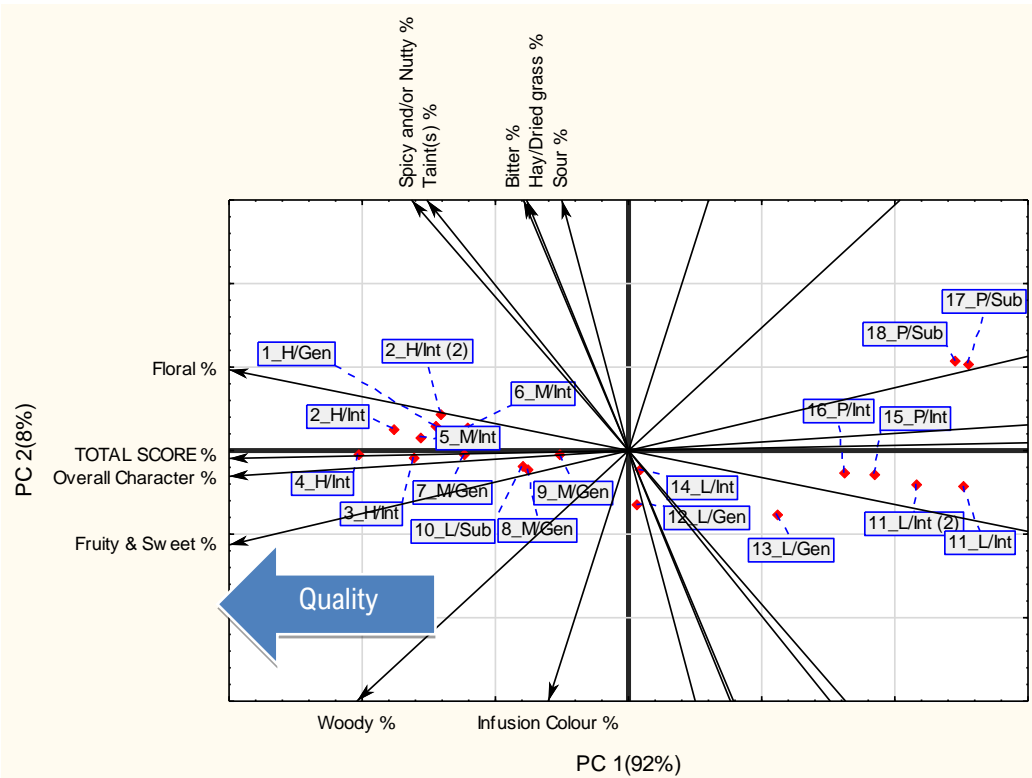


**Figure B7** Fermented honeybush tea plant material studied to establish the criteria for **dry leaf appearance** for scorecard: **A)** even cut, **B)** uneven cut, **C)** presence of undesirable dark particles with very light stem particles, and **D)** presence of undesirable large white stem particles. Digital images **B**, **C** and **D** represent undesirable/inferior dry leaf appearance (images by Anton Jordaan Photography, Stellenbosch, South Africa).

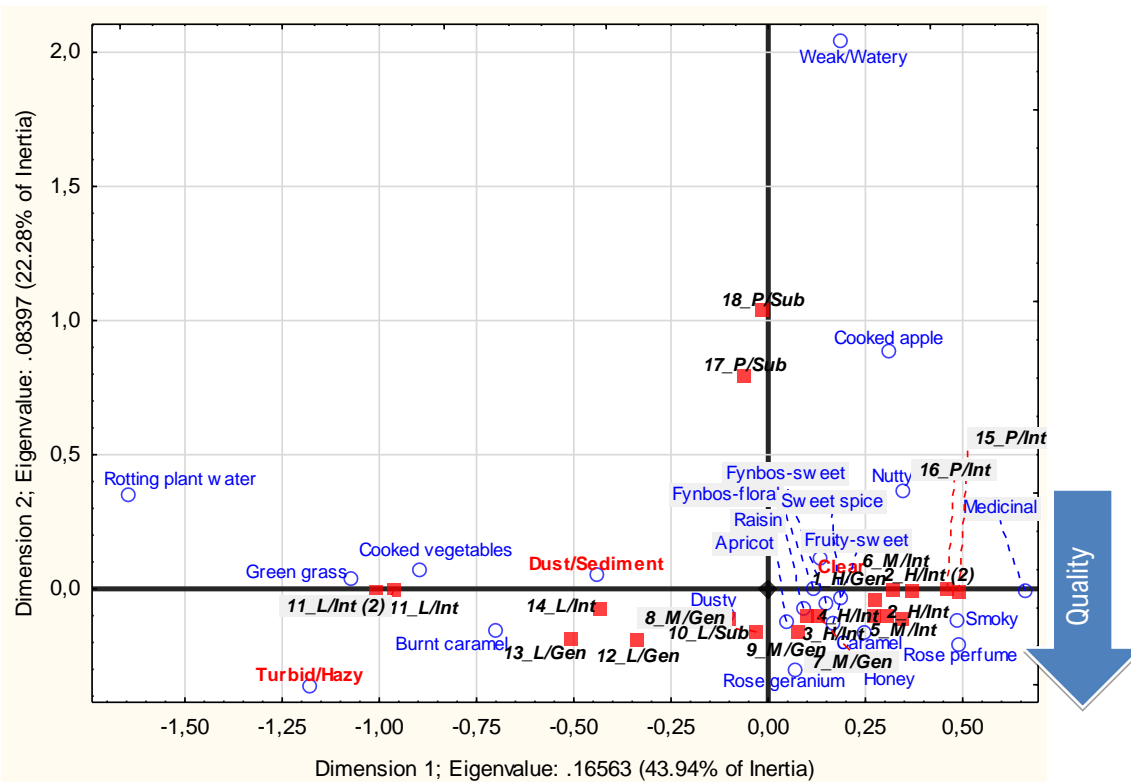


**Figure B8** Infusions of the sample set used for validation of the sensory quality scoring method. Unfiltered aliquots (ca. 100 mL) are presented in white porcelain mugs (diameter 75 mm at 100 mm depth) (images by Anton Jordaan Photography, Stellenbosch, South Africa).

A)

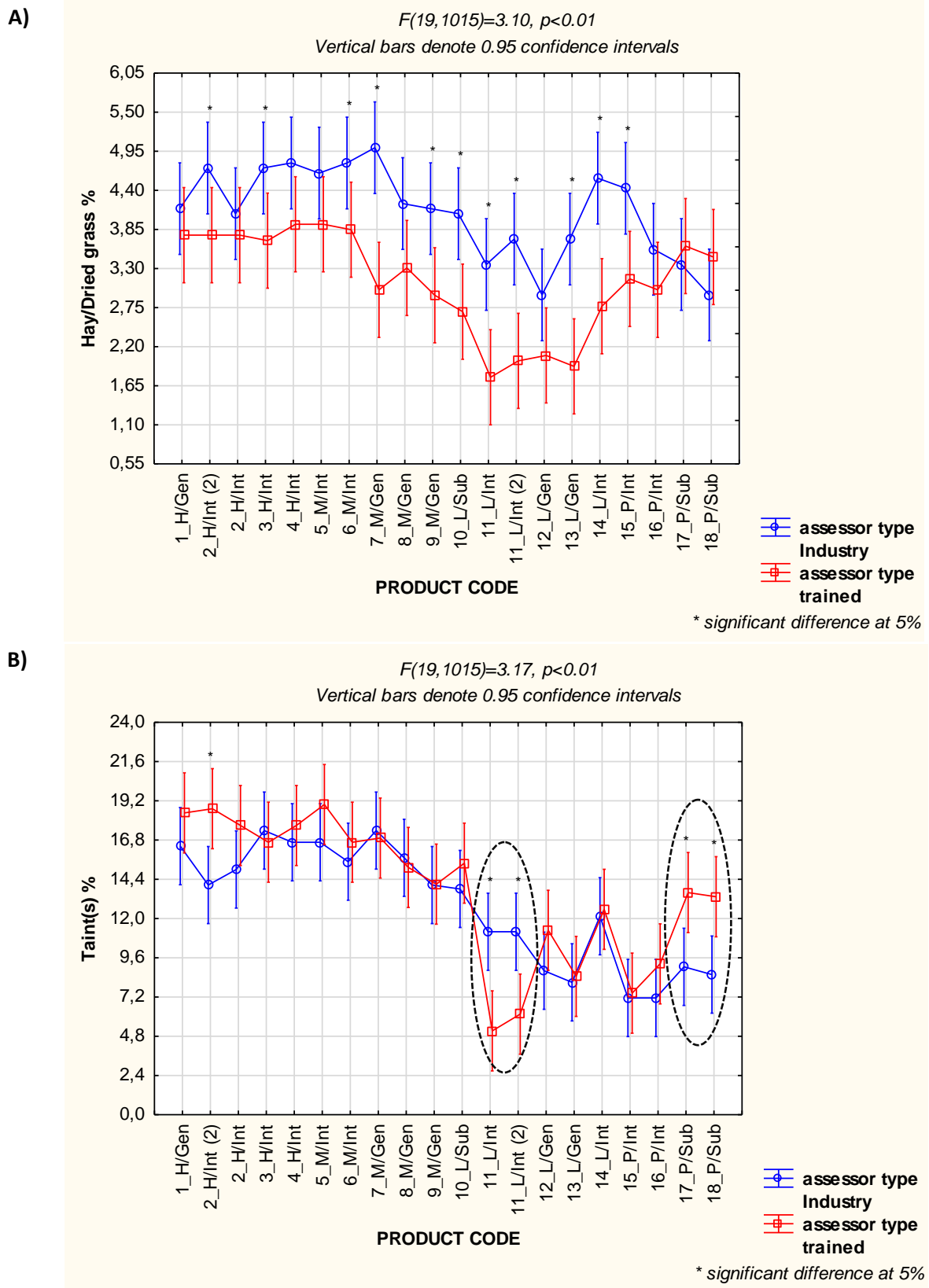


B)



**Figure B9 A) Scorecard PCA bi-plot for total and individual parameter scores<sup>6</sup> of trained panel and B) CA plot of standardised residuals calculated from citation frequencies for trained panel [infusion appearance (red) and sensory attribute parameters (blue); 1–18 represent sample numbers; Int, Sub, Gen represent *C. intermedia*, *C. subternata* and *C. genistoides*, respectively; H, M, L, P represent high, moderate, low and poor quality, respectively; (2) after sample code indicates blind duplicate]. Blue arrow indicates direction of increased sensory quality.**

<sup>6</sup> Reversed scores allocation has been incorporated for negative parameters: therefore, 'hay/dried grass' flavour, bitter taste, sour taste and 'taints' parameter scores are in opposite direction from low and poor quality samples.



**Figure B10** Least squared (LS) means line graphs for **industry vs trained panel** for **A)** ‘hay/dried grass’ flavour scores (%) and **B)** taint(s) scores (%)<sup>7</sup>[1–18 represent sample numbers; Int, Sub, Gen represent *C. intermedia*, *C. subternata* and *C. genistoides*, respectively; H, M, L, P represent high, moderate, low and poor quality, respectively; (2) after sample code indicates blind duplicate].

<sup>7</sup> For negative attributes the scores allocation was reversed, i.e. from 3 to 0 for ‘absent/barely perceptible’ to ‘high’; therefore, samples with high score values indicate low perception of the respective parameter and vice versa.

**Table B1.1** Aroma attribute intensities from DSA of the commercial sample set (n = 36).

SAMPLE CODE	Fynbos-floral aroma	Rose geranium aroma	Rose perfume aroma	Apricot aroma	Apple aroma	Raisin aroma	Woody aroma	Pine aroma	Fruity-sweet aroma	Caramel aroma	Honey aroma	Fynbos-sweet aroma	Sweet spice aroma	Nutty aroma
1_H/Gen	43.4 <sup>bcd</sup>	9.3 <sup>abcde</sup>	14.3 <sup>bcdef</sup>	29.3 <sup>bc</sup>	4.2 <sup>cdef</sup>	31.0 <sup>ab</sup>	49.8 <sup>abcde</sup>	0.5 <sup>efgh</sup>	33.6 <sup>abc</sup>	17.1 <sup>abcd</sup>	7.7 <sup>a</sup>	39.8 <sup>bcdefg</sup>	9.8 <sup>abcdefg</sup>	6.1 <sup>def</sup>
2_H/Int	44.1 <sup>bc</sup>	8.6 <sup>bcdefgh</sup>	16.0 <sup>abc</sup>	25.4 <sup>defghi</sup>	4.0 <sup>cdefgh</sup>	30.8 <sup>abc</sup>	50.9 <sup>abc</sup>	0.8 <sup>defg</sup>	29.7 <sup>defghij</sup>	15.7 <sup>abcdefghi</sup>	6.2 <sup>bcde</sup>	41.6 <sup>abc</sup>	11.2 <sup>ab</sup>	8.0 <sup>bc</sup>
3_H/Int	43.2 <sup>bcd</sup>	8.7 <sup>bcdefg</sup>	15.0 <sup>bcd</sup>	25.7 <sup>defgh</sup>	1.4 <sup>klmn</sup>	29.6 <sup>bcde</sup>	48.3 <sup>defgh</sup>	0.5 <sup>efgh</sup>	28.9 <sup>efghijkl</sup>	14.7 <sup>defghij</sup>	5.3 <sup>cdefg</sup>	39.1 <sup>cdefgh</sup>	10.7 <sup>abcde</sup>	6.8 <sup>cde</sup>
4_H/Int	43.8 <sup>bc</sup>	8.0 <sup>cdefghi</sup>	16.2 <sup>abc</sup>	22.9 <sup>ijkl</sup>	2.9 <sup>defghijkl</sup>	30.0 <sup>bcd</sup>	50.1 <sup>abcd</sup>	0.8 <sup>defg</sup>	26.5 <sup>klmn</sup>	13.9 <sup>efghij</sup>	6.2 <sup>bcde</sup>	40.6 <sup>bcde</sup>	11.0 <sup>abc</sup>	7.8 <sup>bcd</sup>
5_M/Int	39.8 <sup>efghij</sup>	6.1 <sup>ijk</sup>	10.5 <sup>ghijkl</sup>	22.6 <sup>ijklm</sup>	1.3 <sup>klmn</sup>	27.4 <sup>defghi</sup>	47.9 <sup>defgh</sup>	0.9 <sup>cdefg</sup>	26.8 <sup>ijklmn</sup>	14.7 <sup>defghij</sup>	4.2 <sup>ghijk</sup>	37.4 <sup>efghij</sup>	9.5 <sup>bcdefgh</sup>	5.7 <sup>ef</sup>
6_M/Int	42.7 <sup>bcdef</sup>	7.1 <sup>d<sup>efghij</sup></sup>	14.2 <sup>cdef</sup>	23.0 <sup>hijkl</sup>	2.9 <sup>defghijk</sup>	30.6 <sup>abc</sup>	50.9 <sup>ab</sup>	0.5 <sup>efgh</sup>	29.4 <sup>efghijk</sup>	16.8 <sup>abcd</sup>	5.0 <sup>efgh</sup>	40.6 <sup>bcde</sup>	10.3 <sup>abcde</sup>	7.5 <sup>bcd</sup>
7_M/Gen	39.6 <sup>efghij</sup>	8.9 <sup>abcdef</sup>	10.6 <sup>ghijkl</sup>	27.3 <sup>cdef</sup>	2.1 <sup>ghijklm</sup>	27.1 <sup>efghi</sup>	47.7 <sup>efgh</sup>	0.3 <sup>gh</sup>	30.6 <sup>cdefgh</sup>	13.8 <sup>efghij</sup>	4.8 <sup>efghi</sup>	38.7 <sup>defgh</sup>	6.7 <sup>ijklmn</sup>	3.1 <sup>ijkl</sup>
8_M/Gen	39.3 <sup>efghij</sup>	6.8 <sup>efghij</sup>	10.7 <sup>ghijkl</sup>	27.0 <sup>cdef</sup>	1.5 <sup>ijklmn</sup>	26.8 <sup>efghij</sup>	46.8 <sup>gh</sup>	0.0 <sup>h</sup>	27.8 <sup>hijklm</sup>	13.6 <sup>ghij</sup>	4.3 <sup>ghij</sup>	37.7 <sup>efghi</sup>	7.3 <sup>ijkl</sup>	3.0 <sup>ijkl</sup>
9_M/Gen	39.6 <sup>efghij</sup>	8.5 <sup>bcdefghi</sup>	10.0 <sup>hijklm</sup>	28.8 <sup>bc</sup>	3.7 <sup>defghij</sup>	27.5 <sup>defghi</sup>	47.1 <sup>fgh</sup>	0.3 <sup>gh</sup>	30.7 <sup>cdefgh</sup>	16.0 <sup>abcdefg</sup>	5.3 <sup>cdefg</sup>	37.1 <sup>ghij</sup>	8.8 <sup>cdefghi</sup>	4.4 <sup>fghi</sup>
10_L/Sub	36.6 <sup>klm</sup>	8.1 <sup>bcdefghi</sup>	8.8 <sup>klmn</sup>	27.8 <sup>bcd</sup>	1.7 <sup>ijklmn</sup>	25.2 <sup>l</sup>	47.9 <sup>defgh</sup>	2.9 <sup>b</sup>	28.3 <sup>efghijkl</sup>	13.3 <sup>hijk</sup>	3.0 <sup>klmn</sup>	33.8 <sup>klm</sup>	8.5 <sup>efghij</sup>	2.9 <sup>ijkl</sup>
11_L/Int	27.3 <sup>nop</sup>	6.1 <sup>ijk</sup>	3.2 <sup>r</sup>	17.9 <sup>op</sup>	0.0 <sup>n</sup>	17.9 <sup>i</sup>	40.6 <sup>l</sup>	1.5 <sup>cd</sup>	18.6 <sup>q</sup>	6.2 <sup>n</sup>	1.6 <sup>op</sup>	23.9 <sup>p</sup>	1.2 <sup>p</sup>	1.6 <sup>klmn</sup>
12_L/Gen	38.5 <sup>efghij</sup>	8.8 <sup>bcdefg</sup>	9.4 <sup>ijklmn</sup>	33.7 <sup>a</sup>	2.5 <sup>efghijklm</sup>	27.5 <sup>defghi</sup>	48.6 <sup>defg</sup>	0.3 <sup>efgh</sup>	34.5 <sup>ab</sup>	15.9 <sup>abcdefgh</sup>	3.4 <sup>ijklmn</sup>	38.3 <sup>efgh</sup>	7.4 <sup>ijkl</sup>	3.3 <sup>efghij</sup>
13_L/Gen	28.2 <sup>no</sup>	6.1 <sup>ijk</sup>	5.3 <sup>pqr</sup>	30.4 <sup>b</sup>	3.0 <sup>defghijk</sup>	25.3 <sup>hij</sup>	44.2 <sup>jk</sup>	0.3 <sup>gh</sup>	32.8 <sup>abcd</sup>	16.0 <sup>abcdefg</sup>	3.8 <sup>ijklm</sup>	31.1 <sup>mn</sup>	5.0 <sup>mno</sup>	1.1 <sup>lmn</sup>
14_L/Int	34.7 <sup>m</sup>	6.8 <sup>efghij</sup>	6.8 <sup>nopq</sup>	19.7 <sup>no</sup>	2.1 <sup>ghijklmn</sup>	22.3 <sup>k</sup>	44.0 <sup>jk</sup>	3.1 <sup>b</sup>	22.4 <sup>op</sup>	9.9 <sup>lm</sup>	3.3 <sup>ijklmn</sup>	31.7 <sup>m</sup>	5.1 <sup>lmno</sup>	1.5 <sup>lmn</sup>
15_P/Int	35.1 <sup>m</sup>	4.5 <sup>klm</sup>	9.6 <sup>ijklm</sup>	20.0 <sup>mno</sup>	2.2 <sup>efghijklm</sup>	26.9 <sup>efghij</sup>	47.0 <sup>gh</sup>	0.3 <sup>gh</sup>	24.7 <sup>no</sup>	13.8 <sup>efghij</sup>	2.7 <sup>mno</sup>	33.2 <sup>lm</sup>	8.6 <sup>defghi</sup>	5.7 <sup>ef</sup>
16_P/Int	30.1 <sup>n</sup>	5.1 <sup>klm</sup>	7.5 <sup>mnopq</sup>	18.1 <sup>op</sup>	1.8 <sup>hijklmn</sup>	25.9 <sup>efghij</sup>	46.6 <sup>ghi</sup>	0.9 <sup>defg</sup>	25.0 <sup>mno</sup>	13.5 <sup>ghij</sup>	1.5 <sup>opq</sup>	31.5 <sup>m</sup>	6.3 <sup>ijklmn</sup>	4.5 <sup>fgh</sup>
17_P/Sub	28.7 <sup>no</sup>	3.2 <sup>lm</sup>	7.5 <sup>mnopq</sup>	14.3 <sup>q</sup>	8.8 <sup>a</sup>	16.0 <sup>l</sup>	33.7 <sup>n</sup>	0.0 <sup>h</sup>	19.0 <sup>q</sup>	15.8 <sup>abcdefgh</sup>	5.1 <sup>defgh</sup>	28.6 <sup>no</sup>	7.1 <sup>ijklm</sup>	6.8 <sup>cde</sup>
18_P/Sub	25.8 <sup>op</sup>	2.9 <sup>m</sup>	7.6 <sup>mnop</sup>	15.8 <sup>pq</sup>	7.5 <sup>ab</sup>	15.6 <sup>l</sup>	31.7 <sup>n</sup>	0.0 <sup>h</sup>	20.1 <sup>pq</sup>	12.5 <sup>ijkl</sup>	3.9 <sup>hijkl</sup>	24.2 <sup>p</sup>	5.9 <sup>klmno</sup>	6.8 <sup>cde</sup>
19_Sub	24.9 <sup>p</sup>	3.2 <sup>lm</sup>	5.7 <sup>opqr</sup>	14.1 <sup>q</sup>	7.7 <sup>ab</sup>	16.2 <sup>l</sup>	36.8 <sup>m</sup>	0.2 <sup>gh</sup>	19.0 <sup>q</sup>	9.9 <sup>lm</sup>	1.2 <sup>pq</sup>	26.3 <sup>op</sup>	5.4 <sup>lmno</sup>	7.1 <sup>bcde</sup>
20_Gen	38.8 <sup>efghij</sup>	9.9 <sup>abc</sup>	8.9 <sup>klmn</sup>	34.7 <sup>a</sup>	3.7 <sup>cdefghi</sup>	28.6 <sup>bcdefg</sup>	47.8 <sup>defgh</sup>	0.0 <sup>h</sup>	35.6 <sup>a</sup>	17.8 <sup>ab</sup>	5.3 <sup>cdefg</sup>	37.9 <sup>efghi</sup>	8.5 <sup>efghij</sup>	3.3 <sup>efghij</sup>
21_Gen	40.1 <sup>efghij</sup>	10.6 <sup>a</sup>	12.6 <sup>defgh</sup>	29.4 <sup>bc</sup>	3.0 <sup>defghijkl</sup>	30.0 <sup>bcd</sup>	47.7 <sup>efgh</sup>	0.3 <sup>gh</sup>	32.4 <sup>abcde</sup>	15.7 <sup>abcdefgh</sup>	6.2 <sup>bcde</sup>	39.9 <sup>bcdefg</sup>	6.6 <sup>ijklmn</sup>	2.7 <sup>ijklm</sup>
22_Gen	39.3 <sup>efghij</sup>	8.8 <sup>bcdef</sup>	10.8 <sup>efghij</sup>	24.7 <sup>efghij</sup>	1.7 <sup>ijklmn</sup>	28.4 <sup>bcdefg</sup>	47.8 <sup>defgh</sup>	0.3 <sup>gh</sup>	28.6 <sup>efghijkl</sup>	14.6 <sup>defghij</sup>	5.4 <sup>cdefg</sup>	36.5 <sup>hijk</sup>	8.7 <sup>defghi</sup>	3.4 <sup>efghij</sup>
23_Gen	42.9 <sup>bcde</sup>	9.4 <sup>abcd</sup>	15.0 <sup>bcd</sup>	27.9 <sup>bcd</sup>	4.3 <sup>cde</sup>	31.1 <sup>ab</sup>	49.3 <sup>bcdef</sup>	0.3 <sup>gh</sup>	29.8 <sup>defghij</sup>	16.5 <sup>abcde</sup>	5.2 <sup>defg</sup>	41.3 <sup>abcd</sup>	10.0 <sup>abcdef</sup>	6.1 <sup>def</sup>
24_Gen	41.3 <sup>cdefgh</sup>	8.3 <sup>bcdefghi</sup>	11.7 <sup>efghij</sup>	27.5 <sup>cde</sup>	3.9 <sup>cdefgh</sup>	28.7 <sup>bcdef</sup>	46.2 <sup>ijh</sup>	0.0 <sup>h</sup>	30.1 <sup>defghi</sup>	14.7 <sup>defghij</sup>	5.4 <sup>cdefg</sup>	37.2 <sup>ghij</sup>	7.9 <sup>efghij</sup>	4.9 <sup>fg</sup>
25_Gen	29.9 <sup>n</sup>	5.3 <sup>kl</sup>	5.4 <sup>pqr</sup>	30.4 <sup>b</sup>	2.2 <sup>efghijklm</sup>	25.1 <sup>l</sup>	44.4 <sup>ij</sup>	0.3 <sup>gh</sup>	31.9 <sup>bcdef</sup>	15.1 <sup>cdefghi</sup>	2.7 <sup>lmno</sup>	31.7 <sup>m</sup>	4.6 <sup>no</sup>	1.0 <sup>mno</sup>
26_Gen	39.9 <sup>efghij</sup>	8.4 <sup>bcdefghi</sup>	9.2 <sup>ijklmn</sup>	22.9 <sup>ijkl</sup>	2.8 <sup>defghijkl</sup>	28.3 <sup>bcdefg</sup>	49.3 <sup>bcdef</sup>	1.6 <sup>c</sup>	27.7 <sup>hijklmn</sup>	14.0 <sup>efghij</sup>	1.2 <sup>pq</sup>	38.0 <sup>efghi</sup>	10.7 <sup>abcde</sup>	3.0 <sup>hijkl</sup>
27_Gen	38.2 <sup>ijkl</sup>	8.7 <sup>bcdefg</sup>	8.6 <sup>klmn</sup>	24.1 <sup>efghij</sup>	1.6 <sup>ijklmn</sup>	26.3 <sup>efghij</sup>	46.7 <sup>gh</sup>	1.1 <sup>cdef</sup>	26.4 <sup>klmn</sup>	13.2 <sup>ijk</sup>	3.2 <sup>klmn</sup>	35.4 <sup>ijkl</sup>	7.7 <sup>efghij</sup>	3.4 <sup>efghij</sup>
28_Gen	37.3 <sup>klm</sup>	8.2 <sup>bcdefghi</sup>	10.0 <sup>ijklm</sup>	22.7 <sup>klm</sup>	0.6 <sup>mno</sup>	26.5 <sup>efghij</sup>	48.8 <sup>bcdefg</sup>	3.4 <sup>ab</sup>	26.5 <sup>klmn</sup>	12.2 <sup>kl</sup>	4.2 <sup>efghij</sup>	34.5 <sup>ijkl</sup>	6.6 <sup>ijklmn</sup>	2.1 <sup>ijklm</sup>
29_Int	45.2 <sup>ab</sup>	7.4 <sup>defghij</sup>	16.9 <sup>ab</sup>	26.9 <sup>cdefg</sup>	5.8 <sup>bc</sup>	31.0 <sup>ab</sup>	50.0 <sup>abcde</sup>	0.3 <sup>gh</sup>	32.0 <sup>bcdef</sup>	17.4 <sup>abc</sup>	6.5 <sup>abc</sup>	42.0 <sup>ab</sup>	11.8 <sup>a</sup>	8.9 <sup>b</sup>
30_Int	42.3 <sup>bcdefg</sup>	7.1 <sup>defghij</sup>	12.3 <sup>efghi</sup>	25.1 <sup>efghij</sup>	4.3 <sup>cde</sup>	28.6 <sup>bcdef</sup>	48.2 <sup>defgh</sup>	0.0 <sup>h</sup>	27.1 <sup>ijklmn</sup>	16.3 <sup>abcdef</sup>	6.1 <sup>bcde</sup>	40.0 <sup>bcdef</sup>	10.4 <sup>abcde</sup>	7.6 <sup>bcd</sup>
31_Int	40.5 <sup>defghi</sup>	6.4 <sup>efghij</sup>	15.1 <sup>bcd</sup>	21.2 <sup>lmn</sup>	2.6 <sup>defghijklm</sup>	28.1 <sup>cdefgh</sup>	48.2 <sup>defgh</sup>	0.0 <sup>h</sup>	25.8 <sup>lmn</sup>	15.4 <sup>abcdefghi</sup>	6.3 <sup>bcd</sup>	37.6 <sup>efghi</sup>	10.9 <sup>abcd</sup>	10.7 <sup>a</sup>
32_Int	39.8 <sup>efghij</sup>	6.8 <sup>efghij</sup>	12.8 <sup>defg</sup>	24.2 <sup>efghij</sup>	4.4 <sup>cd</sup>	28.6 <sup>bcdefg</sup>	48.7 <sup>cdefg</sup>	0.3 <sup>gh</sup>	27.5 <sup>hijklmn</sup>	15.3 <sup>bcdefghi</sup>	6.7 <sup>ab</sup>	39.0 <sup>cdefgh</sup>	11.5 <sup>ab</sup>	8.3 <sup>bc</sup>
33_Int	42.3 <sup>bcdefg</sup>	6.3 <sup>hijk</sup>	14.8 <sup>bcde</sup>	22.1 <sup>klmn</sup>	4.3 <sup>cde</sup>	28.1 <sup>cdefgh</sup>	48.2 <sup>defgh</sup>	0.0 <sup>h</sup>	27.0 <sup>ijklmn</sup>	14.7 <sup>defghij</sup>	6.8 <sup>ab</sup>	39.1 <sup>cdefgh</sup>	9.8 <sup>abcdefg</sup>	7.9 <sup>bc</sup>
34_Int	47.6 <sup>a</sup>	10.4 <sup>ab</sup>	17.9 <sup>a</sup>	27.5 <sup>cde</sup>	4.1 <sup>cdefg</sup>	33.2 <sup>a</sup>	51.8 <sup>a</sup>	0.5 <sup>efgh</sup>	31.3 <sup>cdefg</sup>	18.0 <sup>a</sup>	5.9 <sup>bcdef</sup>	43.8 <sup>a</sup>	11.6 <sup>ab</sup>	6.9 <sup>cde</sup>
35_Int	35.2 <sup>lm</sup>	7.4 <sup>defghij</sup>	8.1 <sup>lmno</sup>	23.4 <sup>hijkl</sup>	2.3 <sup>efghijklm</sup>	24.4 <sup>jk</sup>	46.8 <sup>gh</sup>	4.1 <sup>a</sup>	25.9 <sup>lmn</sup>	10.9 <sup>kl</sup>	2.3 <sup>nop</sup>	33.1 <sup>lm</sup>	7.8 <sup>efghij</sup>	2.8 <sup>hijkl</sup>
36_Int	29.4 <sup>n</sup>	7.0 <sup>efghij</sup>	4.9 <sup>qr</sup>	19.7 <sup>no</sup>	0.8 <sup>lmn</sup>	18.3 <sup>l</sup>	41.9 <sup>kl</sup>	1.1 <sup>cde</sup>	20.7 <sup>pq</sup>	7.9 <sup>mn</sup>	0.3 <sup>q</sup>	28.1 <sup>o</sup>	3.9 <sup>o</sup>	0.9 <sup>n</sup>

