Effects of steam treatment and storage on green honeybush quality

by

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Declaration

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

Lara Alexander
December 2015

Summary

Application of steam treatment to green *Cyclopia maculata* and *C. longifolia* to modulate their aroma and flavour profile, without detrimental effects on colour and individual phenolic content, was investigated. Steam treatment (96 °C, atmospheric pressure) of different time periods was applied to the shredded, fresh plant material before drying (**STBD**; 0, 30, 60, 90 and 120 s) or the herbal tea product after drying (**STAD**; 0, 1, 2, 3, and 4 min). Steam treatment of 60 s or longer resulted in a significant ($p < 0.05$) decrease in *vegetative* ('green grass' and 'hay/dried grass') and *cereal* ('oats/porridge/grains') aroma and flavour intensities, with an increase in some *fruity* ('tropical fruit' and 'guava') and 'fruity-sweet' aroma intensities. These changes manifested to a greater degree for plant material subjected to STBD, while they were less prominent for *C. longifolia* compared to *C. maculata.* Additionally, 19 aroma compounds were instrumentally identified in the volatile fraction of *C. maculata* infusions prepared from STBD plant material. Of these, seven correlated well with green honeybush *vegetative* and *cereal* aroma attributes associated with unsteamed samples. Steaming thus resulted in the rapid loss of some of these highly volatile 'green'-associated aroma compounds. Steam treatment of more than 60 s, regardless of STBD or STAD, was not detrimental to green colour, and individual phenolic content was not greatly affected. The 30 s STBD treatment of *C. maculata*, however, led to the rapid loss of green colour and oxidation of phenolic compounds, without considerable aroma improvement. This highlights the need for process control during green honeybush production.

Storage stability of steamed (60 s, STBD) and unsteamed green *C. maculata* was investigated under low (0 °C; moisture impermeable packaging) and normal (25 °C at 60% relative humidity (RH); semi-moisture-permeable sachets) temperature storage conditions (**LTS** and **NTS**, respectively) over 6 months and high temperature storage (**HTS**) conditions (40 °C at 75% RH; semi-moisture-permeable sachets) for 1 month. HTS conditions, after 1 month, seemed to emulate changes occurring over 6 months at NTS conditions, with little change detected at LTS conditions. Despite noticeable green colour loss over the respective storage periods at NTS and HTS conditions, individual phenolic compounds were not severely affected. NTS and HTS led to the progressive development of sought-after fermented honeybush sensory attributes, especially in unsteamed samples. These include prominent *fruity* ('stewed fruit', 'apricot jam' and 'marmalade') aromas and flavours, 'general sweet' and 'fruity-sweet' aromas and a sweeter taste.

The collated descriptive sensory data were used to generate a preliminary sensory wheel for green honeybush aroma, and another for flavour, taste and mouthfeel. The sensory profile of green honeybush was finally described as a dominant *vegetative* aroma and flavour, prominent *sweet-associated* and slightly *fruity* aroma, with sweet and notably bitter tastes and an astringent mouthfeel. By combining the sensory data, it was clear that the major effects of storage outweighed those of steam treatments, suggesting that storage of 3 to 6 months may improve sensory quality, although

colour may be slightly compromised. Immediate sensory manipulation and thus improvement, however, may be achieved to a lesser degree in the short term by STBD.

Uittreksel

Die toepassing van stoombehandeling van groen *Cyclopia maculata* en *C. longifolia* vir die modulasie van hul aroma en geur profiel, sonder gepaardgaande nadelige gevolge op kleur en individuele fenoliese inhoud, is ondersoek. Stoombehandeling (96 °C, atmosferiese druk) van verskeie tydstippe is toegepas op die gekerfde, vars plantmateriaal voor droging (**SBVD**; 0, 30, 60, 90 en 120 s) of die groen teeproduk ná droging (**SBND**; 0, 1, 2, 3 en 4 min). Stoombehandeling van 60 s of langer het tot 'n betekenisvolle ($p < 0.05$) afname in *vegetatiewe* ('groen gras' en 'hooi/gedroogde gras') en *graan* ('hawer/pap/graan') aroma en geur intensiteite gelei, met 'n toename in sommige *vrugtige* ('tropiese vrugte' en 'koejawel') en 'vrugtige-soet' aroma intensiteite. Hierdie veranderinge was meer merkbaar in die SBVD behandeling, terwyl *C. longifolia* minder vatbaar vir hierdie veranderinge was as *C. maculata.* Daarbenewens is 19 aromaverbindings in die vlugtige fraksie van *C. maculata* aftreksel voorberei van SBVD plantmateriaal instrumenteel geïdentifiseer. Sewe hiervan het goed gekorreleer met heuningbos *vegetatiewe* en *graan* aromas wat verband hou met ongestoomde monsters. Stoombehandeling het dus gelei tot die vinnige verlies van sommige van hierdie baie vlugtige 'groen'-geassosieërde aromaverbindings. Stoombehandeling vir langer as 60 s, ongeag van SBVD of SBND, het nie die groen kleur en individuele fenoliese inhoud grootliks nadelig beïnvloed nie. 'n SBVD behandeling van 30 s het egter tot die vinnige verlies van groen kleur en oksidasie van fenoliese verbindings, sonder aansienlike aroma verbetering van *C. maculata* gelei. Dít beklemtoon die noodsaaklikheid vir prosesbeheer tydens groen heuningbosproduksie.

Stabiliteit van gestoomde (60 s, SBVD) en ongestoomde groen *C. maculata* tydens opberging onder lae (0 °C; vog-ondeurlaatbare verpakking) en normale (25 °C by 60% relatiewe humiditeit (RH); semi-vogdeurlaatbare sakkies) temperatuur opbergingtoestande (**LTO** en **NTO**, onderskeidelik) oor 6 maande en hoë temperatuur opbergingtoestande (40 °C by 75% RH; semi-vogdeurlaatbare sakkies; **HTO**) vir 1 maand, is ondersoek. HTO het na 1 maand soortgelyke veranderinge teweeggebring as NTO na 6 maande, terwyl min verandering by LTO bespeur is. Ten spyte van merkbare groen kleur verlies oor die onderskeie tydperke by NTO en HTO is individuele fenoliese verbindings min beinvloed. NTO en HTO het gelei tot die progresiewe ontwikkeling van gesogte sensoriese eienskappe van gefermenteerde heuningbos, veral in ongestoomde monsters. Dit sluit in prominente *vrugtige* ('gestoofde vrugte', 'appelkooskonfyt' en 'marmelade') aromas en geure , 'algemene soet' en 'vrugtige-soet' aromas en 'n soeter smaak.

Al die beskrywende sensoriese data is saamgestel om 'n voorlopige sensoriese wiel vir groen heuningbos aroma op te stel, asook een vir geur, smaak en mondgevoel. Die sensoriese profiel van groen heuningbos kan beskryf word as 'n oorheersende *vegetatiewe* aroma en geur, prominente *soet-verwante* en effens *vrugtige* aroma, met soet en veral bitter smaak en 'n vrank mondgevoel. Deur die sensoriese data te kombineer kon duidelik uitgewys word dat die belangrikste gevolge van opberging dié van stoombehandeling oortref. Dit dui daarop dat opberging van 3 tot 6 maande

sensoriese kwaliteit kan verbeter, hoewel kleur effens benadeel kan word. In die kort termyn kan onmiddellike sensoriese manipulasie en dus verbetering egter in 'n mindere mate deur SBVD bereik word.

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‘Where there’s tea, there’s hope.’ – A. W. Pinero

Notes

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

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Chapter 1
General introduction

Green and herbal teas have been a cultural presence in many parts of the world for centuries, largely as a result of the health benefits they are believed to provide. Modern society is interested in the contribution that food and beverage products, such as teas, can deliver towards a healthier lifestyle, as illustrated by the phenomenal success achieved by the South African rooibos (*Aspalathus linearis*) herbal tea industry (Joubert *et al*., 2008a; Joubert *et al*., 2011). By comparison, the South African honeybush (*Cyclopia* spp.) industry is still under-developed, although research into the health-promoting properties of this indigenous herbal tea and the interest generated in the product would suggest that it could emulate the success of rooibos (Joubert *et al*., 2011). The honeybush industry is currently represented by its major product: traditional, 'fermented' honeybush tea. This dark-brown coloured product is produced by subjecting the fresh, shredded leaves and shoots to a high-temperature oxidation process commonly referred to as 'fermentation'. The loss of bioactive compounds that takes place during fermentation (Joubert *et al*., 2008b; Beelders *et al*., 2015) has created a market for green, 'unfermented' honeybush, superior in phenolic content and bioactivity (Joubert *et al*., 2011). Green honeybush, therefore, presents a value-added alternative to fermented honeybush, and is important for market diversification necessary for the sustainability of the honeybush industry.

It is crucial that processes are in place to deliver high quality honeybush and honeybush value-added products, to ensure sustained growth and demand by global and local markets. Process optimisation, and effective quality assurance and control systems are necessary for the production of high quality products (Muñoz, 2002). Development of such systems is supported by the understanding of factors affecting product quality and the identification of measurable parameters for assessment. Despite the knowledge that health-promoting compounds are retained to a higher degree in green honeybush when compared to its fermented counterpart (Joubert *et al.*, 2008b), green honeybush and its production have not received much attention to date. Furthermore, regulatory guidelines are vague regarding green honeybush product quality required for export (Anon., 2002). Regulations are not completely descriptive, with colour guidelines stipulating only that green honeybush "may not contain more than 5% brown fermented leaves per 10 g dry leaf material" (Anon., 2002). The guideline for the sensory quality of green honeybush is equally ambiguous and stipulates, similarly to that of fermented honeybush, that it is required to represent "the clean, characteristic taste and aroma of honeybush" (Anon., 2002). The need for more precise and quantifiable parameters is thus urgent and necessary not only for the production of high quality green honeybush, but also to prevent confusion between green and fermented honeybush quality standards.

Various processing techniques are used for the production of green (*Camellia sinensis*) tea and herbal teas in order to enhance the flavour and aroma profiles or to retain important compounds (as reviewed by Zhao *et al*., 2013). These processes may include roasting and steaming to various degrees and at different processing stages. Steam treatment of the fresh leaves of green (*C. sinensis*) tea not only alters the sensory profile of the product by enhancing aroma and flavour,

but it also preserves the green colour and phenolic composition of the tea material by inactivating enzymes present in the leaves that are responsible for oxidative changes (Chu, 1997; Takeo, 1992; Senanayake, 2013). Similarly, the application of steam also shows potential for enhancing the quality of green honeybush. In the first, and to date the only study conducted on the effects of green honeybush processing, Joubert *et al.* (2010) investigated the effects of various steam treatments on the colour and phenolic composition of green *C. subternata*. It was demonstrated that steam treatment of the shredded, fresh plant material prior to drying effectively prevented the degradation of green colour and phenolic compounds, compared to the shredded plant material not subjected to steam treatment prior to drying. This steam treatment, however, was found to result in an accelerated loss of green colour during storage when compared to plant material that had not undergone steaming. The treated plant material was, however, not subjected to sensory analysis and thus no information is available on the effect of steam treatment on the aroma and flavour profile of green honeybush. Furthermore, the application of steam to the dry material has also been used for the pasteurisation of teas, such as rooibos (Koch *et al*., 2013) and *Lippia multiflora* (Arthur *et al*., 2011a,b) in order to decrease their microbial load, ensuring a product safe for consumption. These positive effects of steam treatment, however, will not be useful if overall quality and purchase-desirability are compromised. For example, Koch *et al*. (2013) demonstrated that steam treatment of the dried, fermented rooibos brought about significant changes in its flavour profile, decreasing negative attributes such as ‘grass’ and ‘hay’, but detrimentally affecting ‘caramel’ aroma, a sought after attribute in rooibos tea of high quality.

Traditionally, the quality assessment of green and herbal teas has largely been of an artisanal approach, with trained and apprenticed tea makers grading tea according to their own subjective sensory testing procedures. Typical parameters would include appearance of the tea leaves in terms of colour as well as infusion colour, aroma, taste and flavour (Wang *et al*., 2000). Where tea products are aimed at health conscious individuals or are to be used for nutraceutical or cosmetic products, phenolic constituents are of importance as these contribute to the bioactive properties of the product. For this reason, additional quality parameters must be considered. Advanced chromatographic techniques such as high-performance liquid chromatography (HPLC) analysis can be used to quantify phenolic compounds of interest. Various methods have been developed specifically for the analysis of honeybush extracts prepared from several *Cyclopia* spp. (De Beer & Joubert, 2010; De Beer *et al*., 2012; Schulze *et al*., 2014; Beelders *et al*., 2014). Colour is a defining characteristic of any product and in many cases a measure of economic worth or the predictor of other quality characteristics (Wei *et al*., 2012). Colour can be defined in terms of the CIE*L*a*b** colour space and is measured using spectrophotometric equipment or a colorimeter. Descriptive sensory analysis (DSA) is the method of choice for the profiling of aroma and flavour attributes (Lawless & Heymann, 2010) and may be used to provide insight into changes in the sensory profile due to treatments. Profiling also enables the development of sensory wheels which may be used in quality assessment. Indeed, recent studies have focussed on the development of sensory wheels for the quality

assessment of fermented rooibos and honeybush (Koch *et al*., 2012; Theron *et al*., 2014; Bergh, 2014; Erasmus, 2015).

In view of the above, the current study investigated the effects of steam processing, applied before or after drying, on the quality of green (unfermented) honeybush. Two species, *C. maculata* and *C. longifolia*, were studied, as both show potential for cultivation by the honeybush industry (Joubert *et al*., 2011). One of the species, *C. maculata*, was further assessed in terms of the effects of steam treatment of the fresh plant material on the stability of the dried product during storage. Quality was assessed in terms of the sensory profile (aroma, flavour, taste and mouthfeel), instrumental colour and individual phenolic content. In addition, preliminary sensory wheels were developed as a quality assessment tool for green honeybush. Overall, the results obtained will aid in understanding the effects of processing and storage on the quality of green honeybush, and aim to provide a basis of insight for further investigation on the consistent production of superior quality green honeybush.

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Chapter 2
Literature review

Contents

1. Introduction

The growing popularity of honeybush as a health-promoting herbal tea and for preparation of food ingredient extracts has prompted the production of green, unfermented honeybush with superior levels of health promoting phenolic constituents (Joubert *et al*., 2011). Local and foreign markets have shown increasing demand for honeybush, although a limited supply is offered by the current industry. This has highlighted the need for defined quality parameters and processing methods in order to decrease losses due to poor quality and to deliver a consistent, high quality product to the market. This chapter aims to present and analyse the current state of knowledge relating to honeybush processing and quality parameters, with emphasis on green honeybush. As background, general aspects of honeybush and its production will be discussed. Furthermore, the limited research conducted on green honeybush to date has prompted review of green tea (*Camellia sinensis*) processing and the effects of processing on end product quality, with the aim of providing insight into the possible behaviour of green honeybush during and as a result of similar processing. The relevance of identified quality parameters and methods by which these can be quantified or analysed will also be considered and discussed.

2. Honeybush (*Cyclopia* spp.)

2.1 History, botany & natural distribution

In literature, the first reference to honeybush (*Cyclopia* spp.) was as early as the year 1705 (Kies, 1951), although popular knowledge of this plant with health-beneficial properties remained limited to its indigenous areas in South Africa until the twentieth century. The honeybush plant has been consumed as a traditional herbal tea in its regional areas and was first recorded as such in the 1770s by a Swedish botanist, C. Thunberg, who noted the Dutch name ‘honigtee’ as used by settlers in the Cape (as reviewed by Joubert *et al*., 2011). These people were aware of the health promoting properties of the honeybush infusion even then, and used it to treat minor intestinal and pulmonary ailments as well as to stimulate the appetite (as reviewed by Joubert *et al*., 2008a). The product used as a herbal tea was processed using various crude techniques including heating in baking ovens (Du Toit & Joubert, 1998), thus forming the basis for the high-temperature oxidation process applied in the production of fermented honeybush today (Joubert *et al*., 2011).

The dicotyledonous *Cyclopia* spp. belong to the family Fabaceae (tribe Podalyrieae) and occur endemically in various areas in the fynbos biome of South Africa, mainly the mountainous and coastal regions of the Western and Eastern Cape Provinces (**Fig. 2.1**). The current 23 species of the *Cyclopia* genus, like many other fynbos plants, have developed strategies to survive the destructive forces of veld-fires by re-seeding or re-sprouting, dividing the genus into two groups (as reviewed by Joubert *et al*., 2011). When observed in the wild, the mature bushes have woody stems and a low leaf-to-stem ratio, growing up to 3 m tall (Joubert *et al*., 2008a). The species all possess tri-foliate leaves often differing in appearance, with leaf shape and size ranging from pubescent,

narrow leaves to flat leaves (**Fig. 2.2**) and deep yellow, sweet (honey-like) smelling flowers with an indented calyx (as reviewed by Joubert *et al*., 2008a). Most plants usually flower during September and October in the spring season (Joubert *et al*., 2008a).

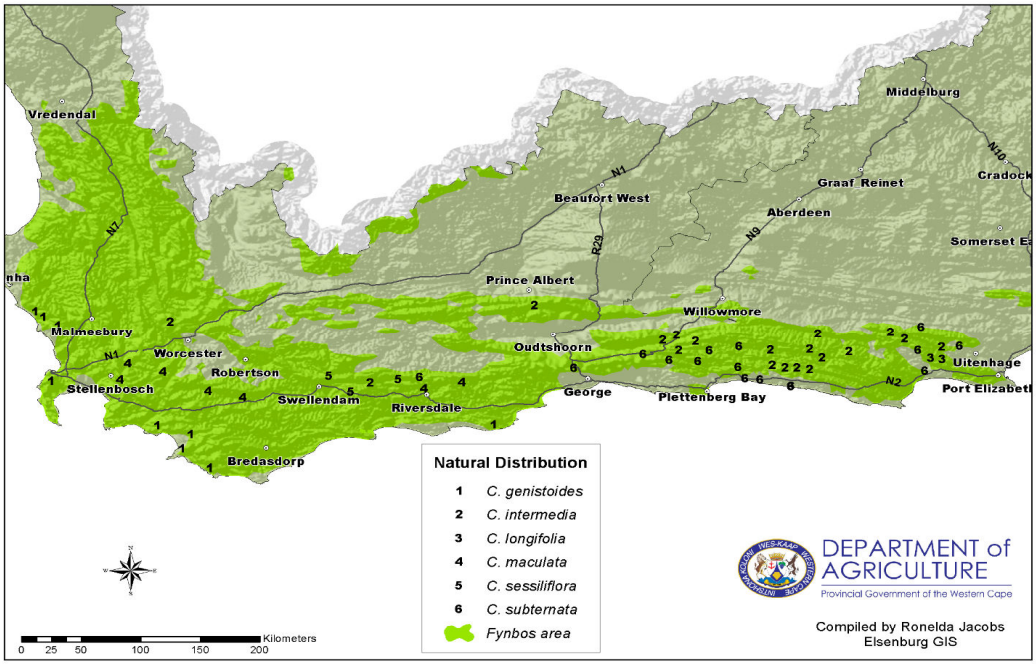

Figure 2.1 Natural occurrence for some *Cyclopia* (honeybush) species within South Africa (Joubert *et al*., 2011).

Figure 2.2 Leaf shape of a) *Cyclopia genistoides*, b) *Cyclopia intermedia*, c) *Cyclopia longifolia* and d) *Cyclopia maculata*.

Since the early 1990s, initial research by the South African National Biodiversity Institute (SANBI), and then later the Agricultural Research Council (ARC), has been committed to the establishment of a formal honeybush industry in South Africa. This follows with the hope of achieving similar success to the rooibos (*Aspalathus linearis*) industry (Joubert *et al*., 2011). Current production of honeybush rests mainly on three species, namely *C. genistoides*, *C. subternata* and *C. intermedia.* The latter species is mostly harvested from the wild (Joubert *et al*., 2011). Other species, either harvested from the wild or under limited commercial cultivation, include *C. maculata*, *C. sessiliflora* and *C. longifolia* (Joubert *et al*., 2011). According to the National Assessment of the Red List of South African Plants, both *C. maculata* and *C. longifolia* are species of conservation concern with declining natural populations and are under threat from alien plant invasion (**Table 2.1**). The

cultivation of these species for commercial use is thus critical. *Cyclopia maculata* is commonly found in the Western Cape, more widely spread than natural populations of *C. longifolia* occurring in the Eastern Cape (**Table 2.1**).

Table 2.1 Natural occurrence of *C. maculata* and *C. longifolia* according to the Red list of South African Plants (Berrington *et al*., 2011; Vlok & Raimondo, 2011)

Scientific name	***Cyclopia maculata* (Andrews) Kies**	***Cyclopia longifolia* Vogel**
National status	Near threatened	Critically endangered
Threats	Water abstraction, alien acacia invasion, vulnerable to overharvesting	Alien plant invasion, inappropriate fire management, afforestation
Range and distribution	Western Cape, Bain's Kloof to Riversdale 11 226 km^2	Eastern Cape, Van Stadens mountains 31 km^2
Habitat	Riverbanks and stream sides in lowland	Moist sandy soil along river banks
Elevation	150 – 830 m	300 – 600 m

2.2 Processing and products

Traditional honeybush is produced after harvesting the shoots, consisting of both stems and leaves, followed by shredding the shoots into small pieces before 'fermentation' (better described as high-temperature oxidation) and drying of the plant material to a moisture content of less than 10%. The dried plant material is sieved to remove coarse pieces, comprised mostly of stems, before packaging. The brown colour of the shredded plant material and infusion as well as the typical sweet aroma and flavour are developed during processing (as reviewed by Joubert *et al*., 2011). Fermentation affects the chemical composition of the plant material by oxidation, decreasing the relative concentrations of phenolic compounds and reducing antioxidant activity (Joubert *et al*., 2008b; Beelders *et al*., 2015). For this reason, green ('unfermented') honeybush was first commercially produced in 2001 by omitting the fermentation step from the production process and drying the plant material before oxidation is allowed to take place (Joubert, E., 2015, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, personal communication, 04 May). A study on *C. subternata* indicated that a steaming step may also be included before drying the unfermented plant material to inactivate enzymes and aid in retaining bioactivity (Joubert *et al*., 2010).

Green honeybush is mostly sold as a flavoured herbal tea or as a blend with other herbs such as buchu or mint. Even the fermented product is often sold as a blend of honeybush and rooibos, mainly due to limited production. When sold in pure form it is commonly a blend of *Cyclopia* spp. and products on the market do not usually indicate the *Cyclopia* spp. that comprise the blend. The processed plant material, especially the unfermented type, may also be used for the preparation of powdered hot water extracts with a high antioxidant activity and polyphenol content for use in foods and cosmetics (Joubert *et al*., 2011). A most recent use of honeybush extract in a common food product is its incorporation in bread. Investigation into extract optimisation with the emphasis on the

phenolic quality of the extracts is underway at the ARC. Various *Cyclopia* spp. and unit operations have been studied or are currently under investigation and include extraction of selected compounds, ultrafiltration for the enrichment of the phenolic content (Bosman, 2014; Von Pressentin Du Preez, 2014) and spray-drying (Joubert, E., 2015, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, personal communication, 04 May).

The packaging of honeybush varies as it is sold not only in supermarkets, but also at farm stalls and by small traders. Supermarkets typically sell it packaged in tea bags placed in cardboard boxes which may or may not be covered with bi-axially oriented polypropylene (BOPP) plastic wrapping. Tea bags may also be sealed in a foil wrapper or, as for high-end products, each tea bag may be sealed individually in an additional paper or laminate pouch. Tea sold by small-scale traders is mostly packed as loose tea in paper packets or even displayed in hessian bags where consumers may scoop the tea out for purchase. These bulk or open packaging conditions are not ideal as volatile aroma compounds may be lost and moisture may permeate through the packaging or lack thereof. This can lead to microbial spoilage in high humidity ambient conditions (De Freitas Araújo & Bauab, 2012) or possible slow oxidation in the case of green honeybush. The risk of pest or insect infestation is also aggravated in such cases. Storage instructions usually recommend that the tea be stored in a dry, cool place. Storage stability studies for honeybush, either as bulk or packed product, are lacking and the effects of moisture and temperature on stability of the phenolic and volatile compounds are not well understood.

2.3 Chemical composition and health-promoting compounds

With the renewed interest in honeybush as a herbal tea in the 1990s its phenolic composition became the focus of several studies in step with world-wide interest in this class of secondary plant metabolites. The first in-depth study was on *C. intermedia* (Ferreira *et al*., 1998; Kamara *et al*., 2003), and major compounds identified included the xanthone, mangiferin, and the flavanone, hesperidin (Ferreira *et al*., 1998). Vyas *et al*. (2012) and Garg *et al*. (2001), respectively, reviewed the pharmacological effects of these two phenolic compounds. Specific activities for mangiferin and hesperidin are summarised in **Table 2.2**. De Nysschen *et al.* (1996), investigating a large number of *Cyclopia* spp., showed these compounds to be ubiquitously present in the genus. Since then, several studies have been undertaken to either quantify the major phenolic compounds and/or to provide a comprehensive profile of the phenolic compounds of several *Cyclopia* spp., including *C. subternata* (Joubert *et al*., 2003; Kamara *et al.,* 2003; De Beer *et al*., 2012; Joubert *et al*., 2012; Kokotkiewicz *et al*., 2012), *C. genistoides* (Joubert *et al*., 2003; Joubert *et al*., 2006; Kokotkiewicz *et al*., 2013; Beelders *et al*., 2014a,b) and *C. maculata* (Schulze *et al*., 2014). Other phenolic compounds of interest that have been identified in these studies include the benzophenones, iriflophenone-3-*C*-glucoside and maclurin-3-*C*-glucoside, the dihydrochalcone, phloretin-3',5'-di-*C*-glucoside, the flavanone, eriocitrin, and the flavone, scolymoside. A recent paper describes the contribution of the consumption of infusions of several *Cyclopia* spp. to the dietary exposure of xanthones,

benzophenones, dihydrochalcones and flavanones (Schulze *et al*., 2015). Inhibition of mammalian α-glucosidase by the benzophenones iriflophenone-3-*C*-glucoside-4-*O*-glucoside, iriflophenone-3-*C*-glucoside and maclurin-3-*C*-glucoside as well as the promotion of *in vitro* glucose uptake in L6 myocytes by iriflophenone-3-*C*-glucoside-4-*O*-glucoside and iriflophenone-3-*C*-glucoside, have been established (Beelders *et al*., 2014a). Malherbe *et al.* (2014) demonstrated that the antioxidant capacity of iriflophenone-3-*C*-glucoside was comparable to that of mangiferin in the oxygen radical antioxidant capacity (ORAC) assay. No information on the potential therapeutic properties of phloretin-3',5'-di-*C*-glucoside could be obtained from current literature, however, another derivative of phloretin, *i.e.* phloretin-2'-*O*-glucoside (phloridzin), served as lead compound for the development of *C*-aryl analogues as sodium glucose transporter 2 inhibitors, and thus for use as anti-diabetic drugs (Xu *et al*., 2011). Additionally, the major rooibos flavonoid and dihydrochalcone, aspalathin (3-hydroxyphloretin-3′-*C*-glucoside), was shown to have anti-diabetic properties in various *in vitro* and *in vivo* models (Kawano *et al*., 2009; Muller *et al*., 2012; Son *et al*., 2013), further indicating that phloretin-3',5'-di-*C*-glucoside may be important as a potential bioactive compound. Anti-inflammatory properties have also been demonstrated for the rooibos dihydrochalcones, aspalathin and nothofagin (phloretin-3′-*C*-glucoside) (Lee & Bae, 2015). Lee *et al.* (2015) showed the potential of scolymoside and another *Cyclopia* flavone, vicenin-2, as therapeutic agents for treatment of various severe vascular inflammatory diseases. Eriocitrin, as for hesperidin, is a well-known citrus flavanone with various health promoting properties including anti-cancer and anti-inflammatory effects (as reviewed by Kahn *et al*., 2014). The potentially bioactive *Cyclopia* compounds and their therapeutic effects noted here are not exhaustive, but serve to emphasise their importance when considering the production of value-added products from *Cyclopia*.

Apart from qualitative differences in phenolic composition between *Cyclopia* spp., quantitative inter-species variation in major compounds has been demonstrated (**Table 2.3**). Plant maturity, climatic conditions, harvesting location and post-harvest treatment, amongst other things, may further contribute to intra-species variation (Joubert *et al*., 2003; De Beer & Joubert, 2010; Joubert *et al*., 2010; Joubert *et al*., 2014). The phenolic content also differs in the different parts of the plant. Both leaves and stems are used for production of honeybush, but sieving removes coarse pieces which are mostly stems. It is has been found that the phenolic composition of *C. subternata* and *C. maculata* leaves display a significantly higher xanthone (mangiferin and isomangiferin) and eriocitrin content than that of the stems (De Beer *et al*., 2012; Von Pressentin Du Preez, 2014). The hesperidin content of their stems, however, is significantly higher than that of the leaves (De Beer *et al*., 2012; Von Pressentin Du Preez, 2014). Furthermore, the presence of dihydrochalcones in *C. subternata* and *C. maculata* (De Beer *et al.*, 2012; Schulze *et al.*, 2014) could potentially contribute to a high susceptibility of the plant material to oxidative changes, considering the susceptibility of the dihydrochalcones, aspalathin in rooibos (Joubert, 1996) and phloridzin in apple (Le Guernevé *et al*., 2004; Guyot *et al*., 2007).

Table 2.2 Studies investigating bioactivity and health-promoting effects of polyphenolic constituents of *Cyclopia* extracts

Compound	Activity	Reference
mangiferin	Anti-diabetic activity by decreasing insulin resistance	Miura *et al*., 2001
	Multiple anti-tumour activity in breast cancer cells	Li *et al*., 2013
	Anti-nociceptive effects in treating inflammatory pains	Izquierdo *et al*., 2013
	Antioxidant and anti-apoptotic properties for attenuation of dopaminergic neurodegeneration	Kavitha *et al*., 2013
	Anti-inflammatory effects in treating sepsis-induced acute lung injury	Gong *et al*., 2013
hesperidin	Promotion of renal and hepatic antioxidant enzyme activity and inhibition of apoptosis and inflammation in kidneys of rats with acetaminophen induced toxicity	Ahmad *et al*., 2012
	Antioxidant and anti-inflammatory properties for amelioration of functional and histological outcomes in MCA occluded rats	Raza *et al*., 2011
	Contributes to vascular protective effects of orange juice	Morand *et al*., 2011
	Protection against γ-irradiation induced hepatocellular damage and oxidative stress in rats	Pradeep *et al*., 2008

Honeybush was first used medicinally to treat, amongst others, minor pulmonary ailments and to alleviate heartburn and nausea (as reviewed by Joubert *et al*., 2008a). However, with scientific insight into the phenolic composition of various *Cyclopia* spp., several studies have been initiated to investigate mechanisms whereby honeybush may have beneficial effects on health (Joubert *et al*., 2008a). Furthermore, growing evidence of the health benefits of consuming diets high in plant polyphenols have led to great interest in the use of polyphenol-rich plant extracts by the food industry, especially as consumers have grown more aware of the advantage that may be gained from eating 'functional' foods (Heinrich & Prieto, 2008). Extracts of honeybush are becoming widely used not only in the food and beverage industry with the production of ready-to-drink iced teas, jams and flavoured teas, but also in the cosmetics industries for lotions and soaps. South African extract manufacturers use total polyphenol content and total antioxidant capacity as quality parameters. Extracts standardised in terms of mangiferin content are also available (Joubert *et al*., 2011). Unfortunately, no current regulations or standards exist for the required polyphenolic composition or antioxidant activity of honeybush extracts. It is thus of importance to gain an understanding of factors that play a role in the phenolic profile and chemical characteristics of honeybush to gain a complete understanding of the product and its potential. Studies are on-going at the ARC to address these concerns (Joubert, E., 2015, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, personal communication, 04 May).

Commonly marketed health benefits of honeybush tea include its low tannin content and lack of caffeine. Labelling regulations prevent health-related claims at present, but promotional material

and web pages emphasise many of its potential health benefits including its antioxidant activity. Investigations have been conducted into these health benefits offered by *Cyclopia*, either as a herbal tea or as an extract. These include phytoestrogenic (Louw *et al*., 2013), anti-diabetic (Muller *et al*., 2011; Chellan *et al*., 2014) and anti-obesity (Dudhia *et al*., 2013; Pheiffer *et al*., 2013) activities for aqueous extracts, thus showing potential for use as nutraceutical extracts or phytopharmaceuticals. Fermented *C. intermedia* extracts evaluated for protection against UVB-induced skin damage displayed significant photoprotective activity (Petrova *et al*., 2011; Im *et al*., 2014). The antioxidant activity of *Cyclopia* extracts before and after fermentation have been established by Joubert *et al*. (2008b). It was found that unfermented aqueous extracts of *Cyclopia* spp. performed significantly better than fermented extracts (**Fig. 2.3**). Evidently, the phenolic composition of honeybush plant material is altered during processing as a result of oxidation of the green plant material to produce the traditional fermented product (**Table 2.3**). The fermentation step results in a decrease of the total polyphenol content of the hot water extract (Joubert *et al.,* 2008b), with the xanthone, mangiferin, and the benzophenone, iriflophenone-3-*C*-glucoside, incurring losses of more than 48% and 62%, respectively (Beelders *et al*., 2015). A study is in progress to provide insight into oxidation and/or degradation products formed from several major compounds (Beelders, T., 2015, PhD student, ARC Infruitec-Nietvoorjib, Stellenbosch, South Africa, personal communication, 05 May). Hubbe (2000) attributed the loss of antioxidant activity or more specifically radical scavenging action as a result of fermentation to the loss of hydroxyl groups due to oxidation and polymerisation. The xanthone glucoside, mangiferin, one of the major phenolics present in *Cyclopia* extracts, was shown to be more effective for radical scavenging ($ABTS^{\bullet+}$ assay), but less effective for inhibition of lipid peroxidation than minor compounds, hesperetin, eriodictyol and luteolin (flavonoid aglycones) (Joubert *et al*., 2008b).

Table 2.3 Average content (g/100 g extract) of the major phenolic compounds in aqueous extracts of unfermented and fermented honeybush

Compound	*C. intermedia*[a]	*C. genistoides*[b]	*C. subternata*[a]	*C. maculata*[c]
mangiferin	4.35 (0.13)	13.79 (6.97)	2.73 (0.06)	5.18 (1.04)
isomangiferin	1.40 (0.26)	1.617 (0.91)	0.86 (0.15)	1.48 (0.62)
eriocitrin	0.13 (0.03)	0.05 (0.04)	0.32 (0.12)	0.33 (0.16)
hesperidin	0.62 (0.27)	0.37 (0.27)	0.62 (0.24)	0.89 (0.51)
scolymoside[d]	0.04 (nd)	ne (ne)	0.68 (0.20)	ne (ne)
iriflophenone 3-*C*-glucoside[e]	1.04 (0.06)	1.22 (0.50)	0.82 (traces)	0.55 (ne)
phloretin-3′,5′-di-*C*-glucoside[f]	0.13 (nd)	0.27 (0.15)	0.86 (0.15)	ne (nd)

Values outside and inside brackets indicate those of unfermented and fermented honeybush, respectively.
[a] De Beer & Joubert (2010), [b] Beelders *et al*., 2014b, [c] Schulze *et al*. (2014)
[d] Previous designation: compound 11, [e] Previous designation: compound 8, [f] Previous designation: compound 12
nd = not detected; ne = not evaluated

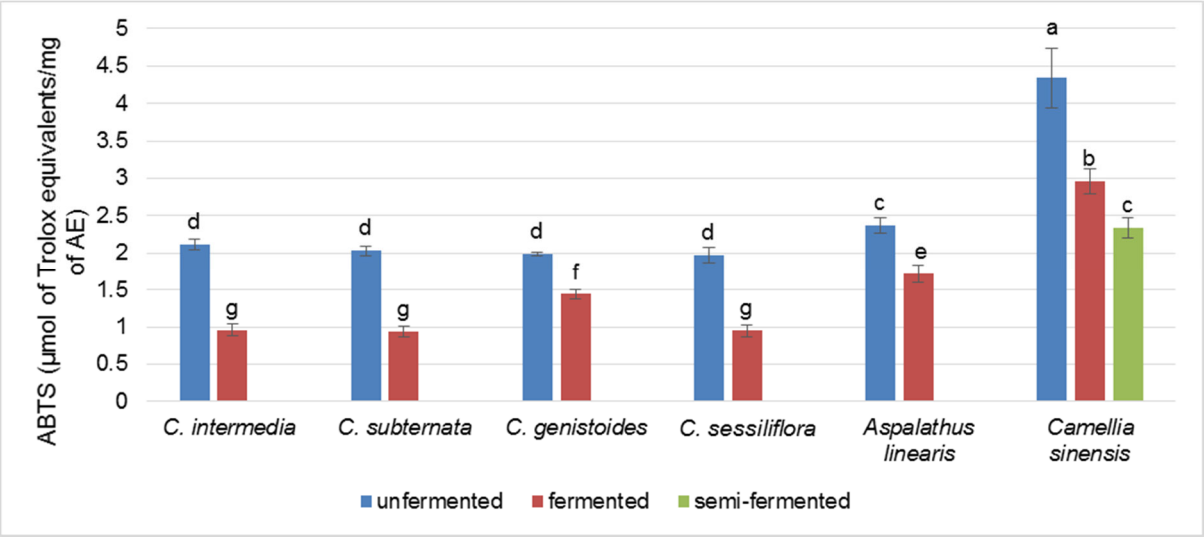

Figure 2.3 Relative ABTS$^{\bullet+}$ activity of dried aqueous extract of *Cyclopia* spp., *Aspalathus linearis* and *Camellia* teas (adapted from Joubert *et al*., 2008b).

2.4 Sensory characteristics and volatile composition

According to the South African Agricultural Products Standards Act, green honeybush should possess "the clean, characteristic taste and aroma of honeybush", with the same description applied to fermented honeybush (Anon., 2002). This vague, ambiguous description is confusing and could result in inferior sensory quality of green as well as fermented honeybush. Quality evaluation is further complicated by the great variation in the sensory characteristics of honeybush infusions produced from different *Cyclopia* spp., as illustrated by Theron *et al*. (2014) and Erasmus (2015). Theron *et al*. (2014) applied descriptive sensory analysis (DSA) to the fermented products of six *Cyclopia* spp., resulting in a clustering effect of certain species due to the prominence of specific flavour and aroma attributes. The study resulted in the development of a generic flavour wheel for honeybush, using 30 attributes including 26 aroma and flavour, 3 taste and 1 mouthfeel attribute (**Fig. 2.4**). Follow-up investigation has led to the construction of species-specific flavour and aroma wheels for fermented *C. genistoides, C. maculata, C. subternata, C. longifolia* and *C. intermedia* using DSA (Bergh, 2014; Erasmus, 2015). Although Erasmus (2015) found great variation between sensory profiles of honeybush produced from plant material harvested in different production years, significant differences in species-specific sensory profiles were still detected. The occurrence of an attribute in the entire species sample set was compared to the average intensity of the specific attribute in order to assess the relative importance of each attribute for each species. Similar to the method used by Theron *et al*. (2014), average attribute intensities of ≥ 10 were considered noteworthy, although smaller average values were not completely disregarded as these may contribute to the overall profile. Studies on volatile compounds and odourants indicated that sub- and peri-threshold odourants and/or even non-aroma active compounds could modify the sensory perception of aroma-active compounds (Delahunty *et al*., 2006; Ryan *et al*., 2008). Species-specific profiles indicated similarity between *C. maculata* and *C. subternata* with high occurrence of 'caramel' notes, and a low occurrence and intensity of bitter taste. *Cyclopia genistoides* and *C. longifolia*

displayed higher occurrences and intensities of 'rose geranium' and 'apricot/apricot jam' notes as well as 'hay/dried grass' aroma. Bitter taste was found to be present in all *C. genistoides* samples at an average intensity of more than 10, distinguishing it from the other three species (Erasmus, 2015).

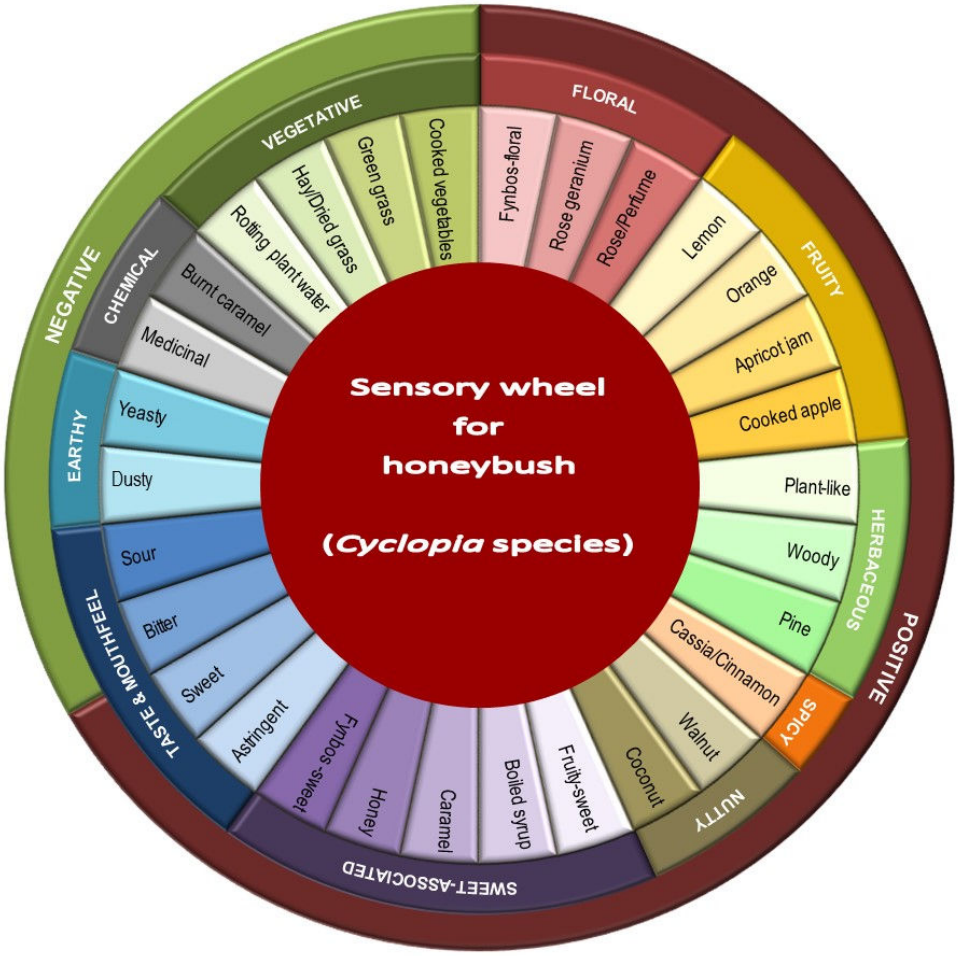

Figure 2.4 Generic wheel for fermented honeybush flavour, taste and mouthfeel (Theron *et al*., 2014).

Similarly to black tea (*C. sinensis*), the characteristic aroma and flavour of fermented honeybush develops during processing and is due to the oxidation processes which takes place during the fermentation step. In industry the plant material is subjected to temperatures of 70 °C and higher for periods longer than 16 h (Joubert *et al*., 2011). The volatile aroma compounds of *C. genistoides* (Le Roux *et al*., 2008) and *C. intermedia* (Cronje, 2010) have been analysed using headspace gas chromatography-mass spectrometry (GC-MS) to investigate the volatile fraction of fermented and unfermented (green) honeybush. Both the green and fermented samples were sub-batches of the same plant material to allow direct comparison. A total of 79 compounds were identified in the aroma fraction of green *C. genistoides* (major components listed in **Table 2.4**). Many alcohols (saturated and unsaturated) as well as aldehydes and methyl ketones were found to be present in substantially higher relative quantities in green than in fermented *C. genistoides* (**Table 2.4**). Most of these compounds are known to be associated with distinctly 'grass-like' odours, especially 6-methyl-5-hepten-2-one, which has been identified as a major constituent (54%) of the

aroma fraction and is described as 'oily, green grass and herbaceous' (Le Roux *et al*., 2008). In contrast, most of the compounds, *i.e.* 46 out of a total of 79 compounds identified and found in fermented *C. genistoides*, were classified as terpenoids, present in much lower concentrations in their green counterparts (Le Roux *et al*., 2008). The main constituent was identified as linalool (36%) which, together with other terpenoid compounds, is known to impart a 'sweet', 'floral' aroma (**Table 2.4**). The volatile fraction of green and fermented *C. intermedia* yielded a total of 244 volatile compounds (**Table 2.4**). Many compounds were described as 'citrus-like', 'herbaceous', 'camphor-like', 'green' and 'woody'. Once again, compounds found in fermented samples had 'sweet', 'floral', 'fruity' and 'woody' aroma descriptors.

Aroma-active compounds present in the volatile fractions of *C. subternata* (Le Roux *et al*., 2012) and *C. maculata* (Theron *et al*., 2014) honeybush were investigated using GC-MS and GC-olfactometry (GC-O). A total of 34 aroma-active compounds were identified in fermented and unfermented *C. subternata*, with the major aroma-active volatile compound in both fermented and unfermented samples identified as linalool (28.8% and 13.1%, respectively), associated with a refreshing, 'floral-woody' aroma. Geraniol (13.8%), α-terpineol (8.7%) and nerol (2.8%) were also more concentrated in the fermented samples and are associated with 'floral' and 'sweet' descriptors (**Table 2.4**). Aroma-active compounds, (*E*)-β-damascenone and terpinolene, occurred in relatively high concentrations in green honeybush compared to fermented samples (4.4% and 2.7%, respectively). They are typically described as 'sweet' and 'fruity'. Analysis of fermented *C. maculata* (Theron *et al*., 2014) led to the identification of 34 odour-active volatile aroma compounds, 14 of which correspond to those identified by Le Roux *et al*. (2012) in *C. subternata*.

The results of volatile analysis of honeybush indicate mostly quantitative variation between *Cyclopia* spp. as well as between fermented and unfermented samples as a result of processing. Variation between species may be affected by parameters similar to those affecting phenolic composition. Gui *et al*. (2015) noted that factors such as biotic and abiotic stress and processing conditions may affect the content of glycosidically bound volatile compounds in *C. sinensis*. Additionally, aroma compounds with higher volatility, such as alcohols and aldehydes with typically 'green' aroma descriptors, may undergo more severe losses as a result of processing than less volatile terpenoid compounds, as indicated by their later elution times during HS-GC-MS analysis (**Table 2.4**).

Table 2.4 Main volatile odour compounds, identified by HS-GC-MS analysis, in the volatile fractions of fermented and unfermented honeybush, *Cyclopia intermedia* (Cronje, 2010), and *Cyclopia genistoides* (Le Roux *et al.*, 2008)

Compound[a]	Aroma descriptors	*C. intermedia*		*C. genistoides*	
		Unferm	Ferm	Unferm	Ferm
hexanal	Fatty-green grassy odour [b,c]	0.48	**0.97**	4.08	1.76
(R)-2-methylbutanoic acid	-	-	-	0.06	0.04
3-methylbutanoic acid	Acid acrid, cheesy, unpleasant [b]	**0.01**	**0.01**	-	-
6-methyl-5-hepten-2-one	Oily-green, pungent-herbaceous, grassy, fresh and green-fruity [b,c]	**2.75**	**2.83**	54.07	14.17
p-cymene	Citrus-like, lemon and bergamot [b]	**1.10**	0.47	0.58	0.34
limonene	Sweet, citrus-like, orange, refreshing, clean, turpentine [b]	8.17	2.06	4.60	3.15
(*Z*)-β-ocimene	Warm-herbaceous, sweet, floral [b]	**0.78**	0.18	0.15	0.17
γ-Terpinene	Refreshing, herbaceous, citrus-like [b]	**1.91**	0.29	0.20	0.12
(*E,E*)-3,5-octadien-2-one	Fatty, fruity, mushroom [b]	0.25	**0.27**	2.42	0.5
terpinolene	Sweet-piney, oily [b]	**2.70**	0.56	-	-
trans-furanoid linalool oxide	Sweet, woody, floral, earthy [c]	-	-	0.93	2.29
cis-furanoid linalool oxide	Sweet, woody, floral, earthy [c]	-	-	0.81	1.67
6-methyl-3,5-heptadien-2-one	Warm spicy, cinnamon-like [c]	-	-	1.43	-
linalool	Refreshing, floral-woody [b,c]	13.16	**28.88**	10.68	35.94
2-phenylethanol	Mild, warm, rose-honey-like [b]	**0.07**	0.04	0.07	0.08
4-acetyl-1-methylcyclohexene†	Spicy [b]	0.03	**0.09**	-	-
(*E,Z*)-2,6-nonadienal	Green-vegetable, cucumber or violet leaf [b]	0.02	**0.12**	0.09	0.01
(*E*)-2-nonenal	Green, cucumber, aldehydic and fatty [b]	0.05	**0.11**	-	-
α-terpineol	Floral, sweet, lilac-type [b,c]	4.12	**8.79**	3.75	17.3
(+)-*p*-menth-1-en-9-al	Powerful spicy, herbaceous odour [b]	0.05	**0.09**	0.02	0.02
β-cyclositral	Green, minty, grassy, hay-like [b,c]	**0.75**	0.69	1.47	0.25
nerol	Fresh, sweet-rosy [b,c]	0.89	**2.83**	0.34	3.49
p-anisaldehyde	Sweet, floral, 'hay-like' [b]	0.04	**0.06**	-	0.01
geraniol	Sweet, floral, rose [b,c]	6.77	**13.90**	0.96	10.80
geranial	Lemon [b]	**0.50**	0.27	0.03	0.06
geranyl formate	Fresh, green-rosy, fruity [b]	0.02	**0.15**	0.03	0.18
(*E,E*)-2,4-decadienal	Fried, waxy, fatty, orange-like [b]	**0.05**	0.04	-	-
eugenol	Warm-spicy, dry [b]	0.15	**0.53**	0.06	0.11
2,3-dehydro-α-ionone†	Tobacco-like [b]	0.03	**0.11**	-	-
(*E*)-β-damascenone	Woody, sweet, fruity, earthy green-floral [b]	**4.48**	1.04	0.09	0.34
(*E*)-β-damascone	Fruity (apple-citrus), tea-like with slight minty notes [b,c]	0.29	**0.74**	-	0.06
geranyl acetone	Floral, sweet-rosy, slightly green [c]			2.33	0.59
2,3-dehydro-γ-ionone	–	0.04	**0.09**	-	-
3,4-dehydro-β-ionone	Ionone-damascone and saffron-like, fruity and slightly leathery [b]	**0.14**	0.13	-	-
(*R*)-decan-5-olide	Sweet, creamy, nut-like, fruity [b]	0.01	**0.02**	-	-
(*E*)-β-ionone	Warm, woody, fruity, raspberry-like; resembles cedarwood [b,c]	**2.25**	1.52	0.74	0.11
bovolide	Celery- and lovage-like, fruity and pleasant [b]	**0.08**	0.07	-	-
dihydroactinidiolide	Sweet, floral, tabacco [c]	-	-	1.02	0.16

(6*E*,8*Z*)-megastigma-4,6,8-trien-3-one	Tobacco-like, woody, balsamic [b]	-	**0.03**	-	-
(6*E*,8*E*)-megastigma-4,6,8-trien-3-one	Tobacco-like, woody, balsamic [b]	0.05	**0.12**	-	-
10-*epi*-γ-eudesmol†	Woody, floral, sweet [b]	0.18	**0.59**	-	-
epi-α-cadinol†	Herbaceous, woody [b]	**0.11**	0.06	-	-
epi-α-muurolol†	Herbaceous, slightly spicy [b]	**0.05**	0.04	-	-
cadalene	–	0.10	**0.11**	-	-

Odour active compounds in bold type (Cronje, 2010).
'Ferm' and 'Unferm' refer to fermented and unfermented plant material, respectively.
[a] In order of elution from the apolar PS-089 column (DB-5 equivalent).
[b] As cited by Cronje, 2010
[c] As cited by Le Roux *et al*., 2008
† Stereochemistry not determined
Values recorded as average percentage area calculated from the total ion chromatogram (n = 3; RSD ≤ 20%).

3. Green and herbal teas

3.1 Green tea

Tea, one of the most popular beverages worldwide, is produced from *Camellia sinensis* (family Theaceae) and may be processed to yield various types of tea including green tea ('unfermented'), oolong (semi-'fermented') and black tea (fully-'fermented') (Chu, 1997). **Figure 2.5** indicates the processing steps commonly applied to yield teas of different fermentation levels. The process yielding black tea is commonly referred to as 'fermentation', since it was initially thought to entail microbial alcoholic fermentation. Advances have since been made, identifying enzymatic and chemical oxidation as the major processes responsible for the conversion of the green plant material into the yellow, red/brown or black tea product. In spite of this, the term 'fermentation' is still used by the tea industry to describe this process of oxidation. Processing is used to manipulate aroma and appearance and has been extensively investigated to understand the underlying mechanism of fermentation, distinguishing between types of tea based on characteristics developed as a result of different extents of fermentation (as reviewed by Pripdeevech & Wongpornchai, 2013).

Fresh leaves of *Camellia sinensis* contain high levels of flavanols, also known as catechins. These compounds are easily oxidised to thearubigins and theaflavins upon processing (Touknekti *et al*., 2013). Other reactions that could take place as a result of inappropriate processing or storage of green tea include thermal degradation, epimerisation and polymerisation (Senanayake, 2013; Ananingsih *et al*., 2013).

Traditional Chinese and Japanese green teas are produced from the variety *Camellia sinensis* var. *sinensis*. The plant material may be cultivated and processed by various methods, namely steaming or pan-firing, to obtain different types of green tea, as indicated in **Fig. 2.6**. Green tea leaves are typically of a green colour and the infusion described as having a 'green' aroma, bitter taste with a sweet, lingering aftertaste and an astringent mouthfeel (Lee & Chambers, 2007; Wang *et al*., 2000). Typical aroma profiles, however, may be associated with specific types of green tea. During the development of general descriptors for green tea, Lee and Chambers (2007) defined the 'green' attribute by breaking it down into various 'plant-like' descriptors as summarised in **Table 2.5**.

Other descriptors were also generated with 'brown', or less common 'fruity/floral' and 'other' associations.

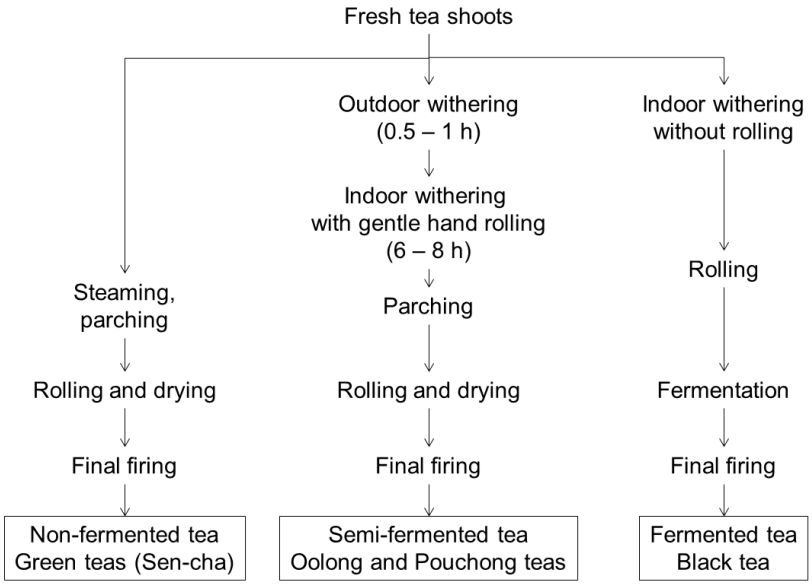

Figure 2.5 Brief outline of processing techniques of green, oolong and black teas (adapted from Takeo, 1992).

As the primary aim during green tea processing is the retention of green leaf properties by prevention of enzymatic activity, green tea cultivars are selected with lower polyphenol oxidase (PPO) activity and lower polyphenol content. Regulating the content of certain phenolic compounds such as flavanols is also important in order to prevent excessively bitter tasting tea infusions associated with high levels of these phenolics (Tounekti *et al*., 2013). This reduction in substrate for enzymatic activity aims to avoid excessive oxidation by endogenous enzymes responsible for the characteristic flavours and appearance associated with fermented or semi-fermented teas (Chu, 1997). Processing techniques employed as methods of enzyme inhibition include steaming (95 – 100 °C, 30 – 45 s; Chu, 1997) and/or pan-firing (200 °C, 10 min; Takeo, 1992) and additionally aid in flavour release or manipulation (Takeo, 1992; Senanayake, 2013). Steam treatment after harvesting prevents loss of vitamins (Vitamins A, B_1 & B_2, Niacin and C) and polyphenols to oxidation, adding to the health benefits of green tea when compared to fermented black teas (Chu, 1997). Roasting of the tea leaves is further known to impart a roasted aroma to the tea as in the case of Kamairicha, a Japanese green tea (Takeo, 1992). This roasted aroma is due to pyrroles and pyrazines formed during heating of amino acids and sugars in the tea (Takeo, 1992). Following investigation of volatile compounds present in pan-fired (Japanese Kamairicha and Chinese Longing tea) and steamed green tea (Sencha), the pan-fired teas were found to contain specific potent odourants, namely 2-acetyl-1-pyrroline and 2-acetyl-2-thiazoline associated with 'popcorn-like' descriptors and 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine associated with 'nutty' descriptors (Kumazawa & Masuda, 2002). Wang *et al*. (2000) found that green tea infusions

prepared from steamed tea material tended to be more bitter and astringent, but less sweet than green tea infusions prepared from roasted tea material.

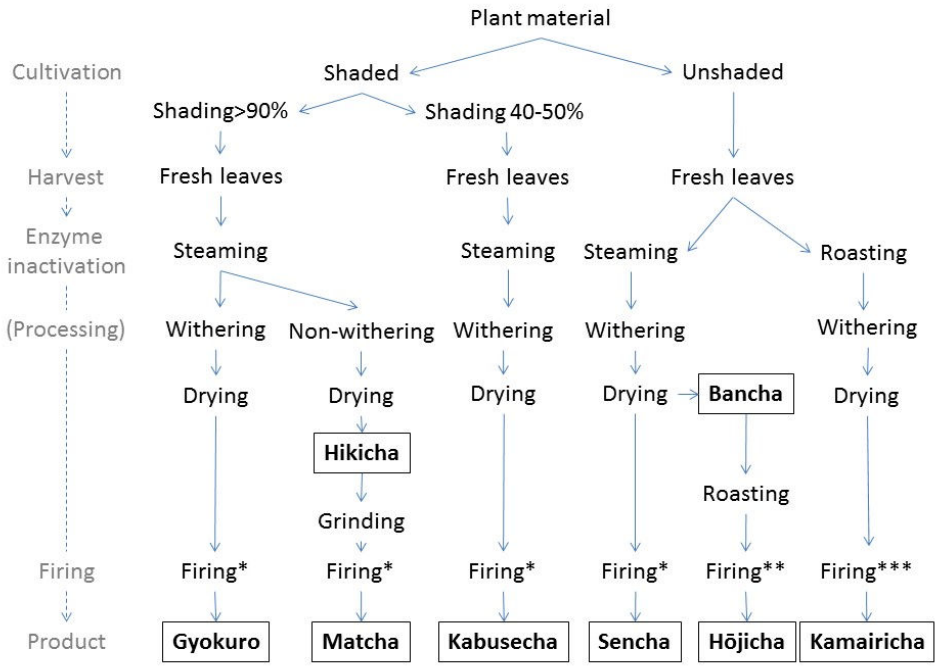

Figure 2.6 Processing of various Japanese green teas (adapted from Chu, 1997).
Degree of firing to produce flavour: *mild, **considerably strong, ***strong

Table 2.5 Attributes for green tea evaluation (Lee & Chambers, 2007)

Green	Brown	Fruity/Floral	Other
Asparagus	Ashy/sooty	Fruity	Grain
Beany	Brown spice	Floral/perfumy	Medicinal
Brussels sprouts	Burnt/scorched	Citrus	Mint
Celery	Nutty	Fermented	Musty/new leather
Green beans	Tobacco		Seaweed
Green herb-like	Almond		Straw-like
Parsley	Animal-like		
Spinach			

Various studies have investigated volatile compounds and their contribution to the flavour of a variety of herbal teas (as reviewed by Lasekan & Lasekan, 2012), however, none have been investigated as thoroughly as those of *C. sinensis* tea. Tea aroma development has been extensively investigated in terms of volatile composition, using GC-MS as a popular technique and is relatively well understood (Pripdeevech & Machan, 2011; Rawat *et al*., 2007; Kim *et al*., 2007). Investigation of black tea has indicated that tea volatiles can be classified in two groups, terpenoids and non-terpenoids (Pripdeevech & Wongpornchai, 2013). Non-terpenoid volatiles are derived from hexenols formed in fresh tea materials and contribute to the 'green' aroma notes in tea. These compounds

are the products of enzymatic lipid degradation which takes place after plucking and during processing (Ravichandran & Parthiban, 2000). Terpenoid tea volatiles may be derived from the thermal degradation or enzymatic oxidation of carotenes or amino-acids found in the tea leaves (Ravichandran, 2002; Gui *et al*., 2015). Most volatiles are lost during the high temperature firing of the tea, but major volatile constituents in most teas include linalool, geraniol, α-damascone, linalool oxide, *cis*-jasmone, maltol, anethole, hotrienol, α-terpeneol and nerolidol, although quantitative differences are evident between fermentation levels and tea types (Pripdeevech & Wongpornchai, 2013). Wang *et al*. (2008) established that close examination of volatile flavour compounds, using solid-phase microextraction – gas chromatography (SPME-GC), can discriminate between teas of differing fermentation levels. The quantification of *trans*-2-hexenal, benzaldehyde, methyl-5-hepten-2-one, methyl salicylate and indole was found to give a clear indication of fermentation differences between fermented and unfermented teas (Wang *et al*., 2008).

3.2 Herbal teas (Tisanes)

Non-*Camellia sinensis* infusions such as camomile and peppermint teas, strictly speaking, are classified as tisanes, despite commonly being referred to as ‘tea’. Tisanes, similarly to tea, have been produced using various methods of processing, for example, rooibos tea is traditionally processed by ‘fermentation’, but has also been produced in unfermented or flavoured forms. Tea or tisane (here forth referred to merely as ‘tea’) drinking holds a place in many cultures across the world where it may have been used as traditional medicine, but are also consumed purely for their sensory properties or health-promoting effects in countries with no specific tea drinking tradition (Lee & Chambers, 2007). Medicinal and health-promoting properties of tea can be attributed to the chemical constituents present in the infusion and may be specific to the species or origin of plant material used. Constituents of particular interest include a variety of polyphenols, especially flavonoids as well as other active compounds such as caffeine.

Steaming of fresh plant material for the production of *Camellia* green tea has been a traditional practice for centuries, however, this treatment is not standard for herbal tea processing. Investigation has been carried out to quantify the effects of steaming on some herbal tea materials, although effects differ with varying plant types or species. In the comprehensive review by Zhao *et al*. (2013), the change in chemical components of various herbal teas and infusions were analysed after treatments of steaming and roasting as well as baking, adjuvant materials processing and fermentation. It was found that results were inconsistent between different kinds of plant material after treatment, indicating the vast impact different heating methods can have on different herbal teas.

Furthermore, steam pasteurisation of dry herbal tea material is common in order to decrease microbial loads, producing tea that is safe to consume. The secondary effects of this treatment with regard to other quality parameters, such as sensory quality and appearance in terms of colour, however, are not well understood. In South Africa, studies have been conducted to evaluate the

effects of steam pasteurisation on both rooibos (Koch *et al*., 2013) and *Lippia multiflora* (Arthur *et al*., 2011a,b) teas, although no such study has been conducted on the dried honeybush product to date.

3.2.1 *Blanching of fresh plant material for enzymatic inactivation*

For the production of yerba-maté tea in South American countries, the plant material undergoes blanching to inactivate enzymes by flash heating of leaves in rotary dryer ovens over direct heat typically between 500 °C and 700 °C for 30 s to 4 min (as reviewed by Berté *et al*., 2014). This may be followed by a roasting or ageing step before commercialisation, depending on the type of tea or maté product produced. Isolabella *et al.* (2010) and Markowicz Bastos *et al.* (2006) both observed increased total soluble solids (TSS) contents in processed maté dried products when compared to fresh leaves. Total polyphenolic (TP) content of infusions was also increased as a result of processing, especially blanching (Markowicz Bastos *et al*., 2006; Valerga *et al*., 2012). Mechanical impact and heat during processing are thought to lead to cell disruption, increasing extractability. Furthermore, caffeoyl derivatives such as chlorogenic acid and isochlorogenic acids (isomers 3,4-dicaffeoylquinic (DCQ), 3,5-DCQ and 4,5-DCQ) were significantly higher in maté tea following various processing steps after blanching than in fresh leaves. Similar effects were observed for the methylxanthines caffeine and theobromine as well as the flavonoid rutin (Isolabella *et al*., 2010).

Investigation of the effect of steam blanching on properties of green honeybush is limited to one study conducted by Joubert *et al*. (2010), investigating the effects of pre-drying steam treatments on unfermented *C. subternata*. This study compared small-scale treatments of leaf samples subjected to different treatment combinations, *i.e.* dried whole to minimize any enzymatic and oxidative changes (T1), shredded and dried (T2), steamed, shredded and dried (T3), and finally, shredded, steamed and dried (T4). Steaming was conducted for 60 s at 96 °C under atmospheric pressure. Steam treatment, irrespective of treatment combinations, had no significant effect on the extractable TSS and TP content of unfermented *C. subternata*, compared to that of the whole dried leaves (T1). Cutting (shredding) and drying (T2) of the leaves with no steam treatment, however, yielded significantly lower extractable TSS and TP contents (**Table 2.6**) (Joubert *et al*., 2010). This indicates that steaming was effective in retaining extractable polyphenols, probably due to inactivation of endogenous enzymes. Conversely, the reduction in extractable TSS and TP contents of cut and dried leaves (T2) may possibly be due to oxidative changes of polyphenolic compounds leading to less soluble compounds. The effect of steam on the cell structure may also have played a role as has been found for the steaming of basil leaves, where steaming was found to result in damage to cell membranes, leading to faster drying rates (Rocha *et al*., 1993). Accelerated drying rates may, in turn, aid in retention of phenolic compounds as enzymes are deactivated at a faster rate as moisture is removed.

For the investigation of steaming pre-treatments on green *C. subternata* (Joubert *et al*., 2010), individual polyphenols were not significantly affected by steaming compared to the control

(T1), but were significantly decreased when the leaves were cut and dried (T2), as observed for mangiferin, isomangiferin and eriocitrin (**Table 2.6**). Conversely, scolymoside was slightly, but not significantly, increased in both the cut and dried (T2) samples as well as the cut and steamed (T4) samples. A significant increase compared to the control (T1) was observed for the leaves steamed before cutting (T3). It was postulated that oxidation of eriocitrin (eriodictyol-7-*O*-rutinoside; flavanone) to scolymoside (luteolin-7-*O*-rutinoside; flavone) occurred under these processing conditions (Joubert *et al*., 2010). A study investigating the effect of steaming on *Eucommia ulmoides* flower tea indicated a significant reduction in chlorogenic acid, a phenolic acid and the iridoid glycosides, aucubin and geniposidic acid, only after steaming for 180 s (Dong *et al*., 2012). Flavonoid content, however, was significantly reduced after 60 s of steaming and still further reduced after 180 s (Dong *et al*., 2012). It is thus possible that compounds quantified in *C. subternata* are more tolerant to steaming conditions than those quantified in *Eucommia ulmoides,* but are still susceptible when endogenous enzymes are not first denatured by heat.

Table 2.6 Effect of pre-drying treatments on chemical properties and composition of dry, milled leaves of *C. subternata* (Joubert *et al*., 2010)

Parameter	**T1** **Dried**	**T2** **Cut-Dried**	**T3** **Steam-Cut-Dried**	**T4** **Cut-Steam-Dried**
Total soluble solids	42.93 ^{a} ± 1.95	40.09 ^{b} ± 1.12	42.11 ^{a} ± 1.38	41.80 ^{a} ± 1.44
Total polyphenol content	12.75 ^{a} ± 0.66	11.72 ^{b} ± 0.57	12.50 ^{a} ± 0.50	12.25 ^{a} ± 0.46
mangiferin	1.22 ^{a} ± 0.35	1. 05 ^{b} ± 0.25	1.11 ^{b} ± 0.22	1.13 ^{ab} ± 0.30
isomangiferin	0.38 ^{a} ± 0.05	0. 34 ^{b} ± 0.04	0.37 ^{a} ± 0.05	0.37 ^{a} ± 0.05
eriocitrin	0.23 ^{a} ± 0.06	0.19 ^{b} ± 0.04	0. 25 ^{a} ± 0.04	0.22 ^{a} ± 0.05
scolymoside	0.48 ^{b} ± 0.32	0.50 ^{ab} ± 0.31	0.53 ^{a} ± 0.28	0.49 ^{ab} ± 0.30
iriflophenone-3-*C*-glucoside†	0.25 ^{a} ± 0.06	0.16 ^{b} ± 0.04	0.24 ^{a} ± 0.08	0.21 ^{a} ± 0.04
phloretin-3',5'-di-*C*-glucoside†	0.41 ^{a} ± 0.01	0.25 ^{c} ± 0.09	0.44 ^{a} ± 0.11	0.35 ^{b} ± 0.13

All values in g/100 g dried plant material.
Means in a row with different letters are significantly different ($p < 0.05$).
†Quantified in terms of eriocitrin equivalents (previously designated compound 1 and compound 6, respectively).

The study by Joubert *et al.* (2010) also assessed the effects of pre-drying treatments on the colour of milled plant material (**Fig. 2.7**). The milled plant material was used to obtain a more homogenous sample for colour measurement (Joubert, E., 2015, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, personal communication, 04 May). Significant differences were detected among treatments in terms of *L*, a** and *b** parameters, although treatment T4 resulted in colour parameter values most similar to that of the leaves dried whole (T1) (**Fig. 2.7**). It may be speculated that the shredded (cut) plant material allowed for quicker steam penetration for enzymatic inactivation, preventing the loss of green colour (*a**) due to chlorophyllase activity (refer to section 5.1), although some degree of darkening (*L**) and yellowing (*b**) were observed. Formation

of chlorophyllide would not greatly affect the green colour as this degradation product of chlorophyll also has a green colour (refer to section 5.1). Furthermore, colour degradation of steamed, shredded and dried, (T3) and unsteamed, whole leaves (T1) of *C. subternata* material stored in sealed containers in the dark at 0° and 30 °C for a period of 6 months was investigated. Browning of the samples stored at 30 °C was observed (Joubert *et al*., 2010). Control samples remained unchanged when stored at 0 °C to serve as benchmark. Initially, however, steaming of the fresh plant material indicated good colour retention (**Fig. 2.7**). It was postulated that the brief steaming process activated the enzymatic conversion of some chlorophylls to chlorophyllides, causing accelerated degradation to olive-brown products during storage at 30 °C (Joubert *et al*., 2010). These results are reflected in the study by Lafeuille *et al.* (2014) on dried culinary herbs, where chlorophyllide was not identified as chlorophyll derivative, except in samples which underwent a mild low temperature blanching treatment before drying, allowing the activation of chlorophyllase.

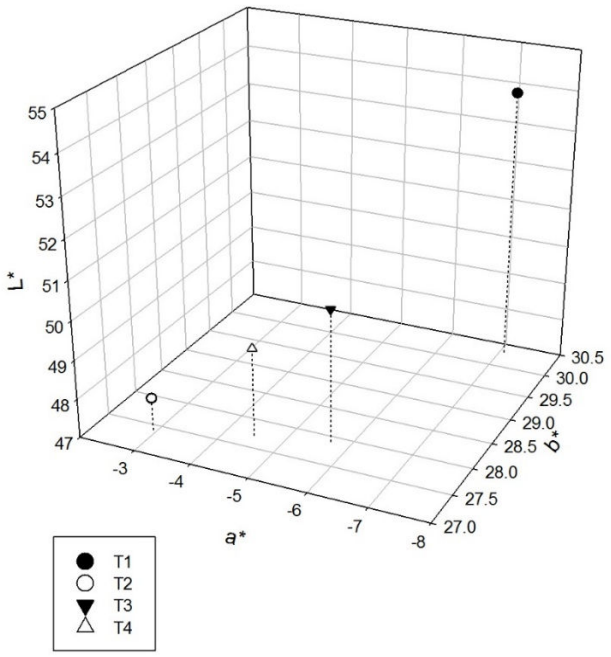

Figure 2.7 CIE*L*a*b** parameters for pre-drying treatment study by Joubert *et al*. (2010).

(T1) dried whole leaves, (T2) shredded and dried, (T3) steamed, shredded and dried, (T4) shredded, streamed and dried

3.2.2 *Microbial decontamination of dry plant material*

Although not currently implemented by the honeybush industry, steam pasteurisation at 96 °C for 60 s is a standard 'decontamination' process in the rooibos tea industry. It was implemented in the 1980s to decrease microbial loads to acceptably safe levels (Koch *et al*., 2013). Steam pasteurisation of fermented rooibos brought about significant changes in the sensory profile and phenolic composition of the tea infusions (Koch *et al*., 2013; Stanimirova *et al*., 2013). Koch *et al*. (2013) also found that steam pasteurisation of 60 s significantly decreased the TSS content of the fermented rooibos infusion from 2240 mg/L before steaming to 2197 mg/L after steaming. The strong correlation between the TSS and TP contents identified the change in polyphenols as the main contributor to

the decreased TSS content. Individual monomeric phenolic components analysed by high-performace liquid chromatography, coupled to a diode array detector (HPLC-DAD), however, were not affected with the exception of aspalathin, a major constituent, which decreased from 16.66 mg/L to 15.83 mg/L. Arthur *et al.* (2011b) investigated chemical changes in *Lippia multiflora* herbal tea as a result of steam pasteurisation for up to 150 s. No significant differences were detected with regard to TSS and TP content after steaming, neither did the contents of the major polyphenolic constituents, *i.e.* phenylethanoid glycosides, differ when compared to the unsteamed control. A slight, but insignificant increase of TSS content from 0.185 g/100mL to 0.192 g/100mL was observed after 30 s of steaming. Steam pasteurisation was found to be an acceptable processing technique for the reduction of microbial loads to acceptable levels (Arthur *et al*., 2011a) whilst maintaining antioxidant activity of the infusions as tested using the 2,2-diphenylpicrylhydrazyl (DPPH) scavenging and ferric reducing antioxidant power (FRAP) assays.

With regard to the effects of steam pasteurisation on the aroma and taste qualities of different quality grades of rooibos (Koch *et al*., 2013), principal component analysis (PCA) was used to interpret the differences due to treatment. There was a shift in position from the upper quadrants to the lower quadrants for all batches of rooibos tea along the 2nd principal component after steam pasteurisation. This indicated a decrease in attribute intensities in pasteurised samples. More specifically, the comparative effects of steam pasteurisation depended largely on the original profile of the tea. For example, lower grade rooibos samples exhibited a greater decrease in negatively associated ‘green’ notes, whereas high grade samples displayed decreases in ‘caramel’ notes which are considered as positive attributes associated with high quality rooibos (Koch *et al*., 2013). Increases in ‘hay’ aroma accompanied the decrease in ‘green’ notes of lower grade teas, although decreases in intensities were observed for most measured attributes.

4. Sensory aspects of tea quality

4.1 Colour and visual appearance

Consumer perception and expectation of food quality are greatly influenced by product appearance, especially colour (Wei *et al*., 2012). Colour may influence a consumer’s decision to either accept or reject the product and may thus determine purchase intent. Furthermore, consumers are more likely to buy a visually attractive product that they feel complies with high quality standards. With regard to green honeybush, South African export regulations state that the product “may not contain more than 5% brown fermented leaves per 10 g dry leaf material” (Anon., 2002). The regulations further stipulate that the tea should have the “clear, distinctive colour of honeybush.” Although these guidelines are vague, they indicate the importance of leaf product colour to the honeybush industry. Regulations regarding colour are not only necessary to ensure green honeybush of high quality reaches the international markets, but they are also crucial to prevent a negative impact on the traditional fermented honeybush market. For example, a batch of green honeybush with substantial browning may be mistaken for fermented honeybush. Such a batch would not meet the expectation

of fermented honeybush in terms of its sensory profile, and thus damage consumer perception as well as consumer expectation of the product (Joubert *et al*., 2010). Given the vague guidelines of the current regulations, it would be beneficial to industry if more exact guidelines, based on objective quality parameters, were developed for green honeybush.

Despite the importance associated with product appearance for consumer acceptance at purchase, infusion colour may also be an important component of product quality, as for traditional green tea (*C. sinensis*). In a study by Wang *et al*. (2004) investigating the compounds responsible for the greenness of green tea leaves and infusions, visual evaluation resulted in a low correlation between the colour of dry leaves and that of the infusion. Chlorophyll, despite its water-insoluble nature, was identified as the main contributor to the green colour of infusions. The colour and turbidity of the infusion were found to increase with longer steam treatments, resulting in an overall loss of quality as turbidity leads to a dull appearance (Wang *et al*., 2004). Green tea infusions of high quality are expected to be bright green and clear. It was proposed that the turbidity of longer steam-treated tea infusions is a result of the extraction of water-insoluble chlorophylls from the leaves, forming an emulsion while the tea is still hot (Wang *et al*., 2004).

Chlorophylls *a* and *b* are the major contributing compounds to green colour in plants and occur in a ratio of 3:1, although several more chlorophylls occur in nature (Schwartz *et al*., 2008). For this reason, chlorophyll content has been used as an indication of green colour of plant products, including green tea, as indicated in **Table 2.7**. The fat-soluble chlorophylls occur as membrane-associated pigments in chloroplast organelles within the plant cell, but they are easily broken down after harvesting of leaves as denaturation of membrane proteins take place (Lafeuille *et al*., 2014). Chlorophyll breakdown is known to follow multiple pathways leading to coloured or non-coloured intermediates or final compounds (Marangoni, 1996). Chlorophylls are classified as porphyrins and consist of a macrocyclic structure of four pyrrole rings linked by four methyne bridges (Lafeuille *et al*., 2014). The porphyrin structure is further linked to a fifth isocyclic ring and associated with a central Mg^{2+} ion (Schwartz *et al*., 2008; Lafeuille *et al*., 2014). The structures of chlorophyll *a* and *b* differ slightly as chlorophyll *a* contains a methyl group at C7 whereas chlorophyll *b* contains a formyl group in this position (**Fig. 2.8**). These constituents result in differences in stability, for instance, chlorophyll *b* has been found to be more heat stable than chlorophyll *a*, possibly due to the electron withdrawing effect of its C7 formyl group (Schwartz *et al*., 2008).

Table 2.7 Studies where chlorophyll content has been used as an indication of green colour or quality parameter

Product/ Plant material	Chlorophyll determination	Colour measurement	Reference
Jalapeño peppers (cv. Marajá)	C_{30} reversed-phase HPLC DAD UV–Vis, MS	Colorimeter, (CIE*L*a*b**)	Cervantes-Paz *et al.*, 2014
Chicory-Hindbeh (*Cichorium intybus* L.)	Spectrophotometric	Colorimeter, (CIE*L*a*b**)	Francis *et al.*, 2014
Pistachio kernels (*Pistacia vera* L.)	C_{30} column HPLC-DAD, MS	Colorimeter, (CIE*L*a*b**)	Pumilia *et al.*, 2014
Spinach	C_{18} column HPLC-DAD	Colorimeter, (CIE*L*a*b**)	Wang *et al.*, 2013
Parsley (*Petroselinum Crispum*); Dill (*Anethum graveolens*)	Spectrophotometric	Colorimeter, (CIE*L*a*b**)	Kamel, 2013
Olive oil	Spectrophotometric	Spectrophotometer, 380–770 nm (CIE*L*a*b** and CIELUV)	Moyano *et al.*, 2008
Green tea (*Camellia sinensis)*	Spectrophotometric	Spectrophotometric absorbance at 420 nm	Huang *et al.*, 2007
	Spectrophotometric; C_{18} column HPLC UV-Vis	Sensory line scale ('no greenness' to 'very green'); Colorimeter, (CIE*L*a*b**)	Wang *et al.*, 2004
Yerba maté (*Ilex paraguariensis*)	C_{18} column HPLC UV-Vis	Colorimeter, (CIE*L*a*b**)	Schmalko & Alzamora, 2001

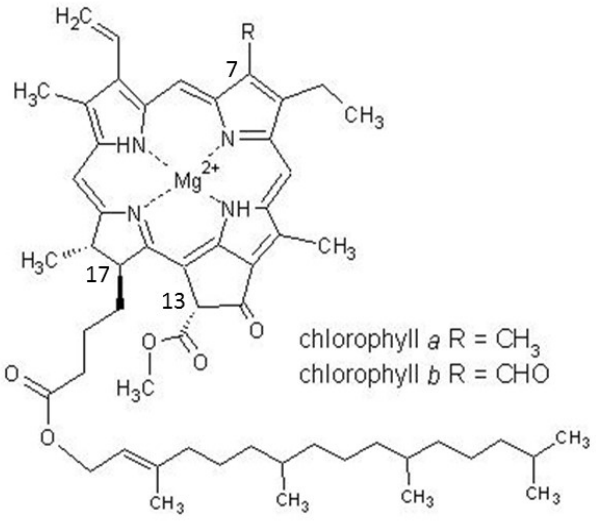

Figure 2.8 Structures of chlorophyll *a* and *b*.