\*means that have not the same letter are significantly different ( $p < 0.05$ )

**Table B1.2** Aroma, flavour, taste and mouthfeel attribute intensities from DSA of the commercial sample set (n = 36).

SAMPLE CODE	Dusty aroma	Medicinal aroma	Burnt caramel aroma	Rotting plant water aroma	Hay/Dried grass aroma	Green grass aroma	Cooked vegetables aroma	Smoky aroma	SOUR taste	BITTER taste	ASTRINGENT mouthfeel	SWEET taste	Fynbos-floral flavour	Rose geranium flavour	Rose perfume flavour	Apricot flavour
1_H/Gen	1.7 <sup>bcdefghi</sup>	2.8 <sup>c</sup>	2.7 <sup>hijk</sup>	0.0 <sup>f</sup>	15.2 <sup>no</sup>	1.2 <sup>ijkl</sup>	0.0 <sup>l</sup>	0.4 <sup>hij</sup>	2.4 <sup>hijklm</sup>	0.6 <sup>n</sup>	28.3 <sup>pqr</sup>	22.6 <sup>abc</sup>	39.9 <sup>a</sup>	6.9 <sup>ab</sup>	11.8 <sup>cd</sup>	2.3 <sup>cd</sup>
2_H/Int	2.1 <sup>bcdefghi</sup>	1.2 <sup>defghi</sup>	1.1 <sup>kl</sup>	0.0 <sup>f</sup>	14.7 <sup>o</sup>	1.1 <sup>ijkl</sup>	0.3 <sup>kl</sup>	0.0 <sup>l</sup>	3.3 <sup>ghijkl</sup>	2.7 <sup>efghijkl</sup>	30.0 <sup>hijklmno</sup>	22.6 <sup>abc</sup>	39.0 <sup>abc</sup>	6.9 <sup>ab</sup>	13.8 <sup>b</sup>	0.3 <sup>fg</sup>
3_H/Int	0.5 <sup>i</sup>	0.6 <sup>defghi</sup>	0.7 <sup>kl</sup>	0.0 <sup>f</sup>	15.3 <sup>no</sup>	0.9 <sup>ijkl</sup>	0.6 <sup>kl</sup>	0.2 <sup>ij</sup>	2.5 <sup>ghijklm</sup>	1.5 <sup>ijklmn</sup>	28.8 <sup>nopqr</sup>	22.3 <sup>abc</sup>	38.7 <sup>abcd</sup>	5.9 <sup>abcdef</sup>	10.7 <sup>cdef</sup>	0.3 <sup>fg</sup>
4_H/Int	1.6 <sup>bcdefghi</sup>	0.3 <sup>hi</sup>	1.4 <sup>hijkl</sup>	0.0 <sup>f</sup>	16.1 <sup>lmno</sup>	0.6 <sup>l</sup>	0.0 <sup>l</sup>	0.0 <sup>l</sup>	1.5 <sup>klm</sup>	1.4 <sup>ijklmn</sup>	29.8 <sup>klmnop</sup>	21.9 <sup>abcd</sup>	39.7 <sup>ab</sup>	6.1 <sup>abcde</sup>	12.1 <sup>bc</sup>	0.3 <sup>fg</sup>
5_M/Int	2.8 <sup>abcd</sup>	0.7 <sup>defghi</sup>	2.3 <sup>hijkl</sup>	0.0 <sup>f</sup>	18.6 <sup>hijk</sup>	0.7 <sup>kl</sup>	0.0 <sup>l</sup>	0.8 <sup>ghij</sup>	1.7 <sup>klm</sup>	1.4 <sup>ijklmn</sup>	29.0 <sup>mno</sup>	21.6 <sup>abcde</sup>	35.3 <sup>efghij</sup>	4.4 <sup>fghij</sup>	8.4 <sup>ghi</sup>	0.0 <sup>g</sup>
6_M/Int	2.0 <sup>bcdefghi</sup>	1.4 <sup>cdefgh</sup>	0.6 <sup>l</sup>	0.0 <sup>f</sup>	15.2 <sup>no</sup>	1.1 <sup>ijkl</sup>	0.5 <sup>kl</sup>	0.9 <sup>fghi</sup>	1.2 <sup>klm</sup>	1.3 <sup>ijklmn</sup>	27.9 <sup>qr</sup>	22.7 <sup>abc</sup>	37.5 <sup>abcde</sup>	4.0 <sup>ghijk</sup>	8.8 <sup>fgh</sup>	0.3 <sup>fg</sup>
7_M/Gen	2.0 <sup>bcdefghi</sup>	0.5 <sup>fghi</sup>	3.0 <sup>ghij</sup>	0.0 <sup>f</sup>	16.6 <sup>lmno</sup>	2.6 <sup>ghi</sup>	1.9 <sup>ijk</sup>	0.0 <sup>l</sup>	3.6 <sup>fghi</sup>	2.5 <sup>fghijklm</sup>	31.5 <sup>defghij</sup>	21.2 <sup>bcdef</sup>	36.4 <sup>defghi</sup>	6.6 <sup>abc</sup>	8.7 <sup>gh</sup>	1.7 <sup>de</sup>
8_M/Gen	2.3 <sup>bcdefgh</sup>	2.0 <sup>cd</sup>	3.4 <sup>gh</sup>	0.0 <sup>f</sup>	16.9 <sup>klmn</sup>	0.6 <sup>l</sup>	1.1 <sup>kl</sup>	0.3 <sup>hij</sup>	3.5 <sup>fghi</sup>	2.1 <sup>ghijklmn</sup>	31.4 <sup>defghijk</sup>	19.3 <sup>gh</sup>	35.2 <sup>efghij</sup>	4.8 <sup>defghij</sup>	6.4 <sup>ijklm</sup>	1.4 <sup>def</sup>
9_M/Gen	2.3 <sup>bcdefg</sup>	1.2 <sup>defghi</sup>	2.8 <sup>hijk</sup>	0.0 <sup>f</sup>	17.8 <sup>ijkl</sup>	1.1 <sup>ijkl</sup>	1.0 <sup>kl</sup>	0.3 <sup>hij</sup>	5.9 <sup>bcde</sup>	4.3 <sup>cde</sup>	32.5 <sup>cde</sup>	19.2 <sup>gh</sup>	34.8 <sup>fghij</sup>	6.2 <sup>abcde</sup>	6.4 <sup>klm</sup>	2.3 <sup>d</sup>
10_L/Sub	0.9 <sup>fghi</sup>	1.0 <sup>defghi</sup>	7.8 <sup>cd</sup>	0.4 <sup>de</sup>	20.8 <sup>efg</sup>	4.6 <sup>ef</sup>	3.5 <sup>fghi</sup>	4.9 <sup>b</sup>	4.5 <sup>defgh</sup>	4.7 <sup>c</sup>	33.2 <sup>bcd</sup>	18.9 <sup>gh</sup>	31.8 <sup>kl</sup>	5.1 <sup>cdefghi</sup>	7.5 <sup>hijkl</sup>	4.0 <sup>ab</sup>
11_L/Int	2.0 <sup>bcdefghi</sup>	1.5 <sup>cdefgh</sup>	5.5 <sup>ef</sup>	8.1 <sup>a</sup>	24.1 <sup>ab</sup>	13.6 <sup>b</sup>	7.8 <sup>c</sup>	1.7 <sup>def</sup>	7.7 <sup>b</sup>	7.7 <sup>b</sup>	33.8 <sup>bc</sup>	15.1 <sup>k</sup>	23.6 <sup>no</sup>	4.5 <sup>efghij</sup>	2.3 <sup>p</sup>	0.3 <sup>fg</sup>
12_L/Gen	2.3 <sup>bcdefg</sup>	1.9 <sup>cde</sup>	11.4 <sup>b</sup>	0.0 <sup>f</sup>	18.7 <sup>hijk</sup>	4.2 <sup>efg</sup>	4.5 <sup>efg</sup>	1.1 <sup>efgh</sup>	6.5 <sup>bcd</sup>	2.7 <sup>efghijklm</sup>	31.8 <sup>defgh</sup>	19.7 <sup>fgh</sup>	34.0 <sup>ijk</sup>	5.9 <sup>abcdef</sup>	8.1 <sup>hij</sup>	4.6 <sup>ab</sup>
13_L/Gen	1.1 <sup>efghi</sup>	1.0 <sup>defghi</sup>	17.5 <sup>a</sup>	0.2 <sup>ef</sup>	22.1 <sup>cde</sup>	5.9 <sup>e</sup>	15.0 <sup>a</sup>	0.0 <sup>l</sup>	11.5 <sup>a</sup>	9.7 <sup>a</sup>	35.6 <sup>a</sup>	16.0 <sup>k</sup>	27.4 <sup>m</sup>	6.3 <sup>abcd</sup>	2.9 <sup>op</sup>	4.8 <sup>a</sup>
14_L/Int	1.7 <sup>bcdefghi</sup>	1.8 <sup>cdef</sup>	3.3 <sup>ghi</sup>	1.6 <sup>b</sup>	21.4 <sup>def</sup>	7.8 <sup>d</sup>	6.8 <sup>cd</sup>	1.4 <sup>efg</sup>	4.5 <sup>defg</sup>	4.5 <sup>cd</sup>	32.0 <sup>cdefg</sup>	18.6 <sup>ghi</sup>	30.9 <sup>l</sup>	5.3 <sup>abcdefg</sup>	5.6 <sup>lmn</sup>	0.0 <sup>g</sup>
15_P/Int	2.3 <sup>bcdefgh</sup>	16.9 <sup>b</sup>	2.3 <sup>hijkl</sup>	0.0 <sup>f</sup>	22.0 <sup>cde</sup>	1.1 <sup>ijkl</sup>	1.2 <sup>kl</sup>	4.9 <sup>b</sup>	2.4 <sup>ghijklm</sup>	4.0 <sup>cdef</sup>	31.1 <sup>efghijkl</sup>	19.0 <sup>gh</sup>	30.9 <sup>l</sup>	3.3 <sup>k</sup>	7.4 <sup>hijkl</sup>	0.0 <sup>g</sup>
16_P/Int	2.1 <sup>bcdefghi</sup>	20.1 <sup>a</sup>	5.7 <sup>de</sup>	0.0 <sup>f</sup>	23.8 <sup>bc</sup>	1.2 <sup>ijkl</sup>	2.9 <sup>ghi</sup>	8.3 <sup>a</sup>	3.5 <sup>fghi</sup>	3.8 <sup>cdefg</sup>	31.5 <sup>defghijk</sup>	18.9 <sup>gh</sup>	27.8 <sup>m</sup>	2.5 <sup>kl</sup>	5.8 <sup>klmn</sup>	0.6 <sup>efg</sup>
17_P/Sub	2.1 <sup>bcdefghi</sup>	0.0 <sup>i</sup>	1.0 <sup>kl</sup>	0.0 <sup>f</sup>	19.5 <sup>ghi</sup>	5.8 <sup>e</sup>	2.3 <sup>hij</sup>	0.2 <sup>ij</sup>	0.6 <sup>m</sup>	0.9 <sup>mn</sup>	27.7 <sup>r</sup>	21.1 <sup>bcdef</sup>	21.6 <sup>op</sup>	1.3 <sup>l</sup>	5.0 <sup>mn</sup>	0.0 <sup>g</sup>
18_P/Sub	0.8 <sup>ghi</sup>	0.0 <sup>i</sup>	1.2 <sup>ijkl</sup>	0.0 <sup>f</sup>	19.3 <sup>ghi</sup>	5.6 <sup>e</sup>	4.4 <sup>efg</sup>	0.0 <sup>l</sup>	1.0 <sup>m</sup>	2.6 <sup>efghijklm</sup>	28.9 <sup>mno</sup>	19.0 <sup>gh</sup>	20.2 <sup>p</sup>	1.4 <sup>l</sup>	4.5 <sup>no</sup>	0.2 <sup>g</sup>
19_Sub	2.8 <sup>abcde</sup>	0.5 <sup>fghi</sup>	1.7 <sup>hijkl</sup>	0.0 <sup>f</sup>	19.2 <sup>ghi</sup>	11.3 <sup>c</sup>	6.0 <sup>de</sup>	0.2 <sup>ij</sup>	2.2 <sup>ijklm</sup>	2.6 <sup>efghijklm</sup>	28.4 <sup>opqr</sup>	19.8 <sup>fgh</sup>	19.8 <sup>p</sup>	2.5 <sup>kl</sup>	4.1 <sup>nop</sup>	0.0 <sup>g</sup>
20_Gen	0.6 <sup>hi</sup>	1.1 <sup>defghi</sup>	10.4 <sup>b</sup>	0.0 <sup>f</sup>	19.1 <sup>ghij</sup>	3.3 <sup>fgh</sup>	6.1 <sup>de</sup>	0.2 <sup>ij</sup>	4.5 <sup>cdef</sup>	3.1 <sup>cdefghij</sup>	31.6 <sup>defghi</sup>	20.0 <sup>efgh</sup>	34.8 <sup>hij</sup>	6.9 <sup>ab</sup>	5.8 <sup>klmn</sup>	4.3 <sup>ab</sup>
21_Gen	1.2 <sup>defghi</sup>	0.2 <sup>hi</sup>	2.8 <sup>hijk</sup>	0.0 <sup>f</sup>	17.4 <sup>ijklm</sup>	2.4 <sup>hijk</sup>	1.0 <sup>kl</sup>	0.2 <sup>ij</sup>	2.8 <sup>fghijkl</sup>	1.2 <sup>klmn</sup>	29.7 <sup>klmnopq</sup>	22.5 <sup>abc</sup>	37.3 <sup>bcdef</sup>	6.9 <sup>ab</sup>	7.9 <sup>hij</sup>	2.2 <sup>d</sup>
22_Gen	3.1 <sup>abc</sup>	1.6 <sup>cdefg</sup>	3.1 <sup>ghij</sup>	0.0 <sup>f</sup>	18.6 <sup>hijk</sup>	2.5 <sup>hij</sup>	2.3 <sup>hij</sup>	0.8 <sup>ghij</sup>	1.7 <sup>klm</sup>	1.8 <sup>hijklmn</sup>	30.7 <sup>fghijklm</sup>	20.2 <sup>defg</sup>	35.0 <sup>fghij</sup>	4.4 <sup>fghij</sup>	7.3 <sup>hijkl</sup>	0.9 <sup>efg</sup>
23_Gen	4.1 <sup>a</sup>	1.2 <sup>defghi</sup>	2.0 <sup>hijkl</sup>	0.0 <sup>f</sup>	17.7 <sup>ijklm</sup>	2.5 <sup>hij</sup>	0.6 <sup>kl</sup>	0.3 <sup>hij</sup>	2.9 <sup>fghijkl</sup>	3.1 <sup>cdefghi</sup>	29.8 <sup>ijklmnop</sup>	21.3 <sup>bcdef</sup>	36.9 <sup>cdefgh</sup>	5.3 <sup>abcdefg</sup>	10.8 <sup>cde</sup>	0.7 <sup>efg</sup>
24_Gen	2.5 <sup>abcdefg</sup>	0.6 <sup>efghi</sup>	2.4 <sup>hijkl</sup>	0.0 <sup>f</sup>	16.3 <sup>lmno</sup>	1.2 <sup>ijkl</sup>	1.1 <sup>kl</sup>	0.0 <sup>l</sup>	3.4 <sup>fghi</sup>	1.5 <sup>ijklmn</sup>	30.3 <sup>ghijklmn</sup>	21.2 <sup>bcdef</sup>	36.4 <sup>defghi</sup>	5.5 <sup>abcdefg</sup>	8.3 <sup>ghij</sup>	2.1 <sup>d</sup>
25_Gen	1.3 <sup>defghi</sup>	0.6 <sup>efghi</sup>	16.8 <sup>a</sup>	0.0 <sup>f</sup>	22.6 <sup>bcde</sup>	5.1 <sup>e</sup>	14.3 <sup>a</sup>	0.5 <sup>hij</sup>	7.8 <sup>b</sup>	7.4 <sup>b</sup>	35.5 <sup>a</sup>	17.1 <sup>ij</sup>	25.8 <sup>m</sup>	5.2 <sup>cdefghi</sup>	3.0 <sup>op</sup>	3.5 <sup>bc</sup>
26_Gen	3.2 <sup>ab</sup>	0.6 <sup>defghi</sup>	6.4 <sup>de</sup>	0.0 <sup>f</sup>	23.2 <sup>bcd</sup>	7.6 <sup>d</sup>	4.9 <sup>ef</sup>	1.9 <sup>de</sup>	4.5 <sup>defg</sup>	2.8 <sup>defghijk</sup>	32.4 <sup>cdef</sup>	19.0 <sup>gh</sup>	33.7 <sup>jk</sup>	5.6 <sup>abcdefg</sup>	7.2 <sup>hijkl</sup>	0.7 <sup>efg</sup>
27_Gen	1.3 <sup>defghi</sup>	0.9 <sup>defghi</sup>	2.6 <sup>hijkl</sup>	0.7 <sup>d</sup>	19.8 <sup>fgh</sup>	3.0 <sup>fgh</sup>	3.8 <sup>fgh</sup>	1.7 <sup>def</sup>	4.1 <sup>efghi</sup>	4.0 <sup>cdef</sup>	31.7 <sup>defgh</sup>	20.2 <sup>defg</sup>	34.5 <sup>hij</sup>	7.0 <sup>a</sup>	7.3 <sup>hijkl</sup>	0.3 <sup>fg</sup>
28_Gen	0.9 <sup>fghi</sup>	0.0 <sup>i</sup>	4.8 <sup>efg</sup>	1.3 <sup>c</sup>	20.8 <sup>efg</sup>	8.5 <sup>d</sup>	3.5 <sup>fghi</sup>	3.4 <sup>c</sup>	6.7 <sup>bc</sup>	3.9 <sup>cdefg</sup>	31.9 <sup>cdefg</sup>	18.8 <sup>gh</sup>	31.5 <sup>kl</sup>	5.3 <sup>abcdefg</sup>	7.6 <sup>hijk</sup>	0.7 <sup>efg</sup>
29_Int	0.9 <sup>fghi</sup>	1.0 <sup>defghi</sup>	1.8 <sup>hijkl</sup>	0.2 <sup>ef</sup>	16.9 <sup>klmn</sup>	0.9 <sup>kl</sup>	0.3 <sup>kl</sup>	0.5 <sup>hij</sup>	0.6 <sup>m</sup>	1.5 <sup>ijklmn</sup>	28.8 <sup>nopqr</sup>	23.1 <sup>a</sup>	39.5 <sup>ab</sup>	5.6 <sup>abcdefg</sup>	16.6 <sup>a</sup>	0.3 <sup>fg</sup>
30_Int	1.8 <sup>bcdefghi</sup>	0.6 <sup>defghi</sup>	2.1 <sup>hijkl</sup>	0.0 <sup>f</sup>	16.0 <sup>lmno</sup>	0.6 <sup>l</sup>	0.2 <sup>kl</sup>	0.0 <sup>l</sup>	1.7 <sup>klm</sup>	1.3 <sup>ijklmn</sup>	29.3 <sup>lmnopqr</sup>	22.1 <sup>abc</sup>	38.3 <sup>abcd</sup>	3.6 <sup>jk</sup>	10.6 <sup>cdef</sup>	0.5 <sup>fg</sup>
31_Int	2.5 <sup>abcdefg</sup>	1.8 <sup>cdef</sup>	1.1 <sup>kl</sup>	0.1 <sup>ef</sup>	17.3 <sup>ijklm</sup>	0.0 <sup>l</sup>	0.6 <sup>kl</sup>	0.6 <sup>ghij</sup>	2.6 <sup>fghijklm</sup>	2.8 <sup>efghijkl</sup>	31.5 <sup>defghij</sup>	21.0 <sup>cdef</sup>	35.2 <sup>efghij</sup>	3.3 <sup>k</sup>	10.1 <sup>defg</sup>	0.0 <sup>g</sup>
32_Int	1.3 <sup>defghi</sup>	0.7 <sup>defghi</sup>	1.1 <sup>kl</sup>	0.0 <sup>f</sup>	16.1 <sup>lmno</sup>	1.3 <sup>ijkl</sup>	0.0 <sup>l</sup>	0.0 <sup>l</sup>	0.9 <sup>lm</sup>	0.4 <sup>n</sup>	28.2 <sup>pqr</sup>	21.9 <sup>abcd</sup>	37.2 <sup>bcdefg</sup>	3.9 <sup>hijk</sup>	9.0 <sup>efgh</sup>	0.0 <sup>g</sup>
33_Int	1.6 <sup>bcdefghi</sup>	0.2 <sup>hi</sup>	1.6 <sup>hijkl</sup>	0.0 <sup>f</sup>	15.8 <sup>mno</sup>	0.9 <sup>kl</sup>	0.3 <sup>kl</sup>	0.3 <sup>hij</sup>	0.6 <sup>m</sup>	1.0 <sup>lmn</sup>	28.8 <sup>mno</sup>	21.5 <sup>abcde</sup>	36.4 <sup>defghi</sup>	3.2 <sup>k</sup>	8.7 <sup>gh</sup>	0.0 <sup>g</sup>
34_Int	1.4 <sup>cdefghi</sup>	0.4 <sup>ghi</sup>	1.7 <sup>hijkl</sup>	0.0 <sup>f</sup>	16.2 <sup>lmno</sup>	0.9 <sup>ijkl</sup>	0.0 <sup>l</sup>	0.0 <sup>l</sup>	2.6 <sup>fghijklm</sup>	1.7 <sup>hijklmn</sup>	29.5 <sup>lmnopq</sup>	22.7 <sup>ab</sup>	39.6 <sup>ab</sup>	5.9 <sup>abcdef</sup>	13.9 <sup>b</sup>	0.2 <sup>g</sup>
35_Int	1.0 <sup>fghi</sup>	0.8 <sup>defghi</sup>	3.4 <sup>gh</sup>	0.5 <sup>de</sup>	20.8 <sup>efg</sup>	8.2 <sup>d</sup>	2.9 <sup>ghi</sup>	2.3 <sup>d</sup>	6.6 <sup>bcd</sup>	3.3 <sup>cdefgh</sup>	32.4 <sup>cdef</sup>	18.5 <sup>hi</sup>	32.0 <sup>kl</sup>	5.6 <sup>abcdefg</sup>	7.3 <sup>hijkl</sup>	0.3 <sup>fg</sup>
36_Int	2.6 <sup>abcdef</sup>	0.2 <sup>hi</sup>	9.6 <sup>bc</sup>	0.6 <sup>d</sup>	25.8 <sup>a</sup>	19.2 <sup>a</sup>	10.2 <sup>b</sup>	3.4 <sup>c</sup>	6.5 <sup>bcd</sup>	7.3 <sup>b</sup>	34.8 <sup>ab</sup>	15.2 <sup>k</sup>	26.7 <sup>m</sup>	5.1 <sup>cdefghi</sup>	2.7 <sup>op</sup>	0.9 <sup>efg</sup>

\*means that have not the same letter are significantly different ( $p < 0.05$ )



**Table B1.3** Attribute intensities from DSA of the commercial sample set (n = 36).

SAMPLE CODE	Raisin flavour	Woody flavour	Pine flavour	Sweet spice flavour	Nutty flavour	Dusty flavour	Medicinal flavour	Burnt caramel flavour	Rotting plant water flavour	Hay/Dried grass flavour	Green grass flavour	Cooked vegetables flavour	Smoky flavour	Overall character on palate	Infusion strength
1_H/Gen	0.6 <sup>cdefgh</sup>	46.0 <sup>abcdef</sup>	0.4 <sup>efg</sup>	4.4 <sup>bcde</sup>	4.8 <sup>ab</sup>	1.2 <sup>efghi</sup>	1.1 <sup>def</sup>	1.6 <sup>ijklmn</sup>	0.0 <sup>e</sup>	16.5 <sup>no</sup>	0.3 <sup>l</sup>	0.0 <sup>m</sup>	0.0 <sup>l</sup>	58.7 <sup>a</sup>	30.6 <sup>pq</sup>
2_H/Int	1.3 <sup>bc</sup>	47.6 <sup>a</sup>	0.6 <sup>defg</sup>	4.8 <sup>abcd</sup>	4.3 <sup>abcd</sup>	1.3 <sup>cdefghi</sup>	1.3 <sup>cde</sup>	0.5 <sup>klmn</sup>	0.0 <sup>e</sup>	17.1 <sup>mno</sup>	1.1 <sup>kl</sup>	0.0 <sup>m</sup>	0.0 <sup>l</sup>	57.1 <sup>abcd</sup>	32.2 <sup>op</sup>
3_H/Int	1.3 <sup>bc</sup>	46.4 <sup>abcd</sup>	0.0 <sup>g</sup>	4.9 <sup>abc</sup>	3.6 <sup>cdefg</sup>	0.9 <sup>ghi</sup>	0.0 <sup>f</sup>	0.2 <sup>mn</sup>	0.0 <sup>e</sup>	17.2 <sup>lmno</sup>	0.8 <sup>kl</sup>	0.3 <sup>m</sup>	0.0 <sup>l</sup>	59.0 <sup>a</sup>	41.1 <sup>ijk</sup>
4_H/Int	1.4 <sup>b</sup>	47.4 <sup>ab</sup>	0.6 <sup>defg</sup>	5.0 <sup>ab</sup>	4.5 <sup>abc</sup>	1.2 <sup>defghi</sup>	0.3 <sup>ef</sup>	0.3 <sup>lmn</sup>	0.0 <sup>e</sup>	16.7 <sup>no</sup>	0.3 <sup>l</sup>	0.3 <sup>m</sup>	0.0 <sup>l</sup>	58.7 <sup>a</sup>	40.0 <sup>kl</sup>
5_M/Int	0.8 <sup>bcdefg</sup>	44.4 <sup>defgh</sup>	1.1 <sup>cdef</sup>	2.8 <sup>ghijkl</sup>	3.6 <sup>cdefg</sup>	2.1 <sup>abcdefgh</sup>	0.0 <sup>f</sup>	2.0 <sup>ghijkl</sup>	0.0 <sup>e</sup>	18.3 <sup>klmn</sup>	1.6 <sup>ijkl</sup>	0.0 <sup>m</sup>	0.0 <sup>l</sup>	53.1 <sup>cdef</sup>	38.2 <sup>klmn</sup>
6_M/Int	1.2 <sup>bcd</sup>	46.2 <sup>abcde</sup>	0.2 <sup>fg</sup>	5.1 <sup>ab</sup>	5.4 <sup>a</sup>	1.4 <sup>cdefghi</sup>	1.3 <sup>de</sup>	0.4 <sup>klmn</sup>	0.0 <sup>e</sup>	16.8 <sup>no</sup>	0.8 <sup>kl</sup>	0.3 <sup>m</sup>	0.7 <sup>fgh</sup>	48.4 <sup>ghi</sup>	32.2 <sup>op</sup>
7_M/Gen	0.6 <sup>cdefgh</sup>	46.2 <sup>abcde</sup>	1.3 <sup>cde</sup>	2.0 <sup>klm</sup>	1.8 <sup>ijklmno</sup>	1.5 <sup>bcdefghi</sup>	0.3 <sup>ef</sup>	3.8 <sup>ef</sup>	0.0 <sup>e</sup>	19.6 <sup>ghijk</sup>	1.9 <sup>ijkl</sup>	2.1 <sup>ghijk</sup>	0.0 <sup>l</sup>	51.4 <sup>efg</sup>	43.6 <sup>ghi</sup>
8_M/Gen	0.3 <sup>fgh</sup>	43.8 <sup>fghi</sup>	0.3 <sup>fg</sup>	1.8 <sup>klmn</sup>	1.6 <sup>klmno</sup>	1.5 <sup>bcdefghi</sup>	1.1 <sup>def</sup>	1.9 <sup>hijkl</sup>	0.2 <sup>e</sup>	19.3 <sup>ijk</sup>	1.4 <sup>kl</sup>	2.4 <sup>ghij</sup>	0.0 <sup>l</sup>	50.5 <sup>efgh</sup>	42.3 <sup>hij</sup>
9_M/Gen	0.3 <sup>fgh</sup>	45.6 <sup>abcdef</sup>	0.0 <sup>g</sup>	2.8 <sup>ghijkl</sup>	1.9 <sup>ijklmno</sup>	1.1 <sup>fghi</sup>	1.0 <sup>def</sup>	1.8 <sup>hijklm</sup>	0.0 <sup>e</sup>	19.5 <sup>hijk</sup>	1.6 <sup>kl</sup>	1.4 <sup>ijklm</sup>	0.3 <sup>hi</sup>	51.2 <sup>efg</sup>	38.3 <sup>klm</sup>
10_L/Sub	0.9 <sup>bcdef</sup>	46.7 <sup>abc</sup>	2.7 <sup>b</sup>	3.3 <sup>efghij</sup>	1.9 <sup>ijklmno</sup>	1.1 <sup>fghi</sup>	0.9 <sup>def</sup>	4.9 <sup>e</sup>	0.7 <sup>d</sup>	21.0 <sup>defghi</sup>	4.2 <sup>fgh</sup>	3.0 <sup>fgh</sup>	2.9 <sup>b</sup>	44.6 <sup>ij</sup>	52.8 <sup>cd</sup>
11_L/Int	0.0 <sup>h</sup>	39.3 <sup>k</sup>	1.5 <sup>cd</sup>	0.6 <sup>o</sup>	1.0 <sup>o</sup>	0.6 <sup>hi</sup>	1.5 <sup>cd</sup>	3.6 <sup>efg</sup>	10.8 <sup>a</sup>	24.1 <sup>ab</sup>	10.7 <sup>b</sup>	7.4 <sup>c</sup>	1.0 <sup>efg</sup>	27.6 <sup>mno</sup>	62.0 <sup>b</sup>
12_L/Gen	0.0 <sup>h</sup>	45.7 <sup>abcdef</sup>	1.3 <sup>cde</sup>	1.7 <sup>lmno</sup>	2.5 <sup>ghijkl</sup>	1.4 <sup>cdefghi</sup>	0.8 <sup>def</sup>	6.9 <sup>cd</sup>	0.0 <sup>e</sup>	21.5 <sup>cdefg</sup>	3.9 <sup>gh</sup>	5.5 <sup>de</sup>	1.4 <sup>de</sup>	44.7 <sup>ij</sup>	48.0 <sup>ef</sup>
13_L/Gen	0.9 <sup>bcdef</sup>	42.8 <sup>hij</sup>	0.5 <sup>defg</sup>	0.5 <sup>o</sup>	1.3 <sup>mno</sup>	0.9 <sup>ghi</sup>	0.6 <sup>def</sup>	13.0 <sup>b</sup>	0.2 <sup>e</sup>	23.2 <sup>bc</sup>	6.1 <sup>cde</sup>	14.2 <sup>a</sup>	0.0 <sup>l</sup>	29.4 <sup>mn</sup>	47.7 <sup>ef</sup>
14_L/Int	0.1 <sup>gh</sup>	43.1 <sup>ghij</sup>	3.2 <sup>ab</sup>	3.6 <sup>defgh</sup>	2.4 <sup>hijklm</sup>	1.3 <sup>cdefghi</sup>	1.2 <sup>def</sup>	3.3 <sup>efgh</sup>	1.5 <sup>b</sup>	20.7 <sup>efghij</sup>	4.2 <sup>fgh</sup>	6.1 <sup>cd</sup>	0.6 <sup>gh</sup>	40.0 <sup>kl</sup>	49.5 <sup>e</sup>
15_P/Int	0.7 <sup>cdefgh</sup>	44.0 <sup>efghi</sup>	0.0 <sup>g</sup>	1.8 <sup>klmn</sup>	2.7 <sup>fghijk</sup>	1.3 <sup>cdefghi</sup>	13.3 <sup>b</sup>	1.5 <sup>ijklmn</sup>	0.0 <sup>e</sup>	21.5 <sup>cdef</sup>	1.4 <sup>kl</sup>	1.4 <sup>ijklm</sup>	4.7 <sup>a</sup>	37.9 <sup>l</sup>	30.6 <sup>pq</sup>
16_P/Int	0.6 <sup>defgh</sup>	44.5 <sup>cdefgh</sup>	0.0 <sup>g</sup>	2.9 <sup>ghijk</sup>	2.9 <sup>efghij</sup>	1.7 <sup>bcdefghi</sup>	16.8 <sup>a</sup>	3.8 <sup>ef</sup>	0.0 <sup>e</sup>	22.8 <sup>bcd</sup>	1.4 <sup>kl</sup>	2.7 <sup>ghi</sup>	4.1 <sup>a</sup>	23.7 <sup>o</sup>	30.9 <sup>pq</sup>
17_P/Sub	0.0 <sup>h</sup>	30.1 <sup>m</sup>	0.0 <sup>g</sup>	3.7 <sup>defgh</sup>	4.4 <sup>abcd</sup>	2.7 <sup>abcde</sup>	0.0 <sup>f</sup>	0.3 <sup>lmn</sup>	0.0 <sup>e</sup>	19.4 <sup>ijk</sup>	3.9 <sup>gh</sup>	1.9 <sup>hijkl</sup>	0.0 <sup>l</sup>	30.0 <sup>m</sup>	13.4 <sup>s</sup>
18_P/Sub	0.0 <sup>h</sup>	28.5 <sup>m</sup>	0.3 <sup>fg</sup>	2.9 <sup>fghijk</sup>	4.5 <sup>abc</sup>	0.6 <sup>hi</sup>	0.0 <sup>f</sup>	0.4 <sup>klmn</sup>	0.0 <sup>e</sup>	19.1 <sup>kl</sup>	5.7 <sup>def</sup>	3.2 <sup>fgh</sup>	0.0 <sup>l</sup>	23.4 <sup>o</sup>	12.7 <sup>s</sup>
19_Sub	0.0 <sup>h</sup>	33.4 <sup>l</sup>	0.0 <sup>g</sup>	1.6 <sup>mno</sup>	3.3 <sup>defgh</sup>	0.8 <sup>ghi</sup>	0.3 <sup>ef</sup>	1.3 <sup>ijklmn</sup>	0.2 <sup>e</sup>	19.4 <sup>ijk</sup>	7.4 <sup>c</sup>	4.1 <sup>ef</sup>	0.0 <sup>l</sup>	23.8 <sup>o</sup>	17.8 <sup>t</sup>
20_Gen	0.2 <sup>fgh</sup>	44.3 <sup>defgh</sup>	0.0 <sup>g</sup>	2.0 <sup>klm</sup>	1.5 <sup>lmno</sup>	0.3 <sup>i</sup>	0.0 <sup>f</sup>	6.8 <sup>d</sup>	0.0 <sup>e</sup>	21.2 <sup>defgh</sup>	3.1 <sup>ghij</sup>	5.7 <sup>d</sup>	0.0 <sup>l</sup>	45.3 <sup>ij</sup>	44.4 <sup>gh</sup>
21_Gen	0.9 <sup>bcdef</sup>	45.8 <sup>abcdef</sup>	0.3 <sup>fg</sup>	2.3 <sup>ijklm</sup>	2.3 <sup>hijklmn</sup>	0.8 <sup>ghi</sup>	0.3 <sup>ef</sup>	1.4 <sup>ijklmn</sup>	0.0 <sup>e</sup>	18.7 <sup>klm</sup>	2.0 <sup>ijkl</sup>	1.2 <sup>klm</sup>	0.0 <sup>l</sup>	56.0 <sup>abcd</sup>	37.5 <sup>lmn</sup>
22_Gen	0.3 <sup>fgh</sup>	44.7 <sup>cdefgh</sup>	0.7 <sup>cdefg</sup>	2.5 <sup>ijklm</sup>	1.7 <sup>klmno</sup>	3.5 <sup>a</sup>	0.6 <sup>def</sup>	2.7 <sup>fghi</sup>	0.2 <sup>e</sup>	20.0 <sup>fghijk</sup>	1.5 <sup>kl</sup>	3.1 <sup>fgh</sup>	0.0 <sup>l</sup>	52.8 <sup>defg</sup>	38.4 <sup>klm</sup>
23_Gen	0.3 <sup>fgh</sup>	46.4 <sup>abcd</sup>	1.1 <sup>cdef</sup>	4.0 <sup>bcdef</sup>	2.9 <sup>efghi</sup>	2.9 <sup>abc</sup>	0.8 <sup>def</sup>	1.9 <sup>hijk</sup>	0.0 <sup>e</sup>	19.5 <sup>hijk</sup>	2.6 <sup>hijk</sup>	0.2 <sup>m</sup>	0.0 <sup>l</sup>	54.1 <sup>bcdef</sup>	46.5 <sup>fg</sup>
24_Gen	0.8 <sup>bcdefg</sup>	45.4 <sup>abcdefg</sup>	0.0 <sup>g</sup>	2.7 <sup>hijklm</sup>	1.8 <sup>ijklmno</sup>	2.3 <sup>abcdefg</sup>	0.5 <sup>def</sup>	1.5 <sup>ijklmn</sup>	0.0 <sup>e</sup>	17.2 <sup>lmno</sup>	1.2 <sup>kl</sup>	1.2 <sup>klm</sup>	0.0 <sup>l</sup>	54.5 <sup>abcdef</sup>	38.9 <sup>klm</sup>
25_Gen	0.0 <sup>h</sup>	41.8 <sup>ij</sup>	0.6 <sup>defg</sup>	0.8 <sup>no</sup>	1.3 <sup>mno</sup>	0.6 <sup>hi</sup>	0.5 <sup>def</sup>	14.7 <sup>a</sup>	0.0 <sup>e</sup>	24.1 <sup>ab</sup>	4.4 <sup>efg</sup>	13.1 <sup>a</sup>	0.3 <sup>hi</sup>	24.9 <sup>no</sup>	44.0 <sup>ghi</sup>
26_Gen	1.1 <sup>bcd</sup>	44.4 <sup>defgh</sup>	1.6 <sup>c</sup>	5.8 <sup>a</sup>	2.0 <sup>ijklmno</sup>	2.0 <sup>abcdefgh</sup>	0.0 <sup>f</sup>	2.4 <sup>fghij</sup>	0.0 <sup>e</sup>	21.9 <sup>cde</sup>	3.6 <sup>ghi</sup>	3.2 <sup>fgh</sup>	1.2 <sup>ef</sup>	43.4 <sup>jk</sup>	49.9 <sup>de</sup>
27_Gen	0.0 <sup>h</sup>	45.4 <sup>abcdef</sup>	1.4 <sup>cd</sup>	3.6 <sup>efgh</sup>	2.6 <sup>fghijk</sup>	2.0 <sup>abcdefgh</sup>	1.4 <sup>cde</sup>	2.6 <sup>fghij</sup>	0.8 <sup>d</sup>	21.2 <sup>defghi</sup>	4.2 <sup>fgh</sup>	3.6 <sup>fg</sup>	1.4 <sup>de</sup>	46.0 <sup>hij</sup>	55.2 <sup>c</sup>
28_Gen	0.3 <sup>efgh</sup>	45.9 <sup>abcdef</sup>	4.1 <sup>a</sup>	3.5 <sup>efghi</sup>	2.3 <sup>hijklmn</sup>	1.5 <sup>cdefghi</sup>	0.3 <sup>ef</sup>	1.9 <sup>hijk</sup>	0.7 <sup>d</sup>	22.3 <sup>bcd</sup>	6.0 <sup>cde</sup>	2.7 <sup>fghi</sup>	1.9 <sup>cd</sup>	45.1 <sup>ij</sup>	65.2 <sup>b</sup>
29_Int	1.5 <sup>b</sup>	46.7 <sup>abc</sup>	0.3 <sup>fg</sup>	3.5 <sup>efghi</sup>	5.2 <sup>a</sup>	1.8 <sup>bcdefghi</sup>	1.2 <sup>de</sup>	0.5 <sup>klmn</sup>	0.0 <sup>e</sup>	16.4 <sup>o</sup>	0.9 <sup>kl</sup>	0.6 <sup>lm</sup>	0.2 <sup>hi</sup>	57.4 <sup>abc</sup>	31.3 <sup>pq</sup>
30_Int	1.0 <sup>bcde</sup>	45.3 <sup>bcdefg</sup>	0.0 <sup>g</sup>	3.8 <sup>cdefgh</sup>	2.6 <sup>fghijkl</sup>	1.8 <sup>bcdefghi</sup>	0.6 <sup>def</sup>	1.1 <sup>ijklmn</sup>	0.0 <sup>e</sup>	17.1 <sup>mno</sup>	1.0 <sup>kl</sup>	0.5 <sup>lm</sup>	0.0 <sup>l</sup>	57.6 <sup>abc</sup>	43.1 <sup>hij</sup>
31_Int	1.0 <sup>bcdef</sup>	46.2 <sup>abcde</sup>	0.6 <sup>cdefg</sup>	3.8 <sup>cdefgh</sup>	4.0 <sup>bcde</sup>	2.8 <sup>abcd</sup>	2.5 <sup>c</sup>	1.1 <sup>ijklmn</sup>	0.0 <sup>e</sup>	18.9 <sup>klm</sup>	1.4 <sup>kl</sup>	1.1 <sup>klm</sup>	0.7 <sup>fgh</sup>	50.1 <sup>fgh</sup>	28.7 <sup>q</sup>
32_Int	0.9 <sup>bcdef</sup>	46.0 <sup>abcde</sup>	0.7 <sup>cdefg</sup>	4.1 <sup>bcde</sup>	3.7 <sup>bcdef</sup>	3.1 <sup>ab</sup>	0.7 <sup>def</sup>	0.2 <sup>mn</sup>	0.0 <sup>e</sup>	17.4 <sup>lmno</sup>	1.2 <sup>kl</sup>	0.8 <sup>klm</sup>	0.3 <sup>hi</sup>	56.7 <sup>abcd</sup>	35.0 <sup>no</sup>
33_Int	1.0 <sup>bcdef</sup>	45.7 <sup>abcdef</sup>	0.3 <sup>fg</sup>	4.3 <sup>bcde</sup>	4.0 <sup>bcde</sup>	2.7 <sup>abcdef</sup>	1.0 <sup>def</sup>	0.1 <sup>n</sup>	0.0 <sup>e</sup>	16.4 <sup>no</sup>	0.5 <sup>l</sup>	0.3 <sup>m</sup>	0.0 <sup>l</sup>	54.8 <sup>abcde</sup>	36.8 <sup>mn</sup>
34_Int	2.2 <sup>a</sup>	46.9 <sup>ab</sup>	0.5 <sup>defg</sup>	5.8 <sup>a</sup>	3.3 <sup>defgh</sup>	0.5 <sup>hi</sup>	0.7 <sup>def</sup>	0.5 <sup>klmn</sup>	0.0 <sup>e</sup>	17.4 <sup>lmno</sup>	0.8 <sup>l</sup>	0.0 <sup>m</sup>	0.0 <sup>l</sup>	58.2 <sup>ab</sup>	40.1 <sup>ijkl</sup>
35_Int	0.0 <sup>h</sup>	45.7 <sup>abcdef</sup>	3.5 <sup>ab</sup>	3.9 <sup>cdefg</sup>	1.5 <sup>lmno</sup>	1.7 <sup>bcdefghi</sup>	0.9 <sup>def</sup>	2.3 <sup>fghij</sup>	0.6 <sup>d</sup>	21.6 <sup>cdef</sup>	7.0 <sup>cd</sup>	2.9 <sup>fgh</sup>	2.1 <sup>c</sup>	45.2 <sup>ij</sup>	50.8 <sup>de</sup>
36_Int	0.0 <sup>h</sup>	41.3 <sup>k</sup>	0.8 <sup>cdefg</sup>	2.7 <sup>hijklm</sup>	1.2 <sup>no</sup>	1.7 <sup>bcdefghi</sup>	0.0 <sup>f</sup>	8.7 <sup>c</sup>	1.2 <sup>c</sup>	25.2 <sup>a</sup>	17.5 <sup>a</sup>	10.2 <sup>b</sup>	2.4 <sup>bc</sup>	27.5 <sup>mno</sup>	78.2 <sup>a</sup>

\*means that have not the same letter are significantly different ( $p < 0.05$ )



**Table B1.4** CIEL\*a\*b\* colour space and turbidity (NTU) values of the commercial sample set (n = 36).

SAMPLE CODE	L*	a*	b*	C	h	NTU
1_H/Gen	88.23	6.65	29.22	29.97	77.19	17.17
2_H/Int	88.82	6.93	26.52	27.41	75.35	12.60
3_H/Int	81.86	14.61	42.10	44.57	70.85	24.93
4_H/Int	83.96	11.62	36.50	38.31	72.35	24.00
5_M/Int	84.74	10.68	37.20	38.70	73.99	27.83
6_M/Int	88.38	7.56	28.92	29.89	75.36	11.33
7_M/Gen	82.44	11.72	40.33	42.00	73.80	46.53
8_M/Gen	80.53	11.04	44.47	45.81	76.06	71.33
9_M/Gen	79.86	10.23	46.27	47.39	77.54	87.40
10_L/Sub	82.42	11.30	36.90	38.59	72.98	144.67
11_L/Int	77.88	17.01	50.08	52.89	71.24	300.33
12_L/Gen	76.92	15.11	53.32	55.41	74.18	91.90
13_L/Gen	77.75	14.71	52.89	54.89	74.46	124.33
14_L/Int	83.87	10.67	35.58	37.15	73.32	166.00
15_P/Int	88.85	6.94	27.75	28.61	75.96	13.67
16_P/Int	88.36	7.58	28.66	29.64	75.19	13.50
17_P/Sub	96.28	0.37	11.07	11.08	88.07	9.87
18_P/Sub	96.48	0.57	10.30	10.32	86.85	7.51
19_Sub	95.19	1.58	13.19	13.28	83.15	7.21
20_Gen	78.92	14.85	51.30	53.41	73.85	52.00
21_Gen	84.26	9.31	37.51	38.65	76.06	31.40
22_Gen	82.64	11.11	42.35	43.78	75.29	39.77
23_Gen	77.43	15.14	51.35	53.54	73.58	69.47
24_Gen	83.81	9.80	38.16	39.39	75.60	32.57
25_Gen	78.54	13.40	51.57	53.28	75.44	94.43
26_Gen	83.91	9.95	33.71	35.14	73.55	161.33
27_Gen	79.88	13.28	39.20	41.39	71.29	131.33
28_Gen	77.95	16.04	39.49	42.62	67.89	243.67
29_Int	88.93	6.23	27.14	27.85	77.07	26.30
30_Int	81.14	14.86	42.90	45.40	70.91	27.23
31_Int	91.05	4.76	22.49	22.99	78.05	15.23
32_Int	86.11	9.42	34.23	35.50	74.62	22.03
33_Int	84.88	10.30	36.36	37.78	74.19	27.50
34_Int	83.57	12.56	37.96	39.99	71.69	28.90
35_Int	81.89	12.82	39.21	41.25	71.89	200.33
36_Int	73.01	20.12	50.51	54.37	68.29	481.33

# Chapter 5

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## Polarised sensory positioning and polarised projective mapping: application as rapid sensory quality classification tools

### Abstract

The efficacy of rapid sensory profiling methods to classify honeybush (*Cyclopia* spp.) tea according to sensory quality was investigated. The present study evaluated two reference-based rapid sensory profiling methods in which products are compared to a fixed set of reference samples or 'poles', namely polarised sensory positioning (PSP) and an extension thereof, polarised projective mapping (PPM). An advantage of PSP and PPM for quality control is that data can be aggregated over consecutive sessions for batch comparison over time. The discrimination ability of PSP and PPM to classify tea products according to high, moderate, poor and low sensory quality was evaluated. Two variations per method were compared, firstly the use of conventional *physical* poles, i.e. honeybush tea infusions (PSP-*p* and PPM-*p*), and secondly *theoretical* poles, i.e. descriptions of the key sensory attributes per sensory quality class (PSP-*t* and PPM-*t*). Descriptive sensory analysis (DSA) was performed on infusions of commercially processed batches of *C. intermedia*, *C. subternata* and *C. genistoides* of varying sensory quality. Four sensory quality classes, previously established for fermented honeybush tea, were pre-assigned to the respective samples based on their DSA data, and test samples and *physical* poles were selected accordingly. Product configurations similar to that of DSA demonstrated the validity of the method variations for broad quality classification of honeybush tea based on key sensory quality parameters. PPM-*p* indicated the highest discrimination ability. RV coefficients of  $\geq 0.7$  substantiated acceptable agreement between the sample configurations of the methods. Samples were classified into 'moderate to high', 'low' and 'poor' quality groupings. Indistinct classification for high and moderate sensory quality classes could be ascribed to overlapping of positive sensory attributes associated with the *physical* and *theoretical* poles. PPM-*t* was selected to test its discrimination ability by a panel of honeybush industry representatives. No distinct classification was obtained and the need for industry assessor training on honeybush sensory lexicon attributes to facilitate assessment was emphasised. Methodological factors that could influence method efficacy such as pole selection, modality, verbalisation step and assessors' expertise level were highlighted.

# 1 Introduction

Honeybush (*Cyclopia spp.*), an indigenous South African commodity, is sold on local and international markets, with the latter comprising the bulk of sales (Joubert et al., 2019; Joubert, Joubert, Bester, De Beer, & De Lange, 2011). Present export regulations only specify food safety standards in terms of microbial, agro-chemical residue and foreign matter content (DAFF, 2019) but do not make provision for sensory quality. Honeybush tea is an agricultural product with natural variation in plant material, apart from differences in *Cyclopia* species. As for commercial black tea (Alasalvar et al., 2012; Joliffe, 2003; Liang, Lu, Zhang, Wu, & Wu, 2003) and wine (Cáceres-Mella et al., 2014), honeybush tea is blended to achieve a product of consistent sensory quality. Currently, no standardised method for sensory quality assessment within the honeybush industry exists. Honeybush packing companies blend batches of varying sensory quality to supply a commercial product that is of consistent quality according to in-house specifications.

The pressing need for assessing the sensory quality of honeybush tea to limit inferior and inconsistent quality, was recently addressed through the development of a quality scoring method that encompasses a quality scorecard (Chapter 4). Although this method proved to be effective in classifying production batches of honeybush tea in terms of sensory quality, the investigation of alternative methods for the rapid screening of large sample sets is still required. Rapid methods should simultaneously screen and classify numerous production batches within a commercial quality control system of herbal tea.

In addition to the requirement for commercial use, the need for a time- and cost-efficient sensory quality classification method has also been identified for honeybush tea research. Cultivation/plant improvement research on different *Cyclopia* species was initiated by the Agricultural Research Council (ARC) to address the need for stable and sustainable sources of high quality plant material (Bester, Joubert, & Joubert, 2016; Joubert et al., 2011). The on-going ARC honeybush breeding research programme aims to assess cultivars with improved intrinsic quality and horticultural properties (Bester et al., 2016). Sensory analysis of selections and progenies form part of the assessment to ensure that the quality of the plant material, selected on horticultural traits (e.g. biomass yield), is not compromised. The high cost of descriptive sensory analysis (DSA) and the quantity of plant material required for processing into herbal tea, limits the number of selections that can be analysed (Bester et al., 2016; Robertson et al., 2018). Therefore, the need for a rapid classification tool to screen a large number of genotypes and selections in terms of sensory quality has been identified.

Rapid sensory profiling methods have become one of the most dynamic areas of sensory science research in recent years, as more time- and cost-effective alternatives to classical DSA. These rapid methods have found application within research and commercial context to determine the relative positioning of products within a sensory space (Ares, Antúnez, De Saldamando, & Giménez, 2018; Horita et al., 2017; Valentin, Chollet, Lelièvre, & Abdi, 2012). Rapid methods can be categorised into three different

groups, namely 1) verbal-based methods, e.g. flash-profile (FP) and check-all-that-apply (CATA), for the assessment of individual attributes, 2) holistic similarity-based methods, e.g. sorting and projective mapping (PM) (aka Napping®) for the assessment of global similarities and differences among samples, and 3) reference-based methods, e.g. polarised sensory positioning (PSP) and polarised projective mapping (PPM) for the comparison with product references (Ares et al., 2013; Valentin et al., 2012; Varela & Ares, 2014).

Projective mapping (PM) has become prominent to obtain a quick overview of the similarities/dissimilarities in a sample set by projecting samples onto a two-dimensional space. The comparison of samples is based on a holistic assessment as the assessor evaluates global differences, according to his/her own criteria, without any prior indication on which sensory attributes should be focussed on, or their relative importance. In PM each assessor is instructed to position samples onto a paper sheet (Valentin et al., 2012). PM is also referred to as Napping® (Pagès, 2005; Perrin & Pagès, 2009; Perrin et al., 2008), with 'nappe' meaning 'tablecloth' in French. A subsequent descriptive step, namely ultra-flash profiling (UFP), may be added in which descriptors are written on the sheet to describe the product samples (Perrin & Pagès, 2009; Perrin et al., 2008).

The comparative nature of PM requires that all samples in a test set need to be evaluated simultaneously. Therefore, conclusions derived about samples in different sessions in terms of their main sensory attributes, cannot be compared to one another (Ares et al., 2018; Hopfer & Heymann, 2013). PM also limits the size of the sample set that can be assessed within a session, especially for sensory-complex products that may affect assessors' sensory fatigue (e.g. wine), products with intense or persistent sensory attributes (e.g. chilli or distillates), or products that necessitate strict temperature-control (Ares et al., 2018). Subsequently, PSP was developed to address this disadvantage. In PSP samples are compared with a fixed set of product references, so-called 'poles', which allow assessors to quantify the overall degree of difference between a sample and each of the poles, ranging from 'exactly the same' to 'totally different' (Teillet, 2014; Teillet, Schlich, Urbano, Cordelle, & Guichard, 2010). When using triad PSP assessors are instructed to simply indicate to which pole a sample is most similar and least similar, without indicating the distance from the pole (Teillet, 2015). PSP using continuous scales has been recommended for industry application such as product development (prototype comparison) and quality control (batch control), specifically for its advantage of aggregating data of different sessions (Antúnez et al., 2015; Teillet, 2014).