Chlorophyll degradation is of concern in the food industry, especially due to the instability of chlorophyll under processing conditions with a resulting detrimental effect on product quality. Green honeybush is no exception. Various factors contribute to chlorophyll degradation and result in the formation of chlorophyll derivatives (**Fig. 2.9**). Derivatives are identified according to amendments to the original chlorophyll structure as indicated in **Table 2.8** (Lafeuille *et al*., 2014). Furthermore, exposure to mild heat, as experienced during even the mildest processing of tea, causes the chlorophyll or chlorophyllide molecule to isomerise as the C13 carbomethoxy group is inverted, leading to the formation of *a′* or *b′* epimers (Schwartz *et al*., 2008; Lafeuille *et al*., 2014). Using UV-Vis absorption properties of extracts of dried herbs, Lafeuille *et al*. (2014) found that chlorophylls and their coloured derivatives may be broadly divided into green and olive-brown groups. Accordingly, chlorophyll, chlorophyllide, pyrochlorophyllide and pyrochlorophyll constitute the green group and pheophytin, pheophorbide, pyropheophytin and pytopheophorbide the olive-brown group.

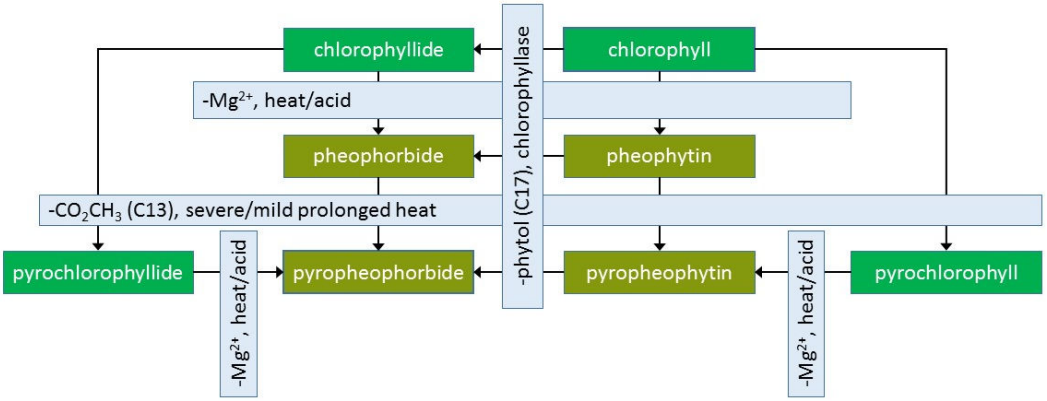

Figure 2.9 Degradation of chlorophyll to coloured chlorophyll derivatives (compiled from Lafeuille *et al*., 2014).

Table 2.8 Processes and nomenclature for chlorophyll degradation derivatives

Process	Mechanism	Nomenclature	Example
Hydrolytic removal of phytol chain (C17)	Chlorophyllase	-ide	chlorophyll → chlorophyllide
Decarboxymethyllation (C13)	Heat	pyro-	pheophorbide → pyropheophorbide
Removal of chelated Mg^{2+}	Acid substitution	pheo-	chorophyll → pheophytin
Epimerisation (C13)	Isomerisation	′	chlorophyll *a* → chlorophyll *a*′

The blue-green colour of chlorophyll is retained in its enzymatically cleaved derivative, chlorophyllide. Chlorophyllase, an esterase enzyme occurring in plant cell vacuoles and active in water, alcohols and acetone, is activated during heat processing and causes cleavage of the phytol chain. The resulting derivatives are termed with the suffix *–ide*, for example, the conversion of chlorophyll to chlorophyllide (**Table 2.8**). Chlorophyllides are slightly more water-soluble than chlorophylls (Schwartz *et al*., 2008). Despite this, chlorophyllides were not found to be a major

contributor to the infusion colour of green tea (as analysed for individual compounds using HPLC), as the chlorophyllase enzyme is deactivated upon fixing (the initial steaming or pan-firing treatment) and chlorophyllide itself is also highly heat labile (Wang *et al*., 2004). Thermal stability of chlorophyllides has been shown to be less than that of chlorophyll (Canjura *et al*., 1991). Rapid degradation of chlorophyllide could be due to the increased likelihood that this more hydrophyllic compound would come into contact with hydrogen ions when in solution, leading to the loss of the Mg^{2+} ion (Schwartz *et al*., 2008).

As mentioned, the chlorophyllase enzymatic reaction itself does not facilitate the colour change from green to olive-brown. The colour change is caused by loss of the Mg^{2+} ion ligand associated with the tetrapyrrole centre with the application of heat and acid. Heat stability of chlorophylls has been shown to be influenced by pH, with greater stability at basic pH values (Ryan-Stoneham & Tong, 2000; Schwartz *et al*., 2008). In the presence of acid, including plant acids released during cutting of the plant material and concomitant cell disruption, the Mg^{2+} ion associated with the chlorophyll molecule or derivative is replaced by two H^+ ions. The derivatives with the prefix *pheo*-, for example, pheophytin from chlorophyll or pheophorbide from chlorophyllide, are thus formed. These derivatives may be further heat-degraded by the removal of the C13 carbomethoxy group to form derivatives with the prefix *pyro*-, which are similarly coloured to their preceding pigments. It was previously thought that these compounds are only formed as a result of severe heat treatment such as canning, but a study by Lafeuille *et al*. (2014) found that *pyro*- derivatives of chlorophylls are also as a result of long-exposure, milder heat treatments such as sun-drying of fresh culinary herbs.

Heat stability and degradation pathways for chlorophyll *a* and *b* do not necessarily favour the formation of the same types of compounds, as was found by Lafeuille *et al.* (2014). Their study conducted on dried culinary herbs showed pheophytin *b* was only found in samples which had undergone sun-drying. Pheophytin *a*, however, was detected in all samples, indicating different pathways for chlorophyll *a* and *b*. It was postulated that chlorophyll *b* follows a degradation pathway via pyrochlorophyll *b* to form pyropheophytin *b*, whereas chlorophyll *a* was degraded to pyropheophytin *a* via the formation of pheophytin *a* (**Fig. 2.9**).

In addition to the above processes induced by heat and/or acidic conditions, a further phenomenon, that of photo-bleaching, where chlorophyll derivatives are finally broken down to colourless compounds with exposure to light, has also been identified. This would be particularly relevant during storage when the green honeybush product could be exposed to light. Significant losses of chlorophyll and carotenoids as a result of photo-bleaching were observed for photosystem I particles from spinach leaves during exposure to high light intensities over the course of 120 min (Andreeva *et al*., 2007). Similarly, blanched broccoli florets stored for two days with exposure to light showed significant decreases in chlorophylls *a* and *b* content, despite successful enzymatic inactivation, whereas similar samples with no light exposure maintained constant chlorophyll levels over the same time period (Kotani *et al*., 1999).

Although comparably little is known about the mechanism of photo-bleaching, Ginsburg and Matile (1993) and Ginsburg *et al.* (1994) have proposed the enzymatic oxygenolytic cleavage of the pheophorbide porphyrin ring as a possible mechanism. This process was found to produce colourless fluorescent compounds which may be further degraded to form colourless non-fluorescent compounds (Ginsburg *et al*., 1994).

In black tea, the fermentation and firing processes result in a red/dark brown to black product. The black colour of black tea is accompanied by a decrease in chlorophyll and its derivatives as well as the formation of flavanol oxidation products, theaflavins and thearubigins, which have been correlated with the increase in darkness of infusion colour and brightness (Kim *et al*., 2011). This oxidation is influenced by the presence of oxygen, temperature, pH and polyphenol composition of the green leaf as well as the event of coupled oxidation (Robertson, 1992). Similar reactions may also play a significant role in the colour development of herbal teas.

Due to the sensitive nature of the green colour components of green tea, processing techniques to prevent the breakdown of chlorophyll are necessary. Early or immediate steaming and/or drying after plucking of green or herbal teas are necessary to prevent the degradation of chlorophyll by chlorophyllase and rapid oxidation of polyphenols by enzymes such as polyphenol oxidase, forming brown pigments. Effective drying is immensely important as even low water activity (A_w 0.32) has been shown to allow slow chlorophyll degradation (LaJollo *et al*., 1971). In fact, LaJollo *et al*. (1971) showed that chlorophyll losses in dehydrated spinach can be predicted as a function of water activity (A_w 0.32 to 0.75, 37 °C) as water availability is required for conversion from chlorophyll to pheophytin. Careful consideration of packaging and storage conditions are thus of importance as further degradation of both chlorophyll and polyphenol compounds may persist during storage as a result of residual enzymatic activity aggravated due to moisture permeation, or non-enzymatic degradation (Robertson, 1992).

4.2 Aroma, flavour, taste and mouthfeel perception

4.2.1 *Aroma and flavour*

In humans, three chemoreceptor systems work together to provide us with the sense of flavour. Gustation (taste on the tongue), trigeminal (sense of irritation) and olfaction (sense of smell) all form part of flavour perception, however, the sense of smell or olfaction makes the largest contribution to flavour perception (Dutta *et al*., 2003). Volatile or aroma compounds are detected in the rear of the nasal cavity on the olfactory mucosa where volatile compounds bind to G-protein coupled receptor molecules to elicit a sensorial response (Sanz *et al*., 2006; Diaz, 2004). Volatile compounds may reach this area via the nose (orthonasal) or the mouth (retronasal), and in both cases some compounds may be absorbed by the mucosa before reaching the point of detection (Dutta *et al*., 2003, **Fig. 2.10**). For an orthonasal stimulus, an adequate number of volatile molecules are inhaled and travel from the anterior nares toward the olfactory mucosa (Diaz, 2004). Alternatively, if these molecules are ingested via the mouth, retronasal stimulus occurs during respiratory exhalation or

after swallowing with the volatiles reaching the olfactory mucosa by ascent through the posterior nares of the nasopharynx (Diaz, 2004). Receptors fall into either class I or II olfactory receptors, of which about 350 have been identified (Sanz *et al*., 2006). These receptors combine to allow humans the ability to detect and discriminate a variety of flavours and odours elicited from a range of structurally diverse odorants (Sanz *et al*., 2006).

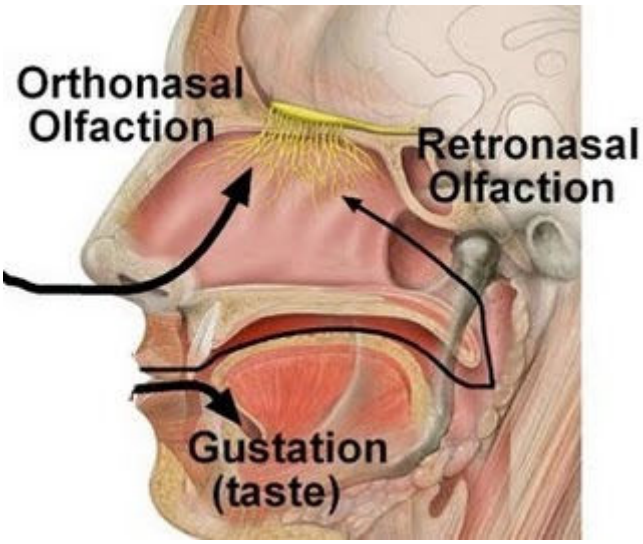

Figure 2.10 Schematic of orthonasal and retronasal olfaction,

(http://www.drsharma.ca/why-hunger-lets-you-smel-through-doors).

Perception of aroma and flavour may be further affected by interactions between volatiles or interactions of volatiles with other dietary compounds. For example, Goldner *et al.* (2011) found that increased polyphenol content of Malbec wines significantly decreased intensities of 'fruity', 'citrus', 'strawberry', 'cooked fruit' and 'floral' aromas without significant differences in volatile composition. The review by Chambers and Koppel (2013) addressed the shortcomings of instrumental and sensory flavour and aroma analysis and correlations drawn between the two. These difficulties may be illustrated by the aroma associations of hexanal, where this compound has been associated with 'hay' aromatics in honey (Castro-Vazquez *et al*., 2009) and 'green' aromatics in tomatoes (Abegaz *et al*., 2004; Baldwin *et al*., 2004). Other associations also included 'rancid' or 'oxidized' aromatics in walnuts (Lee *et al.*, 2011) and dry dog foods (Koppel *et al*., 2013) as well as 'cheese' aromatics in honey (Castro-Vazquez *et al*., 2009) and 'woody' aromatics in cheese (Ercan *et al*., 2011). This range of associations in different food matrixes may be influenced by interactions with other volatiles such as *trans*-2-nonanal, where the two combined volatiles produced a 'beany' character not represented by either compound in isolation (Bott & Chambers, 2006). Furthermore, volatile compound concentration may also affect flavour perception as found by the investigation of Hongsoongnern and Chambers (2008), where low concentrations from 10 to 100 ppm hexanal produced 'musty/earthy' odours and higher concentrations of 5000 to 100000 ppm produced 'green-grassy/leafy', 'green-viney', 'musty-earthy' and 'pungent' aromas.

4.2.2 *Taste and mouthfeel*

Not all individuals experience taste to the same degree, possibly due to a combination of environmental (as reviewed by Duffy, 2007) and genetic factors such as age, gender, hormonal status (as reviewed by Reed *et al*., 2006). For example, receptor genes for bitterness induced by specific bitter compounds (phenylthiocarbamine and 6-n-propylthiouracil) have been identified, dividing the population into the so-called non-, medium- and super-tasters and influencing individual liking or preference for bitter food or beverage products (Dinehart *et al*., 2006; Duffy & Bartoshuk, 2000; as reviewed by Feeny, 2011). Familiarity with the product or variation in individual saliva characteristics due to morphological characteristics of the parotid glands or physiological differences in protein secretory pathways may influence perceived astringency and taste in consumers (Dinella *et al*., 2011; Dinella *et al*., 2009).

Additionally, there exists the possibility of taste modulation by olfaction as observed by Labbe *et al*. (2006). The addition of cocoa or vanilla flavouring to a cocoa-water base analysed with and without a nose clip resulted in the enhancement of bitter taste by the addition of cocoa and the enhancement of sweetness by vanilla flavouring when analysed without the nose-clip. A similar effect was observed by Lavin and Lawless (1998), where vanilla flavouring added to milk resulted in enhanced sweet taste perception. In model solutions, the enhancement of sweet taste by fruity odour has been shown, although sweet taste did not affect fruity odour (Bonnans & Noble, 1993; Cliff & Noble, 1990). Individual taste/aroma associations may thus influence the perceived attributes of a product according to the individual's own experiences (Köster, 2005; Köster *et al*., 2004).

4.2.2.1 *Sweet taste*

The mechanism of sweet taste lies in genetically coded protein-bound taste receptors described as G protein-coupled receptors (GPCRs) (Hoon *et al*., 1999; Nelson *et al*., 2001; Nelson *et al*., 2002; Li *et al*., 2002). Three subunits (T1R1, T1R2 and T1R3) have been identified as affecting sweet taste (Hoon *et al*., 1999; Montmayeur *et al*., 2001; Nelson *et al*., 2002; Sainz *et al*., 2001). Studies using cell-based assays have demonstrated that sweet taste is detected through a heterodimeric receptor comprised of the T1R2 and T1R3 subunits (Nelson *et al*., 2001; Li *et al*., 2002; Zhao *et al*., 2003). The subunits each have multiple ligand binding sites (Zhang *et al*. 2008) possibly providing indication as to the variety of compounds besides sugar which can elicit a sweet taste in the mouth. Many natural botanicals have been found to elicit a sweet taste attributed to components other than sugar (sucrose), namely, some mono-, sesqui-, di-, and triterpenoids, steroidal saponins, some phenolic compounds and proteins (as reviewed by Behrens *et al*., 2011). Sweet-tasting phenolics are of interest as low kilojoule, high intensity natural sweeteners. Bitter-tasting flavanone glycosides, naringin and neohesperidin, have been used to produce highly sweet dihydrochalcones by treatment with alkali and hydrogenation (Behrens *et al*., 2011; Montijano & Borrego, 1999). Von Pressentin Du Preez (2014) achieved conversion of hesperidin extracted from *C. maculata* to hesperetin, its corresponding aglycone known to exert a sweet taste modulation effect. Furthermore,

dihydrochalcones or glycosidic forms of phloretin are thought to contribute to the sweet taste of apples (Kinghorn & Soejarto, 1989). The presence of dihydrochalcones in honeybush extracts (De Beer *et al*., 2012; Schulze *et al*., 2014) may therefore contribute to the sweet taste of infusions. Partial least squares (PLS) regression of the taste modalities and individual phenolic compound content showed that phloretin-3′-5′-di-*C*-glucoside, 3-hydroxyphloretin-3′-5′-di-*C*-hexoside, scolymoside, eriocitrin and eriodictyol-*O*-glucoside associated positively with the sweet taste of honeybush infusions (Erasmus, 2015). Moderate, but significant positive correlations ($r > 0.3$, $p < 0.05$) between sweet taste and phloretin-3′-5′-di-*C*-glucoside, 3-hydroxyphloretin-3′-5′-di-*C*-hexoside, scolymoside and eriocitrin were also identified by PCA. These correlations were, however, not necessarily causative and taste modulation due to compound interaction may play a role. This was demonstrated for Z-2-(β-D-glucopyranosyloxy)-3-phenylpropenoic acid (PPAG), a compound suspected to contribute toward sweet taste of rooibos (*Aspalathus linearis*) infusions, with a significant positive correlation coefficient ($r = 0.356$) (Koch, 2011). When assessed in isolation, however, PPAG was found to exhibit a slightly bitter to astringent taste with a detection threshold of 0.4 mg/L in water (Joubert *et al*., 2013).

4.2.2.2 *Sour taste*

The perception of sour taste is dependent on the activation of the ion channels through the receptor cell membrane (Jackson, 2009). These pH sensitive channels are encoded by the ASIC family of genes and involve direct entry of ions through the channels after activation by a drop in extracellular pH (as reviewed by Kellenberger & Schild, 2002). However, as reviewed by Da Conceicao Neta *et al*. (2007), sour taste cannot be directly related to pH as hydrogen ions are not solely responsible for sour taste perception. It is hypothesised that sour transduction may also be due to proton blockages of K^+ channels (Kinnamon *et al*., 1988). Sour or acid taste has been mostly identified with protons or inorganic cations in solution, as is the case with organic acids present in foodstuffs, although sour taste characteristics have been observed for some phenolic acids (Huang & Zayas, 2001; Peleg & Noble, 1995). Despite much research on the mechanism of sour taste intensity, no simple relationship has been established between this attribute and hydrogen ion concentration, nor sour taste intensity of acids and titratable acidity, buffer capacity, molar concentration or physical and chemical structure (as reviewed by Da Conceicao Neta *et al*., 2007).

4.2.2.3 *Bitter taste*

Bitter taste is also perceived through the activation of GPCRs, but contrary to sweet taste receptors (T1R group subunits with large N-terminal domain) (Hoon *et al*., 1999), the bitter taste receptors are comprised of the T2R group of protein subunits (with a short extracellular N terminus) (Alder *et al*., 2000; Chandrashekar *et al*., 2000). Additionally, the T2R group of subunits is much more diverse than the T1R group, with an expected number of about 40 to 80 different subunits and the expression

of multiple T2R protein subunits in single receptor cells (Alder *et al.*, 2000). This may allow for a broader range of detection of bitter taste from a variety of bitter tasting compounds.

Compounds known to elicit a bitter response include many beneficial dietary compounds such as flavonoids, isoflavones, terpenes and glucosinolates (Drewnowski & Gomez-Carneros, 2000). Monomers of flavanols (Peleg *et al*., 1999) and polymeric tannic acid and grape seed tannin (Robichaud & Noble, 1990) were found to elicit a stronger bitter response than higher polymers. The loss of perceived bitterness for the highly polymerised molecules may be as a result of a higher degree of steric hindrance, reducing the interaction between the molecule and bitter receptor or receptor membrane (Peleg *et al*., 1999).

According to the study by Theron (2012), among the variety of polyphenols present in honeybush infusions, mangiferin was the only compound that showed a strong correlation ($r = 0.74$) to bitter taste. However, a subsequent study by Erasmus (2015) indicated significant ($p < 0.05$) strong correlations between bitter taste perception and mangiferin ($r = 0.755$), maclurin-di-*O,C*-hexoside ($r = 0.737$) and maclurin-3-*C*-glucoside ($r = 0.706$). Additionally, other bitter compounds such as specific amino acids may contribute to the bitter taste of honeybush (Solms, 1969).

It is possible, however, that some compounds induce a bitter modulating effect such as homoeriodictyol and eriodictyol (Ley *et al*., 2005). Eriodictyol has been shown to mask bitter taste in yerba santa (*Eriodictyon angustifolium* and *E. californicum*) extracts (Reichelt *et al.,* 2010a,b). This compound has been successfully produced by hydrolysis of the glycoside moiety from eriocitrin present in *C. maculata* (Von Pressentin Du Preez, 2014). Non-bitter flavanol-3-glycosides were also found to contribute to bitter taste in black tea by modulation, amplifying caffeine bitterness (Hofmann *et al*., 2006).

4.2.2.4 *Astringency*

Astringency is commonly associated with foods and beverages such as teas containing high levels of polyphenols. It is perceived as the tight, puckering sensation of the mucosa and muscles around the mouth as well as dryness of the oral surface (Lee & Lawless, 1991). Astringency in tea is commonly associated with bitter taste (Thorngate & Noble, 1995) and evolves in the mouth with a slow onset, peaking at 20 – 30 s after tasting (Rossetti *et al*., 2009; Joslyn & Goldstein, 1964). A carry-over or build-up effect is also common for astringency as well as bitterness, resulting in the development of various testing methods to isolate perceived attributes for each sample (Lesschaeve & Noble, 2005).

The resulting physical perception of astringency is commonly believed to be due to interactions between lubricating salivary proteins and polyphenols (Rosetti *et al*., 2009; Rossetti *et al*., 2008; Lu & Bennick, 1998; Charlton *et al*., 2002), although it has also been suggested that the inhibition of sodium ion channels on the oral epithelia may contribute to an astringent mouthfeel (Simon *et al*., 1992). According to the review by Gibbins and Carpenter (2013), the two proposed mechanisms of astringent mouthfeel include the possibility of increased friction caused by a

disruption in the mucosal film in the oral cavity due to interactions between tannins and saliva, or the proposed direct interaction of the protein-tannin aggregates with the exposed oral tissue through receptors. The intensity of astringency may be due to, amongst others, the size or degree of polymerisation (Peleg *et al*., 1999), isomerisation (Thorngate & Noble, 1995) or substitution (Rossetti *et al*., 2008; Rossetti *et al*., 2009) of the polyphenolic molecule involved.

Binding of salivary proteins have been observed for polyphenols of molecular weights of 500 Da to 3000 Da, with binding ability increased with greater molecular weight (Bakker, 1998; Lesschaeve & Noble, 2005). This degree of binding is not typical for polyphenols below molecular weights of 500 Da (Bate-Smith, 1973). Despite this select categorisation, astringent mouthfeel has been reported for smaller compounds including flavanol monomers, dimers and trimers (Peleg *et al*., 1999) as well as smaller substituted benzoic acids (Peleg & Noble, 1995). It is postulated that strong binding and cross linking through adjacent hydroxyl groups of the phenolic compounds to salivary proteins may induce the sensation of astringency (McManus *et al*., 1981). This may well be the case for honeybush infusions rich in xanthones (mangiferin and isomangiferin) as well as benzophenones (iriflophenone-3-*C*-glucoside), with molecular weights slightly less than 500 Da, which have been associated with astringency in honeybush infusions (Erasmus, 2015). Some major compounds including hesperidin, eriocitrin, scolymoside and vicenin-2 commonly present in honeybush have molecular weights slightly higher than 500 Da, although of these, only vicenin-2 has been linked to astringency in honeybush (Erasmus, 2015). Larger polymerised molecules may be more effective in binding salivary proteins through increased hydroxyl sites available for hydrogen bonding to carbonyl groups on proteins, eliciting an increase in the perception of astringency (Peleg *et al*., 1999). In the study conducted by Peleg *et al*. (1999) on sensorial bitterness and astringency of flavanols, smaller monomeric flavanol units were found to have higher intensity and persistence of bitterness, but lower intensity of astringency than the larger dimers and trimers. This effect was also observed by Robichaud and Noble (1990) with polymeric tannic acid and grape seed tannin.

Small differences in molecular structure may have an impact on bitter intensity and astringency. A good example is the monomeric diastereomers, 3S(+)-catechin and 3R(–)-epicatechin, both of which are found in *C. sinensis* teas and red wine. These compounds differing in the configuration of one hydroxyl group also differ in bitterness and perceived astringency (Thorngate & Noble, 1995; Kallithraka & Bakker, 1996). The epicatechin diastereomer has been identified as having a significantly higher maximum intensity and longer persistence of bitterness and astringency than catechin. This effect is expected to be, to some degree, as a result of the increased lipophilicity of epicatechin due to the more planar heterocyclic C-ring structure, allowing for easier intermolecular hydrogen bonding through the hydroxyl group (Thorngate & Noble, 1995).

Rossetti *et al*. (2008) investigated the effect of different tea catechins on human saliva to determine the influence these polyphenols have on the tactile perception of tea astringency. They found that a stronger intermolecular network was formed by epigallocatechin gallate (EGCG) than by epicatechin (EC), indicating that the exposed galloyl ring of EGCG promotes preferential

hydrophobic complexation with the pyrrolidine ring of basic proline-rich salivary proteins (Lu & Bennick, 1998; Charlton *et al*., 2002) and may thus result in a heightened perception of astringency. Rossetti *et al*. (2009) established a linear correlation (R^2 = 0.863) between sensory astringency and instrumental friction measurements for EGCG and EC solutions at increasing bitterness threshold concentrations. This supports the hypothesis that an increase in friction due to precipitation of saliva-polyphenol complexes may be responsible for the dry astringent mouthfeel as detected by mechanoreceptors within the mucosa.

Furthermore, modulation of astringent mouthfeel by organic and inorganic acids has been linked to increased perception of astringent mouthfeel, possibly by intensifying interactions between salivary proteins and polyphenols (Siebert & Chassy, 2003). It has also been proposed that a lowered pH may denature some salivary proteins, enabling or preventing effective lubrication (Lawless *et al*., 1996). Contrary to this amplification of astringency, polysaccharides have been linked to inhibition of astringency in wine (Quijada-Morín *et al*., 2014), possibly by disrupting or inhibiting protein-tannin interactions (Mateus *et al*., 2004; Carvalho *et al*., 2006; Escot *et al*., 2001) or by inhibiting protein-tannin complex precipitation (De Freitas *et al*., 2003).

5. Methods for quality assessment of green tea

5.1 Instrumental colour measurement

A product with such a descriptive name as green tea is known for, and expected to have a green colour. A consumer would thus expect the leaf product to appear distinctly green. Colour, however, is perceived subjectively as it is influenced by light source, sample surface condition, the direction of observation and light source position, sample size, background differences as well as the individual observer (Pathare *et al*., 2013). For effective comparable quality assessment it is thus necessary to standardise the conditions under which colour is analysed and measured. Although visual assessment is still commonly used, instrumental colour analysis by tristimulus colorimetry or spectrophotometric measurement is popular in many food and non-food applications (**Table 2.7**). The benefit in using such instrumental techniques is the ease of standardisation, where light source and viewing angle are specified without other environmental disturbances. In order to describe colour objectively and numerically, the Commission *Internationale de l'Eclairage's* (CIE) *L*a*b** colour space is commonly used (**Table 2.7**).

Spectrophotometric measurement quantifies the spectral distribution of the sample from which colour is calculated according to measurement conditions (Pathare *et al*., 2013). Spectral distribution is measured in terms of transmittance and reflectance which are properties inherent to the material measured (Pathare *et al*., 2013). For translucent samples such as liquids or extracts, transmittance is measured as the ratio of transmitted spectral flux to the initial incident spectral flux (Palmer, 1995). Light is thus transmitted through the sample for measurement to be collected on the other side. Reflectance is used for non-translucent samples, where light is reflected off the surface of the sample and returned back into the incident hemisphere, for the collection of the measurement

data (Palmer, 1995). Once again, the measurement is calculated as a ratio of reflected radiant flux to the incident radiant flux (Palmer, 1995)

The CIE*L*a*b** colour space (**Fig. 2.11**) is useful for the interpretation of colour data calculated from spectral distributions and comprises a three dimensional coordinate system indicating specific points in the colour space to accurately identify a colour (Pathare *et al*., 2013). This colour space was developed in 1976 in response to a limitation with other colour spaces such as the *XYZ* and *L*C*h* colour spaces, where measured distances on the x, y chromaticity diagram were not comparable to equal perceived colour differences (Pathare *et al*., 2013).

In order to measure colour, an object (the sample), an illuminant and an observer are required (Pathare *et al*., 2013). The CIE*L*a*b** colour space makes use of a standard illuminating light source and observer at a specified angle to directly measure *a** and *b** as an indication of colour and *L** as a psychometric index of lightness (Pathare *et al*., 2013). Hue or hue angle (h) and chroma (C*;* saturation) are calculated from *a** and *b*,* which together with *L** are used to accurately quantify a reproducible colour measurement (Pathare *et al*., 2013). The value *a** describes colour along an axis with positive values indicating reddish colours and negative values indicating greenish colours. The *b** value runs along a similar perpendicular axis with positive values for yellowish colours and negative values for blueish colours (Pathare *et al*., 2013).

Chroma and hue angle are calculated as indicated in the following two formulae, and deliver a quantitative measure of colourfulness and qualitative measure of colour, respectively (Pathare *et al*., 2013):

$$\mathrm{C} = \sqrt{a^{*2} + b^{*2}}$$

$$\mathrm{h} = tan^{-1}\left(\frac{b^*}{a^*}\right)$$

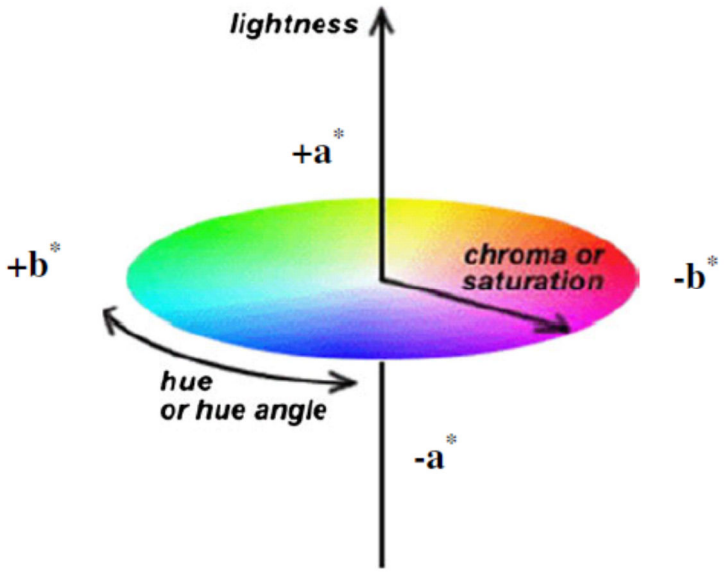

Figure 2.11 CIE*L*a*b** colour space (Pathare *et al*., 2013).

Analytical equipment to measure CIE*L*a*b**, however, is not always available to quality assessors, especially in small, developing industries. In such cases, visual assessment is more appropriate and cost effective. For effective visual assessment a reference colour or range of colours such as the Munsell colour system or colour chips would be used for comparison against the current sample in order to make a less subjective analysis. Colour charts are useful for colour comparison as a reference can be specified once and compared to all other samples for acceptance or rejection relative to the quality standard.

5.2 Chlorophyll determination

Chlorophyll *a* and *b* are the main contributing compounds toward the green colour of vascular plants (Ritchie, 2006). Quantification of these compounds can thus serve as an indication of the green colour quality parameter. Degradation of chlorophyll, as discussed in section 4.1, is associated with a colour change from green to olive-brown due to the conversion of chlorophyll *a* and *b* to pheophytins or pyropheophytins. A decrease in the green colour of unfermented honeybush is associated with a perceived loss of quality (Joubert *et al*., 2010). The labile nature of chlorophyll and its derivatives creates difficulty in analysis as measures must be taken to prevent breakdown, especially during extraction. Hydrophobic chlorophyll may be extracted from plant material using acetone (a popular choice), ethanol or methanol (which often provides better extraction) as solvent and analysed spectrophotometrically by measuring the red absorption peaks of chlorophyll *a* and *b* at the appropriate wavelengths (Richie, 2006; Carvalho *et al*., 2011). This method, however, will only allow for quantification of green chlorophyll and chlorophyllide and not of the olive-brown or colourless derivatives. As previously discussed, the stability of chlorophyll is compromised in acidic conditions encountered in solution when plant acids are released due to cell disruption (cutting or shredding) (Lichtenthaler & Buschmann, 2001). Chlorophyll may thus be stabilised by increasing the pH of the extracting solution with addition of $MgCO_3$. Its use has the additional benefit of increasing the Mg^{2+} concentration to prevent disassociation of the Mg^{2+} ion chelated in the central cavity (Ritchie, 2006; Lichtenthaler & Buschmann, 2001; Schwartz *et al*., 2008).

HPLC has been used effectively to gain insight into the degradation of chlorophylls and its derivatives during food processing (**Table 2.7**), but it is an expensive technique, especially for industrial application. Chlorophyll determination by spectrophotometric methods has been applied successfully to various plant materials (**Table 2.7**) and is a more cost-effective method for routine analysis, where total chlorophyll content or its correlation to green colour is of interest. Spectrophotometric equations (as indicated below) are used to determine chlorophyll content:

$$Chl\ a\ \left(^{\mu g}/_{mL}\right) = \left(\mathrm{E}_{\lambda \mathrm{a,a}} \times A_{\lambda a}\right) + \left(\mathrm{E}_{\lambda \mathrm{b,a}} \times A_{\lambda b}\right)$$

$$Chl\ b\ \left(^{\mu g}/_{mL}\right) = \left(\mathrm{E}_{\lambda \mathrm{a,b}} \times A_{\lambda a}\right) + \left(\mathrm{E}_{\lambda \mathrm{b,b}} \times A_{\lambda b}\right)$$

where Chl a = chlorophyll a, Chl b = chlorophyll b, and $E_{\lambda a,a}$ is the absorbance coefficient (mg 1^{-1} cm A^{-1}) for chlorophyll a at the red peak (λa) of chlorophyll a, $E_{\lambda a,b}$ is the absorbance coefficient (mg 1^{-1} cm A^{-1}) for chlorophyll b at the red peak (λa) of chlorophyll a. $A_{\lambda a}$ is the absorbance of the pigment extract at wavelength (λa) nm.

These equations make use of coefficients for solvent used and chlorophyll species present in the material (**Table 2.9**) as these determinations rely on the unique absorbance of chlorophylls at blue and red regions of the UV-Vis spectrum (Ritchie, 2006). Measured wavelengths are adjusted according to these parameters as shifts in absorbance depend on both solvent and chlorophyll species (**Table 2.9**).

Table 2.9 Coefficients (E_λ) for spectrophotometric equations for chlorophyll *a* and *b* at their respective red absorption peaks (Ritchie, 2006)

Solvent	**Chorophyll**	**λa**	**λb**
		647 nm	*664 nm*
90% acetone	a	-1.7858	11.8668
	b	18.9775	-4.895
		652 nm	*665 nm*
methanol	a	-8.0962	16.5169
	b	27.4405	-12.1688
		649 nm	*665 nm*
ethanol	a	-5.2007	13.5275
	b	22.4327	-7.0741

5.3 Phenolic quantification

Polyphenols are some of the major constituents of *Cyclopia* spp. In addition to their contribution to sensory characteristics such as taste and colour of the infusion, bioactivity is increasingly important as the nutraceutical value of honeybush, and in particular green honeybush, is emphasised for market exploitation. In terms of processing optimisation or development, it is necessary to investigate the effect that processing has on the phenolic constituents of the plant material and final product.

The preferred method of choice to routinely quantify polyphenols is by HPLC combined with UV-Vis or diode array detection (DAD). Many examples of relevant applications can be found in literature. It is of importance that the applied method should provide a comprehensive phenolic profile so that changes in both major and minor compounds can be quantified. For the development of useful HPLC methods, effective separation is required by manipulation of temperature, and composition and gradient of mobile phase, besides column (stationary phase) specifications which affect selectivity (Snyder *et al*., 2010). Method validation is required to ensure the reliability of the method in terms of accuracy, specificity, precision, detection and quantification limits, linearity, range and robustness (Snyder *et al*., 2010). It is also important to confirm that the correct compounds have

been identified and selected for separation. This is commonly performed using liquid chromatography coupled to mass spectrometry (LC-MS) in order to accurately identify the structures of unknown compounds, or to confirm peak identity (Snyder *et al*., 2010).

Given these requirements, the ARC is in the process of developing and validating species-specific HPLC-DAD methods as previous HPLC methods were suitable only for quantification of a limited number of compounds (**Table 2.10**). The method had several shortcomings, including co-elution of unidentified compound(s) with isomangiferin and poor separation of minor constituents, with a complicated integration of eriocitrin, scolymoside and phloretin-3',5'-di-*C*-β-glucoside (De Beer *et al*., 2012; Beelders *et al*., 2014b). To date species-specific methods have been developed for *C. subternata*, *C. maculata*, *C. genistoides* and *C. longifolia* to improve phenolic quantification of honeybush extracts.

Table 2.10 Development of HPLC quantification methods for honeybush phenolic compounds

Species	Compounds quantified	Reference
General *Cyclopia* spp. (*C. intermedia*, *C. sessiliflora*, *C. genistoides* and *C. maculata)*	mangiferin, isomangiferin and hesperidin	Joubert *et al*., 2003
Cyclopia subternata (applied to *C. genistoides, C. longifolia, C. intermedia* and *C. sessiliflora*),	mangiferin, isomangiferin, hesperidin, eriocitrin and scolymoside	De Beer & Joubert, 2010
C. subternata	mangiferin, isomangiferin, hesperidin, eriocitrin, scolymoside, iriflophenone-3-*C*-β-glucoside, phloretin-3',5'-di-*C*-β-glucoside and 3-hydroxyphloretin-3',5'-di-*C*-hexoside	De Beer *et al*., 2012
C. maculata	mangiferin, isomangiferin, hesperidin, eriocitrin, iriflophenone-3-*C*-β-glucoside, vicenin-2, eriodictyol-*O*-hexoside and maclurin-3-*C*-glucoside	Schulze *et al*., 2014
C. genistoides	mangiferin, isomangiferin, hesperidin, eriocitrin, iriflophenone-3-*C*-glucoside, phloretin-3',5'-di-*C*-glucoside, vicenin-2, eriodictyol-*O*-hexose-*O*-deoxyhexose, maclurin-3-*C*-glucoside, maclurin-di-*O,C*-hexoside, iriflophenone-di-*O,C*-hexoside, tetrahydroxyxanthone-*C*-hexoside dimer, tetrahydroxyxanthone di-*O,C*-hexoside, naringenin-*O*-hexose-*O*-deoxyhexose, naringenin-*O*-hexose-*O*-deoxyhexose, 3-hydroxyphloretin-3',5'-di-*C*-hexoside, tetrahydroxyxanthone-*C*-hexoside isomer	Beelders *et al*., 2014b
C. longifolia	mangiferin, isomangiferin, hesperidin, eriocitrin, scolymoside, iriflophenone-3-*C*-glucoside, vicenin-2, maclurin-3-*C*-glucoside, iriflophenone-3-*C*-glucoside-4-*O*-glucoside and narirutin	Schulze *et al*., 2015

5.4 Sensory analysis

Sensory analysis can be used in industry to obtain a detailed description of the sensory profile of a product to establish parameters for quality. It may also be applied for continuous quality assessment and grading. DSA is necessary to accurately and reproducibly define the sensory profile of a product (Lawless & Heymann, 2010). Extensive DSA tests can be used in the development of aroma and flavour lexicons. Lexicons and flavour wheels are useful tools commonly applied in research and quality control for product categorisation and quality assessment in a variety of products, including coffee, tea, honey and cheese, amongst others (**Table 2.11**). A lexicon is, in essence, a list of descriptive words which apply to attributes that can be present in a product and serves as a guideline for quality control assessors to determine or classify a product by its flavour profile (Drake & Civille, 2002). Flavour wheels are graphic representations of a product lexicon and is thus a simplified version of a product lexicon.

Table 2.11 Development of DSA lexicons for some vegetative or tea products

Product	**Reference**
Yerba maté (*Ilex paraguariensis* Saint Hilaire)	Santa Cruz *et al*., 2002
Green tea (*Camellia sinensis*)	Lee & Chambers, 2007
Fresh leafy vegetables	Bianchi, 2009
Dried spices and herbs	Lawless *et al*., 2012
Rooibos tea (*Aspalathus linearis*)	Koch *et al*., 2012
Honeybush tea (*Cyclopia* spp. general)	Theron *et al*., 2014
Honeybush tea (*C. intermedia*)	Bergh, 2014
Honeybush tea (*C. subternata, C. genistoides, C. maculata, C. longifolia*)	Erasmus, 2015

DSA is a sensory profiling method which makes use of a relatively large panel of judges where the individual panellist's scores are used to calculate an overall panel mean score for each attribute (Drake & Civille, 2002). The panel is trained by a professional sensory leader, guiding the panel to generate a list of attributes which describe the products and may further be anchored by references or reference definitions for each attribute (Drake & Civille, 2002). The aim of this is to ensure the reproducibility and precision of analysis to yield accurate results. The DSA method, however, can be regarded as expensive and extensive with regard to necessary resources, especially in an industry environment. Rapid methods are also used in the food industry, including rapid instructed sorting which has been successfully applied to fermented honeybush of different *Cyclopia* spp. (Erasmus, 2015). Despite the benefits of rapid methods, these have not been validated for green honeybush and they do not produce a full product profile lexicon, as is the case with DSA.

For effective sensory analysis, samples should be prepared and presented according to a standardised protocol in order to minimise intra-sample variation. In the case of green tea, the concentration of the infusion is essential for standardisation and is influenced by a variety of factors

including infusion time, water type, amount and temperature (Lee *et al*., 2008; Lee & Chambers, 2009). An effective method for DSA of rooibos was developed by Koch *et al*. (2012) entailing the use of pre-heated flasks for brewing, pre-heated cups and water baths for infusion preparation and subsequent sample temperature control. The method was developed following previous investigation into quantitative descriptive analysis (QDA) of green tea by Lee *et al*. (2008). This method of sample preparation and presentation was also used for the development of the general (Theron *et al*., 2014) and species-specific (Bergh, 2014; Erasmus, 2015) honeybush lexicons and sensory wheels.

As mentioned previously, a general sensory wheel for honeybush has been developed, characterising it as "*floral, sweet-associated, fruity, plant-like* and *woody* with a sweet taste and a slightly astringent mouthfeel" (Theron *et al*., 2014). Flavour, taste and mouthfeel attributes were sectioned into 10 categories further divided into positive and negative sections. The same classification, however, may not stand for green honeybush, where attributes such as 'green grass' or 'hay/dried grass' aromas (considered to be negative attributes in fermented honeybush) may be characteristic and even desirable. The target market to which green honeybush is presented will affect the desired sensory profile. This said, honeybush is currently being exported from South Africa to a variety of countries with differing consumer profiles such as the Netherlands, Germany, the United Kingdom and Japan (DAFF, 2011), further complicating the sensory grading of green honeybush. For example, Asian countries such as Japan might especially favour *vegetative* sensory profiles as consumers are well acquainted with the 'green' sensory profile of *C. sinensis* green teas.

The sensory analysis and characterisation of green honeybush poses several challenges. Due to the limited retail availability of green honeybush, familiarity with the product is limited and acquiring a range of products with characteristic profiles would not be possible. There is also a lack of knowledge on what South African consumers would view as positive or negative attributes with regard to green honeybush. This makes the sensory characterisation and quality optimisation of green honeybush challenging as acceptance criteria are not available.

6. Conclusions

Despite the great potential identified for green honeybush and the export market value of such a product, optimal processing parameters have not been investigated adequately and product losses due to inferior quality and quality deterioration are still a reality. As a result of intensive research, it has been established that great differences occur in the phenolic composition of a number of *Cyclopia* spp. and their subsequent fermented or unfermented plant material. At present, in-depth studies of the phenolic composition of *C. longifolia* are limited. Furthermore, the response of *C. maculata* and *C. longifolia* to processing and storage in terms of end product quality are hereto un-investigated with regard to green honeybush. Insight into the processing of traditional Japanese green tea (*Camellia sinensis*) and rooibos tea provide indications that steaming may be used to improve green honeybush quality. This may include the retention of green colour and phenolic

compounds as well as the development or alteration of the volatile odour compounds in the tea, leading to manipulation or alteration of the sensory profile. The sensory profile of green honeybush has also not yet been characterised and is required to develop a sensory wheel that in future can be used in research and as a quality control tool by industry.

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Chapter 3

*Manipulating the sensory profile of green honeybush (*C. maculata *and* C. longifolia*) using steam treatment, and the effect on colour and phenolic content*

Contents

Abstract

The growth of the honeybush industry and increased demand for honeybush products have led to an interest in the cultivation of additional *Cyclopia* spp. and improvement of product quality. Traditionally, 'fermented' (oxidised) honeybush is consumed. Although this high-temperature oxidation process is responsible for aroma, flavour and brown colour development, it also leads to the degradation of bioactive compounds, with a subsequent detrimental effect on the health-promoting properties of honeybush. The 'unfermented' product, also known as green honeybush, presents a healthier alternative. The predominantly *vegetative* ('green grass', 'cooked vegetables' and 'hay/dried grass') sensory profile of green honeybush may be unacceptable to some consumers, especially those acquainted with the sensory profile of the traditional fermented product. Sensory attributes of commercial product quality (colour, aroma, flavour, taste and mouthfeel) affect commercial value as they are typically of great importance to consumers and may influence purchase intent. Two distinct *Cyclopia* spp. showing potential for commercial development, *C. longifolia* and *C. maculata*, were used for the present investigation on the application of steam treatment to improve the sensory profile of green honeybush without detrimentally affecting colour and phenolic compounds. Steam treatments, applied either before or after drying of the plant material, were incorporated into the processing procedure. For investigation of steam treatment before drying, the fresh, shredded plant material was subjected to 0, 30, 60, 90 or 120 s steaming. Steam treatment after drying entailed steaming of the dried, green honeybush material for 0, 1, 2, 3 or 4 min. Descriptive sensory analysis indicated significant treatment effects ($p < 0.05$) on retro- and orthonasal attributes of the herbal tea infusion yielding increased intensities of *fruity* ('guava' and 'tropical fruit') and decreased intensities of *vegetative* ('green grass' and 'hay/dried grass') sensory attributes. Although steaming resulted in a minor loss of green colour, as assessed using the CIE*L*a*b** colour space, bioactive compounds (xanthones, benzophenones and flavanones) as determined by high-performance liquid chromatography (HPLC) analysis were not detrimentally affected. The shortest steam treatment before drying (30 s) of *C. maculata*, however, resulted in distinctly different sensory developments, visual browning and loss of phenolic compounds. Steam treatment of at least 60 s may thus be useful for the manipulation of the sensory profile of green honeybush to ensure that a marketable product with an enhanced *fruity* aroma and decreased *vegetative* notes is produced, while retaining colour and phenolic content.

1. Introduction

Traditional honeybush processing entails a high-temperature oxidation step, also known as 'fermentation', responsible for the development of the characteristic *floral*, *fruity*, *woody*, *plant-like* and *sweet-associated* aromas, sweet taste and slightly astringent mouthfeel of the infusions (Theron *et al*., 2014). Fermentation, however, leads to the oxidation and degradation of health-promoting phenolic compounds, lowering the total polyphenol content by approximately 23% to 46% (Joubert *et al*., 2008b). Recently, Beelders *et al*. (2015) demonstrated that fermentation of *Cyclopia genistoides* plant material decreased the content of the major xanthone and benzophenone, mangiferin and iriflophenone-3-*C*-glucoside, by more than 48% and 62%, respectively. With no high-temperature oxidative processing step, green, 'unfermented' honeybush is superior in phenolic content and associated health-promoting benefits (Joubert *et al*., 2008a). In spite of these superior characteristics, green honeybush comprises only a small percentage of the total annual honeybush production and caters mainly for the production of extracts for food ingredient, nutraceutical or cosmetic use (Joubert *et al*., 2011). When sold for consumption as herbal tea, the predominant *vegetative* aroma and flavour of green honeybush is masked by the addition of other flavour ingredients such as mint or berry flavours to increase consumer acceptance of the product. Studies by Le Roux *et al*. (2008) and Cronje (2010) on the volatile fraction of *Cyclopia* demonstrated quantitative, rather than qualitative differences, as the major factor distinguishing the aroma chemistry of fermented and unfermented honeybush.

The aim of the present study was to improve the sensory profile of green honeybush through steam treatment, whilst minimising detrimental changes to the green colour and phenolic content of the leaf product. These changes are initiated when the plant is shredded into small pieces for herbal tea production and accelerated by exposure to heat, as demonstrated for *C. subternata* (Joubert *et al*., 2010). Traditional Chinese and Japanese green tea processing makes use of pan firing and steaming of the picked leaves, respectively, to achieve inactivation of oxidative enzymes prior to further processing (Takeo, 1992). Steaming prior to shredding, however, is not practical in the case of honeybush due to the nature of the harvested plant material, consisting of woody stems with leaves attached. This study followed a two-pronged approach, *i.e.* both fresh and dried, shredded plant material of *C. maculata* and *C. longifolia* were subjected to various durations of steam treatment. The effect on the sensory profile and colour of the infusion prepared from the treated plant material as well as the colour and phenolic content of the plant material were determined. The colour was deemed an important parameter as it plays an important role in product quality. South African export regulations account for the importance of colour in quality standards and state that green honeybush "may not contain more than 5% brown fermented leaves per 10 g dry leaf material" (Anon., 2002). Apart from health benefits, the phenolic content of the plant material is relevant in terms of colour, taste and astringency of the infusion (Theron, 2012; Erasmus, 2015). A preliminary study on the volatile faction of *C. maculata* infusions was also included to gain some insight into perceived aroma changes as a result of steam treatment. The two species investigated represent

different sensory profiles when the fermented product is considered (Erasmus, 2015), suggesting that steam treatment could affect their respective sensory profiles differently. Furthermore, the two species also differ in phenolic compound composition (Schulze *et al*., 2014; Schulze *et al*., 2015) as well as in leaf surface area-to-volume ratio with *C. longifolia* having flat, linear leaves and *C. maculata* thin, needle-like leaves (Joubert *et al*., 2011; **Fig. 3.1**). These differences may affect their susceptibility to changes introduced by steam treatment.

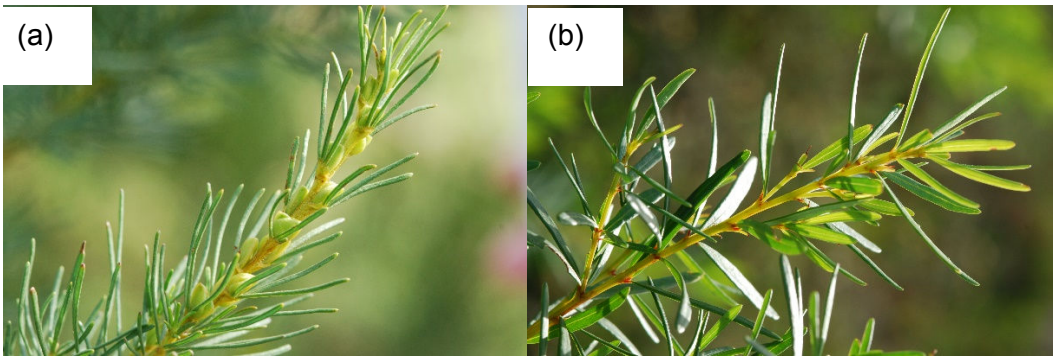


Figure 3.1 Leaf shapes of (a) *C. maculata* and (b) *C. longifolia*.

2. Materials and methods

2.1 Plant material

Shoots of each species (*C. longifolia* and *C. maculata*) were harvested manually according to industry practice and grouped in batches with shoots of several bushes constituting each batch. The plant material was kept in a cold room at *ca.* 0 – 5 °C and processed within 5 days. Specifics of the harvested plant material are presented in **Table 3.1**.

Table 3.1 Plant material harvested for experiments 1 and 2

Experiment	Species	Harvesting date	Harvesting site	Other information
1	*C. maculata*	20 April 2014	Koksrivier (F. Joubert), Pearly Beach	Cultivated; ± 2 yr old
	C. longifolia	08 May 2014	Toekomst (Van Zyl Joubert), Bredasdorp	Cultivated; ± 5 yr old
2	*C. maculata*	29 July 2014	Nietvoorbij farm (ARC), Stellenbosch	Cultivated; ± 2 yr old; Budding plants
	C. longifolia	04 August 2014	Toekomst (Van Zyl Joubert), Bredasdorp	Cultivated; ± 5 yr old

2.2 Chemicals

High-performance liquid chromatography (HPLC) gradient grade acetonitrile and analytical grade glacial acetic acid, dimethyl sulfoxide (DMSO), L-ascorbic acid, $MgCO_3$ hydrate (40 – 43.5% Mg) and NaCl (99.5%) were purchased from Sigma-Aldrich (St. Louis, USA). Analytical grade formic acid and methanol were sourced from Merck Millipore (Darmstadt, Germany). Authentic reference

standards with purity > 95% were sourced from Sigma-Aldrich (mangiferin, hesperidin, maclurin and iriflophenone-3-*C*-glucoside), Extrasynthese (Genay, France: luteolin) and Phytolab (Vestenbergsgreuth, Germany: vicenin-2 and eriocitrin). Stock solutions of the phenolic standards were prepared in DMSO at *ca.* 1 mg/mL and aliquots were kept frozen (-20 °C) until analysis. For sample analyses, deionised water was prepared using an Elix water purification system (Merck Millipore), which was further purified to HPLC grade, using a Milli-Q Academic water purification system (Merck Millipore). Distilled water was used for the preparation of infusions.

2.3 Experiment 1: Effect of steam treatment before drying (STBD) on green honeybush quality

The experimental procedure outline of experiment 1 is presented in **Fig. 3.2**. Five batches (5 – 6 kg/batch) of fresh plant material were harvested for each species, and very thick or leafless stems removed. The remaining shoots were then cut (shredded) to 2 – 3 mm lengths using a mechanised fodder cutter. The shredded plant material of each batch was thoroughly mixed to improve homogeneity of the material. Each batch was then sub-divided into treatment samples by thinly spreading the fresh, shredded plant material across 10 stainless steel trays (20 x 30 cm, 30 mesh; *ca.* 300 g fresh plant material per tray), with two trays placed on a larger single stainless steel mesh tray (43.3 x 60 cm) and allocated per treatment (*ca.* 600 g fresh plant material per treatment). This accommodated five treatments comprising one untreated control (t = 0 s) and four steam treatments (t = 30, 60, 90 and 120 s). Steaming was performed in a steam cabinet at ± 96 °C under atmospheric pressure. All trays allocated to steam treatment entered the steam cabinet at time zero, followed by removal of one large tray (containing *ca.* 600 g plant material comprising a single treatment allocation) at every 30 s interval (t = 30, 60, 90 and 120 s). The control sample was placed directly in a laboratory cross-flow drying tunnel and dried at 40 °C for 6 h to a moisture content of < 10% (Du Toit & Joubert, 1999). Upon removal from the steam cabinet, the treated plant material was similarly dried. The dried plant material of the two trays (of a single treatment allocation) was then pooled and sieved (30 s at 90 rpm; 12 mesh < fraction > 40 mesh), using a SMC Mini-sifter (JM Quality Services, Cape Town, South Africa). The samples were finally sealed in glass jars, boxed and stored in a temperature-controlled cold room in the dark at 0 °C until analysis.

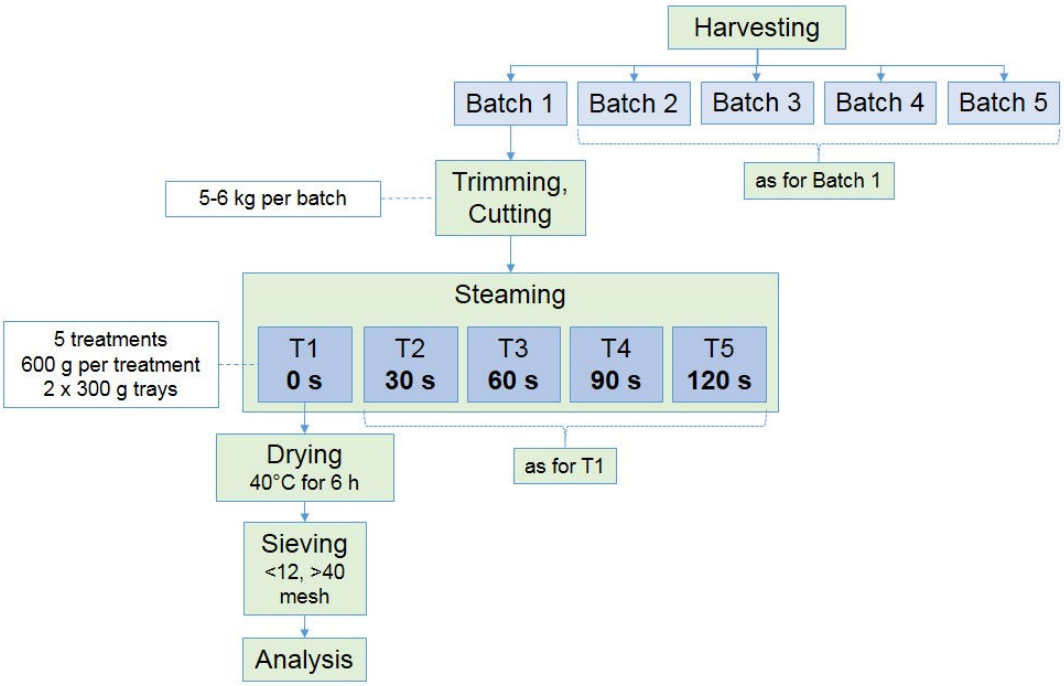


Figure 3.2 Experimental process to evaluate the effects of steam treatment before drying on green honeybush (experiment 1).

2.4 Experiment 2: Effect of steam treatment after drying (STAD) on green honeybush quality

According to the experimental procedure outline in **Fig. 3.3**, five batches (5 – 6 kg/batch) of plant material for each species were harvested, trimmed, shredded and mixed as described in experiment 1. Before drying, each batch of shredded and mixed plant material was divided into sub-batches of approximately 600 g each, with each sub-batch spread across two drying trays (39.5 x 56.5 cm, 30 mesh, Polymon; Swiss Silk Bolting Cloth Mfg. Co. Ltd., Zurich, Switzerland). The plant material was dried overnight at 40 °C in the cross-flow laboratory drying tunnel to a moisture content of < 10%. The dried material was then sieved as described for the previous experiment and divided across two stainless steel trays (20 x 30 cm; 30 mesh) per treatment (70 g dry tea material per tray) before exposure to steam treatment (t = 0, 1, 2, 3 or 4 min), similar to experiment 1. Treated samples were again dried for 30 min at 40 °C in the laboratory cross-flow drying tunnel to a moisture content of < 10% following steaming and sealed in glass jars, boxed and stored in the dark at 0 °C until analysis.

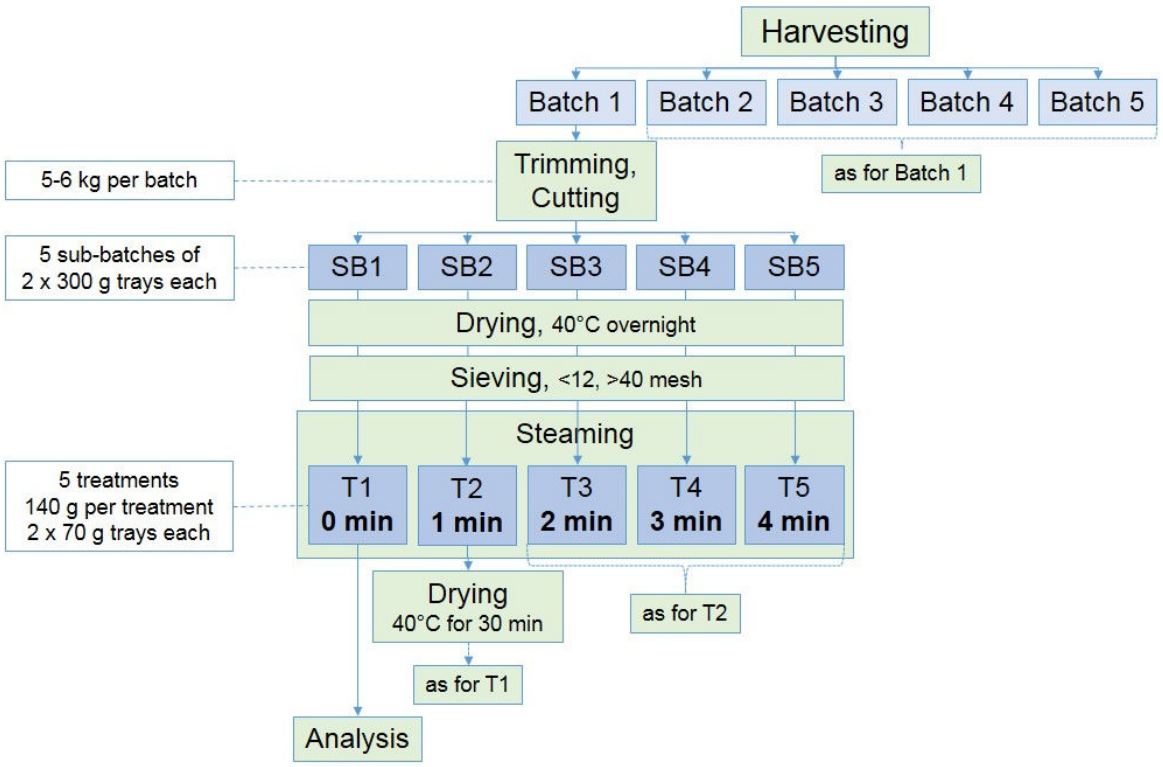


Figure 3.3 Experimental process to evaluate the effects of steam treatment after drying on green honeybush (experiment 2).

2.5 Preparation of infusions

Infusions of each sample were prepared as described by Theron *et al.* (2014). Freshly boiled distilled water (1000 g) was accurately weighed to one decimal as it was poured into a glass jug containing 12.50 g sieved plant material. The plant material was infused for 5 min before straining into a 1 L pre-heated thermos flask (Woolworths, Belville, South Africa). For determination of colour and total soluble solids (TSS) content, *ca*. 100 mL infusion was filtered through a Whatman no. 4 filter paper before cooling to room temperature and analysed.

Measures used by Koch *et al*. (2012) and Theron *et al.* (2014) to keep the infusion warm prior to and during descriptive sensory analysis (DSA) were followed. The infusions were poured into pre-heated white porcelain mugs, covered with plastic lids, and placed in hot water baths controlled at 65 °C (SMC, Cape Town, South Africa) for DSA.

2.6 Milling and extraction of plant material

An aliquot (10 g) of plant material for each treatment sample was finely milled using a ball mill (1 min; 20 s^{-1}; MM301; Retch, Haan, Germany) in order to obtain a homogenous sample. The milled material was used for colour measurements, total chlorophyll (TC) content determination and preparation of an aqueous-organic extract for HPLC analysis.

The aqueous-organic extract of each sample for HPLC analysis was prepared by a small-scale extraction procedure. For each sample, the finely-milled plant material (40 mg) was weighed off into a glass vial before the addition of 3 mL 33% (v/v) acetonitrile. Vials were then capped, mixed

using a vortex mixer and heated for 20 min at 100 °C in a heating block (Stuart, SBH200D/3, Barloworld Ltd., Johannesburg, South Africa). Samples were removed from the heating block after 10 min and mixed in the vortex mixer for 2 s before returning to the heating block to complete extraction. Following heating, the samples were sonicated for 5 min (Bransonic 12, Mechanical ultrasonic bath, Emerson Electric Co., St. Louis, USA) and then rapidly cooled in an ice bath. An ascorbic acid solution (2% m/v; 1 mL) was added to the cooled extract to prevent oxidation of the phenolic compounds during storage and analysis. This extract was then filtered through a 33 mm 0.22 µm pore Millipore PVDF syringe filter (Merck Millipore) before freezing of 1.2 mL aliquots diluted with HPLC grade water in a 1:2 ratio.

2.7 Individual phenolic compound content

The phenolic composition of the aqueous-organic extracts of the plant material was determined according to validated HPLC methods developed for *C. maculata* (Schulze *et al*., 2014) and *C. longifolia* (Schulze *et al*., 2015). Analyses were conducted on an Agilent 1200 series HPLC instrument consisting of an in-line degasser, quaternary pump, autosampler, column thermostat and diode array detector (DAD), all controlled by Chemstation software (Ver. C.01.04, Agilent Technologies Inc., Santa Clara, USA).

Stock solutions of standards, prepared in DMSO, were defrosted and standard mixtures prepared using an aqueous solution of ascorbic acid (*ca*. 5 mg/mL final concentration). Both the standards and defrosted samples were filtered directly into autosampler vials, using 4 mm and 33 mm 0.22 µm pore size Millipore-PVDF syringe filters (Merck Millipore), respectively. A calibration curve was constructed with injection volumes ranging from 5 to 20 µL. Calculated conversion factors were used to quantify a similar class of compound where authentic reference standards were not available (**Table 3.2**). Sample injection was performed in duplicate and injection volumes for aqueous-organic extracts were adjusted between 5 and 30 µL to allow effective quantification of relevant compounds as sample composition varied. For both species, separation was carried out at 30 °C and a flow rate of 1 mL/min.

For extracts of *C. maculata*, separation was achieved on a Gemini-NX C18 (150 × 4.6 mm; 3 µm; 100 Å; Phenomenex, Torrance, USA) column. The mobile phase comprised the following gradient of 2% acetic acid (A) and acetonitrile (B): 0 – 2 min (8% B), 2 – 31 min (8% – 38% B), 31 – 32 min (38% – 50% B), 32 – 33 min (50% B), 33 – 34 (50% – 8% B) and 34 – 44 min (8% B). *Cyclopia longifolia* extracts were analysed on a Kinetex C18 (150 × 4.6 mm; 2.6 µm; 100 Å; Phenomenex) column, using 0.1% formic acid (A) and acetonitrile (B) as solvents and the following mobile phase gradient: 0 – 4 min (4.5% B), 4 – 22 min (4.5% – 8% B), 22 – 49 min (8% – 20% B), 49 – 51 min (20% – 50% B), 51 – 52 min (50% B), 52 – 53 min (50% – 4.5% B) and 53 – 59 min (4.5% B). UV spectra were recorded for all samples from 200 to 550 nm and peaks quantified as indicated in **Table 3.2**.

Table 3.2 Compounds quantified in *C. maculata* and *C. longifolia,* using HPLC-DAD analysis

nm	***C. maculata*** Class	Quantified compound	Quantified as
322	xanthone	mangiferin	mangiferin[a]
	xanthone	isomangiferin	isomangiferin[b]
	flavone	vicenin-2	vicenin-2[a]
	benzophenone	maclurin-3-*C*-glucoside	maclurin-3-*C*-glucoside[b]
288	flavanone	eriocitrin	eriocitrin[a]
	flavanone	hesperidin	hesperidin[a]
	benzophenone	iriflophenone-3-*C*-glucoside	iriflophenone-3-*C*-glucoside[a]
	flavanone	eriodictyol-*O*-glucoside	eriocitrin[c]
nm	***C. longifolia*** **Class**	**Quantified compound**	**Quantified as:**
320	xanthone	mangiferin	mangiferin[a]
	xanthone	isomangiferin	isomangiferin[b]
	xanthone	norathyriol-di-hexoside	mangiferin[c]
	xanthone	hydroxy-mangiferin	mangiferin[c]
	xanthone	hydroxy-isomangiferin	mangiferin[c]
	flavone	vicenin-2	vicenin-2[a]
	benzophenone	maclurin-3-*C*-glucoside	maclurin-3-*C*-glucoside[b]
	flavone	scolymoside	scolymoside[b]
288	flavanone	hesperidin	hesperidin[a]
	flavanone	eriocitrin	eriocitrin[a]
	benzophenone	iriflophenone-3-*C*-glucoside-4-*O*-glucoside	iriflophenone-3-*C*-glucoside-4-*O*-glucoside[c]
	benzophenone	iriflophenone-3-*C*-glucoside	iriflophenone-3-*C*-glucoside[a]
		iriflophenone-di-*C*-glucoside[d]	iriflophenone-3-*C*-glucoside[c]

[a] authentic reference standard
[b] response factor with other compound
[c] quantified in equivalents
[d] tentative identification, sugar position not confirmed

2.8 Total chlorophyll (TC) content

The TC content of the plant material was determined by solvent extraction and spectrophotometric analysis. Briefly, the milled plant material (*ca.* 80 mg) was weighed into a 20 mL amber glass vial before solvent extraction. Avoiding direct light, the material was suspended in 4 mL pre-treated methanol to prevent the acid-induced degradation of chlorophyll in solution. Pre-treatment included addition of 0.1 g $MgCO_3$ to 100 mL 100% methanol, which was then filtered (no. 4 Whatman filter paper) before use (Ritchie, 2006; Lichtenthaler & Bushmann, 2001). Extraction proceeded for 10 min at 30 °C in a temperature-controlled heating block (Stuart, SBH 200D/3). Eppendorf microfuge tubes containing 2 mL extract were then centrifuged for 5 min at 16 000 RCF (Hettich, Micro 120 Microliter Centrifuge, Germany; Rotor: 1224, 24 x 3 g, 14 000 RPM). Aliquots of the supernatant (200 µL) were pipetted in triplicate into a 96-well flat bottom plate (Greiner Bio-one, LASEC, Cape Town, South Africa) and the absorbance measured at 652, 665 and 750 nm using a Biotek Synergy HT microplate reader, equipped with Genstat 5 software (Biotek, Winooski, USA). TC content, expressed in mg/g, was calculated as follows (Ritchie, 2006; Huang *et al*., 2007), with a factor of 1.8 to compensate for the experimental path-length:

$$OD_a = A_a - A_{750}$$

$$Chl\ A\ \left(\mu g/mL\right) = [(-8.0962 \times OD_{652}) + (16.5169 \times OD_{665})] \times 1.8$$

$$Chl\ B\ \left(\mu g/mL\right) = [(27.4405 \times OD_{652}) + (-12.1688 \times OD_{665})] \times 1.8$$

$$Total\ Chl\ \left(\mu g/mL\right) = Chl\ A + Chl\ B$$

$$Total\ Chl\ \left(mg/g\right) = \frac{Chl\ total\left(\mu g/mL\right)}{1000 \times mL / sample\ mass\ (g)}$$

where OD = optical density, A = absorbance, Chl = chlorophyll and numbers or letters in subscript refer to wavelength.

2.9 Total soluble solids (TSS) content

The TSS content of the honeybush infusions prepared for sensory analysis was determined gravimetrically in triplicate. Each 15 mL aliquot was evaporated in pre-weighed nickel moisture dishes on a steam bath, followed by final drying in a forced air laboratory oven at 100 °C for 1 h. The dishes were allowed to cool to room temperature in a desiccator before weighing to calculate the TSS content (expressed as mg/L of infusion).

2.10 Moisture content

The moisture content of the plant material (expressed as g/100 g plant material) was determined gravimetrically using a halogen moisture analyser (HR73, Mettler Toledo, Greifensee, Switzerland). A 2 g sample was spread on an aluminium foil dish and dried using the gentle drying function at 100 °C for 60 min.

2.11 Instrumental colour

CIE*L*a*b** colour measurements of both the unmilled and milled shredded leaf material were conducted for each sample. A Konica Minolta Chroma meter (CM-5, Osaka, Japan) was used to measure *L*, a** and *b** values directly in reflectance mode using a 152 mm integrating sphere. Measuring conditions were standardised on illuminant D65, diffuse illumination, 8° viewing and 10° observer. Auto-calibration was performed using the built-in standard white calibration plate and manual zero calibration with the zero calibration box (black inverted cone cylinder). The unmilled samples (*ca.* 8 g or 70 mL of sieved leaf material) and the milled samples (*ca.* 10 g or 20 mL) were illuminated (30 mm diameter measurement area) from the bottom of a optical glass tube cell cup (CR-A502, Ø 60 mm, 40 mm depth; Konica Minolta) for analysis. Each reading was repeated in triplicate and an average calculated by the instrument software. After each triplicate reading, the sample was poured out of the cup and mixed before filling the cup again with the same material for the next reading (n = 5 for unmilled; n = 3 for milled material). The colour of infusions (prepared for sensory analysis) was measured in triplicate in transmittance mode using 10 mm path length

disposable plastic cuvettes (Greiner Bio-one, macro, Lasec, Cape Town, South Africa). Deionised water served as blank.

Chroma (C) and hue (h) values of the plant material and infusions were generated using SpectraMagic NX software (Ver 2.5, Konica Minolta Inc.). Colour difference (Δ*E**), indicating the magnitude of total difference in colour between a treatment and control sample was calculated using the following equation (Patras *et al*., 2011):

$$\Delta E* = \sqrt{\Delta a*^2 + \Delta b*^2 + \Delta L*^2}$$

2.12 Descriptive sensory analysis (DSA)

During the initial training phase, a panel of 9 female assessors with prior experience of herbal tea (rooibos and fermented honeybush) sensory analysis was chosen. The panel was initially trained by a professional panel leader over a period of eight (*C. maculata*) and six (*C. longifolia*) 40 min sessions for the first experiment. For samples of the second experiment, training sessions consisted of one hour-long session and three 40 min sessions for *C. maculata*, and six 40 min sessions for *C. longifolia*. During training, suitable aroma, flavour, taste and mouthfeel descriptors were generated for all samples. A list of preliminary trial descriptors generated for green *C. maculata, C. intermedia, C. longifolia and C. subternata* honeybush (29 aroma descriptors), descriptors for green tea by Lee and Chambers (2007) (25 aroma descriptors) as well as a descriptor list of fermented *C. maculata* (4 taste and mouthfeel, 29 aroma and 23 flavour descriptors), as described by Erasmus (2015), were assessed as a basis for the descriptors of green honeybush. From these, a list of 21 aroma, 4 taste and mouthfeel and 19 flavour descriptors were selected for initial assessment (**ADDENDUM A**, **Table A.1**). Preceding each subsequent assessment, the list of descriptors were re-evaluated and narrowed down. Some reference standards were identified and used during training in the first experiment to define attributes that were not familiar to all panel members (**ADDENDUM A**, **Table A.2**). Some descriptors were removed based on the low occurrences and intensities of the attributes, while others were grouped together to avoid panel discrepancies or exceedingly long evaluation times. For example, the *vegetative* attributes ‘green herbs’, ‘cooked green beans and/or potato’ and ‘cooked spinach’ were finally added to the ‘cooked vegetables’ descriptor to simplify evaluation. Final lists of attribute descriptors for green *C. maculata* and *C. longifolia* herbal tea consisted of 15 aroma, 3 taste, 1 mouthfeel and 13 flavour attributes grouped into attribute groups indicated in italic script (**Table 3.3**). Samples were analysed first in terms of aroma by removing the plastic lid and swirling the cup to release the volatile compounds before sniffing the infusion. Next, the infusion was tasted by sucking a spoonful (round tablespoon) of the infusion to analyse flavour, taste and mouthfeel. Each attribute was discussed by the panel and consensus reached before the next sample was analysed. Unsalted water biscuits (Woolworths, Stellenbosch, South Africa) and distilled water were supplied as palate cleansers between samples.

Following consensus and product familiarisation during training, the testing phase continued over five days with five samples tested per day. Three consecutive 30 min sessions were performed daily, representing triplicate measurements. Samples were labelled with three-digit codes and presented in a randomised order to each panellist. Short 10 min breaks were scheduled between sessions to prevent panel fatigue. Panellists were assigned testing booths fitted with controlled lighting for the duration of the analysis. The *Compusense® five* software program (Compusense, Guelph, Canada) was used to record panellist scores, where each individual rated the intensities of aroma, taste, mouthfeel and flavour attributes on an anchored, unstructured line scale (0 = low; 100 = prominent).

Table 3.3 Descriptive lexicon for sensory evaluation of green honeybush

Attribute group	Attribute	Description
Vegetative	'Green grass'	Aromatic associated with fresh or stale cut green grass
	'Cooked vegetables'	An overall aroma note associated with cooked green vegetables or green beans
	'Hay/Dried grass'	Slightly sweet aromatics associated with dried grass or hay
Cereal	'Oats/Porridge/Grains'	An overall grain or porridge impression typical of oats or ProNutro™ (raw or cooked)
Fruity	'Stewed fruit'	Sweet, syrup-like aroma of stewed fruit such as peach, raisins, apples and prunes
	'Tropical fruit'	A sweet, aromatic blend, reminiscent of a variety of fresh ripe tropical fruits for example, pineapple, mango or granadilla
	'Marmalade'	Aromatics associated with citrus fruits (orange & lemon) or marmalade
	'Apricot jam'	Sweet aroma or flavour reminiscent of apricot jam
	'Guava'	Aroma reminiscent of fresh, over-ripe or juiced guava
Sweet-associated	'General sweet'	Non-specific sweet aroma or impression
	'Fruity-sweet'	Sweet aromatic reminiscent of non-specific fruit especially berries and apricot jam
	'Caramel'	Sweet aromatic characteristic of molten sugar or caramel pudding
Taints	'Dusty'	Earthy aromatic associated with dry dirt roads
	'Musty'	Earthy aromatic associated with damp hessian or cardboard
	'Seaweed/Oceanic'	Pungent fishy or seaweed-like aroma

2.13 Identification and quantification of volatile compounds

Infusions of one batch (batch 3) of steam-treated (t = 0, 30, 60, 90 and 120 s) unfermented *C. maculata* were prepared as described for sensory analysis, and analysed in triplicate using head space – solid phase micro-extraction (HS-SPME) followed by gas chromatography – mass spectrometry (GC-MS). HS-SPME was performed using a conditioned fibre (100 µm PDMS red fibre; Supelco, Bellefonte, USA) exposed to the headspace of 10 mL tea containing 2 g of NaCl at 21 ± 1 °C for 1 h with stirring. Subsequently, the fibre was introduced into the GC injector (250 °C, splitless, 2 min) for thermal desorption. GC separation was performed on a Hewlett-Packard 5890 model GC system coupled with a 5973 quadruple mass spectrometer and equipped with a high-

efficiency non-polar column (PDMS phase, 50 m x 0.18 mm x 0.1 µm film). The MS was operated in the full scan mode from 35 to 350 amu at a scan rate of 4.5 scans/s using electron ionisation (EI) at 70 eV. The MS source and quadrupole temperatures were 230˚C and 150˚C, respectively. Helium was used as a carrier gas with constant flow of 2 mL/min and split ratio of 1:50. The oven program used was as follows: initial temperature 40˚C (5 min), 5˚C/min – 120˚C (0 min), 10˚C/min – 280˚C (5 min). Identification of compounds was based on comparison of retention times (RTs) and mass spectra with authentic standards and comparison of calculated retention indices (RIs) relative to a series of linear alkanes (Vandendool & Kratz, 1963) with literature values (NIST library, 2015).

2.14 Statistical procedures

Both experiments of each *Cyclopia* spp. were set up as randomised block designs with each of the five steam treatments replicated at random on five batches (blocks) of plant material. For sensory data analysis, individual sample and panellist scores were collected using *Compusense*® *five* software and panel performance monitored using *Panelcheck* software (Version 1.4.0, Nofima Mat, Ås, Norway). The sensory data were pre-processed by subjecting it to a test-retest analysis of variance (ANOVA) using SAS® software (Version 9.2, SAS Institute Inc., Cary, USA) to test for panel reliability. The Shapiro-Wilk test was performed on the standardised residuals from the model to test for normality (Shapiro & Wilk, 1965). In cases where there was significant deviation from normality outliers were removed when the standardised residual for an observation deviated with more than three standard deviations from the model value. After panel reliability was confirmed, ANOVA was conducted according to the experimental design on means over judges for each treatment and batch. Fisher's t-least significant difference was calculated at the 5% level to compare treatment means. Attributes occurring at mean intensities of < 5 were considered negligible and removed from further data analyses. Principal component analysis (PCA) and discriminant analysis (DA) were performed on the average DSA data of triplicate evaluations of a sample replicate (n = 5) by 9 judges, using XLStat (Version 2015.1.01, Addinsoft, New York, USA). DA was supplemented with forward stepwise model selection in order to select attributes necessary for sample classification.

Instrumental data were subjected to ANOVA to test for treatment differences, using SAS® software. The Shapiro-Wilk test was performed to test for normality. Fisher's least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). Graphical representations of the data were generated using Microsoft Exel (2013).

3. Results

3.1 *Cyclopia maculata*

3.1.1 *Effect of steam treatment before drying (STBD) on green* C. maculata *quality*

3.1.1.1 *Sensory profile of infusions*

Data of the sensory attributes of each sample replicate (n = 5) were used to prepare PCA scores and loadings plots (**Fig. 3.4**). Pearson's (n-1) correlations between attributes are also provided in **ADDENDUM A** (**Table A.3**). The PCA scores plot (**Fig. 3.4 a**) indicates a distinction between the steam treatments of 60 s and longer on the left and control (0 s) and 30 s treatments on the right along the first principal component (F1, 42.84%). Along the second principal component (F2, 15.87%), a distinction between the control (upper right quadrant) and 30 s (lower right quadrant) treatments is observed. Some samples of each treatment did not fit this pattern as batches varied greatly in bitterness intensities, causing inconsistent positioning in the PCA scores plots. The PCA loadings plot (**Fig. 3.4 b**) shows that 'tropical fruit' and 'guava' *fruity* aromas associated with samples on the left, while 'apricot jam' and 'stewed fruit' aromas in the right lower quadrant associated with the 30 s steam treatment. *Vegetative* ('green grass', 'cooked vegetables' and 'hay/dried grass') aroma and flavour attributes and *cereal* aroma are positioned in the upper right quadrant of the plot, associating with the control treatment. The 'green beans and/or potato' flavour and aroma attributes were positioned in the upper left and right quadrants, respectively. Sour taste, bitter taste and astringency clustered in the upper left quadrant, while sweet taste is positioned in lower right quadrant.