Polarised projective mapping (PPM) was proposed as an extension of PSP. The proposed method combines the advantage of data aggregation over consecutive sessions of PSP and the holistic character and intuitive association of conventional PM (Ares et al., 2018, 2013). In PPM assessors firstly evaluate the respective poles that have been placed onto a two-dimensional map, and then position the test samples relative to the poles and each other. To date, only one study on the discrimination ability of PSP compared to PPM has been published (Ares et al., 2013). PPM has been applied on a limited range of products, e.g.

orange-flavoured powdered drink samples (Ares et al., 2013), low sodium frankfurter products (Horita et al., 2017) and old-vine Chenin Blanc wine (Wilson, Brand, Du Toit, & Buica, 2018). Wilson et al. (2018) used UFP as complementary descriptive task for PPM for rapid sensory characterisation of wine by industry professionals.

The selection criteria of poles is important for the implementation of reference-based rapid sensory methods (Ares et al., 2018, 2015; Teillet, 2014). The prerequisites include that poles should be stable over time, representative of one/two key sensory product characteristics per pole, representative of the complete sensory space and perceived as different from each other, at least to an intermediate degree (Ares et al., 2018; De Saldamando, Antúnez, Giménez, Varela, & Ares, 2015). In addition, the number of poles should be at least the same as the number of sensory dimensions required to present the perceptual space (Ares et al., 2018, 2015).

Recent evaluation of PM (Moelich, Muller, Joubert, Næs, & Kidd, 2017) and PSP (Moelich, 2018) to distinguish between honeybush infusions of different *Cyclophia* species, indicated the potential of these methods for quality control purposes. Furthermore, PSP with poles representative of different honeybush quality classes was recommended (Moelich, 2018). Fermented honeybush tea has a shelf-life of a minimum two years (Le Roux, Cronje, Burger, & Joubert, 2012), which further grants the potential application of reference-based methods.

Although the application of rapid sensory profiling methods by untrained, semi-trained and/or trained assessors have been indicated, studies using industry professionals, has been limited to specifically wine experts in sorting (Ballester, Patris, Symoneaux, & Valentin, 2008; Brand et al., 2018; Parr, Valentin, Green, & Dacremont, 2010) and PM (Perrin et al., 2008; Torri et al., 2013). To the authors' knowledge no studies on the application of PSP or PPM using industry professionals have been published to date. In addition, to date, only physical products and not theoretical descriptions have been used as poles in PSP and PPM tasks.

The aim of the present study was to investigate the efficacy of two referenced-based rapid characterisation methods, PSP and PPM, for classification of the sensory quality of honeybush tea. Furthermore, comparison of methods based on *physical* poles (product references) and *theoretical* poles (descriptions of poles) was investigated. DSA data were used to validate the discrimination ability of these methods. In addition, the discrimination ability of PPM with *theoretical* poles was evaluated using a panel of industry representatives.

## 2 Materials and methods

The efficacy of two reference-based rapid methods, namely PSP and PPM to classify honeybush tea according to sensory quality was assessed. For each method, two types of references, namely *physical* poles (tea infusions) and *theoretical* poles (descriptions), representative of high, moderate, low and poor sensory quality classes, was selected. The validity of both methods with variation of poles were tested using DSA data as 'gold standard'. PPM with *theoretical* poles was performed by a panel of industry professionals to evaluate this method for application in industry. The experimental lay-out is presented in **Fig. 1**.

### 2.1 Descriptive sensory analysis

Descriptive sensory analysis (DSA) was performed by a trained panel (N = 12) for the profiling of independent commercially processed honeybush tea batches (n = 36), as described in Chapter 4. Samples of *C. intermedia* (n = 17), *C. genistoides* (n = 15) and *C. subternata* (n = 4) with variable sensory attribute intensities, colour (CIEL\*a\*b\*) and turbidity (NTU) values were selected from the in-house sample library. Hot water infusions of the samples were prepared and presented in porcelain mugs (ca. 100 mL aliquots), according to a standard protocol (Erasmus, Theron, Muller, Van der Rijst, & Joubert, 2017).

As described in Chapter 4, assessors (aged 35 to 65) with several years of experience in the DSA of fermented honeybush (Bergh, Muller, Van der Rijst, & Joubert, 2017; Erasmus et al., 2017; Theron et al., 2014) were trained to recognise and score on unstructured line scales, the positive and negative attributes in the fermented honeybush tea. Additional to the 46 aroma, flavour, taste and mouthfeel descriptors, the panel was trained to score the intensity of 'infusion strength' and 'overall positive character on palate'. Assessors were trained in the sensory attributes in four 1 h sessions before testing commenced. Samples were tested in triplicate, as indicated in Chapter 4.

### 2.2 Reference-based rapid sensory methodologies

#### 2.2.1 Sample selection

A total of 18 samples were selected as test set based on the product configuration obtained through principle component analysis (PCA) of the DSA data. Sensory quality classes were assigned to these samples, namely high (n = 4), moderate (n = 5), low (n = 5) and poor (n = 4) quality (**Figs. 2 and 3**). Assigned quality classes were according to the definitions established by expert focus groups, described in Chapter 4. Two blind duplicates representing high and low quality, were included to evaluate panel intra reliability within a test replicate of the respective tasks (Hopfer & Heymann, 2013). Sample codes used for the PSP and PPM tasks are listed in **Table 1** (the same sample set and blind duplicates were used for the quality scoring method validation, described in Chapter 4). Hot water infusions of the test samples were prepared and presented according to a standard protocol (Erasmus et al., 2017).

## 2.2.2 Pole selection

### 2.2.2.1 Physical poles

*Physical* poles were selected from the samples as product references for one variation of each of the PSP and PPM tasks, respectively. Selection was based on the product configuration in the PCA bi-plot (**Fig. 2**). The respective poles were selected to represent the four quality classes (high, moderate, low and poor) within the total sensory space of honeybush quality, as defined in Chapter 4. Selection was based on the key sensory attributes that would discriminate among batches of different sensory quality, as proposed by previous research on pole selection (Ares et al., 2015; De Saldamando, Delgado, Herencia, Giménez, & Ares, 2013). The four poles were prepared from blends of three different batches of fermented honeybush tea. These batches were representative of the quality classes as defined in Chapter 4, namely 1) *high quality class* representing products with high intensities of positive aromas, flavours and ‘overall character on palate’, and the absence of taints; 2) *moderate quality class* representing products with moderate intensities of positive aromas, flavours and ‘overall character on palate’, and the absence of taints; 3) *low quality class* representing products with low intensities of positive aromas, flavours and ‘overall character on palate’, and the presence of taints, including ‘green grass’, ‘cooked vegetables’, ‘rotting plant water’, ‘burnt caramel’ and ‘dusty’ flavours; and 4) *poor quality class* representing products with an insipid ‘overall character on palate’ and high presence of taints, specifically ‘smoky’ and ‘medicinal’ flavours.

Samples selected for the four *physical* poles are indicated in **Table 1**. Hot water tea infusions of the *physical* poles were prepared according to a standard protocol (Erasmus et al., 2017). *Physical* poles were presented in white porcelain mugs with ca. 100 mL aliquots of the respective infusions. Mugs were covered with plastic lids and labelled.

### 2.2.2.2 Theoretical poles

Definitions for the respective sensory quality classes of fermented honeybush tea, as determined by the expert focus group sessions (Chapter 4), were used as *theoretical* poles. These definitions were simplified by excluding the words ‘aroma’, ‘flavour’, ‘taste’ and ‘mouthfeel’ to allow for uninstructed assessment by assessors. Assessors were free to evaluate samples intuitively and choose on which modality/modalities (aroma, flavour, taste and/or mouthfeel) and sensory attributes they would base their decision. Definitions were also further condensed for practicality in terms of digital screen space for the PSP line scales and PPM two-dimensional space on a computer screen. Definitions for the four *theoretical* poles, describing the key sensory attributes represented by each quality class, are provided in **Table 1**.



### 2.2.3 Trained assessors

A trained panel (N = 13) (the same assessors used for DSA, except for omission of one member, and the addition of two trained female members) was acquired to perform the four PSP and PPM variations, which commenced two weeks after completion of the DSA.

### 2.2.4 Polarised sensory positioning (PSP) and polarised projective mapping (PPM)

Two variations for both PSP and PPM were studied, namely 1) PSP with four *physical* poles representing each quality class (PSP-*p*), 2) PSP with four *theoretical* poles representing a definition of each quality class (PSP-*t*), 3) PPM with four *physical* poles (PPM-*p*) and 4) PPM with four *theoretical* poles (PPM-*t*). The PSP and PPM tasks were conducted in individual computerised tasting booths using EyeQuestion® software (Elt, The Netherlands) in a temperature- (21 °C) and light-controlled sensory analysis laboratory. Test samples and *physical* poles (for PSP-*p* and PPM-*p*) were kept in water baths at a constant temperature of 65°C throughout an assessment session. Two assessors were assigned to a water bath to assess one test sample set per test replicate. This would simulate the honeybush industry QC set-up in which assessors from a small industry panel would assess an infusion sample from the same single porcelain cup. Assessors were instructed to place the covered mug back in the water bath after assessing a sample or *physical* pole.

Two replicates for each variation of the PSP and PPM tasks were performed. For each replicate session of each rapid method, three-digit codes were randomly assigned to the set of samples. For all tasks, samples were assessed in a monadic sequence, in a random order, following a balanced experimental design. For both PSP and PPM tasks, test samples were placed in numerical order (i.e. from lowest to highest three-digit number) in a water bath. This allowed for easier and rapid identification of the three-digit code that corresponds to the code indicated on the computer screen.

Assessment of poles and test samples were uninstructed, i.e. assessors could assess infusions based on aroma and/or taste modalities ('taste' refers to industry terminology describing the overall/collective perception of the palate attributes, i.e. flavour, basic taste and mouthfeel attributes). Repeated evaluation of samples and poles during assessment were permitted. Assessors were informed that there exists no correct or wrong answer for the respective tasks. Unsalted water biscuits (Woolworths, Stellenbosch, South Africa) and distilled water (Stellenbosch University, Stellenbosch) were used as palate cleansers between samples. All the assessors completed each replicate of a PSP and PPM task within a one-hour period.

After completion of each variation of the PSP and PPM tasks, assessors were instructed to complete a short questionnaire on the ease of performance of a task (from 'very easy' to 'very difficult'), the modality/modalities that were assessed (aroma and/or taste), and the strategy followed during assessment (open-ended question). Assessors were instructed to indicate their preferred method (PSP-*p*, PSP-*t*, PPM-*p* and PPM-*t*) and provide a reason(s) after completion of the final task. Assessors completed the replicates of

each method variation in separate sessions over two consecutive days. PSP-*p*, PSP-*t*, PPM-*p* and PPM-*t* tasks were performed with a week in between each of the rapid method variations.

As the validity of data aggregation over consecutive sessions has been demonstrated for PSP for honeybush (Moelich, 2018) and for PPM for another sensory-complex product, Chenin Blanc wine (Wilson et al., 2018), the focus of the present study was on the efficacy of sensory quality classification, and not on that of data aggregation. Similar to the current study, Ares et al. (2013) did not study the efficacy of data aggregation on these two methods.

#### 2.2.4.1 PSP with physical poles (PSP-*p*)

Each assessor was presented with their own set of four *physical* poles in the water bath, marked with the letters Q, R, S and T printed on white labels, and additional different coloured labels, representing high, moderate, low and poor sensory quality. The PSP task was briefly explained but no re-training on honeybush sensory attributes was conducted. Certain trained assessors have been exposed to this method previously. Assessors were instructed to smell and/or taste each pole and thereafter assess the (dis)similarity of each test sample to each of the four poles on an unstructured line scale, indicating a sample to be similar to the pole (0 = 'exactly the same') or dissimilar to the pole (100 = 'totally different'). In order to avoid any ambiguity, the numbers '0' and '100' were not indicated under each line scale. Assessors were instructed to assess the first ten samples, by selecting the sample from the water bath that corresponds to the three-digit code that appeared on the computer screen, followed by a 15 min break, before the next 10 samples were assessed. An example of the digital instructions and questionnaire is provided in **Addendum C, Fig. C1**.

#### 2.2.4.2 PSP with theoretical poles (PSP-*t*)

Instructions and assessment procedure were similar as described in Section 2.2.4.1. Assessors were instructed to smell and/or taste each test sample and assess its similarity or dissimilarity on an unstructured line scale to the description of each of the theoretical poles, E, F, G and H, provided on the computer screen. An example of the digital instructions and questionnaire is provided in **Addendum C, Fig. C2**.

#### 2.2.4.3 PPM with physical poles (PPM-*p*) and ultra-flash profiling (UFP)

Each assessor was presented with their own set of four *physical* poles in the water bath, marked with the letters W, X, Y and Z printed on white labels, and additional different coloured labels, representing high, moderate, low and poor sensory quality. The relative distances between the poles were pre-determined, considering the product configuration on the first and second dimensions of the PCA bi-plot (**Fig. 2**). Following the instruction screen, a digital PPM map appeared with a fixed product configuration of the poles indicated by the letters, W, X, Y and Z. The three-digit codes of the 20 test samples and letters for the

poles were presented simultaneously on the screen, next to the PPM map. Assessors were instructed firstly to drag the letters of the respective poles onto the digital two-dimensional map and place them onto the dots next to the corresponding letters representing the poles. This was required to ensure that the software programme records the X and Y coordinates of the respective poles. Assessors were then instructed to smell and/or taste each pole, followed by assessment of each sample according to their own criteria. Assessors who shared a sample set per water bath were verbally instructed to either assess samples from the lowest to the highest three-digit codes, or *vice versa*. Assessors were instructed to place each sample on the digital two-dimensional map according to perceived similarities or dissimilarities to each pole. This was done by dragging the corresponding three-digit code onto the digital map.

UFP was performed as complimentary step to PPM-*p*, to obtain free descriptions for samples associated with the respective poles. The UFP step was performed simultaneously with the PPM task to aid in the assessor's memory of the key attributes perceived in each pole and test sample, as performed by Wilson et al. (2018). Assessors were instructed to record descriptor(s) to samples or groupings of similar samples. Each group of similar samples and where applicable pole(s) were encircled into a 'region' for which descriptor(s) had to be provided. No break was indicated as assessors were instructed to perform the task on each sample of the entire sample set, successively. The PPM and UFP tasks were briefly explained and demonstrated but no re-training on honeybush sensory attributes was conducted. An example of the digital instructions and questionnaire is provided in **Addendum C, Fig. C3**.

#### 2.2.4.4 PPM with theoretical poles (PPM-*t*)

Instructions and assessment procedure were similar as described in Section 2.2.4.3, but no *physical* poles were provided, and no UFP task was performed. The same descriptions used for PSP task with *theoretical* poles were indicated on the digital two-dimensional map under each respective *theoretical* pole, labelled Q, R, S, T, respectively. The exact same pole configuration and relative distances between poles on the digital two-dimensional map were used as for PPM-*p*. Assessors were instructed to drag firstly the letters of the respective poles onto the digital two-dimensional map and place them on the dots next to the corresponding letters. Assessors were instructed to smell and/or taste each test sample and to place each test sample on the digital two-dimensional map according to perceived similarities or dissimilarities to each pole description. This was done by dragging the corresponding three-digit code onto the map relative to the respective *theoretical* pole and sample codes. An example of the digital instructions and questionnaire is provided in **Addendum C, Fig. C4**.

### 2.3 Industry test

The panel of honeybush industry representatives (N = 14), as described in Chapter 4, was invited to perform the PPM-*t* methodology. The level of expertise in general sensory analyses practices, and sensory

quality assessment of fermented honeybush tea specifically, varied extensively within the panel. Prior to assessment, the panel participated in a short briefing session, in which they were introduced to the method using the software programme. No training on honeybush sensory attributes was provided. Assessors completed both replicates of PPM-*t* on the same day, with a 20 min break between sessions. On completion of the PPM-*t* task, assessors were instructed to complete a short questionnaire on the ease of performance of the test (from 'very easy' to 'very difficult'), the modality/modalities that were assessed (aroma and/or taste), and the approach/strategy followed during assessment.

## 2.4 Data analyses

### 2.4.1 Descriptive sensory analysis

The statistical analysis of the DSA data was previously described in Chapter 4.

### 2.4.2 Polarised sensory positioning

Data consisted of distances measured on the continuous scale representing the degree of difference between a sample and pole. Data was captured electronically using EyeQuestion® software. Data was analysed using multiple factor analysis (MFA) in which data from each assessor were regarded as a separate group of variables, to preserve individual data, as well as to compensate for individual assessor differences when rating global differences between products and poles (Teillet, 2014). The data matrix of each variation of the PSP task comprised of ratings per assessor per sample, across each of the four poles and two replicate sessions. An extract of the data matrix used for analysing PSP-*p* is presented in **Addendum C, Table C1 (I)**. MFA comprises of a two-step analysis in which firstly PCA of each set of variables is conducted, and each data table normalised, followed by PCA on the concatenated results (i.e. all normalised data tables are aggregated into one data table) to project a global configuration (Abdi, Williams, & Valentin, 2013). For the identification of significant differences among samples, confidence ellipses were constructed using parametric bootstrapping (Dehlholm, Brockhoff, & Bredie, 2012). Hierarchical cluster analysis (HCA) was conducted on the MFA data used for computing the global product configuration as an alternative method to visualise groupings (Berget, Varela, & Næs, 2019). Ward's method was used, based on minimum variance cluster analysis, and designed to generate clusters with minimum within-cluster variance (Punji & Stewart, 1983). For MFA only the first 2 or 3 dimensions were considered, whereas up to 5 dimensions were considered for HCA. Dendrograms, constructed from HCA, were used to identify groups of samples that associate with specific poles.

### 2.4.3 Polarised projective mapping

The *X* and *Y* coordinate data for each sample placement per assessor was captured electronically using EyeQuestion® software, for which the top left corner of the digital two-dimensional PPM map served as the

zero point (0,0). Data for each of the PPM tasks were analysed using MFA, as proposed by Pagès (2005) for PM. Data from each assessor were regarded as a separate group of variables. The data matrix of each variation of the PPM task comprised of *X* and *Y* coordinates per assessor per sample, across each of the four poles and two replicate sessions. An extract of the data matrix used for analysing PPM with physical poles is presented in **Addendum C, Table C1 (II)**. Confidence ellipses were calculated using parametric bootstrapping (Dehlholm, Brockhoff, & Bredie, 2012). HCA was conducted as described in Section 2.4.2.

UFP free description data from the PPM-*p* task, was captured electronically. Qualitative analysis was performed to construct a frequency table with assessors' descriptions (Ares et al., 2013). In a pre-processing step, the list of descriptors generated was condensed by grouping synonyms. The list of descriptors was condensed by firstly combining linguistic synonyms, e.g. 'smoke' and 'smoky', under a common synonym 'smoky'. The list was further condensed by combining semantic synonyms, e.g. 'veg-like', 'veggies' and 'cooked veg', together under a common synonym, 'cooked vegetables'. Subsequently, similar adjectives of the same descriptor were grouped together, e.g. 'high', 'lots of', 'strong' and 'very' were condensed to 'high' and 'lower', 'slight', 'some' and 'less' were condensed to 'low'. Descriptors cited less than 20 times were excluded for further analysis. Final descriptive data, i.e. citation frequency table of the condensed descriptors list, was considered as a set of supplementary variables and did not contribute to the construction of the MFA dimensions. Standardised residuals for these descriptors were calculated to project the association between products (test samples and poles) and descriptors on the MFA correlation plot.

#### *2.4.4 Method validation*

To assess the validity of the respective PSP and PPM tasks as rapid sensory methods for classification of honeybush sensory quality, product configurations of the MFA maps obtained were visually compared to that of the PCA bi-plot based on DSA data. Regression vector (RV) coefficients were calculated between the first two dimensions of the product configurations of PCA bi-plot and respective MFA individual factor plots. RV coefficients are multivariate similarity (correlation) coefficients that measure the similarities between two-factorial product configurations (Abdi, 2010). High similarity between respective configurations is indicated by RV coefficients closer to 1, whereas 0 indicates uncorrelated configurations. The RV coefficient depends on the relative position of the points in the product configuration and is therefore independent of rotation and translation (Robert & Escoufier, 1976). Assessor inter- and intra-reliability were assessed by comparing RV coefficients between the respective test replicates of each of the PSP and PPM tasks, and the placement of the two blind duplicates relative to their respective duplicate samples.

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). To determine whether RV coefficients

differ significantly from each other, RV coefficients with 95% confidence intervals (R package iTOP) were computed between the PCA bi-plot and MFA plots of the respective PSP and PPM tasks. Overlapping of confidence intervals is indicative of non-significant differences between RV coefficients. DSA data analyses were conducted using Statistica data analysis software system (Statistica, version 13, 2018, TIBCO Software Inc., Palo Alto, CA, USA).

## 3 Results

### 3.1 Descriptive sensory analysis

The product configuration of the honeybush samples ( $n = 36$ ) obtained from the PCA bi-plot, based on the correlation matrix, is presented in **Fig. 2**. The DSA data used for PCA are presented in Chapter 4 (**Addendum B, Table B1**). The first two components of the PCA bi-plot explained 62.58% of the variability. Samples regarded as high and moderate quality associated with positive aroma, flavour and taste attributes (including 'overall character on palate') towards the positive end of principle component 1 (PC1). DSA data indicated that high quality samples associated with higher intensities of positive attributes such as 'fynbos-floral', 'rose perfume' and 'fynbos-sweet' aromas and 'overall character on palate' compared to that of moderate quality samples. Distinction between low and poor quality samples was observed towards the negative end of PC1, for which low quality samples associated with vegetative taints and 'burnt caramel' aroma and flavour, as well as bitter and sour taste and astringent mouthfeel. Poor quality samples associated with 'smoky' and 'medicinal' aroma and flavour (specifically along PC3, as described in Chapter 4). Certain poor quality samples were also situated opposite 'overall quality on palate' and 'infusion strength' (towards the negative end of PC2), indicating the insipid sensory character of these samples.

Samples selected for the respective *physical* pole blends were based on the PCA bi-plot in **Fig. 2**. Samples selected for high, moderate, and low sensory quality poles were blended in a 1:1:1 ratio, whereas samples for the poor sensory quality pole were blended in a 5:1:1 ratio, to ensure that 'medicinal' and 'smoky' aromas and flavours were well-represented in this pole (**Table 1**). Blending of different samples per pole was required to address the variation of attributes and the intensities thereof, especially negative attributes, within a sensory quality class.

**Figure 3** presents the PCA bi-plot computed from the data of the selected test samples ( $n = 18$ ) representative of the respective four sensory quality classes. This PCA bi-plot was used for comparison to the respective product configurations obtained through MFA of the PSP and PPM tasks in method validation.

## 3.2 Polarised sensory positioning

### 3.2.1 Product configuration based on MFA

**Figure 4** presents the sample configurations in the first two dimensions of MFA performed on PSP with *physical* poles (PSP-*p*) and *theoretical* poles (PSP-*t*), respectively. The first two dimensions of MFA explained 48.0% and 47.1% of the variance in the PSP-*p* data and PSP-*t* data, respectively.

For PSP-*p* three groupings were observed, namely ‘moderate to high’, ‘low’ and ‘poor’ quality, although overlapping confidence ellipses for poor quality samples with ‘moderate to high’ and ‘low’ quality were observed (**Fig. 4A**). Two groups within low quality grouping were observed, although their confidence ellipses were overlapping. Samples 13\_L/Gen and 11\_L/Int and its blind duplicate were situated the furthest towards the positive end of dimension 1. From the PCA bi-plot of the DSA data (**Fig. 3**), 13\_L/Gen associated strongly with ‘cooked vegetables’ and ‘burnt caramel’ aroma and flavour, sour and bitter taste and astringent mouthfeel, whereas 11\_L/Int associated with vegetative taints, especially ‘green grass’ aroma and flavour. There is not a clear distinction between quality classes for PSP-*t* as clear from **Fig. 4B**. Samples representing ‘moderate to high’ quality are situated towards the negative side of dimension 1, while an overlap ‘moderate’, ‘low’ and ‘poor’ are situated more to the positive side of this dimension. Samples 15\_P/Int and 16\_P/Int, associating with ‘smoky’ and ‘medicinal’ aroma and flavour (**Fig. 3**), formed a separate group along the positive end of dimension 2, although overlapping of confidence ellipses with that of the other two poor quality samples, 17\_P/Sub and 18\_P/Sub, is indicated. Similar to PSP-*p*, sample 11\_L/Int and its blind duplicate, were positioned the furthest towards the positive end of dimension 1. Moderate quality samples, 8\_M/Gen and 9\_M/Gen, associated with the ‘low’ quality grouping. From their DSA data (Chapter 4, **Addendum B, Table B1**), slightly higher intensities in ‘hay/dried grass’ aroma and flavour, sour and bitter taste, and astringent mouthfeel, and slightly lower intensities in sweet taste were observed for these samples compared to the other moderate quality samples, which may have influenced their positioning towards the low quality grouping.

Tighter group formation usually indicates a higher degree of similar assessments among assessors. Assessors were not able to differentiate between high and moderate samples for both PSP-*p* and PSP-*t*. Although low quality samples did not form a tight group for both variations of PSP, better distinction of the low quality group was obtained for PSP-*p*.

### 3.2.2 Assessor strategy and ease of performance

Results for the questionnaire on modality used during assessment indicated that for both PSP-*p* and PSP-*t* assessors based their decisions predominantly on assessment of aroma attributes rather than on palate, i.e. flavour, taste and mouthfeel. Only 3 of 13 assessors evaluated samples on both aroma and palate. Two assessors indicated that samples were evaluated on both aroma and infusion appearance (colour) modalities. A few assessors specifically commented on the difficulty to rate samples 17\_P/Sub and



18\_P/Sub. From the PCA bi-plot of the DSA data (**Fig. 3**), these samples associated strongly with ‘nutty’ and ‘apple’ aroma but poorly with ‘overall quality on palate’ (i.e. samples were insipid) and ‘infusion strength’ (i.e. samples were light yellow in colour). Assessors therefore might have found it difficult to evaluate the quality of these samples and struggled with the placement thereof.

Results for the questionnaire on assessment strategy followed, indicated that assessors used different approaches for evaluation. Rating of test samples in PSP-*p* task was predominantly based on the aroma of two/three selected key positive and negative attributes followed by taste (i.e. flavour, taste and mouthfeel) if uncertain of negative attributes of the respective *physical* poles. Assessors mainly indicated that *physical* poles for high and moderate quality were associated with positive attributes, with the moderate quality pole representing lower intensities, as well as presence of ‘hay’ or ‘plant-like’ notes. Assessors indicated that they associated the *physical* pole representative of low quality with vegetative aromas/flavours, ‘burnt caramel’ aroma/flavour and high intensities of ‘hay’ aroma/flavour, whereas poor quality was associated with ‘smoky’, ‘medicinal’ and ‘band-aid’ aromas/flavours. Assessors indicated that descriptions of poles in the PSP-*t* task gave better guidance for comparison of test samples to poles, and certain assessors commented that *theoretical* poles were preferred, especially as the descriptions were more representative of specific attributes related to a quality class, than the actual infusion references (i.e. *physical* poles). Furthermore, in terms of user-friendliness, most assessors indicated that both variations of PSP were ‘easy’ to perform (**Fig. 7B**).

### 3.3 Polarised projective mapping

#### 3.3.1 Product configuration based on MFA

The product configurations in the first two dimensions of MFA performed on PPM with *physical* poles (PPM-*p*) and *theoretical* poles (PPM-*t*) are presented in **Figs. 5A and 6A**, respectively. The first and second dimensions of MFA explained 60.1% and 56.5% of the variance in the PPM-*p* and PPM-*t* data, respectively.

Similar to PSP-*p*, three distinct groupings for PPM-*p* was observed, namely ‘moderate to high’, ‘low’ and ‘poor’ quality (**Fig. 5A**). However, computed confidence ellipses for PPM-*p* sample groupings did not overlap as for PSP-*p* indicating significant differences between these groupings. These groupings also distinctively associated with their respective poles, i.e. ‘moderate to high’ quality grouping with Pole H and Pole M, ‘low’ quality grouping with Pole L and ‘poor’ quality grouping with Pole P.