Similarly, DA (**Fig. 3.5 a**) formed two clusters along F1 (80.12%), comprising the longer steam treatments (60, 90 and 120 s) on the right and the control and 30 s treatments on the left of the scores plot. The split along F2 (12.57%) separated the control and 30 s treatments as well as the 120 s treatment from the 60 and 90 s treatments. 'Green grass', 'oats/porridge/grains', 'caramel' and 'guava' aroma and astringent mouthfeel were identified as the drivers of discrimination between treatments (indicated in red on **Fig. 3.5 b**).

The DSA data were subjected to ANOVA to determine whether changes caused by treatments were significant ($p < 0.05$) (**ADDENDUM A**, **Tables A.4** – **A.6**). Not all attributes were found to be prominent in all batches. Aroma, flavour, taste and mouthfeel attributes present in notable intensities (> 5) are depicted in **Fig. 3.6** (**a** – **c**) and include the aroma attributes 'green grass', 'cooked vegetables', 'cooked green beans and/or potato', 'hay/dried grass', 'oats/porridge/grains', 'stewed fruit', 'tropical fruit', 'guava', 'apricot jam', 'general sweet', 'fruity-sweet' and 'caramel'. Of these, only 'apricot jam' aroma intensity did not differ significantly ($p \geq 0.05$) between treatments. Certain general trends can be observed for groups of attributes. *Vegetative* and *cereal* aroma attribute intensities tended to decrease after steam treatment. The attributes were not

all affected to the same extent, with the intensities of 'green grass' and 'cooked green beans and/or potato' decreasing significantly ($p < 0.05$) from 0 s to 30 s to 60 s, whereafter their intensities remained unchanged with longer steam treatments. The aroma intensities of 'hay/dried grass' and 'oats/porridge/grains' were significantly decreased when the samples were subjected to a steam treatment of 60 s. Prolonging the steam treatment did not further significantly ($p \geq 0.05$) affect the intensities of these two aroma attributes. The intensity of 'cooked vegetables' aroma was significantly ($p < 0.05$) affected by steam treatment, but not by the duration of the treatment.

On one hand, 'tropical fruit', 'guava' and 'fruity-sweet' aroma showed a general increase in intensity, reaching the maximum after a 90 s steam treatment. The intensity of 'stewed fruit', on the other hand, remained stable for the first 60 s of steam treatment, only significantly ($p < 0.05$) decreasing after 90 s steam treatment. 'Caramel' intensity clearly peaks after 30 s stream treatment, with longer treatments decreasing the intensity below that of the control.

The mean intensities of the flavour attributes were notably lower than that of the aroma attributes and indicated less clear differences between treatments. Only the *vegetative* and *cereal* aromas were detected at intensities > 5. Of these, 'cooked vegetables' and 'cooked green beans and/or potato' flavours did not show significant ($p < 0.05$) differences between treatments. Similar to the aroma, the intensity of 'green grass' flavour was significantly ($p < 0.05$) lower after steam treatment of 30 s to 120 s, 'oats/porridge/grains' after 60 s to 120 s, and 'hay/dried grass' after 90 s and 120 s compared to the control.

Bitter and sour taste as well as astringency showed a similar trend, with samples steamed for 30 s having significantly lower intensities ($p < 0.05$) than control samples or samples steamed for 60 s and longer. Sweet taste intensity was the highest for the control and 30 s steam-treated samples.

3.1.1.2 *Colour of tea material and infusions*

The colour (CIE$L^*a^*b^*$) of dry leaf material (unmilled and milled) as well as that of the infusions were measured. Additional colour parameters (C, h and ΔE^*) were calculated. Mean values (L^*, a^*, b^*, C, h and ΔE^*) for each treatment are presented in **Fig. 3.7 a** (milled and unmilled leaf material) and **Fig. 3.7 b** (infusions as prepared for sensory analysis). Furthermore, TC and TSS contents of milled material and infusions, respectively, are depicted in **Fig. 3.8**. Tabulated colour, TC and TSS data may be found in **ADDENDUM A** (**Table A.7**).

Similar trends for all colour parameters were observed for milled and unmilled leaf material as a function of steaming time (**Fig. 3.7 a**). In all cases, except for the green/red parameter a^*, the milled material had higher values than the corresponding unmilled material. Since the plant material is sold in unmilled form, the results of the unmilled samples will be highlighted here. The values for L^*, b^* and C increased significantly ($p < 0.05$) for steam treatments of 60 s and longer, while a^* and h values were not significantly different ($p \geq 0.05$), when compared to the control. Steam treatment of 30 s consistently produced significantly different ($p < 0.05$) results from the control and other

treatments. Compared to the control, these samples were darker in colour (lower *L**), less yellow (lower *b**) and less green (positive *a**). Lower C and h values indicated lower colour saturation and a change from the yellow-green quadrant (> 90°) to the yellow-red quadrant (< 90°). Total colour difference (Δ*E**), combining *L**, *a** and *b**, was the largest for samples treated for 30 s (Δ*E** > 3). The other treatments resulted in Δ*E** < 1.5.

The colour parameters of the infusions, prepared from the unmilled plant material, also indicated that 30 s steam treatment affected colour the most, with *L** and h increasing significantly and *a**, *b** and C decreasing significantly ($p < 0.05$). Longer steam treatments reversed this effect, resulting in higher *L** and h values and lower *a**, *b** and C values than that of the control.

Significant chlorophyll degradation ($p < 0.05$) was observed for all steam treatments. Furthermore, the control and 30 s steam treatment produced infusions with the lowest TSS content. Samples treated for 60 and 90 s had significantly ($p < 0.05$) higher TSS content than the control. TSS thus indicated similar trends to the *L** and h values of the infusion.

3.1.1.3 *Phenolics of aqueous-organic extracts*

Results of HPLC analysis for the content of specific individual phenolic compounds in STBD green *C. maculata* plant material are summarised in **Table 3.4** as treatment means. Overall, limited decreases were observed as a result of STBD. Steam treatment of 30 s significantly decreased the xanthone (mangiferin, isomangiferin) and iriflophenone-3-*C*-glucoside content of the samples. These decreases were not observed for the samples subjected to longer steam treatments. The hesperidin content of steam-treated samples was significantly lower ($p < 0.05$) than that of the control, irrespective of the duration of the treatment. Longer steam treatments seemed to aid in retention of the other flavanones, *i.e.* eriodictyol-*O*-glucoside and, to a greater extent, eriocitrin, which showed a significant ($p < 0.05$) increase in content after 60, 90 and 120 s of steam exposure. In the case of the flavone, vicenin-2, no significant difference ($p \geq 0.05$) was observed between the control and the steam-treated samples.

3.1.1.4 *Volatiles of infusions*

Preliminary data obtained by HS-SPME-GC-MS analysis of the volatile fraction of green *C. maculata* samples subjected to the different STBD treatments (t = 0, 30, 60, 90 and 120 s), indicated some notable differences between the different treatments. Despite this, no clear trends could be observed as the 60 s treatment sample seemed to indicate unexpectedly low levels of many of the identified volatile compounds. An example of the chromatograms (representing the unsteamed control) is provided in **Fig. 3.9**. Peak numbers correspond to those indicated in **Table 3.5**, listing the compounds that have been identified based on their RTs and their calculated RI values together with RI values obtained from literature (NIST library, 2015). **Table 3.5** also provides relative peak areas for each identified compound per treatment. The majority of the compounds detected comprised of aldehydes, ketones and terpenes.

PCA was used on the aroma attribute means obtained from the DSA data of the same batch of green *C. maculata* with relative volatile compound peak areas as supplementary variables. The scores and loadings plots are provided in **Fig. 3.10** and Pearson's (n-1) correlations in **Table 3.6**. As previously established, the greatest distinction in treatments is between the control and 30 s steam-treated samples with their accompanying *vegetative* and *cereal* aroma attributes on the right and the samples subjected to longer steamed treatments (60, 90 and 120 s) on the left, with the *fruity* ('guava', 'stewed fruit' and 'tropical fruit') aroma attributes (F1, 67.93%). It may also be noted that 6 out of 8 identified volatile compounds with 'green' or 'herbaceous' descriptors are positioned in the lower right quadrant (**Fig. 3.10**, **Table 3.5**). Furthermore, significant ($p < 0.05$) strong correlations ($r \geq 0.9$) were found between *vegetative* ('hay/dried grass', 'green grass' and 'cooked vegetables') and *cereal* ('oats/porridge/grains') aroma attributes and the volatile compounds with 'green' related descriptors (hexanal, *cis*-3-hexenol, 1-octen-3-ol, 6-methyl-5-heptene-2-one, phenylacetaldehyde, isophorone and γ-terpinene). As indicated in **Fig. 3.11**, these selected volatile compounds tend to be present to a lesser degree in steam-treated samples. Furthermore, 6-methyl-5-heptene-2-one and 1-octen-3-ol are the overwhelmingly predominant volatiles present in the unsteamed control, however, both compounds decrease with steaming time. No significant positive correlations were detected between *fruity* aroma attributes and identified volatiles, although some volatile compounds (n-heptanal, benzaldehyde, octanal, 2,4-heptadienal, (E,E), linalool, benzaldehyde, 4-ethyl, isobornylacetate, and the unidentified norisoprenoids) seemed to be more concentrated in the steamed samples (**Table 3.5**).

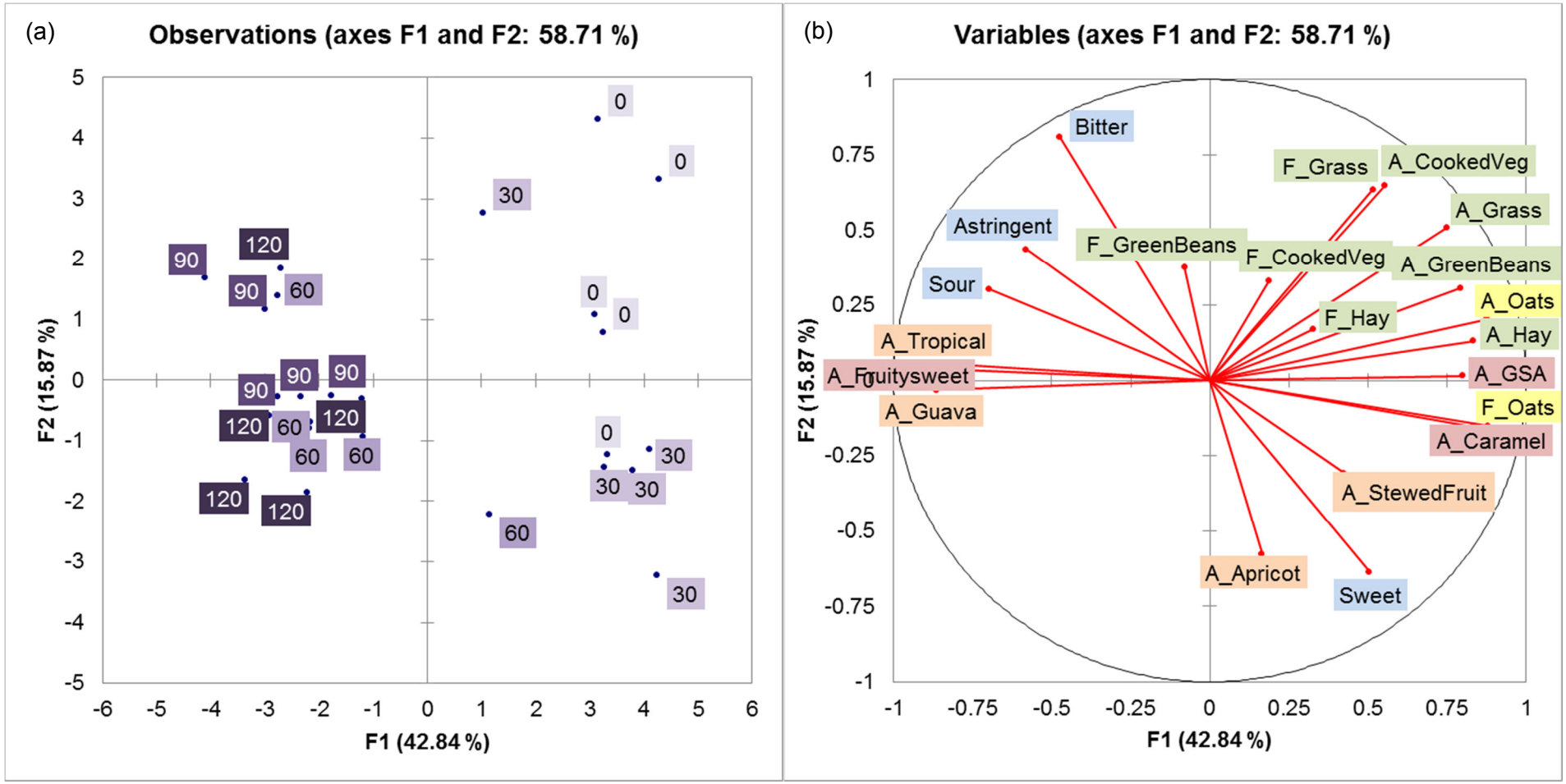


Figure 3.4 PCA (a) scores and (b) loadings plots of all detected (intensity > 5) aroma, flavour, taste and mouthfeel attributes of STBD green *C. maculata*, n = 25.

Steaming times = 0, 30, 60, 90 and 120 s. 'A' and 'F' prefixes refer to aroma and flavour attributes, respectively. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'GreenBeans' = cooked green beans and/or potato, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet, 'Bitter' = bitter taste, 'Sour' = sour taste, 'Sweet' = sweet taste. Variables attribute colours: green = *vegetative*, yellow = *cereal*, pink = *sweet-associated*, orange = *fruity*, blue= taste and mouthfeel.

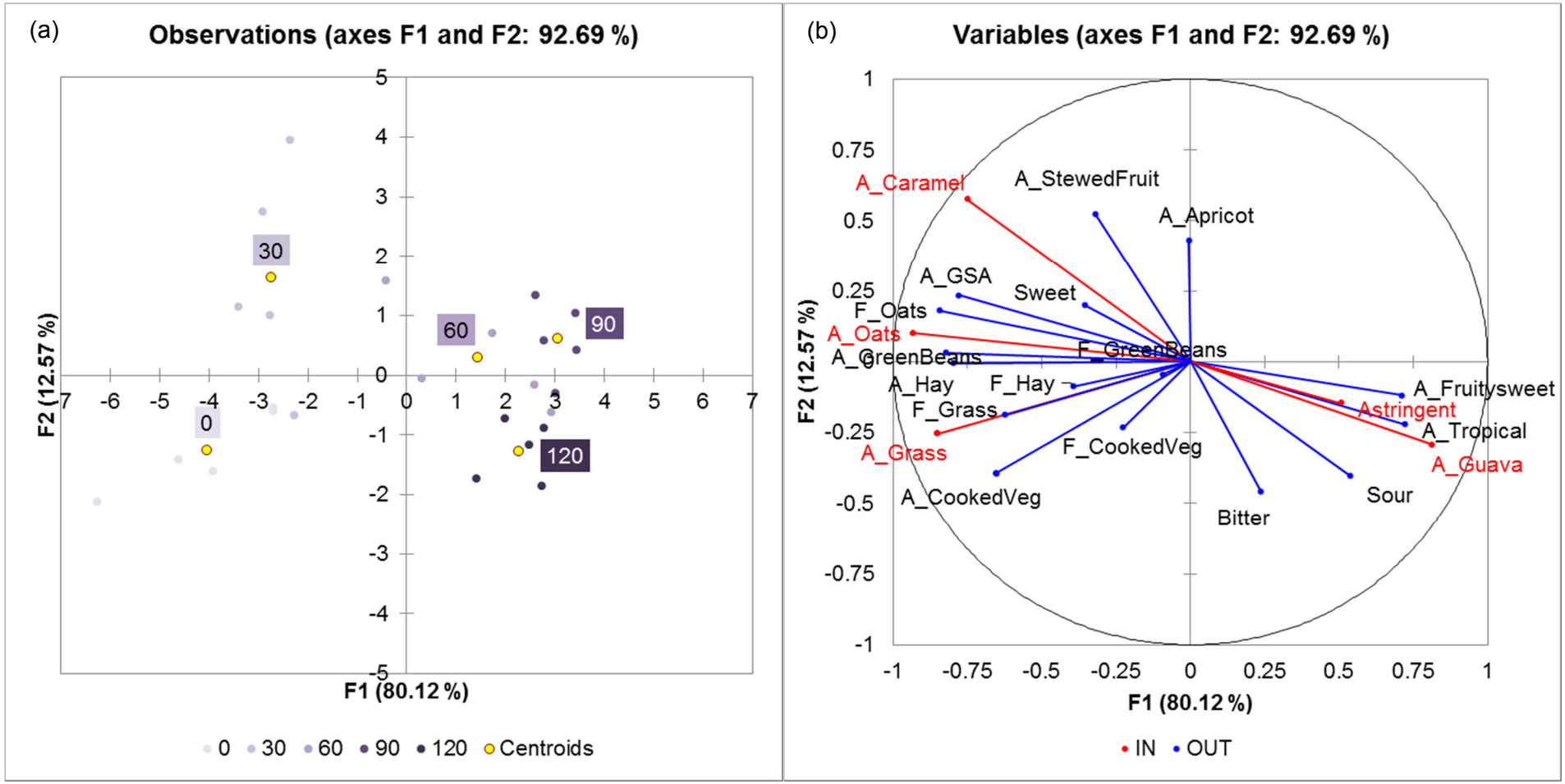
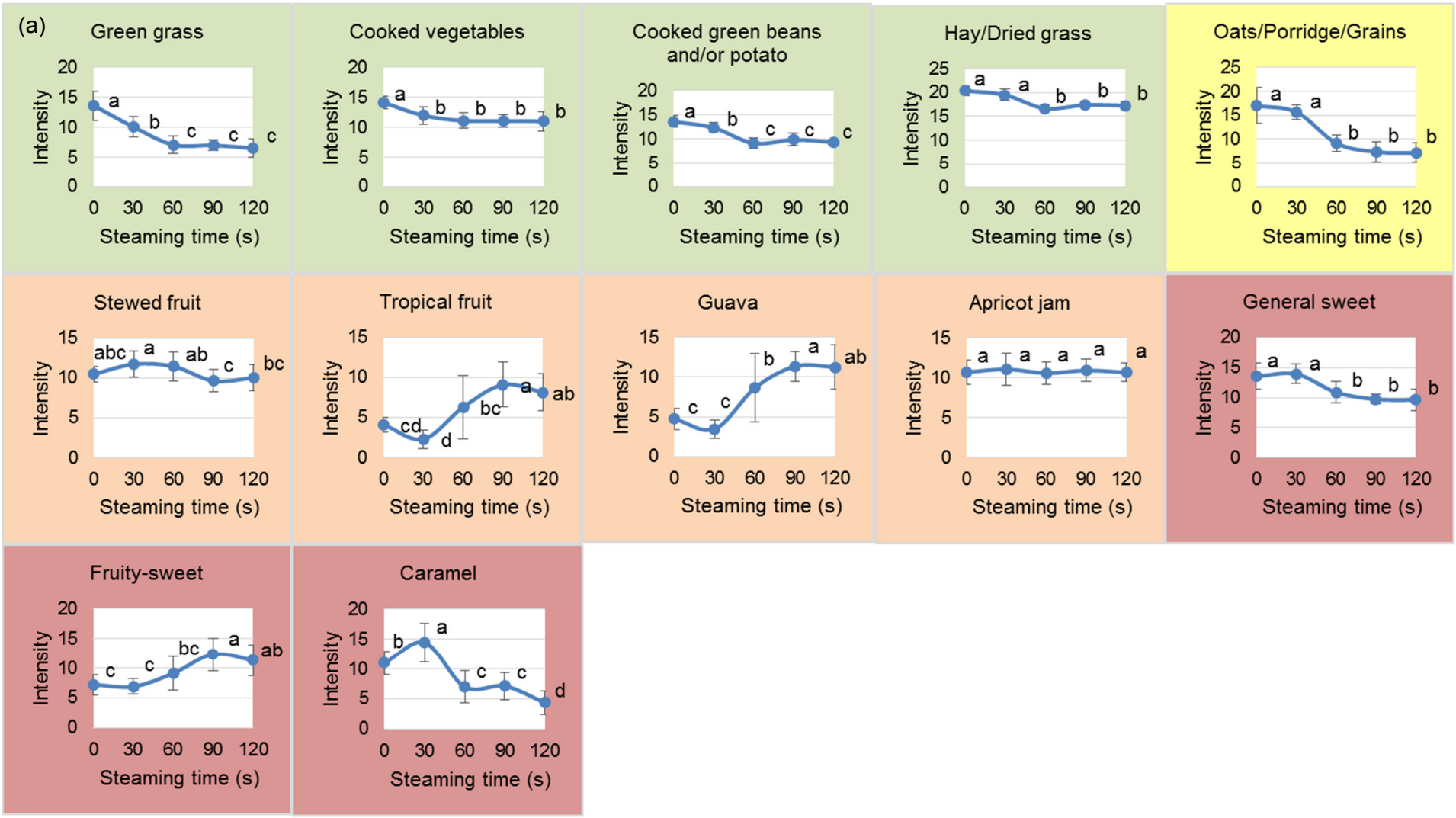


Figure 3.5 DA (a) observations and (b) variables loadings plots of STBD green *C. maculata*, n = 25.

Steaming times = 0, 30, 60, 90 and 120 s. 'A' and 'F' prefixes refer to aroma and flavour attributes, respectively. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'GreenBeans' =cooked green beans and/or potato, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet, 'Bitter' = bitter taste, 'Sour' = sour taste, 'Sweet' = sweet taste.



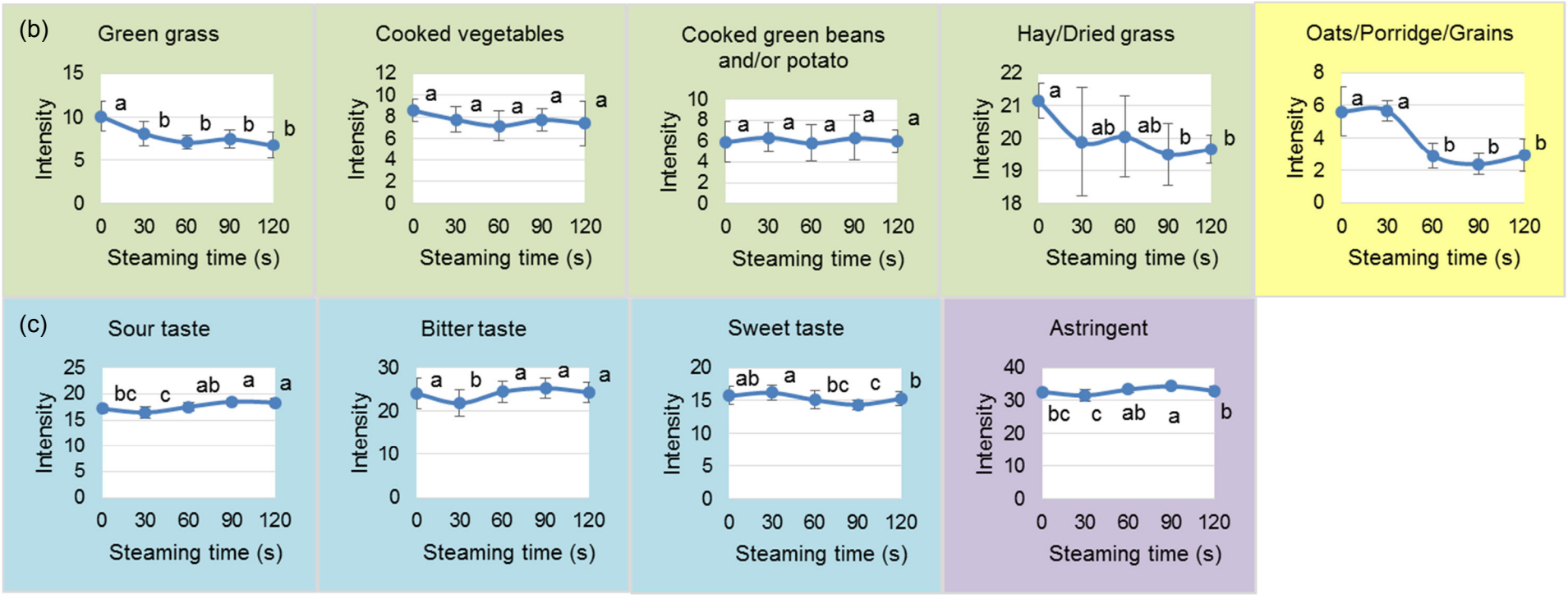


Figure 3.6 Mean intensities of (a) aroma, (b) flavour, (c) taste and mouthfeel attributes of STBD green *C. maculata*.

Bars associated with the same letters do not differ significantly ($p \geq 0.05$). Error bars indicate standard deviation. Green plots = *vegetative* attributes, yellow plots = *cereal* attributes, orange plots = *fruity* attributes, pink plots = *sweet-associated* attributes, blue plots = taste attributes, purple plots = mouthfeel attributes.

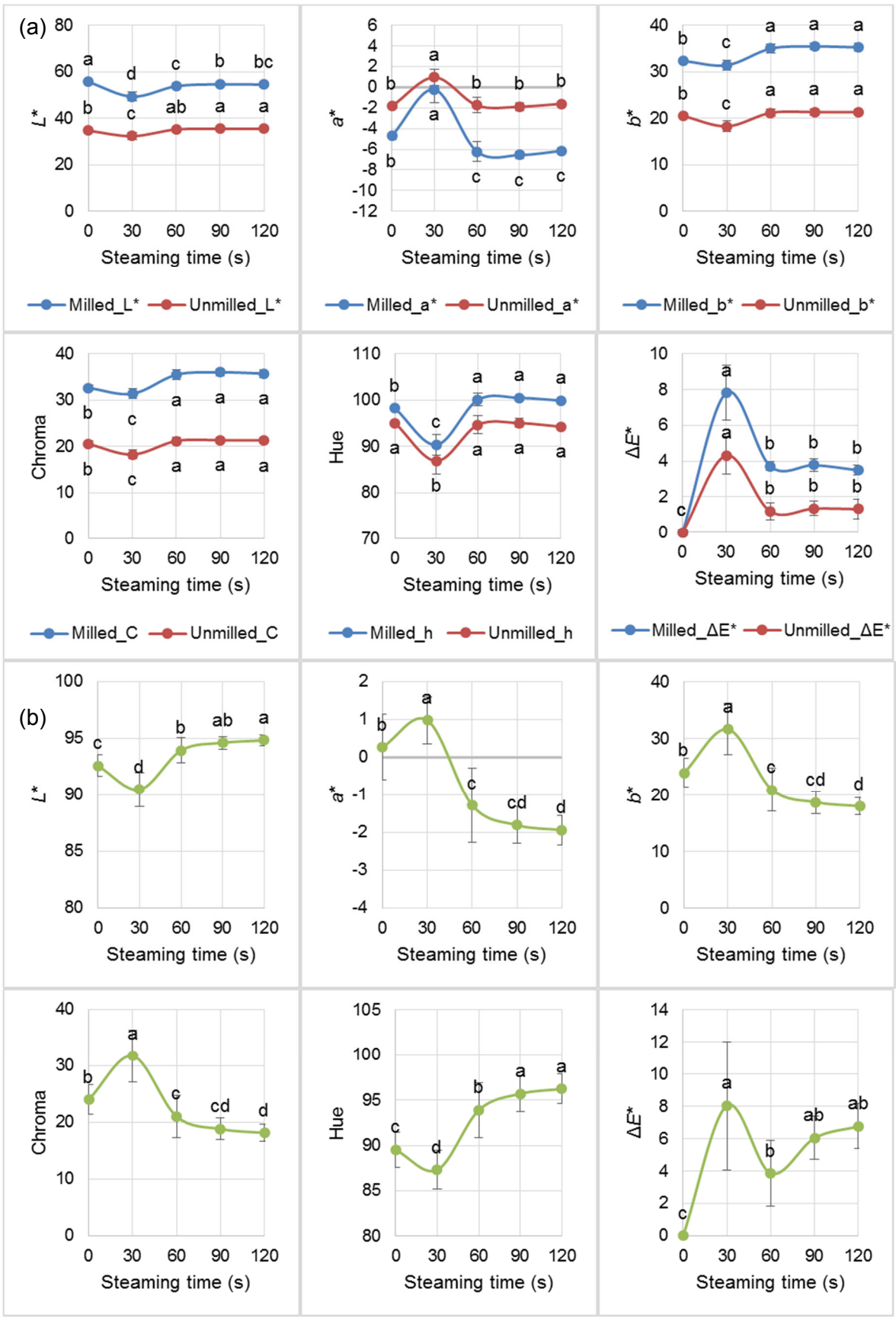


Figure 3.7 Objective colour parameters (CIE*L*a*b**) of (a) milled and unmilled leaf material samples and (b) infusions of STBD green *C. maculata*.

Series points associated with the same letters do not differ significantly ($p \geq 0.05$). Error bars indicate standard deviation.

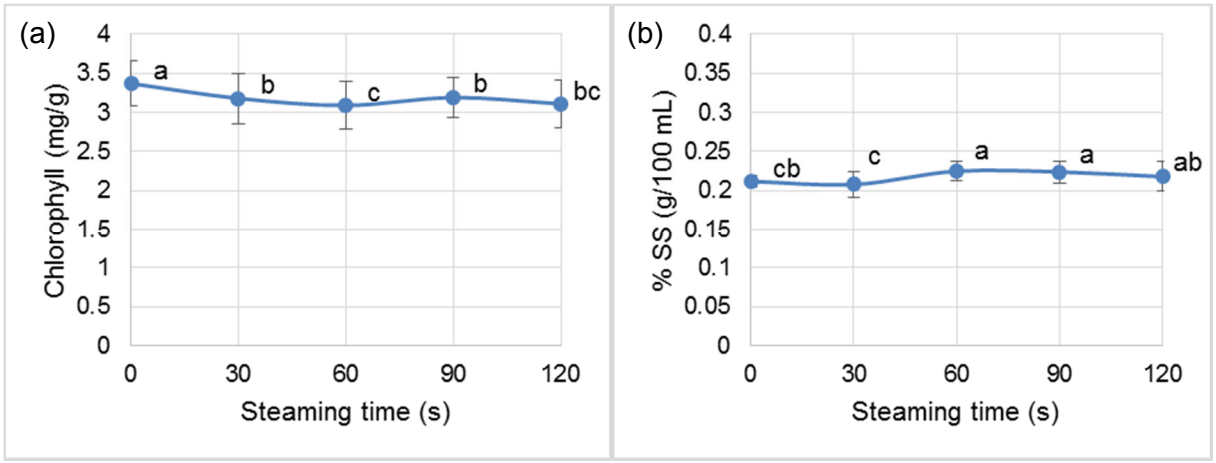


Figure 3.8 (a) Total chlorophyll content and (b) total soluble solids content of STBD green *C. maculata* milled leaf material and infusions, respectively.

Series points associated with the same letters do not differ significantly ($p \geq 0.05$). Error bars indicate standard deviation.

Table 3.4 Mean individual phenolic content (g/100 g plant material) of STBD green *C. maculata*

Compound	Steaming time (s)																			
	0				30				60				90				120			
mangiferin	3.234	a	±	0.290	2.877	b	±	0.330	3.225	a	±	0.352	3.235	a	±	0.347	3.181	a	±	0.241
isomangiferin	0.775	a	±	0.082	0.691	b	±	0.092	0.777	a	±	0.102	0.784	a	±	0.099	0.775	a	±	0.077
vicenin-2	0.110	ab	±	0.009	0.112	a	±	0.009	0.111	ab	±	0.007	0.109	ab	±	0.009	0.108	b	±	0.007
eriodictyol-*O*-glucoside	0.049	ab	±	0.010	0.042	b	±	0.005	0.053	a	±	0.009	0.055	a	±	0.009	0.056	a	±	0.009
iriflophenone-3-*C*-glucoside	0.144	a	±	0.024	0.095	b	±	0.009	0.139	a	±	0.015	0.142	a	±	0.012	0.144	a	±	0.012
eriocitrin	0.285	b	±	0.021	0.257	c	±	0.024	0.303	a	±	0.021	0.306	a	±	0.022	0.303	a	±	0.018
hesperidin	1.636	a	±	0.133	1.497	b	±	0.129	1.424	b	±	0.071	1.447	b	±	0.088	1.449	b	±	0.133

Means associated with the same letters do not differ significantly ($p \geq 0.05$). Values indicated as (mean) ± (standard deviation).

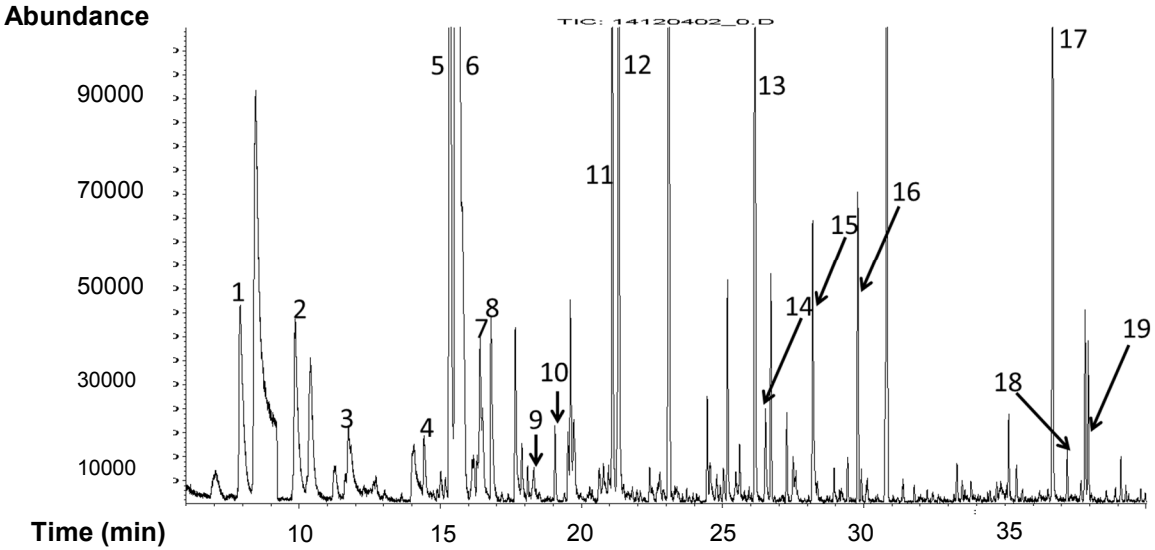


Figure 3.9 GC-MS chromatogram for infusion of unsteamed green *C. maculata*.

Peak numbers correspond to **Table 3.5**.

Table 3.5 Volatile compounds identified in infusions of STBD green *C. maculata*, with relative peak areas per treatment

No.	Compound	Descriptors	RT	RI (calc)	RI (lit)	Relative peak areas per treatment (s) 0	30	60	90	120
1	hexanal [a]	Fatty, green [e]	7.91	820	805	3855374	3358892	1511185	2820954	2167332
2	*cis*-3-hexenol [b]	Green [e]	9.90	857	857	3086381	1942603	983131	607374	656620
3	n-heptanal [a]	Oily, fruity, woody, fatty, nutty [e]	11.77	903	903	1596190	1317377	1239463	1949409	2137920
4	benzaldehyde [a]	Almond, cherry, sweet [e]	14.45	961	962	643668	689011	200498	544387	1263463
5	1-octen-3-ol [b]	Cheese; creamy; earthy; herbaceous; vegetable; meaty; fishy [e]	15.35	977	972	11572974	7537455	1718812	2048139	1501977
6	6-methyl-5-heptene-2-one [c]	Oily, herbaceous, green [e]	15.54	985	985	21058044	22011914	12489837	12178291	12224135
7	octanal [a]	Honey; fruity; fatty; citrus [e]	16.37	1002	1002	1042655	681689	499744	2116106	1770887
8	2,4-heptadienal, (*E,E*) [a]	Cinnamon, hazelnut, fatty [e]	16.82	1012	1012	1575278	1176474	358390	445810	1678174
9	phenyl acetaldehyde [a]	Apple; apricot; berry; cherry; chocolate; grape; grapefruit; honey; hyacinth; lemon; melon; orange; fruity; green; nutty; peach; peanut; vegetable; wine-like [e]	18.32	1042	1046	295187	260832	132896	238415	114678
10	isophorone [c]	Berry, woody, herbaceous, nutty, citrus, sweet, vegetable, green, camphoraceous, wine-like, waxy, tobacco [e]	19.07	1058	1096	451650	414528	195068	268423	276001
11	linalool [b d]	Lemon, orange, floral, citrus, sweet [e]	21.06	1099	1097	3202435	1661657	1414658	2853298	4029211
12	nonanal [a]	Apple; coconut; grape; grapefruit; lemon; lime; melon; oily; orange; nutty; citrus; waxy; fatty; peach; rose; vegetable; fishy; meaty [e]	21.33	1104	1104	5145365	3283071	1948779	6229051	5072136
13	decanal [a]	Waxy, floral, citrus, sweet [e]	26.15	1206	1206	5916796	3416662	823457	6777348	3640701
14	benzaldehyde,4-ethyl [a]	Almond, sweet [e]	26.53	1214	1207	474355	456929	172287	508581	2502916
15	γ-terpinene [d]	Herbaceous; citrus [e]	28.20	1250	1200	1655329	1027096	253182	655603	453969
16	isobornyl acetate [d]	Herbaceous, pine [f]	29.79	1285	1240	1575720	1135021	1144239	1361069	1799830
17	geranyl acetone [d]	Fresh, rose, magnolia [g]	36.64	1435	1453	4033362	1751582	1071059	3436924	3415285
18	unidentified norisoprenoid 1[d]		37.20	1455		247672	270649	243254	238266	812126
19	unidentified norisoprenoid 2[d]		37.90	1477		755106	321485	130006	463860	2503223

a aldehyde, b alcohol, c ketone, d terpene, e Sigma Aldrich, 2011, f Advanced Biotech, 2015, g Bedoukian Research, 2014
RT = retention time (min), RI (calc) = calculated retention index, RI (lit) = retention index from literature.

Table 3.6 Pearson's (n-1) correlations matrix for aroma attributes and GC-MS identified volatile compounds in STBD green *C. maculata* infusions

	Grass	Cooked veg	Beans	Stewed fruit	Tropical	Apricot	Guava	Hay	Oats	GSA	Fruity-sweet	Caramel
hexanal	**0.898**	0.876	**0.963**	-0.708	-0.278	0.331	-0.495	**0.893**	0.685	0.530	-0.577	0.600
cis-3-hexenol	**0.920**	**0.931**	0.852	-0.535	-0.582	0.157	-0.790	**0.901**	**0.932**	**0.933**	-0.777	0.495
n-heptanal	-0.420	-0.069	-0.233	-0.346	**0.961**	-0.232	0.802	-0.334	-0.681	-0.634	0.823	-0.579
benzaldehyde	-0.074	0.202	0.078	-0.062	0.662	0.325	0.289	-0.259	-0.273	-0.212	0.522	-0.208
1-octen-3-ol	**0.962**	**0.953**	**0.926**	-0.580	-0.542	0.242	-0.764	**0.933**	**0.917**	0.872	-0.770	0.568
6-methyl-5-heptene-2-one	**0.979**	0.815	**0.943**	-0.280	-0.680	0.578	**-0.893**	0.819	**0.945**	0.828	-0.856	0.809
octanal	-0.399	-0.156	-0.204	-0.409	0.873	-0.264	0.835	-0.244	-0.695	-0.740	0.733	-0.459
2,4-heptadienal,(E,E)	0.411	0.690	0.480	-0.294	0.234	0.323	-0.220	0.229	0.297	0.404	0.032	0.021
phenylacetaldehyde	0.878	0.739	**0.904**	-0.675	-0.468	0.225	-0.529	**0.946**	0.711	0.508	-0.716	0.675
isophorone	**0.951**	**0.930**	**0.980**	-0.536	-0.383	0.470	-0.676	0.844	0.810	0.708	-0.648	0.644
linalool	-0.161	0.289	-0.009	-0.518	0.842	-0.251	0.553	-0.093	-0.382	-0.256	0.645	-0.559
nonanal	0.026	0.288	0.213	-0.743	0.673	-0.216	0.541	0.200	-0.309	-0.374	0.415	-0.243
decanal	0.331	0.473	0.487	-0.861	0.347	-0.136	0.255	0.523	0.004	-0.133	0.055	0.053
benzaldehyde,4-ethyl	-0.424	-0.094	-0.300	0.122	0.799	0.090	0.510	-0.566	-0.538	-0.393	0.762	-0.513
γ-terpinene	**0.919**	**0.971**	**0.931**	-0.746	-0.352	0.163	-0.585	**0.944**	0.791	0.728	-0.633	0.480
isobornylacetate	-0.171	0.310	-0.052	-0.440	0.793	-0.270	0.476	-0.131	-0.328	-0.141	0.626	-0.611
geranylacetone	0.176	0.551	0.322	-0.855	0.600	-0.299	0.363	0.343	-0.096	-0.060	0.314	-0.311
unidentified norisoprenoid 1	-0.470	-0.156	-0.370	0.235	0.747	0.095	0.474	-0.633	-0.535	-0.367	0.751	-0.527
unidentified norisoprenoid 2	-0.354	0.032	-0.235	-0.012	0.801	0.003	0.484	-0.461	-0.470	-0.297	0.731	-0.553

Values in bold are different from 0 with a significance level alpha = 0.05. 'Grass' = green grass, 'Cooked veg' = cooked vegetables, 'Beans' = green beans and/or potato, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet. Compound labels coloured in green indicate accompanying 'green' or 'herbaceous' aroma descriptors (**Table 3.5**).

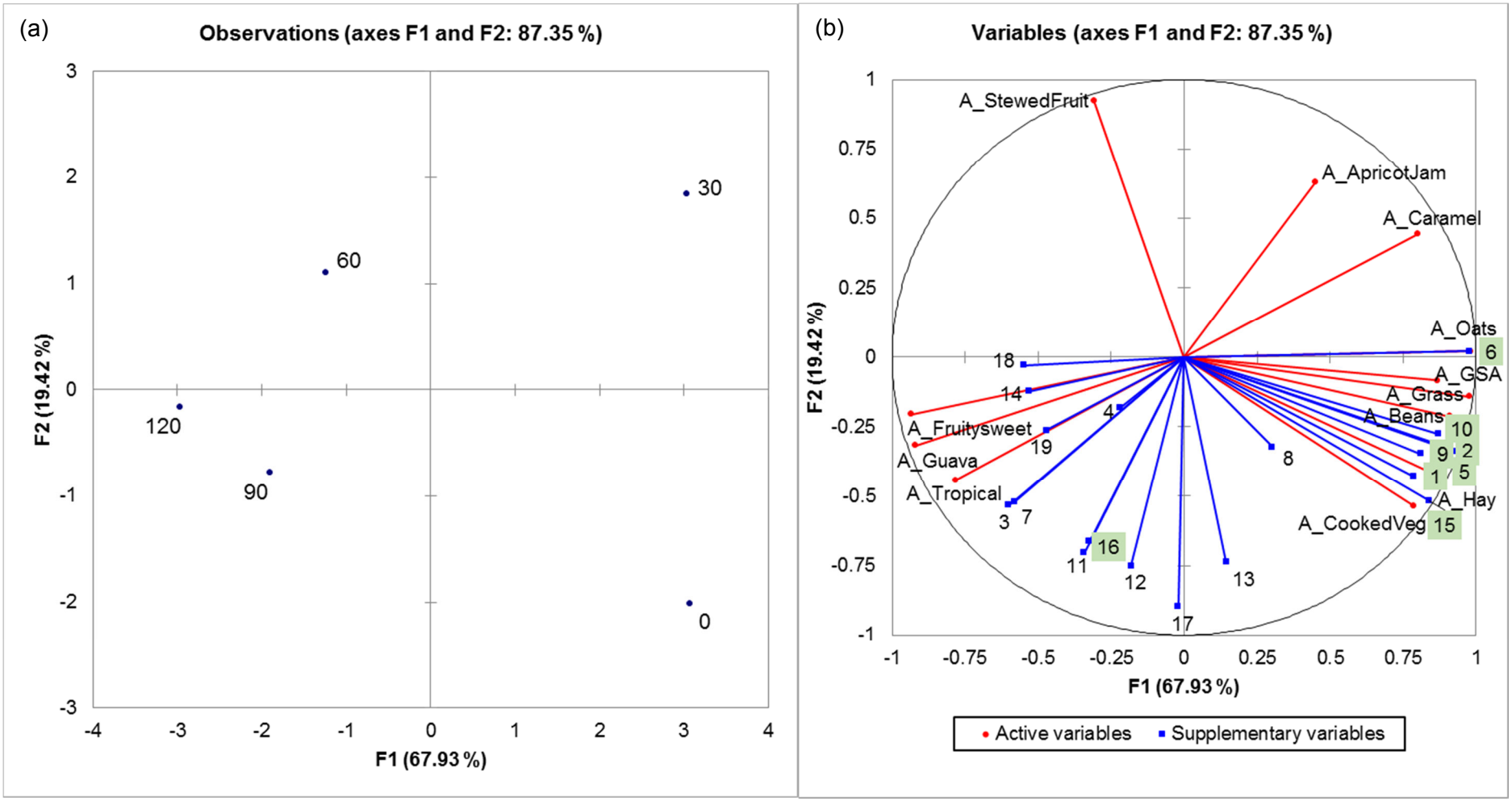


Figure 3.10 PCA (a) scores and (b) loadings plots for detected (intensity > 5) aroma attributes from DSA of STBD green *C. maculata* infusions (batch 3) with GC-MS volatile relative peak areas as supplementary variables.

'A_' prefix indicates aroma attributes. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Beans' = green beans and/or potato, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'GSA' = general sweet. Compound number labels correspond to **Table 3.5**. Compound labels coloured in green indicate accompanying 'green' or 'herbaceous' aroma descriptors.

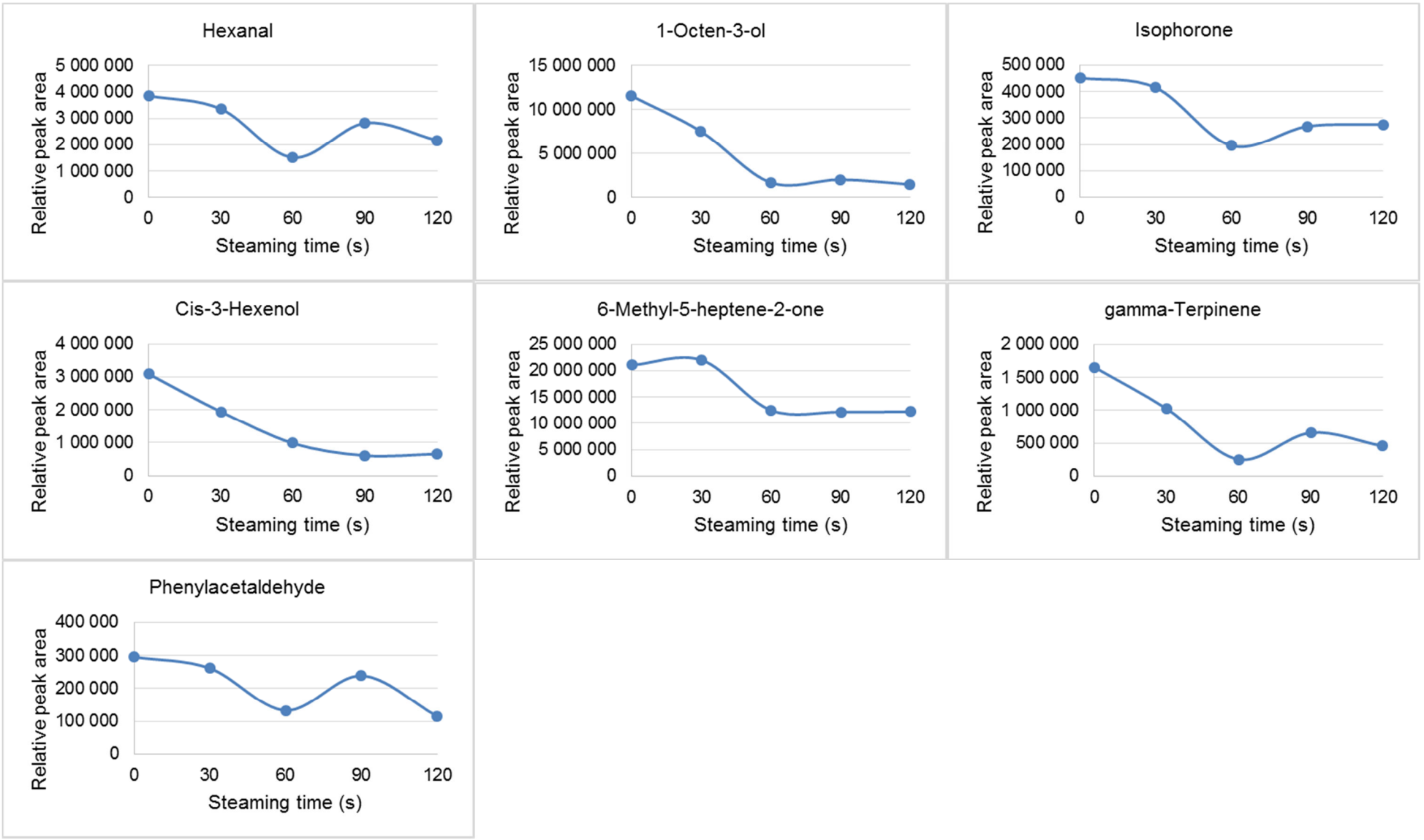


Figure 3.11 Relative peak areas of GC-MS identified volatile compounds with 'green' aroma descriptors, and significant ($p < 0.05$) correlations (**Table 3.6**) with *vegetative* and *cereal* aroma attributes present in infusions of STBD green *C. maculata*.

3.1.2 *Effect of steam treatment after drying (STAD) on green* C. maculata *quality*

3.1.2.1 *Sensory profile of infusions*

PCA scores and loadings plots are provided in **Fig. 3.12** (**a** & **b**) with corresponding Pearson's (n-1) correlations available in **ADDENDUM A** (**Table A.8**). There is a progressive positioning of treatments along the first principal component (F1, 41.14%) in the PCA scores plot, with batches spread along the second principal component (F2, 21.00%). The PCA loadings plot shows a clustering of 'guava', 'apricot', 'fruity-sweet', 'general sweet' and 'stewed fruit' aroma attributes as well as sweet taste on the left, associating with steam-treated samples (2, 3 and 4 min). Bitter taste, sour taste, astringent mouthfeel, 'caramel' aroma as well as *vegetative* and *cereal* attributes are positioned on the right associating with unsteamed control samples, except for 'cooked vegetables' aroma and flavour that was positioned in the left upper quadrant. Significant ($p < 0.05$) correlations were established between corresponding flavour and aroma attributes, ranging from moderate ('stewed fruit', 'hay/dried grass' and 'oats/porridge/grains') to strong ('green grass,' 'cooked vegetables' and 'guava').

The DA plots (**Fig. 3.13 a** & **b**) again indicated a progressive grouping of the longer steaming times (t = 2, 3 and 4 min), and the control and 1 min treatment along F1 (95.60%). Minor batch variation was indicated by a spread along F2 (4.4%). The DA variables loadings plot (**Fig. 3.13 b**) indicated a similar positioning to that of the PCA plot (**Fig. 3.12 b**), with *fruity* and *sweet-associated* attributes to the left and *vegetative* and 'caramel' attributes to the right of F1. 'Green grass' flavour and 'caramel' aroma were selected for treatment discrimination.

Furthermore, individual attributes were analysed by ANOVA (**ADDENDUM A**, **Tables A.9 – A.11**) and are presented in **Fig. 3.14** (**a** – **c**). The aroma profile of *C. maculata* was significantly ($p < 0.05$) affected by STAD in terms of 'green grass', 'cooked vegetables', 'hay/dried grass', 'oats/porridge/grains', 'guava', 'fruity-sweet' and 'caramel' notes. 'Stewed fruit', 'apricot jam' and 'general sweet' aroma intensities were unaffected by STAD ($p \geq 0.05$). All *vegetative* and *cereal* aroma attributes, except 'cooked vegetables' aroma, showed a significant ($p < 0.05$) decrease in intensity after steam treatment of 1 min. 'Green grass' aroma intensity further decreased after 3 min and that of 'oats/porridge/grains' aroma after 2 min. 'Cooked vegetables' aroma intensity, however, seemed to increase at 2 and 3 min of steaming when compared to that of the control, but the duration of steam treatment did not have a significant effect ($p \geq 0.05$). 'Fruity-sweet' aroma intensity was increased by all steam treatments, while a 2 min treatment or longer increased that of 'guava' aroma significantly ($p < 0.05$), compared to the control. 'Caramel' aroma intensity was significantly higher in control than steam-treated samples.

Flavour attributes affected by STAD included 'green grass', 'cooked vegetables', 'stewed fruit' and 'guava'. 'Hay/dried grass' flavour intensity did not show a significant change as a result of steaming ($p \geq 0.05$). Similar to the corresponding aroma attributes, the intensity of 'green grass'

flavours progressively decreased with steam treatment. 'Cooked vegetables' flavour intensity peaked at 2 min, differing significantly from the control, but not from the other steam treatments. 'Guava' flavour intensity peaked at 3 min, after initial increases after 1 min. 'Stewed fruit' flavour intensity remained stable after 1 and 2 min steam treatment, increasing significantly only after 3 min. Taste and mouthfeel intensities were not affected by steaming, except for a significant ($p < 0.05$) decrease in bitter taste intensity, and increase in sweet taste intensity after 4 min steaming, compared to that of the control.

3.1.2.2 *Colour of tea material and infusions*

Instrumental colour measurements of STAD samples are presented in **Fig. 3.15**, with TC and TSS content in **Fig. 3.16**. Tabulated results for all colour, TSS and TC determinations are provided in **ADDENDUM A** (**Table A.12**). Similar to STBD samples of *C. maculata* (section 3.1.1.2), milled and unmilled material produced similar results, once again only differing slightly in lightness (L^*) trends. As before, colour parameters for unmilled plant material and infusions will be discussed.

Steam treatments of 1, 2 and 4 min resulted in a significant ($p < 0.05$) lightening of unmilled plant material. Hue values decreased significantly with increasing steam treatment, while the opposite trend was observed for a^* values which changed from negative for the control, to positive values for the steam-treated samples. Chroma and b^* increased significantly ($p < 0.05$) as a result of steam treatments. In all cases the changes were small. A progressive significant ($p < 0.05$) increase in ΔE^* can be observed for unmilled samples after 1 and 4 min with a final value of < 3. ΔE^* of the infusions remained < 3 as they showed very little change in colour. Significant ($p < 0.05$), but slight decreases in h and C values of the infusions were observed only for the 4 min steam treatment compared to the control.

Plant material TC content followed a gradual decline over the course of steaming, decreasing significantly ($p < 0.05$) after 1, 2 and 4 min. Infusion TSS was not affected by STAD.

3.1.2.3 *Phenolics of aqueous-organic extracts*

The individual phenolic contents of the plant material are summarised per treatment in **Table 3.7**. STAD did not affect the phenolic composition of *C. maculata*, with one exception. Eriodictyol-*O*-glucoside content was significantly ($p < 0.05$) decreased by the 4 min steam treatment compared to the control.

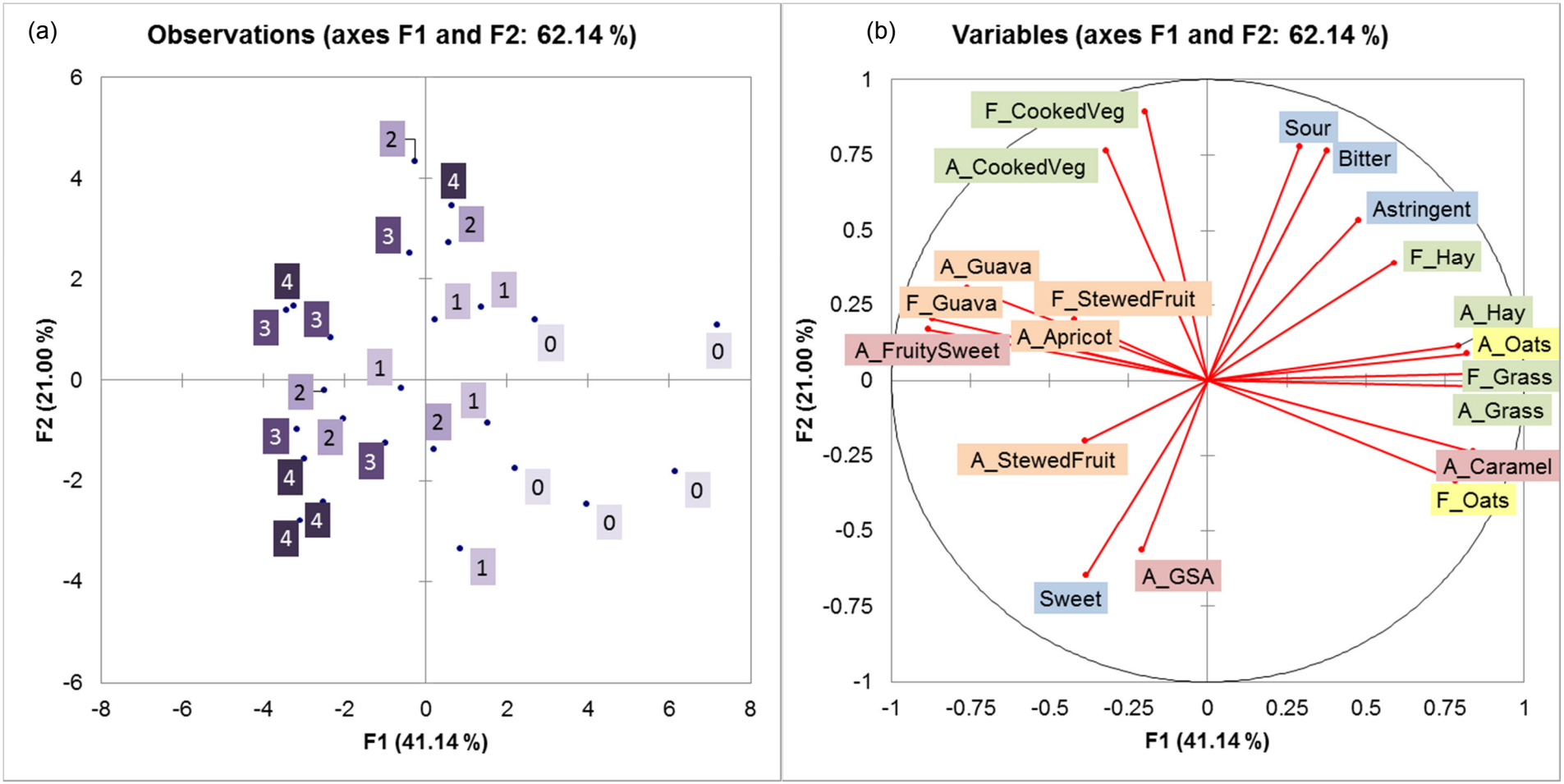


Figure 3.12 PCA (a) scores and (b) loadings plots of all detected (intensity > 5) aroma, flavour, taste and mouthfeel attributes of STAD green *C. maculata*, n = 25.

Steaming times = 0, 1, 2, 3 and 4 min. 'A' and 'F' prefixes refer to aroma and flavour attributes, respectively. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Apricot' = apricot jam, 'GSA' = general sweet, 'Sour' = sour taste, 'Bitter' = bitter taste, 'Sweet' = sweet taste. Variables attribute colours: green = *vegetative*, yellow = *cereal*, pink = *sweet-associated*, orange = *fruity*, blue = taste and mouthfeel.

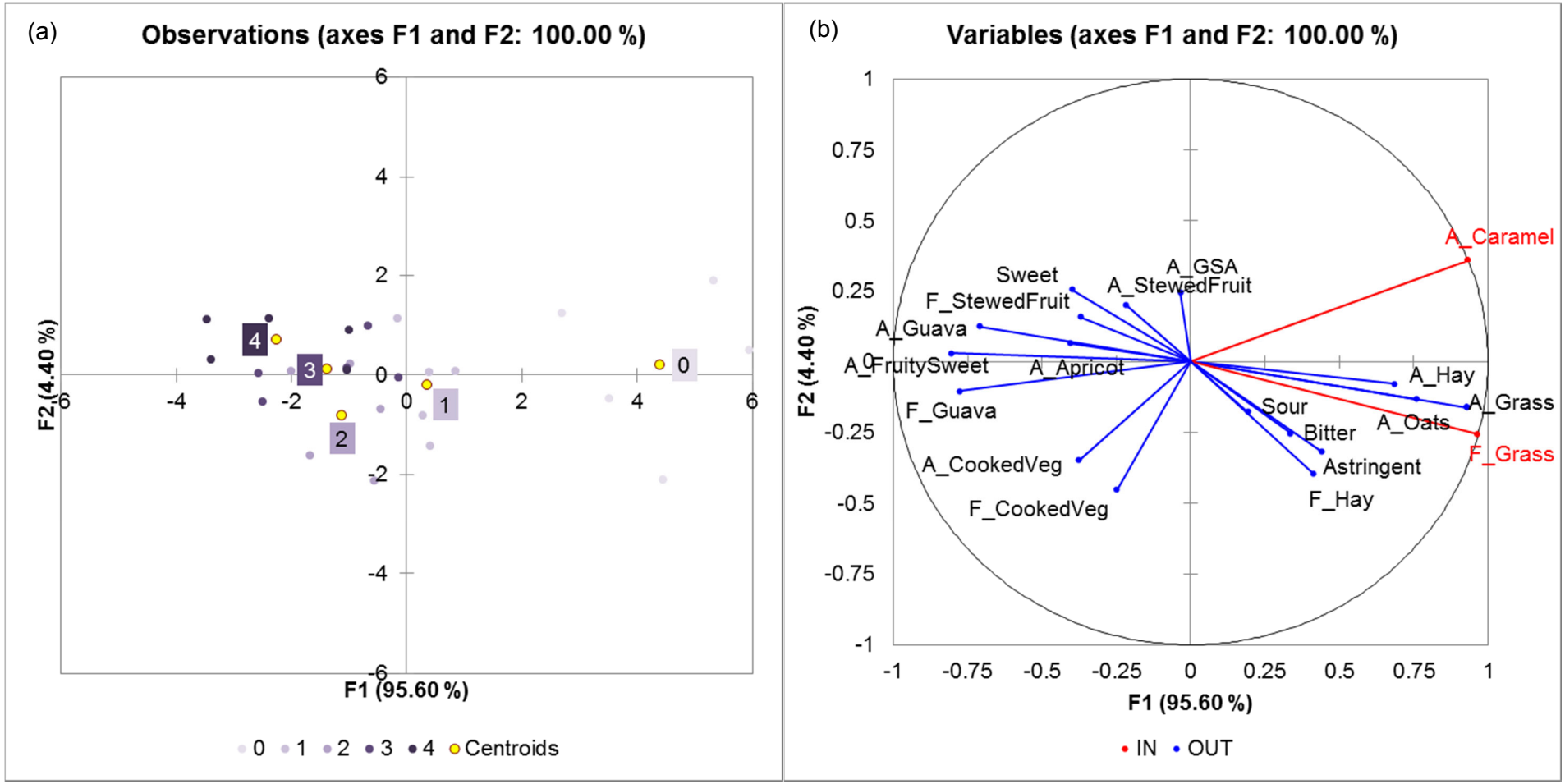
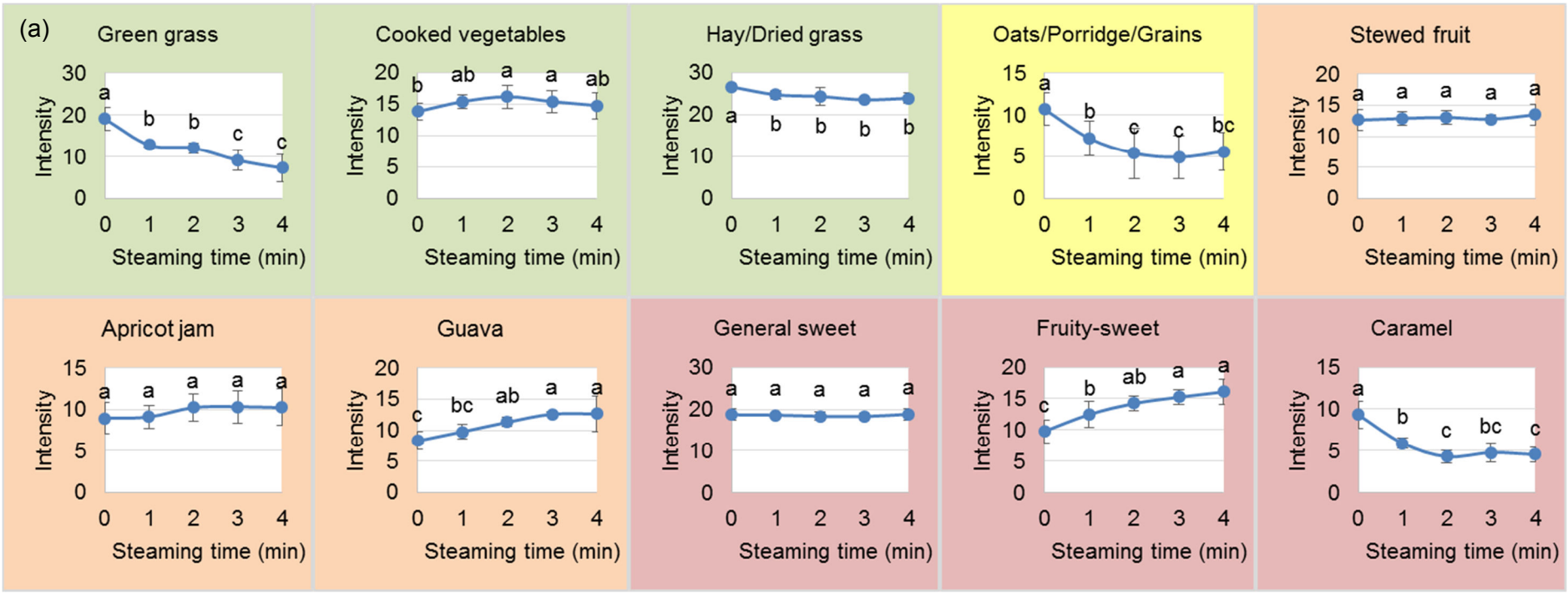


Figure 3.13 DA (a) observations and (b) variables loadings plots of green honeybush lexicon attributes of STAD green *C. maculata*, n = 25.

Steaming times = 0, 1, 2, 3 and 4 min. 'A' and 'F' prefixes refer to aroma and flavour attributes respectively. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet, 'Sweet' = sweet taste, 'Bitter' = bitter taste, 'Sour' = sour taste.



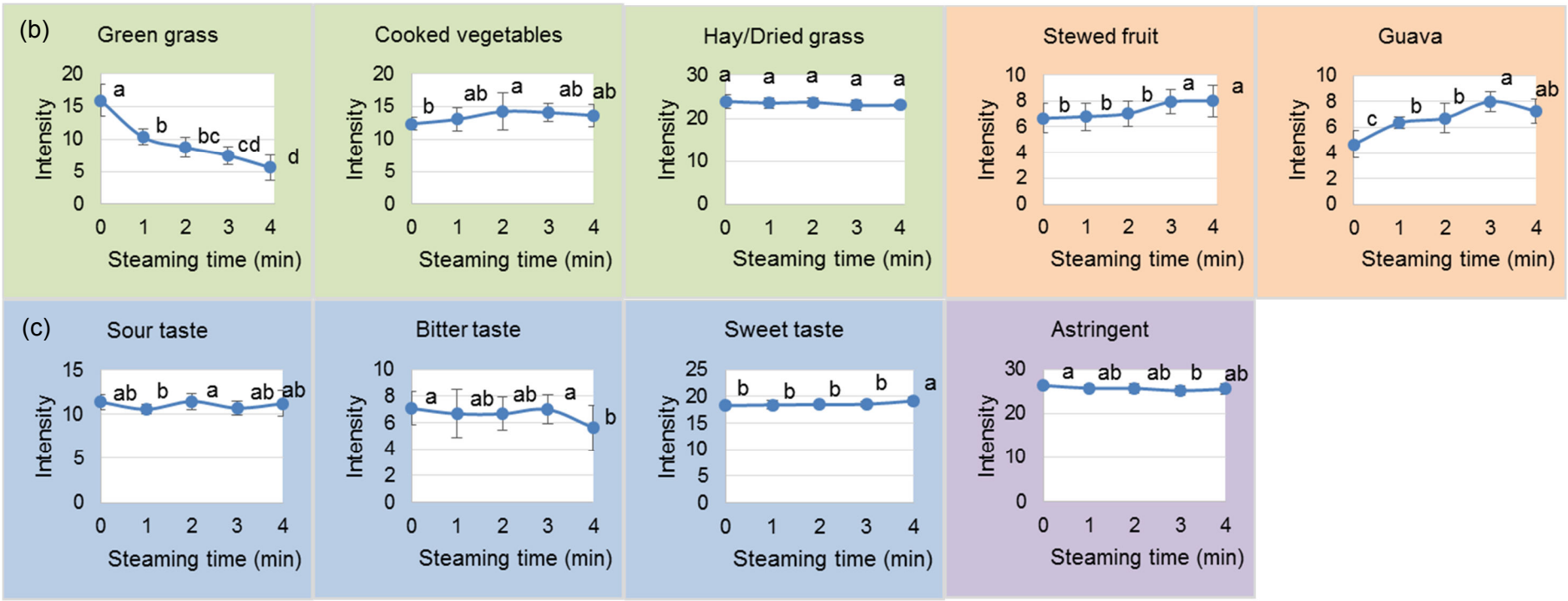


Figure 3.14 Mean intensities of (a) aroma, (b) flavour attributes, (c) taste and mouthfeel of STAD green *C. maculata*.

Bars associated with the same letters do not differ significantly ($p \geq 0.05$). Error bars indicate standard deviation. Green plots = *vegetative* attributes, yellow plots = *cereal* attributes, orange plots = *fruity* attributes, pink plots = *sweet-associated* attributes, blue plots = taste attributes, purple plots = mouthfeel attributes.

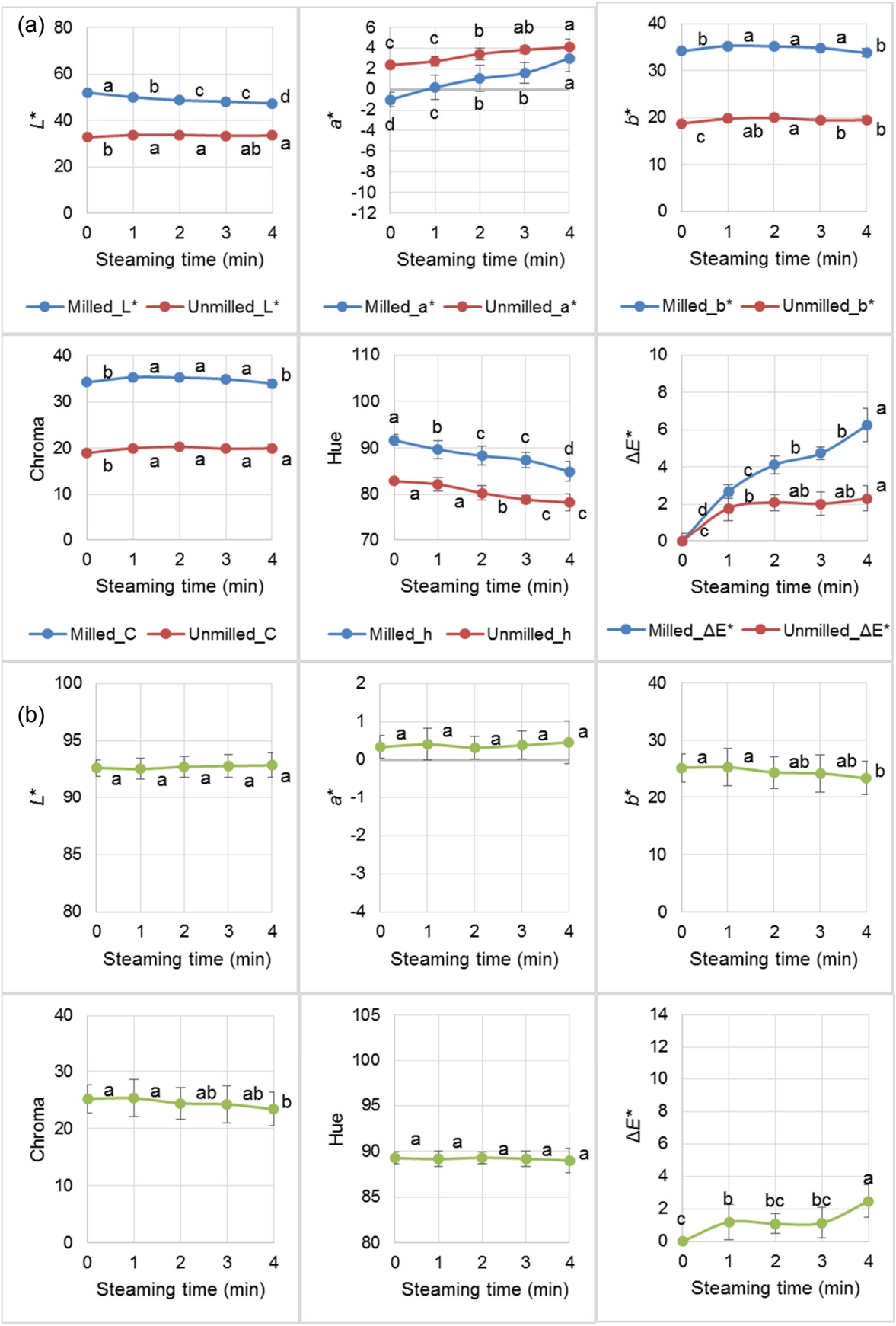


Figure 3.15 Objective colour parameters (CIE*L*a*b**) of (a) milled and unmilled leaf material and (b) infusions samples of STAD green *C. maculata*.

Series points associated with the same letters do not differ significantly ($p \geq 0.05$). Error bars indicate standard deviation.

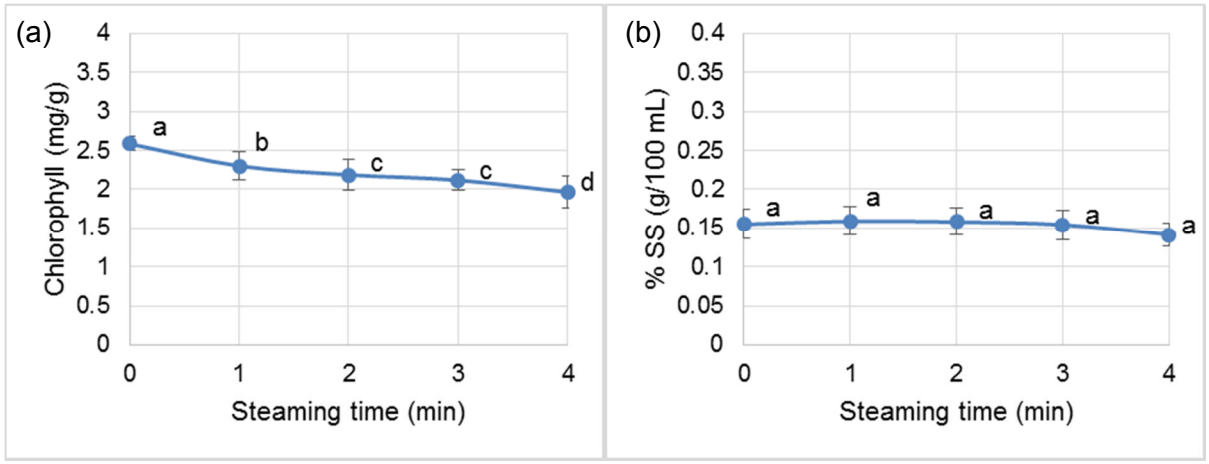


Figure 3.16 (a) Total chlorophyll content and (b) total soluble solids content of STAD green *C. maculata* milled plant material and infusions, respectively.

Series points associated with the same letters do not differ significantly ($p \geq 0.05$). Error bars indicate standard deviation.

Table 3.7 Mean individual phenolic content (g/100 g plant material) of STAD green *C. maculata*

Compound	Steaming time (min)																			
	0				1				2				3				4			
mangiferin	2.044	a	±	0.286	2.034	a	±	0.260	2.020	a	±	0.243	1.964	a	±	0.289	2.082	a	±	0.295
isomangiferin	0.566	a	±	0.062	0.543	a	±	0.059	0.551	a	±	0.062	0.536	a	±	0.070	0.559	a	±	0.076
vicenin-2	0.072	a	±	0.009	0.070	a	±	0.008	0.070	a	±	0.007	0.072	a	±	0.006	0.076	a	±	0.013
eriodictyol-*O*-glucoside	0.029	a	±	0.011	0.027	ab	±	0.008	0.025	ab	±	0.007	0.024	ab	±	0.008	0.021	b	±	0.003
iriflophenone-3-*C*-glucoside	0.060	a	±	0.014	0.056	a	±	0.023	0.055	a	±	0.015	0.056	a	±	0.019	0.056	a	±	0.019
eriocitrin	0.169	a	±	0.023	0.163	a	±	0.041	0.157	a	±	0.039	0.154	a	±	0.039	0.161	a	±	0.021
maclurin-3-*C*-glucoside	0.028	a	±	0.005	0.027	a	±	0.007	0.028	a	±	0.006	0.028	a	±	0.006	0.029	a	±	0.010
hesperidin	1.238	a	±	0.577	1.120	a	±	0.460	0.959	a	±	0.434	0.943	a	±	0.372	1.218	a	±	0.284

Means with the same letters do not differ significantly ($p \geq 0.05$). Values indicated as (mean) ± (standard deviation).

3.2 *Cyclopia longifolia*

3.2.1 *Effect of steam treatment before drying (STBD) on green* C. longifolia *quality*

3.2.1.1 *Sensory profile of infusions*

The PCA results of the STBD green *C. longifolia* data are presented in **Fig. 3.17**. Pearson's (n-1) correlation coefficients for PCA may be found in **ADDENDUM A** (**Table A.13**). The PCA scores plot (**Fig. 3.17 a**) shows a clear grouping of control samples in the lower right quadrant and a vague diagonal positioning from shorter to progressively longer steaming times, with the 120 s treatment samples at the furthest top left periphery. The accompanying PCA loadings plot (**Fig. 3.17 b**) indicates the association of 'caramel' and 'general sweet' aroma attributes as well as *vegetative* and *cereal* attributes (except for 'cooked vegetables' flavour) with control samples.

The DA observations plot (**Fig. 3.18 a**) indicates a clear classification of control samples on the right, separated from the other treatment samples on the left along F1 (92.29%). There is a progressive sample positioning with the 120 s treatment samples furthest to the left. The DA variables loadings plot (**Fig. 3.18 b**) indicates the selection of 'green grass', 'general sweet' and 'caramel' aromas to the right of the plot as necessary for treatment discrimination.

Mean intensities per treatment for each attribute were subjected to ANOVA (**ADDENDUM A**, **Table A.14** – **A.16**) and are presented in **Fig. 3.19** (**a** – **c**). *Vegetative* and *cereal* aroma intensities decreased significantly ($p < 0.05$) as a result of steaming, although the duration of the steam treatment was not significant ($p \geq 0.05$). This was not the case for 'green grass' aroma. *Fruity* ('tropical fruit' and 'guava') and 'fruity-sweet' aroma intensities increased as a result of steam treatment, except for 'apricot jam'. 'Caramel' aroma intensity decreased with increasing steam treatment.

Only *vegetative* flavour attributes were notably present in STBD green *C. longifolia*. 'Green grass' flavour intensity decreased with steam treatment, although the duration had no significant effect ($p \geq 0.05$). The intensity of 'hay/dried grass' flavour decreased slightly after 60 and 90 s, while that of 'cooked vegetables' flavour remained unchanged. Taste and mouthfeel intensities were not affected by steaming ($p \geq 0.05$).

3.2.1.2 *Colour of tea material and infusions*

The effect of STBD on the colour of the green *C. longifolia* plant material (milled and unmilled) and infusions is depicted in **Fig. 3.20**. The TC and TSS contents of milled plant material and infusions are presented in **Fig. 3.21**. A tabulated summary of these results are provided in **ADDENDUM A** (**Table A.17**). Similar trends were observed in terms of colour for the milled and unmilled plant material, with the plant material sold in unmilled form. The results will thus focus on the changes in the colour of the unmilled plant material.

The STBD of *C. longifolia* seems to result in a small, but progressive colour change with longer steaming times. The *L**, *b**, C and h values of the unmilled plant material decreased, while *a** increased progressively and significantly ($p < 0.05$) as a result of steaming. Although the *a** value increased, it remained negative (green), while h shifted within the yellow-green (90° – 180°) quadrant to a less intense green. These changes resulted in $\Delta E^* > 3$ for all steam-treated samples. The loss of green colour was also reflected in the gradual decrease in TC content.

The *L** value of the infusion increased slightly with steam treatment ($p < 0.05$), indicating a slight lightening, while *b** and C remained unaffected overall ($p \geq 0.05$). Notable is that the decrease in *a** (increase in green colour) and increase in h did not lead to a change in the colour quadrant. The colour differences of infusions were small ($\Delta E^* < 2$), yet significant ($p < 0.05$). Infusion TSS increased significantly ($p < 0.05$) as a result of steaming.

3.2.1.3 *Phenolics of aqueous-organic extracts*

The phenolic content of the STBD samples with regard to hesperidin, maclurin-3-*C*-glucoside, mangiferin, hydroxy-mangiferin, vicenin-2 and hydroxy-isomangiferin either did not change significantly ($p \geq 0.05$), or did not show a consistent trend (**Table 3.8**). However, the content of the benzophenone, iriflophenone-di-*C*-glucoside, increased, and that of the flavone, scolymoside, decreased with steam treatment ($p < 0.05$).