The condensed list of descriptors obtained from PPM-*p* task with UFP is presented in **Addendum C, Table C1 (III)**. The descriptors were similar to the attributes used in the updated fermented honeybush lexicon (Chapter 3). **Figure 5B** presents the correlation plot with supplementary UFP descriptors. Samples grouped with Pole H and Pole M towards the positive end of dimension 1, associated with ‘floral high’, ‘fruity high’, ‘woody’, ‘apricot’, ‘spicy high’, ‘nutty high’, ‘caramel’, ‘honey’ and ‘sweet high’, and opposite

to 'floral low'. Samples grouped with Pole L towards the negative end of dimension 1, associated with 'hay/dried grass', 'cooked vegetables', 'green grass high', and 'burnt caramel high'. Samples grouped with Pole P towards the positive end of dimension 2, associated with 'medicinal', 'band aid', 'smoky high' and 'infusion strength low'.

Distinct groupings for the different quality classes were not clear in **Fig. 6A** for PPM-*t*. Samples 11\_L/Int (and its blind duplicate), 13\_L/Gen and 14\_L/Int associated with Pole L towards the negative end of dimension 1, whereas an overlapping of confidence ellipses for samples 10\_L/Sub and 12\_L/Gen with 'moderate to high' quality grouping was observed. The association of these two low quality samples with the 'moderate to high' quality grouping is also visible in the dendrogram based on the MFA data (**Fig. 6B**). Cluster analysis represents an alternative way to study groupings or clusters within the sample set. The DSA data of 10\_L/Sub and 12\_L/Gen indicated that these samples had higher intensities of 'infusion strength' (i.e. darker red-brown in colour), astringent mouthfeel (for 10\_L/Sub), 'burnt caramel' aroma and flavour compared to moderate quality samples. However, these samples indicated higher intensities of positive 'apricot' aroma and flavour compared to most of the moderate quality samples, which may have caused certain assessors to regard these samples as moderate quality. Samples 15\_P/Int and 16\_P/Int, associating with 'medicinal' and 'smoky' aroma and flavour (**Fig. 3**), distinctly associated with Pole P towards the positive end of dimension 2 (**Fig. 6A**). Samples 17\_P/Sub and 18\_P/Sub (with confidence ellipses overlapping with 15\_P/Int and 16\_P/Int) also associated, but to a lesser extent, to Pole P. As mentioned previously (Section 3.2.2), the DSA data of 17\_P/Sub and 18\_P/Sub indicate low intensities of 'overall positive character on palate' and 'infusion strength' but high intensities of 'apple' and 'nutty' aroma which could have influenced the association with the 'moderate to high' quality grouping observed from the overlapping confidence ellipses.

### 3.3.2 Assessor strategy and ease of performance

Results of the questionnaire on modalities used to assess samples, indicated that for both PPM-*p* and PPM-*t*, the placement of samples on the two-dimensional maps was based on assessment of aroma of samples and poles (for PPM-*p*). Similar to the PSP tasks, only 3 assessors selected an approach of assessment on both aroma and palate modalities. A few assessors also noted that they would taste the samples if they were uncertain of their decision. The majority of assessors specifically indicated that for PPM-*p* they evaluated the *physical* poles by identifying one or two attributes that distinguished one pole from another, and then aimed to link one or two prominent attributes in a test sample to one of the poles. Four assessors specifically noted that decisions for placement close to Pole H representing high quality were based on the 'floral' aroma intensities of a sample. Samples associated with positive aroma descriptors but with higher intensity in 'hay' were placed closer to Pole M representing moderate quality. Furthermore, placement of low and poor quality samples was based on the presence of specific negative sensory attributes (taints).

One assessor commented that it was difficult to decide in which spatial direction to place a sample that is similar to a pole, i.e. left, right, above or below relative to a pole.

In terms of user-friendliness, more assessors indicated PPM-*t* ( $n = 9$ ) as 'easy' to perform, compared to PPM-*p* ( $n = 7$ ), whereas the remaining assessors regarded the tasks as 'neither easy nor difficult' (**Fig. 7B**). Five assessors specifically commented that they preferred PPM-*t* (results not shown), as the descriptions of the respective poles guided them in the placement of test samples relative to the poles.

### 3.4 Comparison of methodologies

Assessment of the validity of the respective PSP and PPM tasks as methods for sensory quality classification of fermented honeybush tea infusions was performed by visually comparing the product configuration obtained for the respective PSP and PPM tasks with that of the PCA bi-plot obtained from DSA data (**Fig. 3**). In addition, RV coefficients for the PCA and the product configurations obtained with MFA (**Figs. 4, 5 and 6**) of the respective PSP and PPM data, were calculated to further demonstrate method validity.

#### 3.4.1 Product configuration (MFA)

Visual comparison of the PCA bi-plot obtained from DSA data (**Fig. 3**) and the MFA plots (**Figs. 4, 5 and 6**) obtained from the two variations on the PSP and PPM tasks, respectively, showed similar product configurations in terms of groupings formed. The first two dimensions of the PCA bi-plot (**Fig. 3**) accounted for 64.8% of the explained variance in the DSA data, whereas the first two dimensions of the maps obtained from MFA accounted for > 47% and > 56% of the explained variance in the data for the PSP and PPM task variations, respectively (**Figs. 4, 5 and 6**). Three groupings were observed in the respective MFA product configurations, namely two separate groupings for 'low' and 'poor' quality, and one grouping for 'moderate' and 'high' quality samples combined towards the opposite side of the map. Overlapping of confidence ellipses around samples of the respected groupings were indicated for PSP-*p*, PSP-*t* and PPM-*t*. Significant different groupings were only observed for PPM-*p* since the confidence ellipses of the respective groupings did not overlap, as indicated in **Fig. 5A**. Similar groupings were observed in the PCA bi-plot, although better distinction between 'moderate' and 'high' quality groupings was obtained in the PCA bi-plot. As for the PCA bi-plot, 15\_P/Int and 16\_P/Int, situated towards the centre of the PCA bi-plot, associated with 'medicinal' and/or 'smoky' aroma and flavour and 17\_P/Sub and 18\_P/Sub, towards the negative end of PC2, formed two separate sub-groupings within the 'poor' quality group, for both PSP-*t* and PPM-*t*. In addition, for both variations of PSP and PPM, blind duplicates 2\_H/Int(2) and 11\_L/Sub(2) and their corresponding samples were placed in close proximity with each other (**Figs. 4, 5 and 6**).

### 3.4.2 RV coefficients

The RV coefficients for the product configurations of the PCA bi-plot obtained from DSA data and MFA plots for the respective PSP and PPM tasks are presented in **Table 2A**. The first two dimensions of the respective plots were considered when computing the RV coefficients. The RV coefficients between the PCA of the DSA data and the MFA for the different reference-based rapid methods were considered high ( $RV \geq 0.7$ ). The highest RV coefficient was obtained for PSP-*p* (0.83), followed by PPM-*p* (0.74), PSP-*t* (0.73) and PPM-*t* (0.68). **Figure 7A** shows that the afore-mentioned RV coefficients do not differ significantly from each other, as indicated by overlapping of confidence intervals for the RV coefficients of the respective PSP and PPM tasks. RV coefficients of 0.88 and 0.89 for the correlation between product configurations for PSP-*p* and PSP-*t*, and for PPM-*p* and PPM-*t*, respectively, were obtained (**Table 2A**).

In the present study, two replicates per variation of each method was performed. Product configurations were stable between replicates of both variations of PSP and PPM (**Table 2B**). High RV coefficients between replicates of the individual tasks were obtained, which is further indicative of good panel intra-reliability. High RV coefficients between test replicates for PSP-*p* (0.90) and PSP-*t* (0.86) compared to that of PPM-*p* (0.93) and PPM-*t* (0.94) were obtained (**Table 2B**).

### 3.4.3 Assessor strategy and ease of performance

From the assessor strategy questionnaire that was completed after the last task of each method, most assessors indicated that samples and poles (for PSP-*p* and PPM-*p*) were evaluated on aroma, and not holistically, i.e. on both aroma and palate. Furthermore, assessors predominantly indicated that both variations of PSP and PPM tasks were 'easy' to perform, whereas the remaining assessors indicated that that the tasks were 'neither easy nor difficult' to perform (**Fig. 7B**).

## 3.5 Industry test

To test the efficacy of a reference-based rapid sensory method as a tool to assess and classify batches of fermented honeybush tea according to sensory quality within commercial context, one method applied by the trained panel in the current study, was considered based on ease of implementation in the herbal tea industry. Universally available and stable written standards for *theoretical* poles may be more feasible for QC laboratories of honeybush processing/packing facilities as access to standard tea batches as *physical* poles that correctly represent the sensory space of each quality class, may be a limiting factor. In addition, Ares et al. (2013) considered the holistic approach of PPM in which a test sample is projected relative to all four poles on the two-dimensional map, as an advantage over PSP for which a sample has to be compared to each of the four poles on line scales. PPM-*t* was therefore selected to be performed by the panel of industry professionals.

**Figure 8A** indicates the product configuration obtained from MFA of PPM-*t* by the industry panel. No distinct groupings with the respective poles were obtained. However, samples representative of high and moderate quality associated closer to Pole H and M towards the positive end of dimension 1, whereas samples representative of low and poor quality associated more to the negative end of dimension 1. Samples 17\_P/Sub and 18\_P/Sub for which their DSA data indicated low intensities in ‘infusion strength’ and ‘overall positive character on palate’, formed a separate grouping towards the negative end of dimension 1, between Pole P and Pole L. In addition, 16\_P/Int for which DSA data indicated high intensities in ‘smoky’ and ‘medicinal’ aroma and flavour, associated more with Pole P towards the positive end of dimension 2. These less distinct groupings were better visualised by the dendrogram obtained from hierarchical cluster analysis, in which groupings for ‘moderate to high’, ‘low’ and ‘poor quality’ could be observed (**Fig. 8B**). A RV coefficient of 0.46 for the product configurations obtained with DSA and MFA was computed. Furthermore, a RV coefficient of 0.60 between test replicates was computed. Better distinction for the ‘low’ and ‘poor’ quality sample groupings was obtained in the second test replicate (individual factor maps of replicates are not shown).

Results for the questionnaire on the modality and strategy used during assessment indicated that all assessors used a holistic approach, i.e. samples were assessed on both aroma and palate. Certain assessors commented that the strategy followed included the identification and placement of samples with negative attributes (taints) firstly, followed by placement of samples with positive attributes. A few assessors (N = 4) indicated that if samples have qualities of two or more poles, samples were projected in between the respective poles. Furthermore, assessors rated the method as ‘very easy’ (N = 4), ‘easy’ (N = 8) and ‘neither easy nor difficult’ (N = 2) to perform. Assessors commented that with training, more experience and frequent tasting, PPM-*t* would be a very valuable method within commercial context. Assessors commented that PPM-*t* may be used as a rapid screening tool of bulk supply from honeybush tea processors.

## 4 Discussion

The present study evaluated the efficacy of reference-based rapid sensory profiling methods, PSP and PPM, using two variations of references (poles) to differentiate between four sensory quality classes of honeybush tea. Previously, Moelich (2018; 2017) successfully demonstrated the effectiveness of PSP and projective mapping (PM) for the sensory characterisation of honeybush infusions representing a sensory-complex product for which temperature-control is crucial. The application of rapid sensory profiling methods as quality classification tool within an integrated quality control system of an agricultural commodity, is presented here for the first time. To the author’s knowledge only one study on the efficacy of PSP in assessing the sensory quality of coffee using consumers has been published (De Alcantara &

Freitas-Sá, 2018). For the current study, the efficacy of PSP and PPM to classify infusions of commercially processed honeybush tea in terms of sensory quality was investigated. The efficacy of *physical* poles (tea infusions) and *theoretical* poles (descriptions of poles), representing the respective sensory quality classes established for honeybush tea, was also tested. The validity of these rapid methods was determined by comparing the results of the rapid method against that of conventional DSA. The use of *theoretical* poles for reference-based rapid sensory profiling methods is presented here for the first time. Recommendations in terms of pole selection, modality, description step, test replicates and assessors' expertise level will be addressed.

#### 4.1 Efficacy of PSP and PPM as quality classification tools

Visual inspection of the product configurations obtained from the PCA of the DSA data and MFA plots of the PSP and PPM data using trained assessors, indicated similar configurations in terms of the sensory quality groupings formed. Conventional DSA, a robust and valid profiling method, is generally selected as reference ('gold standard') to determine the efficacy and validity of rapid sensory methods (Dehlholm, Brockhoff, Meinert, Aaslyng, & Bredie, 2012). Based on the similar groupings formed for DSA, PSP and PPM, both PSP and PPM appeared to be valid methods for the classification of honeybush sensory quality by a trained panel. However, on close inspection, better discrimination between quality groupings along the first dimension for PSP-*p* (compared to PSP-*t*), and PPM-*p* (compared to PPM-*t*), were obtained. From visual inspection of computed confidence ellipses, 'moderate to high' and 'low' groupings differed significantly for PSP-*p* (**Fig. 4A**), whereas three significantly different groupings were obtained only for PPM-*p* (**Fig. 5A**). Therefore, PPM-*p* indicated the highest discrimination ability to classify production batches of honeybush tea according to sensory quality.

Similarity of the respective product configurations obtained with DSA and PSP or PPM, was substantiated with RV coefficients  $\geq 0.7$ . RV coefficient values greater than 0.7 have been regarded as an indication of a good level of agreement (Cartier et al., 2006), although researchers have cautioned that interpretation of RV coefficients should be done in combination with visual inspection of product configurations, and not in isolation (Tomic, Berget, & Næs, 2015; Varela & Ares, 2015). RV coefficient values depend on the number of objects (products) and variables (attributes) when comparing two data matrices (Smilde, Kiers, Bijlsma, Rubingh, & Van Erk, 2009) and may be subjected to a centring effect, especially for data matrices with a low number products ( $n < 7$ ) combined with a high number of variables ( $n \geq 20$ ) (Tomic, Forde, Delahunty, & Næs, 2013). In addition, the RV coefficient places the greatest importance on the dimension with the largest explained variance. Even with a high RV coefficient value, low visual correspondence along dimension 2 may be observed, ascribed to its greater emphasis on the first dimension than on the second dimension (Tomic et al., 2015). For the present study, the highest RV value (0.83) was computed for PSP-*p*, followed by a RV value of 0.74 for PPM-*p*. However, better discrimination

between quality groupings were obtained for PPM-*p* on the second dimension. This confirms the importance of interpreting RV coefficients in combination with visual inspection of product configurations. RV coefficients did not differ significantly from each other for the respective PSP and PPM tasks compared to DSA (**Fig. 7A**).

These results indicate that PPM-*p* and PSP-*p* showed a better ability to differentiate between samples representative of the different honeybush sensory quality classes, compared to the respective methods using *theoretical* poles as references. However, the selection of poles, as well as choice of modality and assessor's strategy used during assessment within a method, should also be considered when providing recommendations for industry application. This will be discussed in the following section (Section 4.2). Although trained assessors regarded the respective variations of the PSP and PPM tasks as 'easy' or 'neither easy nor difficult' (**Fig. 7B**), they indicated highest preference for PPM, compared to PSP (results not shown). The holistic approach of PPM may have placed less cognitive strain on trained assessors compared to the analytical approach of PSP, as suggested by Ares et al. (2013).

## 4.2 Considerations for PSP and PPM

### 4.2.1 Pole selection

The selection of poles or reference products is regarded as the most critical step for the implementation of reference-based rapid sensory methodologies (Ares et al., 2018, 2015; Teillet, 2014). Poles should represent the main sensory characteristics responsible for the anticipated similarities and differences among samples (De Saldamando et al., 2015) to ensure that the complete sensory space is represented in the two-dimensional map determined by the poles (Ares et al., 2015).

#### 4.2.1.1 Pole selection for quality discrimination

The present study indicated that good distinction was obtained for low and poor quality samples. This may be ascribed to the selection of representative tea batches for blending the *physical* poles and distinctively different taints highlighted in the descriptions of the *theoretical* poles (**Table 1**), i.e. 'green grass', 'burnt caramel' and 'cooked vegetables' for Pole L, and 'medicinal' and 'smoky' for Pole P. However, limited distinction between high and moderate sensory quality infusions could be made, irrespective of method, or type of poles used. This could be ascribed to the fact that the average intensities of majority of the positive attributes of majority of these samples (from DSA data), as well as samples selected for Pole H and Pole M blends, did not differ significantly from each other.

The sensory characteristics responsible for the main similarities and differences among samples of each of the four poles were considered for pole selection. However, Pole H and Pole M represented similar sections within the total sensory space, i.e. positive aroma and flavour attributes and 'overall character on palate' at high and moderate intensities. Therefore, an overlap of these attributes exists as Pole H and Pole



M only differ in intensities and not in attributes. The rapid methodologies applied in the present study do not account for differences in intensities but only for broad characterisation. Therefore, method variations within PSP and PPM for both trained and industry assessors, were not able to discriminate effectively between samples of high and moderate quality. De Saldamando and co-workers (2015) studied the effect of pole selection for PPM applied to orange-flavoured powdered drinks. The effect of different sets of poles ( $n = 3$ ) representing the main distinctive characteristics, namely 'sweetness', 'sourness' and 'total flavour' intensity, and variations thereof, including sets of poles representing a narrower part of the sensory space, was studied. Results indicated that the degree of difference among the poles can affect discrimination among samples and the authors recommended that apart from selecting poles that represent the entire sensory space, there should be an intermediate degree of difference among poles. These results were also regarded as applicable to other reference-based methodologies, such as PSP. In a study on pole selection for PSP, Ares et al. (2015) advised the selection of poles that are distinctly different and that each pole should be markedly representative of one or two sensory characteristics.

For the present study, two improvements on selection of poles representing high and moderate sensory quality is recommended. Firstly, the selection of a *physical* Pole M with a notably presence of 'hay/dried grass' aroma and flavour, may aid assessors in better distinction between samples of high and moderate quality. For *theoretical* Pole M the term 'hay' could be included in the description, as well as 'Moderate HB character on palate' (Table 1). This may guide assessors to focus on this specific modality, as the positive flavours, sweet taste and astringent mouthfeel would be less pronounced in moderate than high quality samples. Although 'hay/dried grass' aroma and flavour are perceived as intrinsic to the sensory profile of honeybush at lower intensities, it is perceived as a taint at higher intensities (Bergh et al., 2017). 'Hay/dried grass' aroma and flavour, and 'overall character on palate' could therefore be the distinguishing sensory characteristic between Pole H and Pole M for both *physical* and *theoretical* poles.

Secondly, as an alternative, Pole H and Pole M could be combined into only one pole representing 'moderate to high' honeybush sensory quality. Considering the potential application of PSP and PPM as quality screening tools for blending of fermented honeybush batches of different quality levels within commercial context, only three distinctively different poles may be adequate to distinguish between good/acceptable ('moderate to high'), inferior ('low') and rejectable ('poor') quality. In addition, inclusion of a smaller number of poles that are distinctly different in terms of specific sensory characteristics may limit assessors' sensory and cognitive fatigue, especially for PSP in which assessors have to taste and re-taste a sample many times for placement against each of the respective poles. Previously, Moelich (2018) studied the application of PSP on honeybush tea by selecting five poles, representing five different *Cyclopia* species. Poles for *C. subternata* and *C. intermedia* were distinctly different in sensory profiles and represented specific sensory characteristic, whereas poles for *C. genistoides*, *C. maculata* and *C. longifolia* did not differ distinctly and indicated an overlap of sensory characteristics. As assessors could not

distinguish between samples of the afore-mentioned three species, the author recommended the selection of only one pole for each of *C. genistoides*, *C. subternata* and *C. intermedia*, respectively, since each of these species represented high intensities of one or two specific distinctly different sensory characteristics. Correspondingly, Teillet (2014) recommended that if two poles are very close to one another within the sensory space, one of the poles may be omitted. Generally, the number of poles required for a reference-based method is at least equal to the number of sensory dimensions required to present the perceptual space (Ares et al., 2018).

#### 4.2.1.2 Physical vs. theoretical poles

PPM-*p* indicated the highest discrimination ability between the three quality groupings ‘moderate to high’, ‘low’ and ‘poor’ sensory quality. The product configurations for PPM-*p* (Fig. 5A) and PPM-*t* (Fig. 6A) indicate that *physical* poles were more representative of the respective sensory quality classes, than the descriptions of *theoretical* poles. Assessors were able to position samples onto the two-dimensional map on the computer screen, relative to the respective *physical* poles, based on the key sensory attributes they evaluated in each of the tea infusions representative of high, moderate, low and poor quality. Poor discrimination between high and moderate sensory quality was addressed in Section 4.2.1.1. Higher discrimination between samples was also obtained for PSP-*p* compared to PSP-*t*, which confirms that the *physical* poles, i.e. tea infusions, used in the present study were more representative of the respective quality classes than the descriptions for the *theoretical* poles.

Product configurations based on MFA of the data of both PSP and PPM indicated more distinct groupings for poor quality samples for tasks with *physical* poles, compared to tasks using *theoretical* poles. Compared to PPM-*p* (Fig. 5A), PPM-*t* (Fig. 4B) indicated lower discrimination ability between samples 15\_P/Int and 16\_P/Int and samples 17\_P/Sub and 18\_P/Sub. As mentioned previously, the DSA data of samples 15\_P/Int and 16\_P/Int indicated high intensities in ‘medicinal’ and/or ‘smoky’ aroma and flavour, whereas the DSA data of samples 17\_P/Sub and 18\_P/Sub indicated a low intensity in ‘overall positive character on palate’ and high intensities in ‘apple’ and ‘nutty’ aromas. The higher discrimination ability of PPM-*p* may be ascribed to the fact that the *physical* Pole P, i.e. the tea infusion, was more representative of the sensory space associated with poor quality samples. The description of the *theoretical* Pole P (Table 1) may have restricted trained assessors in positioning samples with only ‘medicinal’ and/or ‘smoky’ aroma/flavour (and not insipid or poor ‘overall character on palate’) close to Pole P. Similarly, compared to PSP-*p* (Fig. 4A), PSP-*t* (Fig. 4B) indicated lower discrimination ability between samples 15\_P/Int and 16\_P/Int and samples 17\_P/Sub and 18\_P/Sub. These results indicate that for the selection of *theoretical* poles in a PSP or PPM task, great care should be taken in formulating the description of each of the respective *theoretical* poles. It is recommended that description of *theoretical* poles should be improved to be more representative of the respective sensory quality classes, specifically for *theoretical* Pole P.

Inclusion of the terms 'poor infusion strength' and 'character on palate' for *theoretical* Pole P is recommended. It should therefore encompass all possible descriptions of poor quality although this might have certain practical implications in terms of space on the digital screen.

One disadvantage of reference-based methodologies is that the sensory space of selected *physical* poles should be known prior to the task (Valentin et al., 2012). However, the advantage of PPM or PSP with *theoretical* poles would be that such prior knowledge would not be required. *Theoretical* poles would be pre-determined definitions and would remain constant for all consecutive sessions within a company, and throughout the industry, as they are linked to the sensory quality standard and specifications for fermented honeybush tea (described in Chapter 4). Furthermore, the evaluation of samples against *theoretical* poles requires less tasting compared to the use of *physical* poles, which may limit assessors' sensory fatigue. Pre-defined global summaries of the sensory characteristics of *theoretical* poles may guide assessors in their cognitive decision-making process, compared to *physical* poles. This was also indicated from the questionnaire responses by trained assessors. Both trained assessors and untrained industry assessors indicated the PPM-*t* task predominantly as 'easy' to perform.

Although universally available and stable written standards for *theoretical* poles would be more feasible within a commercial environment, the use of *physical* poles for which the sensory space is available, may be granted for application within a research environment.

#### 4.2.2 Modality

The modality used by assessors for comparing samples relative to the respective poles for the PSP and PPM tasks, may have influenced assessors' cognitive decision-making process. Trained assessors indicated that they based their decisions mainly on infusion aroma, and not on palate, compared to the industry panel who indicated that poles and samples were evaluated on both aroma and palate. The latter approach is also as per general practice within the honeybush industry, as discussed in Chapter 4. This may elucidate the positioning of specifically poor quality samples, 17\_P/Sub and 18\_P/Sub, relative to the Poles H, M and P, as well as the overlapping of confidence ellipses for these samples on the respective MFA plots for the trained panel (**Figs. 4, 5A and 6A**). Even for PPM-*p*, which showed the highest discrimination ability, elongated confidence ellipses for these samples between Pole P and Poles H and M were computed, although no overlapping was observed (**Fig. 5A**). As mentioned previously, the DSA data of these two samples showed low intensities of 'infusion strength' (light yellow in colour) and 'overall positive character on palate' but relatively high intensities in positive 'apple' and 'nutty' aromas. Questionnaire responses from several trained assessors indicated that they were uncertain how to rate or where to place these two samples for both PSP and PPM. This may be ascribed to the assessment of predominantly aroma only by the trained panel. From the DSA data, the intensities of 'overall character on palate' differed significantly for high and moderate sensory quality infusions (apart from 5\_M/Int), as well as samples selected for Pole

H and Pole M blends. Assessment on both aroma and palate by the trained panel may have resulted in better discrimination between moderate and high quality samples.

For PPM-*t* by the industry panel, samples 17\_P/Sub and 18\_P/Sub were grouped the furthest from the cluster of high, moderate and low samples (**Fig. 8A**) compared to PPM-*t* by the trained panel (**Fig. 6A**). These results could be ascribed to assessment on both aroma and palate by industry assessors. However, as their DSA data indicated very low intensities of 'infusion strength' (i.e. light yellow colour compared to the desired red-brown colour for honeybush tea, as described in Chapter 4), one could argue that infusion appearance modality may have influenced their decisions, rather than the aroma and/or palate modality used.

Key sensory quality parameters, i.e. infusion appearance, aroma and taste, were regarded as important by the honeybush industry, and included in the developed scorecard for honeybush tea, as discussed in Chapter 4. In view of the results, instructed (and not 'free') global quality assessment based on infusion appearance, aroma and taste, would be recommended for PSP and PPM for application in a commercial quality control environment for honeybush tea.

#### 4.2.3 Description (verbalisation) step

The UFP task allowed for identification of the main sensory characteristics (quality drivers) associated with samples and *physical* poles of the respective four sensory quality classes, as perceived by the trained assessors (**Fig. 5B**). The descriptors given to sample groupings were similar to the attributes associated with the samples of the respective quality classes as depicted in the PCA bi-plot of their DSA data (**Fig. 3**). This was to be expected as these assessors had in-depth training in the terminology of the specific honeybush sensory attributes. Assessors assigned descriptors that were similar to the vocabulary used in DSA of honeybush tea, even though a list of DSA attributes were not provided prior to the PPM-*p* with UFP task. Furthermore, the descriptors provided to groupings associated with the respective poles were relatively similar to that of the descriptions used for the *theoretical* poles (**Table 1**). Descriptors provided for the negative aromas/flavours (taints) perceived in the 'low' and 'poor' quality groupings were similar to the taints described in the respective *theoretical* poles, i.e. 'green grass', 'burnt caramel' and 'cooked vegetables' for Pole L, and 'medicinal' and 'smoky' for Pole P (**Fig. 5B**). These results provide confirmation that batch blends for the respective *physical* poles were representative of the sensory space of the respective quality classes.

The significantly different groupings resulting only from PPM-*p* (**Fig. 5A**) may be partly ascribed to the inclusion of the UFP task. Although the descriptors assigned were considered as supplementary variables in the construction of MFA maps, the UFP task may have guided assessors in grouping of test samples relative to the respective poles. Assessors were instructed to encircle similar samples (and respective pole where applicable) and give relevant descriptor(s) to groupings, and only when all samples and poles have been

included in a grouping with descriptor(s), the software programme could register the task as complete (**Addendum C, Fig. C3**). Therefore, assessors may have been directed to project samples closer to the respective poles than for PPM-*t* without an UFP task.

PSP-*p* with an UFP task was not investigated for the present study. Generally, a subsequent description task after PSP is not applied, and therefore information on sensory characteristics accountable for similarities and dissimilarities between test samples and poles cannot be collected (Ares et al., 2018). However, assessors may be asked to describe each pole or test sample after the PSP task, as typically performed after completion of a PM task (Varela & Ares, 2012) or PPM task (Ares et al., 2013; Wilson et al., 2018). Ares et al. (2013) highlighted the advantage of PPM with UFP compared to PSP. Considering PSP followed by UFP, descriptions for sensory characteristics of products are only relative to the reference products (poles), and the cognitive process of one-on-one comparison to evaluate samples might potentially limit the obtained description.