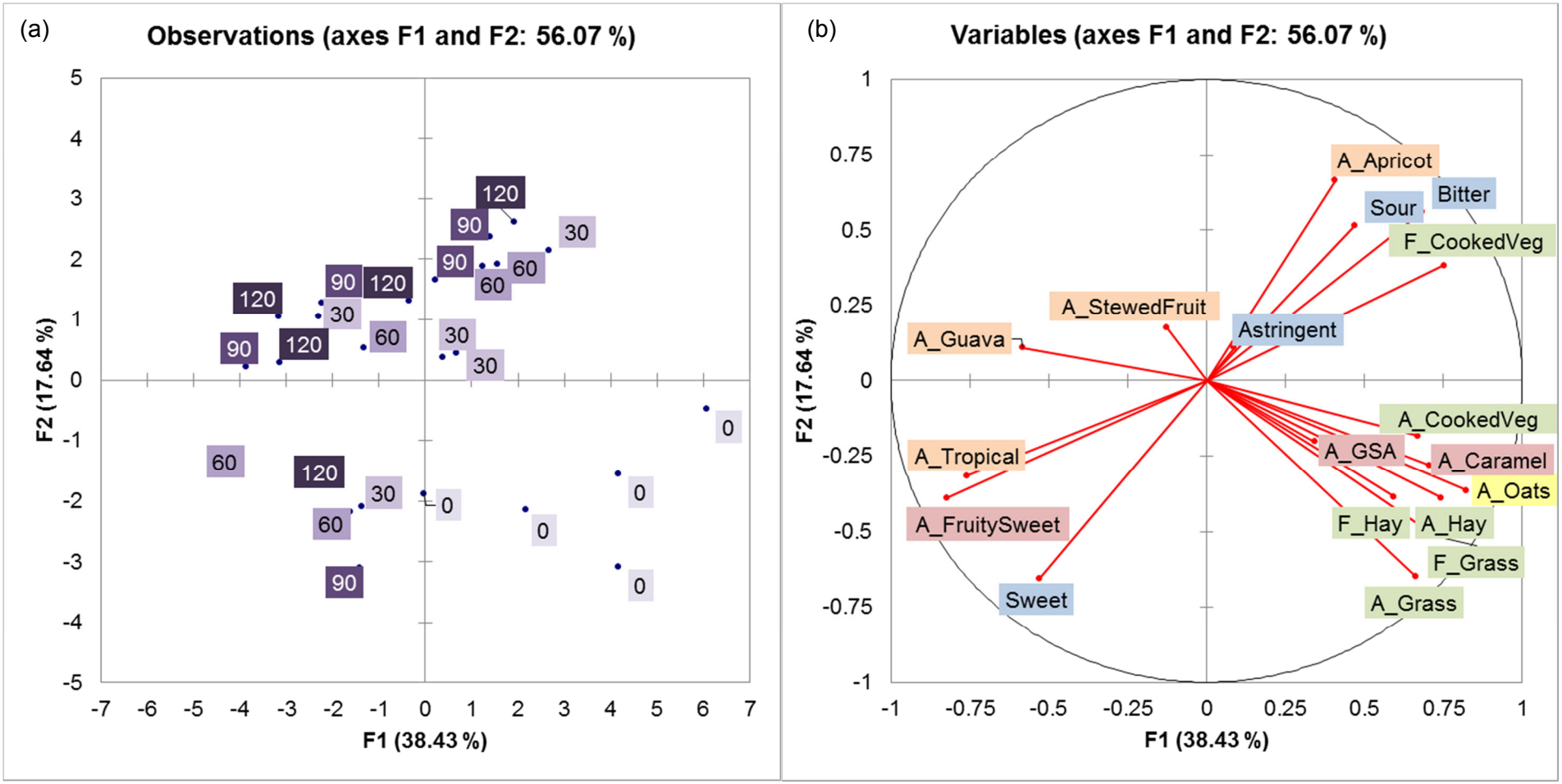


Figure 3.17 PCA (a) scores and (b) loadings plots of all detected (intensity > 5) aroma, flavour, taste and mouthfeel attributes of STBD green *C. longifolia*, n = 25.

Steaming times = 0, 30, 60, 90 and 120 s. 'A' and 'F' prefixes refer to aroma and flavour attributes, respectively. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet, 'Sweet' = sweet taste, 'Bitter' = bitter taste, 'Sour' = sour taste. Variables attribute colours: green = *vegetative*, yellow = *cereal*, pink = *sweet-associated*, orange = *fruity*, blue = taste and mouthfeel.

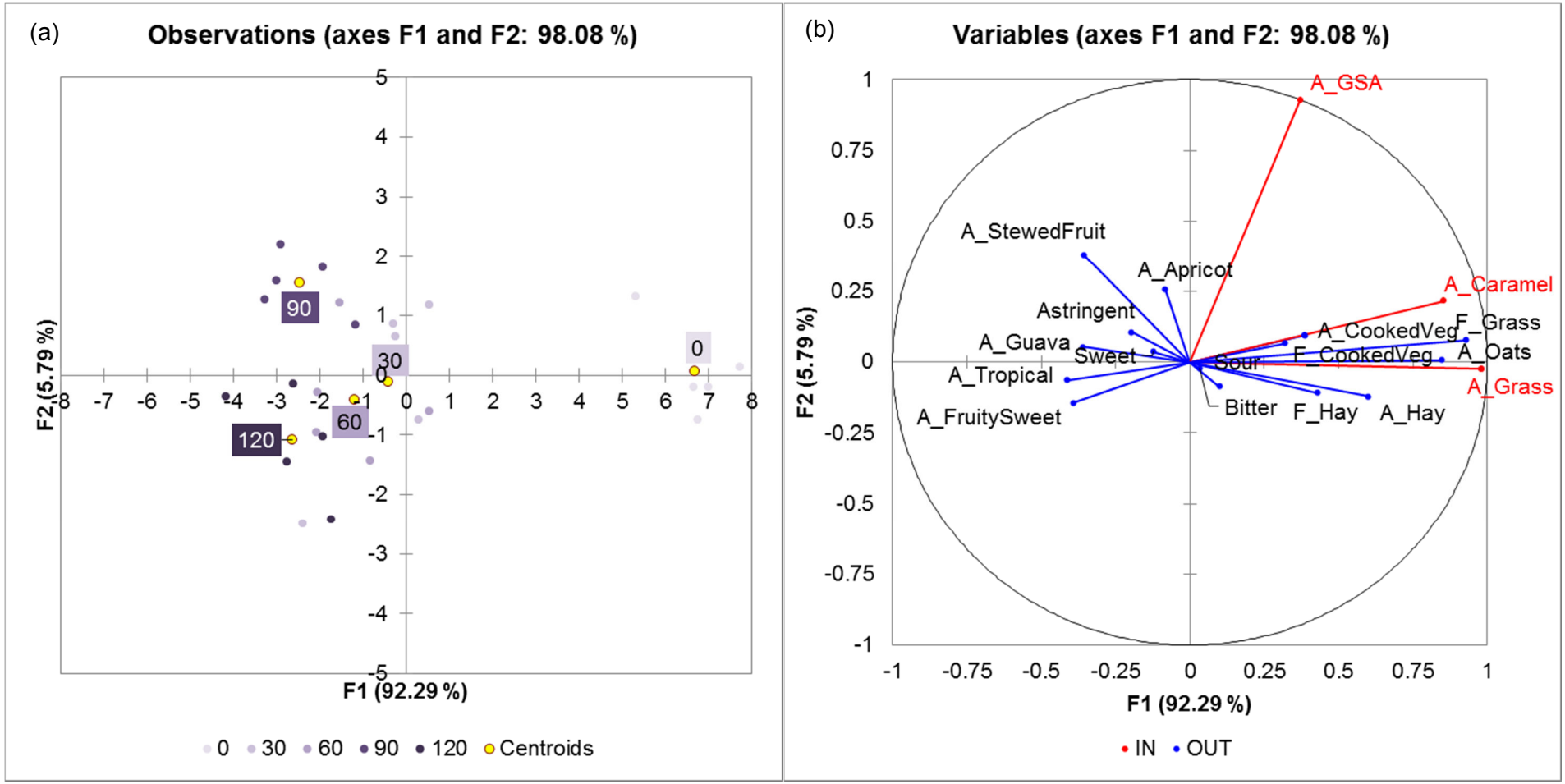
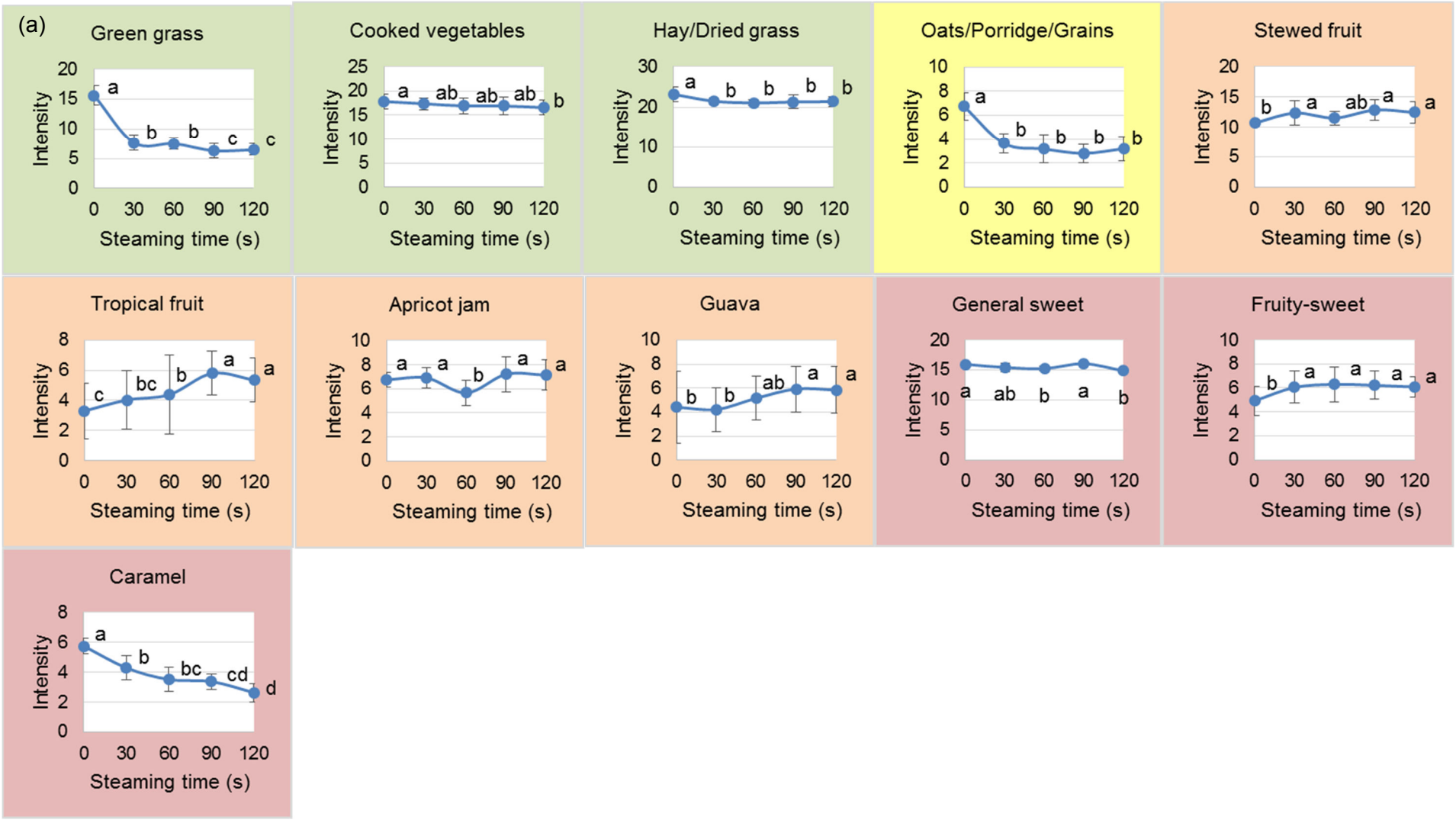


Figure 3.18 DA (a) observations and (b) variables loadings plots of green honeybush lexicon attributes of STBD green *C. longifolia*, n = 25.

Steaming times = 0, 30, 60, 90 and 120 s. 'A' and 'F' prefixes refer to aroma and flavour attributes respectively. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet, 'Sweet' = sweet taste, 'Bitter' = bitter taste, 'Sour' = sour taste.



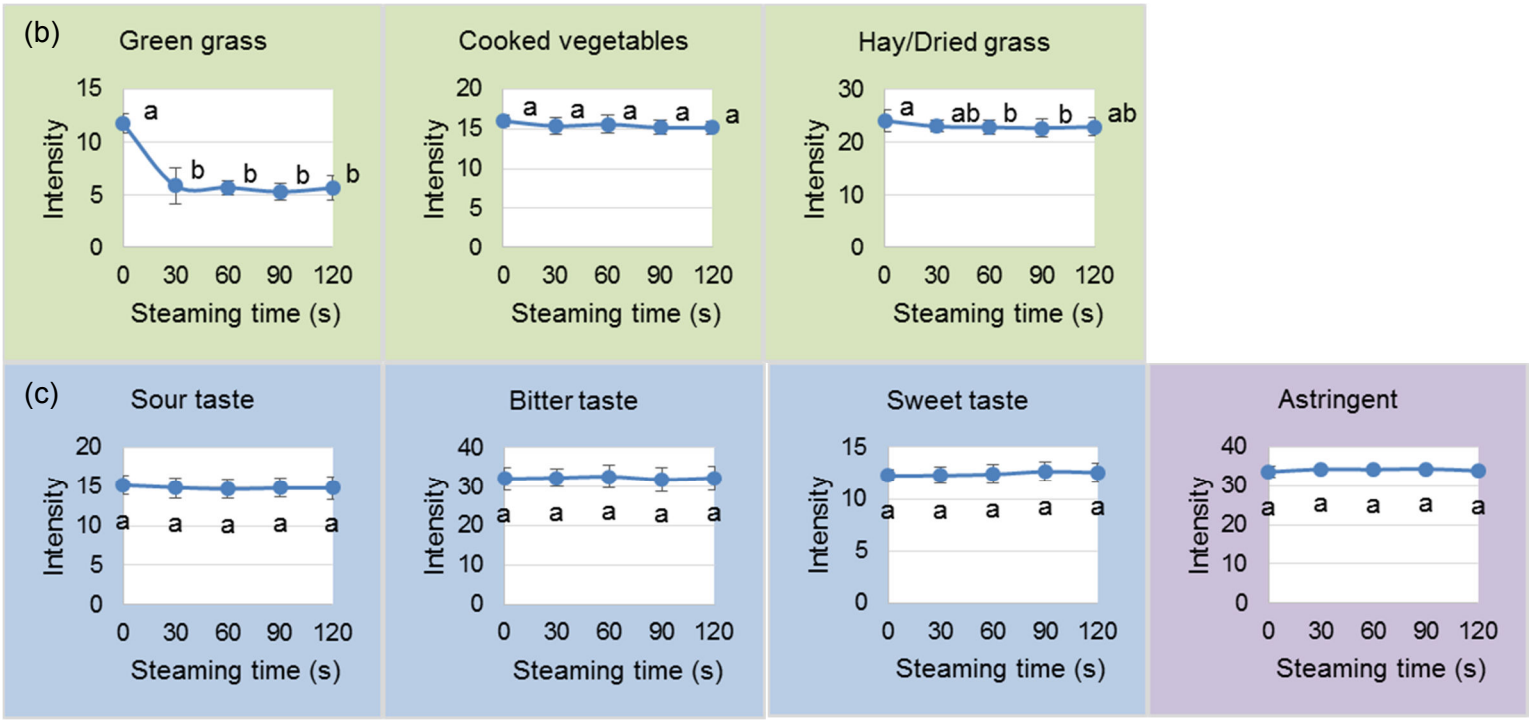


Figure 3.19 Mean intensities of (a) aroma, (b) flavour attributes, (c) taste and mouthfeel in STBD green *C. longifolia*.

Bars associated with the same letters do not differ significantly ($p \geq 0.05$). Error bars indicate standard deviation. Green plots = *vegetative* attributes, yellow plots = *cereal* attributes, orange plots = *fruity* attributes, pink plots = *sweet-associated* attributes, blue plots = taste attributes, purple plots = mouthfeel attributes.

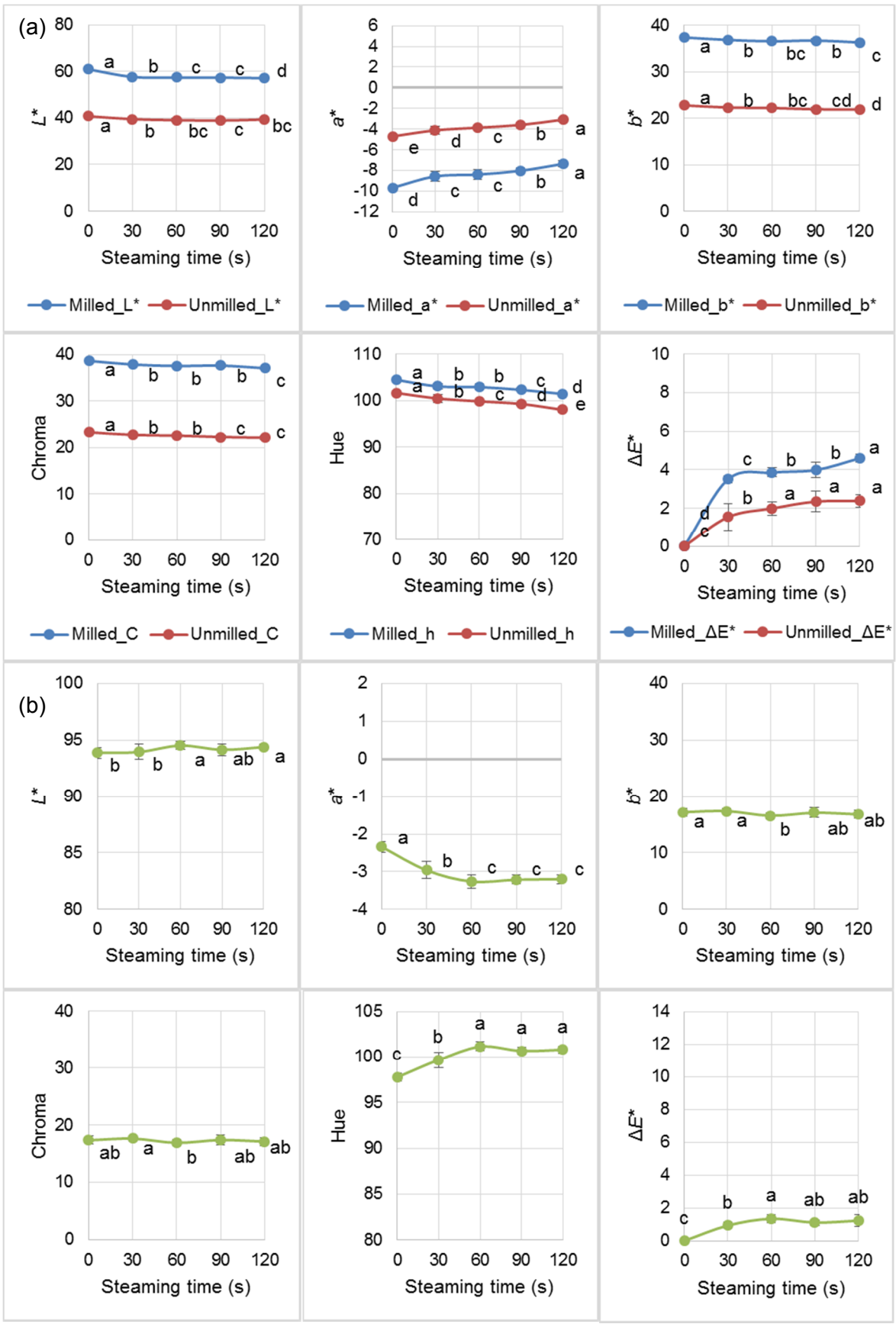


Figure 3.20 Objective colour parameters (CIE*L*a*b**) of (a) milled and unmilled leaf material and (b) infusion samples of STBD green *C. longifolia*.

Series points associated with the same letters do not differ significantly ($p \geq 0.05$). Error bars indicate standard deviation.

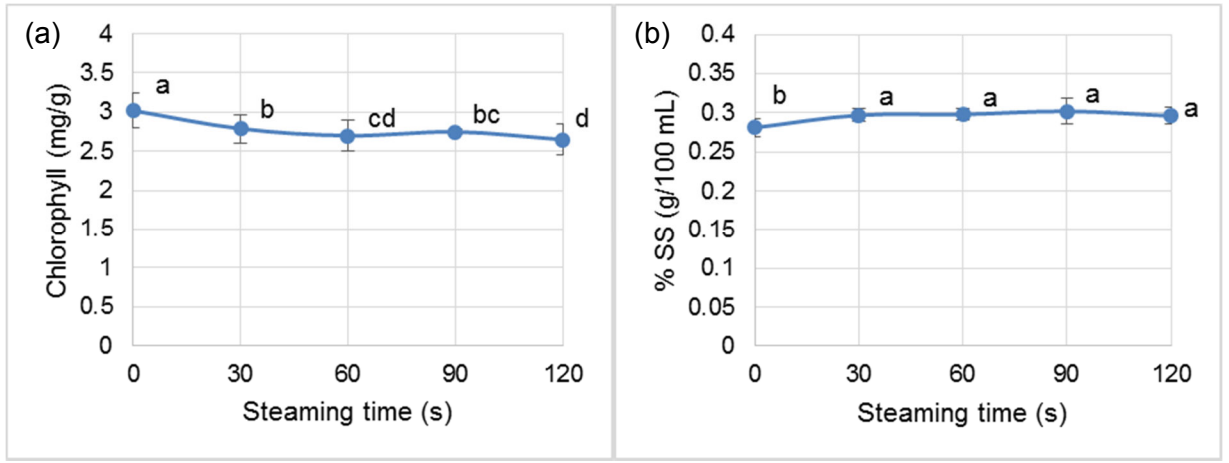


Figure 3.21 (a) Total chlorophyll content and (b) total soluble solids content of STBD green *C. longifolia* milled plant material and infusions, respectively.

Series points associated with the same letters do not differ significantly ($p \geq 0.05$). Error bars indicate standard deviation.

Table 3.8 Mean individual phenolic contents (g/100 g plant material) of STBD green *C. longifolia*

Compound	Steaming time (s)																			
	0				30				60				90				120			
iriflophenone-3-*C*-glucoside	0.386	a	±	0.103	0.330	b	±	0.096	0.372	a	±	0.096	0.334	b	±	0.078	0.373	a	±	0.106
iriflophenone-di-*C*-glucoside	0.031	bc	±	0.003	0.030	c	±	0.002	0.031	abc	±	0.003	0.032	ab	±	0.003	0.032	a	±	0.003
eriocitrin	0.067	b	±	0.012	0.066	b	±	0.012	0.067	b	±	0.013	0.073	a	±	0.010	0.069	b	±	0.013
hesperidin	0.204	a	±	0.056	0.207	a	±	0.033	0.204	a	±	0.042	0.231	a	±	0.039	0.219	a	±	0.042
maclurin-3-*C*-glucoside	0.076	a	±	0.014	0.073	a	±	0.014	0.079	a	±	0.010	0.073	a	±	0.007	0.077	a	±	0.012
norathyriol-di-hexoside	0.040	a	±	0.003	0.039	b	±	0.003	0.039	b	±	0.002	0.040	a	±	0.002	0.038	b	±	0.003
mangiferin	1.777	a	±	0.144	1.780	a	±	0.436	1.836	a	±	0.315	1.818	a	±	0.196	1.546	a	±	0.500
hydroxy-mangiferin	0.100	a	±	0.006	0.102	a	±	0.008	0.103	a	±	0.007	0.103	a	±	0.005	0.100	a	±	0.009
isomangiferin	0.522	a	±	0.065	0.503	b	±	0.050	0.516	ab	±	0.053	0.517	ab	±	0.057	0.510	ab	±	0.066
vicenin-2	0.056	a	±	0.005	0.055	a	±	0.004	0.056	a	±	0.004	0.056	a	±	0.005	0.056	a	±	0.004
hydroxy-isomangiferin	0.035	a	±	0.002	0.035	a	±	0.002	0.035	a	±	0.001	0.035	a	±	0.001	0.034	a	±	0.001
scolymoside	0.043	a	±	0.015	0.041	ab	±	0.012	0.040	ab	±	0.013	0.042	ab	±	0.013	0.039	b	±	0.013

Means with the same letters do not differ significantly ($p \geq 0.05$). Values indicated as (mean) ± (standard deviation).

3.2.2 *Effect of steam treatment after drying (STAD) on green* C. longifolia *quality*

3.2.2.1 *Sensory profile of infusions*

PCA scores and loadings plots are presented in **Fig. 3.22** (**a** & **b**), with Pearson's (n-1) correlation coefficients provided in **ADDENDUM A** (**Table A.18**). The PCA scores plot shows no clear trends, except that the control samples are mostly grouped in the upper right quadrant with a diagonal positioning of progressively longer steaming times towards the lower left quadrant. Control samples associated with *vegetative* aromas and flavour, with *fruity*, 'fruity-sweet' and 'general sweet' aromas in the opposite quadrant.

Using DA (**Fig. 3.23**), control samples grouped to the right and steamed-treated samples to the left along F1 (98.96%) with the 4 min steam treatment positioned furthest to the left (**Fig. 3.23 a**). The DA variables loadings plot (**Fig. 3.23 b**) indicated 'green grass' and 'caramel' aromas to the right as necessary for treatment discrimination.

Results of the univariate ANOVA of DSA are provided in **ADDENDUM A** (**Tables A.19 – A.21**) and the changes in attribute intensities with steam treatments are presented in **Fig. 3.24**. The only aroma attributes significantly ($p < 0.05$) affected by steaming included 'green grass', 'hay/dried grass', 'stewed fruit', 'fruity-sweet' and 'caramel' aroma. Other detectable aroma attributes included 'cooked vegetables', 'apricot jam', 'oats/porridge/grains' and 'general sweet' aromas. 'Green grass' and 'caramel' aroma intensity decreased significantly ($p < 0.05$) with steam treatment, but duration had no effect. The respective intensities of 'hay/dried grass' and 'fruity-sweet' decreased and increased progressively with an increase in the duration of the steam treatment. 'Stewed fruit' aroma intensity was significantly ($p < 0.05$) increased after 2 – 4 min steaming.

Only *vegetative* and *cereal* flavours were detectable, with the intensities of 'green grass' and 'hay/dried grass' significantly ($p < 0.05$) decreased after 1 min steam treatment. Longer steam treatments had no further effect on the latter flavour intensities. Of the taste and mouthfeel attributes only bitter taste showed a slight change in intensity, with samples steam-treated for 4 min having a slightly less bitter taste than the control ($p < 0.05$).

3.2.2.2 *Colour of tea material and infusions*

Data for the objective colour parameters of milled and unmilled plant material and infusions are presented in **Fig. 3.25**, with TC and TSS contents of the milled plant material and infusions presented in **Fig. 3.26**. These results are also presented in tabulated form in **ADDENDUM A** (**Table A.22**). Results for milled and unmilled material were similar with the exception of lightness. The colour data of the unmilled plant material and infusion are summarised below.

Steaming resulted in a slight, but significant ($p < 0.05$) lightening of unmilled material, and increased the b^* (yellow) and C values significantly ($p < 0.05$). The duration of the treatment had no effect ($p \geq 0.05$). An increase in the duration of the steam treatment ($p < 0.05$) resulted in a loss of

green colour, by the gradual increase in *a** and decrease in h. This decrease in green colour was reflected in the gradual loss of TC content. Total colour differences of steamed samples reached $\Delta E^* > 3$ after a steam treatment of 2 min and longer.

The *L**, *a** and *b** values of infusions were not affected by steaming ($p \geq 0.05$). ΔE^* increased slightly with steam treatment, but remained < 3. The TSS content of the infusions did not show a clear trend with steam treatment.

3.2.2.3 *Phenolics of aqueous-organic extracts*

Table 3.9 presents the individual phenolic content for each treatment of STAD green *C. longifolia*. Steaming did not have a significant effect on the individual phenolic compounds of the plant material, when compared to the control ($p \geq 0.05$).

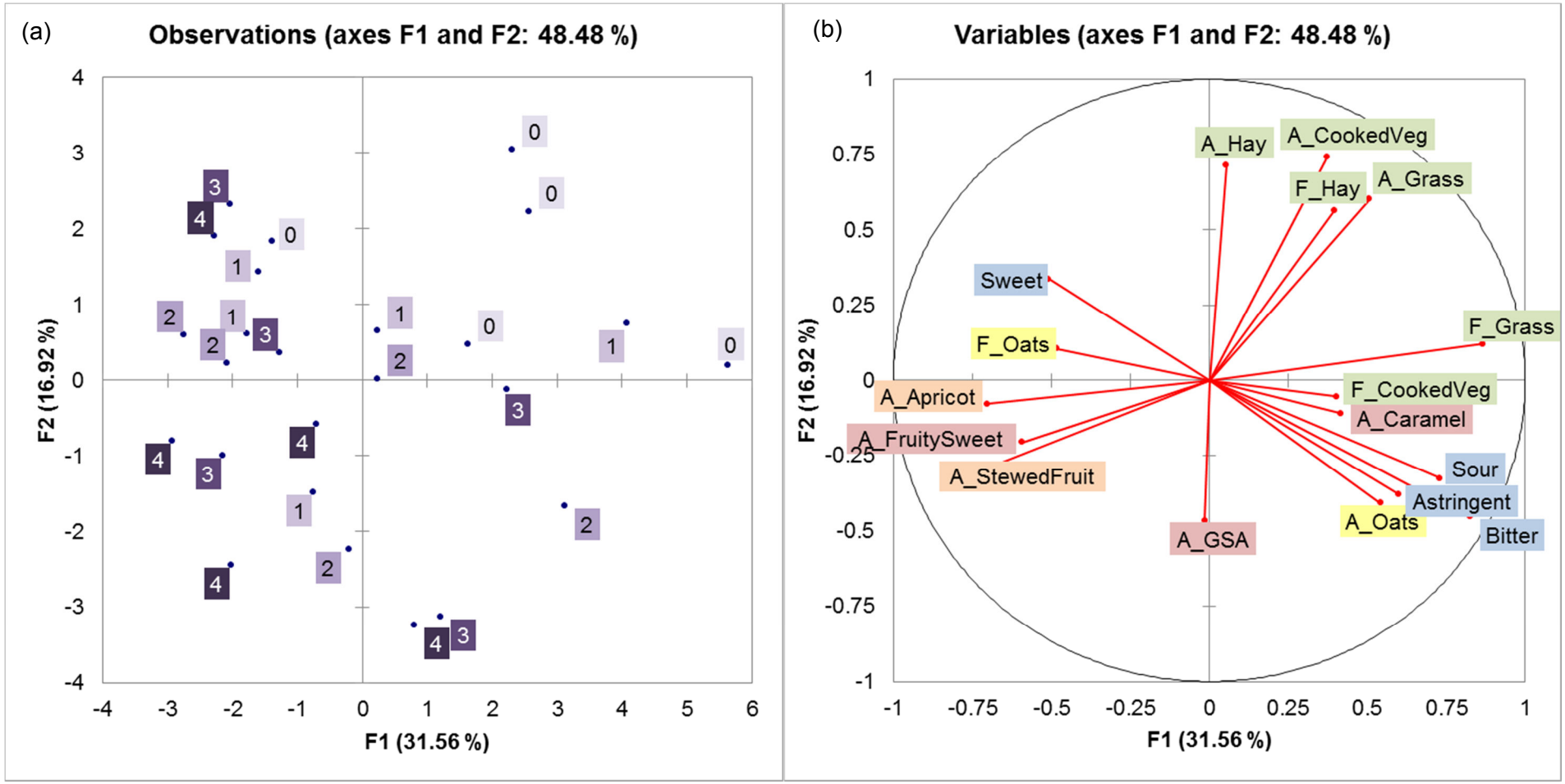


Figure 3.22 PCA (a) scores and (b) loadings plots of all detected (intensity > 5) aroma, flavour, taste and mouthfeel attributes of STAD green *C. longifolia*, n = 25.

Steaming times = 0, 1, 2, 3 and 4 min. 'A' and 'F' prefixes refer to aroma and flavour attributes, respectively. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Apricot' = apricot jam, 'GSA' = general sweet, 'Sweet' = sweet taste, 'Bitter' = bitter taste, 'Sour' = sour taste. Variables attribute colours: green = *vegetative*, yellow = *cereal*, pink = *sweet-associated*, orange = *fruity*, blue = taste and mouthfeel.

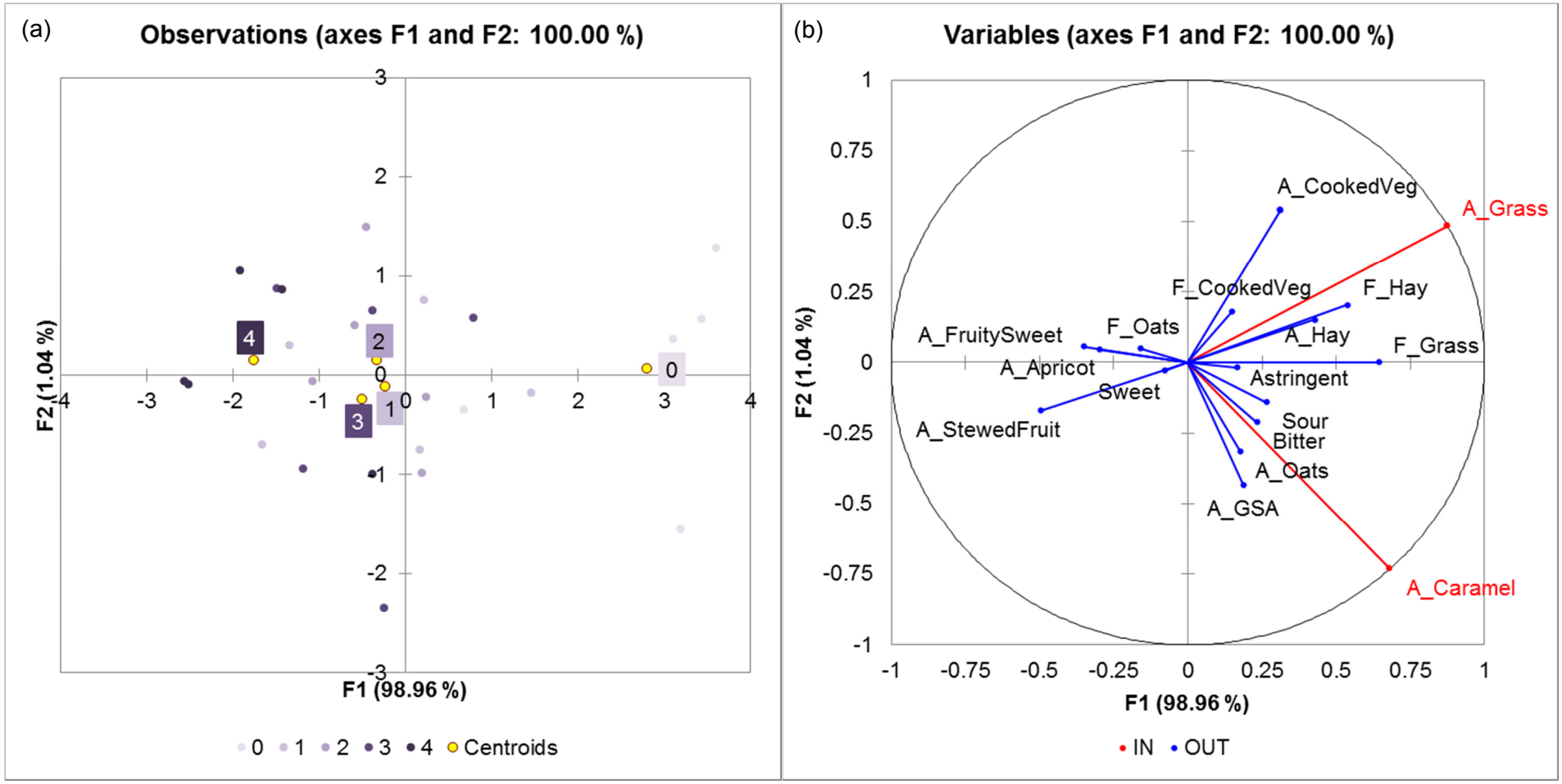
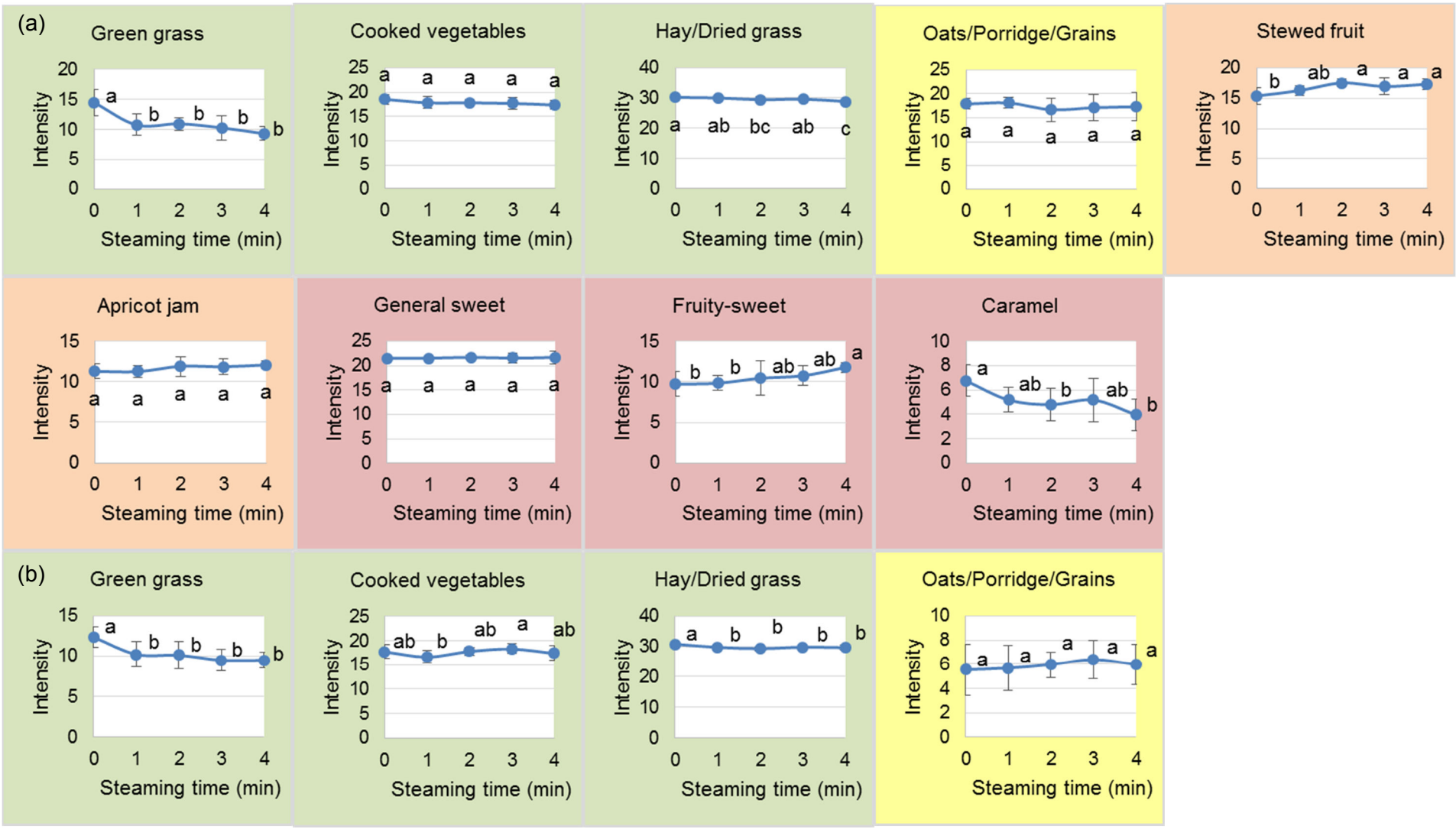


Figure 3.23 DA (a) observations and (b) variables loadings plots of green honeybush lexicon attributes of STAD green *C. longifolia*, n = 25.

Steaming times = 0, 1, 2, 3 and 4 min. 'A' and 'F' prefixes refer to aroma and flavour attributes, respectively. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Apricot' = apricot jam, 'GSA' = general sweet, 'Sweet' = sweet taste, 'Bitter' = bitter taste, 'Sour' = sour taste.



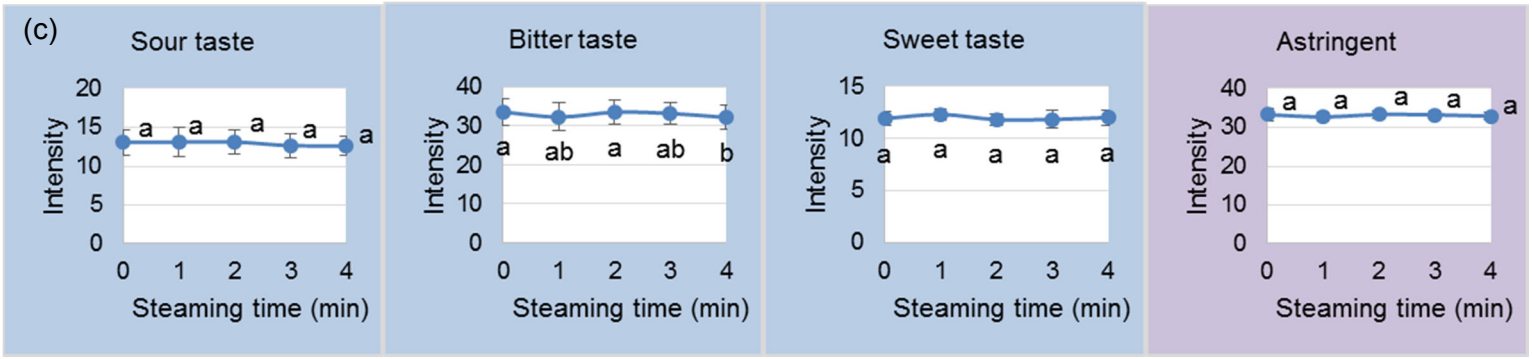


Figure 3.24 Mean intensities of (a) aroma, (b) flavour, (c) taste and mouthfeel attributes in STAD green *C. longifolia*.

Bars associated with the same letters do not differ significantly ($p \geq 0.05$). Error bars indicate standard deviation. Green plots = *vegetative* attributes, yellow plots = *cereal* attributes, orange plots = *fruity* attributes, pink plots = *sweet-associated* attributes, blue plots = taste attributes, purple plots = mouthfeel attributes.

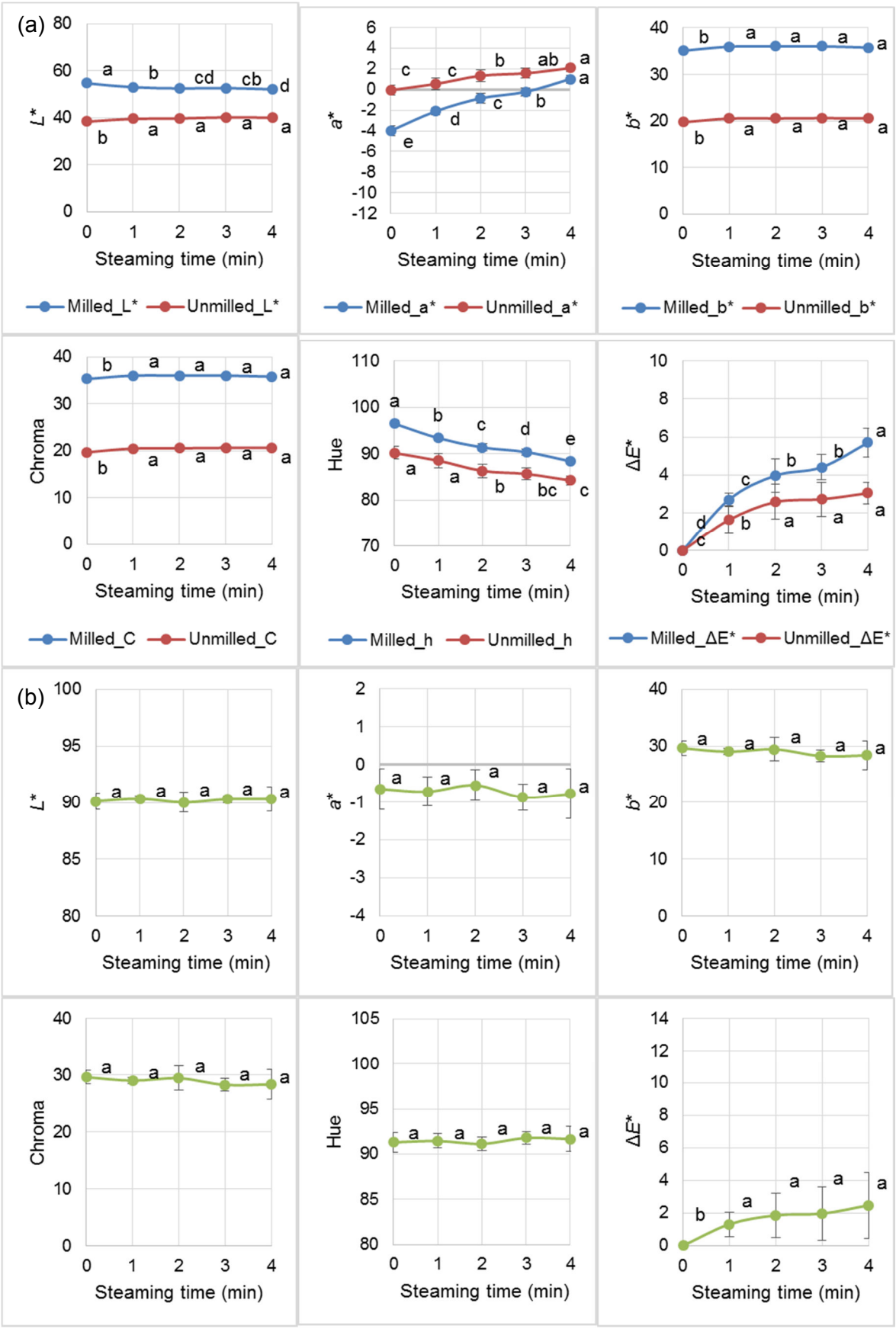


Figure 3.25 Objective colour parameters (CIE*L*a*b**) of (a) milled and unmilled leaf material and (b) infusion samples of STAD green *C. longifolia*.

Series points associated with the same letters do not differ significantly ($p \geq 0.05$). Error bars indicate standard deviation.

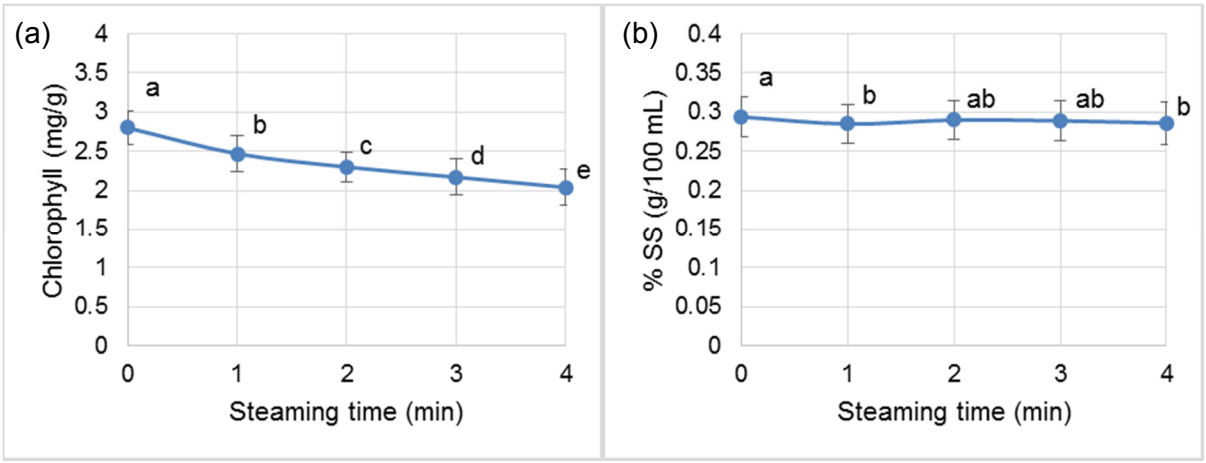


Figure 3.26 (a) Total chlorophyll content and (b) total soluble solids content of STAD green *C. longifolia* milled plant material and infusions, respectively.

Series points associated with the same letters do not differ significantly ($p \geq 0.05$). Error bars indicate standard deviation.

Table 3.9 Mean individual phenolic content (g/100 g plant material) of STAD green *C. longifolia*

Compound	Steaming time (min)																			
	0				1				2				3				4			
iriflophenone-3-*C*-glucoside-4-*O*-glucoside	0.389	a	±	0.074	0.392	a	±	0.067	0.389	a	±	0.071	0.390	a	±	0.072	0.392	a	±	0.073
iriflophenone-3-*C*-glucoside	0.219	a	±	0.071	0.224	a	±	0.075	0.216	a	±	0.056	0.220	a	±	0.074	0.220	a	±	0.078
iriflophenone-di-*C*-glucoside	0.0445	a	±	0.006	0.0445	a	±	0.004	0.0429	a	±	0.006	0.0450	a	±	0.006	0.0435	a	±	0.006
eriocitrin	0.129	a	±	0.019	0.129	a	±	0.021	0.132	a	±	0.018	0.132	a	±	0.016	0.125	a	±	0.020
hesperidin	0.545	a	±	0.096	0.553	a	±	0.095	0.550	a	±	0.103	0.564	a	±	0.088	0.545	a	±	0.094
maclurin-3-*C*-glucoside	0.050	a	±	0.015	0.051	a	±	0.015	0.049	a	±	0.010	0.051	a	±	0.014	0.050	a	±	0.015
norathyriol-di-hexoside	0.081	a	±	0.004	0.082	a	±	0.008	0.084	a	±	0.009	0.084	a	±	0.008	0.081	a	±	0.009
mangiferin	3.864	a	±	0.608	3.829	a	±	0.877	4.225	a	±	0.638	3.762	a	±	0.677	4.126	a	±	0.403
hydroxy-mangiferin	0.238	a	±	0.050	0.228	a	±	0.031	0.229	a	±	0.018	0.234	a	±	0.033	0.242	a	±	0.063
isomangiferin	1.128	ab	±	0.105	1.105	ab	±	0.095	1.141	a	±	0.078	1.123	ab	±	0.105	1.094	b	±	0.109
vicenin-2	0.108	a	±	0.003	0.108	a	±	0.003	0.106	a	±	0.007	0.106	a	±	0.009	0.105	a	±	0.007
hydroxy-isomangiferin	0.071	a	±	0.007	0.072	a	±	0.007	0.074	a	±	0.007	0.076	a	±	0.011	0.072	a	±	0.010
scolymoside	0.119	a	±	0.013	0.117	a	±	0.012	0.116	a	±	0.016	0.113	a	±	0.016	0.112	a	±	0.015

Means with the same letters do not differ significantly ($p \geq 0.05$). Values indicated as (mean) ± (standard deviation).

4. Discussion

4.1 Variation in plant material

Following the processing and analysis of all plant material, intra-species variation was observed between the different plant batches and harvests. Such intra-species variation has previously been established as a result of plant maturity, climatic conditions and harvesting location, amongst others (Joubert *et al*., 2003; Joubert *et al*., 2014).

In terms of green colour, plant material harvested during winter for the second experiment consistently appeared visually considerably less green, compared to the plant material harvested in autumn for the first experiment (**Table 3.1**). This was confirmed by lower TC content, higher *a** values and lower hue values for the winter-harvested plant material. Species differences were also evident. Green colour was more prominent in *C. longifolia* plant material with lower initial *a** values for the untreated samples (*ca*. -5 and 0) compared to that of *C. maculata* (*ca*. -2 and 2, for the first and second experiments, respectively). It is possible that harvesting season had an effect on the green colour of the plant material, as the summer season provides high temperatures and high intensity light for chlorophyll production in plant leaves (Mishra, 2004).

The effect of seasonal variation on the phenolic composition of honeybush was observed by Joubert *et al*. (2014), where *C. genistoides* harvested in summer contained higher mangiferin, isomangiferin and iriflophenone-3-*C*-glucoside levels. Several factors could have affected the variation in xanthone (mangiferin and isomangiferin) content of the plant material in the present study, *i.e.* area, genotype, season, time interval between harvests, *etc.* (Joubert *et al*., 2014). In the current investigation, *C. longifolia* harvested in winter contained the highest levels of these xanthones (4.992 g/100 g plant material), followed by *C. maculata* harvested in autumn (4.009 g/100 g plant material). These groups of plant material also produced infusions with the highest bitterness and astringency intensities per species. *Cyclopia longifolia* harvested in autumn, however, produced infusions with similar bitterness and astringency intensities to that of the material harvested in winter, despite having the lowest xanthone levels (2.300 g/100 g plant material). This indicates that these xanthones might not be solely responsible for taste and mouthfeel of green honeybush infusions.

Variation in bitter taste and astringent mouthfeel intensity was most evident for *C. maculata* plant material harvested from separate locations in autumn and winter. Infusions of *C. maculata* plant material harvested in autumn at Koksrivier, Pearly Beach, had higher intensities for both these attributes (*ca*. 25 and 30, respectively) than infusions of plant material harvested in winter at Nietvoorbij, Stellenbosch (*ca*. 7 and 20, respectively). Once again, variation in individual phenolics gave no indication of their effect on these taste and mouthfeel attributes. Both bitterness and astringency of *C. maculata* infusions were less than those of *C. longifolia*.

4.2 Effect of steam treatment on sensory profile, colour and phenolic content of green *C. maculata*

Similar to the decrease in 'green' aroma intensity of steam-pasteurised rooibos (Koch *et al.,* 2013), steaming of 60 s or longer of green *C. maculata* resulted in a significant reduction in *vegetative* and *cereal* attribute intensities. This was observed regardless of STBD or STAD, with some attributes affected to a greater extent than others. 'Green grass' attributes were affected the most, and were consistently selected for classification of unsteamed control samples using DA.

When comparing the GC-MS identified volatiles present in some STBD samples, it is clear that the predominant volatiles are associated with *vegetative* descriptors such as 'green' and 'herbaceous' (Sigma Aldrich, 2011), especially 6-methyl-5-hepten-3-one and 1-octen-3-one. These volatile compounds represented the largest relative peak areas. Both peak areas decreased as a result of steaming, reaching a plateau when samples were steamed for 60 s or longer (**Fig. 3.11**). This corresponds to the trends observed for the DSA of STBD *C. maculata*, where treatments of 60 s and longer did not associate with *vegetative* aroma attributes (**Fig. 3.6**). Indeed, the results showed significant strong positive correlations ($p < 0.05$, $r > 0.9$), between these 'herbaceous' and 'green' volatiles and the *vegetative* and *cereal* aroma attributes as well as close association with unsteamed (control) and samples treated for 30 s (**Fig. 3.10**). Aroma compounds affecting overall aroma perception may be of high, medium or low volatility (Mamede & Pastore, 2006). Compounds with relatively low boiling points and subsequent high volatility, such as aldehydes, ketones and alcohols corresponding with the earliest elution times in the current GC-MS data (**Table 3.5**), describe most of volatile aroma compounds identified in green *C. maculata*. The exceedingly volatile nature of these compounds may thus contribute to the considerable losses during steaming at high temperature (96 °C) for 60 s and longer, as encountered in the present experiments. Furthermore, the polar nature of these compounds as based on the relative retention times on the non-polar stationary phase of the GC column, especially of alcohols and aldehydes, would allow greater solubility in the infusions. This may result in enhanced association by retro- (flavour) and orthonasal (aroma) perception.

Fruity and *sweet-associated* attribute intensities were also significantly affected by steaming (**Figs. 3.6** & **3.14**). A general increase in *fruity* ('tropical fruit', 'guava') aroma and flavour intensity was observed for both STAD and STBD treatments, although flavour intensities were often lower than 5. All samples had high intensities of 'general sweet' aroma, minimally affected by steaming, with 'fruity-sweet' aroma intensity increasing and that of 'caramel' aroma decreasing. Steam-pasteurisation of rooibos tea also resulted in a marked decrease in 'caramel' aroma intensity (Koch *et al*., 2013). This could indicate that the volatile(s) responsible for this aroma attribute are very susceptible to steam treatment. It was noted that 'caramel' aroma, closely associated with unsteamed samples and consistently used for treatment discrimination in DA, was most prominent in samples subjected to a 30 s STBD treatment. Terpenoids or terpenes, identified in the volatile fraction of honeybush infusions prepared from several *Cyclopia* spp., including *C. maculata* (Le Roux

et al., 2008; Cronje, 2010; Theron *et al*., 2014), are typically associated with 'fruity' or 'floral' aromas and may contribute to the perception of these aroma attributes in green honeybush. However, significantly strong correlations were not found between *fruity* aroma attributes and the identified volatile compounds in this study (**Table 3.6**). This could be due to the sensory and instrumental analysis having been conducted on distinctly separate infusions. Alternatively, it may simply be because the identified compounds do not quantitatively contribute to the development of the *fruity* aroma attributes analysed during DSA. It is thus possible that the increase in the intensities of *fruity* sensory attributes is the result of the modulation of sensory perception due to a decrease in the overpowering *vegetative* impression caused by the more volatile 'green'-associated volatile aroma compounds (Jackson, 2009).

Aroma perception is dependant both on the concentration of the volatile compound, and the threshold at which the volatile compound is perceptible by human olfaction (Sikorski *et al.*, 2007). A higher relative concentration, therefore, does not necessarily imply a greater contribution to aroma. The aroma perception of volatile compounds at any given temperature is affected by the volatility as well as associations between the volatile compounds and the food matrix, *e.g.* solubility, and interactions with proteins, lipids or polysaccharides (Jackson, 2009; Sikorski *et al.*, 2007). The lack of intense *fruity* flavour perception when compared to the intensity of *vegetative* attributes may therefore also be linked to the non-polar nature of associated flavour or aroma compounds (terpenoids) which may be less soluble in the water infusion.

Differences between treatments for taste and mouthfeel, although often significant, were very small with no clear trends followed over time, except for the 30 s STBD treatment group. A significant decrease in bitter taste intensity as well as minor decreases in sour taste and astringent mouthfeel intensities and a slight increase in sweet taste intensity was observed in these samples. The same treatment also caused a decrease in the content of some individual phenolic compounds in the plant material, including mangiferin, isomangiferin, iriflophenone-3-*C*-glucoside and hesperidin. Of these, mangiferin has been linked to bitter taste (Erasmus, 2015, Theron, 2012), and mangiferin, isomangiferin and iriflophenone-3-*C*-glucoside have been linked to astringent mouthfeel in fermented honeybush infusions (Erasmus, 2015). A decrease in their content due to degradation or conversion may have contributed to the perceptible change in bitter or astringent attributes, causing a heightened sweet taste perception.

Overall changes in the phenolic content of the plant material due to STBD and STAD of 60 s or longer were small or not significant. Similar steam exposure in rooibos (Koch *et al*., 2013) and *Lippia multiflora* (Arthur *et al*., 2011) had little to no effect on their phenolic composition and antioxidant activity, respectively. STBD of green *C. subternata* by Joubert *et al.* (2010) induced little change in the individual phenolic content, except for a notable decrease in the flavanone, eriocitrin, and an increase in its corresponding flavone, scolymoside. In the current study the flavanone (eriocitrin and eriodictyol-*O*-glucoside) content was slightly increased by STBD. The apparent stability of individual phenolic contents at longer steaming times may be linked to the effects of

steaming on membrane permeability and enzymatic processes. As noted in the review by Irina and Mohamed (2012), increased extraction efficacy due to alterations to membrane permeability during steaming (as will be discussed with regard to colour degradation) should be considered. This phenomenon is suspected to cause increases in volatile and phenolic concentrations in solution, masking degradation when compared to the unsteamed material. Indeed, STBD of more than 60 s significantly increased the TSS content of the infusions, possibly also resulting in the distinctly greener colour of corresponding infusions. Wang *et al*. (2004) speculated that chlorophyll may be responsible for the green colour of infusions, despite its hydrophobicity, by forming an emulsion in infusions when extracted effectively.

The anomaly of the 30 s STBD treatment group of *C. maculata* is further emphasised by the significant impact on the colour of the leaf (unmilled) material and infusions. Green colour (a^* and hue) was affected to the greatest degree, with significant darkening and a loss of green and yellow colour observed, although TC did not show the same trend. Consistently higher ΔE^* values of well above 3 were observed for plant material as well as infusions, indicating very distinct perceivable colour differences (Adekunte *et al*., 2010) when compared to the unsteamed control. The effect of the 30 s STBD treatment on infusions, however, was manifested as a significant increase in yellow colour with a minor loss of green colour, although TSS was not significantly different from the control.

Product degradation visually manifested as loss of green colour (chlorophyll destruction) or darkening is common as a result of heat processing of fresh vegetables (Sánchez *et al*., 2014; Cervantes-Paz *et al*., 2014). Mechanical processing is further destructive to product colour as plant acids and substances are liberated when membranes are injured, allowing interaction between substrates such as chlorophyll and phenolic acids and active enzymes (Rocha *et al*., 1993). This promotes enzymatic reactions leading to the loss of chlorophyll and oxidation of polyphenols. However, initial heat treatment (steam or water blanching) of fresh green vegetables for the inactivation of degradative enzymes, has proven useful in preventing the loss of green colour during processing or storage (Koca *et al*., 2006; Ruiz-Ojeda & Peñas, 2013). This considered, steam treatment insufficient for the denaturation of oxidative enzymes may promote the activity of these enzymes by affecting cell wall integrity and instantly providing the optimal temperature and moisture necessary for accelerated reaction rates. Loss of green colour as a result of chlorophyll breakdown by chlorophyllase or darkening due to polyphenolic oxidation is desired for the development of the brown colour of fermented honeybush, but is not appropriate in the processing of green honeybush. The increased yellow colour of infusions may also indicate brown pigment formation as a result of phenolic oxidation.

The colour of samples STBD for 60 s and longer appeared stable compared to the untreated samples, indicating successful enzymatic deactivation. Furthermore, steaming may increase cell wall permeability and removal of intercellular air, allowing faster moisture exchange and thus decreasing the time enzymes are provided with sufficient moisture to catalyse degradative reactions (Roca *et al*., 1993). Faster drying rates thus decrease the degradation of compounds, such as

chlorophyll, as has been seen in the oven-drying of basil and sun-drying of grapes (Rocha *et al*., 1993; Mastrocola *et al*., 1988; Bottrill & Hawker, 1970).

Overall, STAD resulted in fewer observed changes in the tea material than for STBD, with maximum total colour difference (ΔE^*) values of less than 3 and no increase in TSS. STAD resulted in a gradual loss of green colour corresponding the progressive decrease in TC content. Plant material sourced for STAD produced fairly high initial a^* values with smaller green colour losses incurred during steam treatment when compared to STBD. This could either be as a result of green colour retention during treatment, or as a result of lower initial green colour levels. Nevertheless these treatments resulted in poor quality green colour with positive a^* values for steamed samples. Non-enzymatic degradation as a result of high temperature processing (Maillard reaction) may play a role, as has been observed for apples (Ibarz *et al*., 2000). Batch variation tended to be more notable than for STBD. Furthermore, moisture penetration during steaming may have been restricted through the dry, hardened material. Inherent moisture is expected to swell the plant matrix and evaporate within plant cells, conducting heat and leading to enzymatic inactivation or possible conformational changes in the membrane. The lack of moisture present within the plant structure may thus lead to lessened effects of the heat of steaming.

4.3 Effect of steam treatment on sensory profile, colour and phenolic content of green *C. longifolia*, in comparison to that of green *C. maculata*

Overall, *C. longifolia* followed similar trends to those observed for *C. maculata*, except for the anomaly observed for the 30 s STBD treatment of *C. maculata*. In addition, *C. longifolia* followed more gradual changes and was less affected by steam treatment in terms of phenolic content and sensory profile. The intensity of aroma and flavour attributes was also generally lower for infusions of *C. longifolia*, especially with regard to the development of ‘guava’ aroma during steam treatment. To date, no investigation has been conducted to investigate the volatiles present in green *C. longifolia* infusions. Based on the DSA results of the current study, however, it may be speculated that this species could have low levels of odour-active aroma compounds present in the leaves.

The total colour difference for *C. longifolia* material was higher at the maximum steaming periods than observed for *C. maculata* subjected to the same treatment. This may indicate that *C. longifolia* plant material is less stable during steam processing than *C. maculata.* This could be due to the difference in leaf shape, where the flatter leaf surface of *C. longifolia* material with a higher surface area-to-volume ratio would enhance exposure of the plant matrix to heat. This may cause heat-induced non-enzymatic degradation after enzymatic de-activation in the early stages of steaming and possible rapid loss of volatiles. It would also explain the effective enzyme-deactivating steam treatment of *C. longifolia* at 30 s STBD compared to the 30 s anomaly of *C. maculata*. Despite the overall greater green colour losses, *C. longifolia* samples still had a convincingly greener colour than *C. maculata*. Further consequences of leaf size and shape variation of *Cyclopia* spp. were

demonstrated by Du Toit and Joubert (1998). They found that the drying rates of *C. genistoides* (thin needle-like leaves, similar to that of *C. maculata*) were slower than for *C. intermedia* (flat, elongated leaves, similar to *C. longifolia*). It could be speculated that factors such as internal structure, porosity and compositional differences in soluble materials (McCormick, 1979) affect internal heat and mass transfer during steaming and drying of *C. maculata* and *C. longifolia* material. This may result in different extents to which steam impact the quality parameters of the two species.

4.4 Chemical composition and sensory profile of green honeybush

As previously confirmed by GC-MS (Cronje, 2010; Le Roux *et al*., 2008), volatile components present in unfermented and fermented honeybush tend to be affected quantitatively rather than qualitatively by fermentation. Since volatile compounds are responsible for aroma and flavour, this effect is carried over to the sensory profiles of the teas. The majority of *vegetative* attributes of infusions of the plant material are associated with volatile compounds of polyunsaturated fatty acid origin such as 6-C aldehydes and consequent alcohols, *e.g.* hexanal and hexenol (Wu & Liou, 1986). These volatiles have been found in higher concentrations in unfermented honeybush than fermented honeybush (Cronje, 2010; Le Roux *et al*., 2008). They are formed in a variety of fresh plant materials upon tissue disruption (such as cutting of the leaves in the case of honeybush) by enzymes of the lipoxygenase (LOX) and hydroperoxide lyase (HPOL) oxidative pathways (Wu & Liou 1986; Azcarate & Baringer 2010). For example, linoleic and linolenic acids are converted to the aldehydes, hexanal and hexenal respectively, followed by conversion to their respective alcohols, hexanol and hexenol (Luning *et al*., 1995; Azcarate & Baringer, 2010), with their typical ‘green’, ‘herbaceous’ aroma attributes (Sigma Aldrich, 2011). Fermentation of honeybush lowers concentrations of these compounds (Cronje, 2010; Le Roux *et al*., 2008), minimizing the *vegetative* aroma and flavour associated with under-fermented honeybush (Bergh, 2014; Erasmus, 2015). Green honeybush is thus expected to retain, to some extent, these aldehyde or alcohol compounds, as found in the present study, with most of these prominently occurring compounds associated with ‘green’ or ‘herbaceous’ attributes (**Table 3.5**, **Fig. 3.11**).

Furthermore, two compounds identified by Cronje (2010) as odour-active in unfermented *C. intermedia* honeybush infusions, namely, 6-methyl-5-heptene-2-one and γ-terpinene, were detected in the present study by GC-MS. These compounds are described in literature as ‘oily, herbaceous, grassy, green’ and ‘herbaceous, citrus’ (Sigma Aldrich, 2011). Many of the volatiles detected in the infusions of unfermented *C. maculata* were also previously identified in fermented *C. maculata* (Theron *et al*., 2014), unfermented *C. intermedia* (Cronje, 2010) and unfermented *C. genistoides* (Le Roux *et al*., 2008).

Foods with substantial levels of polyphenolic substances are expected to be bitter or astringent due to the effect these compounds have on bitter receptors and saliva proteins in the mouth (Peleg *et al*., 1999; Peleg & Noble, 1995; Rosetti *et al*., 2009). High levels of phenolic compounds have been detected in infusions of fermented honeybush prepared from a number of

Cyclopia spp. (Schulze *et al*., 2015). Theron (2012) and Erasmus (2015) presented correlations linking high mangiferin content to bitter taste and astringent mouthfeel. Intense bitter taste (maximum of 26) was observed for some samples, specifically under-fermented samples of *C. genistoides* and *C. longifolia,* which also displayed the highest mangiferin content (Erasmus, 2015). Increases in these attributes of green honeybush infusions in the current study (to an observed maximum of 38 for STAD *C. longifolia*) are thus expected. This is due to the unfermented product typically containing higher concentrations of phenolic compounds than fermented honeybush, as demonstrated for hot water extracts (Joubert *et al*., 2008b; De Beer *et al.,* 2012; Schulze *et al*., 2014). Experimental data on polyphenolic composition in this study, however, were obtained from aqueous-organic extracts to indicate changes in the plant material and not the DSA infusion. This prevents correlations to be made with sensory data and links are purely speculative, based on the findings of previous investigations.

5. Conclusions

Overall, green honeybush with a modified aroma and flavour profile was produced using steam treatments before or after drying. Steam treatment not only had a positive effect on the aroma and flavour, but it had little effect on green colour and the content of the major phenolic compounds. STBD was shown to significantly decrease *vegetative* ('green grass' and 'hay/dried grass') and *cereal* ('oats/porridge/grains') sensory attributes, unmasking *fruity* ('guava' and 'tropical fruit') attributes after steaming of at least 60 s. The effect of STAD was less prominent than for STBD, so that the latter treatment is recommended for the manipulation of the sensory profile of green honeybush. STAD, however, may still be considered a processing option to ensure adequate microbial quality of the material, as it could also have a positive effect on the sensory profile of green honeybush. Furthermore, although both species produced green honeybush of acceptable quality, *C. maculata* seems to be more responsive to steam treatment and may produce a superior *fruity* sensory profile when steamed. This indicated that other *Cyclopia* spp. should also be investigated before blanket recommendations for *Cyclopia* can be made.

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Chapter 4

The effect of storage on the sensory profile, green colour and phenolic content of steam-treated and untreated green honeybush (C. maculata)

Contents

Abstract

The stability of steam-treated green honeybush (*Cyclopia maculata*) was assessed over a storage period of 6 months. The sensory profile of the infusions was assessed in terms of aroma, flavour, taste and mouthfeel using descriptive sensory analysis. High-performance liquid chromatography analysis was employed to quantify the individual phenolic compound content of the infusions, while colour of both the infusions and the plant material was defined in terms of the CIE*L*a*b** colour space. Samples of shredded, fresh plant material, steam-treated for 60 s before drying, were maintained under low temperature (moisture impermeable pouches at 0 °C) and normal temperature (semi-moisture permeable sachets, 25 °C at 65% relative humidity (RH)) storage conditions extending to 6 months, and high temperature (semi-moisture permeable sachets, 40 °C at 75% RH) storage conditions for a period of 1 month. Unsteamed, dried plant material samples were stored under the same conditions for comparison. Low temperature storage conditions did not greatly alter product quality over the 6 month storage period. Exposure of green *C. maculata* to high temperature and relative humidity storage conditions for 1 month produced results similar to accumulated changes in product quality during normal temperature and lower relative humidity storage over 6 months. Storage resulted in significant losses in green colour, especially in steamed samples. Intensities of *fruity* and *sweet-associated* sensory attributes increased progressively during storage, while the opposite was observed for *vegetative* and *cereal* attributes. These changes manifested to a greater extent in unsteamed samples. Changes incurred over time were slight with regard to individual phenolic content of the infusions. Storage of three to six months may thus result in a more appealing sensory profile and enhanced product quality, despite loss of green colour. The latter may not be a major factor in consumer preference if the product is to be packaged in tea bags rather than sold as loose tea, a product where colour is regarded as an important quality parameter.

1. Introduction

The South African honeybush industry shows vast potential. Its current growth requires defined quality parameters as well as knowledge of the effect of processing and storage on these parameters to ensure the promotion of acceptable products to both local and international consumers. One of the honeybush products showing much potential is green honeybush, although this product still possesses a minor market share (Joubert et al., 2011). The superior bioactive content of green honeybush has been established (Joubert *et al*., 2008) allowing access to markets more aware of potential health benefits of herbal tea products, while promoting local produce and economic opportunities.

Product shelf-life is important for the acceptability of food products with regard to food safety as well as sensory and nutritive quality. Shelf-life periods are determined under appropriate packaging and storage environments for the preservation of product quality and are typically affected by temperature as well as humidity. The South African climate varies. According to ICH guidelines for stability testing of pharmaceutical products, South Africa is categorised as a Mediterranean and sub-tropical climate (with mean annual temperatures of 15 °C to 22 °C and mean annual partial water vapour pressure of 11 to 18 hPa) designated as climatic zone II (Q1 Scientific, 2015). With the variation in climate, adverse conditions are commonly met, with high humidity and temperatures occurring in some areas of honeybush production.

Honeybush herbal tea may be bulk packaged and remain on the factory site for several weeks before retail packaging or transportation to packaging facilities or depots from where the product is shipped for export (Joubert, E., 2015, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, personal communication, 03 August). Transportation to facilities or depots by trucks and further transportation to export markets by sea does not employ temperature controlled conditions, and the tea may thus be exposed to high temperature and humidity. It is, therefore, of importance to understand the effect of storage conditions on the quality of honeybush in order to deliver a product with a high market value and quality. This is especially true for green honeybush, which may be more susceptible to degradation and changes in quality. Honeybush is commonly packaged in bulk, with tea stored in sacks or weaved polypropylene bags, weighed out in the required quantity at the point of sale at small farm stalls or traders. High-end packaging may consist of individually wrapped tea bags, presented to the customer in tins or cardboard boxes covered in plastic wrapping. Tea stored in sub-optimal packaging before or after purchase may experience accelerated quality degradation. This may be due to light exposure, release of volatile aroma compounds and/or moisture absorption leading to undesirable chemical changes as well as microbial and/or pest contamination.

As described in the previous chapter, steaming may be used as a processing technique to manipulate the sensory profile of green honeybush. Steaming decreases the intensities of *vegetative* aroma and flavour attributes and increases that of some *fruity* and *sweet-associated* aroma and flavour attributes, whilst retaining individual phenolic compounds and green colour. Current knowledge on the quality parameters of green honeybush after production as affected by processing

techniques and storage conditions, however, is lacking. In order to assess changes during storage, the more 'unstable' species from the investigation in Chapter 3, *Cyclopia maculata*, was selected for the present study. The rationale is that optimal storage conditions determined for this species should be adequate for the more 'stable' species, *C. longifolia*. Furthermore, the 60 s steam treatment before drying was identified (Chapter 3) as the shortest effective steam treatment for significant decrease of *vegetative* and *cereal* attribute intensities, whilst retaining green colour and individual phenolic content. The aim of the present study was therefore to determine the effect of long-term (normal temperature) and accelerated (high temperature) climatic storage conditions of climatic zone II on the aroma, flavour, colour and phenolic stability of green honeybush (*C. maculata*). For the purpose of the study, plant material, steam-treated before drying, was compared to unsteamed, dry plant material. Packaging consisted of semi-moisture permeable sachets for storage under normal and high temperature storage conditions. Low temperature storage was also included with the plant material packed in impermeable metalised pouches and stored under refrigerated conditions for comparison.

2. Materials and methods

2.1 Materials

Shoots of *C. maculata* seedling plants were harvested from a commercial plantation on 3 August 2014 on the Koksrivier farm (F. Joubert) situated in the Pearly Beach area of the Overberg region in the Western Cape Province of South Africa. The plant material was grouped in batches with shoots from several plants constituting a batch, but with shoots from one plant not present in more than one batch. The plant material was stored in a cold room at 0 °C and processed within three days. All chemicals were sourced and prepared as described in Chapter 3.

2.2 Experimental method

Four batches (± 18 – 20 kg/batch) of plant material were trimmed and shredded, steam-treated and dried as described previously (Chapter 3). The shredded, fresh plant material was steamed for 60 s and dried immediately at 40 °C, as outlined in **Fig. 4.1**. Unsteamed samples were also included for comparison. Each treatment of each batch of plant material was sieved to obtain the herbal tea fraction (< 12, > 40 mesh). This fraction was then sub-divided into 12.5 g sub-sample units and stored in individual heat-sealed plastic coated aluminium metal laminated pouches (polyethylene terephthalate 12 µm (PET)/aluminium 7 µm (AL)/linear low density polyethylene 100 µm (PE), impermeable to moisture, light and oxygen), or in 30 µm bi-axially orientated polypropylene (BOPP) plastic sachets (76 x 32 x 216 mm, water vapour permeability at 23 °C/85% relative humidity (RH) = 0.9 g/(m^2d) and at 38 °C/90% RH = 4.6 g/(m^2d) ± 10%), used for low cost retail packaging of tea. The plastic coated aluminium metal laminated pouches were stored in a temperature-controlled cold room at > 0 °C for low temperature storage (**LTS**) conditions. The plastic sachets were stored at 25 °C/60% RH for normal temperature storage (**NTS**) conditions, or 40 °C/75% RH for high

temperature storage (**HTS**) conditions according to ICH guidelines for stability assessments of pharmaceuticals at long term and accelerated storage conditions, respectively (Q1 Scientific, 2015). The sachets were hung from racks in climatic storage chambers (Scientific Manufacturing cc., Cape Town, South Africa). The view window of the chambers were blackened out. Samples stored under HTS conditions were tested only at 0 and 1 months.

2.3 Sample preparation

All samples were prepared as triplicate infusions (for sensory analysis, individual phenolic quantification and colour determination) using three individual sub-sample sachet units. One individual sub-sample sachet unit was subjected to analyses as unmilled material (moisture content and colour measurements), and another prepared and analysed as milled plant material (total chlorophyll (TC) content determinations) as described in Chapter 3.

2.4 Instrumental and sensory analyses

Instrumental analysis, including determination of individual phenolic content (by high-performance liquid chromatography coupled to diode-array detectors (HPLC-DAD)), TC content, total soluble solids (TSS) content, moisture content and objective colour parameters (CIE*L*a*b**) of unmilled plant material and infusions were performed as described in Chapter 3. HPLC-DAD was performed on all the infusion aliquots at the end of the 6 month storage period. Following defrosting of infusion aliquots, 100 uL 10% (m/v) ascorbic acid solution was added to each aliquot (1 mL) to prevent oxidation of phenolic compounds during analysis. Each mixture was filtered through a 0.22 µm pore (33 mm diameter) Millipore-PVDF syringe filter (Merck Millipore; Darmstadt, Germany) before duplicate injection.

Descriptive sensory analysis (DSA) of samples was undertaken at time periods 0 and 1 month (four batches, six treatments, including the HTS samples) as well as at time periods 3 and 6 (four batches, four treatments). No HTS samples were included in the latter analyses as the high humidity and temperature of the HTS conditions favoured moulding and storage was terminated after 1 month. Samples displaying visual microbial degradation were discarded and DSA was limited to aroma analysis only. Panel training took place before each time period. At time 0, training included four sessions of 30 min each with six samples analysed per session. Panel training at 1 month entailed analysis in terms of aroma only and training was conducted in three 30 min sessions on the same day. Testing for both time periods 0 and 1 month included six samples in triplicate across three 30 min sessions per day over four days. Training for time periods 3 and 6 months was conducted over two days in four 30 min sessions with four samples per session. Testing proceeded over four days, in three sessions per day, to analyse four samples a day in triplicate (one repeat set per session).

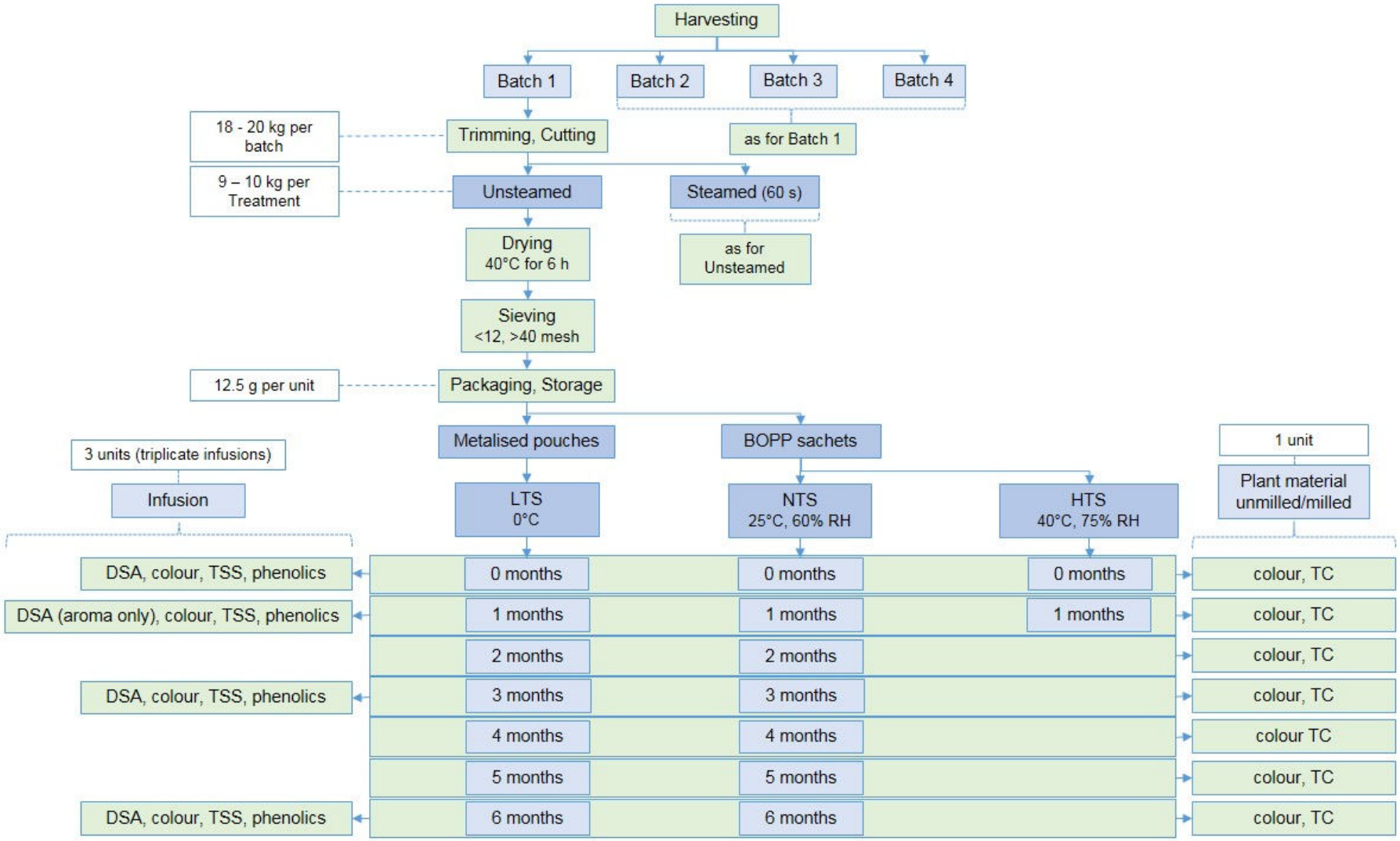


Figure 4.1 Experimental process to evaluate the effects of steam treatment before drying on the sensory, colour and phenolic stability of green *C. maculata* during storage.