One disadvantage of UFP is the difficulty of data analysis and time required for pre-processing of descriptors, especially within a commercial quality control environment. The application of UFP with a pre-determined descriptor list, as proposed by Perrin et al. (2008) for Napping® on wine, and previously applied to honeybush for PM (Moelich et al., 2017), may address this problem. The use of UFP as descriptive step following either PSP or PPM would rather find application within research context than in routine quality screening within commercial context.

Alternatively, PSP in combination with CATA may be investigated to simplify the descriptive task and data analysis thereof. Since each sample in a PSP task is evaluated in a monadic order, a CATA list of the taints in the honeybush lexicon (Chapter 3) may be added for assessors to check the specific taint(s) that apply. Since Pole L and Pole P represent different taints, the additional descriptive step may highlight the specific taints present in batches. Such a description can be valuable in the quality screening process for blending purposes within commercial context. Limited research is available on the combination of PSP with a subsequent CATA step. Previously, Crous (2016) investigated the combination of PSP with CATA to determine the sensory attributes regarded as most important for differentiation of old-vine Chenin Blanc wines.

Further investigation of PSP-*p* or PSP-*t*, followed by a descriptive task such as UFP or CATA, for application as quality classification tool, within both research and commercial context, may be granted. In addition, as very limited consumer studies have been performed on honeybush tea (Moelich, 2018; Vermeulen, 2015), a consumer study using PSP or PPM with UFP or CATA may reveal important information on the consumer's perception and drivers of honeybush sensory quality.

#### 4.2.4 Test replicates

In the present study, two replicates per PSP and PPM task variation were performed to evaluate method reliability and validity. High agreement between sample configurations of the respective replicate sessions per PSP and PPM task variations were obtained and RV coefficients of  $\geq 0.86$  were computed for each set of replicates within a method variation (**Table 2B**). Results demonstrated the validity of these methods for sensory quality discrimination within a honeybush sample set using trained assessors. Contrastingly, low agreement between replicate sample configurations were obtained and a relatively low RV coefficient (0.6) between replicates was computed for PPM-*t* by untrained industry assessors. This may be ascribed to 1) industry assessor sensory fatigue as both replicates for PPM-*t* were performed in two consecutive sessions on the same day, and 2) varying projection strategies between assessors which are largely dependent on the assessor type and level of training (Dehlholm, 2014). This aspect will be addressed in the following section (Section 4.2.5).

Generally, replications are not performed in PSP tasks due to time constraints and/or resources available for the study and data analysis thereof (Ares et al., 2018). Limited studies using replications for individual PSP and PPM tasks have been published, although Hopfer and Heymann (2013) recommended replication in PM to aid in improved discrimination. Alternatively, the use of blind duplicates within the sample set may be used to evaluate assessors' performance (Hopfer & Heymann, 2013), which was similarly applied for the present study. In the present study, trained assessors placed the blind duplicates and their corresponding samples in close proximity with each other, for each variation of the respective PSP and PPM tasks, indicating good panel inter-reliability (Fleming, Ziegler, & Hayes, 2015). In contrast to the trained panel, industry assessors were not able to place samples 2\_H/Int and 11\_L/Int in close proximity to their respective blind duplicates for PPM-*t*, indicating poor repeatability. This further illustrates the importance of industry panel training and calibration in the sensory attributes associated with honeybush quality, which will be highlighted in the following section.

#### 4.2.5 Level of assessor expertise

In the present study, results of trained assessors demonstrated good discrimination ability between samples of different sensory quality for PPM-*p* and acceptable levels for the other tasks. As discussed previously, instances in which the different quality groupings were less distinct or further from their respective quality poles, may be attributed largely to the selection and/or description of poles (*physical* and *theoretical*), the large overlap of sensory attributes for Pole H and Pole M, and the choice of modality (mainly aroma) used, and not to the trained assessors' level of expertise, i.e. their ability to recognise and rate honeybush sensory attributes. Recommended amendments to the respective methods in terms of instructions, pole selection and descriptions for *theoretical* poles, as discussed previously, may aid in improved distinction between the quality classes by trained assessors.

When comparing **Figs. 6A and 8A**, higher discrimination between quality groupings is apparent in **Fig. 6A**. Therefore, higher efficacy of the PPM-*t* task to classify honeybush samples according to sensory quality using trained assessors, was demonstrated. Contrastingly, poor discrimination ability of PPM-*t* when using the panel of industry representatives, was indicated. These results may be mainly ascribed to assessors' limited expertise in honeybush sensory quality and inadequate training in the recognition and rating of the wide range of sensory attributes used to describe honeybush tea. Industry assessors indicated their level of experience in honeybush sensory quality ranging from 'low' to 'moderate'. Therefore, the honeybush industry professionals used in the present study may be regarded as untrained or 'not formally trained' and inexperienced in overall sensory quality of honeybush tea.

Although a low discrimination ability of PPM-*t* using industry assessors was indicated, based on visual inspection of product configurations in **Fig. 6A and 8A**, industry assessors were able to distinguish poor quality samples (17\_P/Sub and 18\_P/Sub) from the other samples. This may be ascribed to their choice of modality (aroma, palate and appearance), therefore their focus on the overall quality, compared to assessment on aroma only, as in the case of the trained assessors. Although these two samples had high intensities of positive aromas, 'nutty' and 'apple', as mentioned previously, the low intensities of 'infusion strength' and 'overall character on palate', and therefore overall quality, may have been the decisive factor for industry assessors. Correspondingly, Torri et al. (2013) indicated in a PM study comparing the efficacy of naive consumers, trained assessors and wine industry professionals, that product discrimination by wine experts was largely based on the concept of perceived overall quality rather than individual sensory differences assessed analytically.

Similarly to the present work, Chollet et al. (2011) and Torri et al. (2013) have attributed different product configurations obtained to assessors' levels of expertise for beer sorting and wine PM tasks, respectively. Trained assessors indicated better discrimination ability between samples than untrained assessors. For these studies untrained assessors represented naive beer or wine consumers, whereas trained assessors represented trained beer industry assessors or wine industry professionals. Furthermore, wine industry professionals have been regarded as experts with a vast product knowledge and good cognitive memory of wine sensory attributes (Torri et al., 2013). Considering the relatively short existence of the formal honeybush industry (Joubert et al., 2019), and the level of experience indicated by honeybush industry assessors, their expertise level cannot be compared to that of wine industry professionals. Torri et al. (2013) specifically noted that provided that assessors share a high level of product knowledge and sensory experience of a product, PM may be applied to differentiate among samples with subtle sensory differences. Trained sensory panels have indicated good discrimination ability in PSP (Moelich, 2018; Teillet et al., 2010) and PPM (Wilson et al., 2018) tasks. Therefore, provided that industry assessors are trained in the sensory attributes for honeybush tea, PSP and PPM could be regarded as efficient quality classification tools for honeybush industry to distinguish between tea batches of variable sensory quality.



With the ongoing debate in literature between the use of untrained consumers vs. trained assessors in sensory analysis, it is important to note that the ultimate goal of a method would determine the choice in assessor type to be used (Ares & Varela, 2017b, 2017a; Varela & Ares, 2012). In the present study, the aim of the reference-based methods was rapid sensory quality classification within research and commercial quality control context, and not rapid sensory profiling for new product development or category appraisal for which consumers have been typically used (Ares & Varela, 2017a). Generally, consumer-based studies have not been recommended for quality control application, or the identification of subtle differences between products (Antúnez, Vidal, De Saldamando, Giménez, & Ares, 2017; Varela & Ares, 2015). Furthermore, consumers or untrained assessors may be unable to detect specific sensory taints or may associate such taints with positive characteristics (Ares & Varela, 2017b, 2017a), which could be detrimental in quality control.

In the present study, it was indicated that only a small number of assessors, often also representatives of company divisions other than the quality control/assurance division, are available for quality assessment of honeybush tea within a commercial environment (Chapter 4). Correspondingly, Ares and Varela (2017b) noted that it is common practice in industrial settings to use internal employees of often different divisions to participate in sensory quality assessment. Ares and Varela (2017b) further commented that important decisions are often made based on results of poorly trained and maintained panels. These assessors may be regarded as 'semi-trained' assessors but often training opportunities are scarce which results in poor panel performance. The importance of the use of trained assessors instead of untrained assessors/consumers within quality control context has been emphasised (Ares & Varela, 2017b). Although the influence of short training sessions on results from novel rapid sensory profiling methods has not been studied (Ares, 2015), the inclusion of short training tasks to familiarise untrained assessors with new methods and/or sample sets has been recommended. This would improve the quality of results in terms of the method's discrimination ability, as well as repeatability (Ares & Varela, 2017a; Hopfer & Heymann, 2013).

Therefore, training opportunities for honeybush industry assessors in the sensory quality of fermented honeybush tea is recommended for the effective application of reference-based rapid methodologies as quality classification tools within a commercial quality control context. The revised lexicon with chemical-based reference standards for fermented honeybush tea (Chapter 3) is recommended for training and calibration of industry assessors in the recognition of positive and negative honeybush sensory attributes. Training would enable industry assessors to rapidly screen and classify honeybush tea batches according to sensory quality in commercial practices.

## 5 Conclusions

Two reference-based rapid sensory methods, PSP and PPM, were validated by trained assessors for their efficacy to differentiate between commercially processed honeybush batches of varying sensory quality. The potential application of *theoretical* poles as an alternative approach to conventional product references was presented for the first time. Although both PSP and PPM could not differentiate between samples of high and moderate sensory quality, recommendations in terms of reducing the number of poles and amendments of *theoretical* pole descriptions were made. Furthermore, the application of a smaller sample size per consecutive test session is recommended, which would be possible due to the advantage of data aggregation in reference-based rapid methods.

Even though higher discrimination between sensory quality classes were obtained for the respective PSP and PPM tasks using *physical* poles, compared to *theoretical* poles, recommended amendments to the *theoretical* poles used in the present study could improve the discrimination ability of the respective methods. Compared to *physical* poles, *theoretical* poles would be more feasible for commercial application as universally available and stable written standards.

The potential application of PSP and PPM as rapid honeybush sensory quality screening tools within commercial quality control context was indicated. It is however important that industry assessors are trained in the specifics of honeybush sensory quality. Both methods have been broadly included into sensory software programmes enabling computers, tablets, and/or other devices to be used for user-friendly application, electronic data capturing and automated data analyses. Despite the cost implication of such software programmes, it would make PSP and PPM attractive tools for future application by large companies. Furthermore, these methods may also find application as rapid sensory quality screening tools within honeybush research, especially the honeybush breeding programme of the ARC for rapid assessment of genotypes and selections. PSP and PPM may also be potentially applied to process optimisation studies for new *Cyclopia* species that enter the market, for which optimum fermentation time/temperature regimes have not yet been established.

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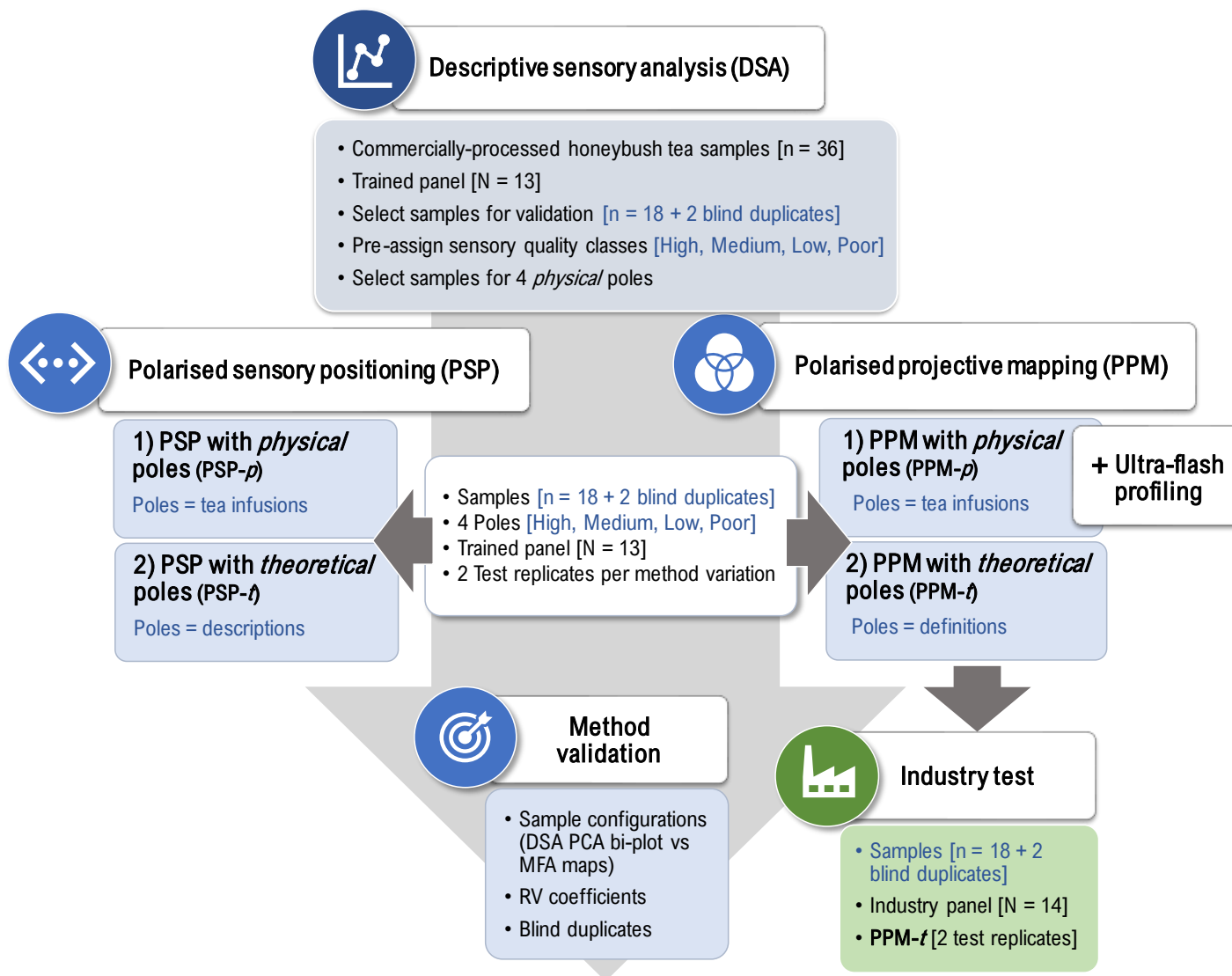
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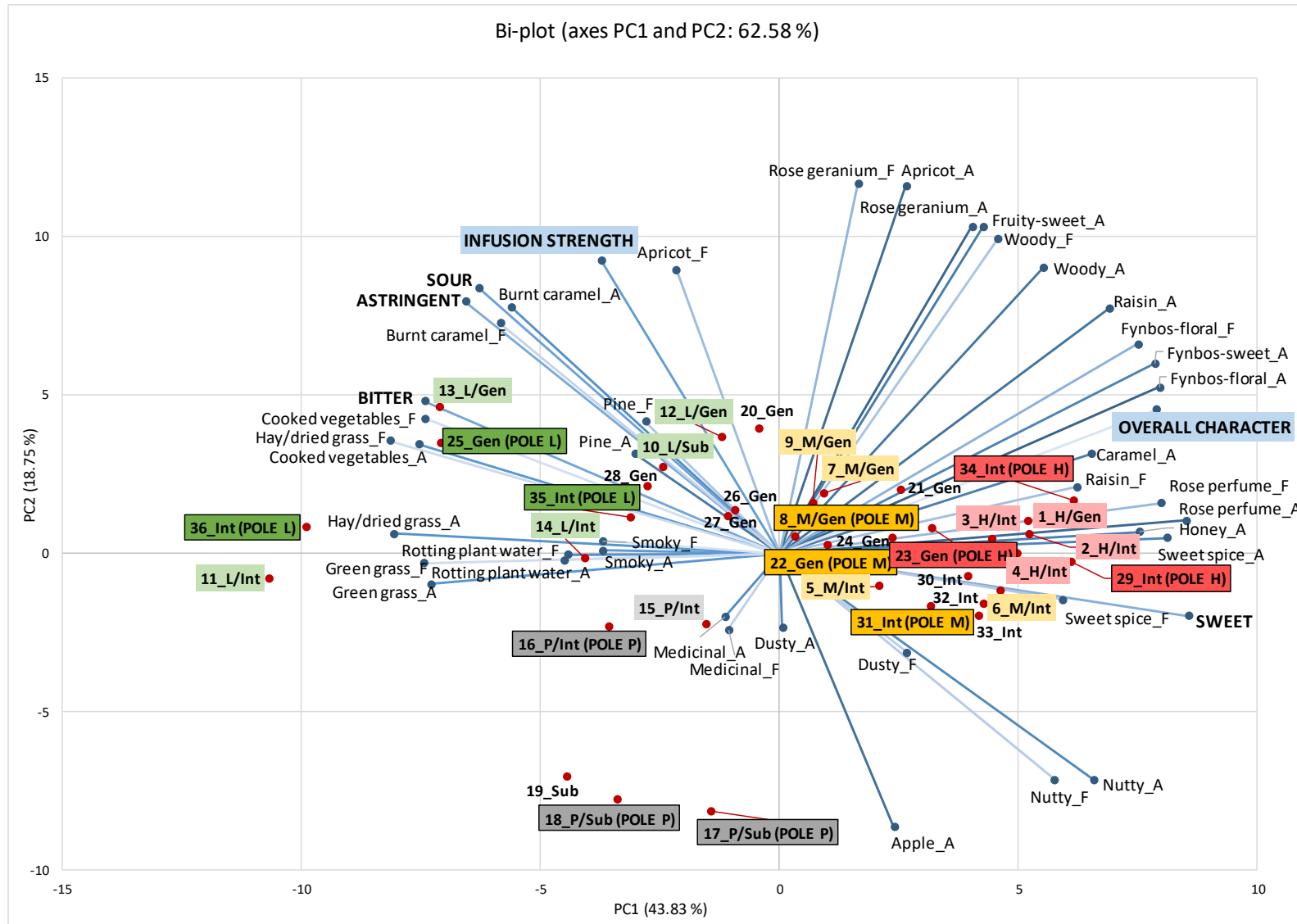
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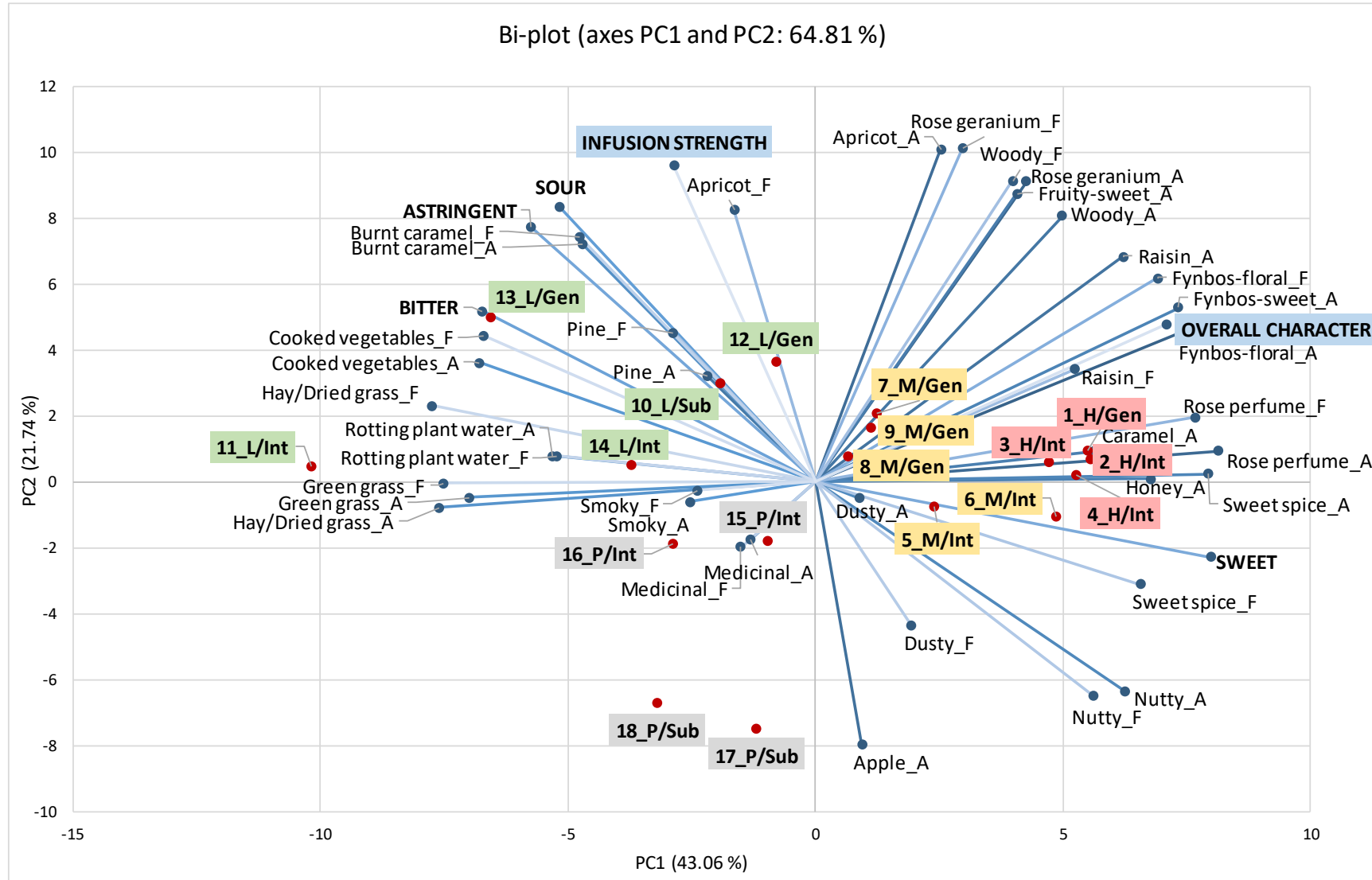




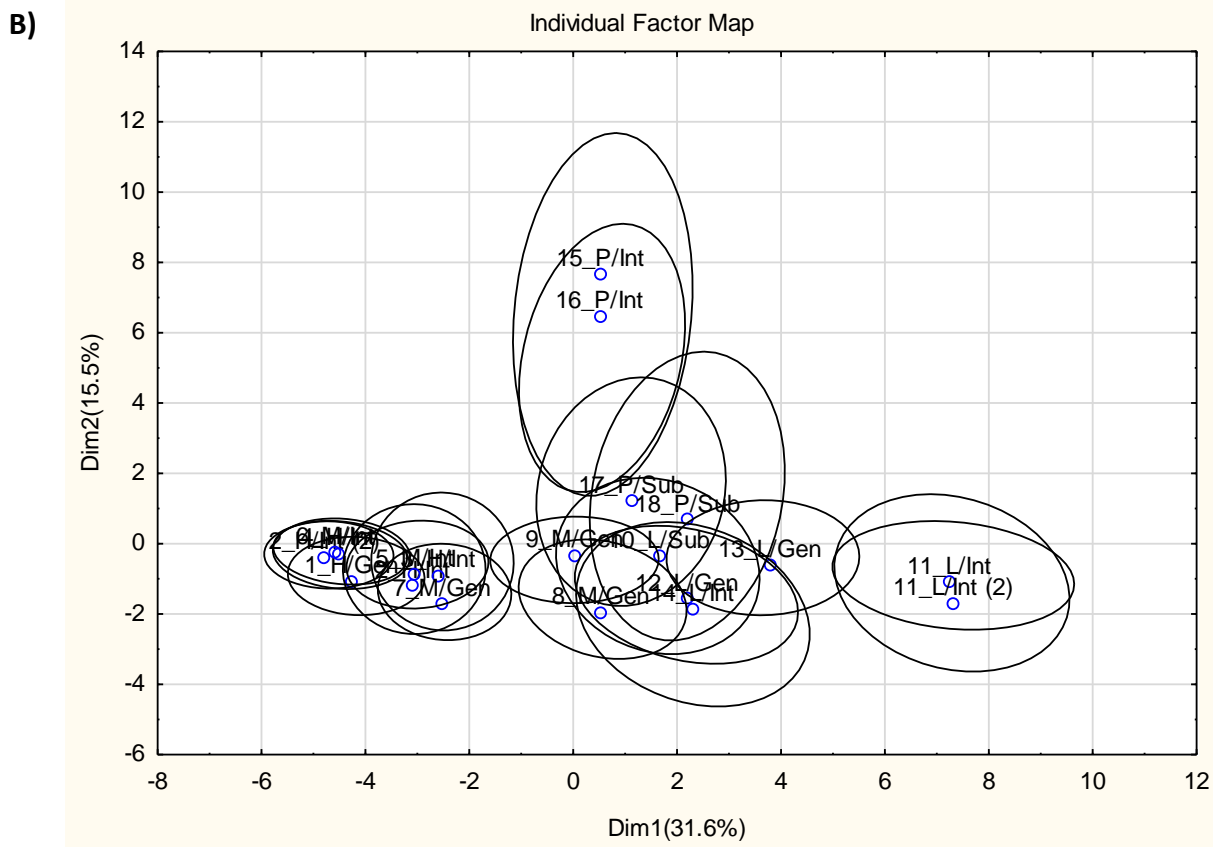
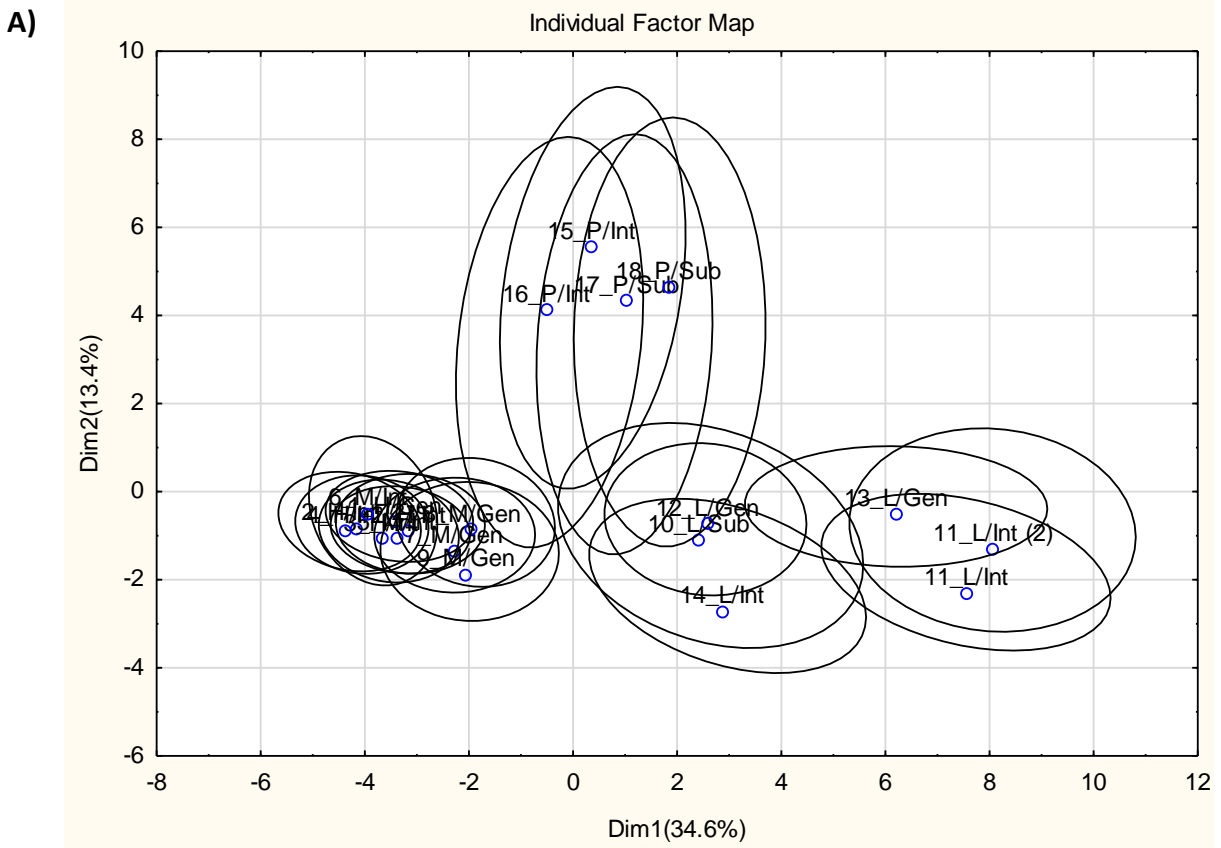
**Figure 1** Experimental lay-out for the application of two reference-based rapid sensory methods for classification of honeybush tea in terms of sensory quality.