LTS = low temperature storage (0 °C), NTS = normal temperature storage (25 °C, 60%RH), HTS = high temperature storage (40 °C, 75%RH), DSA = descriptive sensory analysis, TSS = total soluble solids, TC = total chlorophyll.

2.5 Statistical procedures

Analysis of variance (ANOVA) was performed on all variables obtained with instrumental analysis as described in Chapter 3. ANOVA was conducted across all time periods for LTS and NTS samples. Additionally, data for LTS, NTS and HTS samples were analysed at 0 and 1 months. Sensory data was analysed per separate time period as described in Chapter 3, as panel drift over the six month period was considered likely with no anchoring standard provided other than the samples stored under LTS conditions.

Various methods of data representation were used including principal component analysis (PCA) and discriminant analysis (DA). For the latter method forward stepwise model selection was used on DSA and instrumental data in order to further interpret treatment effects. Supplementary results referred to in this chapter are provided in **ADDENDUM B**.

3. Results

3.1 Effect of steam treatment and storage conditions on overall quality of green *C. maculata*

PCA was used on a combination of DSA and instrumental data for all samples excluding LTS, NTS and HTS samples stored for 1 month that were tested for aroma only. Flavour correlated closely to corresponding aroma attributes (results not shown). Thus, PCA was done again on the full set of DSA results excluding flavour as it allowed inclusion of all samples. The PCA scores and loadings plots of the combined data analysis are depicted in **Fig. 4.2**. Pearson's (n-1) correlations for individual phenolic compounds, moisture content, TC content and objective colour parameters of the plant material, and TSS content and objective colour parameters of the infusions are summarised in **Tables B.1**, **B.2** and **B.3** (**ADDENDUM B**).

A clear discrimination between steamed and unsteamed samples is evident from the PCA scores plot (**Fig. 4.2 a**), with both groups progressively migrating to the lower left quadrant with increasing storage time under NTS and HTS conditions (as indicated by the purple arrows). The unsteamed LTS samples (coded U_0) grouped near the top of the PCA scores plot in the upper left quadrant, separated along the first principal component (F1, 43.06%) from the corresponding steamed LTS samples (coded S_0) on the far right of the plot. All of the steamed LTS samples are placed in the lower right quadrant (except for the t = 3 month storage samples, 3S_0). For both steamed and unsteamed samples, the HTS samples (1 month storage, coded 1U_40 and 1S_40) and NTS samples (6 month storage, coded 6U_25 and 6S_25) are placed close together in the lower left quadrant.

The corresponding PCA loadings plot (**Fig. 4.2 b**) indicates that *vegetative* and *cereal* aroma attributes grouped in the top section of the plot. These thus associated with unsteamed LTS samples as well as NTS and HTS samples stored for 0 months, and NTS samples stored for 1 month. 'Cooked vegetables', 'tropical fruit' and 'guava' aroma attributes positioned toward the right of the plot,

associating with the steam-treated samples of similar storage times and conditions. 'Caramel' aroma associated with unsteamed samples on the left of the PCA loadings plot. *Fruity* and *sweet-associated* aroma attributes positioned in the lower left quadrant, associating with NTS samples stored for 3 and 6 months as well as HTS samples stored for 1 month.

The individual phenolic compounds grouped together in the lower far right side of the plot (**Fig. 4.2 b**), associating with steamed LTS samples. Hesperidin, however, was positioned in the upper left quadrant associating with unsteamed samples. TSS content is also located with the majority of the individual phenolic compounds in the lower right quadrant, indicating moderate to strong positive correlations ($0.650 \leq r \leq 0.908$) with all quantified phenolic compounds except hesperidin. In this case, a moderate negative correlation ($r = -0.721$) was observed between hesperidin content and TSS content (**ADDENDUM B**, **Table B.1**). Furthermore, considering the PCA of all measured DSA attributes (aroma, flavour, taste and mouthfeel) and instrumental parameters for time periods 0, 3 and 6 months (results not shown), significantly strong correlations ($p < 0.05$, $r \geq 0.761$) were identified between some individual phenolic compounds (mangiferin, isomangiferin, iriflophenone-3-*C*-glucoside, eriocitrin and maclurin-3-*C*-glucoside) and astringent mouthfeel.

The colour parameters (a^* and b^*) and chroma (C) of the plant material and total colour difference (ΔE^*) of the plant material and infusions positioned in the lower left quadrant, associating with HTS (t = 1 month) and NTS (t = 6 months) samples (**Fig. 4.2 b**). Total colour difference values and green colour (a^*) of the plant material correlated significantly ($r = 0.989$; $p < 0.05$) (**ADDENDUM B**, **Table B.2**). The green colour parameter of the infusion (Inf_a^*) is positioned in the top left quadrant, associating with unsteamed LTS samples. Hue (h) values of the infusions and plant material correlated negatively ($p < 0.05$) with corresponding a^* values ($r = -0.989$ and -0.998 for the plant material and the infusion, respectively), while b^* and C values also correlated ($p < 0.05$) positively ($r = 0.962$ and 1.000, for the plant material and the infusion, respectively) (**ADDENDUM B**, **Tables B.2** & **B.3**). These parameters are located to the left of the plot, thus associating with unsteamed samples (**Fig. 4.2**). Furthermore, TC content is located in the upper right quadrant, associating with LTS and t = 0 samples. A significant strong negative correlation ($r = -0.957$) (**ADDENDUM B**, **Table B.2**) between TC content and a^* of plant material was established as a higher TC value indicates greener plant material and thus a lower, or more negative a^* value. Moisture content is located in the lower left quadrant, associating with HTS samples (high RH conditions; greater permeability to moisture) or longer storage periods of NTS samples; both these groups of samples were stored in sachets with some permeability to moisture. Moisture also displayed a strong negative correlation ($r = -0.829$) with TC content (**ADDENDUM B**, **Table B.2**).

3.2 Effect of steam treatment and storage conditions on aroma, flavour, taste and mouthfeel of green *C. maculata* after 0, 1, 3 and 6 months of storage

The intensities of the aroma, flavour, taste and mouthfeel attributes were determined for all samples, except for samples stored for 1 month, evaluated for aroma only. LTS and NTS samples were analysed at time points t = 0, 1, 3 and 6 months. HTS was terminated after 1 month of storage due to rapid deterioration of the samples (as indicated by the presence of moulding on some samples).

The PCA scores and loadings plots of the aroma data of all samples, including all time periods, are presented in **Fig. 4.3**. Flavour, taste and mouthfeel attributes are similarly presented in **Fig. B.1** (**ADDENDUM B**). **Figure 4.3** indicates the primary positioning along the first principal component (F1, 49.59%) based on the storage period and the severity of the storage conditions. All LTS samples (t = 0, 1, 3 and 6 months), all samples analysed at t = 0, and NTS samples at t = 1 month positioned to the left of the PCA scores plot with the remaining samples at more severe storage conditions and longer storage times to the right. Positioning along the second principal component (F2, 24.98%) indicates grouping of steamed samples above corresponding unsteamed samples, although not always across the axis. *Vegetative* ('cooked vegetables, 'green grass' and 'hay/dried grass'), 'oats/porridge/grains' and 'tropical fruit' aroma attributes are positioned to the left, while *fruity* and *sweet-associated* aroma attributes are positioned to the right on the PCA loadings plot. The latter attributes associated with NTS (t = 3 and 6 months) and HTS (t = 1 month) samples. Aroma attributes 'cooked vegetables', 'tropical fruit', 'guava' and 'fruity-sweet' are grouped at the top of the PCA loadings plot, associating to a greater degree with steamed samples, especially those stored for t = 0 and 1 month, and LTS conditions. The PCA for flavour, taste and mouthfeel attributes (**ADDENDUM B**, **Fig. B.1**) presents similar patterns of association, while bitter and sweet taste associated with samples stored for t = 6 months, and sour taste and astringent mouthfeel associated with samples stored for t = 0 and 3 months, especially steamed samples.

DA was used on the sensory data per storage time period and the results are presented in **Figs. 4.4**, **4.5**, **4.6** and **4.7**. DA plots for all DSA data obtained at t = 0 (**Fig. 4.4**) indicate complete (100%) discrimination between steamed and unsteamed samples along F1. The DA variables loadings plot indicates grouping of attributes similar to that observed in the previous chapter (Chapter 3), with 'green grass', 'hay/dried grass' and 'oats/porridge/grains' aromas as well as 'caramel' aroma associating with unsteamed samples. On the other end of the spectrum, 'guava', 'tropical fruit', 'fruity-sweet' and 'cooked vegetables' associated with steamed samples. 'Tropical fruit' flavour, 'general sweet' aroma and 'apricot jam' aroma as well as sour taste, bitter taste and astringent mouthfeel were grouped slightly to the left or right of the middle, while sweet taste was placed in the middle of the plot. This indicates that these variables had little effect on the discrimination between steamed and unsteamed samples, and 'caramel' aroma was identified as the only attribute necessary for classification.

DA plots of DSA data of aroma attributes analysed at t = 1 (**Fig. 4.5 a**) discriminate between HTS samples (to the right) and LTS and NTS samples (to the left) along F1 (86.33%). Secondary positioning (F2, 9.79%) discriminated between unsteamed samples (at the top) and steamed samples (at the bottom). The DA variables loadings plot (**Fig. 4.5 b**) indicated grouping similar to that of the PCA (**Fig. 4.3**), with *vegetative* and *cereal* aroma attributes grouped to the left with LTS and NTS samples, and *fruity* and *sweet-associated* aroma attributes grouped to the right with HTS samples. Four attributes, *i.e.* 'caramel' and 'apricot jam' (upper right quadrant), 'marmalade' (lower right quadrant) and 'green grass' (upper left quadrant) aromas, were identified as variables necessary to achieve classification.

DA of DSA data at t = 3 and 6 months (**Figs. 4.6** & **4.7**) were similar and indicated primary discrimination between LTS and NTS samples (F1, 86.11% and 88.86%, respectively), with secondary positioning between steamed (bottom) and unsteamed (top) sample groups (F2, 12.50% and 8.76%, respectively). In spite of no DSA data for the HTS samples at t = 3 and 6 months, the DA variables loadings plots were similar to previous DA plots that included data of the HTS samples, with NTS samples displaying similar associations as were seen for HTS samples (**Figs. 4.3** & **4.5**). V*egetative* and *cereal* attributes associated with unsteamed LTS samples, except for 'cooked vegetables' that associated with steamed LTS samples. *Fruity* and *sweet-associated* attributes associated with samples stored at NTS conditions. The 'guava' attribute associated with steamed samples and 'marmalade' with unsteamed samples. 'Green grass', 'caramel', 'marmalade' and 'apricot jam' aromas were selected as the main variables for discrimination at t = 3 months, with 'marmalade', 'apricot jam', 'oats/porridge/grains' and 'hay/dried grass' aromas selected for discrimination at t = 6 months.

Spider plots of the DSA aroma profiles of samples at LTS conditions (t = 0 months), NTS (t = 6 months) and HTS (t = 1 month) provide another visual overview of the main differences between samples as a result of treatment and storage conditions (**Fig. 4.8**). This representation clearly illustrates the shift from the dominant *vegetative* and *cereal* aroma in the initial profile of LTS (t = 0), to the development of *fruity* and *sweet-associated* attributes in NTS (t = 6 months) and HTS (t = 1 month) samples. It is also visually apparent that storage resulted in similar trends for both steamed and unsteamed samples under NTS and HTS conditions, although intensities of 'stewed fruit', 'marmalade', 'apricot jam', 'general sweet' and 'fruity-sweet' aroma appear more intense in unsteamed samples. Furthermore, the similarity between the aroma profiles of HTS samples after 1 month and NTS samples after 6 months is clearly depicted.

Analysis of variance (ANOVA) was conducted on the data per time period, allowing identification of significant ($p < 0.05$) differences between samples at each time period. These results are summarised for aroma attributes in **Fig. 4.9**, with the attributes for taste and mouthfeel depicted in **Fig. 4.10**. Tables with main effect and interaction p-values are presented in **Tables B.4** – **B.7** (**ADDENDUM B**). Mean values, standard deviations and significant differences for each attribute (steam treatment x storage condition interactions) per time period are provided in **Tables B.8** – **B.17**

(**ADDENDUM B**). Overall, flavour attributes were detected at lower intensities than corresponding aroma attributes, although similar trends were observed. For this reason and since DSA aroma data were available for t = 0, 1, 3 and 6 months, reference will be made to the aroma attributes only. Changes in flavour are depicted in **Fig. B.2** (**ADDENDUM B**). At t = 0 months, storage did not yet have any effect on attribute intensities and steaming was the only significant ($p < 0.05$) main effect (**ADDENDUM B**, **Table B.4**). Similar to results of the previous chapter (Chapter 3), and as indicated by DA (**Fig. 4.4**), *vegetative* and *cereal* attribute intensities were significantly ($p < 0.05$) higher in unsteamed samples, except for the intensity of 'cooked vegetables' which was higher in steamed samples. The intensities of 'tropical fruit', 'guava' and 'fruity-sweet' were significantly ($p < 0.05$) higher in steamed samples than unsteamed samples, while those of 'apricot jam' and 'caramel' were significantly ($p < 0.05$) higher in unsteamed samples at t = 0 months. The intensity of 'general sweet' aroma was not greatly affected by steam treatment (**ADDENDUM B**, **Tables B.4** & **B.8**). The 'stewed fruit' and 'marmalade' intensities were extremely low (< 5). Bitter taste, astringent mouthfeel and sour taste were slightly, albeit significantly ($p < 0.05$) higher in steamed samples and sweet taste was not affected ($p \geq 0.05$).

At t = 1 month similar trends to t = 0 were indicated for steamed and unsteamed LTS and NTS samples, with the main differences indicated for HTS samples. HTS samples displayed significantly ($p < 0.05$) lower intensities for *vegetative* and *cereal* aroma attributes, while the intensities of *fruity* and *sweet-associated* aroma attributes, except 'guava' and 'caramel' were notably higher than the other samples. 'Caramel' aroma was highest for unsteamed HTS samples, followed by unsteamed NTS samples. Comparing just the HTS samples (steamed *vs.* unsteamed) it is clear that the unsteamed samples produced significantly ($p < 0.05$) higher intensities for 'stewed fruit', 'apricot jam', 'general sweet' and 'fruity-sweet' aromas than the steamed samples. The opposite was again observed for 'tropical fruit' and 'marmalade'.

LTS samples at t = 3 months had significantly ($p < 0.05$) higher intensities for *vegetative* and *cereal* attributes, with the intensities of 'green grass', 'hay/dried grass' and 'oats/porridge/grains' higher in unsteamed samples and 'cooked vegetables' intensity higher in steamed samples. 'Green grass' aroma of NTS samples decreased to intensities below 10. The intensities of *fruity* and *sweet-associated* attributes seemed to increase as a result of NTS conditions, with the exception of 'tropical fruit', for which intensities decreased to below 5. The intensities of 'stewed fruit', 'apricot jam', 'marmalade' and 'guava' attributes of NTS samples were significantly ($p < 0.05$) higher than in LTS samples, with those of unsteamed NTS samples at slightly higher intensities than those of steamed NTS samples (except for 'guava'). The intensities of 'general sweet' and 'fruity-sweet' aroma attributes were significantly ($p < 0.05$) higher in NTS than LTS samples. 'Caramel' aroma, however, did not show any drastic increase in intensity, but was significantly ($p < 0.05$) higher for NTS samples than for steamed LTS samples. Differences in taste and mouthfeel were small and not significant ($p \geq 0.05$), except for sweet taste, being slightly higher in NTS than LTS samples.

Results for samples stored for 6 months were similar to those stored for 3 months, except that differences between samples at t = 6 months were more pronounced in many cases. Once again, *vegetative* and *cereal* attributes were significantly ($p < 0.05$) lower for NTS samples, with 'green grass' and 'oats/porridge/grains' aroma intensities decreasing to below 10. 'Cooked vegetables' intensities were lower for unsteamed samples, especially NTS samples. Unsteamed NTS samples scored overall maximum intensities of *ca.* 35, 40, 50 and 25 for 'stewed fruit', 'marmalade', 'apricot jam' and 'guava' aromas, respectively. Similar increases were observed for 'general sweet' and 'fruity-sweet' aromas, reaching intensities of 37 and 45, respectively, in unsteamed NTS samples. 'Caramel' aroma remained higher in NTS samples than in LTS samples. Taste and mouthfeel attributes once again showed only small differences (< 5 units) (**ADDENDUM B**, **Tables B.7** & **B.16**), with NTS samples resulting in slightly higher sour taste scores and unsteamed NTS samples resulting in slightly lower bitter taste and astringent scores. Sweet taste, astringent mouthfeel and bitter taste were all affected by steaming.

At a glance it would seem that storage time affected the intensity of the attributes. It must, however, be stressed that stastistic comparison between time points were not possible as no reference standard that could serve as a fixed point to 're-calibrate' the panel members was available.

3.3 Effect of steam treatment and storage conditions on instrumental quality parameters of green *C. maculata* over 6 months of storage

PCA was used on the complete set of instrumental data (individual phenolics, colour, TC, TSS and moisture content) and is presented in **Fig. 4.11**. The PCA scores plot indicates positioning similar to that found in **Fig. 4.2** (sensory and instrumental data), with a clear grouping of unsteamed samples on the left and steamed samples on the right (with the exception of steamed HTS samples at t = 1 month), along the first principal component (F1, 52.93%). Again, there is a progressive movement from t = 0 months along the second principal component (F2, 28.83%) to t = 6 months for NTS and t = 1 months for HTS samples, positioned towards the bottom of the plot. The PCA loadings plot is also similar to that in **Fig. 4.2**. An additional PCA was used on only plant material and infusion colour parameters (results not shown) with PCA scores and loadings very similar to that of the PCA of all instrumental parameters (**Fig. 4.11**). This indicates the relevance of the colour parameters as drivers of discrimination between steam treatments and storage times and conditions.

Statistical analyses were conducted on samples from t = 0 and 1 month to compare HTS samples to NTS and LTS samples. Data for the LTS and NTS samples were also analysed for the entire storage period (t = 0 to 6 months). ANOVA was employed, and significant differences ($p < 0.05$) are indicated according to main effects and interactions (**ADDENDUM B**, **Tables B.18 – B.21**). Green colour (a^*) of plant material and infusions were affected by steam treatment x storage conditions x time interactions. Individual phenolic compound content of the infusions were affected to a lesser degree by interactions and more by main effects, especially time. Plant material analyses,

i.e. colour, and moisture and chlorophyll content after 1 month, was affected by both steam treatment and storage conditions x time interactions.

3.3.1 *Effect of steam treatment and storage conditions on green colour over 6 months of storage*

Colour analyses were performed on unmilled plant material as well as on infusions. The data collected over storage time is presented in **Figs. B.3** and **B.4** (**ADDENDUM B**). The Pearson's correlation values for the combined PCA (**Fig. 4.2**) indicated strong correlations between *a** and h for unmilled plant material (r = -0.989; **ADDENDUM B**, **Table B.2**) and the infusion (r = -0.998; **ADDENDUM B**, **Table B.3**). The change in green colour is thus the biggest contributor to changes in observed hue. Furthermore, the strong correlation between total colour change (ΔE^*) and green colour (*a**) (r = 0.989) of the unmilled plant material indicates the relevance of green colour as an indicator of visual colour change. ΔE^* reached very discernable levels of > 6 after 1 month for HTS and 6 months for steamed plant material at NTS (**ADDENDUM B**, **Fig. B.4**). The infusion colour parameters, however, did not indicate any strong correlations with ΔE^* which remained below 3 (**ADDENDUM B**, **Fig. B.4**). For these reasons, only changes in green colour (*a**) of the plant material and infusions are presented (**Fig. 4.12**).

The lack of correlation between the *a** values of the plant material and infusion (**Figs. 4.2** & **4.11**) indicates that these two parameters are not closely related. For the plant material, LTS conditions resulted in a convincing retention of the green colour, with NTS resulting in significant losses over the 6 month period (**Fig. 4.12**). This increase in *a** was more pronounced for steam-treated samples, compared to the unsteamed samples. HTS, although applied only for 1 month, resulted in approximately the same absolute change in *a** as NTS storage for 6 months. For infusions, unsteamed samples displayed consistent significantly ($p < 0.05$) higher *a** values than for steamed samples. The infusion colour of the steamed samples was not greatly affected by LTS and NTS over 6 months. In this case HTS of unsteamed samples resulted in a significantly ($p < 0.05$) greener appearance after 1 month, similar to that of unsteamed NTS samples after 6 months.

Furthermore, photographs of the unmilled plant material of steamed and unsteamed treatments at initial conditions (t = 0 months, LTS) and after completed storage at NTS (t = 6 months) and HTS (t = 1 month) conditions are provided in **Fig. 4.13**. It can be seen from the photographs that although colour differences are visually apparent, the differences between NTS and HTS samples and steamed and unsteamed samples were small. It can thus be said that both steamed and unsteamed samples stored under NTS and HTS conditions were of reasonably acceptable colour. The similarity in appearance between HTS (t = 1 month) and NTS (t = 6 months) is also clear.

The change in TC and moisture content of the plant material and the TSS content of the infusion as a result of storage of the plant material are presented in **Fig. B.5** (**ADDENDUM B**). Significant main effects and interactions are indicated in **Fig. 4.14**. Steam treatment decreased TC content, irrespective of storage conditions. LTS had little effect on TC content, but NTS after

6 months and HTS after 1 month resulted in significant ($p < 0.05$) and similar decreases in TC content. Moisture uptake during LTS was not significant ($p \geq 0.05$), but HTS resulted in an increased moisture content of > 10% after 1 month, causing microbial spoilage. The steamed samples had a slightly lower moisture content than unsteamed samples after 1 month storage. NTS samples experienced a gradual moisture uptake over 6 months, equilibrating at a moisture content of *ca.* 10% after 3 months. No visual microbial spoilage was detected. Differences in TSS of the samples were very small, but were consistently higher for steamed samples at all storage conditions. Differences between TSS content of steamed and unsteamed samples were smaller for HTS samples after 1 month than NTS samples after 6 months. Storage time had little effect on TSS, probably due to the relatively low sensitivity of measurement.

3.3.2 *Effect of storage environment and steam treatment on individual phenolic compounds over 6 months of storage*

Fig. 4.15 is a representation of the significant ($p < 0.05$) main effects and interactions observed for the phenolic compounds. The change in individual phenolic content during storage was small and is presented in **Fig. B.6** (**ADDENDUM B**). Steamed samples produced infusions containing consistently higher levels of the phenolic compounds than unsteamed samples, except for hesperidin. The infusions prepared from the plant material contained significantly ($p < 0.05$) higher mangiferin, isomangiferin, vicenin-2, eriocitrin, maclurin-3-*C*-glucoside, eriodictyol-*O*-glucoside and hesperidin content before storage. After 6 months of storage, however, the mangiferin, hesperidin and eriocitrin content of the infusions of the stored plant material did not differ from the initial content before storage. Iriflophenone-3-*C*-glucoside content remained relatively stable, except for a decrease after 6 months of storage at NTS conditions. Other small, but significant ($p < 0.05$) differences were also observed for the compounds when further effects of LTS and NTS were considered. The same general trends were observed for samples stored under HTS conditions for 1 month.

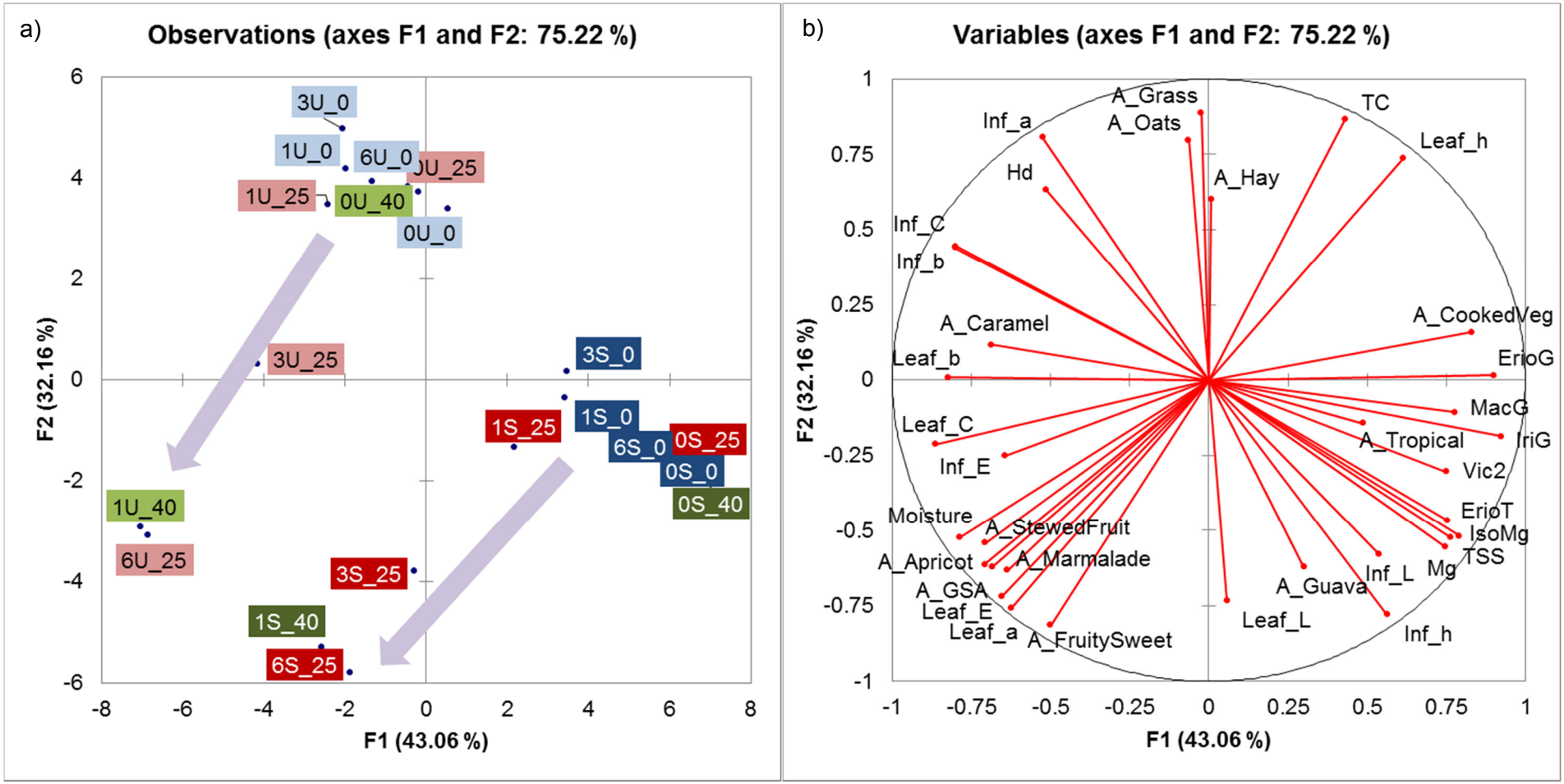


Figure 4.2 PCA (a) scores and (b) loadings plots of all instrumental and aroma attributes of steamed (S) and unsteamed (U) green *C. maculata*, stored under LTS (0), NTS (25) and HTS (40) conditions over the storage period of t = 0 to 6 months, n = 20.

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. PCA scores plot sample codes indicated as (month)(steam treatment)_(storage condition temperature). Light blue = unsteamed LTS, Dark blue = steamed LTS, Light pink = unsteamed NTS, Dark red = steamed NTS, Light green = unsteamed HTS, Dark green = steamed HTS. 'Mg' = mangiferin, 'IsoMg' = isomangiferin, 'Vic2' = vicenin-2, 'ErioT' = eriocitrin, 'IriG' = iriflophenone-3-*C*-glucoside, 'MacG' = maclurin-3-*C*-glucoside, 'ErioG' = eriodictyol-*O*-glucoside, 'Hd' = hesperidin, 'Inf' = infusion, 'Leaf' = plant material. 'L', 'a', 'b', 'C', 'h' and 'E' refer to objective colour parameters. 'TSS' = soluble solids, 'TC' = total chlorophyll content. 'A' prefix refers to aroma attributes. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet.

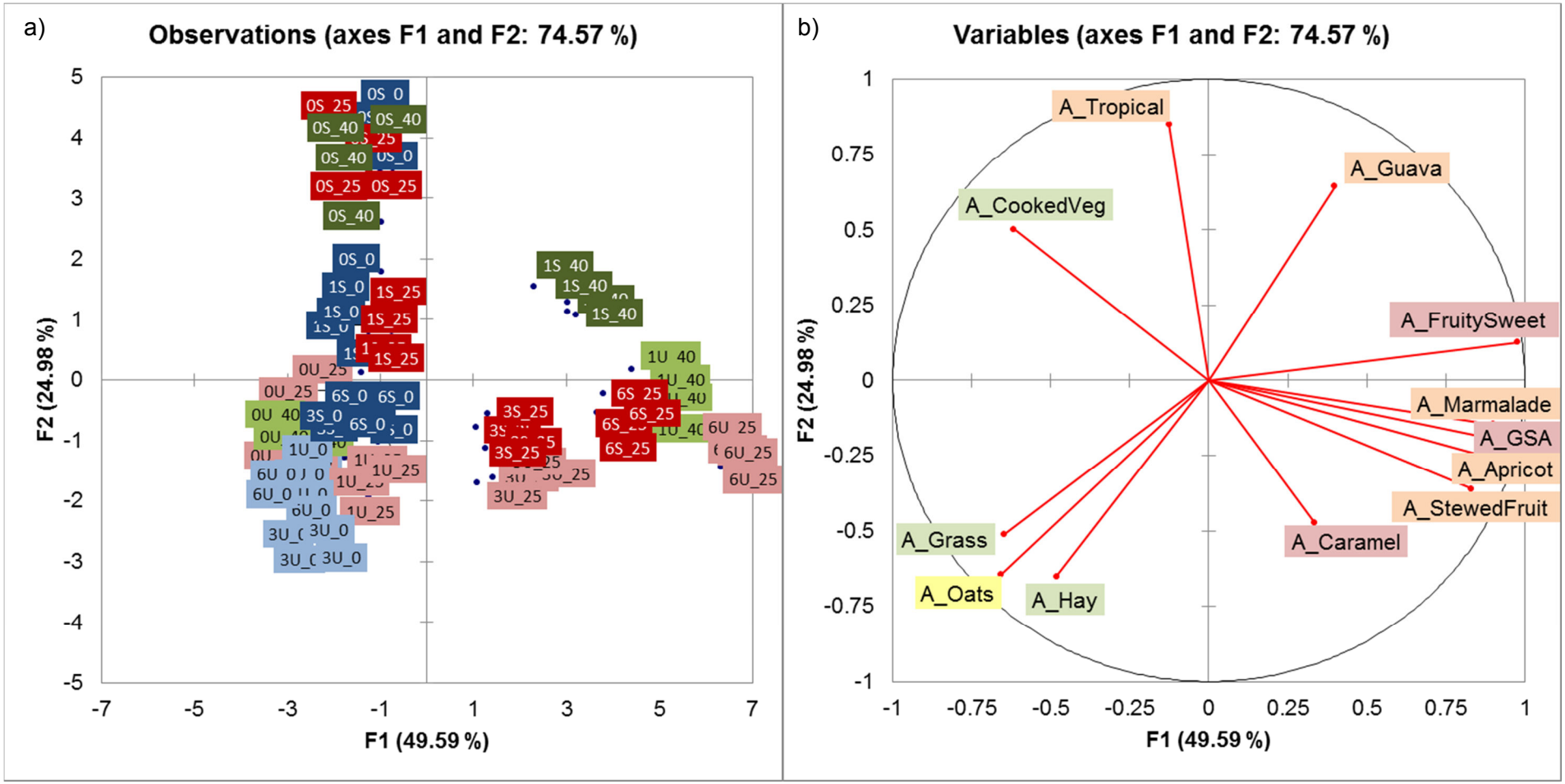


Figure 4.3 PCA (a) scores and (b) loadings plots of aroma attributes of steamed (S) and unsteamed (U) green *C. maculata*, stored under LTS (0), NTS (25) and HTS (40) conditions over the storage period of t = 0 to 6 months, n = 20.

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. PCA scores plot sample codes indicated as (month)(steam treatment)_(storage condition temperature). Light blue = unsteamed LTS, Dark blue = steamed LTS, Light pink = unsteamed NTS, Dark red = steamed NTS, Light green = unsteamed HTS, Dark green = steamed HTS. 'A' prefix refers to aroma attributes. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet.

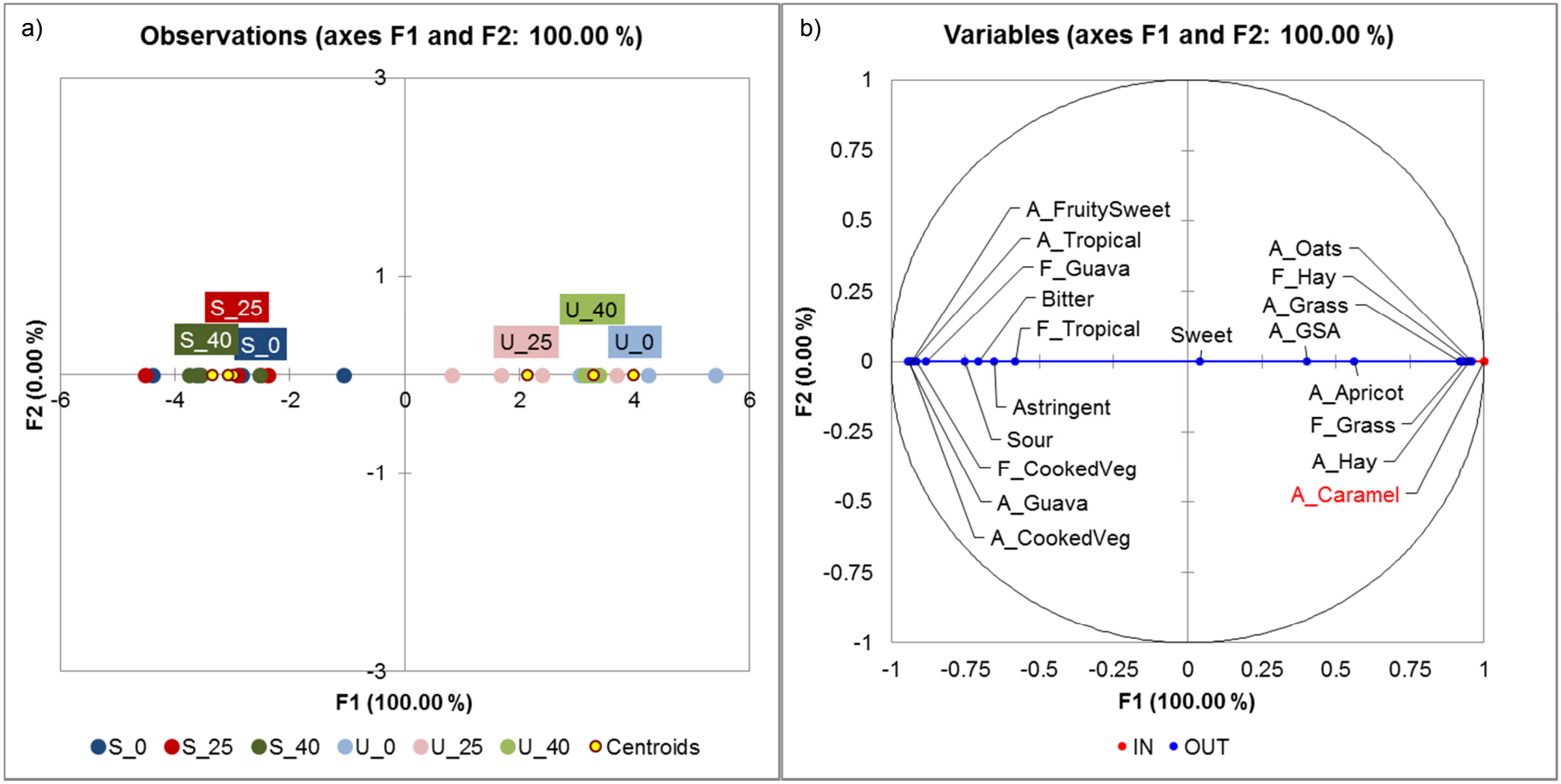


Figure 4.4 DA (a) observations and (b) variables loadings plots with aroma, flavour, taste and mouthfeel attributes of steamed (S) and unsteamed (U) green *C. maculata*, stored under LTS (0), NTS (25) and HTS (40) conditions for t = 0 months, n = 24.

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. DA observations plot sample codes indicated as (steam treatment)_(storage condition temperature). Light blue = unsteamed LTS, Dark blue = steamed LTS, Light pink = unsteamed NTS, Dark red = steamed NTS, Light green = unsteamed HTS, Dark green = steamed HTS. 'A' and 'F' prefixes refer to aroma and flavour attributes respectively. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet, 'Bitter' =bitter taste, 'Sour' =sour taste, 'Sweet' =sweet taste.

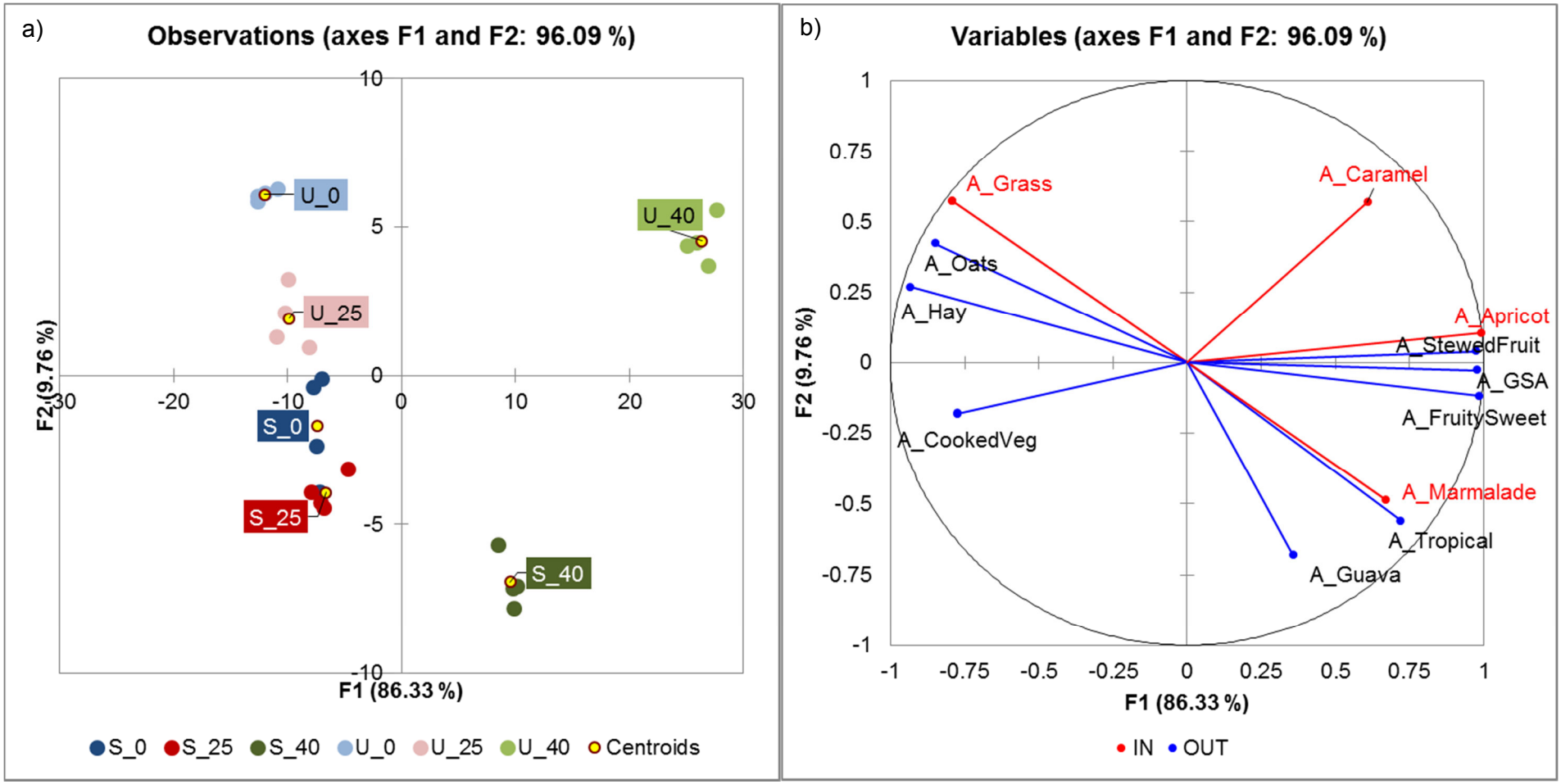


Figure 4.5 DA (a) observations and (b) variables loadings plots with aroma attributes of steamed (S) and unsteamed (U) green *C. maculata*, stored under LTS (0), NTS (25) and HTS (40) conditions for t = 1 month, n = 24.

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. DA observations plot sample codes indicated as (steam treatment)_(storage condition temperature). Light blue = unsteamed LTS, Dark blue = steamed LTS, Light pink = unsteamed NTS, Dark red = steamed NTS, Light green = unsteamed HTS, Dark green = steamed HTS. 'A' prefix refer to aroma attributes. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet, 'Bitter' =bitter taste, 'Sour' =sour taste, 'Sweet' =sweet taste.

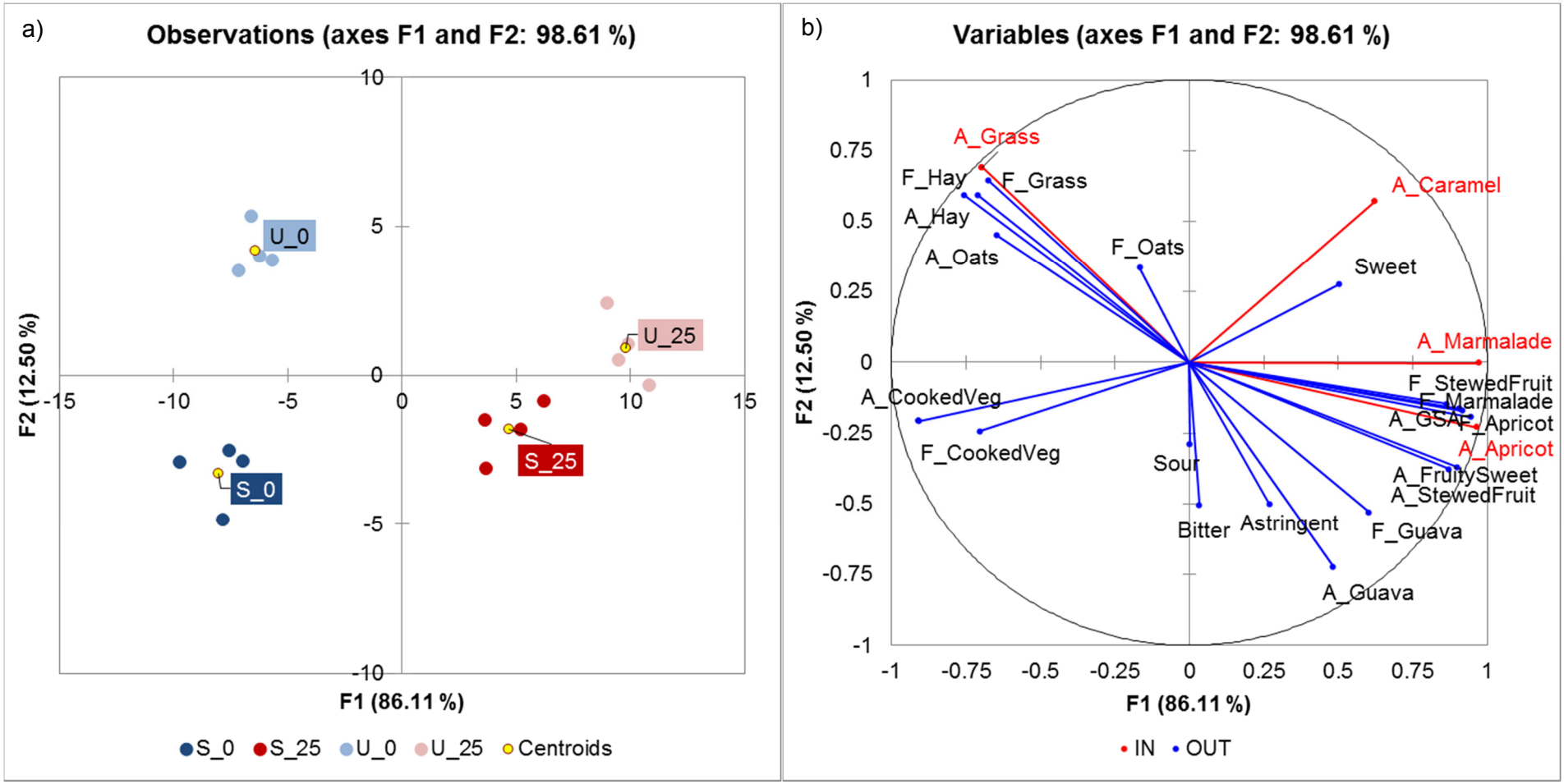


Figure 4.6 DA (a) observations and (b) variables loadings plots with aroma, flavour, taste and mouthfeel attributes of steamed (S) and unsteamed (U) green *C. maculata*, stored under LTS (0) and NTS (25) conditions for t = 3 months, n = 16.

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH. DA observations plot sample codes indicated as (steam treatment)_(storage condition temperature). Light blue = unsteamed LTS, Dark blue = steamed LTS, Light pink = unsteamed NTS, Dark red = steamed NTS. 'A' and 'F' prefix refers to aroma and flavour attributes respectively. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet, 'Bitter' =bitter taste, 'Sour' =sour taste, 'Sweet' =sweet taste.

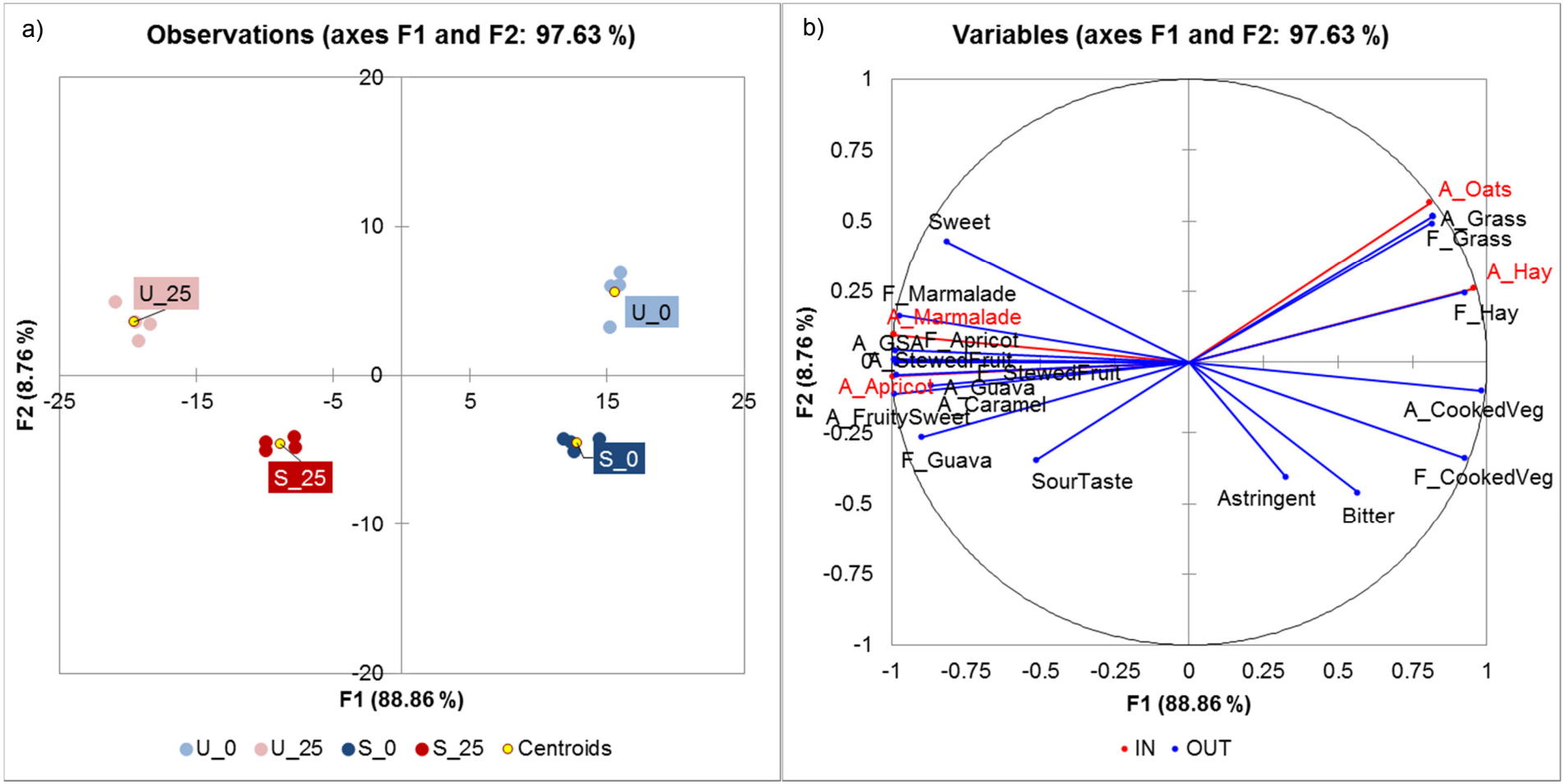


Figure 4.7 DA (a) observations and (b) variables loadings plots with aroma, flavour, taste and mouthfeel attributes of steamed (S) and unsteamed (U) green *C. maculata*, stored under LTS (0) and NTS (25) conditions for t= 6 months, n = 16.

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH. DA observations plot sample codes indicated as (steam treatment)_(storage condition temperature). Light blue = unsteamed LTS, Dark blue = steamed LTS, Light pink = unsteamed NTS, Dark red = steamed NTS. 'A' and 'F' prefix refers to aroma and flavour attributes respectively. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet, 'Bitter' =bitter taste, 'Sour' =sour taste, 'Sweet' =sweet taste.

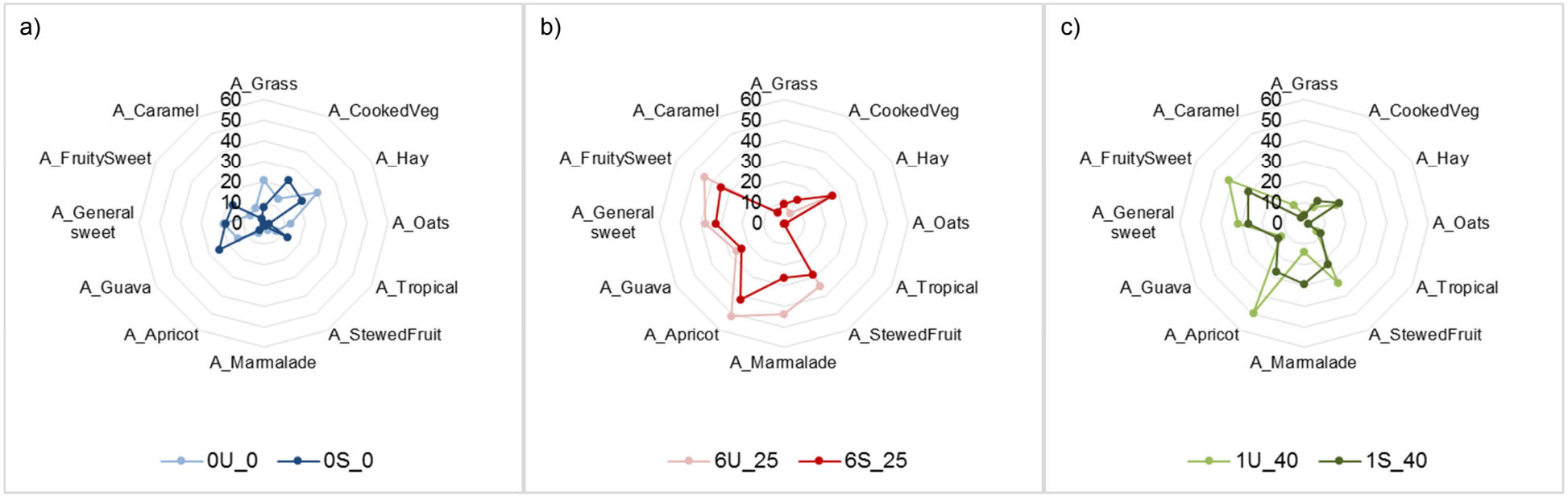
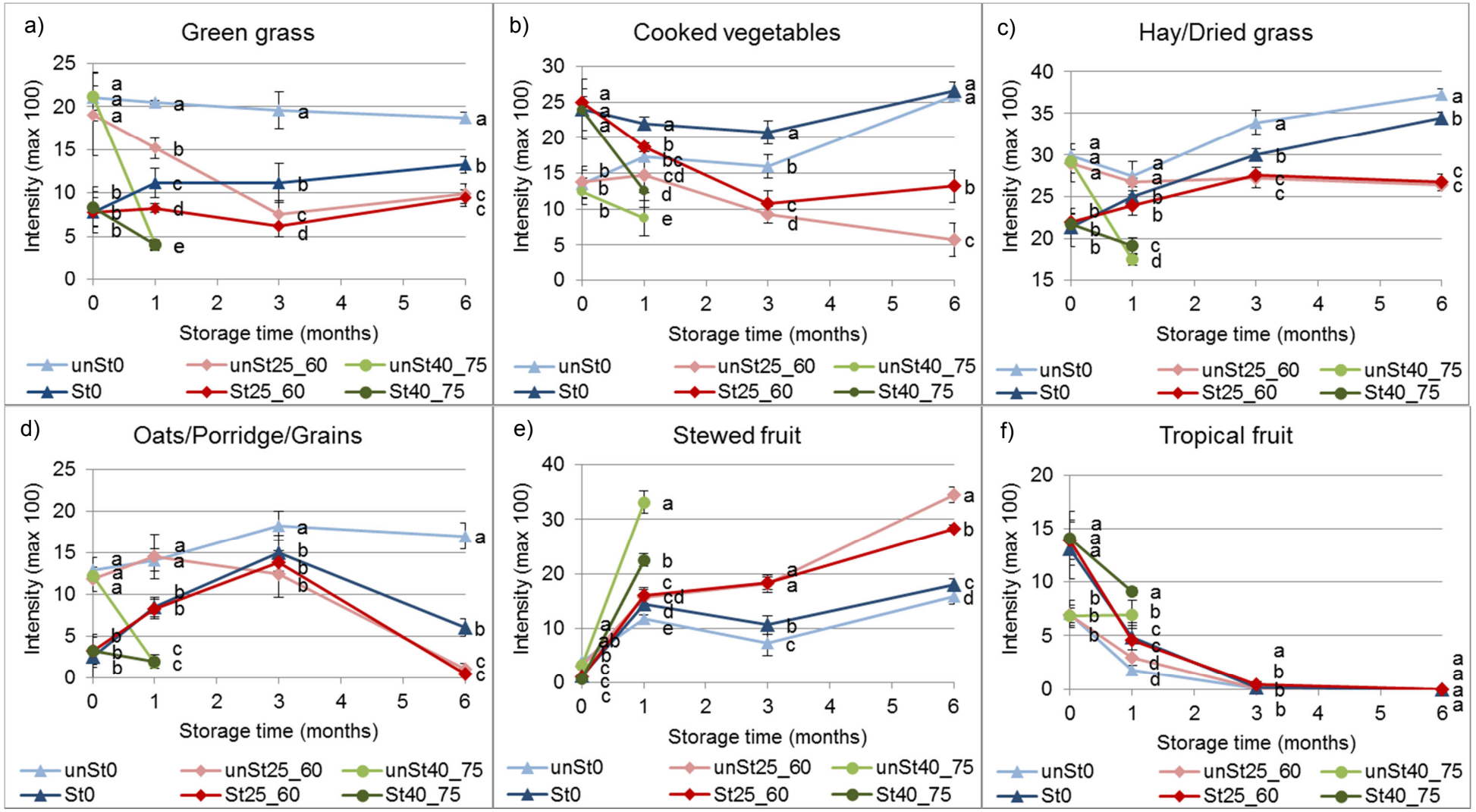


Figure 4.8 Spider plots of DSA aroma intensities of steamed (S) and unsteamed (U) green *C. maculata* at (a) initial conditions (t = 0 months, LTS), and after completed storage under (b) NTS (t = 6 months) and (c) HTS (t = 1 month) conditions.

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. 'A_' prefix refer to aroma attributes. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit.



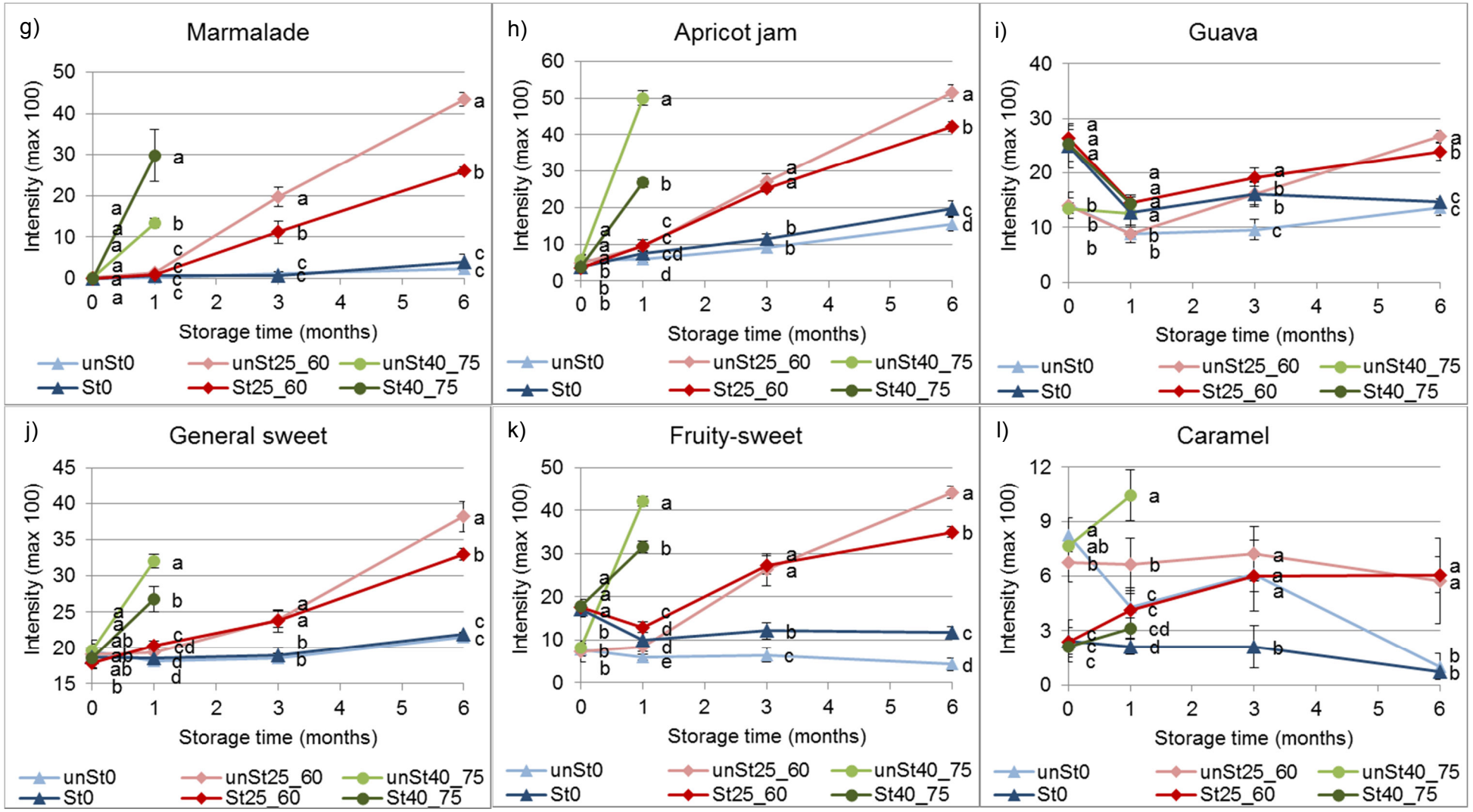


Figure 4.9 *Vegetative* (a – c), *cereal* (d), *fruity* (e – i) and *sweet-associated* (j – l) aroma attributes of infusions prepared from steamed and unsteamed green *C. maculata* after t = 0, 1, 3 and 6 months of storage under LTS, NTS and HTS conditions.

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. Intensity points within a time point do not differ significantly ($p \geq 0.05$) from each other when associated with the same letter/s. 'St' = steamed, 'unSt' = unsteamed, '0' = LTS conditions, '25_60' = NTS conditions, '40_75' = HTS conditions.

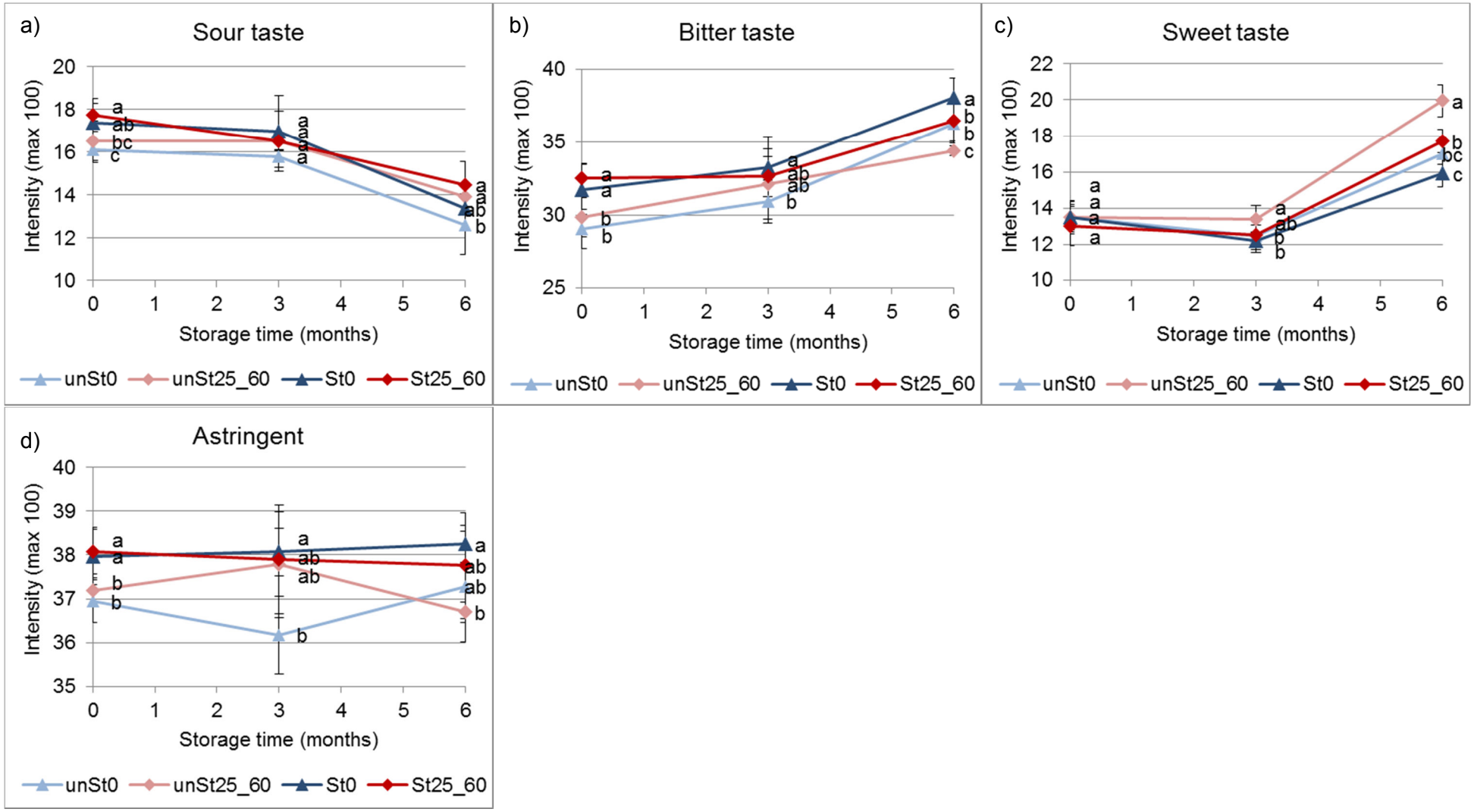


Figure 4.10 Taste (a – c) and mouthfeel (d) attributes of infusions prepared from steamed and unsteamed green *C. maculata* after t = 0, 1 and 6 months of storage under LTS, NTS and HTS conditions.

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. Intensity points within a time point do not differ significantly ($p \geq 0.05$) from each other when associated with the same letter/s. 'St' = steamed, 'unSt' = unsteamed, '0' = LTS conditions, '25_60' = NTS conditions, '40_75' = HTS conditions.

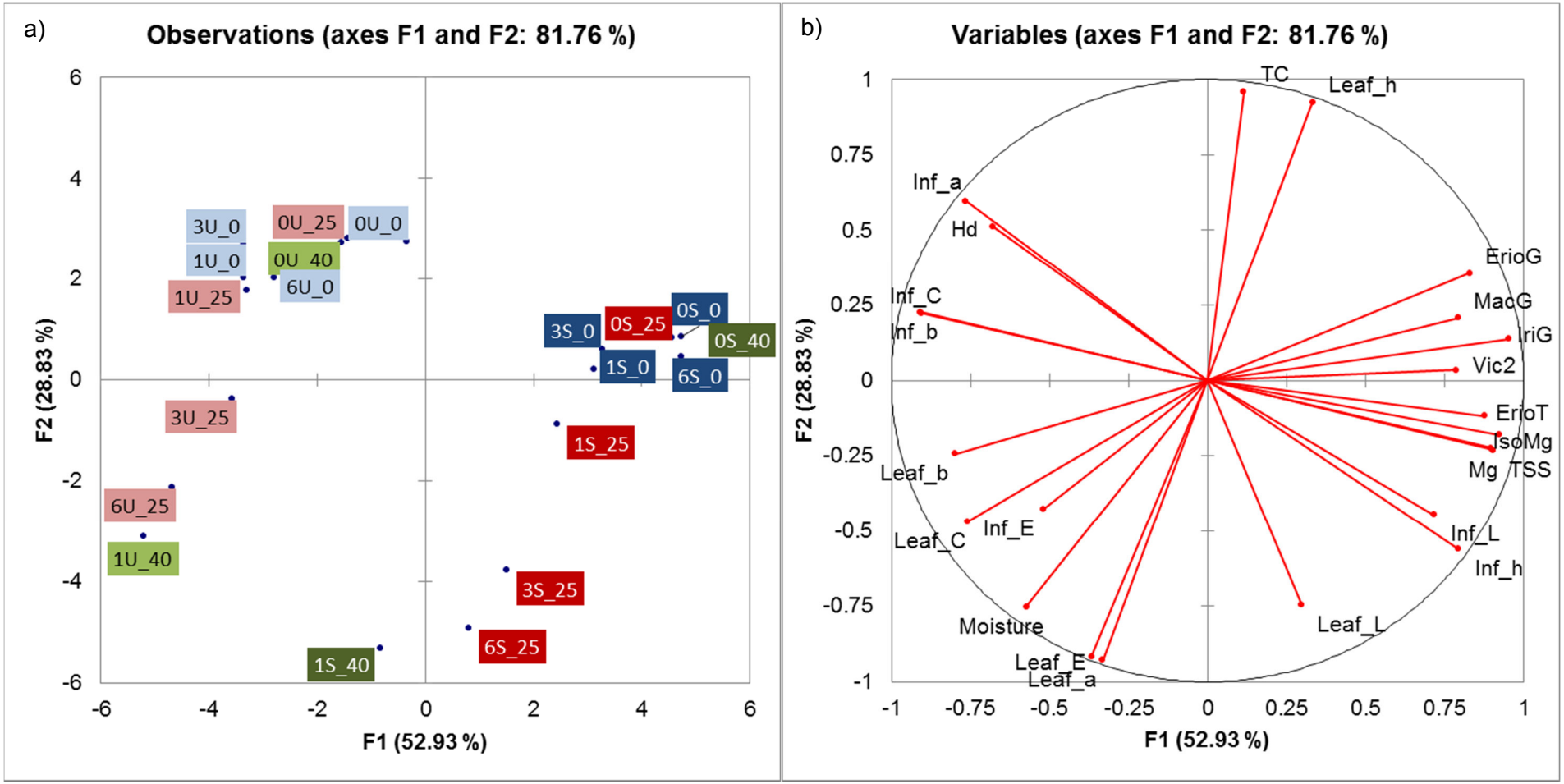


Figure 4.11 PCA (a) scores and (b) loadings plots of all instrumental attributes of steamed (S) and unsteamed (U) green *C. maculata*, stored under LTS (0), NTS (25) and HTS (40) conditions over the storage period of t = 0 to 6 months, n = 20.

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. PCA scores plot sample codes indicated as (month)(steam treatment)_(storage condition temperature). Light blue = unsteamed LTS, Dark blue = steamed LTS, Light pink = unsteamed NTS, Dark red = steamed NTS, Light green = unsteamed HTS, Dark green = steamed HTS. 'Mg' = mangiferin, 'IsoMg' = isomangiferin, 'Vic2' = vicenin-2, 'ErioT' = eriocitrin, 'IriG' = iriflophenone-3-*C*-glucoside, 'MacG' = maclurin-3-*C*-glucoside, 'ErioG' = eriodictyol-*O*-glucoside, 'Hd' = hesperidin, 'Inf' = infusion, 'Leaf' = plant material. 'L', 'a', 'b', 'C', 'h' and 'E' refer to objective colour parameters. 'TSS' = total soluble solids content, 'TC' = total chlorophyll content.

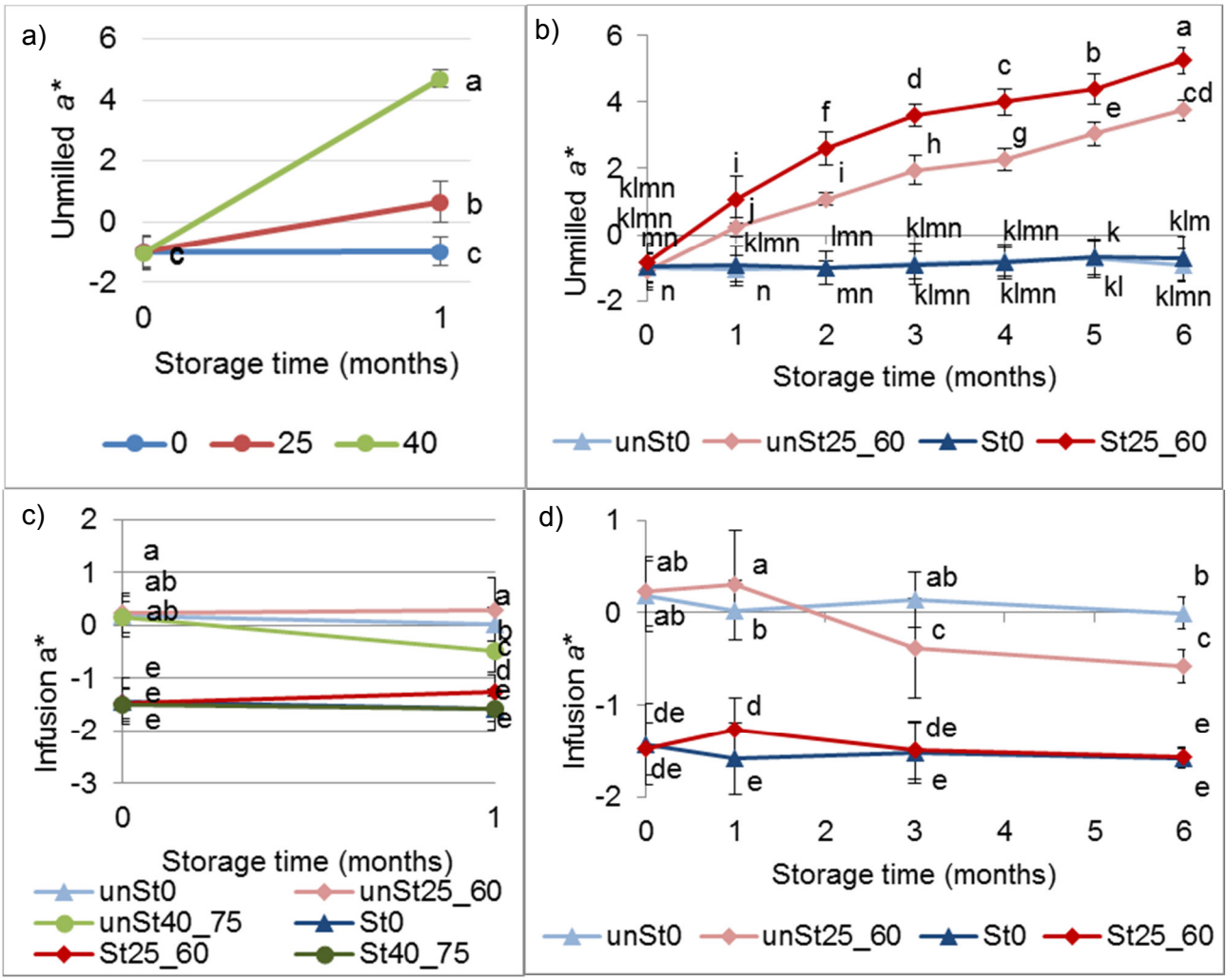


Figure 4.12 Unmilled plant material (a – b) and infusion (c – d) green colour (*a**) of green *C. maculata* stored for t = 0 to 1 month (a & c) and t = 0 to 6 months (b & d) indicating significant differences, main effects and interactions as per ANOVA (**ADDENDUM B**, **Tables B.18** – **B.21**).

'St' = steamed, 'unSt' = unsteamed, '0' = LTS conditions, '25_60' or '25' = NTS conditions, '40_75' or '40' = HTS conditions. LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH.

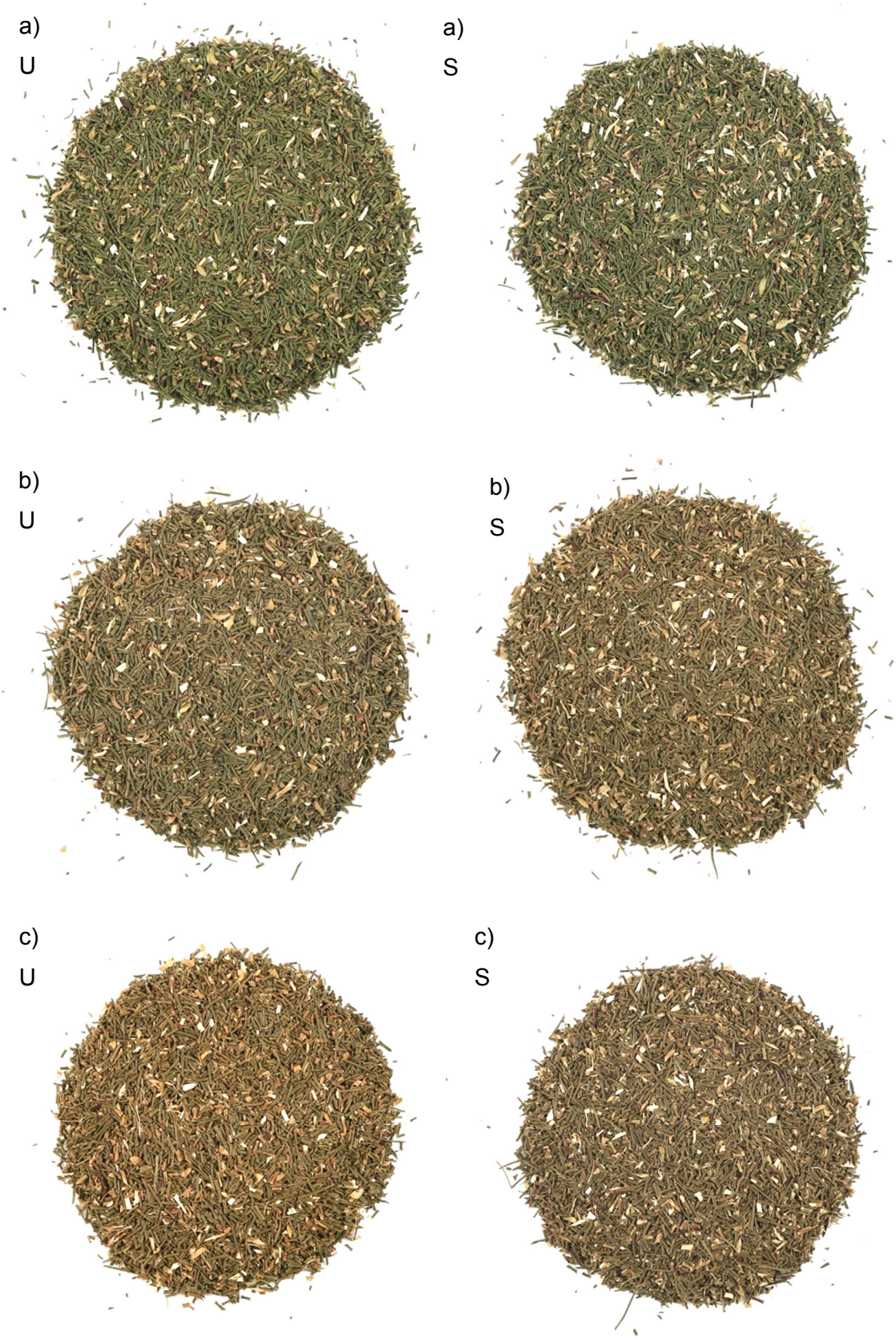


Figure 4.13 Visual indications of unmilled plant material colour of steamed (S) and unsteamed (U) green *C. maculata* at (a) initial conditions (t = 0 months, LTS), and after completed storage under (b) NTS (t = 6 months) and (c) HTS (t = 1 month) conditions.

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH.

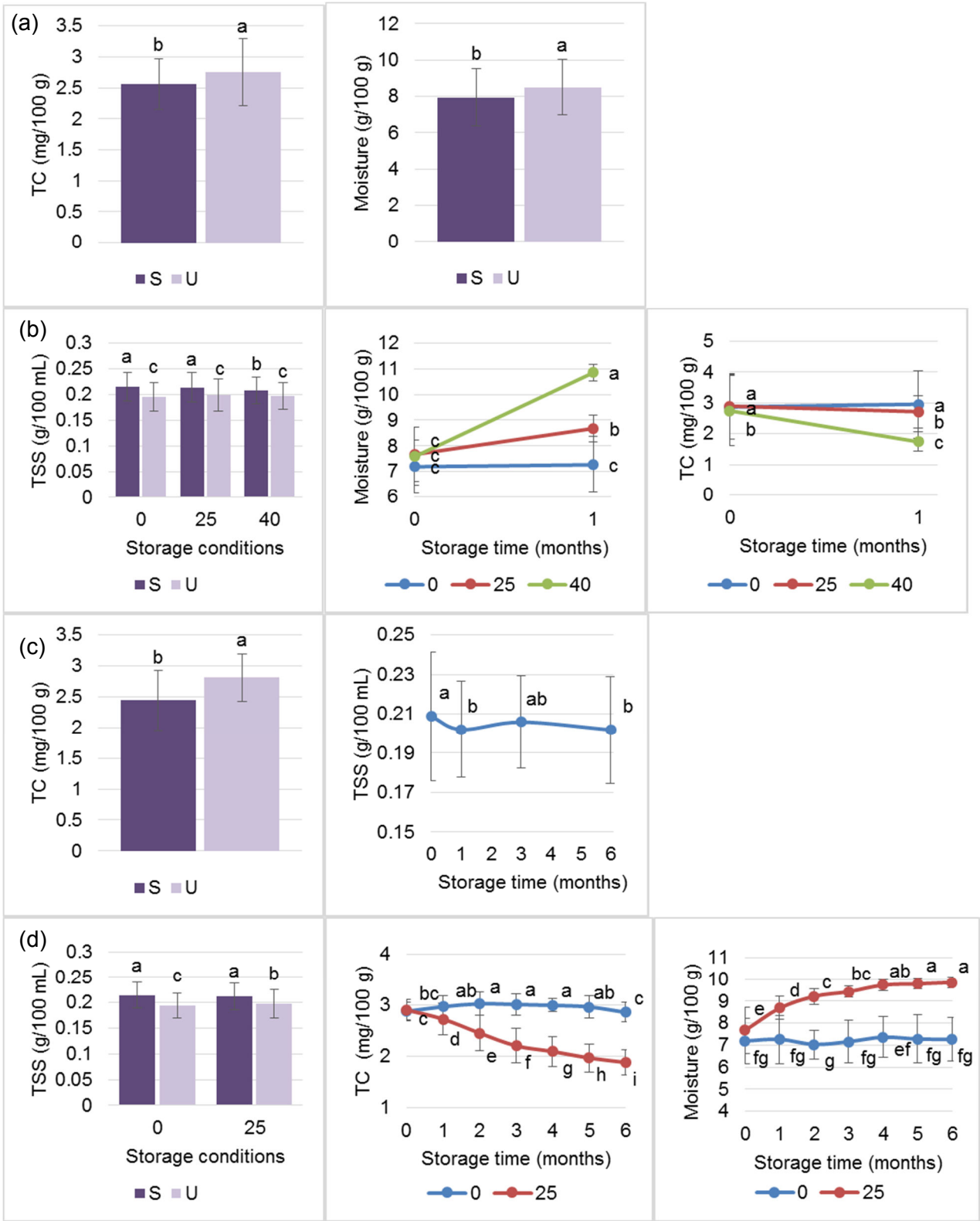


Figure 4.14 Main effects (a) and interactions (b) after t = 0 to 1 month, and main effects (c) and interactions (d) after t = 0 to 6 months of storage for (TC) total chlorophyll, moisture and (TSS) total soluble solids contents of green *C. maculata* indicating significant differences (**ADDENDUM B**, **Tables B.18** – **B.21**).

'St' or 'S' = steamed, 'unSt' or 'U' = unsteamed, '0' = LTS conditions, '25_60' = NTS conditions, '40_75' = HTS conditions, SS = total soluble solids content. LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH.

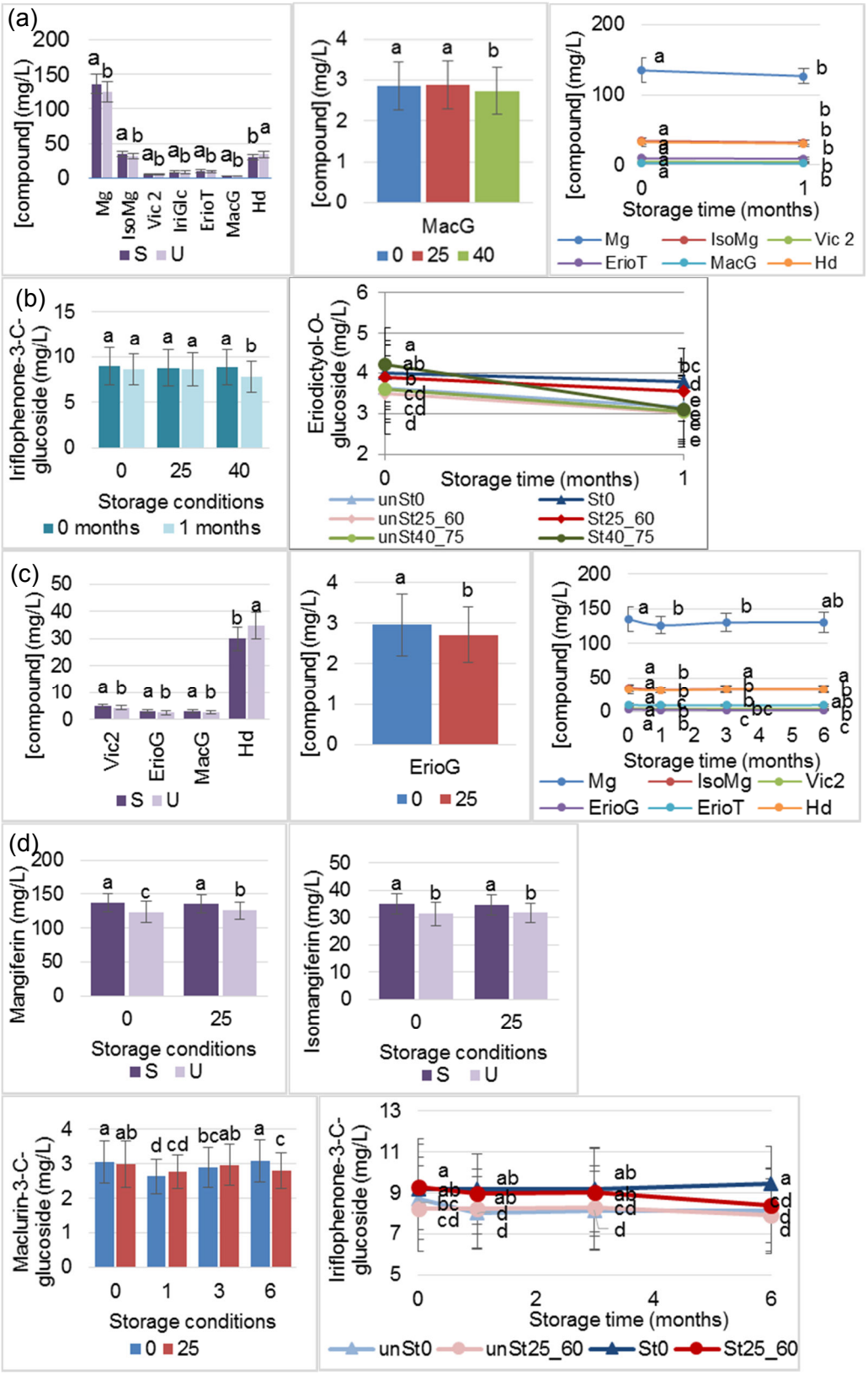


Figure 4.15 Main effects (a) and interactions (b) after t = 0 to 1 month (**ADDENDUM B**, **Table B.19**), and main effects (c) and interactions (d) after t = 0 to 6 months (**ADDENDUM B**, **Table B.21**) of storage for individual phenolic compounds in infusions of green *C. maculata*.

'St' or 'S' = steamed, 'unSt' or 'U' = unsteamed, '0' = LTS conditions, '25_60' or '25' = NTS conditions, '40_75' or '40' = HTS conditions. 'Mg' = mangiferin, 'IsoMg' = isomangiferin, 'Vic2' = vicenin-2, 'ErioT' = eriocitrin, 'IriG' = iriflophenone-3-*C*-glucoside, 'MacG' = maclurin-3-*C*-glucoside, 'ErioG' = eriodictyol-*O*-glucoside, 'Hd' = hesperidin. Standard deviation indicated by error bars. Different letters indicate significant ($p < 0.05$) differences. LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH.

4. Discussion

In the Chapter 3, it was shown that steam treatment of green honeybush has an immediate beneficial effect on its aroma and flavour profile when analysed directly after processing. However, green honeybush can be stored at the production facility or warehouses for several months before it is packed for retail and sold. With no data available on the effect of storage conditions on the overall quality of green honeybush, this investigation addressed this lack of information to enable processors and tea merchants to make informed decisions about the storage of the product.

Interesting trends were observed in the present investigation. In essence, PCA of all aroma and instrumental data collected over the 6 month period (**Fig. 4.2**) provided an excellent overview of the results. Close association between corresponding aroma and flavour attributes and very small changes in taste and mouthfeel intensities (**Fig. 4.10**) validated the exclusion of flavour, taste and mouthfeel attributes from PCA to simplify the dataset. The PCA scores plot of the combined DSA and instrumental dataset indicated a clear distinction between steamed and unsteamed samples. A further positioning separated samples at t = 0 months and LTS samples from increasingly longer and harsher storage times and conditions. Steaming, storage conditions and storage period thus had an impact on the overall results. Similar positioning was observed when PCA of only the instrumental parameters was performed (**Fig. 4.11**), although PCA of DSA aroma attributes did not distinguish as effectively between steamed and unsteamed samples (**Fig. 4.3**). This indicated that instrumental parameters, especially the well distributed colour variables are the main drivers of the overall PCA and can effectively indicate steaming and storage effects (**Fig. 4.2**). These parameters, in particular green colour (a^*), correlated strongly and significantly with many quality parameters (ΔE^*, hue, moisture content and TC content), and may thus be useful for the indication of quality, especially as a first screening procedure. Furthermore, the close and consistent grouping of HTS samples at t = 1 month with those subjected to NTS conditions for 6 months in all the PCA representations indicated the possibility that HTS (t = 1 month) may provide valuable information by emulating changes that take place in the plant material over 6 months of storage at NTS conditions. Further research should be undertaken to determine if this is an effect of both storage temperature and relative humidity, to assess whether it is only valid when semi-permeable packaging is used.

As observed in Chapter 3, steam treatment of fresh, shredded *C. maculata* plant material for 60 s before drying resulted in a significant decrease ($p < 0.05$) in *vegetative* and *cereal* attribute intensities (except for ‘cooked vegetables’) as well as increased ‘guava’, ‘tropical fruit’ and ‘fruity-sweet’ attribute intensities. Treatment discrimination was based on ‘caramel’ aroma intensity which was present in unsteamed samples.

In order to provide a ‘stable’ reference as a sensory anchor, LTS of both the unsteamed and steamed samples at 0 °C in heat-sealed metalised pouches were included. Although these samples associated closely with samples at t = 0 months throughout statistical analyses and thus appeared relatively stable, changes in absolute attribute intensities were observed. The stability of green

honeybush aroma during storage has not been studied to date. It was thus not clear whether these changes were as a result of inherent instability of the green plant material, or due to 'drift' in the scoring by the panel over the 6 month period. For these reasons the attribute intensities were only compared per time point and not between time points. It was, however, clear that these differences between treatments became progressively more prominent over time, indicating that changes took place over time.

One month of storage at HTS conditions resulted in microbial spoilage with visually apparent mould growth in some sub-sample units. Indeed, yeast and mould contamination of medicinal herbs or plants, as observed in the present study, is common, as contamination can take place by air, water or soil environmental pathways before processing (De Freitas Araújo & Bauab, 2012). Poor sealing of the BBOP sachets and the very high relative humidity caused moisture migration into the package, rapidly increasing the moisture content of the plant material to above 10% (**Fig. 4.14**). This created favourable conditions for mould growth, especially when combined with the high storage temperature (40 °C) (De Freitas Araújo & Bauab, 2012). Samples stored for 1 month that did not display visual signs of microbial spoilage were thus analysed sensorially for aroma only. Significant ($p < 0.05$) changes in aroma intensities were observed. Most notable were increases in various *fruity* aroma attribute intensities such as 'stewed fruit' and 'apricot jam' (most prominent in unsteamed HTS samples) and 'marmalade' (most prominent in steamed HTS samples). *Vegetative* and *cereal* aroma intensities decreased markedly resulting in significantly lower ($p < 0.05$) intensities for HTS samples than for LTS or NTS samples. 'Cooked vegetables' and 'hay/dried grass' aroma intensities were slightly lower in unsteamed than in steamed HTS samples (**Fig. 4.9**). Drastic significant ($p < 0.05$) increases in 'general sweet' and 'fruity-sweet' aroma intensities were observed, especially in unsteamed HTS samples (**Fig. 4.9**). These results offer preliminary insight into the comparatively higher susceptibility of unsteamed green honeybush material to sensorial changes during storage. As will be discussed, these differences in susceptibility may be as a result of the loss of volatiles during steam treatment, and subsequent lower concentrations of volatile compounds available for extraction in the tea infusions.

The notable developments in sensory profiles of the samples were once again observed at t = 3 and 6 months of storage between LTS and NTS conditions. Unsteamed NTS samples, followed by steamed NTS samples, produced infusions which scored very high intensities for *fruity* and *sweet-associated* attributes and lower intensities for *vegetative* and *cereal* attributes than LTS samples (**Fig. 4.9**). Increasing *fruity* and *sweet-associated* attribute intensities, and decreasing *vegetative* attribute intensities in green honeybush as a result of prolonged NTS or short term HTS indicate increasing similarities to the sought-after sensory profile of fermented honeybush. The sensory profile of fermented honeybush has prominent *sweet-associated* and *fruity* descriptors (Theron *et al*., 2014), similar to those appearing more prominently as a result of storage of green honeybush.

Decreases in *vegetative* and *cereal* attribute intensities as well as that of 'tropical fruit' and 'guava' aroma in NTS and HTS samples were observed during the course of the present study

(**Fig. 4.9**). The possibility of higher volatility of the compounds responsible for these associations, such as aldehydes, ketones or alcohols (as discussed in the previous chapter with reference to steaming) may explain these losses. Furthermore, unsteamed samples seemed to incur comparatively greater losses than steamed samples, where initial levels of such 'green'-associated volatile compounds may already be driven off by steaming (as discussed in the previous chapter). The marked loss of 'tropical fruit' aroma and flavour after 3 months and the apparent decrease in 'guava' aroma and flavour intensities after 1 month may be due to a similar loss of volatile compounds. Alternatively, it may be due to interaction with lost volatiles, as most commonly it is not one single volatile compound in isolation which is responsible for aroma associations, but rather interaction between two or more compounds (Bott & Chambers, 2006). Specific aroma associations may also change with differing concentrations of volatile constituents, as observed by Hongsoongnern and Chambers (2008) for different concentrations of hexanal.

As discussed, the combined PCA of sensory and instrumental data (**Fig. 4.2**) appeared to be driven by the instrumental parameters. The strong correlations between a^*, h and ΔE^* values of the plant material (**ADDENDUM B**, **Table B.2**) indicate that the change in green colour is the main contributor to the observed colour change of the samples. Furthermore, the strong correlations between these colour parameters and TC content indicate that instrumental colour measurements may be sufficient to indicate TC content. Similar correlations have been demonstrated for a range of plant materials, including green tea (Wang *et al*., 2004), yerba maté (Schmalko & Alzamora, 2001), spinach (Wang *et al*., 2013) and Jalapeño peppers (Cervantes-Paz *et al*., 2014). In practice this could simplify standard quality analyses as these colour measurements are quick and easy to perform, while chlorophyll analyses, by comparison, are relatively expensive, labour-intensive and prone to experimental errors.

Similar to results found by Joubert *et a*l. (2010) for *C. subternata*, green colour remained relatively stable during LTS irrespective of treatment. NTS and HTS, however, resulted in significant ($p < 0.05$) green colour losses over the period of storage. Contradictory to changes in aroma intensities as determined by DSA, loss of green colour was more exaggerated in steamed than unsteamed NTS samples. Despite the minimal effect of steam treatment on a^* values of plant material, steamed samples contained consistently lower levels of chlorophyll, decreasing further at NTS and HTS conditions (**Fig. 4.14**). Various factors contribute to chlorophyll degradation, including high temperatures, light exposure, relative water activity and integrity of cell membranes (LaJollo *et al*., 1971; Lafeuille *et al*., 2014; Rocha *et al*., 1993). Chlorophyll breaks down to pheophytin faster at higher water activities ($A_w > 0.32$) (LaJollo *et al*., 1971). Indeed, a significant negative correlation was found between TC and moisture content (**ADDENDUM B**, **Table B.2**). This indicates that an increase in moisture content, and subsequently water activity, may contribute to chlorophyll losses by providing water available for enzymatically or chemically catalysed reactions (LaJollo *et al*., 1971).

As postulated in Chapter 3, the initial retention of green colour for steam-treated samples, in spite of chlorophyll breakdown, may suggest enzymatic conversion of chlorophyll to chlorophyllide

with a similar green colour (Lafeuille *et al*., 2014). However, steaming of the samples did not accelerate chlorophyll degradation compared to the untreated samples and chlorophyllide is known to degrade at a faster rate at higher temperatures than chlorophyll (Canjura *et al*., 1991, Schwartz *et al*., 2008). This conversion may thus not be the primary effect during steaming of green honeybush. Furthermore, enzymatic deactivation by heating may preclude chlorophyllide formation, as was observed for other plant materials (Hu *et al*., 2013, Wang *et al*., 2004). It is well-established that heating causes degradation of chlorophyll to pheophytin (Schwartz *et al*., 2008), which at low levels may not be measurable by spectrophotometric methods, causing overestimation of chlorophyll (Dos Santos *et al.,* 2003). The initial loss of TC may not have been severe enough to significantly affect green colour. Significant changes in green colour were thus only observed after sufficient build-up of pheophytin with the loss of Mg^{2+} over time. This conversion to pheophytin occurs in an acid environment with applied heat (Schwartz *et al*., 2008). Such conditions would be experienced to a greater degree in steamed samples, due to changes in membrane permeability and increased acid release from plant vacuoles in the shredded material (Ritchie, 2006; Rocha *et al*., 1993) and mild heat exposure over longer storage times. This probability is supported by the higher TSS content of steam-treated samples (**Fig. 4.14**).

Infusion colour did not follow similar trends to that of the plant material. For compounds to make an impact on infusion colour they must be released from the plant matrix into solution. As observed and discussed in Chapter 3, infusions prepared from steamed plant material were greener (or less red) than those of unsteamed material. The *a** values of the infusions indicated no strong correlation to TC content of the plant material, but showed a strong negative correlation to TSS content ($r = -0.851$) (**ADDENDUM B**, **Table B.3**). This indicated higher extractability of plant constituents into the infusions after steaming and when stored over six months, possibly due to physiological changes as discussed in Chapter 3. This could support the suggestion by Wang *et al*. (2004), who considered the possibility of heightened chlorophyll extraction contributing to green colour of infusions. Lipophilic chlorophyll could be suspended in the infusion in an emulsified state at high temperatures, aggregating and precipitating at lower temperatures.

All individual phenolic compounds (except hesperidin) quantified in the infusions were associated with steamed samples and had strong significant positive correlations to TSS content (**Fig. 4.2**, **ADDENDUM B**, **Table B.1**). As noted in Chapter 3, this improved extractability may mask slight phenolic degradation in the material itself. Once again, steaming (96 °C, 60 s), however, did result in a significant ($p < 0.05$) decrease in hesperidin content of the infusions, although it is considered a relatively heat-stabile compound (Agcam *et al*., 2014; Sánchez-Moreno *et al*., 2005). Hesperidin is the most non-polar of the quantified phenolics in the current investigation and is thus weakly soluble in a polar medium such as water (Joubert *et al*., 2012). It is thus possible that the conformational changes in the cellular structure of steamed honeybush may inhibit extraction of hesperidin into solution. Precipitation of hesperidin due to low solubility at low temperatures when the infusion is cooled to room temperature, or due to complexation with unidentified substances

present in the infusion is also possible. This would lead to lower quantified concentrations of hesperidin when such precipitates are removed by filtration using a 0.22 µm pore size filter before HPLC analysis. Another observation which may have been influenced by the strong associations between individual phenolic content and TSS, is the strong correlation observed between some individual phenolics and astringent mouthfeel. As astringency is considered a tactile sensation in the mouth, the increased extraction of some of these compounds into the infusion may have caused excessive interactions in the mouth, causing the heightened sensation of astringency (Rosetti *et al*., 2009; Rossetti *et al*., 2008; Lu & Bennick, 1998; Charlton *et al*., 2002). Indeed, of these correlated phenolics, mangiferin, isomangiferin and iriflophenone-3-*C*-glucoside have been linked to the astringency of fermented honeybush (Erasmus, 2015).

Losses in phenolic compounds have been observed for green and black teas (*Camellia sinensis*) over 6 months, and are accelerated by increasing moisture contents (Friedman *et al*., 2009, Wickremasinghe & Perera, 1972). Green honeybush, however, does not seem to incur great losses in phenolic content over the same storage period, even with significant increases in moisture content over time. The individual phenolics appeared relatively stable after an initial decrease in the content of some compounds after 1 month of storage (**Fig. 4.15**). It must, however, be noted that changes in phenolic content were subject to much variation. This may be due to inherent variation in plant materials as the large batches consisted of varied groups of individual seedling plants (Joubert *et al*., 2003; De Beer & Joubert, 2010; Joubert *et al*., 2010; Joubert *et al*., 2014).

5. Conclusions

The sensory profile of green *C. maculata* was demonstrated to be subject to major aroma changes over the course of 6 months under normal temperature storage conditions (BOPP sachets, 25 °C at 60%RH) likely to be encountered before and during retail. These changes manifest as an increase in the intensity of *fruity* and *sweet-associated* attributes, especially that of ‘apricot jam’, ‘marmalade’, ‘fruity-sweet’ and ‘general sweet’ as well as a decrease in the intensity of *vegetative* and *cereal* attributes. Similar trends were observed for high temperature storage (BOPP sachets, 40 °C at 75% RH) after one month, although microbial spoilage was a concern. Chlorophyll degradation and subsequent loss of green colour was notable, but the product was still green after storage. Steam pre-treatment before drying resulted in a slightly less stable green colour over time, but changes in the sensory profile of these samples were slightly less severe than for unsteamed samples. Overall, however, steamed and unsteamed material stored under normal temperature storage conditions produced green honeybush with improved aroma quality and a green colour. Phenolic degradation as a result of storage was minimal and would therefore not be detrimental to bioactivity. Steaming of green honeybush is thus an effective method of achieving an improved sensory profile without compromising visual or phenolic quality in the short term when the product is to be consumed without a prolonged storage process. Storage of three to six months under normal storage conditions led to an improvent in aroma. Although colour degradation is a reality, it is not necessarily detrimental to

product quality, especially if the product is to be packaged as tea bags and protected from light rather than presented as loose tea in open bulk bags at farm stalls. Future studies should determine CIE*L*a*b** colour difference values required to observe noticeable visual colour changes to establish defined colour specifications for green honeybush.

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Chapter 5

Development of a preliminary sensory lexicon and wheels for green honeybush (C. maculata and C. longifolia)

Contents

Abstract

Generic sensory wheels and lexicons for aroma, flavour, taste and mouthfeel attributes were developed for green honeybush based on descriptive sensory analysis of *Cyclopia maculata* and *C. longifolia.* The sample set was comprised of samples that were processed according to standard industry methods as well as samples that were steam-treated before or after drying. Variability was further enhanced by including samples subjected to low, normal and high temperature storage conditions. Attributes were selected based on percentage occurrence and mean intensity in the complete sample set and evaluated for susceptibility to treatments using principal component analysis. A total of 12 aroma, 9 flavour and 3 taste descriptors were included together with 1 mouthfeel descriptor to generate two-tiered aroma and flavour wheels for green honeybush. Descriptors included *vegetative* ('green grass', 'cooked vegetables' and 'hay/dried grass'), *cereal* ('oats/porridge/grains'), *fruity* ('stewed fruit', 'apricot jam', 'tropical fruit', 'guava' and 'marmalade') and *sweet-associated* ('general sweet', 'fruity-sweet' and 'caramel') as well as sour, sweet and bitter taste and astringent mouthfeel. Green honeybush has a dominant *vegetative* aroma and flavour, with a prominent *sweet-associated* and slight *fruity* aroma, with sweet and notably bitter tastes and an astringent mouthfeel. The susceptibility of the green honeybush sensory profile to processing and storage conditions indicated that these wheels and lexicon may require adaptation in future to fully reflect the full range of aroma and flavour attributes.

1. Introduction

Tools for quality management are becoming ever-more necessary as product competition increases in the market. Food producers are thus required to develop quality control tools in order to set and maintain quality standards. This is especially relevant for small-scale industries such as the green honeybush industry with a major potential for growth on the global market (Joubert *et al*., 2011).

For the production of high quality products, quality is assured by the implementation of a quality control system. Although some industries rely on chemical or physical instrumental analyses to specify quality parameters, food and beverage industries require sensory parameters and specifications for the assessment of aroma and flavour quality. These sensory parameters are of utmost importance for the assessment of food quality and the development of new or improved products (Muñoz, 2002).

Descriptive sensory analysis (DSA) is regarded not only as the cornerstone profiling method in academic research, but also in industry when the aim is to quantify the full sensory profile of a product range (Drake & Civille, 2002). This technique utilises a panel of judges, trained extensively to define the sensory attributes relevant to the product, in order to quantify the aroma, flavour, taste and mouthfeel attributes associated with the products being tested (Lawless & Heymann, 2010). The data obtained from DSA can be used to establish sensory lexicons and wheels which are commonly used in the assessment and quality control of various food and beverage products (Drake & Civille, 2002).

Sensory lexicons serve to establish a common qualitative frame of reference from which quality assessors may be trained to quantify the defined attributes on an intensity scale to reproducibly assess products (Lawless & Civille, 2013). These lexicons provide the necessary terminology which may be accompanied by physical reference standards, whether chemical or non-chemical, to further aid in precise and unambiguous sensory profiling (Drake & Civille, 2002; Lawless & Civille, 2013). Lexicon descriptive sensory terms can be assembled in a sensory wheel with a more convenient format (Lawless & Civille, 2013). Sensory wheels are usually presented in a tiered format, with the most detailed secondary descriptors associated with broader primary attributes (Lawless & Civille, 2013). These may be further categorised into positive or negative attributes (Koch *et al*., 2012; Theron *et al*., 2014). This simple, easy-to-interpret format is useful as it graphically presents a system by which to evaluate product quality. Furthermore, a sensory wheel may be coupled with DSA to quantify the intensity of specific sensory attributes captured in the sensory wheel (EyeQuestion, Logic8 BV, Netherlands).

Data obtained from DSA can be further analysed using multivariate statistical techniques, especially principal component analysis (PCA) to spatially illustrate the association between samples and attributes (Zielinski *et al*., 2014). For example, the development of a descriptive lexicon for Danish honey resulted in a well-defined list of descriptors with reference standards including physical references for texture and appearance as well as chemical and non-chemical references for mouthfeel, flavour and taste (Stolzenbach *et al*., 2011). This lexicon was further used in the DSA of

various Danish honeys and PCA applied to identify seasonal effects as well as sensory characteristics influenced by geographical location (Stolzenbach *et al*., 2011).

Sensory wheels and lexicons are available for tea produced from *Camellia sinensis,* and are used by tea associations and tea merchants (ATM, 2013). Product-specific sensory wheels have been developed for black tea (Bhuyan & Borah, 2001; ATM, 2013), while Lee and Chambers (2007) developed a lexicon for green tea, although few such quality tools exist for the assessment of herbal teas. The lack of sensory quality control tools available to the South African herbal tea industry motivated the development of sensory wheels and lexicons for rooibos (Koch *et al*., 2012; Jolley, 2014) and traditional (fermented) honeybush (Theron *et al*., 2014; Bergh 2014; Erasmus, 2015).

The generic honeybush sensory wheel, based on the sensory evaluation of six *Cyclopia* spp., *i.e. C. sessiliflora, C. longifolia, C. genistoides, C. intermedia, C. subternata* and *C. maculata*, indicates positive (*sweet-associated, nutty, spicy, plant-like, fruity* and *floral* related attributes) and negative (*vegetative, chemical* and *earthy* related attributes) sensory attributes. Given the prominence of some attributes, the typical sensory profile of fermented honeybush can be described as '*floral, sweet-associated, fruity, plant-like* and *woody*' with a sweet taste and slight astringency. Subsequently, the lexicon for fermented honeybush was updated with species-specific aroma and flavour wheels, developed for *C. maculata, C. longifolia, C. subternata, C. genistoides* (Erasmus, 2015) and *C. intermedia* (Bergh, 2014). It was found that 'caramel' aroma was typical of *C. maculata*, while 'rose geranium', 'apricot/apricot jam' and 'hay/dried grass' aroma were typically present in the *C. longifolia* samples. Furthermore, bitter taste was not considered typical of either of these two species, with astringent mouthfeel slightly more intense in infusions of *C. longifolia* than those of *C. maculata*.

Although Theron *et al.* (2014) identified several negative attributes (including bitter and sour taste, 'dusty', 'yeasty', 'medicinal', 'burnt caramel', 'rotting plant water', 'hay/dried grass', 'green grass' and 'cooked vegetables'), only 'hay/dried grass' and 'cooked vegetables' were included in the species-specific sensory wheels for *C. maculata* and *C. longifolia* (**Fig. 5.1**). The latest generic and species-specific sensory wheels developed for fermented honeybush not only indicate the positive and negative sensory attributes associated with the respective *Cyclopia* spp., but they also indicate the average intensity and percentage occurrence of the respective attributes (Erasmus, 2015). These aspects can all be used to determine whether a batch of fermented honeybush can be regarded as superior or inferior quality tea.

Green (unfermented) honeybush, however, cannot be graded according to the same classifications as fermented honeybush. This is becuase negative attributes identified in fermented honeybush, such as *vegetative* aromas and flavours are commonly associated with under-fermented teas, and thus inferior quality (Bergh, 2014; Erasmus, 2015). These *vegetative* attributes are expected to be prominent, or even desirable in green honeybush. It is thus important to distinguish between the requirements for 'good quality' in both fermented and green honeybush. Classification of positive and negative attributes may also be largely market-dependent (Drake & Civille, 2002;

Lawless & Civille, 2013). For example, Asian markets are familiar with green teas with typically *vegetative* or *cereal-like* aromas and bitter tastes (Lee & Chambers, 2007; Wang *et al*., 2000). The majority of local South African consumers, however, have not been exposed to these profiles to the same degree and may prefer a profile more typical of fermented teas that tend to be less bitter, sweeter and with *fruity*, rather than *vegetative* aroma and flavour attributes (Erasmus, 2015). Although the present study is only able to provide general and preliminary insight into the sensory profile of green honeybush, the distinction between green and fermented honeybush, prepared from *C. maculata* and *C. longifolia,* is clearly indicated by the prominent contribution of *vegetative* attributes to the characteristic aroma of green honeybush (Chapters 3 and 4). Furthermore, the low intensities of flavour attributes highlighted the relative contributions made by taste and mouthfeel attributes, especially bitter taste and astringent mouthfeel.

In view of the above, the aim of the current investigation was thus to define the green honeybush sensory profile. This is achieved by collating the data generated in the previous chapters, to create preliminary aroma and flavour wheels and an accompanying lexicon describing typical aroma, flavour, taste and mouthfeel attributes associated with the indigenous South African green honeybush.

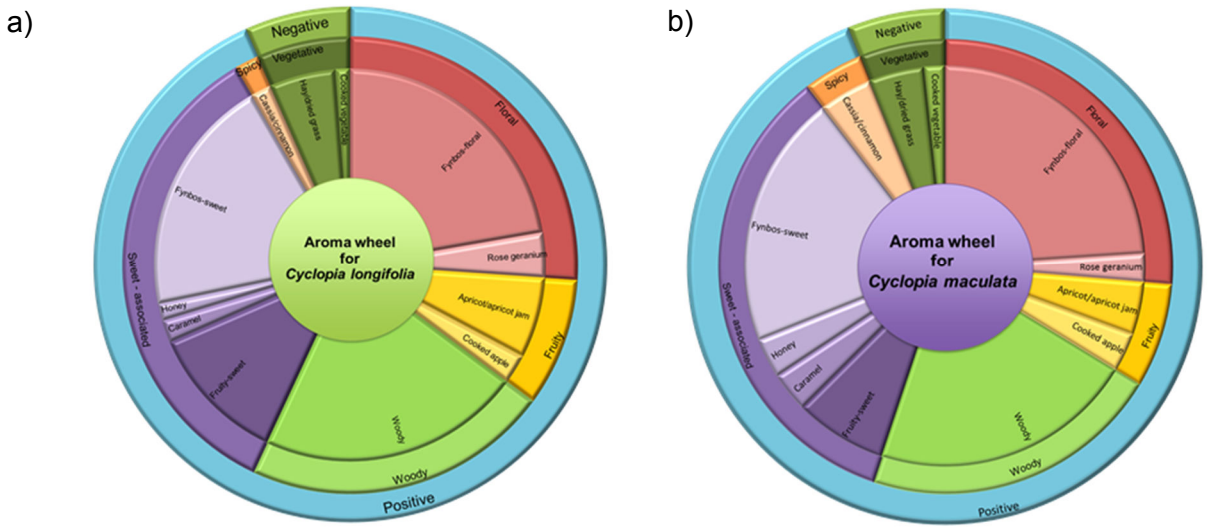


Figure 5.1 Aroma sensory wheels for fermented (a) *C. longifolia* and (b) *C. maculata* honeybush (Erasmus, 2015).

2. Materials and methods

2.1 Materials, data collection and statistical procedures

Plant material (*C. maculata* and *C. longifolia*) was collected, processed and analysed using DSA and statistical procedures as described in Chapter 3. The data from all samples described in Chapters 3 and 4 were included in the current data set as summarised in **Table 5.1** and **Table 5.2**. Experimental setup, treatment, species and numbers of samples are included in the tables. The sample set included *C. maculata* and *C. longifolia* samples steam-treated before drying (STBD, EXP 1) or after

drying (STAD, EXP 2) for various time periods (Chapter 3) as well as *C. maculata* samples STBD for 60 s or unsteamed and stored under low temperature storage conditions (in moisture impermeable pouches at 0° C) (LTS), and normal temperature storage conditions (in semi-moisture permeable sachets at 25 °C and 60% relative humidity (RH); NTS) for 0 (T0), 1 (T1), 3 (T3) and 6 (T6) months. High temperature storage conditions (in semi-moisture permeable sachets at 40 °C and 75% RH; HTS) for 0 and 1 month (EXP3, Chapter 4) were also included. A total of 180 samples was analysed for aroma, but only 156 samples were analysed for flavour, taste and mouthfeel, due to microbial sample degradation during HTS in experiment 3 (Chapter 4). Samples were all tested in triplicate. All attributes and descriptions utilised across the three experiments are provided in **ADDENDUM A** (**Table A.1**). It may be noted, however, that not all attributes were included in every experiment as some attributes (*e.g.* 'cooked spinach', 'green herbs' and 'cooked green beans and/or potato') were vague or ambiguous and replaced by more general terms (*e.g.* 'cooked vegetables') in subsequent analyses. Other attributes (*e.g.* 'cooked apple' and 'honey'), especially *taints* or negative attributes (*e.g.* 'dusty', 'musty' and 'seaweed/oceanic'), were not present at detectable (< 5) intensities (Chapter 3 and 4).

Table 5.1 Samples providing pooled data for analysis of the aroma profile of green honeybush

Chapter	Experiment	Batches	Treatments			*C. longifolia*	*C. maculata*
			Steaming	Storage conditions	Time points		
3	EXP 1	5	5			25	25
3	EXP 2	5	5			25	25
4	EXP 3	4	2	LTS, NTS, HTS	T0, T1		48
4	EXP 3	4	2	LTS, NTS	T3, T6		32
TOTAL						**50**	**130**

Table 5.2 Samples providing pooled data for analysis of the flavour, taste and mouthfeel profile of green honeybush

Chapter	Experiment	Batches	Treatments			*C. longifolia*	*C. maculata*
			Steaming	Storage conditions	Time points		
3	EXP 1	5	5			25	25
3	EXP 2	5	5			25	25
4	EXP 3	4	2	LTS, NTS, HTS	T0		24
4	EXP 3	4	2	LTS, NTS	T3, T6		32
TOTAL						**50**	**106**

2.2 Sensory wheel development

In order to represent typical sensory attributes of green honeybush, the data of all samples described in **Table 5.1** and **Table 5.2** were collected and pooled. The overall mean intensity of each attribute for all samples as well as the tally of samples in which each attribute was present at an intensity of ≥ 5, was calculated. The data were presented as overall mean intensity *vs*. percentage occurrence plots for aroma attributes, and for flavour, taste and mouthfeel attributes. Sensory attributes present

in at least 9% of samples were considered for inclusion in the sensory wheels. Further attribute-limiting criteria included the removal of aroma and flavour attributes present at an overall mean intensity of below 3 and below 1, respectively.

PCA was used on data from all samples pooled for the final list of aroma, flavour taste and mouthfeel attributes included in the sensory lexicon for green honeybush (**Table 5.2**, n = 156). XLStat (Version 2015.1.01, Addinsoft, New York, USA) was used for data analysis in order to assess the legitimacy of the combined percentage occurrence and overall mean intensity results. It also served to test the susceptibility of the data to change as a result of treatments. A similar PCA was used on the data from aroma attributes of the full sample set (**Table 5.1**, n = 180), but resulted in similar positioning to the presented plots (results not shown). Specific mention is also made to maximum and minimum intensities in the complete data set, as provided in Chapters 3 and 4.

Two-tiered aroma and flavour sensory wheels were generated in Microsoft Excel (2013) with overall mean intensities indicated by the relative portion of each attribute in the wheel, similar to the species-specific sensory wheels for fermented honeybush developed by Erasmus (2015).

3. Results and discussion

3.1 Occurrence plots and evaluation of attribute susceptibility

The overall mean intensity (as analysed on a 100-point scale) of each aroma and flavour attribute was plotted against its percentage occurrence to illustrate the selection of attributes for each wheel (**Figs. 5.2** & **5.3**). Mean aroma attribute intensities of more than 3 on a 100 point scale and with percentage occurrences of more than 10% were selected (**Fig. 5.2**). Flavour attributes (**Fig. 5.3**) were similarly selected at mean intensities above 1 on a 100-point scale and percentage occurrences of above 9%. These limits were imposed in order to include a comprehensive set of descriptors considering the vast range of intensities amongst samples as a result of treatments, including steam treatment and changes during storage (Chapter 3 and 4). Although flavour attributes were typically present at noticeably lower intensities than the corresponding aroma attributes, as also demonstrated by Erasmus (2015), they are not less important when considering product quality and were thus included at a lower mean intensity than for aroma attributes. As indicated in **Figs. 5.2** and **5.3**, *vegetative* aromas and flavours were present in > 90% of samples, with ‘hay/dried grass’ present in 100% of samples at an overall mean intensity of *ca*. 25. ‘Green grass’ and ‘cooked vegetables’ followed closely with overall mean intensities of *ca*. 10 and 15, respectively. *Sweet-associated* aroma attributes, *i.e.* ‘general sweet’ and ‘fruity-sweet’ were also present in > 90% of samples at overall mean intensities of 19 and 13, respectively. Most of the taste and mouthfeel descriptors (sweet and sour taste and astringent mouthfeel) were present in all samples, followed by bitter taste in 98% of the samples. Overall mean intensities ranged from 15 for sweet and sour taste, 27 for bitter taste and 34 for astringent mouthfeel. These attributes are the most prominent (high intensity) and common (high percentage occurrence) attributes found in green honeybush. Other common aroma attributes (percentage occurrences > 50% in samples) indicated with percentage occurrence and

overall mean intensity in brackets include: 'apricot jam' (89%; 13), 'stewed fruit' (86%; 13), 'guava' (77%; 11), 'oats/porridge/grains' (70%; 9) and 'caramel' (52%; 5). Minor aroma attributes (present in < 50% of samples) also include 'tropical fruit' (37%; 4) and 'marmalade' (14%; 4) (**Fig. 5.2**). 'Guava' flavour was common (51%, 6) and minor flavour constituents, 'stewed fruit' (30%; 4), 'oats/porridge/grains' (24%; 3), 'apricot jam' (15%; 3), 'tropical fruit' (10%; 2) and 'marmalade' (9%; 2), were less common and less prominent (**Fig. 5.3**).

PCA was used on the data from all samples tested for the full lexicon set of descriptors to allow for a full assessment of the associations between sample 'history' and attributes. The results are presented in **Fig. 5.4** (**a** & **b**). The PCA scores plot indicates that the main separation along the first principal component (F1, 32.23%). Distinction is made between samples (EXP1 *C. maculata* and *C. longifolia*, EXP2 *C. longifolia*, and EXP3 T0) that were not stored (assessed after treatment) to the left and stored samples (EXP3 T3 and T6) as well as samples of STAD *C. maculata* subjected to the longer steam treatments (EXP2, 2 to 4 min STAD) to the right, as indicated by the light green arrows. A secondary positioning of samples also appears along the second principal component (F2, 19.77%). Along this axis, unsteamed samples are located mostly at the top of the plot, except for unsteamed samples subjected to NTS conditions (EXP3 T6). Steamed samples are located predominantly at the bottom of the plot, except STAD *C. longifolia* samples (EXP2), and as STBD *C. maculata* samples stored under LTS (EXP3 T3 and T6) and NTS conditions (EXP 3 T3). The loadings plot follows familiar groupings of *vegetative* and *cereal* attributes in the top left quadrant, associating with unsteamed samples (not stored), except for 'hay/dried grass' which is shifted slightly to the right, indicating slight association with samples stored for longer periods. This quadrant also includes 'stewed fruit', 'apricot jam' and 'general sweet' aromas as well as bitter taste and astringent mouthfeel. In the opposite lower left quadrant lies 'tropical fruit' aroma and flavour together with sour taste, associating with steamed samples that were assessed directly after treatment. The bottom right quadrant groups 'guava', 'fruity-sweet' and 'marmalade' attributes as well as sweet taste and 'stewed fruit' and 'apricot jam' flavour, as associated with samples stored for longer periods. Indeed, factor loadings indicate that *fruity* and *sweet-associated* attributes ('apricot jam', 'marmalade', 'stewed fruit' and 'guava' aroma and flavour as well as 'general sweet' and 'fruity-sweet' aroma) represent the ten highest ranking factor contributions to F1. These attributes thus drive the split due to storage treatments, whereas *vegetative* and *cereal* attributes ('hay/dried grass', 'oats/porridge/grains', 'green grass' and 'cooked vegetables' aroma and flavour as well as bitter taste and 'general sweet' aroma) are the drivers of F2 indicating the positioning due to steam treatments.

With *fruity* and *sweet-associated* attributes driving the first principal component (F1) of the PCA (**Fig. 5.4**), it was also evident from previous chapters (Chapter 3 and 4) that *fruity* and *sweet-associated* aromas are subject to much variation as a result of treatment, especially storage, with intensities ranging from not present (< 5) to more than 50 for *fruity* ('apricot jam') and 45 for *sweet-associated* ('fruity-sweet') aromas after NTS of 6 months. Given the range of intensities for these attributes, the overall intensity means remained fairly low for *fruity* attributes (< 15 aroma, < 10

flavour) and occurrences were low in terms of overall *fruity* flavour (< 55%) (**Figs. 5.2** & **5.3**). 'Apricot jam', 'stewed fruit' and 'guava' aroma were, however, present in more than 75% of the samples, indicating that these attributes can be regarded as common to green honeybush aroma. These attributes are also prominent in the sensory profile of fermented honeybush (Theron *et al*., 2014; Erasmus, 2015; Bergh, 2014) and are thus expected to evolve with processing of green honeybush. Steaming led to increases in some *fruity* and *sweet-associated* attributes, especially 'guava', an attribute not present in fermented honeybush, and 'fruity-sweet' aroma (Chapter 3 and 4), although some attributes such as 'apricot jam' and 'stewed fruit' were affected minimally. 'Tropical fruit' aroma and flavour developed during steaming and were also found to decrease and disappear during storage, accounting for the association with steamed samples that were assessed shortly after treatment, and indicated in the PCA (**Fig. 5.4**). Investigation of NTS and HTS samples revealed drastic increases in 'apricot jam', 'marmalade', 'stewed fruit', 'general sweet' and 'fruity-sweet' aromas and flavours, although storage had a much less notable effect on 'guava' aroma and flavour. The *fruity* attribute, 'marmalade', only became prominent in samples as a result of NTS (or HTS) conditions and was not detected in samples assessed shortly after treatment, as evident in the PCA plots (**Fig. 5.4**). 'Marmalade' intensities reached a maximum of 45 for aroma and 23 for flavour, dominating most other *fruity* attributes after 6 months of storage. The 'collective' *sweet-associated* aroma attribute, 'general-sweet' aroma was present in all samples at fairly high intensities (overall mean intensity of 19), and did not diminish during processing, but rather increased to a maximum intensity of 40 as a result of storage. 'Fruity-sweet' aroma was present in 94% of samples at an overall mean intensity of 13, and was affected to a greater extent by steam treatment as well as storage, increasing the intensity of this attribute to a maximum of 45.

Vegetative aroma and flavour attributes were very common as well as prominent in intensity, especially 'hay/dried grass' (**Figs. 5.2** & **5.3**); this attribute was not greatly affected by the steam or storage treatments (Chapter 3 and 4, with a minimum aroma intensity of 15 and maximum of 38), making it one of the most *characteristic attributes* present in the green honeybush sensory profile. 'Green grass' and 'cooked vegetables' were similarly common and more prominent in untreated samples and some steam-treated samples assessed directly after treatment than in stored treated samples, together with the *cereal* attribute, 'oats/porridge/grains', as reflected in the PCA (**Fig. 5.4**). The *cereal* attribute was not present in all samples and batches, with a percentage occurrence of only 70% for aroma (**Fig. 5.2**) and just more than 20% for flavour (**Fig. 5.3**) at relatively low overall mean intensities (< 10 aroma, < 5 flavour). Steam treatment, as discussed in Chapter 3, decreased the intensity of *vegetative* and *cereal* attributes and evidently drove the discrimination of steamed and unsteamed treatments in the PCA (**Fig. 5.4**). As indicated in Chapter 3, this was especially evident in the drastic decreases in 'green grass' aroma and flavour intensities leading to selection by discriminant analysis (DA) as the major attributes contributing to treatment discrimination of both *C. maculata* and *C. longifolia* STBD and STAD samples. Attributes such as 'cooked vegetables' and 'hay/dried grass' were not affected by steaming to the same degree, despite being very common in

the sample set with slight changes in intensity affecting attribute positioning on the PCA loadings plot (**Fig. 5.4**). Storage of 6 months at NTS conditions (25 °C at 60% RH) and 1 month at HTS conditions (40 °C at 75% RH) resulted in further losses of *vegetative* and *cereal* attributes with distinct losses in 'green grass', 'cooked vegetables' and 'oats/porridge/grains' aromas (Chapter 4), although not to intensities lower than the limit of detection.

The *vegetative* aroma and flavour of green honeybush including 'hay/dried grass', 'green grass' or 'cooked vegetables', is not a result of inferior quality or so-called *taints* or negatrive attirbutes, but are characteristic to the sensory profile of green honeybush. These attributes, however, are regarded as negative in fermented honeybush as they indicate under-fermentation leading to uncharacteristic *vegetative taints* (Erasmus, 2015). Consumer education and exposure would be required to familiarise them with the typical sensory profile of green honeybush.

Taste and mouthfeel attributes were present in nearly all samples (> 97%), indicating the importance of these attributes in the overall sensory profile of green honeybush produced from these *Cyclopia* species. These attributes were not greatly affected by green honeybush processing. The exception is sweet taste, which seemed to increase with prolonged steam treatments and, to a greater degree as a result of prolonged storage at NTS conditions. This may be due to taste modulation by olfaction, where sweet-associated aroma may influence the perceived sweet taste (Bonnans & Noble, 1993; Cliff & Noble, 1990). Consistently high intensities of bitter taste and astringent mouthfeel with overall mean intensities of close to 30 dominate the flavour profile of green honeybush. Green *Camellia sinensis* tea typically has a bitter taste and astringent mouthfeel ranging from mild to intense, and is not considered as a negative attribute (Lee & Chambers, 2007; Wang *et al*., 2000), as in the fermented honeybush sensory profile (Theron et al., 2014). Bitter taste occurs in high intensities in the sensory profile of under-fermented honeybush infusions and is thus associated with inferior quality fermented honeybush (Erasmus, 2015). In the case of green honeybush, however, a bitter taste does not indicate inferior processing as it is inherent to the sensory profile of some batches of high quality green honeybush (ranging from a minimum intensity of *ca*. 4 to a maximum of *ca*. 40, based on batch variation in the current dataset). Sour and sweet taste, although present in all samples, were not dominant in the sensory profile at overall mean intensities of *ca*. 15.

The current representation ascribes equal weight to all samples. Overall mean intensity values and occurrences of the attributes are thus subject to the current data set, compiled of a variety of processing and storage times and conditions. It has been established in previous chapters that the sensory profile of green honeybush is susceptible to various changes as a result of these treatments, although the development of negative attributes or *taints* (such as 'seaweed/oceanic', 'dusty' or 'musty') was not observed. This indicated that minimal processing of green honeybush does not lead to the development of *taints*, as has been observed for the over-fermentation of traditional honeybush (Theron, 2012). Sensory profiles may be further dependent on species, as observed for fermented honeybush (Erasmus, 2015, Theron *et al*., 2014) as well as harvesting period

and season. Some species variation (*C. longifolia* and *C. maculata*) was already observed with regard to sensory occurrences, intensities and response to treatments (**Fig. 5.4**). A preliminary sensory screening of a number of green honeybush infusions prepared from several *Cyclopia* spp. (*C. genistoides, C. maculata, C. longifolia, C. intermedia* and *C. subternata*), prior to the present study, indicated that species differed in major aroma notes (results not shown). It may be proposed that in-depth, species-specific investigations could be conducted on green honeybush of the various commercial species, in order to ascertain the potential these species may have for green honeybush production, as were done for fermented honeybush (Erasmus, 2015).

3.2 Green honeybush sensory wheel

Two separate two-tiered aroma and flavour sensory wheels were generated with the pooled data of *C. maculata* and *C. longifolia* from all treatment studies and are presented in **Figs. 5.5** and **5.6**. The aroma wheel and flavour, taste and mouthfeel wheel attributes and descriptors are summarised in the lexicon provided (**Table 5.3**). The lexicon comprises of 12 aroma, 9 flavour, 3 taste and 1 mouthfeel attributes. These represented *vegetative* ('green grass', 'cooked vegetables' and 'hay/dried grass'), *cereal* ('oats/porridge/grains'), *fruity* ('stewed fruit', 'apricot jam', 'tropical fruit', 'guava' and 'marmalade') and *sweet-associated* ('general sweet', 'fruity-sweet' and 'caramel') attribute groups as well as taste and mouthfeel attributes (sour, sweet and bitter taste and astringent mouthfeel).

The sensory lexicon and wheels for green honeybush (**Table 5.3**, **Figs. 5.5** & **5.6**) represent a preliminary tool for its sensory analysis. As previously discussed, relative intensities may be affected by treatments or storage and adjustment of the wheels and lexicon may be required in future as more data becomes available. Based on the occurrences and intensities of the descriptors from the current data set, green honeybush may be generally described as having a dominant *vegetative* aroma and flavour, prominent *sweet-associated* and slight *fruity* aroma, and sweet and notably bitter tastes and astringent mouthfeel.

Table 5.3 List of aroma and flavour attributes for green honeybush (lexicon descriptions provided in **ADDENDUM A**, **Table A.1**)

Aroma attribute group	Aroma attribute	Flavour, taste or mouthfeel attribute group	Flavour, taste or mouthfeel attribute
Vegetative	'Green grass'	*Vegetative*	'Green grass'
	'Cooked vegetables'		'Cooked vegetables'
	'Hay/Dried grass'		'Hay/Dried grass'
Cereal	'Oats/Porridge/Grains'	*Cereal*	'Oats/Porridge/Grains'
Fruity	'Stewed fruit'	*Fruity*	'Stewed fruit'
	'Tropical fruit'		'Tropical fruit'
	'Apricot jam'		'Apricot jam'
	'Guava'		'Guava'
	'Marmalade'		'Marmalade'
Sweet-associated	'General sweet'	Taste	Sour
	'Fruity-sweet'		Bitter
	'Caramel'		Sweet
		Mouthfeel	Astringent

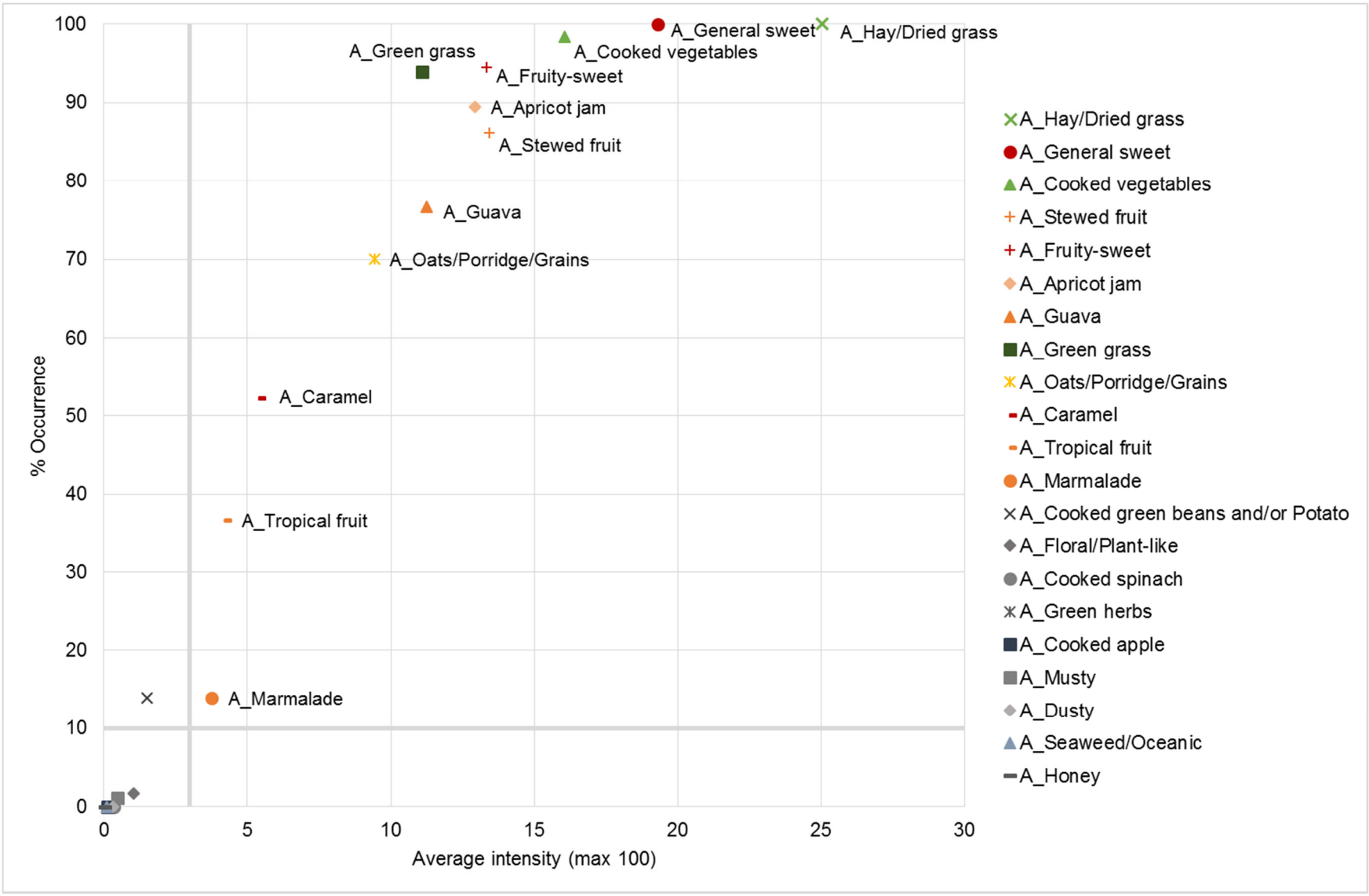


Figure 5.2 Percentage occurrence and overall mean intensity plot for aroma attributes of green honeybush compiled from data for *C. maculata* and *C. longifolia.*

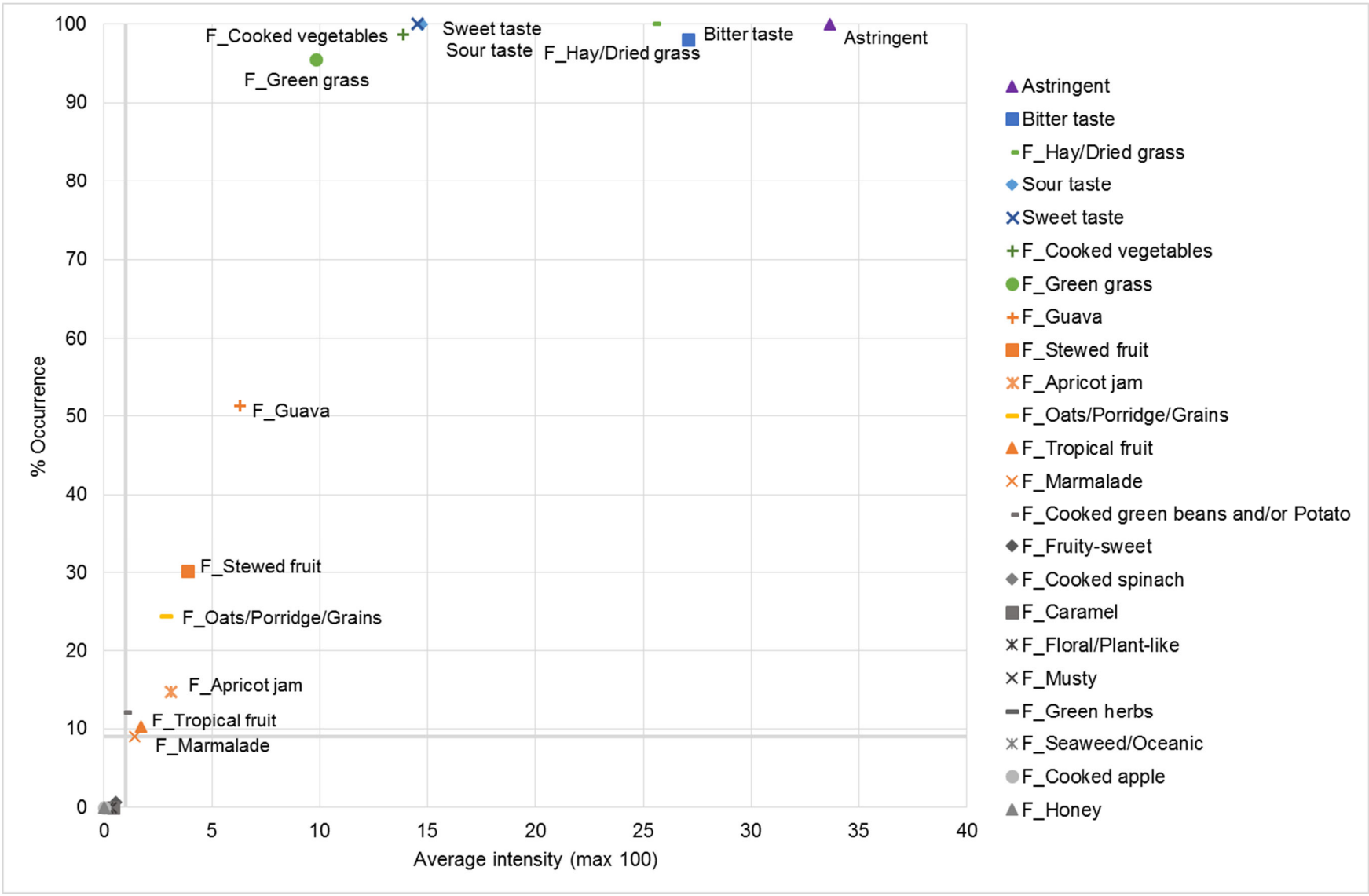


Figure 5.3 Percentage occurrence and overall mean intensity plot for flavour, taste and mouthfeel attributes of green honeybush compiled from data for *C. maculata* and *C. longifolia*.

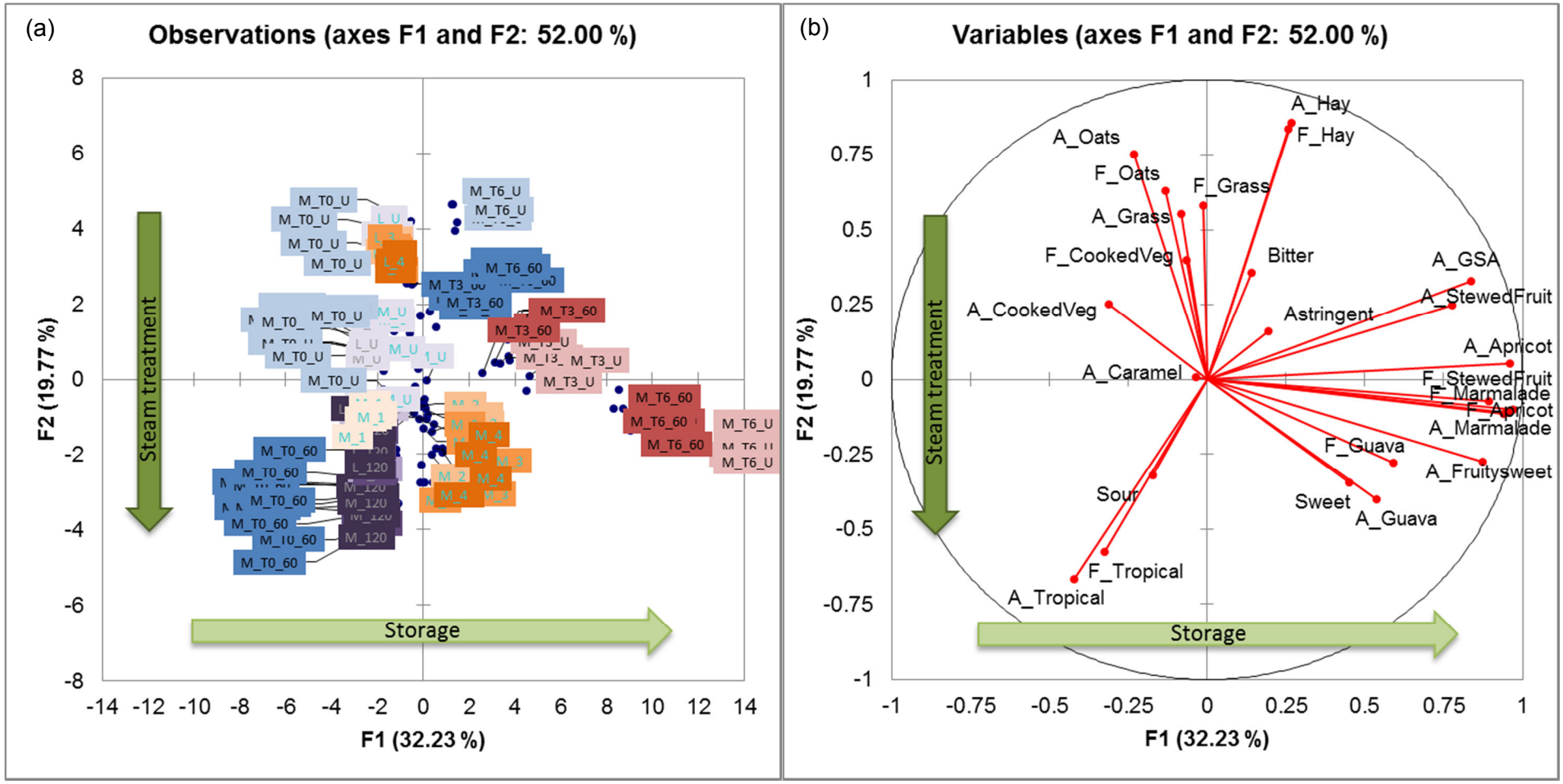
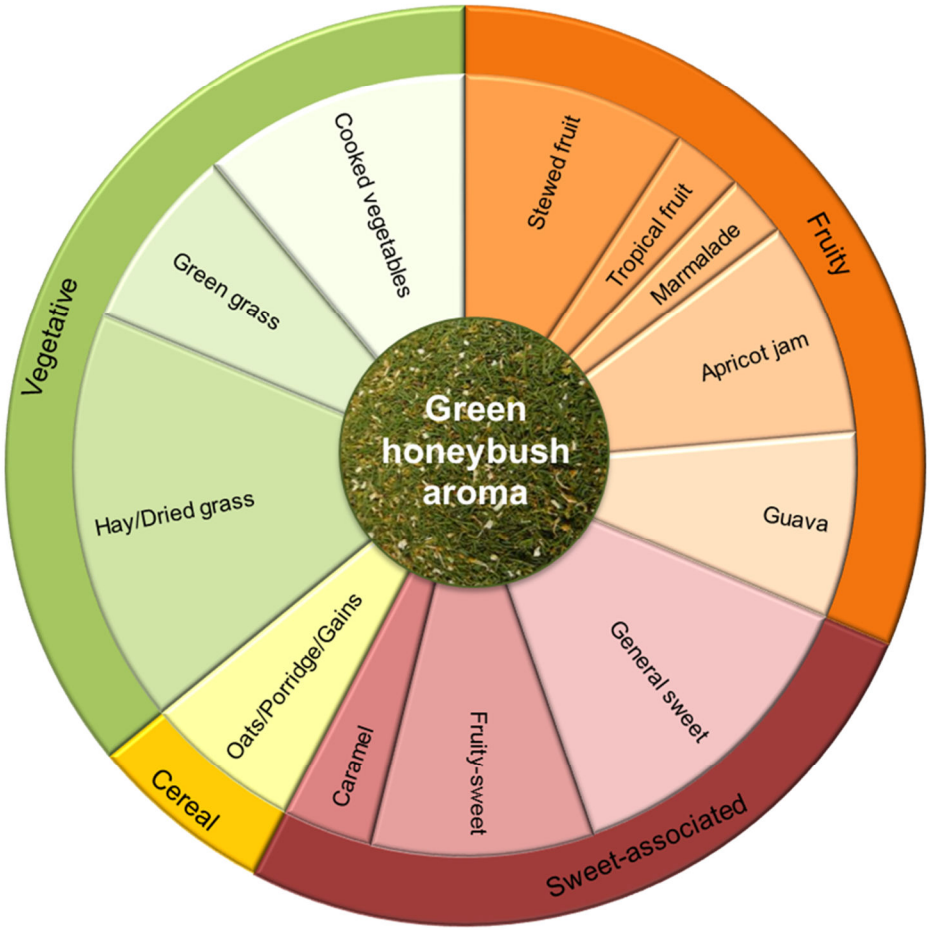


Figure 5.4 PCA (a) scores and (b) loadings plots of all pooled samples for the full set (EXP1, EXP2 and EXP3) of selected green honeybush (*C. maculata* and *C. longifolia*) sensory attributes.

Coloured samples in the PCA scores plot indicate: dark pink = EXP3, steamed NTS, light pink = EXP3, unsteamed NTS, dark blue = EXP3, steamed LTS, light blue = EXP3, unsteamed LTS, light purple = EXP1 and 2, unsteamed, darker purple = EXP1, steaming of 30 s to 120 s before drying, orange = EXP2, steaming 1 to 4 min after drying. 'L' = *C. longifolia*, 'M' = *C. maculata*, T0 – T6 = time periods in months, 'U' = unsteamed. '30' to '120' = steaming before drying times in s. '1' to '4' = steaming after drying time in min. Light green arrow indicates progressive direction of storage treatments (F1). Dark green arrow indicates progressive direction of steam treatments (F2). 'A' or 'F' prefix refers to aroma and flavour, respectively. 'Grass' = green grass, 'Cookedveg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats\porridge\grains, 'Apricot' = apricot jam, 'Tropical' = tropical fruit, 'GSA' = general sweet. 'Bitter' = bitter taste, 'Sour' = sour taste, 'Sweet' = sweet taste. LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH

(a)



(b)

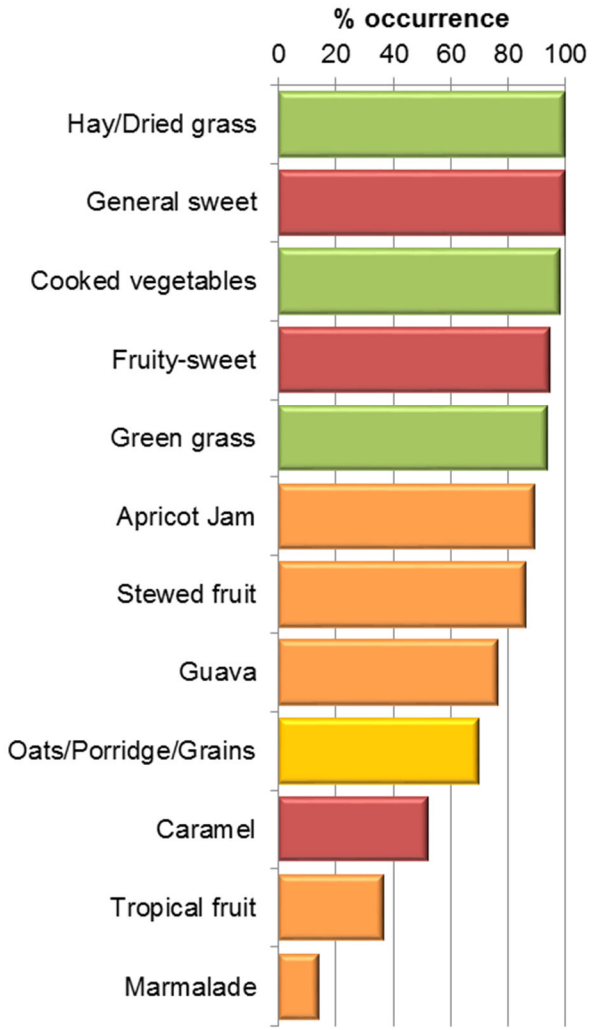


Figure 5.5 Green honeybush (a) aroma wheel and (b) percentage occurrence of aroma attributes in full sample set (n = 180) of *C. maculata* and *C. longifolia*.

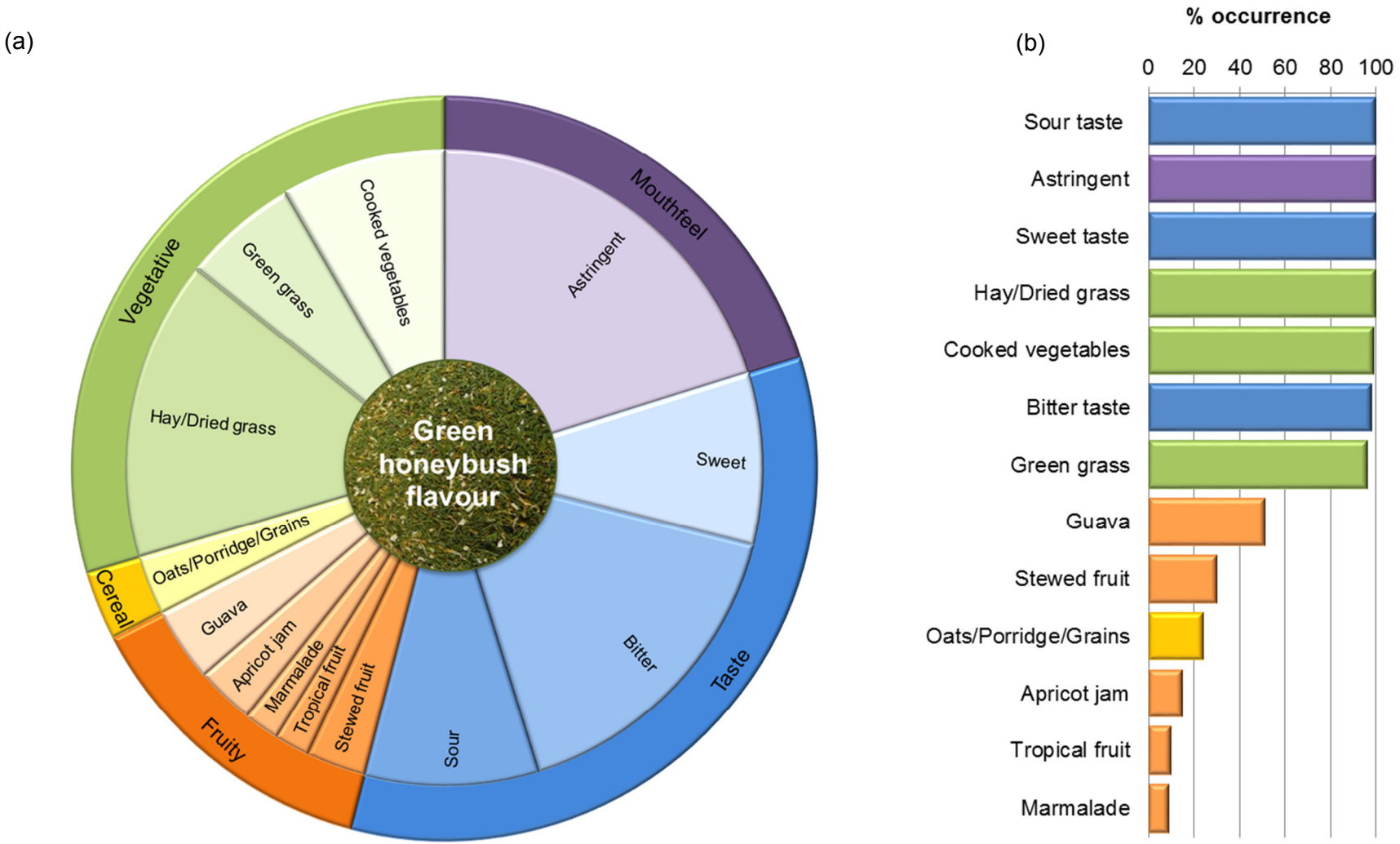


Figure 5.6 Green honeybush (a) flavour wheel and (b) percentage occurrence of flavour, taste and mouthfeel attributes in the sample set (n = 156) of *C. maculata* and *C. longifolia*.

4. Conclusions

The sensory lexicon with aroma and flavour wheels for green honeybush could be considered a preliminary tool for sensory quality assessment. As attribute intensities are expected to depend on processing parameters and treatments, more studies are required to finely adjust and validate these tools. This tool can also be developed further by adding intensity scales to the respective sensory attributes depicted in the sensory wheel for green honeybush. Changes in attribute intensities with processing and storage seem to be species-dependant. Further research should therefore be conducted to assess the potential of commercial *Cyclopia* spp. with favourable sensory profiles for the production of green honeybush, and to assess the effect of processing and storage on their sensory profiles. By taking into account percentage occurrences and mean intensities of the sensory attributes of green honeybush, the product may be described as having a 'dominant *vegetative* aroma and flavour, prominent *sweet-associated* and slight *fruity* aroma, and sweet and notably bitter tastes and astringent mouthfeel'. Careful selection of *Cyclopia* spp. and the use of optimal processing treatments may, in future, eliminate the need for flavour masking, while retaining the advantage of superior bioactivity when compared to traditional fermented honeybush.

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Chapter 6

General discussion, recommendations and conclusions

An increasing local and global demand for honeybush (*Cyclopia* spp.) herbal tea, combined with limited cultivation necessitates the minimisation of product losses due to poor quality. The situation thus demands the optimisation of honeybush processing and storage. Whilst several studies have dealt with the optimisation of the fermentation process of traditional, fermented honeybush (Du Toit & Joubert, 1999; Theron, 2012; Erasmus, 2015; Bergh, 2014), the optimisation of green (unfermented) honeybush production and processing has been largely neglected (Joubert *et al*., 2011). This is partly due to the small market share held by green honeybush, despite its superior health-promoting bioactive content, compared to that of the traditional fermented product (Joubert *et al*., 2008a,b). A major shortcoming of the green honeybush product is its prominent *vegetative* aroma and flavour, as opposed to the '*floral*, *sweet-associated, fruity, plant-like* and *woody*' aroma of the fermented product (Theron *et al*., 2014). This *vegetative* aroma and flavour is generally not well accepted by some consumers, especially if exposure to green tea or similar products has been minimal. For this reason, green honeybush is commonly sold as a flavoured herbal tea, *e.g.* with added berry or mint flavours, in order to mask *vegetative* aromas and flavours, and increase acceptability of the product. Global growth of the herbal and green tea market sectors (Wesgro, 2012), however, indicate increased acceptability of these products with improved consumer awareness of the health-promoting potential of these beverages. Green honeybush production is also less energy-intensive than that of fermented honeybush, which requires long exposure of the plant material to high temperatures (80 °C/24 h or 90 °C/16 h) to develop the dark brown leaf colour and characteristic aroma and flavour (Joubert *et al*., 2011).

A study on the indigenous South African rooibos (*Aspalathus linearis*) herbal tea demonstrated that steam treatment of the dry product could be used to modulate its sensory profile, especially decreasing intensities of 'green' and 'hay' notes in lower quality grades (Koch *et al*., 2013). A previous study on *C. subternata* demonstrated that steam treatment of fresh plant material could be effective to prevent detrimental changes to the green colour and bioactive content of the green honeybush product (Joubert *et al*., 2010). This study, however, did not investigate the effect of steam treatment on the aroma and flavour of green *C. subternata*. A preliminary study was carried out to determine the feasibility of steam treatment (short exposure to moist heat) to improve the aroma and flavour profile of green honeybush. Results indicated that, on the one hand, *C. maculata* and *C. longifolia* both presented prominent 'guava' and 'apricot jam' aromas as a result of steaming, whereas the intensity of 'marmalade' aroma in *C. intermedia* increased compared to untreated plant material. *Cyclopia subternata*, on the other hand, experienced the increase of prominent unpleasant *taints* or negative attribute intensities, such as 'seaweed' and 'animal-like' aromas. This species was thus not considered a likely candidate for the production of good quality green honeybush (unpublished results). The same preliminary study also assessed the effect of pan-roasting (short exposure to dry heat) on the aroma and flavour profile of dried, green honeybush using the aforementioned species. The development of *nutty* ('peanut', 'cashew nut' and 'pine nut') or 'woody' and *sweet-associated* aromas were noted for shorter roasting periods, whereas longer periods

resulted in 'burnt', 'smoky' or 'tobacco' notes (unpublished results). These *nutty*, 'smoky' and 'tobacco'-type attributes are common in *Camellia sinensis* green teas (Lee & Chambers, 2007), and may be useful as an alternative processing method for green honeybush exports to countries in Asia, where these flavours are considered positive in high quality green teas. These treatments, however, resulted in major losses of green colour and led to brown colour development. Loss of green colour indicated chlorophyll breakdown (Lafeuille *et al*., 2014) and/or extensive brown pigment formation due to oxidation of phenolics. Pan-roasting was thus not pursued further in the present investigation.

For the final selection of *Cyclopia* spp. for the current study, differences in leaf shape and phenolic composition were taken into account, as these factors may affect susceptibility to colour change and changes in bioactive content. Not only do *C. maculata* and *C. longifolia* differ in phenolic composition (Schulze *et al*., 2014; Schulze *et al*., 2015), but they represent the spectrum of honeybush leaf shapes with thin, needle-like and linear, flat, oblong leaves, respectively. These differences in leaf shape have implications with regard to thermodynamic heat and mass transfer (Lienhard & Lienhard, 2008).

The current study, therefore, aimed to provide insight into the effects of steam treatment as a processing method on the sensory profile of green honeybush. Desirable changes to the aroma profile should, however, not compromise colour and bioactive content. The shelf-life stability of steam-treated plant material was also investigated as changes could be initiated during steaming, but may not yet be detectable when the product is analysed soon after processing. Phenolic oxidation, for example, is a free radical process, and once initiated, will continue under favourable conditions to form coloured products from colourless intermediates (as reviewed by Tanaka *et al*., 2010). The suitability of steaming as a potential processing step of green honeybush was assessed in terms of its effect on the sensory profile of the infusion as well as the changes introduced to colour and bioactive content of the infusion and/or plant material.

The first objective of the study was to determine the effects of steam treatment on the fresh plant material of *C. maculata* and *C. longifolia* as well as their green honeybush products. This presented two scenarios, *i.e.* one where the fresh plant material was steamed directly after shredding, and one where the dried herbal tea product as currently produced for the herbal tea market, was steam-treated. The latter process is especially relevant if the tea needs to be steam-treated to ensure microbial safety, as for rooibos (Koch *et al*., 2013) and *Lippia multiflora* herbal teas (Arthur *et al*., 2011a,b). Based on the outcome of the first experiment, *C. maculata* was selected to investigate the effect of storage on the shelf-life stability of green honeybush, steamed prior to drying. The third and final objective was to collate the data to create a preliminary sensory quality assessment tool (*i.e.* sensory wheel) to represent the sensory profile of green honeybush. The various methods of analysis applied during the course of the study included descriptive sensory analysis (DSA), high-performance liquid chromatography (HPLC), colour measurement in terms of the CIE*L*a*b** colour space, spectrophotometric determination of total chlorophyll (TC) content and gravimetric determination of the total soluble solids (TSS) content of the infusions and/or plant

material. As part of a preliminary study, tentative identification and quantification of volatiles by gas chromatography-mass spectrometry (GC-MS) of the volatile fraction of infusions were performed. These analytical procedures were employed to assess the varying aspects of green honeybush quality or to provide insight into changes taking place as a result of treatment and/or storage.

Steam treatment lasting at least 60 s, especially when applied to the shredded, fresh plant material, was found to be useful for the manipulation of the sensory profile of green honeybush. This could produce a marketable product with a less prominent *vegetative* aroma and flavour character when compared to the untreated plant material. Steaming also enhanced the intensity of *fruity* and 'fruity-sweet' notes, while retaining green colour and bioactive compound content. Sub-optimal steaming (of 30 s for *C. maculata*, but not for *C. longifolia*) was shown to trigger detrimental changes in product colour and bioactive content, possibly as a result of insufficient heat penetration for the inactivation of enzymes, as affected by leaf shape. This highlights the need for systems control during processing to prevent product loss due to poor quality. Steam treatment of the dried, herbal tea product resulted in comparably less prominent changes in the sensory profile than what was observed for the fresh plant material.

Furthermore, the two *Cyclopia* spp. investigated produced green honeybush of acceptable sensory quality and green colour, with *C. maculata* apparently more responsive to steam treatment than *C. longifolia* in terms of sensory manipulation. The latter species, due to the inherent colour of the leaf, produced herbal tea with a slightly greener colour than *C. maculata*. Consumer sensory preference studies may provide further insight into market opportunities and consumer profiling which may prove useful for selection of and processing of a specific species for the market. For this reason, future species-specific investigations, as previously conducted on the sensory profiles of fermented honeybush (Theron, 2012; Erasmus, 2015) may expand market opportunities as well as provide useful guidelines for blending of various species as is currently practiced with various outcomes.