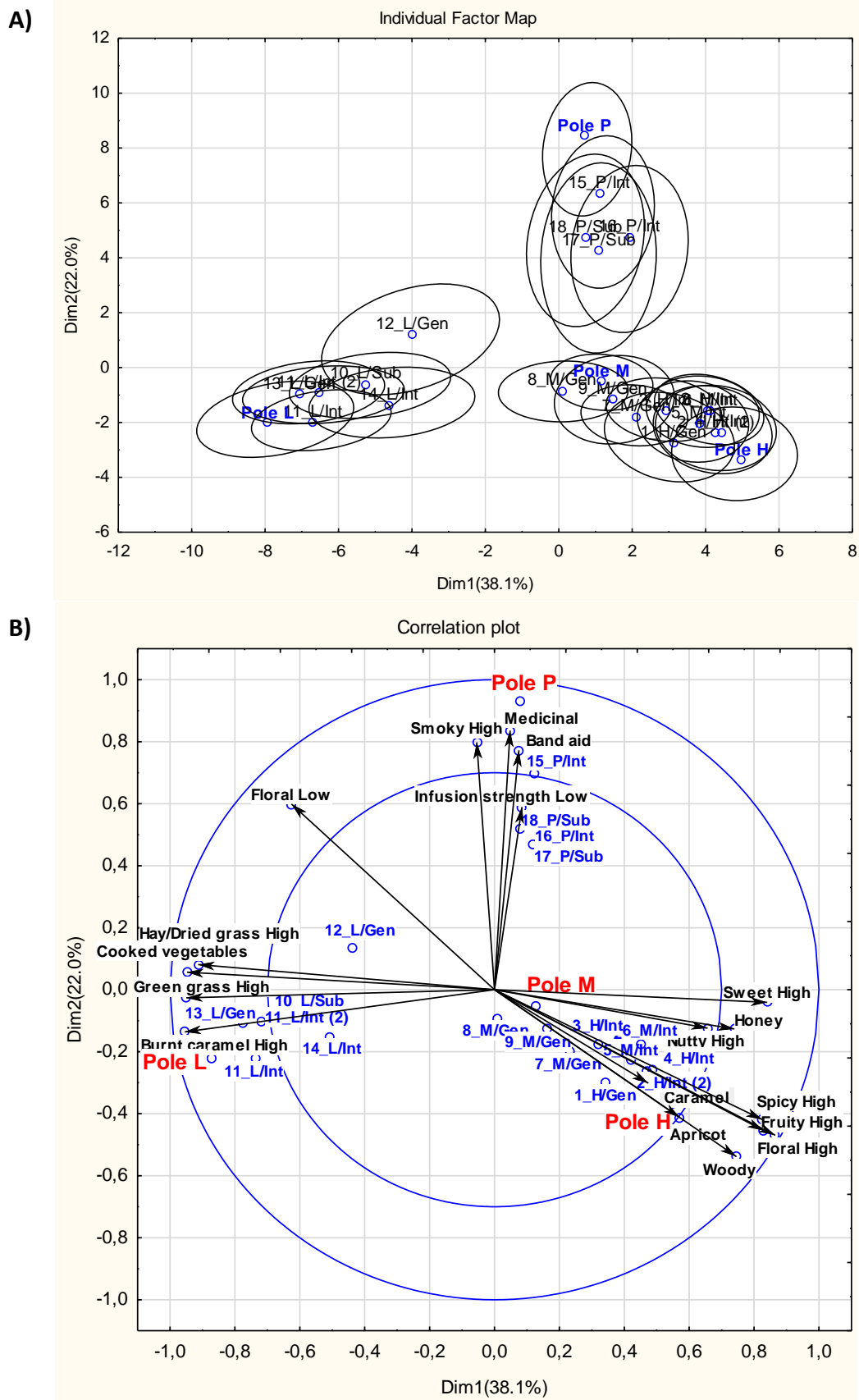
**Figure 2** PCA bi-plot indicating sample configuration of commercially processed honeybush samples (n = 36) [1–36 represent sample numbers; Int, Sub, Gen represent *C. intermedia*, *C. subternata* and *C. genistoides*, respectively; H (red), M (yellow), L (green), P (grey) represent high, moderate, low and poor quality, respectively; aroma (A) or flavour (F) attributes are indicated after descriptors; samples used in blends for physical poles are indicated by 'POLE' in brackets].



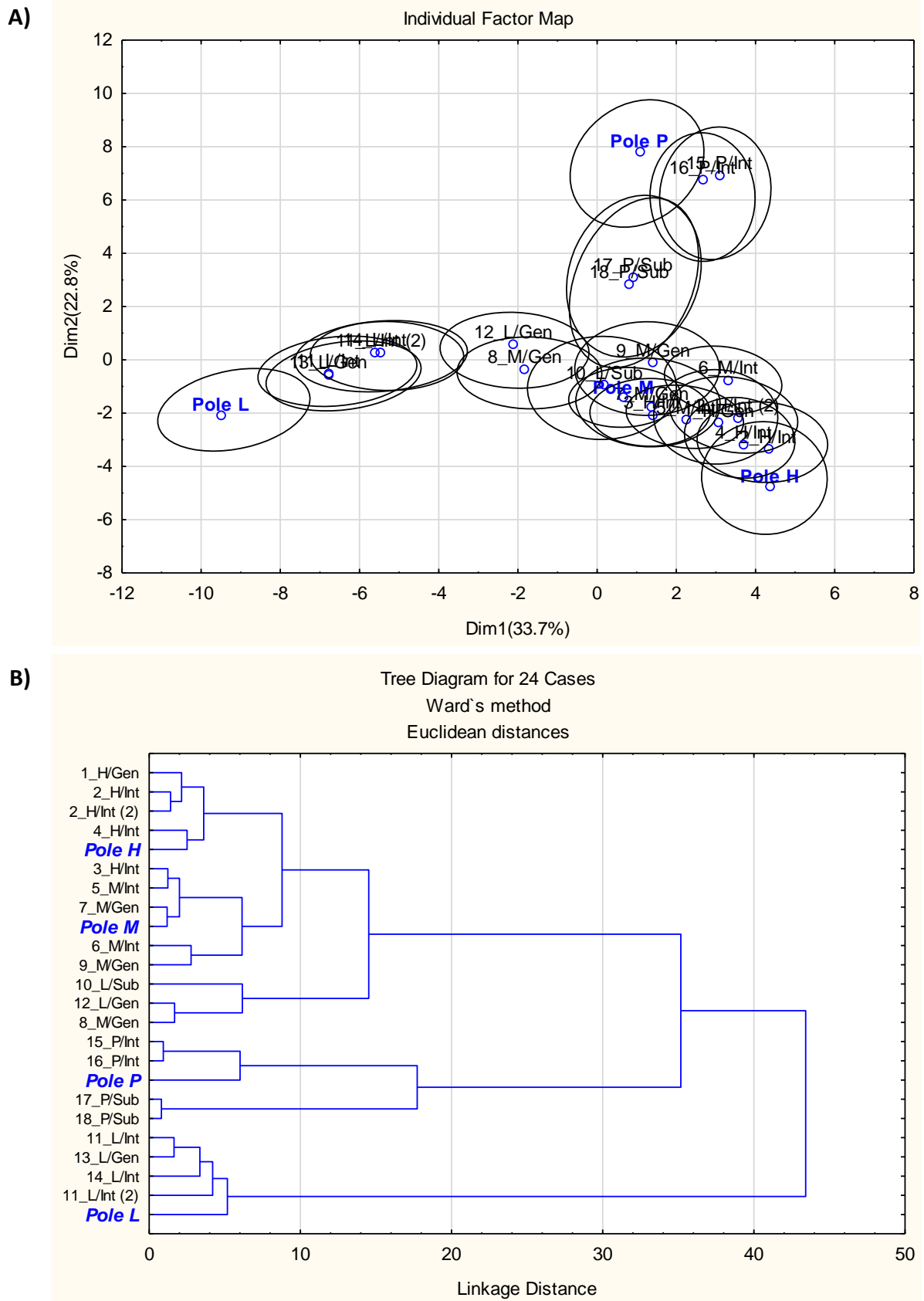
**Figure 3** PCA bi-plot indicating samples (n = 18) selected for rapid sensory profiling method tasks [1–18 represent sample numbers; Int, Sub, Gen represent *C. intermedia*, *C. subternata* and *C. genistoides*, respectively; H (red), M (yellow), L (green), P (grey) represent high, moderate, low and poor quality, respectively; aroma (A) or flavour (F) attributes are indicated after descriptors].



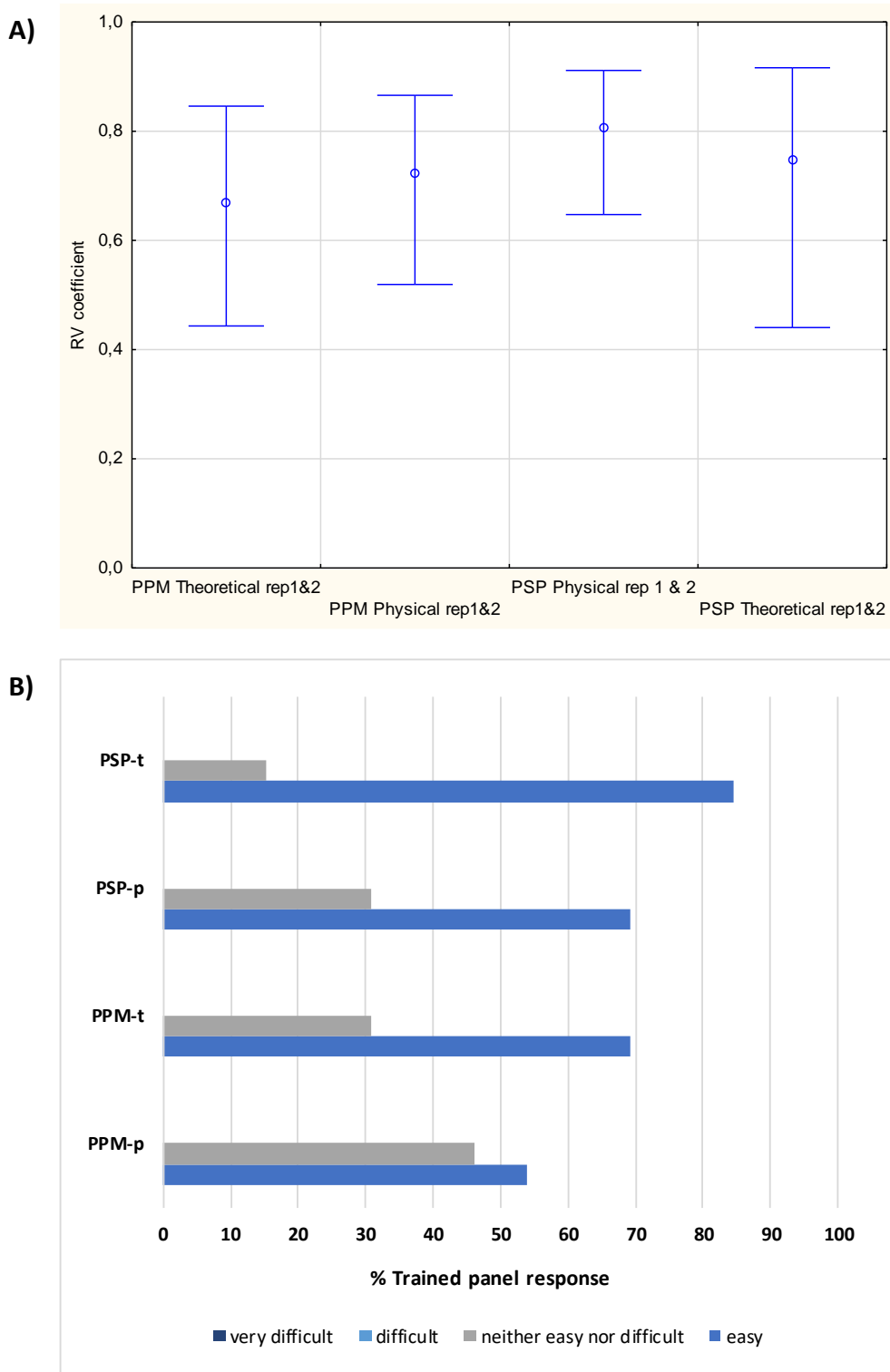
**Figure 4** MFA scores plot with confidence ellipses for **A) PSP-p** and **B) PSP-t** by the **trained panel** [1–18 represent sample numbers; Int, Sub, Gen represent *C. intermedia*, *C. subternata* and *C. genistoides*, respectively; H, M, L, P represent high, moderate, low and poor quality, respectively; (2) after sample code indicates blind duplicate].



**Figure 5** A) MFA scores plot with confidence ellipses and B) correlation plot with supplementary UFP descriptors of data for PPM-p by the **trained panel** [1–18 represent sample numbers; Int, Sub, Gen represent *C. intermedia*, *C. subternata* and *C. genistoides*, respectively; H, M, L, P represent high, moderate, low and poor quality, respectively; (2) after sample code indicates blind duplicate].

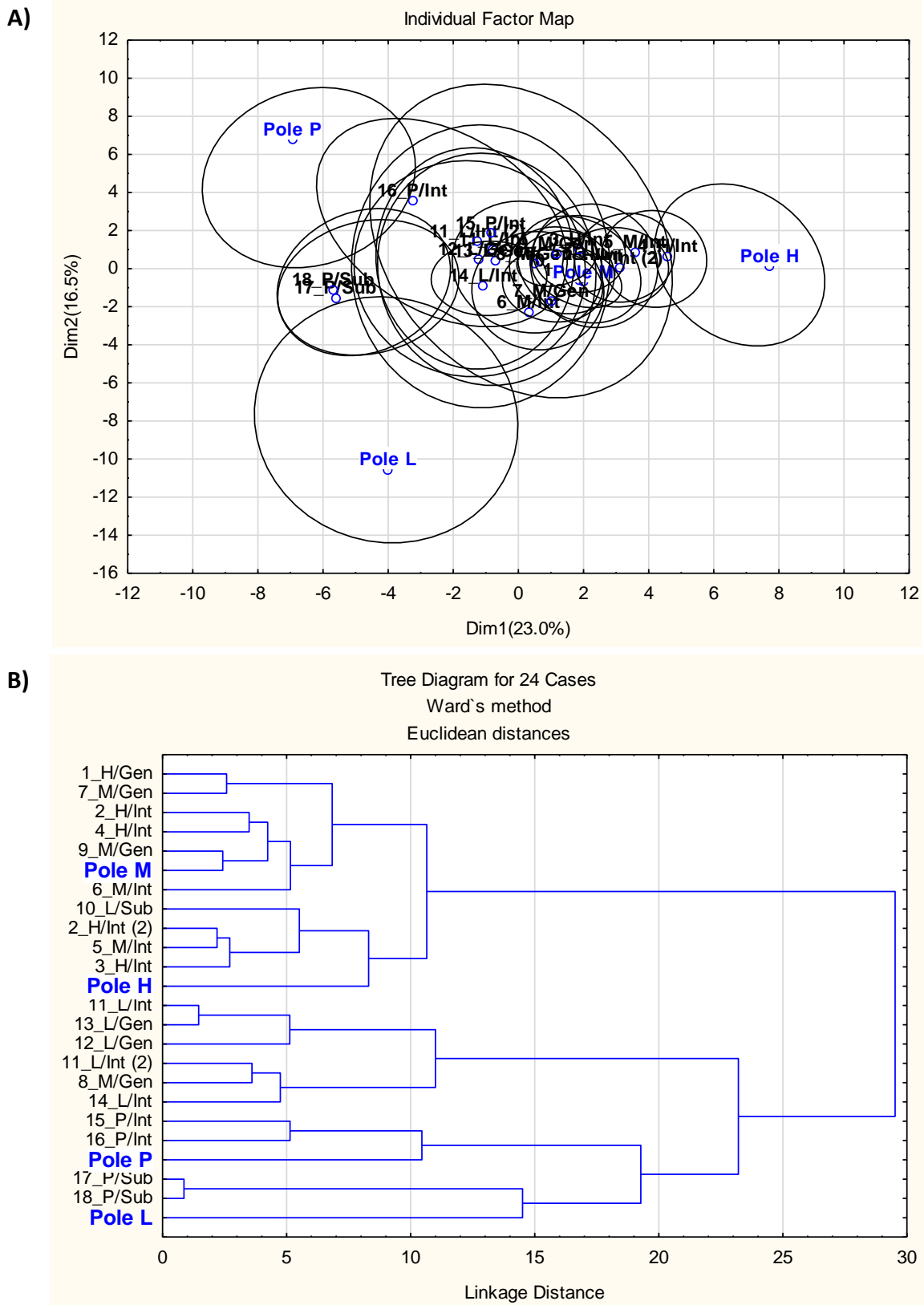


**Figure 6** **A)** Scores plot with confidence ellipses and **B)** cluster plot of MFA data for **PPM-t** performed by the **trained panel** [1–18 represent sample numbers; Int, Sub, Gen represent *C. intermedia*, *C. subternata* and *C. genistoides*, respectively; H, M, L, P represent high, moderate, low and poor quality, respectively; (2) after sample code indicates blind duplicate].



**Figure 7 A)** RV coefficients with 95% confidence intervals computed for product configurations obtained from DSA, PSP and PPM data, and **B)** Method ease of use: responses cited by **trained panel** (%) ( $N = 13$ ) ( $N = 0$  responses were obtained for 'very difficult' and 'difficult' categories).





**Figure 8** A) Scores plot with confidence ellipses and B) cluster analysis plot of MFA data for PPM-t performed by the **industry panel** [1–18 represent sample numbers; Int, Sub, Gen represent *C. intermedia*, *C. subternata* and *C. genistoides*, respectively; H, M, L, P represent high, moderate, low and poor quality, respectively; (2) after sample code indicates blind duplicate].

**Table 1** Sample set (n = 20), physical poles (n = 4) and theoretical poles (n = 4) used in the respective variations of PSP and PPM.

SAMPLES			PHYSICAL POLES			THEORETICAL POLES	
Sensory class <sup>1</sup>	quality	Sample code <sup>2</sup>	Pole	Samples <sup>2</sup> used for blend	Blend ratio	Pole	Pole definition <sup>3</sup>
High		1_H/Gen	POLE High	23_Gen	1	POLE High	'High HB character. High floral, fruity, sweet, woody. No taints.'
		2_H/Int		29_Int	1		
		2_H/Int (2)		34_Int	1		
		3_H/Int					
		4_H/Int					
Moderate		5_M/Int	POLE Moderate	8_M/Gen	1	POLE Moderate	'Moderate HB character. Moderate floral, fruity, sweet, woody. No taints.'
		6_M/Int		22_Gen	1		
		7_M/Gen		31_Int	1		
		8_M/Gen					
		9_M/Gen					
Low		10_L/Sub	POLE Low	36_Int	1	POLE Low	'Low HB character. Low floral, fruity, sweet, woody. Taints: green grass/burnt caramel/cooked vegetable.'
		11_L/Int		25_Gen	1		
		11_L/Int (2)		35_Int	1		
		12_L/Gen					
		13_L/Gen					
		14_L/Int					
Poor		15_P/Int	POLE Poor	16_P/Int	5	POLE Poor	'Poor HB character. Taints: medicinal/smoky.'
		16_P/Int		17_P/Sub	1		
		17_P/Sub		18_P/Sub	1		
		18_P/Sub					

<sup>1</sup> Sensory quality class pre-assigned based on DSA attribute intensities and definitions by expert focus groups (*Chapter 4*)

<sup>2</sup> Int = *C. intermedia*; Sub = *C. subternata*; Gen = *C. genistoides*; Blind duplicates are indicated by '(2)'

<sup>3</sup> Definitions as appeared on digital screens presented to assessors (HB = honeybush)

**Table 2** RV coefficients for the correlation between product configurations of the PCA bi-plot based on DSA data, and MFA plots for PSP with *physical poles* (PSP-p) and *theoretical poles* (PSP-t) and PPM with *physical poles* (PPM-p) and *theoretical poles* (PPM-t).

**A) RV COEFFICIENTS BETWEEN METHODS ( $p \leq 0.01$ )**

	DSA PCA bi-plot	PSP-p MFA map	PSP-t MFA map	PPM-p MFA map	PPM-t MFA map
DSA_PCA bi-plot	1	<b>0.83</b>	<b>0.73</b>	<b>0.74</b>	<b>0.68</b>
PSP-p_MFA map	-	1	<b>0.88</b>	0.91	0.81
PSP-t_MFA map	-	-	1	0.82	0.83
PPM-p_MFA map	-	-	-	1	<b>0.89</b>
PPM-t_MFA map	-	-	-	-	1

**B) RV COEFFICIENTS BETWEEN TEST REPLICATES (Rep1 and Rep2 per method variation) ( $p \leq 0.01$ )**

	DSA PCA bi-plot	PSP-p MFA map Rep1	PSP-p MFA map Rep2	PSP-t MFA map Rep1	PSP-t MFA map Rep2	PPM-p MFA map Rep1	PPM-p MFA map Rep2	PPM-t MFA map Rep1	PPM-t MFA map Rep2
DSA_PCA bi-plot	1	0.83	0.79	0.72	0.68	0.71	0.74	0.68	0.64
PSP-p_MFA map_Rep1	-	1	<b>0.90</b>	<b>0.85</b>	<b>0.85</b>	0.88	0.87	0.77	0.77
PSP-p_MFA map_Rep2	-	-	1	<b>0.79</b>	<b>0.80</b>	0.85	0.87	0.79	0.71
PSP-t_MFA map_Rep1	-	-	-	1	<b>0.86</b>	0.80	0.75	0.74	0.75
PSP-t_MFA map_Rep2	-	-	-	-	1	0.79	0.76	0.80	0.85
PPM-p_MFA map_Rep1	-	-	-	-	-	1	<b>0.93</b>	<b>0.88</b>	<b>0.86</b>
PPM-p_MFA map_Rep2	-	-	-	-	-	-	1	<b>0.85</b>	<b>0.83</b>
PPM-t_MFA map_Rep1	-	-	-	-	-	-	-	1	<b>0.94</b>
PPM-t_MFA map_Rep2	-	-	-	-	-	-	-	-	1

Addendum C (*Supplementary material Chapter 5*)

**Polarised Sensory Positioning Test**

1. You have been presented with four Honeybush samples (poles) labelled Q, R, S and T (coloured labels). These samples are the reference samples or poles, representative of four quality levels.
2. You have further been presented with 10 test samples, labelled with a three-digit code.
3. Mark the similarity or dissimilarity of each test sample relative to each pole using the line scale where 0 = similar to the pole while 100 = totally different from the pole.
4. You have to test each sample against all four poles.
5. Select the sample as indicated on the screen from the water bath.
6. You are allowed to taste and/or smell the samples as many times as you like. Please use the spoon for tasting the samples.
7. Place the samples back in the water-bath to keep the temperature consistent.
8. Take a 15 min break after sample 10, the program will indicate the break.
9. Please evaluate in silence.

You have been presented with test sample 950. Mark the test sample in comparison to each pole.

Pole Q - high quality

Exactly the same

Totally different



Pole R - moderate quality

Exactly the same

Totally different



Pole S - low quality

Exactly the same

Totally different



Pole T - poor quality

Exactly the same

Totally different



Take a break of 15 minutes before completing the next set of 10 samples.

**Figure C1** Instructions and questionnaire used for **PSP-p** task by the trained panel.


## Polarised Sensory Positioning Test

1. Four theoretical poles for Honeybush (HB) have been defined. The description of each pole will appear on the screen.
2. You have been presented with 10 test samples, labelled with a three-digit code.
3. Mark the similarity or dissimilarity of each test sample relative to each pole using the line scale where 0 = similar to the pole while 100 = totally different from the pole.
4. You have to test each sample against all four poles.
5. Select the sample as indicated on the screen from the water bath.
6. You are allowed to taste and/or smell the samples as many times as you like. Please use the spoon for tasting the samples.
7. Place the samples back in the water-bath to keep the temperature consistent.
8. Take a 15 min break after sample 10, the program will indicate the break.
9. Please evaluate in silence.

You have been presented with test sample 286. Mark the test sample in comparison to each pole.

Pole E &#61; **High HB character.** High floral, fruity, sweet, woody. *No taints.*

Exactly the same Totally different



Pole F &#61; **Moderate HB character.** Moderate floral, fruity, sweet, woody. *No taints.*

Exactly the same Totally different



Pole G &#61; **Low HB character.** Low floral, fruity, sweet, woody. *Taints: green grass/ burnt caramel / cooked vegetable.*

Exactly the same Totally different



Pole H &#61; **Poor HB character.** *Taints: medicinal / smokey.*

Exactly the same Totally different

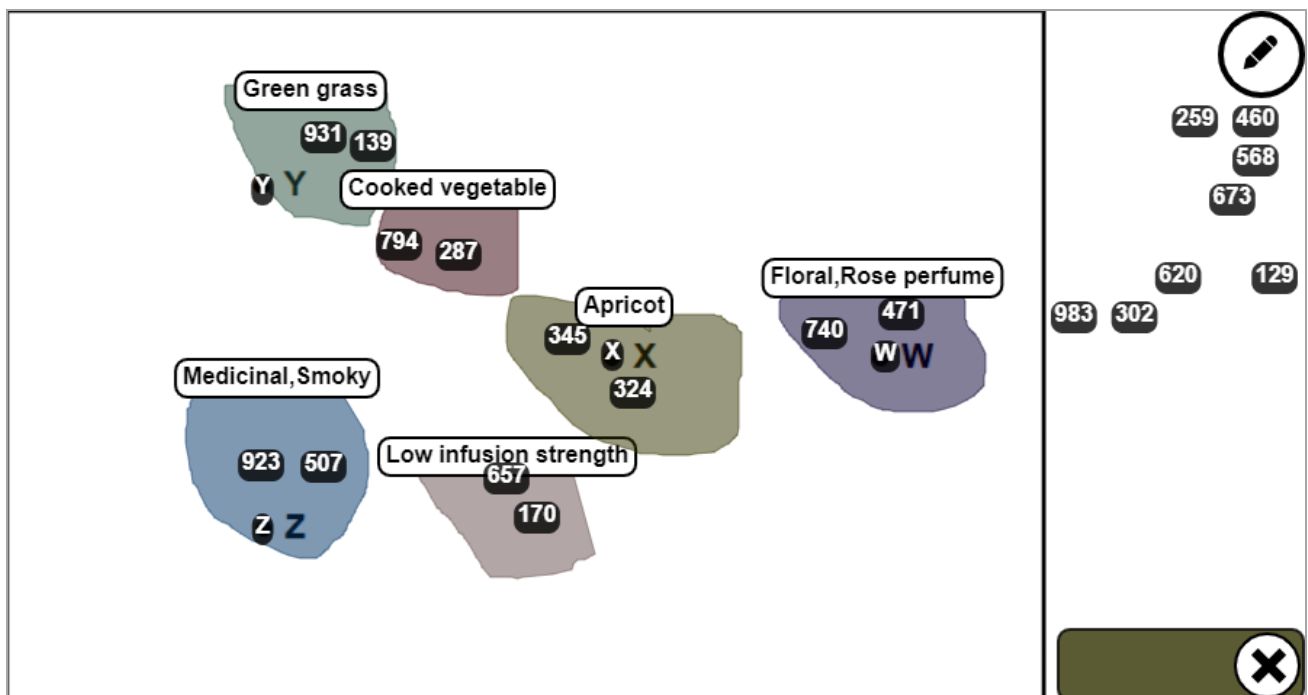


Take a break of 15 minutes before completing the next set of 10 samples.

**Figure C2** Instructions and questionnaire used for **PSP-t** task by the trained panel.

## Polarised Projective Mapping Test

1. You have been presented with four Honeybush samples (poles) labelled **W**, **X**, **Y** and **Z** (coloured labels).
2. These samples are the reference samples or poles, representative of four quality levels: **W=High quality**; **X=Moderate quality**; **Y=Low quality**; **Z=Poor quality**
3. Before evaluation of test samples, move the 4 poles onto the map by **dragging each pole exactly onto the dot (•)** of the corresponding letter on the map.
4. You have further been presented with 20 test samples, labelled with a three-digit code.
5. Mark the similarity or dissimilarity of each test sample relative to the poles by **dragging each test sample code** onto the map. Place test samples in relation to both the poles, and to one another with similar samples being closely located, and different samples being far from one another.
6. Add descriptors to samples or a group of samples by selecting the pencil icon, and highlighting the samples that you would like to describe. Enter descriptor(s) on the screen as indicated.
7. You are allowed to taste and/or smell the samples as many times as you like. Please use the spoon for tasting the samples.
8. Place the samples back in the water-bath to keep the temperature consistent.
9. Please evaluate in silence.



**Figure C3** Instructions used for **PPM-p** and **UFP** tasks by the trained panel and an example of a digital two-dimensional map of a trained assessor.

## Polarised Projective Mapping Test

1. **Four theoretical poles for Honeybush (HB)** have been defined. The description of each pole will appear on the map on the screen.
2. Before evaluation of test samples, move the 4 poles onto the map by **dragging each pole exactly onto the dot (•)** of the corresponding letter on the map.
3. You have further been presented with 20 test samples, labelled with a three-digit code.
4. Mark the similarity or dissimilarity of each test sample relative to the poles by **dragging each test sample code** onto the map. Place test samples in relation to both the poles, and to one another with similar samples being closely located, and different samples being far from one another.
5. You are allowed to taste and/or smell the samples as many times as you like. Please use the spoon for tasting the samples.
6. Place the samples back in the water-bath to keep the temperature consistent.
7. Please evaluate in silence.

<p style="text-align: center;">• <b>S</b></p> <p><b>Low HB Character.</b> Low floral, fruity, sweet, woody. <i>Taints: green grass / burnt caramel / cooked vegetable.</i></p> <p style="text-align: center;">• <b>T</b></p> <p><b>Poor HB Character.</b> <i>Taints: smokey / medicinal.</i></p>	<p style="text-align: center;">• <b>R</b></p> <p><b>Moderate HB Character.</b> Moderate floral, fruity, sweet, woody. <i>No taints.</i></p>	<p style="text-align: center;">• <b>Q</b></p> <p><b>High HB Character.</b> High floral, fruity, sweet, woody. <i>No taints.</i></p>	<table border="1"> <tbody> <tr><td>371</td><td>836</td><td>268</td><td>623</td></tr> <tr><td>176</td><td>705</td><td>431</td><td>591</td></tr> <tr><td>R</td><td>615</td><td>953</td><td>739</td><td>S</td></tr> <tr><td>894</td><td>581</td><td>380</td><td>240</td></tr> <tr><td>479</td><td>Q</td><td>502</td><td>T</td><td>148</td></tr> <tr><td>645</td><td>725</td><td></td><td></td><td></td></tr> </tbody> </table>	371	836	268	623	176	705	431	591	R	615	953	739	S	894	581	380	240	479	Q	502	T	148	645	725			
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645	725																													

**Figure C4** Instructions and digital two-dimensional map used for **PPM-t** task by the trained and industry panel, respectively (*letters for poles and three-digit codes for test samples to be dragged onto the map by the assessor, are indicated on the top right*).



**Table C1** Extracts of data matrixes for PSP and PPM with *physical* poles used for multiple factor analysis.

**I) Degree of distance between samples and respective poles for PSP-*p* task**

Sample	Assessor #1 [REP1]				Assessor #2 [REP1]				...	Assessor #13 [REP2]			
	Pole H	Pole M	Pole L	Pole P	Pole H	Pole M	Pole L	Pole P		Pole H	Pole M	Pole L	Pole P
1_H/Gen	36.8	18.5	43.9	62.2	0	30.1	100	100	...	2.4	100	100	100
2_H/Int	13.8	28.6	51.8	80.5	0	17.5	100	100	...	0.7	99.7	99.5	100
...	...	...	...	...	...	...	...	...	...	...	...	...	...
18_P/Sub	70.3	57.3	40.7	29.4	43.9	20.2	100	100	...	83.4	0	100	100

**II) X and Y coordinates for PPM-*p* task and UFP descriptor<sup>III</sup> citation frequencies**

Sample	Assessor #1 [REP1]		Assessor #2 [REP1]		...	Assessor #13 [REP2]		Cooked vegetables	Hay/Dried grass High	Green grass High	...	Spicy High
	X	Y	X	Y								
1_H/Gen	445	211	394	276	...			1	4	0	...	5
2_H/Int	393	244	648	286	...			0	4	0	...	4
...	...	...	...	...	...			...	...	...	...	...
18_P/Sub	282	353	194	376	...			10	6	9	...	1

**III) UFP descriptors for PPM-*p* task – condensed list in alphabetical order\***

Apricot	Caramel	Fruity High	Herbaceous	Raisin	Smoky Low
Bakelite	Chemical	Fruity Low	Honey	Rose geranium	Spicy High
Band aid	Cooked vegetables	Green grass High	Infusion strength Low	Rose perfume	Sweet High
Burnt	Dusty	Green grass Low	Medicinal	Rotting plant water	Sweet Low
Burnt caramel High	Floral High	Hay/Dried grass High	Nutty High	Seaweed	Under-fermented
Burnt vegetables	Floral Low	Hay/Dried grass Low	Pine High	Smoky High	Woody

\*Descriptors list after data pre-processing (condensing linguistic and semantic synonyms for descriptors and adjectives); descriptors highlighted in blue were cited <20 and excluded from further analyses.

# Chapter 6

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## General discussion and conclusions

The formal honeybush tea industry has rapidly grown over the past two decades from a local cottage industry to a participant in the global herbal tea trade, one of the most rapidly increasing segments of hot beverages (Euromonitor, 2019; Joubert et al., 2019). The sensory appeal of herbal teas is one of the key drivers of choice which creates market opportunities for new entries (Joubert, De Beer, & Malherbe, 2017). However, a major concern is the prevalence of honeybush tea of inconsistent and inferior sensory quality in the value chain (**Fig. 1**) which is detrimental for the reputation of honeybush and consumer confidence in the product (DAFF, 2016). This was also emphasised by various industry role-players when interviewed during the present study.

The need for an effective approach to deliver consistent quality products globally, i.e. universal quality control (QC), has been recognised for numerous agricultural commodities, such as olive oil (Langstaff, 2014) and coffee (Chambers et al., 2016; Feria-Morales, 2002), as well as for products with quality distinctiveness labels (Pérez-Elortondo et al., 2018). In view of its acknowledgement as a South African herbal tea on international level, honeybush has been included in the Geographical Indication (GI) Protocol of the Economic Participation Agreement with the EU. GI recognition enables consumers to trust and distinguish quality products (European Commission, 2019). Application for the registration of honeybush tea as Protected designation of origin (PDO) is in progress (D. Troskie, Department of Agriculture, Western Cape, personal communication, 2019). To provide PDO products of high quality, the definition of specific sensory characteristics and the objective control thereof, are required to assure their authenticity and to differentiate them from similar commercial products (Bertozi, 1995; Bertozi & Panari, 1993).

A general parallel could be drawn between the current status of sensory quality control of EU PDO products and that of traditional (oxidised) honeybush tea. No standardised approach for the development of official sensory quality control methods for PDO products exists, i.e. each PDO decides on the method to fulfil the legal requirement for certification which leads to unfair competition (Ojeda et al., 2015; Pérez-Elortondo et al., 2018). Similarly, no standardised method for sensory quality assessment within the honeybush industry exists, and each processor and packer apply their own sensory quality control measures. Furthermore, current SA export regulations for honeybush tea do not make provision for sensory quality (DAFF, 2019), whereas in many instances EU regulatory bodies stipulate only a general description

of the sensory characteristics that the PDO product in question must present (Ojeda et al., 2015; Pérez-Elortondo et al., 2018). The need for a reference database of sensory descriptors for different PDO products, including defects, to standardise the communication of sensory quality, has been recognised (Pérez-Elortondo et al., 2018). For honeybush, baseline data, generated during previous research, on the sensory attributes obtained through descriptive sensory analysis (DSA) of fermented honeybush tea samples (mainly processed on laboratory-scale) of different *Cyclophia* species exists. However, the need for a comprehensive dataset that encompasses variable sensory quality of the three major commercial *Cyclophia* species (*C. intermedia*, *C. subternata* and *C. genistoides*) has been identified and addressed in the present study (Chapter 3). The existing baseline dataset was combined with a new sensory attribute and physicochemical parameter dataset of samples processed on both laboratory- and commercial-scale. The relevant data were obtained through DSA and CIEL\*a\*b\* colour and turbidity analyses, respectively. The comprehensive dataset formed the foundation for the sensory lexicon/wheel, quality standard and method development in the present study.



**Figure 1** Proposed integration of the developed sensory quality grading system for the honeybush tea industry to ensure that a product of improved and consistent quality reaches the consumer.

Quality control of honeybush is critical, not only to deliver a consistent quality product to ensure the sustained growth of local and international honeybush tea markets, but also to ensure that good quality is not forfeited during breeding of 'superior' plant material (Joubert et al., 2019). The current honeybush breeding programme of the Agricultural Research Council therefore includes sensory quality as second tier evaluation criterium (Bester, Joubert, & Joubert, 2016). The need for a quality grading system that

encompasses a sensory quality standard and standardised methods for the evaluation and classification of fermented honeybush tea in terms of generic sensory quality, i.e. irrespective of *Cyclopia* species, was addressed in the present study. The study aim was to translate outcomes of on-going research focussing on the quality of fermented honeybush tea in terms sensory characterisation and process optimisation, into a user-friendly and scientifically founded quality grading system with newly developed and validated quality control elements. The objectives were to develop the required tools for role-players within commercial, research and regulatory environments to assess, differentiate and communicate the sensory quality of honeybush in a rapid, simple and reliable manner. Key research outcomes, limitations and recommendations for a revised **honeybush lexicon** with **universal chemical reference standards**, a newly developed **quality scoring method** that encompasses a user-friendly scorecard, and suitable **rapid quality classification methods** will be presented.

The first objective (*Chapter 3*) was to address the need for a revised **honeybush aroma lexicon** based on the newly established comprehensive dataset representative of the three major *Cyclopia* species. Additionally, **chemical-based reference standards** to illustrate individual aroma attributes for improved global understanding of the respective descriptions, were identified. A large number of aroma chemicals were screened. This entailed adding of the chemical to a 'base' honeybush tea to allow for matrix effects, followed by scoring of typicality and intensity by a trained panel, comparing the target aroma of the selected chemicals to the aroma perceived in the respective reference teas. The replacement of food-based reference standards in the previous version of the honeybush lexicon (Erasmus, 2014) with more universally available and stable chemicals, underpins the objective to train various industry role-players in a standardised quality grading system for honeybush tea. In addition, based on the newly established comprehensive dataset, the generic **honeybush aroma wheel** (Erasmus, 2014) was revised to represent the positive and negative aroma attributes, and the intensities thereof. The importance of lexicons as training and calibration tools in quality control has also been highlighted in literature on lexicon development for a variety of beverage products, e.g. hibiscus tea (Monteiro et al., 2017), yerba mate tea (Godoy, Chambers, & Yang, 2020) and coffee (Chambers et al., 2016). In addition, the role of lexicons in defining the specific quality characteristics of traditional products with qualitative distinctiveness labels, i.e. to distinguish them from atypical and/or inferior quality products, has been recognised. The universal lexicon would form the basis for training assessors in the recognition of honeybush attributes for quality control, and subsequently the development of a quality scoring method. Sensory lexicon development and reference standard selection were regarded as an essential step in the development of quality scoring methods for PDO wines (Etaio et al., 2010a, 2012).

Sensory lexicons can be regarded as 'living' documents and should be updated regularly as new information becomes available. A 'living' coffee lexicon was developed for the coffee industry (World Coffee Research, 2017) for which several commercial products were replaced by chemicals as lexicon

reference standards. Similarly, the honeybush sensory lexicon is a 'living' document as more typical chemical reference standards could be developed through further research. In the present study, high typicality scores were obtained for the majority of the chemicals selected to effectively represent the respective positive and negative aroma attributes in the lexicon. However, more suitable chemicals for taints such as 'cooked vegetables', 'burnt caramel' and 'rotting plant water' aromas would improve the lexicon even further, thereby improving effective assessor training in taint recognition for quality control. The typicality scores of the aroma of these three chemicals compared to the target aromas of the respective reference teas were low, and GC-O/GC-MS analyses of poor quality tea samples to identify better representative chemicals, is recommended. In addition, it is recommended that the rooibos lexicon attribute, 'musty/mouldy' aroma (Jolley, Van der Rijst, Joubert, & Muller, 2017) and a corresponding chemical reference standard should be included in the honeybush lexicon for quality control purposes. The presence of this negative aroma attribute may be ascribed to poor processing or storage conditions (Du Toit & Joubert, 1999). The revised honeybush sensory lexicon and wheel would serve as valid tools to improve interpretation and communication of the honeybush sensory vocabulary and ultimately, improve sensory quality assessment of honeybush tea across all industry sectors.

The second objective (*Chapter 4*) of the present study was to address the need to establish a **sensory quality standard** for fermented honeybush tea. A panel of honeybush experts was convened to establish key sensory quality parameters for which sound specifications were defined, based on the established comprehensive dataset. Industry responses from interviews and a survey were also considered in the selection of quality parameters. This intricate process signified the link to the important elements in quality control, i.e. representative sampling, incorporation of product variability and consideration of expert input and/or consumer input (Muñoz, 2002). The objective of the envisaged sensory quality grading system was to address quality and not consumer preference. Therefore, considering the relative short existence of the formal honeybush industry (Joubert et al., 2019) in which consumers have been exposed to products of variable quality, input from product experts, and not consumers, was used in the process of identifying quality parameters and defining specifications for the sensory quality classes. The definition of sensory quality standards by product experts, as opposed to consumers, is regarded as being more comprehensive and accurate (Ballester, Dacremont, Le Fur, & Etiévant, 2005; Ojeda et al., 2015). Similar approaches with input from sensory professionals and/or industry experts with thorough product knowledge were followed for the establishment of quality standards for omega-3 fish oil (Larssen et al., 2019), PDO wines (Etaio et al., 2010a, 2012) and cheese (Pérez-Elortondo et al., 2007), and meat (Etaio et al., 2013).

The third objective was to develop and validate **quality control methods** to assess, in a reliable manner, whether production batches comply with the requirements of the developed quality standard, and to classify each batch accordingly. The process of establishing a standard and developing a method to determine whether a product falls within the defined specifications exemplifies the common approach to

food quality control (Costell, 2002; Lawless & Heymann, 2010). The target industry sector and quality control application were considered in the selection and development of these methods, namely 1) the commercial sector that includes primary level processors (QC in on-farm/commercial oxidation and drying practices) and secondary level processors (QC/quality screening in commercial blending and packing practices, both locally and internationally), 2) the research sector (quality screening in e.g. plant breeding/cultivation programmes), and 3) the regulatory sector (non-statutory and GI/PDO regulatory control). All honeybush industry role-players throughout the value chain would contribute in delivering a final product of acceptable and consistent quality (**Fig. 1**).

For quality grading of honeybush tea, sensory classes and specifications for ‘high’, ‘moderate’, ‘low’ and ‘poor’ quality were defined: ‘High’ quality represents premium quality, ‘moderate’ quality represents the minimum accepted standard for a final product for consumption by the consumer, ‘low’ quality represents the minimum accepted standard to be used for batch blending, and ‘poor’ quality represents the level at which batches should be rejected. ‘Poor’ quality was ascribed to an insipid ‘overall character on palate’, high intensities of taints and/or the presence of specific critical taints, i.e. ‘smoky’, ‘medicinal’ and ‘musty’ flavours. The latter flavours were designated as critical taints as ‘smoky’ and ‘medicinal’ flavours cannot be blended with batches of higher quality to mask these taints, even when perceived at low intensity levels, whereas ‘musty’ flavour could be indicative of a potential safety risk due to microbial contamination. Specifications for the optimum quality were defined as 1) dry leaf appearance parameter: *even* cut size and *absence* of light stem particles, 2) infusion appearance parameter: *red-brown* colour and *absence* of haze, 3) infusion aroma parameter: high intensities for ‘floral’, ‘fruity and sweet’, ‘nutty and/or spicy’ and moderate intensity of ‘woody’ aroma, and 4) infusion palate attribute parameter: high intensity for ‘overall character on palate’, low intensity of ‘hay/dried grass’ flavour, low intensity/absence of bitter taste, and absence of sour taste and taints.

Considering application in the commercial and regulatory sectors of the honeybush industry, a user-friendly **quality scoring method** (*Chapter 4*) was developed to assess the sensory quality of production batches in a systematic manner (i.e. assessment of firstly appearance, followed by aroma and palate), and to classify the batch accordingly, based on the total score (%) obtained. Quality scoring methods are the most commonly used in food quality control (Rogers, 2010) and a wide range of linear and category scale types for scoring is applied in scorecards (Beeren, 2010; Lawless & Heymann, 2010). In the present study, the information collected in the development of the quality standard and establishment of the specifications, based on the comprehensive dataset, is encompassed in a scorecard for honeybush tea. The scorecard includes a scoring system with points assigned to parameter sub-categories using semantic category scales, as well as checkboxes for the citation of specific positive and negative attributes. The highest parameter weights (%) were assigned to the most critical parameter sub-categories, namely infusion colour, ‘overall character on palate’ and ‘taints’ (negative flavour attributes), whereas the presence

of infusion haze (indicated by a checkbox) was regarded as unacceptable, which would terminate the quality scoring process until further processing steps have been performed (indicated by action steps on the scorecard). An accompanying colour reference card was developed for the assessment of infusion colour.

The quality scoring method was developed through expert focus groups and information derived from the comprehensive dataset. Industry responses directed the choice of parameters and scales for inclusion in the scorecard. A similar approach, using expert focus groups, was followed for the development of quality scoring methods for the regulatory control of PDO wine and cheese products to ensure the sensory quality and protection of the products (Etaio et al., 2010a, 2012; Pérez-Elortondo et al., 2007).

The quality scoring method was validated by a trained panel, as well as industry representatives with different levels of experience in the assessment of the sensory quality of honeybush tea. The DSA data of the validation sample set, comprising of commercially processed batches of honeybush of variable sensory quality, was used as 'gold standard' for method validation. Quality classes were pre-assigned to the respective samples according to the DSA data. The scorecard results indicated that for both trained and industry panels, the majority of samples were classified correctly, irrespective of expertise' level of assessors. Classification was based on pre-determined total score (%) ranges per sensory quality class. In practice, the obtained total score would serve as a guideline for classification; however, individual parameter scores, as well as the citation frequencies, specifically that of negative attributes (taints), need to be considered before final classification of a production batch can be made. Correspondingly, accredited laboratories that perform sensory assessment for the certification of PDO products, compile a report that includes the mean score of each parameter, as well as citation frequencies of specific negative and positive attributes, to provide an overview of the batch quality (Etaio et al., 2010a, 2012; Ojeda et al., 2015; Pérez-Elortondo et al., 2007).

The present study demonstrated that sensory attribute data obtained through DSA were effectively translated into scorecard parameters to provide representative descriptions of the sensory quality of honeybush tea. The scorecard provides detailed information that may be used for compilation of report documents (certificates of analysis) and for batch traceability in routine QC assessment within a tea processing and blending/packing environment. The provision of checkboxes for citation of specific taints would aid QC assessors in the decision-making process to determine whether a batch may be blended with higher quality batches or should be rejected entirely due to citations for 'medicinal', 'smoky' and/or 'musty' flavours. Furthermore, the provision of checkboxes for citation of specific positive aroma attributes such as 'rose perfume', 'apricot' and 'raisin' aromas may aid as distinguishing factor amongst samples of moderate to high quality. This would also form a foundation for distinguishing and reporting species-specific differences for niche markets that require specific sensory attributes in a production batch.



The quality scoring method could also find application in international herbal tea blending/packing companies. For example, honeybush is listed as herbal plant by the Tea and Herbal Infusions Europe (THIE), which represents the interests of producers and traders of tea (*Camellia sinensis*) and herbal infusions in the EU (THIE, 2019). Although basic guidelines for the assessment of the sensory quality of herbal tea infusions in terms of infusion colour, aroma and flavour are provided (THIE, 2018), currently no THIE sensory specifications for honeybush exists (L. Mönch-Sander, THIE, personal communication, 2018). The newly developed quality standard and scoring method would provide international herbal tea blending and packing companies the required QC tools to distinguish moderate to high quality products from inferior ones, which would contribute to the reputation and market share of honeybush tea. The method would also encourage the effective marketing of honeybush tea based on its unique sensory qualities.

In the present study, industry and trained assessors were able to discriminate samples, similarly, based on critical parameters, 'infusion colour' and 'overall character on palate'. The accurate interpretation of semantic category scales for these two parameters and use of a colour reference card for assessor alignment were indicated. However, higher discrimination between samples was obtained by the trained panel based on parameter scores. The need for industry assessor training in parameter recognition and scale interpretation to facilitate quality scoring and improve batch discrimination, was identified. The revised honeybush lexicon and wheel (*Chapter 3*) will aid in assessor training and calibration. However, the development of additional quantitative reference standards is recommended to aid assessors in anchoring of the scorecard parameter scales. Quantitative reference standards representative of 'moderate' or 'high' intensities, specifically for parameters that are regarded as defects at higher intensities ('woody' and 'hay/dried grass') are recommended. Quantitative reference standards for the more intricate 'overall character on palate' parameter are also required. In the present study, the development of chemical-based reference standards for only aroma attributes was considered. However, from industry responses and focus groups in the development of the quality scoring method, the importance of sensory quality on 'palate', i.e. flavour, taste and mouthfeel, was emphasised. As the developed chemical-based reference standards are food-grade, these chemicals may be used as foundation for the development of certain critical reference standards for palate attributes. The development of defect wheels (Langstaff, 2016) for improved taint recognition may also be considered. In addition, the development of CIEL\*a\*b\* colour specifications for the instrumental analysis of infusion colour could find application in larger companies for supplementary quality control purposes.

For small sized panels (<5 assessors), assessor training and good sensory practices are important (Lawless & Heymann, 2010; Lawless, Liu, & Goldwyn, 1997) for effective sensory quality assessment of honeybush tea. Honeybush industry assessors would benefit from a user-friendly manual that includes general good sensory practice guidelines/protocols for panel training using the honeybush sensory lexicon and wheel, sample preparation and temperature control, randomised sample presentation during testing,

inclusion of blind duplicates, as well as a flow diagram for sample assessment. Furthermore, the limitations of using paper ballots for quality scoring have been recognised and a computerised system is recommended to ensure that scorecards are completed before submission, as well as to ease data capturing and analyses.

Following the development of the quality scoring method for which a high level of detail per production batch is obtained, the need for a more time-efficient quality screening tool for the comparison of batches based on key sensory quality parameters, within commercial and research environments, was addressed (*Chapter 5*). **Figure 2** presents a comparison of the quality scoring method and rapid quality classification methods to DSA in terms of validity, discrimination ability, ease of use, time to conduct test, level of expertise required, method objective and application. The application of two **reference-based rapid sensory methods**, namely polarised sensory positioning (PSP) and polarised projective mapping (PPM), were selected for their advantage of data aggregation for batch comparison over time (Ares, Antúnez, De Saldamando, & Giménez, 2018). Two variations of each method were compared and validated, namely the use of *physical* poles (shelf-stable tea infusions representing each of the four newly established sensory quality classes for fermented honeybush tea), i.e. PSP-*p* and PPM-*p*, and *theoretical* poles (written descriptions of the key sensory attributes represented by each quality class), i.e. PSP-*t* and PPM-*t*. The use of *theoretical* poles for PSP and PPM is presented and validated here for the first time, as well its application within routine sensory QC. These method variations would contribute to the scientific body of knowledge of the dynamic field of rapid sensory profiling methods within sensory science.

FACTOR	DESCRIPTIVE SENSORY ANALYSIS	QUALITY SCORING METHOD	RAPID QUALITY CLASSIFICATION METHODS
1) Validity vs DSA	n/a	Yes	Yes
2) Discrimination ability	High	Moderate – High	Moderate – High
3) Ease of use	Intricate	Easy	Easy
4) Time to conduct test [n = 18 test samples]	ca. 6 hours	1 – 2 hours	< 1 hour
5) Level of expertise required	High	High	High
6) Method objective – <i>To determine:</i>	Full sensory profile [attribute intensities]	Sensory quality [classification based on scores + citation frequencies]	Sensory quality [classification based on key attributes of references]
7) Application	Research	Commercial QC + Regulatory control	Commercial QC + Research

**Figure 2** Comparison of the quality scoring method and rapid quality classification methods, PSP and PPM, to DSA.

Similar to the validation of the quality scoring method, the results of the rapid methods were compared to that of DSA ('gold standard'). Product configurations for PSP and PPM similar to that of DSA demonstrated the validity of the method variations for broad quality classification based on key sensory quality parameters, although PPM-*p* indicated the highest discrimination ability between the quality classes. The results indicated that both variations of PSP and PPM were able to discriminate to a certain extent between test samples according to their pre-assigned quality classes, as for DSA. However, no clear distinction between high and moderate quality classes was obtained and samples were classified into 'moderate to high', 'low' and 'poor' quality groupings with distinction primarily based on the presence of specific taints. For PPM-*p*, three significantly different quality groupings were obtained with visual inspection of the plot. Better discrimination could be ascribed to the complimentary ultra-flash profiling step, which may have guided assessors to position samples closer to the respective poles.

Poor distinction between moderate and high quality was ascribed to the large overlap between attributes in the high and moderate quality classes. The rapid methods applied in the present study do not account for differences in intensities but only for broad characterisation, based on a few key attributes. The importance of good discrimination between high and moderate sensory quality, as well as the selection of a method variation, would depend on the intended application. Firstly, for breeding/cultivation research programs, plant material of selections and genotypes would be processed on laboratory-scale according to optimum processing parameters under highly controlled conditions. These batches would likely be of moderate to high quality. Therefore, for research application, discrimination between high and moderate quality is critical. Amendments to *theoretical* pole descriptions (inclusion of 'hay/dried grass' and 'overall character on palate' for high and moderate poles) and the addition of pre-defined 'CATA' (check-all-that-apply) checklists for both *physical* and *theoretical* poles, are recommended for improved discrimination ability between high and moderate quality. However, in a commercial environment that is exposed to production batches of variable quality from different commercial processors, discrimination between 'poor' and 'low' quality, compared to 'moderate to high' quality, would be more important. Furthermore, stable *physical* poles that correctly represent the respective quality classes would be more readily available in a research environment, than in the commercial environment, for which *theoretical* poles are recommended, provided that assessors are highly trained in the key attributes described in the *theoretical* poles.

The discrimination ability of PPM-*t* was tested by the panel of representatives of the honeybush industry with varying expertise in the evaluation of the sensory quality of honeybush. No distinct classification groupings were obtained and as for the quality scoring method, the need for industry assessor training on the honeybush sensory lexicon attributes to facilitate assessment was clearly indicated. The revised lexicon and wheel, with the improvements recommended in this chapter, would support and equip industry assessors to apply the integrated quality grading system (**Fig.1**). Both PSP and PPM have been broadly included into sensory software programmes which enables the use of computers, tablets, etc. for

user-friendly application, electronic data capturing and automated data analyses. Despite the cost implication of such software programmes, it would make PSP and PPM attractive rapid quality screening tools within research, and future application by large companies.

In future, the developed quality scoring method could be used as basis for the sensory quality certification of fermented honeybush tea, for example within a regulatory control context (GI/PDO control). There exists a demand for the standardisation and accreditation of sensory quality evaluation methods to certify food products, to ensure that the sensory quality of food products with specific sensory characteristics, specifically products with PDO status, are assessed in a reliable manner (Pérez-Elortondo et al., 2007, 2018). Successful accreditation of sensory quality scoring methods for the control and improvement of the quality characteristics of products with PDO status, has been reported (Etaio et al., 2010b; Ojeda et al., 2015; Pérez-Elortondo et al., 2007). Future accreditation would give the quality scoring method for honeybush tea and quality class assigned to a production batch credibility within the national and international herbal tea market, i.e. enhance the 'quality seal' of honeybush tea. In addition, the methodical approach used in the present study could be applied to develop a sensory quality standard and scoring method for classification of green honeybush tea, an increasingly popular product that has seen growth in the herbal tea market alongside green rooibos tea.

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