The variation in sensory profiles and perceived aroma between species as well as between different treatments may indicate differences in volatile compound composition. It has been established that the volatile composition of green honeybush differs quantitatively between species (Cronje, 2010; Le Roux *et al*., 2008). The current preliminary investigation on infusions of *C. maculata* samples that were steam-treated before drying identified 19 volatile compounds that differed quantitatively between treatments. These differences in concentrations may cause changes in aroma perception, which is dependent on the volatility, concentration, threshold level at which a specific compound becomes perceptible as well as combinations with other volatiles (Sikorski *et al*., 2007; Jackson, 2009; Bott & Chambers, 2006; Hongsoongnern & Chambers, 2008). In the current study, principal component analysis (PCA) indicated close association of unsteamed samples with *vegetative* aroma descriptors obtained by DSA, and seven volatile compounds associated with 'herbaceous' or 'vegetative' aromas as determined by GC-MS. This suggests that short exposure to steam provides sufficient heat for the subsequent loss of some highly volatile compounds

contributing to the *vegetative* aroma and flavour of the herbal infusions. Further investigation of the effects of green honeybush processing on its volatile compound composition may prove useful for understanding the scope and extent of the impact of processing conditions on the aroma profile of the herbal tea product.

Honeybush is currently subject to much variation with regard to the period of bulk storage and conditions before packaging and during retail distribution. An understanding of the factors which play a role in deterioration of quality may thus be of great value when determining storage conditions in warehouses or during transport. The present study established that storage conditions (temperature and humidity) have a major impact on green honeybush quality. Low temperature storage (LTS) conditions without exposure to ambient humidity (impermeable metalised pouches at 0 °C) did not greatly alter product quality over the 6 month storage period. Normal temperature storage (NTS) conditions (semi-permeable BOPP sachets stored at 25 °C and 65% relative humidity (RH)) led to significant increases ($p < 0.05$) in the intensities of *fruity* and *sweet-associated* sensory attributes and decreases in the intensities of *vegetative* and *cereal* attributes of infusions, emphasised to a greater extent in unsteamed samples. Progressive losses of green colour of the plant material were also observed, especially in steamed samples. Changes incurred to individual phenolic compounds over time were not severe. The sensory quality of green honeybush, especially unsteamed plant material, may thus well improve over storage time, although steaming may be more beneficial as a treatment for green honeybush with short storage periods. It is thus recommended that steam treatment of green honeybush takes place directly before packaging and shipment for retail, if the storage period is short (less than three months). Alternatively, re-evaluation of the sensory profile after bulk storage may indicate whether steam treatment before packaging is necessary, especially if the untreated product has already been stored for many months under storage conditions conducive to desirable changes in the sensory profile. However, it has yet to be established what the effect of steaming on the dry tea material would be after a considerable period of storage, as decreases in desirable *fruity* and *sweet-associated* aroma attribute intensities might occur.

It is vital, however, that more species be investigated to further validate these findings with other sensory profiles. Additionally, the effects of high temperature storage (HTS) conditions (BOPP sachets stored at 40 °C and 75% RH) after 1 month proved similar to accumulated changes in product quality for NTS conditions over the 6 month period. The development of ‘accelerated’ storage conditions, might thus be possible, and would be of great use for the estimation of shelf-life and changes that might be expected to take place during normal strorage conditions. It may be useful in future to assess the effects of temperature alone on the samples by storing them in sealed, moisture impermeable pouches.

PCA indicated that instrumental parameters, especially colour, distinguished between the effects of both steam treatments and storage of green honeybush. Strong correlations between colour parameters (a^*, hue and ΔE^*) of plant material implicated the loss of green colour as the

contributing factor to observed colour changes. The strong correlations between these parameters and TC content indicated that the loss of chlorophyll is responsible for the loss of green colour, an attribute associated with high quality green tea. Furthermore, the inverse relationship between moisture and TC content as well as the microbial degradation of samples stored under HTS conditions emphasised the importance of preventing moisture uptake during storage. In order to retain green colour, precautions to prevent chlorophyll losses, such as inactivation of chlorophyllase (Schwartz *et al*., 2008), prevention of moisture uptake (LaJollo *et al*., 1971) and minimal exposure to light (Schwartz *et al*., 2008) should thus be employed. Both prevention of moisture uptake and light exposure may be achieved by effective packaging precautions such as the use of metalised pouches that are both impermeable to moisture and light. Highly impermeable packaging such as metal-laminated pouches or lacquered metal tins commonly used for high-end packaging, however, may be very expensive and not suitable for all markets. Investigations of less expensive, but effective packaging materials may thus be useful. For example, plastic covered cardboard boxes (as is commonly used), thicker plastic or laminated plastic re-sealable pouches or plastic-lined cardboard packets may present cheaper options to the industry without incurring product losses due to degradation during storage. Further studies to understand the influence of moisture permeability of packaging materials as well as temperature and light exposure may reveal effective measures for maintaining or even improving product quality after production. The loss of green colour during storage, however, may not necessarily be detrimental to product quality if the product is packed in tea bags, as it will not be seen by the consumer.

Neither steam treatment, nor storage had a notable impact on taste and mouthfeel attributes, although variation between groups of sourced plant material were observed, manifested to a large degree in the excessive differences of bitterness between infusions. As discussed in Chapter 3, variation between groups of plant material may be the result of differences in genetic composition, harvesting location, season as well as other factors which could affect plant composition. Apart from two studies on the variation in phenolic content of honeybush, especially xanthone content with harvest time (Joubert *et al*., 2003; Joubert *et al*., 2014) limited data are available for the respective *Cyclopia* species. Despite this, links have been established between the taste and mouthfeel modalities and the concentration of individual phenolic compounds in fermented honeybush infusions (Erasmus, 2015). *Cyclopia maculata* harvested for the present study in winter at Nietvoorbij in Stellenbosch, had a very low average bitterness intensity (*ca.* < 10), whereas material harvested in autumn at Koksriver in the Pearly Beach area had a high average bitterness intensity (*ca*. 20). For the third experiment (Chapter 4) high bitterness intensities (*ca*. 30) that were not greatly affected by treatments, were once again observed in green *C. maculata*. This plant material was also harvested at Koksriver, but in winter, indicating that the inherent bitterness of the plant material was perhaps affected by the genetic composition and/or location, and not necessarily by harvest season. Few significant ($p < 0.05$) correlations between phenolic compounds and taste and mouthfeel attributes (results not shown) indicate that the currently quantified individual phenolic compounds are not the

sole drivers of the sensory perception of taste and mouthfeel in green honeybush. Furthermore, these variables were subject to much variation in the inherent composition of the plant material and not greatly affected by treatments.

As observed by the sensory panel in the current research, a bitterness intensity of > 20 is regarded as reasonably high, and may thus potentially be unacceptable to some consumers. However, there is no current information available on the consumer acceptance of sensory quality of honeybush, and studies to determine the drivers of liking will be of great use for the development of optimum sensory quality standards. Further insight into the factors which contribute to the sensory perception and thus consumer acceptance or purchase value of the product may provide alternative methods of processing to improve the sensory quality of green honeybush further. For example, assessment of amino acids present in infusions (Solms, 1969) may be helpful in understanding the mechanism of bitter as well as sweet taste in honeybush. Indeed, two aromatic amino acids have recently been tentatively identified in *C. genistoides* (Beelders *et al.*, 2014). The role of amino acids in the taste of traditional *C. sinensis* teas have been demonstrated (Yu *et al.*, 2014; Ekborg-Ott *et al.*, 1997).

No major changes were detected as a result of storage for individual phenolic content in the present study, except for some slight decreases. However, although measures were taken to ensure reasonable homogeneity of sub-samples, natural variation due to the nature of the plant material could not be eliminated (Chapter 4). Furthermore, with the degradation of samples stored under HTS conditions for 1 month, the experimental setup entailing four batches and four treatments resulted in more complex results as differences were not as easily identified as statistically different. Notable sensory changes and green colour loss could be observed, but phenolic degradation may have been underestimated. For a previous study on *C. subternata*, a high degree of homogeneity was achieved by using milled plant material in the storage experiment so that significant treatment effects could be demonstrated (Joubert *et al.*, 2010). It is possible, however, that the storage of milled honeybush material may not accurately describe changes in the unmilled material, as the same trends were not observed in lightness (*L**) between colour measurements of milled and unmilled plant material in the current study (Chapter 3). For this reason, further investigation may be necessary for validation of the current results for the effect of storage on the phenolic composition of green honeybush by using more batches, and possibly more species, as noted before. It has been mentioned in a review by Irina and Mohamed (2012) that a loss in phenolic content is not a direct indication of a loss in antioxidant activity. In fact, breakdown products may possess even higher antioxidant activity than their phenolic precursors. It is thus suggested, due to the health-promoting effects associated with green honeybush and the interest of niche markets in these possibilities, that the effects of steam treatment and storage on antioxidant activity be evaluated, even if only to confirm that no significant decrease in antioxidant capacity is introduced. If steaming results in an enhanced antioxidant capacity, the use of steam treatment for the production of green honeybush will be of even greater

value for both the production of green honeybush as a beverage or for extracts production for nutraceutical use.

The availability of the sensory data generated during the experiments on the effects of steam treatment and storage on green honeybush quality motivated the development of a sensory wheel and lexicon for green honeybush. The sensory wheels and lexicons developed for fermented honeybush (Theron *et al*., 2014; Erasmus, 2015; Bergh, 2014) are not suitable for green honeybush as the characteristic prominence of the *vegetative* aroma and flavour and bitter taste in green honeybush are considered negative attributes in fermented honeybush. The prominent *sweet-associated* and slight *fruity* aroma, notable sweet taste, and the astringent mouthfeel of green honeybush are attributes also characteristic of fermented honeybush. The two, two-tiered aroma and flavour wheels for green honeybush were generated, incorporating *vegetative* ('green grass', 'cooked vegetables' and 'hay/dried grass'), *cereal* ('oats/porridge/grains'), *fruity* ('stewed fruit', 'apricot jam', 'tropical fruit', 'guava' and 'marmalade') and *sweet-associated* ('general sweet', 'fruity-sweet' and 'caramel') attributes. The taste modalities, sour, sweet and bitter taste as well as astringent mouthfeel were also included in the flavour wheel.

The sensory wheels developed in the current study provide the green honeybush industry with a valuable tool, albeit in its preliminary stages, for the assessment of sensory quality. The lexicon provides assessors with a defined vocabulary that can be used to describe and quantify sensory attributes associated with green honeybush. These tools are equally valuable for research-based investigations for further processing optimisation and establishing parameters for green honeybush quality criteria.

The susceptible nature of the sensory profile of green honeybush to inter- and intra-species variation and processing indicates that these wheels and lexicon may require adaptation in future to fully reflect the development of other aroma and flavour attributes. The current preliminary sensory wheels represent overall mean attribute intensity of an attribute in the current dataset and is therefore subject to adjustments, depending on the method and intensity of processing or storage. Subsequently, these sensory wheels would require validation and further investigation to fully capture the entire spectrum of sensory characteristics of green honeybush. As mentioned previously, species variation and alternative processing methods may be investigated to present a better overview of green honeybush aroma and flavour. Furthermore, the presented lexicon lacks defined reference standards for all attributes which are required for universal sensory anchoring. This may be further refined for the development of an industry training flavour kit as is currently under development for fermented rooibos as well as honeybush.

Considering the variation and progressive effects of treatments investigated, this study represents a preliminary investigation into the effects of steam treatment and storage on green honeybush. The results and insights gained indicate that a wealth of valuable information may yet be gained from further investigation in order to optimise the production process of green honeybush for consumption as a beverage, extract or flavour ingredient.

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ADDENDUM A

Supplementary results pertaining to Chapter 3

Table A.1 List of all assessed aroma and flavour attributes in the descriptive sensory analysis of green honeybush

Attributes	Descriptors
'Floral/Plant-like'	Fresh, slightly floral aroma of fresh green plant leaves or shrub, for example, that of fynbos or malva plants
'Green grass'	Aromatic associated with fresh or stale cut green grass
'Cooked vegetables'	An overall aroma note associated with cooked green vegetables or green beans
'Cooked green beans and/or Potato'	Aroma associated with cooked processed green beans and/or cooked green beans and potato
'Green herbs'	Aromatics associated with fresh green herbs
'Cooked spinach'	Green, slightly metallic aromatics associated with cooked spinach
'Stewed fruit'	Sweet, syrup-like aroma of stewed fruit such as peach, raisins, apples and prunes
'Tropical fruit'	A sweet, aromatic blend, reminiscent of a variety of fresh ripe tropical fruits for example, pineapple, mango or granadilla
'Marmalade'	Sweet aromatic reminiscent of citrus fruits (lemon, orange) or marmalade
'Apricot jam'	Sweet aroma or flavour reminiscent of apricot jam
'Cooked apple'	The flat, slightly sour aroma of cooked apples
'Guava'	Aroma reminiscent of fresh, over-ripe or juiced guava
'Hay/Dried grass'	Slightly sweet aromatics associated with dried grass or hay
'Oats/Porridge/Grains'	An overall grain or porridge impression typical of oats or ProNutro™ original (raw or cooked)
'General sweet'	Non-specific sweet aroma or impression
'Fruity-sweet'	Sweet, acidic aromatic reminiscent of non-specific fruit especially berries and apricot jam
'Caramel'	Sweet aromatic characteristic of molten sugar or caramel pudding
'Honey'	Aromatics associated with the sweet fragrance of fynbos honey
'Dusty'	Earthy aromatic associated with dry dirt roads
'Musty'	Earthy aromatic associated with damp hessian or cardboard
'Seaweed/Oceanic'	Pungent fishy or seaweed-like aroma

Attributes assessed on 100-point line scale with 0 = low and 100 = prominent. 'Fynbos' refers to the indigenous group of plants growing in the Cape Floral Kingdom and Fynbos Biome of South Africa characterised by a diverse species range of aromatic heather-like trees or shrubs.

Table A.2 Reference standards for selected attributes (Experiment 1)

Attributes	Reference standards
Cooked vegetables	30 g raw spinach (rinsed, chopped) in 400 mL boiling dH_2O for 10 min, serve water and a leaf piece
Stewed fruit	150 g mixed dried fruit pack [Montagu standard grade mixed dried fruit] (apple, prune, peach, raisin – remove pear and apricot) chopped, boiled in 300 mL dH_2O on stove for 15 min
Tropical fruit	Granadilla juice (Woolworths) diluted with dH_2O (x 6 dilution), served with two $1cm^3$ pieces of fresh pineapple
Marmalade	5 mL Orange marmalade (All Gold Seville Orange Connoisseur's Fruit Marmalade) in 200 mL warm dH_2O
Guava	Whole guava (over-ripe)
Oats/Porridge/Grains	90 mL instant oats (Woolworths) in 300 mL boiling dH_2O

Table A.3 Pearson's (n-1) correlation matrix for PCA of STBD green *C. maculata* sensory attributes

	A_GG	A_CV	A_CGB	A_SF	A_TF	A_AJ	A_G	A_HDG	A_OPG	A_GSA	A_FS	A_C	S	B	AST	SWT	F_GG	F_CV	F_CGB	F_HDG	F_OPG
A_GG	**1**	**0.820**	**0.784**	-0.010	**-0.486**	-0.036	**-0.584**	**0.729**	**0.746**	**0.422**	**-0.476**	**0.586**	-0.318	0.041	-0.365	0.177	**0.770**	0.322	0.106	0.276	**0.623**
A_CV	**0.820**	**1**	**0.591**	-0.122	**-0.408**	-0.290	**-0.459**	**0.551**	**0.518**	0.346	**-0.461**	0.289	-0.320	0.228	-0.131	-0.058	**0.743**	0.314	-0.009	0.036	0.351
A_CGB	**0.784**	**0.591**	**1**	0.051	**-0.400**	0.190	**-0.529**	**0.671**	**0.854**	**0.651**	**-0.463**	**0.717**	**-0.465**	-0.136	**-0.413**	0.301	**0.606**	0.228	0.297	**0.404**	**0.657**
A_SF	-0.010	-0.122	0.051	**1**	**-0.529**	0.113	**-0.588**	0.202	0.280	**0.477**	**-0.452**	**0.401**	-0.315	**-0.429**	-0.129	0.106	0.161	-0.298	-0.055	0.279	0.368
A_TF	**-0.486**	**-0.408**	**-0.400**	**-0.529**	**1**	0.168	**0.915**	**-0.641**	**-0.528**	**-0.622**	**0.924**	**-0.553**	**0.643**	**0.404**	0.392	-0.307	-0.287	-0.304	0.331	-0.094	**-0.567**
A_AJ	-0.036	-0.290	0.190	0.113	0.168	**1**	0.026	0.013	0.084	0.103	0.142	**0.412**	-0.246	**-0.574**	**-0.464**	**0.397**	-0.092	-0.213	0.051	0.063	0.262
A_G	**-0.584**	**-0.459**	**-0.529**	**-0.588**	**0.915**	0.026	**1**	**-0.636**	**-0.635**	**-0.737**	**0.828**	**-0.663**	**0.673**	0.379	0.351	-0.223	**-0.439**	-0.276	0.164	-0.244	**-0.678**
A_HDG	**0.729**	**0.551**	**0.671**	0.202	**-0.641**	0.013	**-0.636**	**1**	**0.747**	**0.603**	**-0.628**	**0.604**	**-0.496**	-0.320	-0.341	**0.442**	**0.463**	0.291	-0.218	0.301	**0.756**
A_OPG	**0.746**	**0.518**	**0.854**	0.280	**-0.528**	0.084	**-0.635**	**0.747**	**1**	**0.783**	**-0.553**	**0.789**	**-0.454**	-0.198	**-0.467**	0.388	**0.553**	0.083	0.239	**0.462**	**0.839**
A_GSA	**0.422**	0.346	**0.651**	**0.477**	**-0.622**	0.103	**-0.737**	**0.603**	**0.783**	**1**	**-0.652**	**0.613**	**-0.512**	-0.356	-0.353	0.245	0.384	0.024	0.093	0.385	**0.722**
A_FS	**-0.476**	**-0.461**	**-0.463**	**-0.452**	**0.924**	0.142	**0.828**	**-0.628**	**-0.553**	**-0.652**	**1**	**-0.484**	**0.667**	0.384	0.347	-0.352	-0.228	-0.307	0.352	-0.210	**-0.538**
A_C	**0.586**	0.289	**0.717**	**0.401**	**-0.553**	**0.412**	**-0.663**	**0.604**	**0.789**	**0.613**	**-0.484**	**1**	**-0.601**	**-0.458**	**-0.630**	**0.483**	**0.404**	-0.021	0.125	0.164	**0.762**
Sour	-0.318	-0.320	**-0.465**	-0.315	**0.643**	-0.246	**0.673**	**-0.496**	**-0.454**	**-0.512**	**0.667**	**-0.601**	**1**	**0.523**	**0.426**	**-0.515**	-0.094	-0.144	0.294	0.017	**-0.500**
Bitter	0.041	0.228	-0.136	**-0.429**	**0.404**	**-0.574**	0.379	-0.320	-0.198	-0.356	0.384	**-0.458**	**0.523**	**1**	**0.619**	**-0.723**	0.216	0.097	**0.404**	-0.036	**-0.568**
Astr	-0.365	-0.131	**-0.413**	-0.129	0.392	**-0.464**	0.351	-0.341	**-0.467**	-0.353	0.347	**-0.630**	**0.426**	**0.619**	**1**	**-0.685**	-0.088	0.104	-0.057	0.119	**-0.597**
Sweet	0.177	-0.058	0.301	0.106	-0.307	**0.397**	-0.223	**0.442**	0.388	0.245	-0.352	**0.483**	**-0.515**	**-0.723**	**-0.685**	**1**	-0.204	-0.114	-0.334	0.092	**0.616**
F_GG	**0.770**	**0.743**	**0.606**	0.161	-0.287	-0.092	**-0.439**	**0.463**	**0.553**	0.384	-0.228	**0.404**	-0.094	0.216	-0.088	-0.204	**1**	0.041	0.220	0.217	0.375
F_CV	0.322	0.314	0.228	-0.298	-0.304	-0.213	-0.276	0.291	0.083	0.024	-0.307	-0.021	-0.144	0.097	0.104	-0.114	0.041	**1**	-0.224	-0.040	0.062
F_CGB	0.106	-0.009	0.297	-0.055	0.331	0.051	0.164	-0.218	0.239	0.093	0.352	0.125	0.294	**0.404**	-0.057	-0.334	0.220	-0.224	**1**	0.234	-0.078
F_HDG	0.276	0.036	**0.404**	0.279	-0.094	0.063	-0.244	0.301	**0.462**	0.385	-0.210	0.164	0.017	-0.036	0.119	0.092	0.217	-0.040	0.234	**1**	0.372
F_OPG	**0.623**	0.351	**0.657**	0.368	**-0.567**	0.262	**-0.678**	**0.756**	**0.839**	**0.722**	**-0.538**	**0.762**	**-0.500**	**-0.568**	**-0.597**	**0.616**	0.375	0.062	-0.078	0.372	**1**

'A' and 'F' prefixes refer to aroma and flavour attributes, respectively. 'GG' = green grass, 'CV' = cooked vegetables, 'CGB' = cooked green beans and/or potato, 'ST' = stewed fruit, 'TF' = tropical fruit, 'AJ' = apricot jam, 'G' = guava, 'HDG' = hay/dried grass, 'OPG' = oats/porridge/grains, 'GSA' = general sweet, 'FS' = fruity-sweet, 'C'= caramel, 'S' = sour taste, 'B' = bitter taste, 'AST' = astringent, 'SWT' = sweet taste. Values in bold are different from 0 with a significance level of α = 0.05.

Table A.4 Aroma attributes of STBD green *C. maculata*

Steaming time (s)	Geranium leaf					Green grass					Cooked vegetables					Cooked green beans and/or potato				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	3.29	ab	1.37	1.27	5.03	13.66	a	2.44	10.42	16.78	14.15	a	1.03	12.68	15.53	13.61	a	1.15	11.97	15.20
30	2.77	b	1.09	1.37	3.87	10.09	b	1.72	7.28	11.52	11.99	b	1.46	10.03	13.60	12.36	b	1.00	11.08	13.47
60	3.01	ab	1.15	1.17	3.93	7.09	c	1.47	4.81	8.73	11.13	b	1.25	8.95	12.05	9.13	c	1.19	8.20	11.08
90	3.35	ab	0.53	2.78	3.93	7.03	c	0.85	5.63	8.73	11.09	b	1.09	9.67	12.05	9.85	c	1.31	8.55	11.08
120	3.98	a	0.99	2.83	5.30	6.53	c	1.52	4.48	8.37	11.03	b	1.64	9.16	12.95	9.36	c	0.54	8.45	9.83
Steaming time (s)	Green herbs					Cooked spinach					Hay/Dried grass					Oats/Porridge/Grains				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	1.10	a	0.48	0.67	1.87	2.37	bc	1.52	1.00	4.41	20.39	a	0.96	19.32	21.68	17.05	a	3.72	13.02	23.05
30	0.92	a	0.72	0.00	1.58	2.02	c	1.04	0.69	3.55	19.51	a	1.16	18.36	21.34	15.67	a	1.43	13.55	17.48
60	0.53	a	0.47	0.00	1.20	3.37	ab	0.65	2.57	4.17	16.67	b	0.86	15.28	17.42	9.19	b	1.80	6.28	11.00
90	1.12	a	0.51	0.48	1.20	4.28	a	1.19	3.16	4.17	17.42	b	0.66	16.52	17.42	7.38	b	2.11	4.53	11.00
120	0.95	a	0.38	0.50	1.47	4.00	a	1.11	2.78	5.78	17.25	b	0.82	16.26	18.25	7.20	b	2.02	4.19	9.87
Steaming time (s)	Stewed fruit					Tropical fruit					Marmalade					Apricot jam				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	10.48	abc	0.94	9.25	11.78	4.11	cd	0.88	3.03	5.12	0.98	a	0.48	0.25	1.48	10.69	a	1.51	9.63	13.37
30	11.72	a	1.68	10.26	14.22	2.30	d	1.16	0.55	3.80	1.09	a	0.49	0.67	1.83	11.06	a	2.00	8.10	12.67
60	11.44	ab	1.85	9.28	13.70	6.29	bc	3.97	1.35	10.91	1.30	a	0.78	0.25	2.08	10.58	a	1.37	8.67	12.47
90	9.62	c	1.41	8.05	13.70	9.09	a	2.79	6.85	10.91	1.32	a	0.98	0.15	2.08	10.92	a	1.39	9.05	12.47
120	10.02	bc	1.62	8.43	12.10	8.15	ab	2.28	6.52	11.73	1.45	a	1.16	0.52	3.41	10.67	a	1.19	8.88	12.12
Steaming time (s)	Cooked apple					Guava					General sweet					Fruity-sweet				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	1.00	bc	0.85	0.35	2.42	4.72	c	1.33	2.95	6.43	13.52	a	2.19	11.50	16.95	7.23	c	1.67	6.36	10.21
30	1.70	a	0.80	0.85	2.83	3.46	c	1.16	1.85	4.63	13.93	a	1.63	12.05	15.33	6.90	c	1.27	5.76	8.95
60	1.23	ab	0.50	0.67	1.88	8.65	b	4.32	2.83	14.26	10.89	b	1.74	9.40	13.87	9.14	bc	2.85	5.63	12.59
90	0.28	d	0.20	0.00	1.88	11.36	a	1.91	9.38	14.26	9.72	b	0.83	8.80	13.87	12.34	a	2.73	9.33	12.59
120	0.52	cd	0.32	0.00	0.83	11.26	ab	2.82	9.66	16.22	9.61	b	1.81	7.28	11.88	11.32	ab	2.62	8.38	15.23
	Caramel					Honey					Dusty					Musty				

Steaming time (s)	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	10.99	b	1.89	8.12	12.98	0.13	a	0.29	0.00	0.65	0.62	a	0.57	0.00	1.16	0.91	a	0.33	0.67	1.29
30	14.42	a	3.23	9.34	16.86	0.27	a	0.22	0.00	0.60	1.14	a	0.66	0.18	1.75	0.32	b	0.46	0.00	1.00
60	6.99	c	2.71	4.26	11.42	0.23	a	0.32	0.00	0.67	0.80	a	0.54	0.23	1.40	0.48	ab	0.40	0.00	0.98
90	7.12	c	2.24	5.32	11.42	0.12	a	0.17	0.00	0.67	0.95	a	0.97	0.33	1.40	0.92	a	0.42	0.34	0.98
120	4.35	d	1.94	2.35	7.25	0.23	a	0.28	0.00	0.67	0.59	a	0.30	0.18	1.03	0.61	ab	0.50	0.00	1.18

Steaming time (s)	Seaweed/Oceanic				
	Mean		SD	Min	Max
0	0.36	a	0.32	0.00	0.83
30	0.25	a	0.47	0.00	1.09
60	0.34	a	0.28	0.00	0.71
90	0.63	a	0.59	0.00	0.71
120	0.27	a	0.25	0.00	0.67

Treatment means corresponding with the same letters do not differ significantly ($p \geq 0.05$). Mean = mean intensity treatment value, SD = standard deviation, Min = minimum batch treatment mean, Max = maximum batch treatment mean. Attributes coloured in red = means < 5, yellow = means > 5 and do not differ significantly ($p \geq 0.05$), green = means > 5 and differ significantly ($p < 0.05$).

Table A.5 Taste and mouthfeel attributes of STBD green *C. maculata*

Steaming time (s)	Sour taste					Bitter taste					Astringent					Sweet taste				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	17.15	bc	0.81	16.03	18.12	24.11	a	3.50	19.27	27.48	32.69	bc	0.97	31.07	33.47	15.75	ab	1.38	13.41	16.98
30	16.44	c	1.18	15.41	18.31	21.84	b	3.05	19.10	27.05	31.62	c	1.78	28.57	33.18	16.22	a	1.11	14.43	17.27
60	17.53	ab	0.88	16.48	18.88	24.47	a	2.34	20.95	27.53	33.41	ab	1.05	31.95	34.92	15.11	bc	1.39	12.84	16.21
90	18.52	a	0.68	17.75	18.88	25.31	a	2.33	22.82	27.53	34.37	a	0.74	33.10	34.92	14.40	c	0.74	13.67	16.21
120	18.35	a	0.84	17.55	19.55	24.36	a	2.37	21.93	27.93	33.02	b	1.39	30.82	34.31	15.32	b	1.05	13.78	16.70

Treatment means corresponding with the same letters do not differ significantly ($p \geq 0.05$). Mean = mean intensity treatment value, SD = standard deviation, Min = minimum batch treatment mean, Max = maximum batch treatment mean. Attributes coloured in red = means < 5, yellow = means > 5 and do not differ significantly ($p \geq 0.05$), green = means > 5 and differ significantly ($p < 0.05$).

Table A.6 Flavour attributes of STBD green *C. maculata*

Steaming time (s)	Geranium leaves					Green grass					Cooked vegetables					Cooked green beans and/or potato				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	1.07	a	0.19	0.83	1.31	10.10	a	1.69	8.12	12.38	8.59	a	1.02	7.29	9.53	5.93	a	1.98	4.10	9.00
30	0.98	a	0.44	0.50	1.62	8.13	b	1.41	6.47	9.88	7.75	a	1.16	6.75	9.60	6.37	a	1.38	5.20	8.38
60	1.21	a	0.55	0.48	1.83	7.11	b	0.78	5.93	8.07	7.17	a	1.37	5.57	9.00	5.82	a	1.79	3.22	7.38
90	1.33	a	0.75	0.33	1.83	7.48	b	1.00	6.58	8.07	7.74	a	1.06	6.60	9.00	6.31	a	2.13	4.68	7.38
120	1.21	a	0.32	0.89	1.57	6.75	b	1.50	5.07	9.00	7.40	a	2.01	4.59	9.60	6.01	a	1.12	4.30	7.36
Steaming time (s)	Green herbs					Cooked spinach					Hay/Dried grass					Oats/Porridge/Grains				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	0.89	a	0.86	0.17	2.37	1.77	b	1.57	0.50	4.28	21.15	a	0.53	20.40	21.83	5.63	a	1.49	3.31	7.00
30	0.52	a	1.00	0.00	2.30	1.31	b	0.74	0.50	2.50	19.89	ab	1.67	17.38	21.25	5.67	a	0.64	4.90	6.43
60	0.57	a	0.53	0.17	1.45	3.02	a	0.89	2.00	3.80	20.06	ab	1.25	18.37	21.55	2.91	b	0.77	1.95	3.88
90	0.68	a	0.42	0.00	1.45	3.57	a	0.40	3.06	3.80	19.51	b	0.96	18.55	21.55	2.39	b	0.65	1.28	3.88
120	0.49	a	0.55	0.00	1.27	3.32	a	0.78	2.14	4.17	19.67	b	0.45	19.03	20.17	2.93	b	0.99	1.82	3.90
Steaming time (s)	Stewed fruit					Tropical fruit					Marmalade					Apricot jam				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	1.73	a	0.70	1.07	2.78	1.54	bc	1.10	0.17	2.76	0.48	a	0.53	0.00	1.28	1.98	a	0.93	1.02	3.10
30	2.50	a	0.70	1.72	3.38	1.12	c	0.47	0.69	1.90	0.44	a	0.34	0.18	1.02	1.99	a	0.98	1.02	3.42
60	2.23	a	0.65	1.21	3.02	2.43	ab	0.79	1.53	3.57	0.89	a	0.72	0.00	1.74	1.88	a	0.25	1.55	2.17
90	2.30	a	0.46	1.80	3.02	2.44	ab	0.55	1.85	3.57	0.59	a	0.65	0.00	1.74	1.67	a	0.45	1.17	2.17
120	1.94	a	0.99	0.88	3.37	3.04	a	1.05	1.91	4.10	0.43	a	0.43	0.00	1.12	1.96	a	0.76	0.78	2.76
Steaming time (s)	Cooked apple					Guava					Fruity-sweet					Caramel				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	0.04	a	0.09	0.00	0.20	2.41	b	1.15	1.00	3.98	1.26	c	0.38	0.65	1.63	2.52	ab	0.71	1.90	3.73
30	0.07	a	0.15	0.00	0.33	2.07	b	0.82	1.28	3.18	2.19	b	0.60	1.42	3.02	3.18	a	0.54	2.47	3.85
60	0.07	a	0.15	0.00	0.33	3.64	a	1.78	2.23	6.67	2.06	bc	0.74	1.20	2.92	2.37	b	1.09	1.67	4.29
90	0.03	a	0.07	0.00	0.33	4.34	a	1.35	3.17	6.67	2.40	ab	0.37	1.83	2.92	1.84	bc	0.23	1.67	4.29
120	0.00	a	0.00	0.00	0.00	4.40	a	0.93	3.10	5.65	3.25	a	1.21	2.10	5.28	1.24	c	0.34	1.00	1.83

Steaming time (s)	Honey					Musty					Seaweed/Oceanic				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	0.10	ab	0.22	0.00	0.50	0.07	b	0.10	0.00	0.20	0.68	a	0.88	0.00	2.17
30	0.03	ab	0.07	0.00	0.17	0.50	a	0.14	0.30	0.67	0.22	a	0.22	0.00	0.52
60	0.00	b	0.00	0.00	0.00	0.23	ab	0.37	0.00	0.85	0.67	a	0.48	0.00	1.28
90	0.00	b	0.00	0.00	0.00	0.37	ab	0.49	0.00	0.85	0.74	a	0.56	0.00	1.28
120	0.20	a	0.22	0.00	0.50	0.21	ab	0.20	0.00	0.45	0.56	a	0.38	0.00	1.00

Treatment means corresponding with the same letters do not differ significantly ($p \geq 0.05$). Mean = mean intensity treatment value, SD = standard deviation, Min = minimum batch treatment mean, Max = maximum batch treatment mean. Attributes coloured in red = means < 5, yellow = means >5 and do not differ significantly ($p \geq 0.05$), green = means > 5 and differ significantly ($p < 0.05$).

Table A.7 Objective colour parameters, total soluble solids (TSS) and total chlorophyll (TC) content of STBD green *C. maculata* plant material and/or infusion

		Steaming time (s)															
		0				30				60				90				120			
Milled	*L**	55.59	a	±	0.90	49.28	d	±	1.97	53.75	c	±	1.48	54.57	b	±	0.84	54.42	bc	±	1.13
	*a**	-4.69	b	±	0.37	-0.21	a	±	1.25	-6.22	c	±	1.00	-6.56	c	±	0.33	-6.15	c	±	0.29
	*b**	32.33	b	±	0.48	31.41	c	±	1.02	34.97	a	±	0.98	35.43	a	±	0.71	35.23	a	±	0.86
	C	32.67	b	±	0.53	31.43	c	±	1.02	35.53	a	±	1.13	36.03	a	±	0.74	35.76	a	±	0.87
	h	98.24	b	±	0.53	90.34	c	±	2.29	100.05	a	±	1.37	100.48	a	±	0.42	99.90	a	±	0.42
	Δ*E**	0.00	c			7.82	a	±	1.54	3.72	b	±	0.26	3.78	b	±	0.33	3.50	b	±	0.29
TC	mg/g	3.37	a	±	0.29	3.18	b	±	0.32	3.09	c	±	0.30	3.19	b	±	0.26	3.11	bc	±	0.31
Leaf	*L**	34.61	b	±	0.88	32.27	c	±	1.55	35.14	ab	±	1.17	35.39	a	±	0.99	35.46	a	±	1.15
	*a**	-1.82	b	±	0.29	0.96	a	±	0.81	-1.75	b	±	0.74	-1.88	b	±	0.41	-1.59	b	±	0.18
	*b**	20.48	b	±	0.68	18.26	c	±	1.11	21.16	a	±	0.77	21.31	a	±	0.62	21.29	a	±	0.71
	C	20.56	b	±	0.70	18.31	c	±	0.92	21.24	a	±	0.81	21.40	a	±	0.65	21.35	a	±	0.71
	h	95.06	a	±	0.69	86.87	b	±	2.76	94.67	a	±	1.93	95.03	a	±	0.97	94.27	a	±	0.46
	Δ*E**	0.00	c			4.32	a	±	1.06	1.19	b	±	0.50	1.35	b	±	0.41	1.32	b	±	0.55
Infusion	*L**	92.58	c	±	0.94	90.48	d	±	1.50	93.95	b	±	1.10	94.62	ab	±	0.56	94.83	a	±	0.44
	*a**	0.27	b	±	0.86	0.98	a	±	0.62	-1.28	c	±	0.98	-1.81	cd	±	0.49	-1.94	d	±	0.39
	*b**	23.98	b	±	2.59	31.67	a	±	4.52	20.97	c	±	3.81	18.71	cd	±	1.97	18.02	d	±	1.54
	C	24.00	b	±	2.60	31.73	a	±	4.61	21.03	c	±	3.76	18.80	cd	±	1.92	18.13	d	±	1.51
	h	89.51	c	±	1.91	87.31	d	±	2.14	93.90	b	±	3.01	95.70	a	±	2.02	96.24	a	±	1.63
	Δ*E**	0.00	c			8.04	a	±	3.98	3.86	b	±	2.03	6.04	ab	±	1.34	6.76	ab	±	1.39
TSS	g/100 mL	0.212	cb	±	0.008	0.208	c	±	0.017	0.225	a	±	0.013	0.224	a	±	0.014	0.218	ab	±	0.019

Means associated with same letters do not differ significantly ($p \geq 0.05$). Values indicated as (mean) ± (standard deviation).

Table A.8 Pearson's (n-1) correlation matrix for STAD green *C. maculata* sensory attributes

	A_GG	A_CV	A_SF	A_AJ	A_G	A_HDG	A_OPG	A_GSA	A_FS	A_C	S	B	AST	SWT	F_GG	F_CV	F_SF	F_G	F_HDG	F_OPG
A_GG	**1**	-0.280	-0.328	-0.375	**-0.653**	**0.648**	**0.626**	-0.195	**-0.797**	**0.811**	0.204	0.298	**0.467**	-0.384	**0.941**	-0.224	**-0.444**	**-0.715**	**0.482**	**0.724**
A_CV	-0.280	**1**	-0.086	0.320	0.358	-0.160	-0.289	**-0.437**	0.272	**-0.476**	**0.447**	0.394	0.156	**-0.431**	-0.275	**0.742**	0.140	0.365	-0.098	**-0.514**
A_SF	-0.328	-0.086	**1**	0.371	0.087	-0.266	-0.079	**0.690**	**0.468**	-0.133	-0.064	-0.289	-0.106	0.124	-0.263	0.011	**0.507**	0.333	**-0.397**	-0.122
A_AJ	-0.375	0.320	0.371	**1**	**0.636**	-0.373	**-0.497**	0.324	**0.607**	-0.353	0.021	-0.144	-0.031	0.075	**-0.407**	0.249	0.256	**0.624**	**-0.417**	-0.308
A_G	**-0.653**	0.358	0.087	**0.636**	**1**	**-0.451**	**-0.561**	-0.066	**0.831**	**-0.618**	0.030	-0.076	-0.073	0.217	**-0.719**	0.353	0.374	**0.781**	-0.278	**-0.562**
A_HDG	**0.648**	-0.160	-0.266	-0.373	**-0.451**	**1**	**0.796**	-0.118	**-0.595**	**0.610**	**0.451**	0.233	**0.630**	-0.140	**0.681**	-0.071	-0.112	**-0.619**	**0.581**	**0.722**
A_OPG	**0.626**	-0.289	-0.079	**-0.497**	**-0.561**	**0.796**	**1**	0.020	**-0.568**	**0.661**	**0.404**	0.353	**0.642**	-0.257	**0.767**	-0.056	-0.032	**-0.637**	**0.575**	**0.692**
A_GSA	-0.195	**-0.437**	**0.690**	0.324	-0.066	-0.118	0.020	**1**	0.241	0.056	**-0.442**	**-0.423**	-0.133	0.320	-0.096	-0.338	0.201	0.115	-0.389	0.139
A_FS	**-0.797**	0.272	**0.468**	**0.607**	**0.831**	**-0.595**	**-0.568**	0.241	**1**	**-0.741**	-0.032	-0.230	-0.121	0.296	**-0.787**	0.301	**0.573**	**0.864**	-0.393	**-0.662**
A_C	**0.811**	**-0.476**	-0.133	-0.353	**-0.618**	**0.610**	**0.661**	0.056	**-0.741**	**1**	0.116	0.222	0.298	-0.281	**0.810**	-0.395	-0.289	**-0.763**	0.242	**0.843**
S	0.204	**0.447**	-0.064	0.021	0.030	**0.451**	**0.404**	**-0.442**	-0.032	0.116	**1**	**0.580**	**0.666**	**-0.470**	0.231	**0.582**	0.264	-0.139	**0.478**	0.074
B	0.298	0.394	-0.289	-0.144	-0.076	0.233	0.353	**-0.423**	-0.230	0.222	**0.580**	**1**	**0.455**	**-0.854**	0.389	**0.636**	-0.108	-0.157	**0.417**	0.007
AST	**0.467**	0.156	-0.106	-0.031	-0.073	**0.630**	**0.642**	-0.133	-0.121	0.298	**0.666**	**0.455**	**1**	-0.312	**0.508**	0.345	0.220	-0.186	**0.547**	0.342
SWT	-0.384	**-0.431**	0.124	0.075	0.217	-0.140	-0.257	0.320	0.296	-0.281	**-0.470**	**-0.854**	-0.312	**1**	**-0.452**	**-0.573**	0.270	0.243	-0.267	-0.032
F_GG	**0.941**	-0.275	-0.263	**-0.407**	**-0.719**	**0.681**	**0.767**	-0.096	**-0.787**	**0.810**	0.231	0.389	**0.508**	**-0.452**	**1**	-0.125	**-0.399**	**-0.725**	**0.498**	**0.723**
F_CV	-0.224	**0.742**	0.011	0.249	0.353	-0.071	-0.056	-0.338	0.301	-0.395	**0.582**	**0.636**	0.345	**-0.573**	-0.125	**1**	0.214	0.355	0.200	**-0.422**
F_SF	**-0.444**	0.140	**0.507**	0.256	0.374	-0.112	-0.032	0.201	**0.573**	-0.289	0.264	-0.108	0.220	0.270	**-0.399**	0.214	**1**	**0.471**	-0.038	-0.327
F_G	**-0.715**	0.365	0.333	**0.624**	**0.781**	**-0.619**	**-0.637**	0.115	**0.864**	**-0.763**	-0.139	-0.157	-0.186	0.243	**-0.725**	0.355	**0.471**	**1**	**-0.423**	**-0.663**
F_HDG	**0.482**	-0.098	**-0.397**	**-0.417**	-0.278	**0.581**	**0.575**	-0.389	-0.393	0.242	**0.478**	**0.417**	**0.547**	-0.267	**0.498**	0.200	-0.038	**-0.423**	**1**	0.192
F_OPG	**0.724**	**-0.514**	-0.122	-0.308	**-0.562**	**0.722**	**0.692**	0.139	**-0.662**	**0.843**	0.074	0.007	0.342	-0.032	**0.723**	**-0.422**	-0.327	**-0.663**	0.192	**1**

'A' and 'F' prefixes refer to aroma and flavour attributes, respectively. 'GG' = green grass, 'CV' = cooked vegetables, 'ST' = stewed fruit, 'TF' = tropical fruit, 'AJ' = apricot jam, 'G' = guava, 'HDG' = hay/dried grass, 'OPG' = oats/porridge/grains, 'GSA' = general sweet, 'FS' = fruity-sweet, 'C' = caramel, 'S' = sour taste, 'B' = bitter taste, 'AST' = astringent, 'SWT' = sweet taste. Values in bold are different from 0 with a significance level of α = 0.05.

Table A.9 Aroma attributes of STAD green *C. maculata*

Steaming time (min)	Geranium leaf					Green grass					Cooked vegetables					Hay/Dried grass				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	0.73	a	0.33	0.37	1.19	19.09	a	2.79	14.98	21.65	13.87	b	1.36	12.89	16.19	26.71	a	0.89	25.46	27.80
1	0.80	a	0.51	0.00	1.37	12.85	b	0.65	11.96	13.54	15.46	ab	1.07	14.37	17.04	24.83	b	1.02	23.83	26.37
2	0.49	a	0.33	0.00	0.81	12.10	b	1.18	10.83	13.48	16.24	a	1.84	13.57	18.41	24.42	b	2.10	21.85	26.44
3	0.90	a	0.88	0.00	2.31	9.20	c	2.33	6.85	12.15	15.47	a	1.75	12.69	17.28	23.62	b	0.59	22.72	24.15
4	1.99	a	3.39	0.00	8.04	7.34	c	3.18	2.37	10.44	14.84	ab	2.09	11.67	17.02	24.02	b	1.35	22.67	25.50
Steaming time (min)	**Oats/Porridge/Grains**					**Stewed fruit**					**Tropical fruit**					**Marmalade**				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	10.70	a	1.94	8.57	13.28	12.65	a	1.76	10.11	14.81	2.59	b	1.65	1.15	4.48	0.19	b	0.19	0.00	0.38
1	7.21	b	2.08	4.96	10.22	12.91	a	1.06	11.23	13.69	2.42	b	1.64	0.50	4.88	0.29	b	0.29	0.00	0.69
2	5.43	c	3.00	1.74	9.04	13.07	a	1.04	12.04	14.59	3.48	ab	1.88	1.19	5.98	0.47	b	0.73	0.00	1.67
3	4.96	c	2.60	1.93	7.33	12.80	a	0.61	12.24	13.81	4.16	a	1.72	2.15	6.11	0.56	ab	0.53	0.00	1.07
4	5.64	bc	2.27	2.31	8.22	13.52	a	1.68	11.50	15.31	4.57	a	1.83	2.22	7.22	1.25	a	1.09	0.00	2.40
Steaming time (min)	**Apricot jam**					**Cooked apple**					**Guava**					**General sweet**				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	8.97	a	1.95	6.41	11.46	nd					8.28	c	1.43	7.17	10.17	18.71	a	1.45	17.22	20.43
1	9.13	a	1.38	7.59	11.26	nd					9.77	bc	1.20	8.78	11.52	18.61	a	0.37	18.09	19.00
2	10.26	a	1.65	8.41	12.04	nd					11.31	ab	0.77	10.61	12.39	18.33	a	0.98	16.70	19.09
3	10.36	a	1.96	8.28	12.72	nd					12.61	a	0.29	12.15	12.89	18.29	a	0.83	17.48	19.52
4	10.24	a	2.22	7.65	12.91	nd					12.66	a	2.82	8.61	16.07	18.83	a	1.35	16.78	20.28

Steaming time (min)	Fruity-sweet					Caramel					Dusty					Musty				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	9.80	c	1.82	7.24	11.52	9.34	a	1.60	7.69	11.30	0.27	a	0.19	0.00	0.50	0.63	a	0.75	0.00	1.81
1	12.49	b	2.15	10.02	14.40	5.92	b	0.66	5.06	6.50	0.30	a	0.33	0.00	0.67	0.19	a	0.18	0.00	0.37
2	14.25	ab	1.11	12.81	15.31	4.31	c	0.78	3.31	5.19	0.09	a	0.21	0.00	0.46	0.24	a	0.38	0.00	0.86
3	15.25	a	1.15	13.72	16.44	4.79	bc	1.12	3.44	5.98	0.22	a	0.49	0.00	1.09	0.31	a	0.49	0.00	1.13
4	16.10	a	2.06	13.33	18.52	4.56	c	0.91	3.39	5.67	0.19	a	0.17	0.00	0.37	0.69	a	1.05	0.00	2.50

Treatment means corresponding with the same letters do not differ significantly ($p \geq 0.05$). Mean = mean intensity treatment value, SD = standard deviation, Min = minimum batch treatment mean, Max = maximum treatment batch mean. Attributes coloured in red = means < 5, yellow = means > 5 and do not differ significantly ($p \geq 0.05$), green = means > 5 and differ significantly ($p < 0.05$). nd = not detected.

Table A.10 Taste and mouthfeel attributes of STAD green *C. maculata*

Steaming time (min)	Sour taste					Bitter taste					Astringent					Sweet taste				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	11.40	ab	0.88	10.57	12.78	7.09	a	1.23	5.83	8.80	26.37	a	0.50	25.59	26.96	18.29	b	0.69	17.72	19.15
1	10.58	b	0.51	9.87	11.17	6.68	ab	1.81	4.19	8.93	25.74	ab	0.76	24.78	26.65	18.44	b	0.83	17.78	19.80
2	11.46	a	0.94	10.37	12.46	6.67	ab	1.26	5.13	8.48	25.70	ab	1.14	24.57	27.50	18.48	b	0.49	17.80	19.09
3	10.71	ab	0.82	9.76	11.43	7.00	a	1.10	5.98	8.46	25.19	b	1.04	23.59	26.50	18.54	b	0.58	17.72	19.30
4	11.21	ab	1.44	9.56	13.04	5.62	b	1.71	4.09	8.39	25.56	ab	1.31	23.78	26.78	19.19	a	0.63	18.30	19.93

Treatment means corresponding with the same letters do not differ significantly ($p \geq 0.05$). Mean = mean intensity treatment value, SD = standard deviation, Min = minimum batch treatment mean, Max = maximum treatment batch mean. Attributes coloured in red = means < 5, yellow = means > 5 and do not differ significantly ($p \geq 0.05$), green = means > 5 and differ significantly ($p < 0.05$).

Table A.11 Flavour attributes of STAD green *C. maculata*

Steaming time (min)	Green grass					Cooked vegetables					Hay/Dried grass					Oats/Porridge/Grains				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	15.95	a	2.37	12.44	18.28	12.37	b	1.00	11.65	13.91	23.96	a	1.60	22.26	26.37	2.87	a	0.65	2.13	3.52
1	10.35	b	1.25	8.31	11.63	13.06	ab	1.77	10.74	14.85	23.58	a	1.18	22.04	25.09	1.44	b	0.59	0.94	2.43
2	8.75	bc	1.62	6.59	10.85	14.26	a	2.87	11.07	18.42	23.74	a	0.98	22.31	24.72	0.63	c	0.45	0.19	1.31
3	7.47	cd	1.43	5.80	9.48	14.09	ab	1.38	11.87	15.27	23.06	a	1.28	21.24	24.72	0.66	c	0.33	0.30	1.09
4	5.60	d	1.96	3.43	8.00	13.65	ab	1.68	12.07	16.39	23.08	a	0.55	22.54	23.94	1.15	bc	0.52	0.37	1.80

Steaming time (min)	Stewed fruit					Tropical fruit					Marmalade					Apricot jam				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	6.67	b	1.13	5.35	8.35	1.32	c	1.13	0.56	3.29	nd					2.14	b	0.81	1.44	3.13
1	6.80	b	1.04	5.09	7.63	1.72	abc	0.94	0.61	3.00	nd					1.99	b	0.48	1.48	2.72
2	7.03	b	0.97	5.52	7.93	1.55	bc	1.26	0.20	2.89	nd					2.10	b	0.63	1.50	3.15
3	7.93	a	0.93	6.35	8.74	2.12	ab	1.45	0.92	3.85	nd					2.57	ab	0.78	1.44	3.60
4	8.00	a	1.20	5.98	8.89	2.33	a	1.44	0.94	4.06	nd					2.96	a	0.42	2.48	3.44

Steaming time (min)	Cooked apple					Guava				
	Mean		SD	Min	Max	Mean		SD	Min	Max
0	nd					4.69	c	1.04	3.24	6.11
1	nd					6.34	b	0.44	5.72	6.83
2	nd					6.71	b	1.12	5.31	7.78
3	nd					7.99	a	0.77	6.98	9.11
4	nd					7.26	ab	0.91	6.56	8.46

Treatment means corresponding with the same letters do not differ significantly ($p \geq 0.05$). Mean = mean intensity treatment value, SD = standard deviation, Min = minimum batch treatment mean, Max = maximum treatment batch mean. Attributes coloured in red = means < 5, yellow = means > 5 and do not differ significantly ($p \geq 0.05$), green = means > 5 and differ significantly ($p < 0.05$). nd = not detected.

Table A.12 Objective colour parameters, total chlorophyll (TC) content and total soluble solids (TSS) content of STAD green *C. maculata* plant material and/or infusions

		Steaming time (min)																			
		0				1				2				3				4			
Milled	*L**	52.00	a	±	1.26	50.03	b	±	0.70	48.68	c	±	0.85	48.12	c	±	0.52	47.34	d	±	0.79
	*a**	-1.00	d	±	0.70	0.19	c	±	1.18	1.06	b	±	1.27	1.59	b	±	1.03	3.02	a	±	1.30
	*b**	34.17	b	±	0.38	35.28	a	±	0.37	35.17	a	±	0.47	34.84	a	±	0.35	33.80	b	±	0.88
	C	34.19	b	±	0.37	35.30	a	±	0.36	35.20	a	±	0.48	34.88	a	±	0.33	33.95	b	±	0.89
	h	91.68	a	±	1.18	89.68	b	±	1.93	88.29	c	±	2.06	87.39	c	±	1.70	84.90	d	±	2.16
	Δ*E**	0	d			2.67	c	±	0.65	4.12	b	±	0.58	4.74	b	±	0.88	6.25	a	±	0.76
TC	mg/g	2.59	a	±	0.09	2.30	b	±	0.18	2.19	c	±	0.19	2.12	c	±	0.13	1.96	d	±	0.20
Leaf	*L**	32.72	b	±	0.92	33.76	a	±	0.83	33.80	a	±	0.49	33.39	ab	±	0.64	33.51	a	±	0.52
	*a**	2.36	c	±	0.22	2.73	c	±	0.51	3.44	b	±	0.59	3.84	ab	±	0.36	4.09	a	±	0.81
	*b**	18.77	c	±	0.67	19.78	ab	±	0.59	20.00	a	±	0.58	19.48	b	±	0.45	19.51	b	±	0.77
	C	18.92	b	±	0.67	19.97	a	±	0.60	20.30	a	±	0.61	19.85	a	±	0.48	19.94	a	±	0.89
	h	82.83	a	±	0.63	82.15	a	±	1.41	80.28	b	±	1.59	78.86	c	±	0.90	78.23	c	±	1.85
	Δ*E**	0	c			1.79	b	±	0.68	2.1	ab	±	0.44	2.03	ab	±	0.62	2.31	a	±	0.67
Infusion	*L**	92.63	a	±	0.73	92.53	a	±	0.91	92.72	a	±	0.88	92.79	a	±	1.00	92.85	a	±	1.07
	*a**	0.33	a	±	0.29	0.41	a	±	0.42	0.32	a	±	0.29	0.38	a	±	0.37	0.46	a	±	0.56
	*b**	25.27	a	±	2.45	25.36	a	±	3.20	24.48	ab	±	2.78	24.30	ab	±	3.22	23.42	b	±	2.92
	C	25.28	a	±	2.45	25.37	a	±	3.21	24.48	ab	±	2.78	24.31	ab	±	3.23	23.43	b	±	2.93
	h	89.27	a	±	0.64	89.16	a	±	0.84	89.31	a	±	0.62	89.19	a	±	0.83	88.99	a	±	1.32
	Δ*E**	0	c			1.19	b	±	1.07	1.08	bc	±	0.62	1.14	bc	±	0.94	2.48	a	±	1.00
TSS	g/100mL	0.155	a	±	0.019	0.159	a	±	0.018	0.158	a	±	0.017	0.154	a	±	0.019	0.141	a	±	0.016

Means with the same letters do not differ significantly ($p \geq 0.05$). Values indicated as (mean) ± (standard deviation).

Table A.13 Pearson's (n-1) correlation matrix for STBD green *C. longifolia* sensory attributes

	A_GG	A_CV	A_SF	A_TF	A_AJ	A_G	A_HDG	A_OPG	A_GSA	A_FS	A_C	S	B	AST	SWT	F_GG	F_CV	F_HDG
A_GG	**1**	0.360	-0.300	-0.357	-0.146	-0.384	**0.636**	**0.832**	0.346	-0.319	**0.738**	0.007	-0.029	-0.240	-0.049	**0.933**	0.274	**0.475**
A_CV	0.360	**1**	0.021	-0.225	0.199	**-0.447**	**0.724**	**0.516**	0.231	-0.366	0.391	0.310	**0.603**	**0.451**	0.023	0.378	0.340	**0.793**
A_SF	-0.300	0.021	**1**	-0.121	0.193	-0.317	-0.105	-0.319	0.225	0.037	-0.369	**-0.410**	0.008	0.082	-0.023	-0.143	-0.106	-0.020
A_TF	-0.357	-0.225	-0.121	**1**	-0.390	**0.637**	-0.355	**-0.524**	-0.209	**0.814**	**-0.481**	-0.252	**-0.583**	0.161	**0.769**	**-0.429**	**-0.687**	-0.121
A_AJ	-0.146	0.199	0.193	-0.390	**1**	-0.086	0.037	0.142	0.202	**-0.590**	0.125	**0.519**	**0.574**	-0.018	**-0.618**	0.035	**0.415**	-0.054
A_G	-0.384	**-0.447**	-0.317	**0.637**	-0.086	**1**	**-0.628**	-0.339	-0.087	0.384	-0.221	0.139	**-0.410**	0.015	0.326	-0.373	-0.267	**-0.525**
A_HDG	**0.636**	**0.724**	-0.105	-0.355	0.037	**-0.628**	**1**	**0.677**	0.117	**-0.427**	0.378	0.152	**0.445**	0.130	-0.018	**0.597**	0.374	**0.849**
A_OPG	**0.832**	**0.516**	-0.319	**-0.524**	0.142	-0.339	**0.677**	**1**	0.323	**-0.565**	**0.724**	0.255	0.280	-0.085	-0.212	**0.830**	**0.500**	**0.503**
A_GSA	0.346	0.231	0.225	-0.209	0.202	-0.087	0.117	0.323	**1**	-0.272	**0.508**	-0.053	-0.014	0.020	-0.004	**0.420**	0.177	0.064
A_FS	-0.319	-0.366	0.037	**0.814**	**-0.590**	0.384	**-0.427**	**-0.565**	-0.272	**1**	**-0.518**	**-0.539**	**-0.720**	0.085	**0.647**	**-0.438**	**-0.765**	-0.187
A_C	**0.738**	0.391	-0.369	**-0.481**	0.125	-0.221	0.378	**0.724**	**0.508**	**-0.518**	**1**	0.292	0.178	-0.036	-0.275	**0.744**	0.379	0.212
S	0.007	0.310	**-0.410**	-0.252	**0.519**	0.139	0.152	0.255	-0.053	**-0.539**	0.292	**1**	**0.683**	0.303	**-0.446**	0.041	**0.516**	0.145
B	-0.029	**0.603**	0.008	**-0.583**	**0.574**	**-0.410**	**0.445**	0.280	-0.014	**-0.720**	0.178	**0.683**	**1**	0.345	**-0.564**	0.055	**0.689**	**0.426**
AST	-0.240	**0.451**	0.082	0.161	-0.018	0.015	0.130	-0.085	0.020	0.085	-0.036	0.303	0.345	**1**	0.026	-0.141	0.071	**0.456**
SWT	-0.049	0.023	-0.023	**0.769**	**-0.618**	0.326	-0.018	-0.212	-0.004	**0.647**	-0.275	**-0.446**	**-0.564**	0.026	**1**	-0.182	**-0.687**	0.090
F_GG	**0.933**	0.378	-0.143	**-0.429**	0.035	-0.373	**0.597**	**0.830**	**0.420**	**-0.438**	**0.744**	0.041	0.055	-0.141	-0.182	**1**	0.368	**0.459**
F_CV	0.274	0.340	-0.106	**-0.687**	**0.415**	-0.267	0.374	**0.500**	0.177	**-0.765**	0.379	**0.516**	**0.689**	0.071	**-0.687**	0.368	**1**	0.313
F_HDG	**0.475**	**0.793**	-0.020	-0.121	-0.054	**-0.525**	**0.849**	**0.503**	0.064	-0.187	0.212	0.145	**0.426**	**0.456**	0.090	**0.459**	0.313	**1**

'A' and 'F' prefixes refer to aroma and flavour attributes, respectively. 'GG' = green grass, 'CV' = cooked vegetables, 'ST' = stewed fruit, 'TF' = tropical fruit, 'AJ' = apricot jam, 'G' = guava, 'HDG' = hay/dried grass, 'OPG' = oats/porridge/grains, 'GSA' = general sweet, 'FS' = fruity-sweet, 'C' = caramel, 'S' = sour taste, 'B' = bitter taste, 'AST' = astringent, 'SWT' = sweet taste. Values in bold are different from 0 with a significance level of α = 0.05.

Table A.14 Aroma attributes of STBD green *C. longifolia*

Steaming time (s)	Plantlike floral					Green grass					Cooked vegetables					Hay/Dried grass				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	3.39	ab	0.73	2.26	4.00	15.63	a	1.57	13.70	17.87	17.86	a	1.58	16.24	19.89	23.19	a	1.77	20.48	24.55
30	3.61	a	0.50	2.95	4.32	7.66	b	1.32	5.65	9.35	17.39	ab	1.19	15.78	18.85	21.49	b	0.83	20.53	22.42
60	2.94	bc	0.90	1.57	3.90	7.51	b	0.96	6.42	8.80	16.94	ab	1.66	14.29	18.45	21.04	b	0.71	19.90	21.83
90	3.09	abc	0.66	2.22	4.07	6.31	c	1.21	5.57	8.43	16.91	ab	1.87	14.75	18.60	21.29	b	1.72	19.47	23.93
120	2.55	c	0.95	1.60	3.78	6.49	c	0.98	5.42	7.42	16.59	b	1.52	14.60	18.45	21.38	b	1.24	19.62	22.47
Steaming time (s)	**Oats/Porridge/Grains**					**Stewed fruit**					**Tropical fruit**					**Marmalade**				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	6.74	a	1.12	5.13	7.88	10.72	b	0.56	10.16	11.52	3.29	c	1.85	1.70	5.86	0.27	b	0.26	0.00	0.57
30	3.64	b	0.80	2.72	4.48	12.36	a	2.10	9.23	14.78	4.03	bc	1.97	2.08	6.19	0.20	b	0.22	0.00	0.50
60	3.17	b	1.19	1.69	5.00	11.54	ab	1.14	9.78	12.45	4.38	b	2.64	1.93	7.58	0.37	b	0.29	0.10	0.83
90	2.81	b	0.79	1.78	3.90	12.86	a	1.69	11.10	15.03	5.79	a	1.45	4.53	7.93	0.25	b	0.17	0.00	0.48
120	3.19	b	1.01	1.63	4.47	12.46	a	1.81	10.14	14.37	5.34	a	1.47	3.40	7.10	0.68	a	0.43	0.00	1.03
Steaming time (s)	**Apricot jam**					**Guava**					**General sweet**					**Fruity-sweet**				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	6.76	a	0.59	5.92	7.48	4.42	b	3.02	2.15	9.55	15.96	a	0.32	15.58	16.43	4.93	b	1.20	3.67	6.35
30	6.92	a	0.87	5.77	8.12	4.23	b	1.81	2.16	7.07	15.42	ab	0.74	14.22	16.05	6.05	a	1.34	4.43	7.73
60	5.67	b	1.06	4.72	7.25	5.16	ab	1.84	3.67	8.34	15.25	b	0.44	14.81	15.95	6.30	a	1.47	4.65	7.87
90	7.22	a	1.47	4.91	8.69	5.93	a	1.90	4.22	9.10	16.05	a	0.21	15.83	16.32	6.22	a	1.17	4.73	7.52
120	7.15	a	1.24	5.57	8.55	5.84	a	1.95	4.12	9.17	14.86	b	0.39	14.31	15.30	6.08	a	0.86	4.75	7.00

Steaming time (s)	Caramel					Dusty					Musty					Seaweed/Oceanic				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	5.75	a	0.50	5.10	6.45	0.31	b	0.30	0.00	0.65	1.44	a	0.67	0.52	1.96	0.12	a	0.17	0.00	0.34
30	4.29	b	0.81	3.38	5.39	0.78	ab	0.56	0.33	1.60	0.46	bc	0.30	0.00	0.67	0.11	a	0.15	0.00	0.27
60	3.52	bc	0.83	2.37	4.35	1.25	a	0.75	0.23	2.09	0.83	b	0.53	0.17	1.65	0.16	a	0.18	0.00	0.45
90	3.38	cd	0.50	2.88	4.14	1.40	a	0.28	1.12	1.73	0.32	c	0.24	0.00	0.67	0.00	a	0.00	0.00	0.00
120	2.64	d	0.63	1.90	3.30	0.92	ab	0.48	0.58	1.73	0.61	bc	0.51	0.00	1.30	0.03	a	0.07	0.00	0.17

Treatment means corresponding with the same letters do not differ significantly ($p \geq 0.05$). Mean = mean intensity treatment value, SD = standard deviation, Min = minimum batch treatment mean, Max = maximum treatment batch mean. Attributes coloured in red = means < 5, yellow = means >5 and do not differ significantly ($p \geq 0.05$), green = means > 5 and differ significantly ($p < 0.05$).

Table A.15 Taste and mouthfeel attributes of STBD green *C. longifolia*

Steaming time (s)	Sour taste					Bitter taste					Astringent					Sweet taste				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	15.17	a	1.20	13.22	16.52	32.03	a	2.88	28.52	36.13	33.64	a	1.32	32.17	35.17	12.27	a	0.55	11.55	13.00
30	14.87	a	1.29	13.30	16.67	32.27	a	2.40	30.65	36.43	34.36	a	0.78	33.02	35.05	12.30	a	0.75	11.55	13.45
60	14.67	a	1.19	13.50	16.09	32.59	a	3.05	28.55	36.40	34.25	a	0.60	33.48	34.77	12.40	a	0.92	11.35	13.42
90	14.81	a	1.23	13.10	16.45	31.85	a	3.10	29.02	36.52	34.44	a	0.67	33.60	35.10	12.68	a	0.93	11.93	14.07
120	14.82	a	1.45	13.47	17.09	32.23	a	3.07	29.33	37.00	33.96	a	0.73	33.33	34.87	12.58	a	0.91	11.72	13.68

Treatment means corresponding with the same letters do not differ significantly ($p \geq 0.05$). Mean = mean intensity treatment value, SD = standard deviation, Min = minimum batch treatment mean, Max = maximum treatment batch mean. Attributes coloured in red = means < 5, yellow = means > 5 and do not differ significantly ($p \geq 0.05$), green = means > 5 and differ significantly ($p < 0.05$).

Table A.16 Flavour attributes of STBD green *C. longifolia*

Steaming time (s)	Floral plantlike					Green grass					Cooked vegetables					Hay/Dried grass				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	1.34	a	0.80	0.33	2.42	11.72	a	0.91	10.98	13.12	16.01	a	0.70	15.05	16.78	24.09	a	2.11	21.50	26.82
30	1.08	a	0.51	0.58	1.80	5.90	b	1.72	3.13	7.47	15.36	a	1.04	13.67	16.15	23.00	ab	1.09	21.64	24.38
60	0.83	a	0.36	0.42	1.28	5.74	b	0.66	5.00	6.40	15.57	a	1.18	14.20	16.97	22.84	b	1.28	21.24	24.40
90	1.05	a	0.70	0.63	2.30	5.32	b	0.82	4.15	6.41	15.15	a	0.90	14.27	16.37	22.68	b	1.72	20.40	24.92
120	0.82	a	0.46	0.17	1.35	5.69	b	1.16	3.68	6.60	15.16	a	0.84	14.13	16.25	22.94	ab	1.72	20.73	24.95
Steaming time (s)	**Oats/Porridge/Grains**					**Stewed fruit**					**Tropical fruit**					**Marmalade**				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	1.55	a	0.67	0.64	2.37	1.30	b	0.49	0.70	2.07	0.89	a	0.72	0.10	1.73	0.23	a	0.09	0.17	0.33
30	0.83	b	0.61	0.33	1.83	1.70	ab	0.44	1.15	2.32	0.89	a	0.66	0.17	1.45	0.31	a	0.21	0.13	0.55
60	0.62	b	0.34	0.17	1.03	1.50	ab	0.43	1.05	2.22	1.00	a	0.66	0.27	1.95	0.31	a	0.37	0.00	0.90
90	0.76	b	0.46	0.33	1.52	1.85	a	0.31	1.47	2.32	1.26	a	0.93	0.17	2.30	0.20	a	0.14	0.00	0.34
120	0.87	b	0.72	0.00	1.85	1.68	ab	0.39	1.18	2.08	1.07	a	0.96	0.00	2.48	0.30	a	0.21	0.00	0.50
Steaming time (s)	**Apricot jam**					**Guava**					**Fruity sweet**					**Caramel**				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	1.24	ab	0.45	0.80	1.92	0.80	b	1.00	0.33	2.60	0.78	b	0.66	0.00	1.70	0.44	ab	0.20	0.28	0.78
30	0.76	c	0.57	0.27	1.46	1.10	ab	0.67	0.52	2.23	1.00	ab	0.65	0.35	1.97	0.25	bc	0.17	0.00	0.40
60	0.88	bc	0.21	0.68	1.18	1.02	ab	0.63	0.47	2.10	0.99	ab	0.81	0.00	2.25	0.50	a	0.32	0.00	0.80
90	1.43	a	0.51	0.75	1.84	1.34	a	0.73	0.57	2.55	1.58	a	0.47	1.08	2.13	0.40	ab	0.26	0.00	0.67
120	0.99	abc	0.38	0.48	1.33	1.37	a	1.18	0.33	3.40	1.18	ab	0.79	0.32	2.40	0.15	c	0.17	0.00	0.43

Steaming time (s)	Musty					Seaweed/Oceanic				
	Mean		SD	Min	Max	Mean		SD	Min	Max
0	0.21	a	0.30	0.00	0.73	0.03	a	0.08	0.00	0.17
30	0.23	a	0.19	0.00	0.53	0.04	a	0.08	0.00	0.18
60	0.37	a	0.29	0.00	0.73	0.10	a	0.09	0.00	0.17
90	0.26	a	0.17	0.00	0.42	0.00	a	0.00	0.00	0.00
120	0.51	a	0.54	0.00	1.10	0.05	a	0.08	0.00	0.17

Treatment means corresponding with the same letters do not differ significantly ($p \geq 0.05$). Mean = mean intensity treatment value, SD = standard deviation, Min = minimum batch treatment mean, Max = maximum treatment batch mean. Attributes coloured in red = means < 5, yellow = means > 5 and do not differ significantly ($p \geq 0.05$), green = means > 5 and differ significantly ($p < 0.05$).

Table A.17 Objective colour parameters, total chlorophyll (TC) content and total soluble solids (TSS) content of STBD green *C. longifolia* plant material and/or infusions

		Steaming time (s)															
		0				30				60				90				120			
Milled	*L**	61.01	a	±	0.68	57.73	b	±	0.59	57.47	c	±	0.50	57.45	c	±	0.48	57.20	d	±	0.51
	*a**	-9.67	d	±	0.30	-8.57	c	±	0.46	-8.40	c	±	0.49	-8.03	b	±	0.25	-7.36	a	±	0.34
	*b**	37.40	a	±	0.19	36.87	b	±	0.15	36.57	bc	±	0.33	36.74	b	±	0.41	36.32	c	±	0.19
	C	38.63	a	±	0.23	37.85	b	±	0.25	37.53	b	±	0.41	37.61	b	±	0.44	37.06	c	±	0.23
	h	104.49	a	±	0.40	103.08	b	±	0.63	102.93	b	±	0.65	102.33	c	±	0.30	101.45	d	±	0.47
	Δ*E**	0.00	d			3.51	c	±	0.20	3.86	b	±	0.23	3.99	b	±	0.39	4.60	a	±	0.23
TC	mg/g	3.02	a	±	0.22	2.79	b	±	0.18	2.70	cd	±	0.20	2.75	bc	±	0.03	2.65	d	±	0.20
Leaf	*L**	40.83	a	±	0.91	39.56	b	±	0.22	39.14	bc	±	0.69	38.99	c	±	0.46	39.42	bc	±	0.72
	*a**	-4.71	e	±	0.18	-4.12	d	±	0.39	-3.86	c	±	0.23	-3.60	b	±	0.17	-3.08	a	±	0.18
	*b**	22.81	a	±	0.26	22.33	b	±	0.36	22.23	bc	±	0.22	21.96	cd	±	0.32	21.89	d	±	0.42
	C	23.29	a	±	0.28	22.71	b	±	0.39	22.56	b	±	0.23	22.26	c	±	0.34	22.11	c	±	0.42
	h	101.68	a	±	0.36	100.46	b	±	0.92	99.86	c	±	0.54	99.30	d	±	0.36	98.03	e	±	0.52
	Δ*E**	0 c	c			1.54	b	±	0.69	1.99	a	±	0.36	2.35	a	±	0.55	2.38	a	±	0.33
Infusion	*L**	93.88	b	±	0.47	93.95	b	±	0.67	94.52	a	±	0.36	94.15	ab	±	0.52	94.39	a	±	0.23
	*a**	-2.34	a	±	0.14	-2.96	b	±	0.23	-3.26	c	±	0.18	-3.21	c	±	0.12	-3.20	c	±	0.12
	*b**	17.18	a	±	0.70	17.38	a	±	0.45	16.57	b	±	0.57	17.08	ab	±	0.88	16.80	ab	±	0.70
	C	17.34	ab	±	0.70	17.63	a	±	0.45	16.89	b	±	0.58	17.38	ab	±	0.88	17.10	ab	±	0.70
	h	97.76	c	±	0.43	99.67	b	±	0.85	101.14	a	±	0.49	100.67	a	±	0.41	100.81	a	±	0.38
	Δ*E**	0.00	c			0.93	b	±	0.20	1.34	a	±	0.26	1.11	ab	±	0.17	1.23	ab	±	0.36
TSS	g/100 mL	0.281	b	±	0.012	0.297	a	±	0.008	0.298	a	±	0.008	0.302	a	±	0.016	0.296	a	±	0.011

Means with the same letters do not differ significantly ($p \geq 0.05$). Values indicated as (mean) ± (standard deviation).

Table A.18 Pearson's (n-1) correlation matrix of STAD green *C. longifolia* sensory attributes

	A_GG	A_CV	A_SF	A_AJ	A_HDG	A_OPG	A_GSA	A_FS	A_C	S	B	AST	SWT	F_GG	F_CV	F_HDG	F_OPG
A_GG	**1**	**0.536**	**-0.517**	-0.237	**0.447**	0.002	-0.046	-0.280	0.240	0.162	0.101	0.139	-0.082	**0.563**	0.218	**0.569**	-0.114
A_CV	**0.536**	**1**	-0.369	-0.211	**0.557**	-0.176	**-0.454**	-0.230	-0.185	0.072	0.064	0.027	-0.074	**0.434**	0.357	**0.469**	-0.157
A_SF	**-0.517**	-0.369	**1**	**0.606**	0.000	-0.303	**0.418**	**0.666**	-0.213	**-0.475**	-0.369	-0.287	0.322	**-0.599**	0.026	**-0.397**	0.075
A_AJ	-0.237	-0.211	**0.606**	**1**	0.041	**-0.429**	0.107	**0.677**	-0.235	**-0.591**	**-0.452**	-0.295	0.002	**-0.492**	-0.111	-0.334	0.232
A_HDG	**0.447**	**0.557**	0.000	0.041	**1**	-0.181	-0.076	-0.076	0.180	-0.272	-0.181	-0.220	0.258	0.108	0.111	**0.414**	-0.239
A_OPG	0.002	-0.176	-0.303	**-0.429**	-0.181	**1**	0.156	-0.214	0.350	0.324	**0.526**	**0.512**	-0.195	**0.501**	0.043	0.009	-0.243
A_GSA	-0.046	**-0.454**	**0.418**	0.107	-0.076	0.156	**1**	0.384	**0.444**	0.213	0.158	0.096	0.056	-0.013	0.223	0.092	-0.078
A_FS	-0.280	-0.230	**0.666**	**0.677**	-0.076	-0.214	0.384	**1**	-0.280	**-0.472**	-0.340	-0.046	0.087	**-0.398**	0.046	-0.020	0.267
A_C	0.240	-0.185	-0.213	-0.235	0.180	0.350	**0.444**	-0.280	**1**	0.283	0.314	0.127	-0.033	**0.438**	-0.031	0.217	-0.144
S	0.162	0.072	**-0.475**	**-0.591**	-0.272	0.324	0.213	**-0.472**	0.283	**1**	**0.744**	**0.433**	**-0.414**	**0.471**	0.301	0.062	-0.263
B	0.101	0.064	-0.369	**-0.452**	-0.181	**0.526**	0.158	-0.340	0.314	**0.744**	**1**	**0.686**	**-0.667**	**0.650**	**0.439**	0.025	**-0.608**
AST	0.139	0.027	-0.287	-0.295	-0.220	**0.512**	0.096	-0.046	0.127	**0.433**	**0.686**	**1**	**-0.408**	**0.545**	0.334	0.080	-0.090
SWT	-0.082	-0.074	0.322	0.002	0.258	-0.195	0.056	0.087	-0.033	**-0.414**	**-0.667**	**-0.408**	**1**	-0.384	**-0.396**	0.012	0.361
F_GG	**0.563**	**0.434**	**-0.599**	**-0.492**	0.108	**0.501**	-0.013	**-0.398**	**0.438**	**0.471**	**0.650**	**0.545**	-0.384	**1**	0.225	**0.483**	-0.371
F_CV	0.218	0.357	0.026	-0.111	0.111	0.043	0.223	0.046	-0.031	0.301	**0.439**	0.334	**-0.396**	0.225	**1**	0.222	**-0.407**
F_HDG	**0.569**	**0.469**	**-0.397**	-0.334	**0.414**	0.009	0.092	-0.020	0.217	0.062	0.025	0.080	0.012	**0.483**	0.222	**1**	0.009
F_OPG	-0.114	-0.157	0.075	0.232	-0.239	-0.243	-0.078	0.267	-0.144	-0.263	**-0.608**	-0.090	0.361	-0.371	**-0.407**	0.009	**1**

'A' and 'F' prefixes refer to aroma and flavour attributes, respectively. 'GG' = green grass, 'CV' = cooked vegetables, 'ST' = stewed fruit, 'TF' = tropical fruit, 'AJ' = apricot jam, 'G' = guava, 'HDG' = hay/dried grass, 'OPG' = oats/porridge/grains, 'GSA' = general sweet, 'FS' = fruity-sweet, 'C' = caramel, 'S' = sour taste, 'B' = bitter taste, 'AST' = astringent, 'SWT' = sweet taste. Values in bold are different from 0 with a significance level of $\alpha = 0.05$.

Table A.19 Aroma attributes of STAD green *C. longifolia*

Steaming time (min)	Green grass					Cooked vegetables					Hay/Dried grass					Oats/Porridge/Grains				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	14.52	a	2.22	11.54	16.98	18.54	a	1.04	17.72	20.17	30.21	a	0.73	29.22	31.04	17.86	a	1.11	16.65	19.59
1	10.77	b	1.77	8.37	12.65	17.85	a	1.26	16.63	19.91	30.02	ab	0.59	29.20	30.67	18.06	a	1.08	16.80	19.50
2	10.97	b	1.04	9.85	12.52	17.85	a	0.57	17.07	18.54	29.45	bc	0.93	28.35	30.35	16.66	a	2.40	12.87	19.46
3	10.30	b	2.01	7.96	12.81	17.70	a	1.18	16.41	18.98	29.66	ab	0.82	28.91	30.85	17.09	a	2.72	14.17	20.09
4	9.32	b	1.16	8.13	10.59	17.36	a	0.96	16.26	18.37	28.86	c	0.55	28.39	29.61	17.28	a	2.87	15.06	21.02
Steaming time (min)	**Stewed fruit**					**Tropical fruit**					**Apricot jam**					**Guava**				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	15.37	b	1.37	12.96	16.28	1.21	a	1.13	0.00	2.67	11.31	a	0.89	10.07	12.56	3.68	b	0.95	2.50	5.06
1	16.26	ab	0.79	14.89	16.85	1.25	a	0.66	0.33	2.17	11.26	a	0.77	10.50	12.33	4.31	ab	0.97	3.06	5.65
2	17.53	a	0.67	16.56	18.26	1.50	a	1.04	0.26	3.11	11.92	a	1.22	9.94	12.96	3.99	ab	1.58	1.57	5.76
3	16.94	a	1.40	15.20	19.04	1.91	a	0.90	0.35	2.52	11.86	a	1.03	10.80	13.56	4.89	ab	1.00	3.89	6.22
4	17.32	a	0.90	16.07	18.20	2.17	a	0.72	1.37	3.11	12.10	a	0.47	11.59	12.83	4.93	a	0.68	3.83	5.67
Steaming time (min)	**General sweet**					**Fruity-sweet**					**Caramel**					**Dusty**				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	21.52	a	0.42	21.02	21.89	9.71	b	1.52	7.28	10.94	6.76	a	1.28	5.73	8.96	0.29	a	0.30	0.00	0.70
1	21.52	a	0.35	20.93	21.83	9.84	b	0.94	8.98	11.07	5.18	ab	1.02	4.02	6.26	0.61	a	0.36	0.35	1.17
2	21.70	a	0.43	21.17	22.30	10.45	ab	2.16	6.70	12.20	4.80	b	1.31	3.07	6.50	0.40	a	0.30	0.00	0.83
3	21.54	a	1.09	20.39	22.98	10.76	ab	1.25	8.70	12.11	5.18	ab	1.77	3.24	7.91	0.43	a	0.40	0.00	0.80
4	21.69	a	1.28	20.28	23.63	11.80	a	0.62	11.19	12.72	3.95	b	1.31	2.76	6.17	0.34	a	0.34	0.00	0.74

Steaming time (min)	Musty				
	Mean		SD	Min	Max
0	2.62	a	1.96	0.69	5.17
1	0.77	a	0.26	0.37	1.04
2	1.33	a	0.85	0.00	2.17
3	1.65	a	2.70	0.00	6.43
4	2.16	a	1.53	0.94	4.72

Treatment means corresponding with the same letters do not differ significantly ($p \geq 0.05$). Mean = mean intensity treatment value, SD = standard deviation, Min = minimum batch treatment mean, Max = maximum treatment batch mean. Attributes coloured in red = means < 5, yellow = means > 5 and do not differ significantly ($p \geq 0.05$), green = means > 5 and differ significantly ($p < 0.05$).

Table A.20 Taste and mouthfeel attributes of STAD green *C. longifolia*

Steaming time (min)	Sour taste					Bitter taste					Astringent					Sweet taste				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	13.10	a	1.63	11.88	15.87	33.59	a	3.32	29.22	38.06	33.24	a	1.62	31.13	35.07	11.94	a	0.70	11.13	12.72
1	13.11	a	1.83	11.81	16.33	32.31	ab	3.52	28.54	37.87	32.66	a	1.13	31.65	33.98	12.33	a	0.54	11.69	13.11
2	13.13	a	1.54	11.48	15.31	33.55	a	2.95	30.09	37.33	33.28	a	1.03	32.33	34.77	11.84	a	0.57	11.19	12.76
3	12.66	a	1.58	11.43	14.67	33.23	ab	2.67	30.33	36.35	33.18	a	0.99	32.11	34.37	11.85	a	0.87	10.65	12.98
4	12.62	a	1.17	11.24	13.94	32.21	b	2.97	29.20	36.50	32.84	a	0.91	31.73	34.13	12.04	a	0.74	11.04	12.72

Treatment means corresponding with the same letters do not differ significantly ($p \geq 0.05$). Mean = mean intensity treatment value, SD = standard deviation, Min = minimum batch treatment mean, Max = maximum treatment batch mean. Attributes coloured in red = means < 5, yellow = means > 5 and do not differ significantly ($p \geq 0.05$), green = means > 5 and differ significantly ($p < 0.05$).

Table A.21 Flavour attributes of STAD green *C. longifolia*

Steaming time (min)	Green grass					Cooked vegetables					Hay/Dried grass					Oats/Porridge/Grains				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
0	12.34	a	1.23	10.72	13.78	17.73	ab	1.42	15.31	18.98	30.79	a	0.56	30.31	31.61	5.55	a	2.13	2.78	8.37
1	10.23	b	1.56	8.72	12.80	16.65	b	1.32	15.33	18.17	29.77	b	0.76	28.57	30.37	5.68	a	1.88	3.30	8.06
2	10.12	b	1.68	7.94	12.30	17.81	ab	0.75	16.93	18.76	29.33	b	0.62	28.76	30.20	5.97	a	1.06	4.35	7.17
3	9.49	b	1.33	7.56	10.98	18.30	a	1.00	16.80	19.22	29.82	b	0.75	29.02	30.81	6.40	a	1.55	4.19	8.22
4	9.49	b	1.01	7.83	10.52	17.39	ab	1.52	15.39	18.87	29.66	b	0.82	28.44	30.48	5.98	a	1.64	4.00	8.09
Steaming time (min)	**Stewed fruit**					**Guava**					**Musty**					**Caramel**				
	Mean		**SD**	**Min**	**Max**	**Mean**		**SD**	**Min**	**Max**	**Mean**		**SD**	**Min**	**Max**	**Mean**		**SD**	**Min**	**Max**
0	1.26	a	1.12	0.33	2.52	0.07	a	0.15	0.00	0.33	0.28	a	0.45	0.00	1.04	nd				
1	1.19	a	0.70	0.20	1.81	0.23	a	0.27	0.00	0.67	0.14	a	0.20	0.00	0.37	nd				
2	1.12	a	0.52	0.50	1.76	0.07	a	0.15	0.00	0.33	0.22	a	0.21	0.00	0.43	nd				
3	1.30	a	0.79	0.00	1.96	0.19	a	0.23	0.00	0.56	0.89	a	1.44	0.00	3.40	nd				
4	1.65	a	1.13	0.67	3.48	0.16	a	0.26	0.00	0.59	0.63	a	0.76	0.00	1.69	nd				

Treatment means corresponding with the same letters do not differ significantly ($p \geq 0.05$). Mean = mean intensity treatment value, SD = standard deviation, Min = minimum batch treatment mean, Max = maximum treatment batch mean. Attributes coloured in red = means < 5, yellow = means > 5 and do not differ significantly ($p \geq 0.05$), green = means > 5 and differ significantly ($p < 0.05$). nd = not detected.

Table A.22 Objective colour parameters, total chlorophyll (TC) content and total soluble solids (TSS) content of STAD green *C. longifolia* plant material and/or infusions

		Steaming time (min)																		
		0				1				2				3				4			
Milled	*L**	54.71	a	±	0.62	53.03	b	±	0.63	52.53	cd	±	0.48	52.66	cb	±	0.44	52.16	d	±	0.40
	*a**	-3.99	e	±	0.48	-2.11	d	±	0.34	-0.85	c	±	0.50	-0.23	b	±	0.42	1.02	a	±	0.39
	*b**	35.09	b	±	0.47	35.98	a	±	0.36	36.03	a	±	0.24	36.05	a	±	0.27	35.78	a	±	0.17
	C	35.32	b	±	0.52	36.04	a	±	0.37	36.04	a	±	0.24	36.05	a	±	0.27	35.80	a	±	0.17
	h	96.49	a	±	0.69	93.36	b	±	0.53	91.35	c	±	0.80	90.36	d	±	0.67	88.37	e	±	0.63
	Δ*E**	0	d			2.71	c	±	0.35	3.97	b	±	0.88	4.41	b	±	0.65	5.7	a	±	0.76
TC	mg/g	2.80	a	±	0.21	2.47	b	±	0.23	2.30	c	±	0.19	2.17	d	±	0.23	2.04	e	±	0.23
Leaf	*L**	38.40	b	±	1.08	39.58	a	±	0.48	39.76	a	±	0.56	40.23	a	±	0.56	40.06	a	±	0.42
	*a**	-0.06	c	±	0.47	0.54	c	±	0.58	1.33	b	±	0.53	1.57	ab	±	0.46	2.08	a	±	0.37
	*b**	19.71	b	±	0.56	20.50	a	±	0.34	20.53	a	±	0.33	20.60	a	±	0.36	20.55	a	±	0.34
	C	19.71	b	±	0.57	20.52	a	±	0.33	20.58	a	±	0.34	20.66	a	±	0.35	20.66	a	±	0.36
	h	90.16	a	±	1.37	88.50	a	±	1.62	86.30	b	±	1.46	85.64	bc	±	1.31	84.24	c	±	0.97
	Δ*E**	0	c			1.64	b	±	0.74	2.58	a	±	0.92	2.73	a	±	0.89	3.05	a	±	0.55
Infusion	*L**	90.12	a	±	0.72	90.33	a	±	0.24	90.02	a	±	0.88	90.30	a	±	0.30	90.30	a	±	1.08
	*a**	-0.65	a	±	0.54	-0.72	a	±	0.38	-0.55	a	±	0.40	-0.87	a	±	0.34	-0.78	a	±	0.66
	*b**	29.62	a	±	1.24	29.01	a	±	0.53	29.44	a	±	2.12	28.25	a	±	1.07	28.34	a	±	2.61
	C	29.63	a	±	1.23	29.02	a	±	0.52	29.45	a	±	2.12	28.27	a	±	1.07	28.36	a	±	2.60
	h	91.30	a	±	1.08	91.45	a	±	0.78	91.13	a	±	0.79	91.78	a	±	0.71	91.67	a	±	1.41
	Δ*E**	0	b			1.32	a	±	0.75	1.86	a	±	1.36	1.97	a	±	1.66	2.48	a	±	2.04
TSS	g/100mL	0.294	a	±	0.025	0.285	b	±	0.025	0.290	ab	±	0.025	0.289	ab	±	0.026	0.286	b	±	0.027

Means with the same letters do not differ significantly ($p \geq 0.05$). Values indicated as (mean) ± (standard deviation).

ADDENDUM B

Supplementary results pertaining to Chapter 4

Table B.1 Pearson's (n-1) correlation matrix for PCA (**Fig. 4.2**) of all quantified individual phenolic compounds of steamed (S) and unsteamed (U) green *C. maculata*, stored under LTS, NTS and HTS conditions over the period of t = 0 to 6 months

Variables	Mg	IsoMg	Vic2	ErioG	IriG	ErioT	MacG	Hd
Mg	**1**	**0.996**	**0.834**	**0.707**	**0.858**	**0.983**	**0.806**	**-0.560**
IsoMg	**0.996**	**1**	**0.867**	**0.761**	**0.875**	**0.981**	**0.797**	**-0.582**
Vic2	**0.834**	**0.867**	**1**	**0.887**	**0.712**	**0.843**	**0.602**	-0.438
ErioG	**0.707**	**0.761**	**0.887**	**1**	**0.781**	**0.731**	**0.620**	-0.381
IriG	**0.858**	**0.875**	**0.712**	**0.781**	**1**	**0.853**	**0.867**	**-0.521**
ErioT	**0.983**	**0.981**	**0.843**	**0.731**	**0.853**	**1**	**0.844**	-0.437
MacG	**0.806**	**0.797**	**0.602**	**0.620**	**0.867**	**0.844**	**1**	-0.194
Hd	**-0.560**	**-0.582**	-0.438	-0.381	**-0.521**	-0.437	-0.194	**1**
A_Grass	**-0.510**	**-0.473**	-0.293	-0.017	-0.198	-0.405	-0.070	**0.620**
A_CookedVeg	0.442	**0.492**	0.415	**0.669**	**0.689**	**0.464**	**0.573**	-0.403
A_StewedFruit	-0.263	-0.315	**-0.539**	**-0.739**	**-0.504**	-0.306	**-0.478**	-0.010
A_Tropical	**0.460**	**0.508**	**0.830**	**0.737**	0.313	**0.456**	0.184	-0.318
A_Marmalade	-0.112	-0.174	-0.323	**-0.637**	-0.440	-0.156	-0.300	0.012
A_Apricot	-0.146	-0.211	-0.406	**-0.680**	**-0.503**	-0.171	-0.364	0.089
A_Guava	**0.681**	**0.653**	**0.563**	0.312	0.411	**0.670**	**0.542**	-0.269
A_Hay	-0.281	-0.290	**-0.469**	-0.228	0.010	-0.200	0.260	**0.487**
A_Oats	**-0.480**	**-0.475**	**-0.472**	-0.203	-0.110	**-0.458**	-0.055	**0.446**
A_General sweet	-0.120	-0.179	-0.321	**-0.623**	**-0.501**	-0.139	-0.343	0.104
A_Fruity-sweet	0.138	0.079	-0.041	-0.405	-0.299	0.084	-0.233	-0.140
A_Caramel	**-0.457**	**-0.480**	-0.267	-0.441	**-0.636**	-0.432	**-0.537**	**0.533**
Moisture	-0.289	-0.338	-0.350	**-0.683**	**-0.656**	-0.349	**-0.590**	0.037
TSS	**0.908**	**0.907**	**0.699**	**0.650**	**0.854**	**0.855**	**0.706**	**-0.721**
TC	-0.171	-0.125	0.028	0.376	0.256	-0.093	0.246	0.332
Leaf_L	0.374	0.373	0.276	0.035	0.110	0.301	0.023	**-0.605**
Leaf_a	-0.050	-0.103	-0.244	**-0.596**	-0.438	-0.130	-0.407	-0.197
Leaf_b	**-0.555**	**-0.597**	**-0.489**	**-0.669**	**-0.734**	**-0.580**	**-0.622**	**0.488**
Leaf_C	**-0.479**	**-0.527**	**-0.467**	**-0.716**	**-0.751**	**-0.520**	**-0.642**	0.352
Leaf_h	0.053	0.104	0.233	**0.581**	0.435	0.139	0.431	0.223
Leaf_E	-0.106	-0.162	-0.346	**-0.665**	**-0.462**	-0.185	-0.429	-0.178
Inf_L	**0.593**	**0.600**	0.284	0.318	**0.635**	**0.507**	0.395	**-0.831**
Inf_a	**-0.792**	**-0.783**	**-0.502**	-0.393	**-0.654**	**-0.704**	**-0.462**	**0.856**
Inf_b	**-0.745**	**-0.772**	**-0.568**	**-0.612**	**-0.816**	**-0.671**	**-0.573**	**0.867**
Inf_C	**-0.743**	**-0.770**	**-0.568**	**-0.613**	**-0.816**	**-0.669**	**-0.573**	**0.866**
Inf_h	**0.804**	**0.798**	**0.522**	0.428	**0.685**	**0.717**	**0.486**	**-0.866**
Inf_E	-0.384	-0.430	**-0.656**	**-0.711**	**-0.501**	-0.397	-0.432	0.167

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. Values in bold are different from 0 with a significance level alpha = 0.05. 'Mg' = mangiferin, 'IsoMg' = isomangiferin, 'Vic2' = vicenin-2, 'ErioT' = eriocitrin, 'IriG' = iriflophenone-3-*C*-glucoside, 'MacG' = maclurin-3-*C*-glucoside, 'ErioG' = eriodictyol-*O*-glucoside, 'Hd' = hesperidin, 'Inf' = infusion, 'Leaf' = unmilled, 'L', 'a', 'b', 'C', 'h' and 'E' refer to objective colour parameters. 'TSS' = total soluble solids, 'TCC' = total chlorophyll content. 'A' prefix refers to aroma attributes. 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'CookedVeg' = cooked vegetables, 'Tropical' = tropical fruit, 'Grass' = green grass, 'GSA' = general sweet, 'Apricot' = apricot jam.

Table B.2 Pearson's (n-1) correlation matrix for PCA (**Fig. 4.2**) of moisture content, chlorophyll content and leaf colour attributes of steamed (S) and unsteamed (U) green *C. maculata*, stored under LTS, NTS and HTS conditions over the period of t = 0 to 6 months

Variables	Moisture	TC	Leaf_L	Leaf_a	Leaf_b	Leaf_C	Leaf_h	Leaf_E
Mg	-0.289	-0.171	0.374	-0.050	**-0.555**	**-0.479**	0.053	-0.106
IsoMg	-0.338	-0.125	0.373	-0.103	**-0.597**	**-0.527**	0.104	-0.162
Vic2	-0.350	0.028	0.276	-0.244	**-0.489**	**-0.467**	0.233	-0.346
ErioG	**-0.683**	0.376	0.035	**-0.596**	**-0.669**	**-0.716**	**0.581**	**-0.665**
IriG	**-0.656**	0.256	0.110	-0.438	**-0.734**	**-0.751**	0.435	**-0.462**
ErioT	-0.349	-0.093	0.301	-0.130	**-0.580**	**-0.520**	0.139	-0.185
MacG	**-0.590**	0.246	0.023	-0.407	**-0.622**	**-0.642**	0.431	-0.429
Hd	0.037	0.332	**-0.605**	-0.197	**0.488**	0.352	0.223	-0.178
A_Grass	**-0.490**	**0.726**	**-0.488**	**-0.642**	-0.063	-0.209	**0.670**	**-0.604**
A_CookedVeg	**-0.802**	**0.531**	-0.101	**-0.662**	**-0.783**	**-0.834**	**0.655**	**-0.644**
A_StewedFruit	**0.778**	**-0.747**	0.261	**0.835**	0.434	**0.595**	**-0.807**	**0.881**
A_Tropical	-0.181	0.071	0.139	-0.221	-0.213	-0.227	0.188	-0.334
A_Marmalade	**0.788**	**-0.762**	0.398	**0.839**	**0.536**	**0.696**	**-0.785**	**0.845**
A_Apricot	**0.812**	**-0.823**	0.322	**0.880**	**0.493**	**0.671**	**-0.839**	**0.905**
A_Guava	0.019	-0.354	0.408	0.227	-0.122	-0.016	-0.155	0.156
A_Hay	-0.431	**0.508**	-0.388	-0.433	-0.157	-0.250	**0.466**	-0.343
A_Oats	-0.370	**0.625**	**-0.526**	**-0.505**	0.115	-0.076	**0.460**	-0.442
A_General sweet	**0.797**	**-0.812**	0.358	**0.862**	**0.488**	**0.672**	**-0.800**	**0.869**
A_Fruity-sweet	**0.800**	**-0.897**	**0.469**	**0.893**	**0.457**	**0.642**	**-0.863**	**0.867**
A_Caramel	**0.582**	-0.284	-0.074	0.394	**0.595**	**0.600**	-0.405	0.357
Moisture	**1**	**-0.829**	0.367	**0.920**	**0.642**	**0.785**	**-0.931**	**0.902**
TSS	-0.314	-0.125	0.323	-0.081	**-0.622**	**-0.555**	0.062	-0.114
TC	**-0.829**	**1**	**-0.696**	**-0.957**	-0.324	**-0.548**	**0.949**	**-0.930**
Leaf_L	0.367	**-0.696**	**1**	**0.597**	-0.075	0.142	**-0.577**	**0.552**
Leaf_a	**0.920**	**-0.957**	**0.597**	**1**	**0.483**	**0.687**	**-0.989**	**0.989**
Leaf_b	**0.642**	-0.324	-0.075	**0.483**	**1**	**0.962**	**-0.500**	**0.477**
Leaf_C	**0.785**	**-0.548**	0.142	**0.687**	**0.962**	**1**	**-0.690**	**0.679**
Leaf_h	**-0.931**	**0.949**	**-0.577**	**-0.989**	**-0.500**	**-0.690**	**1**	**-0.977**
Leaf_E	**0.902**	**-0.930**	**0.552**	**0.989**	**0.477**	**0.679**	**-0.977**	**1**
Inf_L	-0.198	-0.251	**0.538**	0.114	**-0.551**	-0.422	-0.100	0.138
Inf_a	0.032	**0.453**	**-0.592**	-0.273	**0.458**	0.303	0.269	-0.259
Inf_b	0.413	0.050	**-0.483**	0.127	**0.729**	**0.640**	-0.127	0.138
Inf_C	0.416	0.046	**-0.481**	0.131	**0.731**	**0.642**	-0.131	0.142
Inf_h	-0.075	-0.414	**0.585**	0.231	**-0.498**	-0.348	-0.227	0.217
Inf_E	**0.521**	-0.416	-0.112	**0.525**	0.407	**0.483**	**-0.505**	**0.609**

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. Values in bold are different from 0 with a significance level alpha = 0.05. 'Mg' = mangiferin, 'IsoMg' = isomangiferin, 'Vic2' = vicenin-2, 'ErioT' = eriocitrin, 'IriG' = iriflophenone-3-*C*-glucoside, 'MacG' = maclurin-3-*C*-glucoside, 'ErioG' = eriodictyol-*O*-glucoside, 'Hd' = hesperidin, 'Inf' = infusion, 'Leaf' = unmilled, 'L', 'a', 'b', 'C', 'h' and 'E' refer to objective colour parameters. 'TSS' = total soluble solids, 'TC' = total chlorophyll content. 'A' prefix refers to aroma attributes. 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'CookedVeg' = cooked vegetables, 'Tropical' = tropical fruit, 'Grass' = green grass, 'GSA' = general sweet, 'Apricot' = apricot jam.

Table B.3 Pearson's (n-1) correlation matrix for PCA (**Fig. 4.2**) of soluble solids content and infusion colour attributes of steamed (S) and unsteamed (U) green *C. maculata*, stored under LTS, NTS and HTS conditions over the period of t = 0 to 6 months

Variables	TSS	Inf_L	Inf_a	Inf_b	Inf_C	Inf_h	Inf_E
Mg	**0.908**	**0.593**	**-0.792**	**-0.745**	**-0.743**	**0.804**	-0.384
IsoMg	**0.907**	**0.600**	**-0.783**	**-0.772**	**-0.770**	**0.798**	-0.430
Vic2	**0.699**	0.284	**-0.502**	**-0.568**	**-0.568**	**0.522**	**-0.656**
ErioG	**0.650**	0.318	-0.393	**-0.612**	**-0.613**	0.428	**-0.711**
IriG	**0.854**	**0.635**	**-0.654**	**-0.816**	**-0.816**	**0.685**	**-0.501**
ErioT	**0.855**	**0.507**	**-0.704**	**-0.671**	**-0.669**	**0.717**	-0.397
MacG	**0.706**	0.395	**-0.462**	**-0.573**	**-0.573**	**0.486**	-0.432
Hd	**-0.721**	**-0.831**	**0.856**	**0.867**	**0.866**	**-0.866**	0.167
A_Grass	**-0.556**	**-0.459**	**0.749**	0.364	0.359	**-0.720**	-0.239
A_CookedVeg	**0.494**	**0.487**	-0.364	**-0.644**	**-0.646**	0.400	-0.289
A_StewedFruit	-0.247	0.113	-0.133	0.255	0.259	0.092	**0.875**
A_Tropical	0.366	-0.026	-0.220	-0.264	-0.264	0.231	**-0.575**
A_Marmalade	-0.194	0.059	-0.181	0.231	0.235	0.138	**0.615**
A_Apricot	-0.206	-0.009	-0.139	0.328	0.333	0.092	**0.773**
A_Guava	**0.505**	0.296	**-0.567**	-0.352	-0.349	**0.553**	-0.138
A_Hay	-0.291	-0.113	0.404	0.152	0.149	-0.386	0.087
A_Oats	-0.378	-0.331	**0.593**	0.278	0.274	**-0.573**	-0.152
A_General sweet	-0.231	-0.023	-0.122	0.316	0.320	0.077	**0.703**
A_Fruity-sweet	0.059	0.076	-0.365	0.139	0.144	0.317	**0.509**
A_Caramel	**-0.530**	**-0.702**	**0.570**	**0.717**	**0.718**	**-0.591**	0.045
Moisture	-0.314	-0.198	0.032	0.413	0.416	-0.075	**0.521**
TSS	**1**	**0.671**	**-0.851**	**-0.816**	**-0.814**	**0.864**	-0.350
TC	-0.125	-0.251	**0.453**	0.050	0.046	-0.414	-0.416
Leaf_L	0.323	**0.538**	**-0.592**	**-0.483**	**-0.481**	**0.585**	-0.112
Leaf_a	-0.081	0.114	-0.273	0.127	0.131	0.231	**0.525**
Leaf_b	**-0.622**	**-0.551**	**0.458**	**0.729**	**0.731**	**-0.498**	0.407
Leaf_C	**-0.555**	-0.422	0.303	**0.640**	**0.642**	-0.348	**0.483**
Leaf_h	0.062	-0.100	0.269	-0.127	-0.131	-0.227	**-0.505**
Leaf_E	-0.114	0.138	-0.259	0.138	0.142	0.217	**0.609**
Inf_L	**0.671**	**1**	**-0.878**	**-0.905**	**-0.903**	**0.892**	0.015
Inf_a	**-0.851**	**-0.878**	**1**	**0.845**	**0.842**	**-0.998**	0.022
Inf_b	**-0.816**	**-0.905**	**0.845**	**1**	**1.000**	**-0.872**	0.358
Inf_C	**-0.814**	**-0.903**	**0.842**	**1.000**	**1**	**-0.869**	0.361
Inf_h	**0.864**	**0.892**	**-0.998**	**-0.872**	**-0.869**	**1**	-0.059
Inf_E	-0.350	0.015	0.022	0.358	0.361	-0.059	**1**

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. Values in bold are different from 0 with a significance level alpha = 0.05. 'Mg' = mangiferin, 'IsoMg' = isomangiferin, 'Vic2' = vicenin-2, 'ErioT' = eriocitrin, 'IriG' = iriflophenone-3-*C*-glucoside, 'MacG' = maclurin-3-*C*-glucoside, 'ErioG' = eriodictyol-*O*-glucoside, 'Hd' = hesperidin, 'Inf' = infusion, 'Leaf' = unmilled, 'L', 'a', 'b', 'C', 'h' and 'E' refer to objective colour parameters. 'TSS' = total soluble solids, 'TC' = total chlorophyll content. 'A' prefix refers to aroma attributes. 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'CookedVeg' = cooked vegetables, 'Tropical' = tropical fruit, 'Grass' = green grass, 'GSA' = general sweet, 'Apricot' = apricot jam.

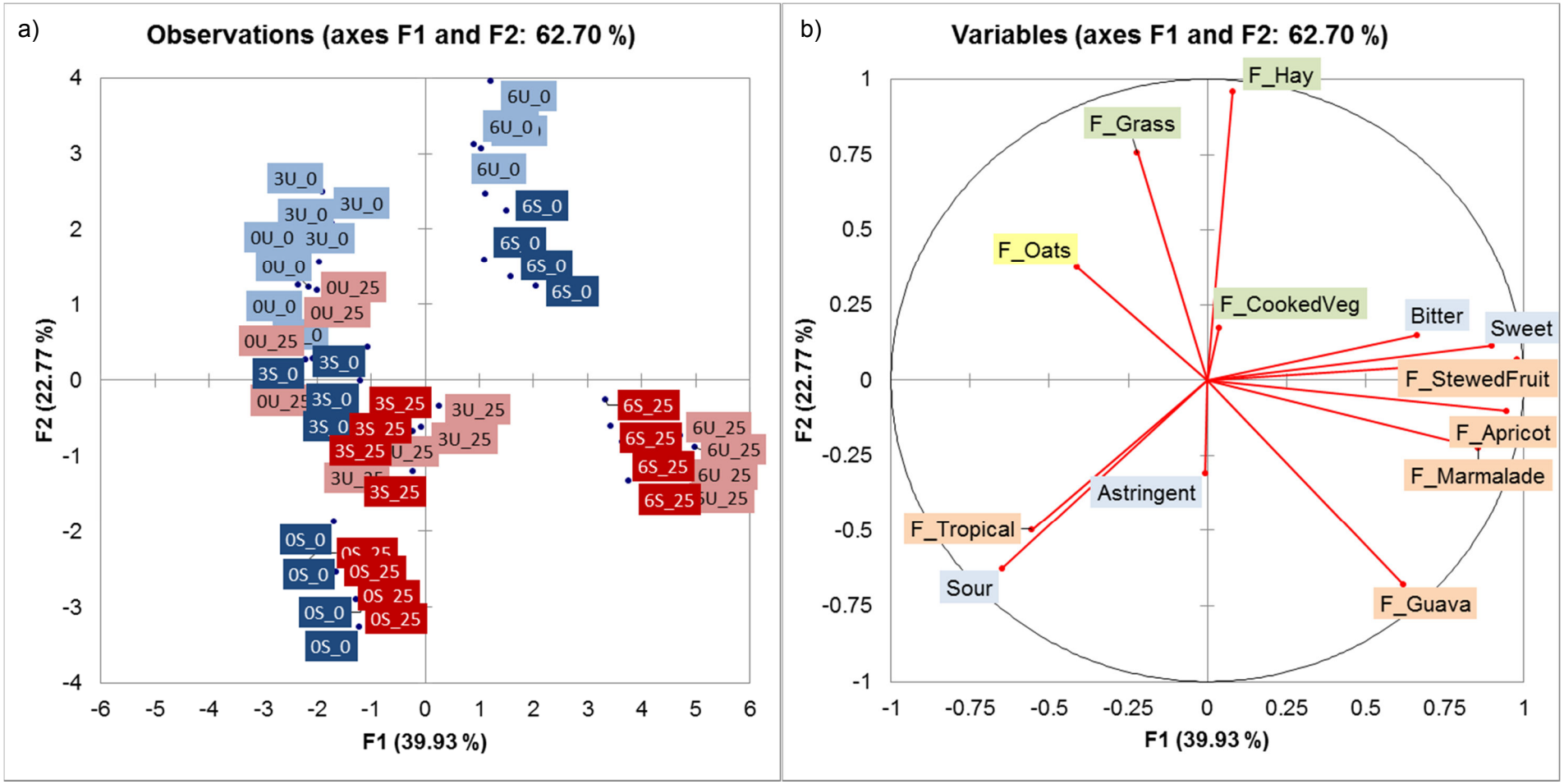


Figure B.1 PCA (a) scores and (b) loadings plots of flavour, taste and mouthfeel attributes of steamed (S) and unsteamed (U) green *C. maculata*, stored under LTS (0) and NTS (25) conditions over the storage period of t = 0 to 6 months, n = 20.

PCA scores plot sample codes indicated as (month)(steam treatment)_(storage condition temperature). Light blue = unsteamed LTS, Dark blue = steamed LTS, Light pink = unsteamed NTS, Dark red = steamed NTS. LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH. 'F' prefix refers to flavour attributes. 'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'Bitter' =bitter taste, 'Sour' =sour taste, 'Sweet' =sweet taste.

Table B.4 ANOVA p-values for DSA at t = 0 months

		AROMA											
Source	DF	Grass	Cooked veg	Stewed fruit	Tropical	Marmalade	Apricot	Guava	Hay	Oats	GSA	Fruity-sweet	Caramel
Batch	3	0.0271	0.0143	0.0306	0.0288	0.0047	<.0001	0.0081	0.5268	0.0866	0.0022	0.494	0.3697
Steam	1	<.0001	<.0001	<.0001	<0.0001	0.0297	<.0001	<.0001	<.0001	<.0001	0.0092	<.0001	<.0001
Temp	2	0.4924	0.5082	0.0827	0.8169	0.3484	0.098	0.7017	0.9771	0.9609	0.2535	0.6776	0.1832
Steam x Temp	2	0.6695	0.8627	0.3934	0.7082	0.648	0.7794	0.6747	0.6488	0.4704	0.9178	0.7911	0.2083
		TASTE AND MOUTHFEEL				FLAVOUR							
Source	DF	Sour	Bitter	Astringent	Sweet	Grass	Cooked veg	Stewed fruit	Tropical	Apricot	Guava	Hay	Oats
Batch	3	0.1351	0.0033	0.0253	0.0003	0.0826	0.0049	0.4687	0.0001	0.0016	0.0029	0.1558	0.1382
Steam	1	0.0002	<.0001	0.0002	0.5895	<.0001	<.0001	0.0008	<.0001	0.5642	<.0001	<.0001	<.0001
Temp	2	0.4391	0.1405	0.6459	0.3899	0.7905	0.1625	0.578	0.6742	0.8917	0.7367	0.7563	0.9093
Steam x Temp	2	0.9953	0.9525	0.8509	0.3931	0.4114	0.5514	0.578	0.3144	0.9922	0.822	0.1951	0.6774

'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet, 'Bitter' =bitter taste, 'Sour' =sour taste, 'Sweet' =sweet taste. Significant ($p < 0.05$) p-values indicated in yellow.

Table B.5 ANOVA p-values for DSA at t = 1 month

		AROMA											
Source	DF	Grass	Cooked veg	Stewed fruit	Tropical	Marmalade	Apricot	Guava	Hay	Oats	GSA	Fruity- sweet	Caramel
Batch	3	0.9756	0.5094	0.0146	0.2049	0.3016	0.3697	0.0991	0.1315	0.0046	0.227	0.577	0.7324
Steam	1	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.006	<.0001	0.0039	0.2322	<.0001
Temp	2	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0128	<.0001	<.0001	<.0001	<.0001	<.0001
Steam x Temp	2	<.0001	0.8503	<.0001	0.3871	<.0001	<.0001	0.0764	0.0003	<.0001	<.0001	<.0001	0.0004

'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet. Significant ($p < 0.05$) p-values indicated in yellow.

Table B.6 ANOVA p-values for DSA at t = 3 months

		AROMA											
Source	DF	Grass	Cooked veg	Stewed fruit	Tropical	Marmalade	Apricot	Guava	Hay	Oats	GSA	Fruity- sweet	Caramel
Batch	3	0.0005	0.0005	0.0043	0.198	0.9171	0.8505	0.0026	0.0297	0.1449	0.0805	0.1467	0.246
Steam	1	<.0001	<.0001	0.0063	0.0548	0.0026	0.831	<.0001	0.001	0.3021	0.8557	0.0121	0.0038
Temp	1	<.0001	<.0001	<.0001	0.1393	<.0001	<.0001	<.0001	<.0001	0.0039	<.0001	<.0001	0.0046
Steam x Temp	1	<.0001	0.0013	0.011	0.1417	0.0042	0.0289	0.0095	0.0004	0.0313	0.5238	0.0568	0.0681

		TASTE AND MOUTHFEEL				FLAVOUR								
Source	DF	Sour	Bitter	Astringent	Sweet	Grass	Cooked veg	Stewed fruit	Tropical	Marmalade	Apricot	Guava	Hay	Oats
Batch	3	0.3103	0.0337	0.6664	0.0529	0.0218	0.0008	0.0272	0.2294	0.2645	0.1	0.0888	0.0223	0.0235
Steam	1	0.3064	0.0646	0.0947	0.076	0.0003	0.0013	0.6885	0.7626	0.358/6	0.9961	0.0082	0.009	0.4988
Temp	1	0.7956	0.7031	0.2174	0.0485	<.0001	<.0001	<.0001	0.0432	<.0001	<.0001	0.0002	<.0001	0.3107
Steam x Temp	1	0.3064	0.2096	0.1346	0.291	0.0029	0.2828	0.0622	0.5644	0.0389	0.0544	0.0088	0.0055	0.3344

'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet, 'Bitter' =bitter taste, 'Sour' =sour taste, 'Sweet' =sweet taste. Significant ($p < 0.05$) p-values indicated in yellow.

Table B.7 ANOVA p-values for DSA at t = 6 months

		AROMA										
Source	DF	Grass	Cooked veg	Stewed fruit	Marmalade	Apricot	Guava	Hay	Oats	GSA	Fruity- sweet	Caramel
Batch	3	0.2493	0.4566	0.2136	0.5163	0.0695	0.9068	<.0001	0.5965	0.141	0.7906	0.3802
Steam	1	<.0001	0.0014	0.0039	<.0001	0.0093	0.2666	<.0001	<.0001	0.0012	0.2984	0.9717
Temp	1	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Steam x Temp	1	0.0003	0.0045	<.0001	<.0001	<.0001	0.0145	<.0001	<.0001	0.0005	<.0001	0.6398

		TASTE AND MOUTHFEEL				FLAVOUR							
Source	DF	Sour	Bitter	Astringent	Sweet	Grass	Cooked veg	Stewed fruit	Marmalade	Apricot	Guava	Hay	Oats
Batch	3	0.0441	0.0491	0.1827	0.9016	0.0135	0.3876	0.8231	0.0109	0.7087	0.1655	0.8848	0.8896
Steam	1	0.1005	0.0024	0.0341	0.0026	<.0001	<.0001	0.0419	<.0001	0.0117	0.1823	0.1995	0.0012
Temp	1	0.0083	0.0048	0.2183	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002
Steam x Temp	1	0.7559	0.7548	0.9081	0.1789	0.07	0.0008	0.0002	<.0001	0.0003	0.0619	0.0119	0.0026

'Grass' = green grass, 'CookedVeg' = cooked vegetables, 'Hay' = hay/dried grass, 'Oats' = oats/porridge/grains, 'Tropical' = tropical fruit, 'Apricot' = apricot jam, 'GSA' = general sweet, 'Bitter' =bitter taste, 'Sour' =sour taste, 'Sweet' =sweet taste. Significant ($p < 0.05$) p-values indicated in yellow.

Table B.8 Means, standard deviation (SD) and significant differences between aroma attributes of steamed and unsteamed green *C. maculata* stored under LTS, NTS and HTS conditions for t = 0 months

		Green grass				Cooked vegetables				Stewed fruit				Tropical fruit				Marmalade			
Steaming time (s)	Storage conditions	Mean			SD	Mean			SD	Mean			SD	Mean			SD	Mean			SD
0	LTS	21.00	a	±	1.36	13.57	b	±	1.91	3.93	a	±	0.58	7.00	b	±	1.29	0.09	a	±	0.10
0	HTS	21.17	a	±	2.84	12.45	b	±	1.84	3.15	ab	±	0.44	6.87	b	±	0.97	0.15	a	±	0.20
0	NTS	19.06	a	±	4.77	13.75	b	±	2.28	2.99	b	±	0.61	6.89	b	±	0.68	0.16	a	±	0.20
60	LTS	7.81	b	±	1.65	24.02	a	±	4.24	1.25	c	±	0.61	13.06	a	±	2.77	0.00	a	±	0.00
60	HTS	8.35	b	±	2.29	23.94	a	±	2.95	0.81	c	±	0.95	14.11	a	±	2.52	0.09	a	±	0.18
60	NTS	7.80	b	±	2.35	25.01	a	±	0.72	1.09	c	±	0.73	13.88	a	±	1.75	0.00	a	±	0.00

		Apricot jam				Guava				Hay/Dried grass				Oats/Porridge/Grains				General sweet			
Steaming time (s)	Storage conditions	Mean			SD	Mean			SD	Mean			SD	Mean			SD	Mean			SD
0	LTS	5.58	a	±	1.47	14.19	b	±	1.19	29.89	a	±	0.67	12.92	a	±	1.46	19.57	a	±	1.06
0	HTS	5.61	a	±	1.74	13.49	b	±	1.04	29.17	a	±	1.41	12.13	a	±	0.70	19.60	a	±	1.46
0	NTS	4.81	a	±	0.92	13.98	b	±	2.39	29.01	a	±	2.27	11.80	a	±	1.44	19.07	ab	±	2.00
60	LTS	3.87	b	±	1.07	24.95	a	±	4.11	21.37	b	±	2.28	2.48	b	±	2.74	18.70	ab	±	0.41
60	HTS	3.90	b	±	1.83	25.38	a	±	3.23	21.76	b	±	1.21	3.23	b	±	2.00	18.55	ab	±	1.28
60	NTS	3.46	b	±	0.86	26.39	a	±	1.54	21.98	b	±	1.11	3.20	b	±	1.62	17.84	b	±	0.72

		Fruity-sweet				Caramel				Seaweed/Oceanic	
Steaming time (s)	Storage conditions	Mean			SD	Mean			SD	Mean	SD
0	LTS	7.70	b	±	1.52	8.26	a	±	0.92	nd	
0	HTS	8.28	b	±	0.83	7.68	ab	±	0.11	nd	
0	NTS	7.27	b	±	2.45	6.72	b	±	1.01	nd	
60	LTS	17.18	a	±	1.79	2.44	c	±	1.19	nd	
60	HTS	17.75	a	±	1.65	2.13	c	±	0.46	nd	
60	NTS	17.62	a	±	1.75	2.36	c	±	0.83	nd	

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. Means associated with different letters differ significantly ($p < 0.05$) for each attribute. nd = not detected.

Table B.9 Means, standard deviation (SD) and significant differences between taste and mouthfeel attributes of steamed and unsteamed green *C. maculata* stored under LTS, NTS and HTS conditions for t = 0 months

		Sour taste				Bitter taste				Astringent				Sweet taste			
Steaming time (s)	Storage conditions	Mean			SD	Mean			SD	Mean			SD	Mean			SD
0	LTS	16.09	c	±	0.46	29.05	b	±	1.33	36.94	b	±	0.49	13.53	a	±	0.81
0	HTS	16.41	c	±	0.56	29.07	b	±	1.02	36.79	b	±	1.01	13.50	a	±	0.32
0	NTS	16.49	bc	±	0.98	29.85	b	±	1.33	37.19	b	±	0.30	13.50	a	±	0.92
60	LTS	17.38	ab	±	0.89	31.75	a	±	1.70	37.97	a	±	0.65	13.50	a	±	0.69
60	HTS	17.72	a	±	0.77	31.51	a	±	1.38	37.98	a	±	0.67	13.68	a	±	0.84
60	NTS	17.75	a	±	0.17	32.53	a	±	0.99	38.07	a	±	0.51	13.02	a	±	1.06

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. Means associated with different letters differ significantly ($p < 0.05$) for each attribute.

Table B.10 Means, standard deviation (SD) and significant differences between flavour attributes of steamed and unsteamed green *C. maculata* stored under LTS, NTS and HTS conditions for t = 0 months

		Green grass				Cooked vegetables				Stewed fruit				Tropical fruit				Marmalade	
Steaming time (s)	Storage conditions	Mean			SD	Mean			SD	Mean			SD	Mean			SD	Mean	SD
0	LTS	17.77	a	±	0.82	8.21	c	±	2.59	0.33	ab	±	0.38	4.46	b	±	1.75	nd	
0	HTS	17.91	a	±	0.70	8.69	c	±	2.28	0.53	a	±	0.39	4.24	b	±	2.00	nd	
0	NTS	16.62	a	±	2.46	9.06	c	±	2.22	0.63	a	±	0.44	4.11	b	±	0.98	nd	
60	LTS	8.22	b	±	1.95	16.62	b	±	4.16	0.00	b	±	0.00	5.95	a	±	1.59	nd	
60	HTS	8.27	b	±	1.23	18.56	ab	±	1.46	0.00	b	±	0.00	6.94	a	±	1.37	nd	
60	NTS	8.66	b	±	1.41	19.42	a	±	1.54	0.00	b	±	0.00	6.69	a	±	1.19	nd	
		Apricot jam				Guava				Hay/Dried grass				Oats/Porridge/Grains				Caramel	
Steaming time (s)	Storage conditions	Mean			SD	Mean			SD	Mean			SD	Mean			SD	Mean	SD
0	LTS	0.89	a	±	0.33	9.15	b	±	2.26	29.61	a	±	0.50	2.50	a	±	0.93	nd	
0	HTS	0.98	a	±	0.67	8.40	b	±	1.90	29.23	a	±	0.63	2.98	a	±	0.87	nd	
0	NTS	0.82	a	±	0.45	9.24	b	±	1.15	28.65	a	±	0.74	2.80	a	±	1.46	nd	
60	LTS	0.75	a	±	0.98	15.42	a	±	2.79	23.82	b	±	1.69	0.17	b	±	0.20	nd	
60	HTS	0.83	a	±	1.00	15.51	a	±	1.16	23.49	b	±	1.21	0.00	b	±	0.00	nd	
60	NTS	0.74	a	±	0.99	15.74	a	±	1.96	24.43	b	±	0.77	0.08	b	±	0.17	nd	

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. Means associated with different letters differ significantly ($p < 0.05$) for each attribute. nd = not detected.

Table B.11 Means, standard deviation (SD) and significant differences between aroma attributes of steamed and unsteamed green *C. maculata* stored under LTS, NTS and HTS conditions for t = 1 month

		Green grass				Cooked vegetables				Stewed fruit				Tropical fruit				Marmalade			
Steaming time (s)	Storage conditions	Mean			SD	Mean			SD	Mean			SD	Mean			SD	Mean			SD
0	LTS	20.49	a	±	0.22	17.35	bc	±	0.64	11.68	e	±	0.82	1.79	d	±	0.46	0.16	c	±	0.21
0	HTS	4.04	e	±	0.54	8.79	e	±	2.48	33.14	a	±	1.96	6.97	b	±	1.33	13.40	b	±	1.15
0	NTS	15.16	b	±	1.21	14.75	cd	±	2.45	15.64	cd	±	1.48	2.92	d	±	0.73	1.31	c	±	0.26
60	LTS	11.17	c	±	1.67	22.03	a	±	0.95	14.37	d	±	0.96	4.81	c	±	1.10	0.81	c	±	0.61
60	HTS	4.01	e	±	0.63	12.50	d	±	2.21	22.48	b	±	1.15	9.11	a	±	0.37	29.78	a	±	6.33
60	NTS	8.26	d	±	0.49	18.65	b	±	0.62	16.01	c	±	1.43	4.55	c	±	1.67	0.95	c	±	0.47

		Apricot jam				Guava				Hay/Dried grass				Oats/Porridge/Grains				General sweet			
Steaming time (s)	Storage conditions	Mean			SD	Mean			SD	Mean			SD	Mean			SD	Mean			SD
0	LTS	6.02	d	±	0.88	8.76	b	±	0.57	27.45	a	±	1.72	14.10	a	±	1.36	18.22	d	±	0.40
0	HTS	50.02	a	±	1.94	12.52	a	±	2.19	17.53	d	±	0.62	1.79	c	±	0.27	32.04	a	±	1.00
0	NTS	9.40	c	±	1.80	8.75	b	±	1.58	26.72	a	±	0.57	14.50	a	±	2.67	19.37	cd	±	0.74
60	LTS	7.63	cd	±	0.77	12.78	a	±	2.67	25.05	b	±	0.18	8.44	b	±	1.13	18.58	d	±	0.93
60	HTS	26.77	b	±	1.26	14.38	a	±	1.48	19.18	c	±	0.94	1.93	c	±	0.81	26.76	b	±	1.75
60	NTS	9.57	c	±	1.58	14.54	a	±	1.42	23.94	b	±	1.14	8.26	b	±	1.15	20.33	c	±	0.60

		Fruity-sweet				Caramel				Seaweed/Oceanic			
Steaming time (s)	Storage conditions	Mean			SD	Mean			SD	Mean			SD
0	LTS	6.01	e	±	0.67	4.32	c	±	1.05	0.01	a	±	0.02
0	HTS	42.01	a	±	1.12	10.43	a	±	1.41	0.00	a	±	0.00
0	NTS	8.45	d	±	1.10	6.65	b	±	1.44	0.13	a	±	0.25
60	LTS	10.07	d	±	1.67	2.13	d	±	0.46	0.00	a	±	0.00
60	HTS	31.51	b	±	1.31	3.15	cd	±	0.59	0.16	a	±	0.32
60	NTS	13.00	c	±	1.16	4.17	c	±	0.90	0.06	a	±	0.11

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. Means associated with different letters differ significantly ($p < 0.05$) for each attribute.

Table B.12 Means, standard deviation (SD) and significant differences between aroma attributes of steamed and unsteamed green *C. maculata* stored under LTS and NTS conditions for t = 3 months

		Green grass				Cooked vegetables				Stewed fruit				Tropical fruit				Marmalade			
Steaming time (s)	Storage conditions	Mean			SD	Mean			SD	Mean			SD	Mean			SD	Mean			SD
0	LTS	19.57	a	±	2.17	16.00	b	±	1.62	7.31	c	±	2.23	0.05	b	±	0.10	1.07	c	±	0.65
0	NTS	7.57	c	±	1.50	9.23	d	±	1.15	18.09	a	±	1.61	0.05	b	±	0.10	19.62	a	±	2.34
60	LTS	11.15	b	±	2.23	20.77	a	±	1.65	10.61	b	±	1.67	0.10	b	±	0.12	0.73	c	±	1.00
60	NTS	6.15	d	±	1.23	10.76	c	±	1.78	18.26	a	±	1.17	0.37	a	±	0.32	11.21	b	±	2.71

		Apricot jam				Guava				Hay/Dried grass				Oats/Porridge/Grains				General sweet			
Steaming time (s)	Storage conditions	Mean			SD	Mean			SD	Mean			SD	Mean			SD	Mean			SD
0	LTS	9.14	b	±	1.34	9.53	c	±	1.88	33.93	a	±	1.51	18.25	a	±	1.77	18.57	b	±	0.67
0	NTS	27.17	a	±	1.98	16.05	b	±	2.28	27.29	c	±	1.25	12.46	b	±	2.78	24.00	a	±	1.15
60	LTS	11.46	b	±	1.35	16.16	b	±	2.04	29.98	b	±	0.75	14.95	b	±	2.12	18.97	b	±	1.07
60	NTS	25.21	a	±	1.14	19.17	a	±	1.66	27.54	c	±	0.41	13.77	b	±	1.42	23.77	a	±	1.59

		Fruity-sweet				Caramel				Seaweed/Oceanic			
Steaming time (s)	Storage conditions	Mean			SD	Mean			SD	Mean			SD
0	LTS	6.46	c	±	1.63	6.11	a	±	0.98	0.25	a	±	0.31
0	NTS	26.31	a	±	3.64	7.23	a	±	1.50	0.08	a	±	0.16
60	LTS	12.13	b	±	1.89	2.11	b	±	1.18	0.54	a	±	0.68
60	NTS	27.32	a	±	2.15	6.02	a	±	1.96	0.21	a	±	0.24

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH. Means associated with different letters differ significantly ($p < 0.05$) for each attribute.

Table B.13 Means, standard deviation (SD) and significant differences between taste and mouthfeel attributes of steamed and unsteamed green *C. maculata* stored under LTS and NTS conditions for t = 3 months

Steaming time (s)	Storage conditions	Sour taste Mean			SD	Bitter taste Mean			SD	Astringent Mean			SD	Sweet taste Mean			SD
0	LTS	15.79	a	±	0.28	30.94	b	±	1.45	36.18	b	±	0.89	12.46	b	±	0.59
0	NTS	16.52	a	±	1.40	32.12	ab	±	2.37	37.78	ab	±	1.21	13.40	a	±	0.77
60	LTS	16.96	a	±	1.69	33.28	a	±	2.05	38.07	a	±	0.54	12.22	b	±	0.46
60	NTS	16.52	a	±	0.41	32.63	ab	±	1.35	37.90	ab	±	1.25	12.53	ab	±	0.95

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH. Means associated with different letters differ significantly ($p < 0.05$) for each attribute.

Table B.14 Means, standard deviation (SD) and significant differences between flavour attributes of steamed and unsteamed green *C. maculata* stored under LTS and NTS conditions for t = 3 months

Steaming time (s)	Storage conditions	Green grass Mean			SD	Cooked vegetables Mean			SD	Stewed fruit Mean			SD	Tropical fruit Mean			SD	Marmalade Mean			SD
0	LTS	15.71	a	±	2.45	13.22	b	±	2.45	1.74	b	±	0.36	0.43	a	±	0.50	0.24	c	±	0.18
0	NTS	7.10	c	±	1.88	9.91	c	±	2.04	6.48	a	±	1.89	0.00	a	±	0.00	7.13	a	±	2.49
60	LTS	9.50	b	±	1.83	16.57	a	±	2.24	2.50	b	±	0.51	0.30	a	±	0.38	1.25	c	±	1.07
60	NTS	6.08	c	±	0.87	11.94	b	±	3.02	5.36	a	±	1.50	0.04	a	±	0.08	4.76	b	±	1.26

Steaming time (s)	Storage conditions	Apricot jam Mean			SD	Guava Mean			SD	Hay/Dried grass Mean			SD	Oats/Porridge/Grains Mean			SD	Caramel Mean	SD
0	LTS	1.93	b	±	0.51	7.46	b	±	0.87	30.26	a	±	1.08	7.02	a	±	1.75	nd	
0	NTS	9.51	a	±	1.07	12.69	a	±	1.99	27.16	c	±	0.61	6.03	a	±	0.78	nd	
60	LTS	2.96	b	±	0.96	11.25	a	±	0.74	28.39	b	±	0.83	6.20	a	±	1.13	nd	
60	NTS	8.49	a	±	1.65	12.72	a	±	1.55	27.24	c	±	0.41	6.18	a	±	1.57	nd	

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH. Means associated with different letters differ significantly ($p < 0.05$) for each attribute. nd = not detected.

Table B.15 Means, standard deviation (SD) and significant differences between aroma attributes of steamed and unsteamed green *C. maculata* stored under LTS and NTS conditions for t = 6 months

		Green grass				Cooked vegetables				Stewed fruit				Marmalade				Apricot jam			
Steaming time (s)	Storage conditions	Mean			SD	Mean			SD	Mean			SD	Mean			SD	Mean			SD
0	LTS	18.71	a	±	0.66	25.90	a	±	0.69	15.76	d	±	1.33	2.32	c	±	0.51	15.50	d	±	1.84
0	NTS	9.93	c	±	1.10	5.77	c	±	2.32	34.55	a	±	1.44	43.42	a	±	1.59	51.40	a	±	2.25
60	LTS	13.25	b	±	0.94	26.61	a	±	1.26	17.89	c	±	1.14	4.04	c	±	1.74	19.64	c	±	2.07
60	NTS	9.41	c	±	0.92	13.21	b	±	2.28	28.30	b	±	0.63	26.04	b	±	0.90	42.30	b	±	1.29

		Guava				Hay/Dried grass				Oats/Porridge/Grains				General sweet				Fruity-sweet			
Steaming time (s)	Storage conditions	Mean			SD	Mean			SD	Mean			SD	Mean			SD	Mean			SD
0	LTS	13.51	c	±	1.38	37.24	a	±	0.71	17.00	a	±	1.55	21.51	c	±	0.66	4.29	d	±	1.45
0	NTS	26.73	a	±	1.12	26.45	c	±	0.41	0.99	c	±	0.70	38.19	a	±	2.13	44.20	a	±	1.49
60	LTS	14.74	c	±	0.37	34.46	b	±	0.71	6.00	b	±	1.02	21.87	c	±	0.22	11.84	c	±	1.39
60	NTS	23.90	b	±	1.57	26.73	c	±	0.96	0.43	c	±	0.18	33.00	b	±	0.83	35.00	b	±	1.16

		Caramel			
Steaming time (s)	Storage conditions	Mean			SD
0	LTS	1.02	b	±	0.74
0	NTS	5.74	a	±	2.33
60	LTS	0.73	b	±	0.35
60	NTS	6.08	a	±	0.99

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH. Means associated with different letters differ significantly ($p < 0.05$) for each attribute.

Table B.16 Means, standard deviation (SD) and significant differences between taste and mouthfeel attributes of steamed and unsteamed green *C. maculata* stored under LTS and NTS conditions for t = 6 months

Steaming time (s)	Storage conditions	Sour taste Mean			SD	Bitter taste Mean			SD	Astringent Mean			SD	Sweet taste Mean			SD
0	LTS	12.59	b	±	1.37	36.25	b	±	1.37	37.28	ab	±	1.26	17.01	bc	±	0.58
0	NTS	13.94	a	±	0.50	34.39	c	±	0.32	36.70	b	±	0.23	19.95	a	±	0.87
60	LTS	13.38	ab	±	0.70	38.03	a	±	1.36	38.25	a	±	0.42	15.93	c	±	0.75
60	NTS	14.49	a	±	1.07	36.46	b	±	1.41	37.76	ab	±	1.21	17.69	b	±	0.64

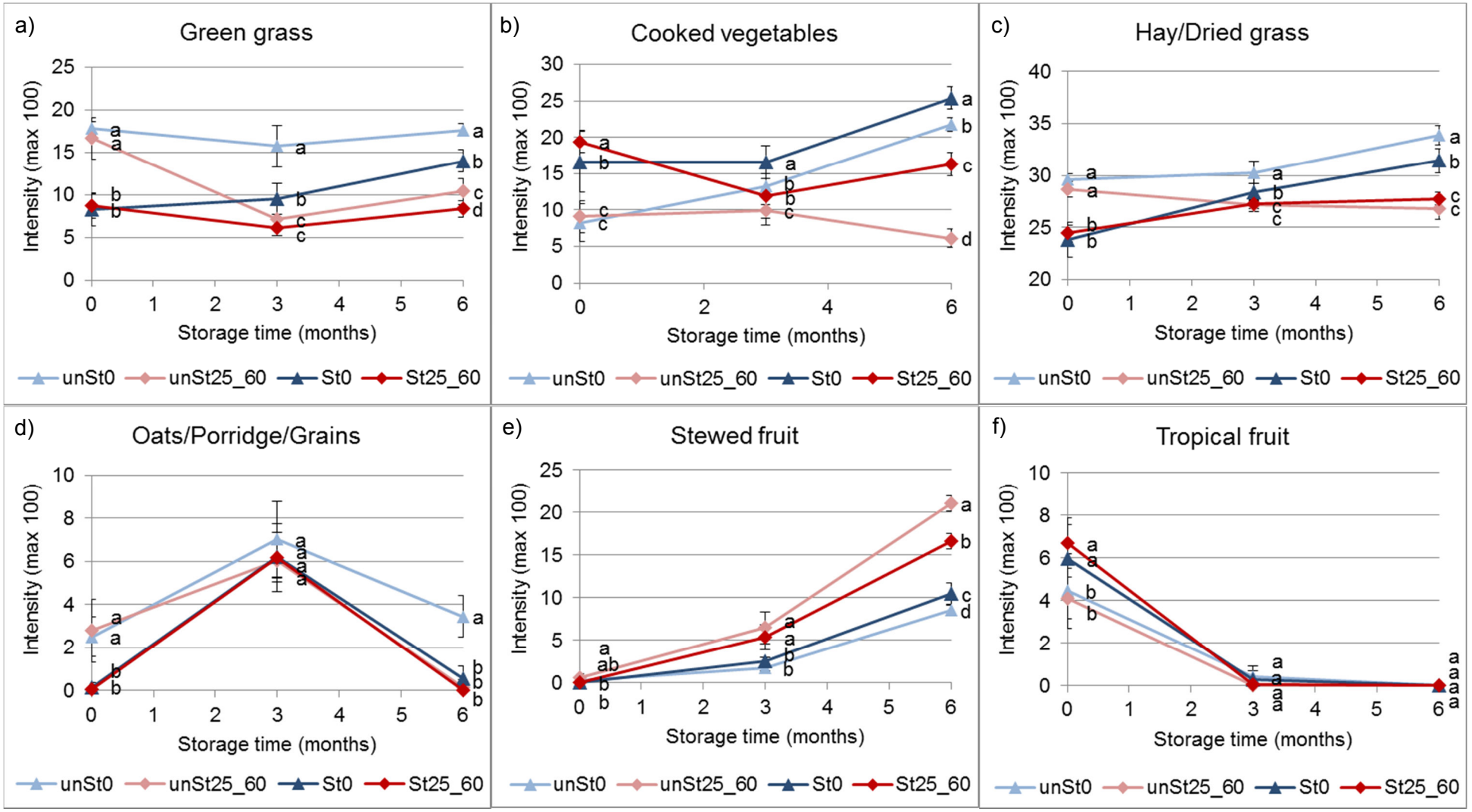
LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH. Means associated with different letters differ significantly ($p < 0.05$) for each attribute.

Table B.17 Means, standard deviation (SD) and significant differences between flavour attributes of steamed and unsteamed green *C. maculata* stored under LTS and NTS conditions for t = 6 months

Steaming time (s)	Storage conditions	Green grass Mean			SD	Cooked vegetables Mean			SD	Stewed fruit Mean			SD	Marmalade Mean			SD	Apricot jam Mean			SD
0	LTS	17.60	a	±	0.75	21.77	b	±	0.89	8.57	d	±	0.59	1.31	d	±	0.58	6.20	c	±	1.30
0	NTS	10.36	c	±	1.50	6.12	d	±	1.30	21.04	a	±	0.88	22.39	a	±	1.22	26.23	a	±	1.53
60	LTS	13.97	b	±	1.27	25.42	a	±	1.52	10.48	c	±	1.26	1.29	c	±	1.52	8.03	c	±	0.80
60	NTS	8.31	d	±	0.99	16.29	c	±	1.57	16.66	b	±	0.93	11.85	b	±	1.44	19.80	b	±	1.64

Steaming time (s)	Storage conditions	Guava Mean			SD	Hay/Dried grass Mean			SD	Oats/Porridge/Grains Mean			SD
0	LTS	10.75	c	±	0.96	33.86	a	±	0.95	3.44	a	±	0.97
0	NTS	17.39	a	±	1.17	26.82	c	±	1.05	0.17	b	±	0.33
60	LTS	12.70	b	±	1.66	31.42	b	±	1.15	0.58	b	±	0.55
60	NTS	17.02	a	±	1.04	27.76	c	±	0.63	0.00	b	±	0.00

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH. Means associated with different letters differ significantly ($p < 0.05$) for each attribute.



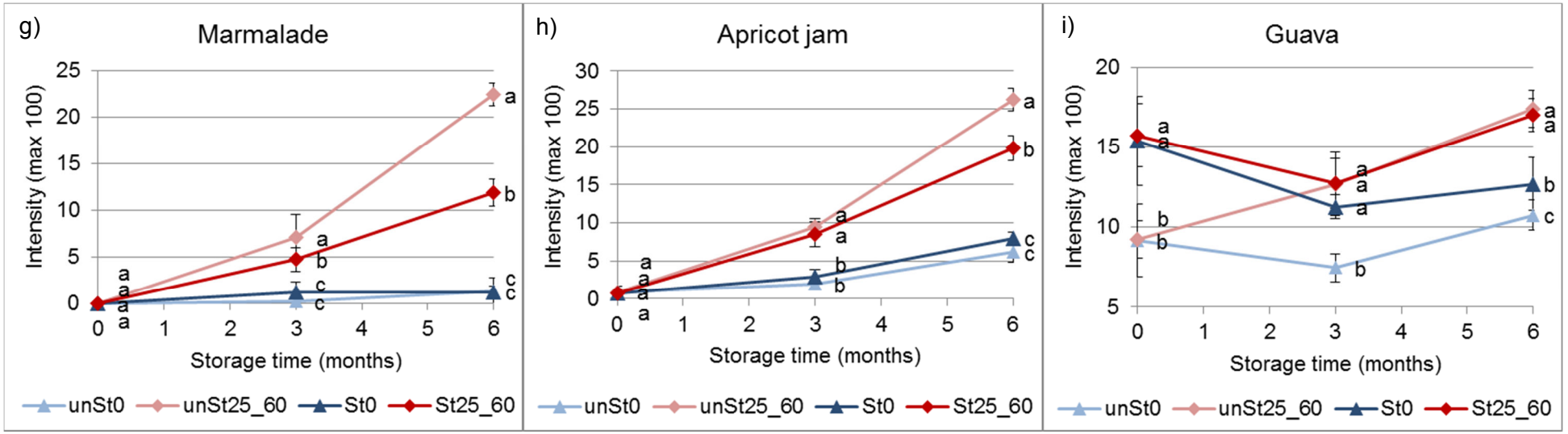


Figure B.2 *Vegetative* (a – c), *cereal* (d) and *fruity* (e – i) flavour attributes of infusions, prepared from steamed and unsteamed green *C. maculata* after t = 0, 3 and 6 months of storage under LTS, NTS and HTS conditions.

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. Intensity points within a time point do not differ significantly ($p \geq 0.05$) from each other when associated with the same letter/s. 'St' = steamed, 'unSt' = unsteamed, '0' = LTS conditions, '25_60' = NTS conditions, '40_75' = HTS conditions.

Table B.18 ANOVA of green *C. maculata* plant material attributes after t = 0 to 1 months of storage under LTS, NTS and HTS conditions

Source	DF	Moisture	TC	Leaf L	Leaf a	Leaf b	Leaf C	Leaf h	Leaf E
Batch	3	<.0001	0.0035	<.0001	0.0016	<.0001	<.0001	0.0015	0.1748
Steam	1	0.0025	0.0065	0.0177	0.0772	<.0001	<.0001	0.0848	0.3032
Temp	2	<.0001	<.0001	0.1695	<.0001	0.0004	<.0001	<.0001	<.0001
Steam x Temp	2	0.7721	0.0655	0.187	0.2792	0.1345	0.1027	0.2701	0.1645
Time	1	<.0001	<.0001	0.5708	<.0001	0.0014	<.0001	<.0001	<.0001
Steam x Time	1	0.0533	0.0708	0.1971	0.2324	0.9454	0.8446	0.1683	0.3329
Temp x Time	2	<.0001	<.0001	0.0234	<.0001	0.0051	<.0001	<.0001	<.0001
Steam x Temp x Time	2	0.5117	0.0786	0.4976	0.3827	0.9433	0.9637	0.3959	0.1975

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH. 'TC' = total chlorophyll content, 'Leaf' = unmilled plant material. Significant ($p < 0.05$) p-values indicated in yellow.

Table B.19 ANOVA of infusion attributes, as prepared from green *C. maculata* after t = 0 to 1 months of storage under LTS, NTS and HTS conditions

Source	DF	Mg	IsoMg	Vic2	ErioG	IriGlc	ErioT	MacG	Hd	TSS	Inf L	Inf a	Inf b	Inf C	Inf h	Inf E
Batch	3	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0005	<.0001	<.0001	<.0001	0.7957
Steam	1	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2496
Temp	2	0.9882	0.9521	0.8873	0.1526	0.0004	0.8182	0.0047	0.7118	0.0729	0.3056	0.0526	0.4297	0.4259	0.0666	0.0668
Steam x Temp	2	0.9526	0.9503	0.6245	0.6111	0.9104	0.7936	0.478	0.8983	0.0235	0.7924	0.4383	0.6834	0.6804	0.5017	0.2529
Time	1	0.0041	0.0012	<.0001	<.0001	0.0004	0.0017	<.0001	0.0026	0.0737	0.0012	0.0014	0.4129	0.4082	0.0115	<.0001
Steam x Time	1	0.6928	0.6578	0.4733	0.5119	0.6834	0.7085	0.9412	0.4088	0.7162	0.356	0.0018	0.339	0.3319	0.0111	0.2204
Temp x Time	2	0.5877	0.5568	0.3965	0.0009	0.0226	0.5559	0.127	0.4227	0.7219	0.0229	<.0001	0.0014	0.0013	0.0006	0.0463
Steam x Temp x Time	2	0.2109	0.1561	0.1222	0.0038	0.0956	0.1718	0.3645	0.0807	0.721	0.8768	0.0064	0.3554	0.3524	0.0098	0.2116

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. 'Mg' = mangiferin, 'IsoMg' = isomangiferin, 'Vic2' = vicenin-2, 'ErioG' = eridictyol-*O*-glucoside, 'IriGlc' = iriflophenone-3-*C*-glucoside, 'ErioT' = eriocitrin, 'MacG' = maclurin-3-*C*-glucoside, 'Hd' = hesperidin, 'TSS' = total soluble solids, 'Inf' = infusion. Significant ($p < 0.05$) p-values indicated in yellow.

Table B.20 ANOVA of green *C. maculata* plant material attributes after t = 0 to 6 months of storage under LTS and NTS conditions

Source	DF	Moisture	TC	Leaf L	Leaf a	Leaf b	Leaf C	Leaf h	Leaf E
Batch	3	0.0069	<.0001	<.0001	0.0052	0.0009	0.0012	0.0049	0.3308
Steam	1	0.1072	<.0001	0.0017	0.0006	<.0001	0.0003	0.0006	0.006
Temp	1	<.0001	<.0001	0.0152	<.0001	<.0001	<.0001	<.0001	<.0001
Steam x Temp	1	0.8164	0.0001	0.0113	0.0011	0.0533	0.0187	0.0008	0.0019
Time	6	<.0001	<.0001	0.502	<.0001	<.0001	<.0001	<.0001	<.0001
Steam x Time	6	0.2767	0.0002	0.3862	<.0001	<.0001	0.0003	0.0001	0.0002
Temp x Time	6	<.0001	<.0001	0.0131	<.0001	<.0001	<.0001	<.0001	<.0001
Steam x Temp x Time	6	0.5586	0.0839	0.322	<.0001	0.14	0.0761	<.0001	<.0001

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH. 'TC' = total chlorophyll content, 'Leaf' = unmilled plant material. Significant ($p < 0.05$) p-values indicated in yellow.

Table B.21 ANOVA of infusion attributes, as prepared from green *C. maculata* after t = 0 to 6 months of storage under LTS and NTS conditions

Source	DF	Mg	IsoMg	Vic2	ErioG	IriGlc	ErioT	MacG	Hd	TSS	Inf L	Inf a	Inf b	Inf C	Inf h	Inf E
Batch	3	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0045	<.0001	<.0001	0.0006	0.3851
Steam	1	<.0001	<.0001	<.0001	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.3133
Temp	1	0.1885	0.839	0.5644	0.0068	0.0031	0.8923	0.3229	0.6002	0.7191	0.146	0.5443	0.1442	0.1443	0.7583	0.9897
Steam x Temp	1	0.0099	0.0305	0.7075	0.4594	0.0382	0.0605	0.0301	0.2342	0.0115	0.9017	0.1736	0.4248	0.4125	0.1166	0.3251
Time	3	0.0073	0.002	<.0001	<.0001	0.0251	0.0025	<.0001	0.005	0.0306	0.0009	<.0001	0.2613	0.2538	0.0002	<.0001
Steam x Time	3	0.7479	0.7349	0.9161	0.5334	0.8923	0.7472	0.8714	0.0972	0.9754	0.9767	0.0115	0.8747	0.8779	0.0517	0.1498
Temp x Time	3	0.3692	0.2965	0.3807	0.1973	0.053	0.2635	0.0029	0.6229	0.477	0.6799	<.0001	0.5595	0.5485	0.0005	0.9932
Steam x Temp x Time	3	0.5035	0.4517	0.3094	0.8292	0.0473	0.3415	0.3688	0.6142	0.6434	0.9117	0.0132	0.8563	0.8588	0.0513	0.5267

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH. 'Mg' = mangiferin, 'IsoMg' = isomangiferin, 'Vic2' = vicenin-2, 'ErioG' = eridictyol-*O*-glucoside, 'IriGlc' = iriflophenone-3-*C*-glucoside, 'ErioT' = eriocitrin, 'MacG' = maclurin-3-*C*-glucoside, 'Hd' = hesperidin, 'TSS' = total soluble solids, 'Inf' = infusion. Significant ($p < 0.05$) p-values indicated in yellow.

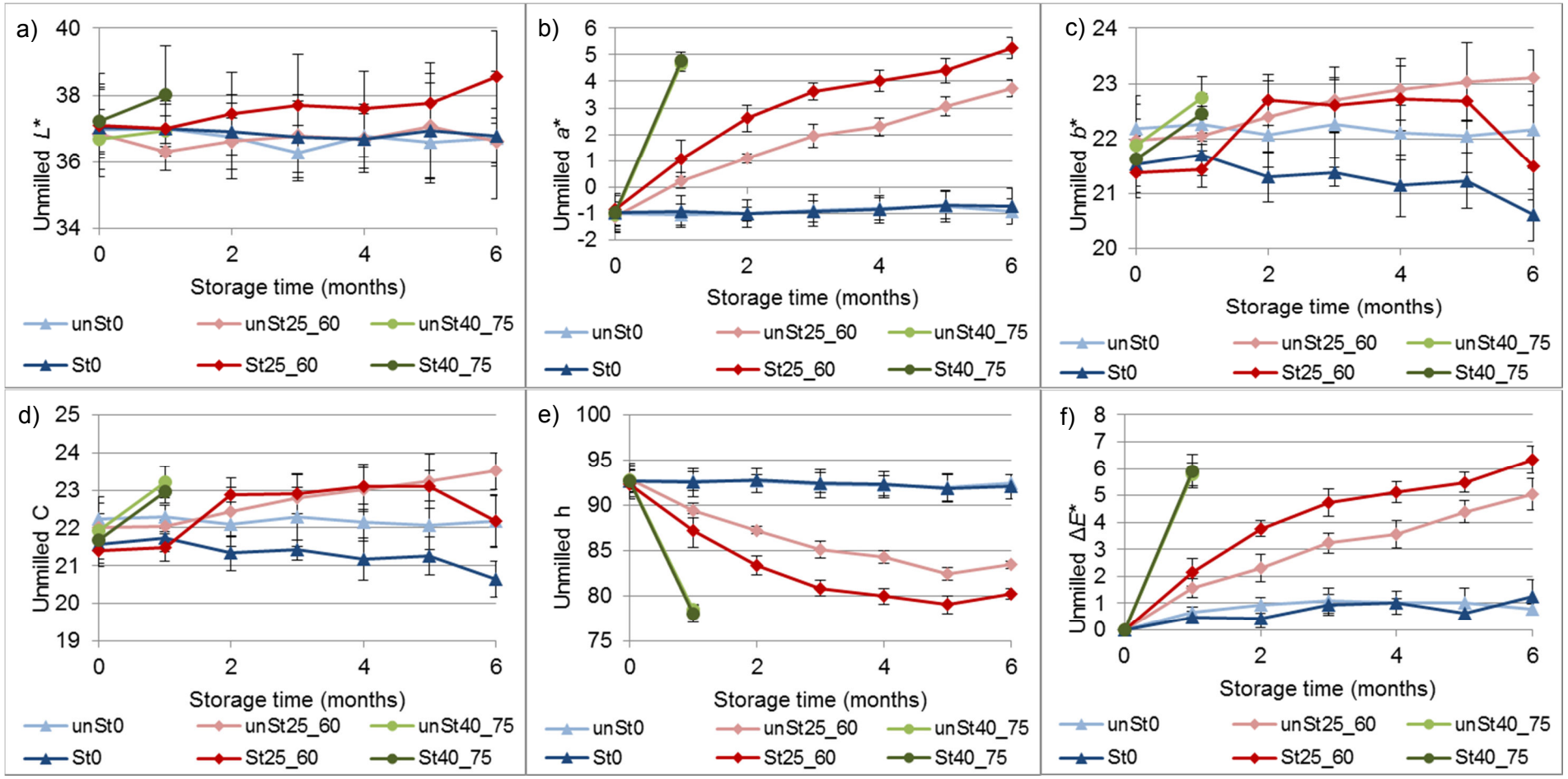


Figure B.3 Measured (a – c) and calculated (d – f) objective colour parameters of unmilled plant material of steamed and unsteamed green *C. maculata* over storage of 6 months under LTS (0), NTS (25_60) and HTS (40_75).

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH.

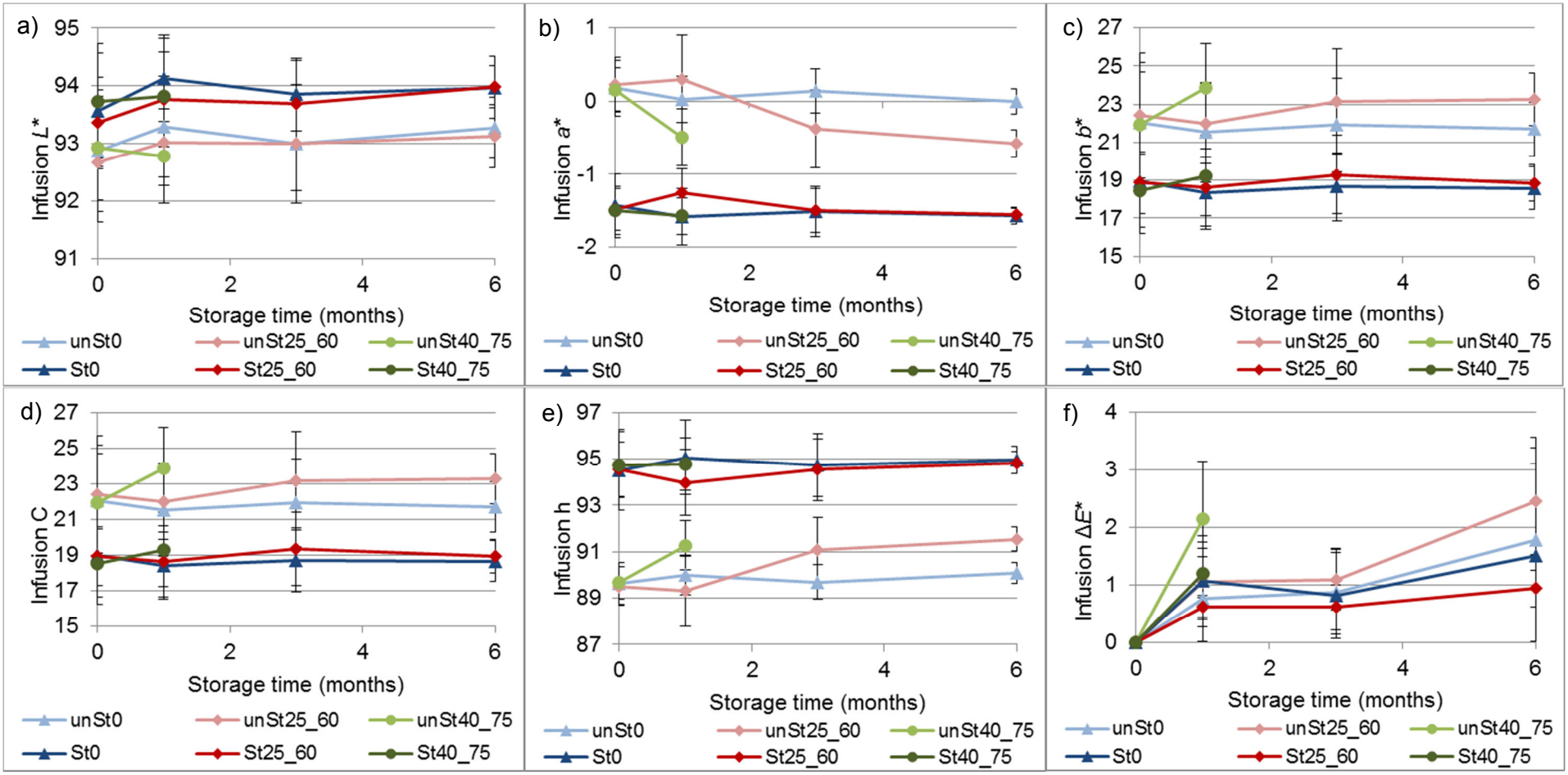


Figure B.4 Measured (a – c) and calculated (d – f) objective colour parameters of infusions of steamed and unsteamed green *C. maculata* over storage of 6 months under LTS (0), NTS (25_60) and HTS (40_75).

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH.

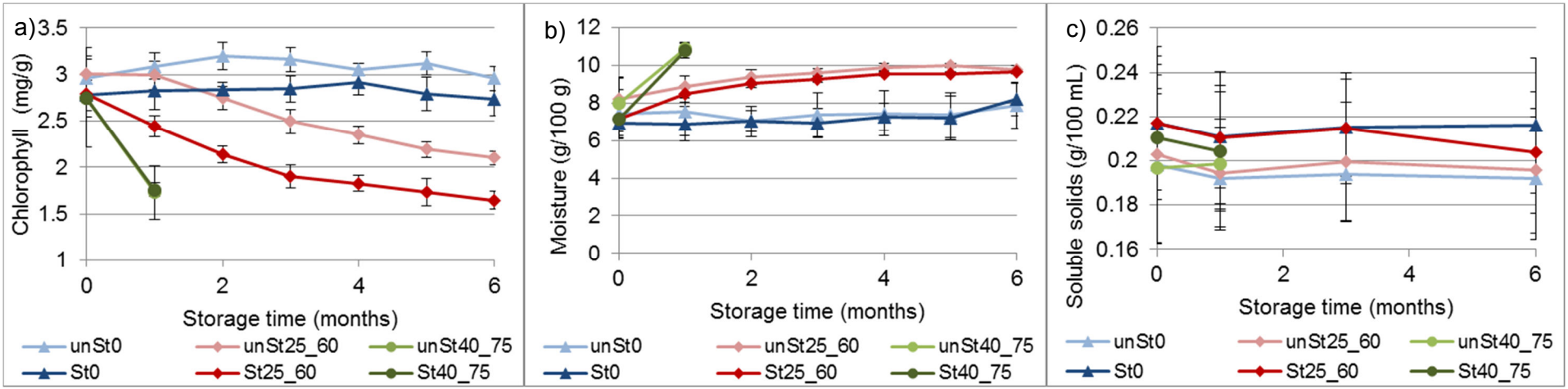


Figure B.5 (a) Total chlorophyll content and (b) percentage moisture content of plant material and (c) total soluble solids of infusions of steamed and unsteamed green *C. maculata* over storage of 6 months under LTS (0), NTS (25_60) and HTS (40_75).

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH.

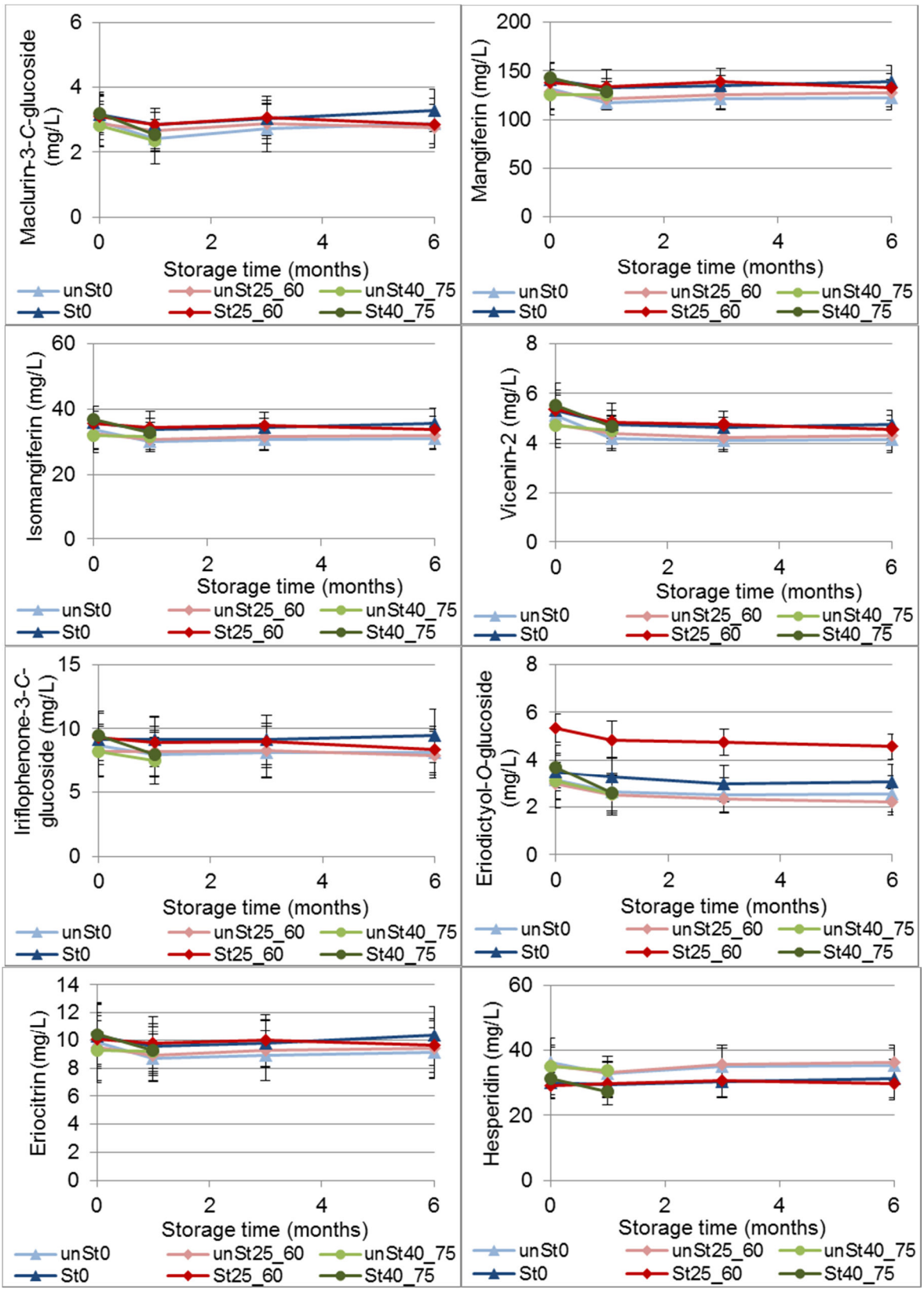


Figure B.6 Individual phenolic contents of infusions of steamed and unsteamed green *C. maculata* over storage of 6 months under LTS (0), NTS (25_60) and HTS (40_75).

LTS = low temperature storage in heat-sealed plastic coated aluminium metal laminated pouches at 0° C, NTS = normal temperature storage in BOPP plastic sachets at 25 °C and 60% RH, HTS = high temperature storage in BOPP plastic sachets at 40 °C and 75% RH.